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Systematics of Multillidae (Hymenoptera) With Special Emphasis on Dasymutilla and Their Allies

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SYSTEMATICS OF MUTILLIDAE (HYMENOPTERA) WITH SPECIAL EMPHASIS ON *DASYMUTILLA* AND THEIR ALLIES

by

Kevin A. Williams

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

of

DOCTOR OF PHILOSOPHY

in

Biology

Approved:

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2012

ABSTRACT

Systematics of Mutillidae (Hymenoptera)

with special emphasis on *Dasymutilla* and their allies

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Kevin A. Williams, Doctor of Philosophy

Utah State University, 2012

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

Velvet ants are solitary wasps that have been virtually ignored in the past. Although over 4200 species have been described, fewer than 10 articles are published on these wasps each year. Their research potential is hindered by lack of phylogenetic and taxonomic data and lack of interest in the scientific community. In this dissertation, I sought to overcome the hindrances to mutillid research with a holistic systematic research model. By reconstructing phylogenies using molecular methods and correcting taxonomy based on the phylogenetic reconstructions, I was able to diminish the barriers to velvet ant research while concurrently presenting broadly interesting hypotheses. I applied this model to multiple hierarchical levels within the most widely studied velvet ant genus, *Dasymutilla*, and its allies. Molecular phylogenetic reconstructions, particularly those using the internal transcribed spacer units of ribosomal DNA (ITS1 and

ITS2), were effective under Bayesian criteria. My results reveal the utility of velvet ants for studying biogeography and mimicry. I specifically determined that velvet ants dispersed between North and South America prior to the Great American Biotic Interchange and members of the genus *Dasymutilla* form the world's largest known Müllerian mimicry complex. Taxonomic problems in *Dasymutilla* and their allies are addressed in the remaining sections of the dissertation. Overall, 35 species were treated taxonomically. Seven new sex associations were discovered, 22 species were recognized as synonyms, and nine new species were described. In each taxonomic treatment, hypotheses concerning biogeography, mimicry, and host selection were discussed briefly.

(341 pages)

PUBLIC ABSTRACT

Systematics of Mutillidae (Hymenoptera)
with Special Emphasis on *Dasymutilla* and Their Allies

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Velvet ants are solitary wasps that are excellent candidates as model organisms for studying evolutionary phenomena like mimicry and biogeography. They can also be used in conservation studies because of their local abundance. Their abundance, coupled with parasitic lifestyle on native pollinators, suggests they will be economically important as native pollinators are implemented more widely. Velvet ants, however, are rarely studied and their research potential is drastically hindered by lack of phylogenetic and taxonomic data and lack of interest in the scientific community. In this dissertation, I sought to overcome the hindrances to mutillid research with a holistic systematic research model. By reconstructing the phylogeny using molecular methods and correcting taxonomy based on the phylogenetic reconstructions, I was able to overcome the barriers to velvet ant research while concurrently presenting broadly interesting hypotheses. I applied this model to multiple hierarchical levels within the most widely studied velvet ant genus, *Dasymutilla*, and its allies. My results reveal the utility of velvet ants for studying biogeography and mimicry. I specifically determined that velvet ants dispersed between North and South America prior to the Great American Biotic Interchange and members of the genus *Dasymutilla* form the world's largest known Müllerian mimicry complex. Taxonomic problems in *Dasymutilla* and their allies are tackled in the remaining sections of the dissertation. Overall, 32 species were treated taxonomically. Seven new sex associations were discovered, 22 species were recognized as synonyms of previously known species, and nine new species were described. In each taxonomic treatment, hypotheses concerning biogeography, mimicry, and host selection were discussed briefly. This dissertation permits study of the species included therein and promotes new studies in multiple facets by future collaborators.

(341 pages)

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CHAPTER 1

INTRODUCTION

Without question or dispute, the most interesting and beautiful animals on earth are members of the wasp family Mutillidae. These solitary vespoid wasps are commonly known as velvet ants. They are typically external parasitoids on the larvae or pupae of apoid Hymenoptera. Female mutillids are completely apterous, while males are typically fully winged and capable of flight. Over 4,000 species have been described (Lelej 2005). Mutillids occur on each habitable continent and in virtually every habitat type. They are especially abundant in tropical forests and deserts. These wasps have the potential to be model organisms for biogeography and mimicry research and they are important natural enemies of pollinating insects. Research on these wasps is hindered by taxonomic uncertainty and limited availability of natural history and phylogenetic information.

Many of the taxonomic issues in Mutillidae are common to all insect groups. Descriptions and diagnoses of more than half of Mutillidae are incomplete and inadequate for accurate identification (e.g. Schuster 1958, Casal 1969a). Many type specimens are lost (Pitts, pers. obs.), or were never designated (e.g. Schuster 1958). In many cases lectotypes were improperly designated (e.g. Mickel 1928) and subsequent authors have not addressed these improper type designations (e.g. Manley and Pitts 2007). Because velvet ants are marked by setal and integumental color patterns, consistent structural characteristics have been virtually ignored in favor of coloration for diagnosing species (e.g., Manley and Pitts 2007). When structurally diagnostic features, like male genitalia, were discussed and illustrated, often a single view was drawn and diagnostic features were not effectively discussed (e.g. Mickel 1928). Mutillidae are marked by extreme sexual dimorphism, which further complicates taxonomy. Coloration and size often differ between the sexes as well; therefore, many species and genera are known only from a single sex (Krombein 1979, Nonveiller 1990). In many instances, associating the sexes necessitates synonymy of previously recognized forms (e.g. Pilgrim et al. 2008). In some cases, sexes were mis-associated and incorrect synonymies were made (e.g. Mickel 1934), which necessitated subsequent species resurrection (e.g. Brabant et al. 2010). Undescribed new species are also common (KAW, pers.obs.).

Velvet ant defenses, mating systems, and host selection are fertile grounds for experimental research, but remain largely unstudied. Velvet ants are protected by a suite of defense mechanisms, which often include stridulation, pungent exudate secretions, a strong slippery cuticle, and a painful sting (Manley 2000, Schmidt and Blum 1977). These defenses are usually accompanied by brilliant aposematic color patterns, which are often shared by multiple unrelated velvet ant species. These overlapping color patterns may indicate mimicry complexes, but few trials have been done to see if mutillids are attacked by any predators (Vitt and Cooper 1988, Manley and Sherbrooke 2001) and no experiments have been done to compare the effectiveness of warning color in velvet ants. The wingless nature of females reduces their dispersal capabilities. These can be partially overcome through phoretic copulation, wherein winged males carry the females during courtship and mating. In all known mutillids with phoretic copulation, males are significantly larger than females (Brothers 1989). Fewer than 20% of velvet ant species are known from both sexes, though, and observations of mating behavior have been published for fewer than 20 species (e.g. Brothers 1972; Bayliss and Brothers 1996, 2001; Tormos et al. 2010). As parasitoids of bees and apoid wasps, mutillids could become economic pests when native pollinators are used more frequently. Multiple genera have broad host ranges (Pitts and Manley 2004, Pitts et al. 2004) and many species are locally or seasonally abundant (Manley 1980, Schmidt and Hook 1979, Schmidt and Bachmann 1986). Host information, however, is known for only a few species.

Molecular data have greatly advanced velvet ant research in recent years. Genetic distance data, particularly from the internal transcribed spacer units (ITS1 and ITS2) of the ribosomal DNA have been used effectively to associate the dimorphic velvet ant sexes (Pilgrim and Pitts 2006). These techniques have allowed sex associations to be made in multiple genera (Pilgrim et al. 2008, Pitts et al. 2009). Genetic distances in ITS1 and ITS2 sequences also allowed species limits to be tested, leading to synonymy of conspecific color forms that had been treated as valid species in the past (Pilgrim et al. 2008). Perhaps even more effectively, molecular data have also been used to reconstruct mutillid phylogenies. Phylogeny reconstructions have allowed synonymy of color forms when genetic distances alone did not provide clear answers (Pilgrim et al. 2009). Phylogeny reconstructions have also allowed hypothesis testing in velvet ants. Molecular phylogenies have allowed velvet ants to become model organisms for historical biogeography. These studies have revealed both Pleistocene glaciation and Neogene mountain building events as driving factors in warm desert diversification (Pitts et al. 2010; Wilson and Pitts 2010a, 2010b, 2011). These studies have also revealed the first cryptic mutillid species (Wilson et al. 2012).

In light of limitations to velvet ant research caused by taxonomic uncertainty and inadequate natural history data, and in light of the advancements that have already been made in this group using molecular techniques, this dissertation addresses taxonomic and evolutionary hypotheses using molecular phylogenetics at multiple hierarchical levels. First, two broad molecular phylogenies are used to address biogeography and mimicry systems at the genus level. Second, three narrower molecular phylogenies of species-groups within these genera are used to address taxonomy, mimicry systems, and natural history patterns. Third, the taxonomy of one species-group and one small genus is revised using morphological data. All of these studies were done on the most conspicuous New World velvet ants, the dasymutilline genera.

Dasymutillines make up roughly 25% of the New World mutillid species (427 of 1717) and include seven genera: *Cephalomutilla* André, *Dasymutilla* Ashmead, *Leucospilomutilla* Ashmead, *Reedomutilla* Mickel, *Suareztilla* Casal, *Tobantilla* Casal and *Traumatomutilla* André (Krombein 1979, Nonveiller 1990; Table 1.1). These genera are allied by the following traits: the first metasomal segment is narrow and usually petiolate, the pygidium in both sexes is defined by lateral carinae, the males have dentate or truncate projections on the axillae, both sexes lack definite bands of plumose setae on the metasomal fringes. Additionally, almost all members of these genera are diurnal and brightly colored. Although their monophyly has not been tested directly, we assume that they form a natural grouping based on their shared morphology.

Dasymutilla is the most frequently studied genus in Mutillidae and it currently includes 202 species. More sex associations are known in Dasymutilla than other mutillid genera; fifteen such associations were made since 2000 (Manley and Radke 2002, 2006;

Table 1.1. Overview of diversity in dasymutilline genera. Numbers in parentheses represent undescribed morphospecies.

Genus	**Male only**	**Female only**	**Both sexes**	**Total**
Cephalomutilla	0	11(1)	0	11(1)
Dasymutilla	61	84	57	202
Leucospilomutilla	0	2	1	3
Reedomutilla	1	1	2	4
Suareztilla	4	5	0	9
Tobantilla	4	6	0	10
Traumatomutilla	49(31)	137(23)	2	188(54)
Total	119	246	62	427

Manley 2003a, 2003b; Manley and Yanega 2005; Manley and Pitts 2007; Pilgrim et al., 2008). Even with this recent work, only 57 *Dasymutilla* species are known from both sexes (Krombein 1979, Manley and Pitts 2007). It should be noted, however, that this is much better than most North American genera where fewer than 25% are known from both sexes (Krombein 1979). Dasymutillines are typically large and colorful, which makes them easier to observe in nature. There is more available information about courtship behavior, host selection, parasitic behavior, activity periods, and natural enemies than in other genera (e.g. Mickel 1928, Manley 1977, Manley and Taber 1978, Manley 1984, Manley and Deyrup 1987).

The brilliant coloration and conspicuous behavior that attracted abundant taxonomic and biological research on *Dasymutilla* has played too conspicuous a role in systematics. *Dasymutilla* is one of the only genera of Mutillidae where subspecific classification and rampant, over-splitting based on minor coloration differences has occurred. For example, *Dasymutilla vesta* (Cresson), the most widespread species in the United States, has 17 synonyms (Manley and Pitts 2007). Recent phylogenetic revision of

D. quadriguttata (Say) revealed 18 synonyms of this species (Pilgrim et al. 2009). In Mickel's (1928, 1936) initial revisions, the *D. foxi* (Cockerell) species-group included three species known from both sexes; more recent publications, however, show that these species are all members of a single widespread species with variable coloration (Manley 1980, Pilgrim et al. 2008). Preliminary molecular and morphological data suggest over 80 other *Dasymutilla* species are synonymous forms (KAW pers. obs.). For better or for worse, *Dasymutilla* has received more taxonomic and biological attention than any other American genus of Mutillidae, making it an excellent candidate for phylogenetic research.

The closely related, and potentially synonymous, *Traumatomutilla* present a completely different suite of taxonomic problems, stemming from being virtually ignored taxonomically. This exclusively Neotropical genus is nearly as species-rich as *Dasymutilla*, with over 200 known species. Only two of these species are known from both sexes. Like *Dasymutilla*, many of the species are diagnosed by color only, suggesting numerous conspecific forms and synonyms will be necessary. Unlike *Dasymutilla*, however, it has been over 40 years since the most recent publication on *Traumatomutilla* (Casal 1969a) and numerous undescribed species likely exist. Also, the biology and behavior of *Traumatomutilla* is virtually unknown.

Species-group nomenclature has been used to varying degrees in both *Dasymutilla* and *Traumatomutilla* (e.g. Mickel 1928, 1952; Casal, 1969a). In *Dasymutilla*, groups were proposed by Mickel (1928), where they were described and catalogued well. Many of these species-groups, however, actually represent variable single species (e.g., *D. foxi*), and the Neotropical species of *Dasymutilla* have not been placed into groups (Manley and Pitts 2007). In *Traumatomutilla* the species-groups are not easily recognizable, and most species have never been formally placed into a species-group (*e.g.*: Burmeister 1854, Gerstaecker 1874, Cresson 1902, André 1908, Casal 1969a). Many characters used to separate these species-groups, such as truncate femora and tubercles on the head, are apparently not autapomorphic, as they are seen in both genera and multiple species-groups. Even with their shortcomings, species-groups are valuable for categorizing the diversity in dasymutilline genera, but new diagnostic features and rearrangement of included species are necessary.

The other five genera in this complex are easily diagnosed, but their validity as isolated monophyletic taxa is questionable. *Suareztilla* and *Reedmutilla* are obviously sister genera and the males can be recognized easily by the strongly upcurved gonoforceps of the genitalia. *Leucospilomutilla* are also separated from other genera by the male gonoforceps, which are shortened drastically and truncate apically (Williams, *pers. obs.*). *Tobantilla* is known from females only, and is separated from other Neotropical genera by size and coloration (Casal 1965). *Cephalomutilla* is separated from closely related South American genera by its large head, and is only known from the female sex (Mickel 1960). The defining characters of *Cephalomutilla* and *Tobantilla* are homoplasious, occurring in numerous Nearctic species of *Dasymutilla*. Both *Dasymutilla* and *Traumatomutilla* are variable morphologically, and lack adequate distinguishing characters (Williams, *pers. obs.*). Although *Dasymutilla* is typically found in North America, and *Traumatomutilla* lives throughout South America, there is considerable overlap in ranges (Nonveiller 1990). Regional keys typically separate these genera by coloration, but none of the characters used in these keys account for all of the variation

(Cambra and Quintero 1992, Quintero and Cambra 1997). The most recent key to Neotropical genera does not even attempt to separate *Dasymutilla* and *Traumatomutilla* (Brothers 2006). It is unlikely that *Cephalomutilla*, *Tobantilla*, *Dasymutilla* and *Traumatomutilla* will show reciprocal monophyly. Additionally, some species-groups in *Dasymutilla* and *Traumatomutilla* could prove to be discrete genera. Molecular phylogenetics, coupled with new sex associations, will be necessary to determine valid generic limits.

Throughout this dissertation, I aimed to use phylogenetic reconstructions at varied hierarchical levels to address evolutionary, biogeographical, and taxonomic hypotheses in dasymutilline velvet ants. Chapter 2 of this dissertation sought to elucidate biogeographic patterns and provide a starting phylogeny for dasymutillines. Formation of the Isthmus of Panama in the Pliocene facilitated the Great American Biotic Interchange, widely informed by the mammalian fossil record (Simpson 1980). Recent studies, however, found that intercontinental exchange preceded closure of the Isthmus of Panama in many groups (Cody et al. 2010, Pinto-Sánchez et al. 2011), implicating overwater dispersal and migration via a historically peninsular Lower Central America (e.g. Kirby et al. 2008). Intercontinental exchange could also have occurred across the Caribbean region, either through GAARlandia (Greater Antilles + Aves Ridge; Iturralde-Vincent and MacPhee, 1999) or overwater dispersal between islands and the mainland (e.g., Sturge et al. 2009). Phylogenetic studies, coupled with molecular dating and ancestral area reconstructions, have informed biogeographic research in groups without an extensive fossil record (Zeh et al. 2003, Weigt et al. 2005) and are revealing repeated biogeographic patterns in unrelated groups (Cody et al. 2010). Dasymutillines can inform intercontinental interchange in the New World because of their distribution and natural history. Dasymutillines are known from virtually every habitat in both the Nearctic and Neotropical biomes, including Lower Central America and the Greater Antilles. Because females are apterous and phoretic copulation is unlikely (Deyrup and Manley 1986), long distance dispersal is improbable, closely linking current dasymutilline distributions to historical vicariant events. I used phylogenetic reconstructions to determine where dasymutillines originated and how they colonized the entire New World. In addition to intercontinental patterns, I studied dasymutilline communities in the Caribbean Islands, the Isthmus of Panama, and Nearctic North America. I also determined the age of diversification for most extant dasymutilline species, in order to predict the predominant forces that drove speciation. The results of this chapter were also valuable for determining the validity of the dasymutilline genera.

Chapter 3 of the dissertation examined Müllerian mimicry in *Dasymutilla* females. Müllerian mimicry is a mutualism in which two or more species with effective secondary defenses share a similar appearance for mutual benefit (Müller 1879). Wingless female *Dasymutilla* species are the some of the most brightly colored and well defended insects in North America. Although few have been carried out, feeding trials revealed that predatory lizards avoid larger velvet ants (Vitt and Cooper 1988, Manley 2000, Manley and Sherbrooke 2001) or are quickly trained to recognize and avoid them (Schmidt and Blum 1977, Vitt and Cooper 1988). Several authors have noted the phenotypic similarity among unrelated velvet ant species (Ferguson 1966, Pilgrim and Pitts 2006) and even documented high amounts of color polymorphism within a widespread species (Pilgrim et al. 2008, 2009), yet mimicry within velvet ants has only been reported from selected species that are thought to mimic ants (Yanega 1994, Manley 2000). In this chapter, I identified and described Müllerian mimicry complexes in *Dasymutilla* females and determined whether the mimetic forms resulted from shared ancestry or convergent evolution.

Chapter 4 addressed taxonomy of the *D. monticola* species-group. Of the Nearctic members of *Dasymutilla*, many of the smallest species belong to the *D. monticola* and *D. caneo* species-groups (Mickel 1928). These species appear to form a natural group based on shared morphology and are clearly members of *Dasymutilla*, but it is unclear which *Dasymutilla* taxa are their closest relatives. The *D. monticola* and *D. caneo* species-groups have had reticulating taxonomic history, being initially linked four years ago (Pilgrim et al. 2008) and having members of the *D. subhyalina* species-group (Mickel 1974) and *D. quadriguttata* (Krombein 1951, 1954) species-group linked with them. The usefulness of species-groups in Dasymutilla has already been discussed, but most species described since Mickel's first revision (1928) were never conclusively placed into species-groups (Mickel 1936, 1938; Manley and Pitts 2007). Typically, rather than explicitly conjecturing about inter-specific relationships or species-group placement, authors have implied relations by discussing species identification and providing characters to separate new taxa from previously established species (e.g. Mickel 1938, Schmidt and Mickel 1979, Manley and Radke 2006). In the Neotropical region, there appears to be a homogenous mixture of species that readily fit into Mickel's species-groups and other species that readily fit into undefined species-groups comprised of closely related Neotropical taxa (Manley and Pitts 2007). *Dasymutilla archboldi* Schmidt and Mickel, the only *Dasymutilla* with males significantly larger than females (Deyrup and Manley 1986), is apparently a member of this species-group and could be the only *Dasymutilla* species with phoretic copulation. In this chapter, I used molecular data to reconstruct the phylogeny of the *D. monticola* species-group. I aimed to determine the affiliation of multiple *Dasymutilla* species to the *D. monticola* species-group. I also tested the monophyly of the *D. monticola* species-group. Necessary taxonomic changes, including male genitalia descriptions, sex associations, synonymies, and species-group placement were addressed in light of these results. Finally, implications on natural history were discussed in light of the phylogenetic results.

Chapter 5 examined the phylogeny of thistledown velvet ants and their mimicry patterns. Three Nearctic *Dasymutilla* species are marked by remarkable female coloration, wherein they are entirely clothed with long white setae that give them an almost seed-like appearance. They closely resemble the fruit of Creosote Bush, *Larrea tridentata* (Sesse and Moc. *ex* DC) Coville, with which they share an overlapping geographic range in the warm deserts of North America. Some authors suggested these species use a form of crypsis by camouflaging themselves as Creosote fruit (Ferguson 1967, Williams and Manley 2006). The males of these species, where known, do not resemble females, because they have black ventral and lateral setae and contrasting white, yellow, or red dorsal setae. Because the sexes display different aposematic signals, we consider this an example of dual sex-limited mimicry. In this chapter, I used phylogenetic and morphological methods to study these wasps. Species limits were determined for each thistledown velvet ant. I determined whether these species obtained their female color pattern through shared ancestry or convergence. Based on distribution and color patterns, I identified the mimicry system and potential models for each sex. Additionally, I determined whether dual sex-limited mimicry was driven by selection pressure on males or females.

Chapter 6 used a holistic approach to investigate the species-limits of *Dasymutilla bioculata*. Numerous *Dasymutilla* species were previously differentiated by color characters alone (*e.g.,* Rohwer 1912; Mickel 1928, 1936; Manley and Pitts 2007). Recent studies used ecological, morphological, and molecular methods to show that these "species" were just variants of a single widespread species (*e.g.,* Manley 1980, 1999; Pilgrim et al. 2008, 2009). These studies showed that characters were considered as "discrete" only because of insufficient sample size. As sample size was increased, intermediate forms were discovered, discrediting the validity of those characters. The *D. bioculata* species-group currently includes ten species and eight subspecies that are diagnosed mainly by coloration. Mickel (1928) illustrated the male genitalia of only one species, because he found all of the species to have identical genitalia. These species are often notoriously difficult to identify due to the existence of specimens with intermediate coloration or structural characters. Most of the characters used to separate these taxa are polymorphic in other *Dasymutilla* species. The tenuous nature of characters used to differentiate the species of the *D. bioculata* species-group, coupled with the identical male genitalia, suggests that some, if not all, of these species may be conspecific. Molecular sequence data also suggested that some of the species of the *D. bioculata* species-groups could be conspecific. In the study that initially displayed the effectiveness of ITS1 and ITS2 for testing species limits, Pilgrim and Pitts (2006) compared sequences of *D. ch. ursula* (Cresson) and *D. lepeletierii* (Fox) and found the sequences completely identical in ITS1 and 99.6-99.7% identical in ITS2 (Pilgrim and Pitts 2006). In this chapter, I evaluated the species validity of 14 taxa using four lines of evidence: structural and color-based morphology, field observations of mating pairs, geographical distribution, and genetic distances using ITS1 and ITS2.

The results of Chapter 6 allowed taxonomy of Latin American members of the *D. bioculata* species-group to be studied in Chapter 7. The shape of the genitalic cuspis in *D. bioculata* was found to be unique among Nearctic *Dasymutilla*. Five Latin American species were found with similar male genitalia. Three of these species, *D. nocticaro* Manley and Pitts, *D. truxali* Manley and Pitts, and *D. veracruz* Manley and Pitts, are found in Mexico and known exclusively from the male sex; these species have never been placed in a species-group. The other two taxa, *D. bouvieri* (Andre) and *D. insulana* Mickel, are found in the Greater Antilles and are known from both sexes; Genaro (1997) placed these species in his Group II based on similarities in coloration. Genaro's (1997) species-groups, however, were only applicable to the Caribbean fauna and not compared to the species-groups of Mickel (1928). Although the males of *D. bouvieri* and *D. insulana* were recognized and keyed by Genaro (1997), full descriptions were never proposed. Based on structural similarities to *D. bioculata*, the five aforementioned species and three species known only from females (*D. chionothrix* Manley and Pitts, *D. concordia* Manley and Pitts, and *D. latebalteata* Manley and Pitts) should be transferred to the *D. bioculata* species-group. Using morphological and distributional data, new sex associations were made in the Mexican fauna. This study, coupled with Chapter 6, provides a full synthesis of the *D. bioculata* species-group throughout its range and displays the relevance of previous molecular data to morphological studies of related taxa.

Chapter 8 revises the South American genus *Tobantilla*. This genus was erected to include a single new species, *T. montonera*, which was known from females only (Casal 1965). Casal (1969b) described a second species, *T. charrasca*, which was also known only from females. In this same paper, Casal discussed males that had been collected in the same locality as *T. charrasca* and generally resembled the putative relatives of *Tobantilla*: *Traumatomutilla* André, *Reedomutilla* Mickel, and *Suareztilla* Casal. Although they have been discussed in the literature briefly, males of *Tobantilla* remain undescribed and the genus is still recorded only from Argentina (Nonveiller 1990). In Chapter 8, I described seven new species of *Tobantilla* from Argentina, Colombia and Venezuela and redescribed the previously known species to provide a consolidated account. During this study, we also found and described a new Argentinean genus and species that resembles *Tobantilla* in both sexes.

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CHAPTER 2

HISTORICAL BIOGEOGRAPHY OF DASYMUTILLINE VELVET ANTS (HYMENOPTERA: MUTILLIDAE): RETHINKING THE GREAT AMERICAN BIOTIC INTERCHANGE[1]

1. Introduction

Interchange of biotic lineages between North and South America shaped the faunal composition of both continents. Formation of the Isthmus of Panama in the Pliocene facilitated the Great American Biotic Interchange, widely based on the mammalian fossil record (Simpson, 1980). Recent studies, however, have found that intercontinental exchange preceded closure of the Isthmus of Panama in many groups (Cody et al., 2010; Pinto-Sánchez et al., 2011), implicating overwater dispersal and migration via a historically peninsular Lower Central America (e.g. Kirby et al., 2008). Intercontinental exchange could also have occurred across the Caribbean region, either through GAARlandia (Greater Antilles + Aves Ridge; Iturralde-Vincent & MacPhee, 1999) or overwater dispersal between islands and the mainland (e.g., Sturge et al., 2009). Phylogenetic studies, coupled with molecular dating and ancestral area reconstructions, have informed biogeographic research in groups without an extensive fossil record (Weigt et al., 2005; Zeh et al., 2003) and are revealing repeated biogeographic patterns in unrelated groups (Cody et al., 2010).

Phylogenetic studies of unrelated groups with varying natural histories contribute to a more complete understanding of New World biogeography. Velvet ants (Mutillidae)

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[1] This manuscript is formatted for submission to *Molecular Phylogenetics and Evolution*.

are solitary wasps that usually parasitize apoid Hymenoptera. Sexual dimorphism is extreme in this group, as females always lack wings and males are usually fully winged. Nocturnal members of this family have been used extensively to study biogeographic patterns in western North American deserts (Pitts et al., 2010; Wilson and Pitts, 2008, 2010a, 2010b, 2011). Roughly 20% of New World velvet ants form a complex of seven genera (Krombein, 1979; Nonveiller, 1990; KAW pers. obs.), hereafter referred to as “the dasymutilline genera” (Table 2.1, 2.2). These insects share the following characters: the first metasomal segment is narrow and usually petiolate, the pygidium in both sexes is defined by lateral carinae, the males have dentate or truncate projections on the axillae, both sexes lack definite bands of plumose setae on the metasomal fringes. Almost all members of these genera are diurnal and brightly colored. Although the phylogeny of these insects is unknown, they form a putative natural grouping based on their shared morphology.

Dasymutillines can inform intercontinental interchange in the New World because of their distribution and natural history. Over 200 species are known from South America and over 150 occur in North America (Krombein, 1979; Manley & Pitts, 2007; Nonveiller, 1990; Table 2.1). Dasymutillines are known from virtually every habitat in both the Nearctic and Neotropical biomes. Over 20 species are recorded from the Isthmus of Panama and nine species live on Caribbean islands (6 in Greater Antilles, 3 in Trinidad) (Manley & Pitts, 2007; Nonveiller, 1990;). Because females are apterous and phoretic (airborne) copulation is unlikely (Deyrup & Manley, 1986), long-distance dispersal over water is improbable, closely linking current dasymutilline distributions to historical vicariant events.

Table 2.1
List of dasymutilline species, which sexes they are known from, and their distributions based on Fig. 1. Subspecific epithets are listed alphabetically excluding their current specific epithets. Asterisks (*) denote species that we have not examined.

Genus	species	Sex	Area	Genus	species	Sex	Area
Cephalomutilla	*albicalcaris*	♀	A	*Dasymutilla*	*chamela*	♂	CD
Cephalomutilla	*argyrostictia*	♀	A	*Dasymutilla*	*chilcotti*	♂	D
Cephalomutilla	*confluenta**	♀	A	*Dasymutilla*	*chionothrix*	♀♂	D
Cephalomutilla	*distincta*	♀	A	*Dasymutilla*	*chisos*	♂	D
Cephalomutilla	*fasciata*	♀	A	*Dasymutilla*	*cirrhomeris*	♀	CD
Cephalomutilla	*flavigastra*	♀	A	*Dasymutilla*	*citromaculosa*	♀	CD
Cephalomutilla	*graviceps*	♀	A	*Dasymutilla*	*clotho*	♀	D
Cephalomutilla	*haematodes*	♀	A	*Dasymutilla*	*coccineohirta*	♀♂	D
Cephalomutilla	*transversa*	♀	A	*Dasymutilla*	*combusta*	♀	D
Cephalomutilla	*vivata*	♀	A	*Dasymutilla*	*cotulla*	♂	D
Cephalomutilla	*zelichi*	♀	A	*Dasymutilla*	*creon*	♀♂	D
Dasymutilla	*aequitorialis**	♂	A	*Dasymutilla*	*cressoni*	♀	C
Dasymutilla	*albiceris*	♀♂	D	*Dasymutilla*	*curialis*	♂	D
Dasymutilla	*altamira*	♀	C	*Dasymutilla*	*dammersi*	♀	D
Dasymutilla	*andreniformis**	♂	C	*Dasymutilla*	*dawsoni*	♀	D
Dasymutilla	*angulata*	♀	D	*Dasymutilla*	*deyrollesi*	♂	BC
Dasymutilla	*apicalata*	♂	D	*Dasymutilla*	*digressa*	♂	D
Dasymutilla	*arachnoides*	♀	CD	*Dasymutilla*	*dilucida*	♀	D
Dasymutilla	*araneoides*	♀♂	BC	*Dasymutilla*	*dionysia*	♀	D
Dasymutilla	*archboldi*	♀♂	D	*Dasymutilla*	*dorippa*	♂	D
Dasymutilla	*arenivaga*	♀♂	D	*Dasymutilla*	*eminentia*	♀♂	D
Dasymutilla	*arenneronea*	♀♂	D	*Dasymutilla*	*erythrina*	♀♂	D
Dasymutilla	*argentea*	♀	C	*Dasymutilla*	*eurynome*	♀	D
Dasymutilla	*asopus*	♀♂	D	*Dasymutilla*	*fasciventris*	♂	CD
Dasymutilla	*asteria*	♀	D	*Dasymutilla*	*ferruginea*	♀	CD
Dasymutilla	*atricauda*	♀♂	D	*Dasymutilla*	*fimbrialis*	♂	D
Dasymutilla	*aureola*	♀♂	D	*Dasymutilla*	*fimbriata*	♂	C
Dasymutilla	*azteca*	♂	BC	*Dasymutilla*	*flammifera*	♀♂	D
Dasymutilla	*baleygi*	♂	C	*Dasymutilla*	*formosa*	♀	CD
Dasymutilla	*batui*	♀	C	*Dasymutilla*	*foxi*	♀♂	D
Dasymutilla	*bellatrix*	♀	A	*Dasymutilla*	*furina*	♀♂	D
Dasymutilla	*bioculata*	♀♂	D	*Dasymutilla*	*gentilicia*	♂	D
Dasymutilla	*birkmani*	♀♂	D	*Dasymutilla*	*gibbosa*	♀♂	D
Dasymutilla	*blattoserica*	♀♂	A	*Dasymutilla*	*gloriosa*	♀♂	D
Dasymutilla	*boharti*	♂	D	*Dasymutilla*	*glycera*	♂	D
Dasymutilla	*bonita*	♀♂	CD	*Dasymutilla*	*gorgon*	♀	D
Dasymutilla	*bouvierii*	♀♂	E	*Dasymutilla*	*griswoldi*	♀	C
Dasymutilla	*brazillia*	♂	A	*Dasymutilla*	*guanacaste*	♂	BC
Dasymutilla	*buenavista*	♂	C	*Dasymutilla*	*hector*	♂	D
Dasymutilla	*californica*	♀♂	D	*Dasymutilla*	*heliophila*	♀♂	D
Dasymutilla	*calorata*	♀♂	D	*Dasymutilla*	*hispidaria*	♀	D
Dasymutilla	*campanula*	♀	D	*Dasymutilla*	*holotricha*	♀	C

Genus	**species**	**Sex**	**Area**	**Genus**	**species**	**Sex**	**Area**
Dasymutilla	*campogrande*	♀	A	*Dasymutilla*	*homochroma*	♀	A
Dasymutilla	*canella*	♀♂	D	*Dasymutilla*	*homole*	♀	D
Dasymutilla	*canina*	♀	BC	*Dasymutilla*	*icaris*	♀	AB
Dasymutilla	*cardinalis*	♀	D	*Dasymutilla*	*impressa*	♂	D
Dasymutilla	*chalcocephala*	♀	BC	*Dasymutilla*	*insulana*	♀♂	E
Dasymutilla	*ionothorax*	♂	B	*Dasymutilla*	*rutilliventris**	♂	CD
Dasymutilla	*iztapa*	♂	BC	*Dasymutilla*	*sackenii*	♀♂	D
Dasymutilla	*jalisco*	♀♂	C	*Dasymutilla*	*saetigera*	♀♂	D
Dasymutilla	*klugii*	♀♂	D	*Dasymutilla*	*satanas*	♀♂	D
Dasymutilla	*klugiodes*	♀	D	*Dasymutilla*	*scabra*	♀♂	D
Dasymutilla	*lachesis*	♀	D	*Dasymutilla*	*scaevola*	♀♂	D
Dasymutilla	*latebalteata*	♀♂	C	*Dasymutilla*	*schumanni**	♀	C
Dasymutilla	*leda*	♀	D	*Dasymutilla*	*scitula*	♀♂	D
Dasymutilla	*leve*	♀	D	*Dasymutilla*	*serenitas*	♂	D
Dasymutilla	*loreto*	♂	D	*Dasymutilla*	*sicheliana*	♀♂	CD
Dasymutilla	*macilenta*	♀♂	D	*Dasymutilla*	*sinaloa*	♂	D
Dasymutilla	*macra*	♂	D	*Dasymutilla*	*sleipniri*	♂	B
Dasymutilla	*magna*	♀♂	D	*Dasymutilla*	*snoworum*	♀♂	D
Dasymutilla	*magnifica*	♀♂	D	*Dasymutilla*	*sophrona*	♂	D
Dasymutilla	*mazatlanorum*	♂	D	*Dasymutilla*	*spilota*	♀	B
Dasymutilla	*melanargyrea*	♀	C	*Dasymutilla*	*spiniscapula*	♂	E
Dasymutilla	*melancholica*	♀	E	*Dasymutilla*	*stevensi*	♀♂	D
Dasymutilla	*militaris*	♀	E	*Dasymutilla*	*terminata*	♂	C
Dasymutilla	*mirabilis*	♀♂	B	*Dasymutilla*	*testaceiventris*	♂	D
Dasymutilla	*mirapenis*	♂	B	*Dasymutilla*	*texanella*	♀	D
Dasymutilla	*modgudi*	♂	C	*Dasymutilla*	*thetis*	♀♂	D
Dasymutilla	*monstrosa*	♀	D	*Dasymutilla*	*toluca*	♀	CD
Dasymutilla	*monticola*	♀♂	D	*Dasymutilla*	*tomasi*	♀	D
Dasymutilla	*montivagoides*	♀	D	*Dasymutilla*	*twegeni*	♀	C
Dasymutilla	*munifica*	♀	BC	*Dasymutilla*	*ulkei*	♀	D
Dasymutilla	*myrice*	♂	D	*Dasymutilla*	*uniguttata*	♀	D
Dasymutilla	*naranjo*	♂	BC	*Dasymutilla*	*vandala*	♂	D
Dasymutilla	*neomexicana*	♂	D	*Dasymutilla*	*vesta*	♀♂	D
Dasymutilla	*nigriceps*	♀	E	*Dasymutilla*	*vestita*	♀♂	D
Dasymutilla	*nigripes*	♀♂	D	*Dasymutilla*	*vulpina*	♀	CD
Dasymutilla	*niphopilis*	♀	D	*Dasymutilla*	*waco*	♀♂	D
Dasymutilla	*nocticaro*	♀♂	C	*Dasymutilla*	*wasbaueri*	♂	BC
Dasymutilla	*nocturna*	♀♂	D	*Dasymutilla*	*whartoni*	♀	D
Dasymutilla	*nupera*	♀	D	*Dasymutilla*	*wileyae*	♀	D
Dasymutilla	*occidentalis*	♀♂	D	*Dasymutilla*	*xalisco*	♀	C
Dasymutilla	*ocyrae*	♀	BC	*Dasymutilla*	*yucatana*	♂	C
Dasymutilla	*parkerorum*	♀	C	*Dasymutilla*	*zelaya*	♀♂	D
Dasymutilla	*parksi*	♀	D	*Dasymutilla*	*zoster*	♀	BC
Dasymutilla	*perilla*	♂	D	*Leucospilomutilla*	*cerbera*	♀♂	A
Dasymutilla	*personata*	♂	C	*Leucospilomutilla*	*chemea*	♀	A
Dasymutilla	*peruviana**	♂	A	*Leucospilomutilla*	*staurogastra*	♀	A
Dasymutilla	*phaon*	♂	D	*Reedomutilla*	*dureti*	♀♂	A

Genus	**species**	**Sex**	**Area**	**Genus**	**species**	**Sex**	**Area**
Dasymutilla	*phya*	♀	BC	*Reedomutilla*	*gayi*	♀♂	A
Dasymutilla	*proclea*	♀	C	*Reedomutilla*	*heraldica*	♀	A
Dasymutilla	*pseudopappus*	♀	D	*Reedomutilla*	*pubescens*	♂	A
Dasymutilla	*pulchra*	♀♂	BC	*Suareztilla*	*bimaculata*	♂	A
Dasymutilla	*quadriguttata*	♀♂	D	*Suareztilla*	*calycina*	♀	A
Dasymutilla	*radkei*	♀	D	*Suareztilla*	*centrolineata*	♀	A
Dasymutilla	*relata**	♂	C	*Suareztilla*	*centrovittata*	♀	A
Dasymutilla	*rubicunda*	♀	D	*Suareztilla*	*clypeata*	♂	A
Dasymutilla	*rufopilis*	♀	D	*Suareztilla*	*colorata*	♂	A
Suareztilla	*gazagnairei*	♀	A	*Traumatomutilla*	*chasca*	♀	A
Suareztilla	*leucotaenia*	♀	A	*Traumatomutilla*	*chilca*	♀	A
Suareztilla	*vulneriventris*	♂	A	*Traumatomutilla*	*chingona*	♀	A
Tobantilla	*aleatrix*	♀	A	*Traumatomutilla*	*chrysozona*	♂	A
Tobantilla	*andrikos*	♂	A	*Traumatomutilla*	*chusa*	♀	A
Tobantilla	*charrasca*	♀	A	*Traumatomutilla*	*comata**	♂	A
Tobantilla	*drosos*	♂	A	*Traumatomutilla*	*compar*	♀	A
Tobantilla	*ephemeros*	♂	A	*Traumatomutilla*	*confluens*	♀	A
Tobantilla	*frigidula*	♀	A	*Traumatomutilla*	*consimilis**	♀	A
Tobantilla	*kolasma*	♀	A	*Traumatomutilla*	*contempta*	♀	A
Tobantilla	*krima*	♀	A	*Traumatomutilla*	*coya*	♀	A
Tobantilla	*montonera*	♀	A	*Traumatomutilla*	*cristata*	♂	A
Tobantilla	*xouthos*	♂	A	*Traumatomutilla*	*crixa*	♀	A
Traumatomutilla	*abrupta*	♀	A	*Traumatomutilla*	*crona*	♀	A
Traumatomutilla	*acara*	♂	A	*Traumatomutilla*	*cuiba*	♂	A
Traumatomutilla	*aemulata*	♀	A	*Traumatomutilla*	*cuyana*	♀	A
Traumatomutilla	*aequinoctialis*	♀	A	*Traumatomutilla*	*demissa*	♀	A
Traumatomutilla	*aethiops*	♂	A	*Traumatomutilla*	*dentata**	♂	A
Traumatomutilla	*affinis*	♀	A	*Traumatomutilla*	*diabolica*	♀	A
Traumatomutilla	*albata*	♀	A	*Traumatomutilla*	*dictynna*	♀	B
Traumatomutilla	*alhuampa*	♀	A	*Traumatomutilla*	*dignitosa*	♂	A
Traumatomutilla	*almada*	♂	A	*Traumatomutilla*	*diopthalma*	♀	A
Traumatomutilla	*ameliae*	♀	A	*Traumatomutilla*	*dives*	♂	A
Traumatomutilla	*americana*	♀	A	*Traumatomutilla*	*dubia*	♀♂	A
Traumatomutilla	*andrei*	♀	A	*Traumatomutilla*	*duplicata*	♀	A
Traumatomutilla	*angustata*	♀	A	*Traumatomutilla*	*estrella*	♀	A
Traumatomutilla	*aterrima*	♂	A	*Traumatomutilla*	*fascinata*	♂	A
Traumatomutilla	*auriculata*	♀	A	*Traumatomutilla*	*feia*	♀	A
Traumatomutilla	*austera*	♀	A	*Traumatomutilla*	*fissiventris*	♂	A
Traumatomutilla	*auxiliaris*	♀	A	*Traumatomutilla*	*floccosa*	♂	A
Traumatomutilla	*baguala*	♀	A	*Traumatomutilla*	*foveiventris**	♂	A
Traumatomutilla	*barra*	♂	A	*Traumatomutilla*	*funebris**	♀	A
Traumatomutilla	*bartica*	♂	A	*Traumatomutilla*	*funesta*	♂	A
Traumatomutilla	*bellica*	♀	A	*Traumatomutilla*	*gausapata*	♀	A
Traumatomutilla	*bellicosa*	♀	A	*Traumatomutilla*	*gemella*	♀	A
Traumatomutilla	*bellifera*	♀	A	*Traumatomutilla*	*gemina*	♀	A
Traumatomutilla	*bifurca*	♀	A	*Traumatomutilla*	*geographica*	♀	A
Traumatomutilla	*bispiculata*	♀♂	A	*Traumatomutilla*	*graphica*	♀	A

Genus	species	Sex	Area	Genus	species	Sex	Area
Traumatomutilla	*bivittata*	♀	A	*Traumatomutilla*	*grossa*	♀	A
Traumatomutilla	*boliviana*	♀	A	*Traumatomutilla*	*guarata*	♀	A
Traumatomutilla	*borba*	♀	A	*Traumatomutilla*	*guayaca*	♀	A
Traumatomutilla	*borrosa*	♀	A	*Traumatomutilla*	*gurisa*	♀	A
Traumatomutilla	*bruchi*	♀	A	*Traumatomutilla*	*hemicycla**	♀	A
Traumatomutilla	*burmeisteri**	♂	A	*Traumatomutilla*	*immaculiceps*	♀	A
Traumatomutilla	*cachimba*	♀	A	*Traumatomutilla*	*impetuosa**	♂	A
Traumatomutilla	*caipira*	♀	A	*Traumatomutilla*	*incerta*	♀	A
Traumatomutilla	*caneta*	♂	A	*Traumatomutilla*	*indica*	♀	A
Traumatomutilla	*caxara*	♂	A	*Traumatomutilla*	*indicoides*	♀	A
Traumatomutilla	*centralis*	♀	A	*Traumatomutilla*	*inermis*	♀	A
Traumatomutilla	*chapada*	♀	A	*Traumatomutilla*	*infernalis*	♂	A
Traumatomutilla	*characterea*	♂	A	*Traumatomutilla*	*ingens*	♀	A
Traumatomutilla	*integella*	♀	A	*Traumatomutilla*	*quadrinotata*	♀	A
Traumatomutilla	*ipanema*	♀	A	*Traumatomutilla*	*quadripustulata*	♀	A
Traumatomutilla	*ira*	♀	A	*Traumatomutilla*	*quadrum*	♀	A
Traumatomutilla	*ispiala*	♀	A	*Traumatomutilla*	*rastra*	♀	A
Traumatomutilla	*juvenilis*	♀	A	*Traumatomutilla*	*rectilineata*	♀	A
Traumatomutilla	*laida*	♀	A	*Traumatomutilla*	*rorida*	♂	A
Traumatomutilla	*lasiogastra*	♀	A	*Traumatomutilla*	*rubroguttata*	♀	A
Traumatomutilla	*latevittata*	♀	A	*Traumatomutilla*	*sancta*	♀	A
Traumatomutilla	*latona*	♀	A	*Traumatomutilla*	*scripta*	♀	A
Traumatomutilla	*lineifera**	♀	A	*Traumatomutilla*	*seabrai*	♀	A
Traumatomutilla	*lorena*	♂	A	*Traumatomutilla*	*selligera*	♂	A
Traumatomutilla	*lugubrina*	♀	A	*Traumatomutilla*	*serra*	♂	A
Traumatomutilla	*lunigera**	♀	A	*Traumatomutilla*	*sigillata**	♀	A
Traumatomutilla	*lusca*	♀	A	*Traumatomutilla*	*simulans*	♀	A
Traumatomutilla	*luscoides**	♀	A	*Traumatomutilla*	*sodalicia**	♀	A
Traumatomutilla	*maipa*	♀	A	*Traumatomutilla*	*solemnis*	♀	A
Traumatomutilla	*maligna**	♂	A	*Traumatomutilla*	*soriciana*	♂	A
Traumatomutilla	*manca*	♀	A	*Traumatomutilla*	*spectabilis*	♀	A
Traumatomutilla	*maraca*	♂	A	*Traumatomutilla*	*sphegea*	♂	A
Traumatomutilla	*maula*	♀	A	*Traumatomutilla*	*tabapua*	♀	A
Traumatomutilla	*melaleuca*	♂	A	*Traumatomutilla*	*tabatinga*	♀	A
Traumatomutilla	*mesolueca*	♂	A	*Traumatomutilla*	*taboca*	♂	A
Traumatomutilla	*miniata*	♀	A	*Traumatomutilla*	*tayguaya*	♀	A
Traumatomutilla	*mirina*	♀	A	*Traumatomutilla*	*tenuis**	♂	A
Traumatomutilla	*moesta*	♀	A	*Traumatomutilla*	*tetrastigma*	♀	A
Traumatomutilla	*moinga*	♀	A	*Traumatomutilla*	*tijuca*	♀	A
Traumatomutilla	*mundula*	♀	A	*Traumatomutilla*	*triangulifera**	♀	A
Traumatomutilla	*musculus**	♂	A	*Traumatomutilla*	*simulans*	♀	A
Traumatomutilla	*obsoleta*	♀	A	*Traumatomutilla*	*sodalicia*	♀	A
Traumatomutilla	*ocellaris*	♀	A	*Traumatomutilla*	*solemnis*	♀	A
Traumatomutilla	*oculifera*	♂	A	*Traumatomutilla*	*soriciana*	♂	A
Traumatomutilla	*orbana*	♂	A	*Traumatomutilla*	*spectabilis*	♀	A
Traumatomutilla	*ormena*	♀	A	*Traumatomutilla*	*sphegea*	♂	A
Traumatomutilla	*oxira*	♀	A	*Traumatomutilla*	*tabapua*	♀	A

Genus	species	Sex	Area	Genus	species	Sex	Area
Traumatomutilla	*paraiba*	♀	A	*Traumatomutilla*	*tabatinga*	♀	A
Traumatomutilla	*parallela*	♀	A	*Traumatomutilla*	*taboca*	♂	A
Traumatomutilla	*peperina*	♀	A	*Traumatomutilla*	*tayguaya*	♀	A
Traumatomutilla	*pereirai**	♀	A	*Traumatomutilla*	*tenuis*	♂	A
Traumatomutilla	*pertela*	♀	A	*Traumatomutilla*	*tetrastigma*	♀	A
Traumatomutilla	*piasta*	♀	A	*Traumatomutilla*	*tijuca*	♀	A
Traumatomutilla	*picada*	♂	A	*Traumatomutilla*	*triangulifera*	♀	A
Traumatomutilla	*pillinata*	♀	A	*Traumatomutilla*	*trinacria*	♀	A
Traumatomutilla	*polita**	♂	A	*Traumatomutilla*	*tristis*	♀	A
Traumatomutilla	*pomba*	♂	A	*Traumatomutilla*	*trivirgata*	♀	A
Traumatomutilla	*pompiliformis**	♂	A	*Traumatomutilla*	*trochantera*	♀	A
Traumatomutilla	*preta*	♀	A	*Traumatomutilla*	*tulumba*	♀	A
Traumatomutilla	*protuberans**	♂	A	*Traumatomutilla*	*unimarginata*	♀	A
Traumatomutilla	*proxima*	♂	A	*Traumatomutilla*	*ursina*	♀	A
Traumatomutilla	*pruinosa*	♂	A	*Traumatomutilla*	*urupema*	♀	A
Traumatomutilla	*puella**	♀	A	*Traumatomutilla*	*vagabunda*	♀	A
Traumatomutilla	*punctosignata*	♀	A	*Traumatomutilla*	*valuta*	♀	A
Traumatomutilla	*verecunda*	♀	A	*Traumatomutilla*	*vulnerata*	♂	A
Traumatomutilla	*viana*	♂	A	*Traumatomutilla*	*vulnerifera*	♂	A
Traumatomutilla	*vidua*	♂	A	*Traumatomutilla*	*weyrauchi*	♀	A
Traumatomutilla	*virginalis*	♀	A	*Traumatomutilla*	*xiringa*	♀	A
Traumatomutilla	*vitelligera*	♀	A	*Traumatomutilla*	*zayapa*	♀	A
Traumatomutilla	*vivax*	♀	A	*Traumatomutilla*	*zebrata*	♀	A

Table 2.2. Overview of diversity in dasymutilline genera. Numbers in parentheses represent undescribed morphospecies. List of species from which we obtained DNA provided in Table 2.4.

Genus	Male only	Female only	Both sexes	Total	DNA obtained
Cephalomutilla	0	11(1)	0	11(1)	3(1)
Dasymutilla	49	70	53	172	90
Leucospilomutilla	0	2	1	3	0
Reedomutilla	1	1	2	4	2
Suareztilla	4	5	0	9	4
Tobantilla	4	6	0	10	2
Traumatomutilla	49(31)	137(23)	2	188(54)	20(12)

Fig. 2.1. Geopolitical map of dasymutilline areas of endemism.

Using phylogenetic reconstructions, we aimed to determine where dasymutillines originated and how they arrived at their present distribution in the New World. We addressed the following questions: 1) did dasymutillines disperse between continents before, or after, the Isthmus of Panama was fully formed? 2) Which corridors did they use to disperse between continents? 3) How many lineages participated in intercontinental biotic exchanges? In addition to continental reconstructions, we studied dasymutilline communities in the Caribbean Islands, the Isthmus of Panama, and Nearctic North America. By determining the number of lineages, their ancestral areas, and their diversification dates in each region, we developed hypotheses concerning how these faunas were assembled. Finally, because both Pleistocene glacial cycles and Neogene mountain building events were shown to drive speciation in other velvet ant lineages (Pitts et al., 2010), we determined the age of diversification for most extant dasymutilline species.

2. Materials and Methods

2.1 Taxon Sampling

At least one member of each dasymutilline genus was included in the analysis, except *Leucospilomutilla* Ashmead, of which we could not find suitable specimens (Table 2.3). *Dasymutilla* Ashmead and *Traumatomutilla* André, as the most diverse dasymutilline genera (Table 2.2), were most heavily sampled (Table 2.4). Eighty-six species were selected for the phylogenetic analysis (Table 2.3). These species were chosen because sequence data was obtained successfully from all three targeted molecular markers (except two for ITS1 and five for wg; Table 2.3). We chose

Table 2.3

Molecular vouchers used in this study. Superscripts in Sex column indicate type of sex association used to determine opposite sex characters for morphological data. 1=species known from both sexes; 2=association based on unpublished molecular comparisons; 3=association based on morphological similarities and overlapping distribution; 4=species placed into species-group, opposite sex characters based on putative relatives; 5=opposite sex unknown.

Genus	species	Voucher	Sex	Clade (Fig.3)	Genbank ITS1	Genbank ITS2	Genbank wg
Tobantilla	*drosos*	JP606	male[3]	1	JQ945825	JX002750	JQ945855
Traumatomutilla	*species*	JP1779	female[5]	2	JQ945822	JX002751	JQ945901
Dasymutilla	*blattoserica*	JP841	male[1]	3	JQ945774	JX002752	JQ945841
Suareztilla	*clypeata*	JP1773, JP1774	male[2]	4	JQ945813	JX002753	JQ945853
Reedomutilla	*pubescens*	JP1123	male[3]	5	JQ945812	JX002754	JQ945875
Cephalomutilla	*proxima*	JP1900	male[2]	6	JQ945766	JX002755	JQ945882
Traumatomutilla	*valuta*	JP1776	female[5]	7	JQ945823	JX002756	JQ945900
Tobantilla	*frigidula*	JP1918	female[5]	8	JQ945824	JX002757	JX002749
Traumatomutilla	*c.f. sabara*	JP906	male[4]	9	JQ945833	JX002758	JX002744
Traumatomutilla	*bispiculata*	JP899	male[1]	9	JQ945830	JX002759	JQ945905
Traumatomutilla	*rubroguttata*	JP876	female[4]	9	JQ945816	JX002760	n/a
Traumatomutilla	*fascinata*	JP1852	male[3]	9	JQ945827	JX002761	JQ945903
Traumatomutilla	*c.f. orbana*	JP891	male[4]	9	JQ945828	JX002762	JQ945904
Traumatomutilla	*pertela*	JP1777	female[4]	9	JQ945821	JX002763	JQ945880
Traumatomutilla	*species*	JP901	male[4]	9	JQ945831	JX002764	JQ945902
Traumatomutilla	*inermis*	JP1085	female[3]	9	JQ945818	JX002765	JQ945877
Traumatomutilla	*barra*	JP902	male[5]	9	JQ945832	JX002766	JQ945906
Traumatomutilla	*lasiogastra*	JP898	male[3]	9	JQ945829	JX002767	JQ945881
Traumatomutilla	*obsoleta*	JP877	female[4]	9	JQ945817	JX002768	JQ945876
Traumatomutilla	*dubia*	JP843	male[1]	9	JQ945826	JX002769	JQ945856
Traumatomutilla	*americana*	JP1158	female[3]	9	JQ945815	JX002770	n/a
Traumatomutilla	*peperina*	JP879	female[4]	9	JQ945819	JX002771	JQ945878
Traumatomutilla	*centralis*	JP842	female[2]	9	JQ945814	JX002772	JQ945854
Traumatomutilla	*punctosignata*	JP1778	female[3]	9	JQ945820	JX002773	JQ945879
Dasymutilla	*brazillia*	JP608	male[3]	9	JQ945775	JX002774	JQ945842
Dasymutilla	*arenivaga*	JP460	female[1]	10A	JQ945770	JX002775	JQ945835

Genus	species	Voucher	Sex	Clade (Fig.3)	Genbank ITS1	Genbank ITS2	Genbank wg
Dasymutilla	*texanella*	JP381	female[3]	10A	JX002853	JX002776	JQ945893
Dasymutilla	*nigripes*	JP463, JP464	female[1]	10A	DQ408502	DQ408502	JQ945871
Dasymutilla	*coccineohirta*	JP99	female[1]	10A	JQ945779	JX002777	JX002743
Dasymutilla	*satanas*	JP603	female[1]	10A	JQ945800	JX002778	JQ945885
Dasymutilla	*gloriosa*	JP241	female[1]	10A	HQ317244	HQ317288	JQ945859
Dasymutilla	*thetis*	KW04	female[1]	10A	EF433453	EF433453	JQ945845
Dasymutilla	*stevensi*	JP461	male[1]	10A	JQ945807	JX002779	JQ945892
Dasymutilla	*pallene*	JP671	female[3]	10A	JQ945780	JX002780	JQ945873
Dasymutilla	*magna*	JP599	female[1]	10A	JQ945792	JX002771	JQ945868
Dasymutilla	*occidentalis*	MoccF	female[1]	10A	HQ317246	HQ317268	JQ945909
Dasymutilla	*klugii*	JP437	male[1]	10A	JQ945790	JX002782	JQ945866
Dasymutilla	*sackenii*	JP602	male[1]	10A	JQ945799	JX002783	JQ945884
Dasymutilla	*gorgon*	JP383	female[1]	10A	HQ317247	HQ317289	JQ945860
Dasymutilla	*californica*	JP164	female[1]	10A	EU627568	EU627568	JQ945843
Dasymutilla	*arachnoides*	JP883	female[3]	10A	JQ945767	JX002784	JQ945907
Dasymutilla	*araneoides*	JP832	female[1]	10A	JQ945768	JX002785	JQ945834
Dasymutilla	*sicheliana*	JP238	female[1]	10A	JQ945804	JX002786	JQ945889
Dasymutilla	*asteria*	JP237	female[1]	10A	JQ945772	JX002787	JQ945838
Dasymutilla	*pseudopappus*	JP1246	female[2]	10A	JQ945796	JX002788	n/a
Dasymutilla	*scitula*	JP924	male[1]	10A	JQ945803	JX002789	JQ945888
Dasymutilla	*magnifica*	JP259	female[1]	10A	DQ408497	DQ408497	JQ945869
Dasymutilla	*saetigera*	JP1208	male[1]	10A	JQ945797	JX002790	JQ945883
Dasymutilla	*vesta*	JP1233, JP225	male[1]	10A	DQ408496	JX002791	JQ945894
Dasymutilla	*macilenta*	JP1077	female[1]	10A	JQ945791	JX002792	JQ945867
Dasymutilla	*canella*	JP1081	female[1]	10A	JQ945777	JX002793	JQ945846
Dasymutilla	*monticola*	JP316	male[1]	10A	EF433448	EF433448	JQ945870
Dasymutilla	*birkmani*	JP380	male[1]	10A	EF433443	EF433443	JQ945908
Dasymutilla	*archboldi*	JP1240	female[1]	10A	JQ945769	JX002794	n/a
Dasymutilla	*bioculata*	JP1189	male[1]	10A	HQ317263	HQ317277	JQ945840
Dasymutilla	*insulana*	JP1893	male[1]	10A	JQ945787	JX002795	JQ945840
Dasymutilla	*cirrhomeris*	JP840	male[3]	10A	JQ945785	JX002796	JQ945858
Dasymutilla	*campanula*	JP911	female[2]	10A	JQ945776	JX002797	JQ945844

Genus	species	Voucher	Sex	Clade (Fig.3)	Genbank ITS1	Genbank ITS2	Genbank wg
Dasymutilla	*quadriguttata*	JP159	male[1]	10A	HQ317245	HQ317267	JQ945910
Dasymutilla	*parksi*	JP1431	female[2]	10A	JQ945795	JX002798	n/a
Dasymutilla	*creon*	JP670	male[1]	10A	JQ945781	JX002799	JQ945849
Dasymutilla	*wileyae*	JP370	female[3]	10A	EU627572	EU627572	JQ945896
Dasymutilla	*impressa*	JP672	male[3]	10A	JQ945786	JX002800	JQ945862
Dasymutilla	*scabra*	JP1896	female[1]	10A	JQ945801	JX002801	JQ945886
Dasymutilla	*erythrina*	JP881	male[1]	10A	JQ945784	JX002802	JQ945911
Dasymutilla	*cressoni*	JP885	female[2]	10A	JQ945782	JX002803	JQ945850
Dasymutilla	*iztapa*	JP612	male[2]	10A	JQ945789	JX002804	JQ945865
Dasymutilla	*zoster*	JP836	female[2]	10A	JQ945811	JX002805	JQ945899
Dasymutilla	*vestita*	JP19	male[1]	10A	JQ945808	JX002806	JQ945895
Dasymutilla	*foxi*	JP454	female[1]	10A	EF433450	EF433450	JQ945857
Dasymutilla	*canina*	JP909	female[2]	10A	JQ945778	JX002807	JQ945847
Dasymutilla	*asopus*	JP1080	male[1]	10A	JQ945771	JX002808	JQ945837
Dasymutilla	*pulchra*	JP1904	male[1]	10B	JQ945798	JX002809	JQ945874
Dasymutilla	*sleipniri*	JP611	male[3]	10B	JQ945805	JX002810	JQ945890
Dasymutilla	*yucatana*	JP610	male[3]	10B	n/a	JX002811	JQ945898
Dasymutilla	*chamela*	JP913	male[3]	10B	n/a	JX002812	JQ945848
Dasymutilla	*ionothorax*	JP1307	female[2]	10C	JQ945788	JX002813	JQ945864
Dasymutilla	*xalisco*	JP1898	female[4]	10C	JQ945810	JX002814	JQ945897
Dasymutilla	*snoworum*	JP443	female[1]	10D	GU814282	GU814407	JQ945891
Dasymutilla	*scaevola*	JP1196	female[1]	10D	JQ945802	JX002815	JQ945887
Dasymutilla	*heliophila*	JP321	female[1]	10D	EF433445	EF433445	JQ945861
Dasymutilla	*ocyrae*	JP835	female[2]	10D	JQ945794	JX002816	JQ945872
Dasymutilla	*dilucida*	JP479	female[2]	10D	JQ945783	JX002817	JQ945851
Dasymutilla	*sophrona*	JP1207	female[2]	10D	JQ945806	JX002818	JQ945912
Dasymutilla	*militaris*	JP888	male[1]	10E	JQ945793	JX002819	JX002748
Dilophotopsis	*concolor*	JP263	male[1]	n/a	EU369210	EU369225	EU367352
Sphaeropthalma	*pensylvanica*	JP245	male[1]	n/a	GU814354	GU814479	EU367377

Table 2.4
Molecular vouchers from which at least ITS1 was sequenced. Asterisk (*) indicates putative generic placement based on preliminary morphological and molecular comparisons, not based on previous catalogs (Krombein, 1979; Nonveiller, 1990). Bolded entries indicate species that were not included in the phylogenetic due to morphological and molecular similarity to included species coupled with inability to amplify additional molecular markers.

Genus	species	Voucher	Locality
Cephalomutilla	***distincta***	**JP1112**	PARAGUAY: Paraguari
Cephalomutilla	*proxima**	JP1900	BRAZIL: Minas Gerais
Cephalomutilla	***vivata***	**JP1166**	BRAZIL: Minas Gerais
Cephalomutilla	***species****	**JP838**	COLOMBIA: Vichada
Dasymutilla	***apicalata***	**JP1302**	MEXICO: Sonora
Dasymutilla	*arachnoides*	JP883	MEXICO: Guerrero
Dasymutilla	*araneoides*	JP832	NICARAGUA: Granada
Dasymutilla	*archboldi*	JP1240	USA: FL: Polk Co.
Dasymutilla	*arenivaga*	JP460	USA: AZ: Maricopa Co.
Dasymutilla	*asopus*	JP1080	USA: FL: Orange Co.
Dasymutilla	*asteria*	JP237	USA: AZ: Santa Cruz Co.
Dasymutilla	*atricauda*	JP487	USA: CA: Inyo Co.
Dasymutilla	*aureola*	JP915	USA: OR: Lake Co.
Dasymutilla	*bioculata*	JP1189	USA: NE: Morrill Co.
Dasymutilla	*birkmani*	JP380	USA: TX: Potter Co.
Dasymutilla	*blattoserica*	JP841	PERU: Sechura
Dasymutilla	*brazillia*	JP608	FRENCH GUIANA: Korou
Dasymutilla	*californica*	JP164	USA: CA: San Diego Co.
Dasymutilla	***calorata***	**JP438**	USA: TX: Randall Co.
Dasymutilla	*campanula*	JP911	USA: CO: Weld Co.
Dasymutilla	*canella*	JP1081	USA: MA: Plymouth Co.
Dasymutilla	*canina*	JP909	NICARAGUA: Granada
Dasymutilla	*chamela*	JP913	MEXICO: Sonora
Dasymutilla	***chisos***	**Dsp05**	USA: TX: Presidio Co.
Dasymutilla	*cirrhomeris*	JP840	MEXICO: Sonora
Dasymutilla	***clotho***	**JP590**	USA: OK: Comanche Co.
Dasymutilla	*coccineohirta*	JP99	USA: CA: Riverside Co.
Dasymutilla	***cotulla***	**JP448**	USA: TX: Presidio Co.
Dasymutilla	*creon*	JP670	USA: TX: Harris Co.
Dasymutilla	*cressoni*	JP885	MEXICO: Guerrero
Dasymutilla	***digressa***	**JP442**	USA: TX: Cameron Co.
Dasymutilla	*dilucida*	JP479	USA: AZ: Cochise Co.
Dasymutilla	*eminentia*	Demi01	USA: AZ: Cochise Co.
Dasymutilla	*erythrina*	JP881	MEXICO: Michoacan
Dasymutilla	*ferruginea*	JP667	MEXICO: Sonora
Dasymutilla	*foxi*	JP454	USA: AZ: Cochise Co.
Dasymutilla	***furina***	**Dfur01**	USA: AZ: Cochise Co.
Dasymutilla	*gloriosa*	JP241	USA: AZ: Cochise Co.
Dasymutilla	***glycera***	**Dper01**	USA: AZ: Cochise Co.
Dasymutilla	*gorgon*	JP383	USA: TX: La Salle Co.
Dasymutilla	*heliophila*	JP321	USA: AZ: Cochise Co.

Genus	species	Voucher	Locality
Dasymutilla	*impressa*	JP672	MEXICO: Sonora
Dasymutilla	*insulana*	JP1893	CUBA: Guantanamo Bay
Dasymutilla	*ionothorax*	JP1307	NICARAGUA: Granada
Dasymutilla	*iztapa*	JP612	COSTA RICA: Guanacaste
Dasymutilla	***jalisco***	**JP1899**	MEXICO: Jalisco
Dasymutilla	*klugii*	JP437	USA: TX: La Salle Co.
Dasymutilla	***klugiodes***	**JP910**	USA: NM: Lea Co.
Dasymutilla	***leda***	**JP384**	USA: TX: Randall Co.
Dasymutilla	*macilenta*	JP1077	USA: FL: Orange Co.
Dasymutilla	***macra***	**JP204**	USA: CO: Sedgwick Co.
Dasymutilla	*magna*	JP599	USA: AZ: Santa Rita Co.
Dasymutilla	*magnifica*	JP259	USA: AZ: Cochise Co.
Dasymutilla	*militaris*	JP888	REP. DOMINICANA: Punta Cana
Dasymutilla	***m. nigriceps***	**JP1799**	BAHAMAS: Great Exuma Island
Dasymutilla	*monticola*	JP316	USA: CA: San Bernardino Co.
Dasymutilla	*munifica*	JP834	NICARAGUA: Granada
Dasymutilla	***myrice***	**JP385**	USA: TX: Potter Co.
Dasymutilla	***nigricauda***	**JP439**	USA: TX: Randall Co.
Dasymutilla	*nigripes*	JP464	USA:TX: Randall Co.
Dasymutilla	***nocturna***	**JP266**	USA: CA: Imperial Co.
Dasymutilla	***nogalensis***	**JP488**	USA: AZ: Cochise Co.
Dasymutilla	***nupera***	**JP449**	USA: TX: LaSalle Co.
Dasymutilla	*occidentalis*	MoccF	USA: SC: Florence Co.
Dasymutilla	***ocydrome***	**JP1866**	MEXICO: Sonora
Dasymutilla	*ocyrae*	JP835	NICARAGUA: Granada
Dasymutilla	*pallene*	JP671	MEXICO: Sonora
Dasymutilla	*parksi*	JP1431	USA: TX: Leon Co.
Dasymutilla	***phaon***	**JP675**	USA: CA: Kern Co.
Dasymutilla	***p. fimbrialis***	**JP1246**	USA: NV: Clark Co.
Dasymutilla	*pseudopappus*	JP1300	USA: CA: Riverside Co.
Dasymutilla	*pulchra*	JP1904	MEXICO: Guerrero
Dasymutilla	*quadriguttata*	JP159	USA: AR: Pulaski Co.
Dasymutilla	*sackenii*	JP602	USA: CA: Siskiyou Co.
Dasymutilla	*saetigera*	JP1208	MEXICO: Sonora
Dasymutilla	*satanas*	JP603	USA: UT: Washington Co.
Dasymutilla	*scabra*	JP1896	MEXICO: Baja California Sur
Dasymutilla	*scaevola*	JP1196	USA: FL: Alachua Co.
Dasymutilla	*scitula*	JP924	USA: UT: Garfield Co.
Dasymutilla	*sicheliana*	JP238	USA: AZ: Cochise Co.
Dasymutilla	*sleipniri*	JP611	PANAMA: Barro Colorado Island
Dasymutilla	*snoworum*	JP443	USA: TX: Cameron Co.
Dasymutilla	***sophrona***	**JP1207**	MEXICO: Sonora
Dasymutilla	*spilota*	JP833	NICARAGUA: Granada
Dasymutilla	*stevensi*	JP461	USA: TX: Presidio Co.
Dasymutilla	*texanella*	JP381	USA: TX: LaSalle Co.
Dasymutilla	*thetis*	KW04	USA: AZ: Cochise Co.
Dasymutilla	*vesta*	JP1233	USA: FL: Polk Co.
Dasymutilla	*vestita*	JP19	USA: UT: Cache Co.
Dasymutilla	*waco*	JP914	USA: TX: Walker Co.
Dasymutilla	*wasbaueri*	JP1299	COSTA RICA: Guanacaste

Genus	species	Voucher	Locality
Dasymutilla	*wileyae*	JP370	USA: TX: LaSalle Co.
Dasymutilla	*xalisco*	JP1898	MEXICO: Jalisco
Dasymutilla	***zelaya***	**JP450**	USA: TX: Val Verde Co.
Dasymutilla	*zoster*	JP836	NICARAGUA: Granada
Reedomutilla	***gayi***	**JP607**	CH1
Reedomutilla	*pubescens*	JP1123	AR051214
Suareztilla	***bimaculata***	**JP1119**	BOLIVIA: Santa Cruz
Suareztilla	***calycina***	**JP1130**	PARAGUAY: Puerto Galileo
Suareztilla	*clypeata*	JP1774	BRAZIL: Ceara
Suareztilla	***colorata***	**JP1869**	BRAZIL: Sao Paulo
Tobantilla	*drosos*	JP606	CH41
Tobantilla	*frigidula*	JP1918	BRAZIL: Amazonas
Traumatomutilla	*americana*	JP1158	BRAZIL: Roraima
Traumatomutilla	*barra*	JP902	CH41
Traumatomutilla	***bifurca***	**JP1163**	BRAZIL: Minas Gerais
Traumatomutilla	*bispiculata*	JP899	CH41
Traumatomutilla	*centralis*	JP842	ARGENTINA: La Rioja
Traumatomutilla	***cuyana***	**JP671**	BOLIVIA: Santa Cruz
Traumatomutilla	*dubia*	JP843	GUYANA
Traumatomutilla	*fascinata*	JP1852	PERU: Lambayeque
Traumatomutilla	***gausapata***	**JP1156**	BRAZIL: Roraima
Traumatomutilla	*inermis*	JP1085	BRAZIL: Espiritu Santo
Traumatomutilla	*lasiogastra*	JP898	CH41
Traumatomutilla	*obsoleta*	JP877	PARAGUAY: Kanindeyu
Traumatomutilla	*peperina*	JP879	PARAGUAY: Kanindeyu
Traumatomutilla	*pertela*	JP1777	BRAZIL: Ceara
Traumatomutilla	*punctosignata*	JP1778	BRAZIL: Ceara
Traumatomutilla	*rubroguttata*	JP876	PARAGUAY: Kanindeyu
Traumatomutilla	***spectabilis***	**JP1116**	PARAGUAY: Paraguari
Traumatomutilla	***tristis***	**JP1197**	BOLIVIA: Santa Cruz
Traumatomutilla	*valuta*	JP1776	BRAZIL: Ceara
Traumatomutilla	***weyrauchi***	**JP1154**	ECUADOR: Napo
Traumatomutilla	*species**	JP906	BOLIVIA: Villa Tunari
Traumatomutilla	*species**	JP1779	BRAZIL: Ceara
Traumatomutilla	*species**	JP891	CH50
Traumatomutilla	*species**	JP901	BOLIVIA: Santa Cruz
Traumatomutilla	***species****	**JP1121**	PERU: Biodiversidad 10
Traumatomutilla	***species****	**JP1192**	COLOMBIA: Vichada
Traumatomutilla	***species****	**JP1194**	CH47
Traumatomutilla	***species****	**JP1195**	PERU: 11-02
Traumatomutilla	***species****	**JP1786**	BRAZIL: Pará
Traumatomutilla	***species****	**JP1787**	BRAZIL: Pará
Traumatomutilla	***species****	**JP1788**	BRAZIL: Pará
Traumatomutilla	***species****	**JP1800**	BRAZIL: Goias

Sphaeropthalma pensylvanica (Lepeletier) and *Dilophotopsis concolor* (Cresson) for outgroups.

2.2 DNA sampling

DNA was extracted from ethanol-preserved specimens or recently collected dried specimens using the High Pure PCR Template Preparation Kit (Roche Pharmaceuticals, Indianapolis, IN). DNA was extracted from multiple legs of larger specimens or from the entire individual of smaller specimens after piercing the mesosoma. This process preserved the external anatomy of the individual for identification and vouchering. Voucher specimens were deposited in the Department of Biology Insect Collection, Utah State University, Logan, UT. The two internal transcribed spacers (ITS1 and ITS2) of the nuclear ribosomal RNA cistron and the nuclear gene wingless (wg) were sequenced for each species. PCR conditions for ITS1 and ITS2 followed the molecular protocols described in Pilgrim and Pitts (2006). PCR conditions for wg follow Brower & DeSalle (1998).

Contigs were assembled and edited using Geneious 4.7.5 (Drummond et al., 2009). Corrected sequences from all samples were aligned separately for ITS1, ITS2, and wg using the default parameters of the Geneious alignment program. Alignments were visually inspected and errors were corrected in Geneious. The alignments of ITS1 and ITS2 contained some areas that were conserved across all taxa and other areas with insertion and deletion polymorphisms (indels). While all of the taxa had indels—which is not unexpected given the non-coding nature of these intergenic regions—the alignment was informative and we included the entire alignment in the phylogenetic analyses rather than excluding the less conserved areas, except in rare cases of long, unique indels that we excluded from the alignment. As a nuclear coding gene, the wingless dataset was devoid of indels and easily aligned in Geneious. All sequences were deposited in GenBank (see Table 2.3 for accession numbers).

2.3 Morphological characters

Morphological characters were coded for both sexes. Fifteen female characters and 17 male characters were used (Table 2.5, 2.6). Only 39 of the species included in this analysis are currently known from both sexes, leaving 50 species that are known from only a single sex (Table 2.3). Complete datasets for both sexes, however, were compiled for all but four of the species. Preliminary molecular data have revealed the putative opposite sex for 25 of the single sex species. Shared morphology and distribution permitted us to predict of the opposite sex of 12 more species. Finally, putative species-group placement of nine species has allowed us to predict most character states of the unknown sex. This left five species with morphological data for a single sex (Table 2.3).

2.4 Phylogeny reconstruction

Bayesian inference of phylogeny was performed with mrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Each alignment was analyzed separately with MrModeltest v2.2 (Nylander, 2004) to find appropriate models of molecular evolution. The following models were used for each molecular marker: GTR+I for ITS1, GTR+G for ITS2, and GTR+I for wg. Alignments of the three molecular markers were combined analyzed as a single dataset. Twenty-four phylogenetically informative indels were coded for ITS1 and 67 were coded for ITS2 (Table 2.7). Because it is a coding nuclear gene, the wg alignment did not contain any indels. These binary characters were partitioned from the molecular data and analyzed under a restricted binary model. Morphological traits were analyzed under the Markov morphology model suggested by Lewis (2001). Molecular loci were partitioned by gene and set to the appropriate model, with all parameters set as unlinked across partitions. The analysis included four independent runs with three heated chains and one cold chain in each run. The MCMC chains were set for 10,000,000 generations and sampled every 100 generations. A majority-rule consensus tree was obtained from the tree data after removing the burnin period, which was identified by examining the substitution model parameters (.p files) in Microsoft Excel.

2.5 Molecular dating

Strict molecular clock approaches to calculate divergence dates are not appropriate for Mutillidae due to rate heterogeneity among lineages (Pitts et al., 2010). Instead, we determined divergence dates using penalized likelihood (PL) approach to rate smoothing with r8s 1.71 (Sanderson, 2002), and a Bayesian MCMC averaging approach to rate smoothing using the program BEAST v1.6.2 (Drummond and Rambaut, 2007). Very few mutillid fossils are known, but there are two fossil species within the genus *Dasymutilla*, *D. dominicana* Manley & Poinar and *D. albofasciata* Manley & Poinar. Williams et al. (2011a) recognize these fossils as relatives of the extant Caribbean species *D. militaris*. The most commonly used range for Dominican Amber is 15 to 20 Ma (Iturralde-Vincent and MacPhee, 1996), leading us to estimate divergence dates separately for the minimum and maximum of this range. These fossil ages were treated as a minimum age for the ancestor of *D. militaris*. Two additional calibration points were based on a broader dating analysis of vespoid Hymenoptera (Wilson et al., in review). In

Table 2.5
Morphological characters used in mrBayes reconstruction: females.

Characters		States
1	Antennal scrobe	**0**=Absent; **1**=Present
2	Genal carina	**0**=Absent; **1**=Present
3	Gular carina	**0**=Evenly raised throughout; **1**=Sharply raised beneath mandibular base
4	Paired tubercles on vertex	**0**=Absent; **1**=Present
5	Humeral carina	**0**=Carina absent, pronotum simply punctate; **1**=Low rounded carina; **2**=Incomplete, sharply raised carina; **3**=Complete and sharply raised carina
6	Lateral pronotal face	**0**=Lacking any carina; **1**=Carina present, but incomplete or interrupted by punctation; **2**=Carina complete and virtually straight
7	Scutellar scale	**0**=Absent; **1**=Present
8	Propodeal sculpture	**0=**Dorsal and lateral faces with contiguous punctures; **1=**Lateral face sparsely punctured, differentiated from dorsal face
9	Tibial spurs	**0=**Black or concolorous with tibia; **1=**White
10	Metasomal tergum I: width	**0**=Greater than 0.35 × T2 width; **1**=Narrower than 0.35 × T2 width
11	Metasomal tergum II: basal shape	**0**=Lowly rounded; **1**=Swollen anteriorly over base of T1
12	Metasomal tergum II: coloration	**0**=T2 concolorous with remainder of metasoma, lacking distinct pattern; **1**=T2 with integumental spots, spots punctured throughout; **2**=T2 with integumental spots, spots lacking punctures
13	Metasomal tergum II: width	**0**=Length greater than width; **1**=Length and width subequal; **2**=Length shorter than width
14	Setae of terga III-V	**0**=Unicolorous; **1**=Having some complete black bands and some complete silver or golden bands; **2**=Each tergum with medial spot of white setae; **3**=Having lateral silver spots, black medial setae on each segment
15	Pygidial carinae	**0**=Pygidium apparently undefined; **1**=Lateral carinae weak, pygidium granulate; **2**=Lateral and apical carinae continuous; **3**=Lateral carinae highly raised above weak apical carina

Table 2.6

Morphological characters used in mrBayes reconstruction: males.

Characters		States
1	Clypeus	**0**=Rounded apically; **1**=Bidentate; **2**=Raised and shelf-like
2	Flagellomere I	**0**=Shorter than F2; **1**=Subequal to or longer than F2
3	Axillae	**0**=Unarmed; **1**=Truncate; **2**=Dentate; **3**=Forming long lateral arms; **4**=Rounded apically
4	Metasternum	**0**=Having elongate medial tooth, lateral teeth small; **1**=Lateral teeth equal or larger than medial tooth
5	Middle and hind femora	**0**=Rounded apically; **1**=Truncate apically
6	Tibial spurs	**0**=Black or concolorous with tibia; **1**=White
7	Wing color	**0**=Unicolorous; **1**=Having basal hyaline areas and darker color apically
8	Marginal cell	**0=**Shorter than 1.5 × stigma length; **1=**Longer than 1.5 × stigma length
9	Dorsal face of metasomal tergum I	**0=**Shorter than 0.3 × T1 width; **1=**0.3-0.5 × T1 width; 2=Longer than 0.5 × T1 width
10	Length of metasomal tergum I	**0**=Shorter than 0.9 × T1 width; **1**=Greater than 0.9 × T1 width
11	Tergum I shape	**0**=Rounded, T1 dorsal face less than 0.67 × T1 lateral length; **1**= Nodose, T1 dorsal face greater than 0.67 × T1 lateral length
12	Carina of metasomal sternum I	**0**=Carina low or lacking; **2**=Carina forming highly raised tooth medially
13	Setae of metasomal tergum II	**0**=Relatively uniform on disc; **1**=Having dense basal patches of white setae
14	Metasomal sternum II	**0**=Lacking seta-filled pit or midification; **1**=Having seta-filled pit, dense patch of short setae, or longitudinal row of setae medially
15	Metasomal sternum II: shape	**0**=Evenly convex; **1**=Medially flattened or slightly concave
16	Paramere apex	**0**=Virtually straight or slightly upcurved; **1**=Sharply upcurved
17	Genitalic setae	**0**=Entirely simple; **1**=Having plumose setae, at least on cuspis

Table 2.7
Indel characters and their position in the data matrix.

Indel	Position	Marker
1	19	ITS1
2	21	ITS1
3	48	ITS1
4	50	ITS1
5	52	ITS1
6	54	ITS1
7	56	ITS1
8	58	ITS1
9	63	ITS1
10	74	ITS1
11	76	ITS1
12	102	ITS1
13	106	ITS1
14	141	ITS1
15	142	ITS1
16	143	ITS1
17	151	ITS1
18	160	ITS1
19	287	ITS1
20	346	ITS1
21	396	ITS1
22	400	ITS1
23	473	ITS1
24	484	ITS1
25	710	ITS2
26	716	ITS2
27	772	ITS2
28	773	ITS2
29	774	ITS2
30	807	ITS2
31	814	ITS2
32	819	ITS2
33	835	ITS2
34	837	ITS2
35	843	ITS2
36	871	ITS2
37	898	ITS2
38	899	ITS2
39	906	ITS2
40	913	ITS2
41	927	ITS2
42	954	ITS2
43	979	ITS2
44	1015	ITS2
45	1017	ITS2
46	1018	ITS2
47	1038	ITS2
48	1048	ITS2
49	1080	ITS2
50	1091	ITS2
51	1107	ITS2
52	1144	ITS2
53	1160	ITS2
54	1263	ITS2
55	1269	ITS2
56	1282	ITS2
57	1402	ITS2
58	1403	ITS2
59	1404	ITS2
60	1405	ITS2
61	1425	ITS2
62	1468	ITS2
63	1476	ITS2
64	1498	ITS2
65	1513	ITS2
66	1514	ITS2
67	1519	ITS2
68	1536	ITS2
69	1563	ITS2
70	1575	ITS2
71	1577	ITS2
72	1580	ITS2
79	1639	ITS2
80	1655	ITS2
81	1660	ITS2
82	1662	ITS2
83	1699	ITS2
84	1711	ITS2
85	1712	ITS2
86	1751	ITS2
87	1809	ITS2
88	1854	ITS2
89	1871	ITS2
90	1873	ITS2
91	1894	ITS2

this analysis, the date of the node connecting *Dilophotopsis* and *Sphaeropthalma* was 15 MYA. The node connecting these genera with *Dasymutilla* was dated to 31 MYA.

2.6 Ancestral Area Reconstructions

A condensed tree was compiled in RASP 2.0 (Yu et al., 2010, 2011) using 10000 of the post-burnin trees from the mrBayes phylogenetic reconstruction. This tree was analyzed using Binary Bayesian MCMC criteria for 50,000 generations. We used the

F81+G model, because geographic distributions were not evenly distributed through the tree and did not have equal frequencies. Five areas were coded in the analysis (Fig. 2.1): South America, Lower Central America (Panama, Costa Rica, and southern Nicaragua), northern Neotropical North America (Neotropical Mexico, Guatemala, Belize, Honduras, El Salvador, northern Nicaragua), Nearctic North America (D), and the Greater Antilles (E). In tectonic nomenclature, the North American Neotropical zones can also be defined by their blocks on the Caribbean and North American plates: southern Central America includes the Chocó and Chorotega blocks; northern Neotropical North America includes the Chortis, Maya, and Oaxaca blocks (Fig. 2.2). The Chocó and Chorotega blocks define the modern Isthmus of Panama.

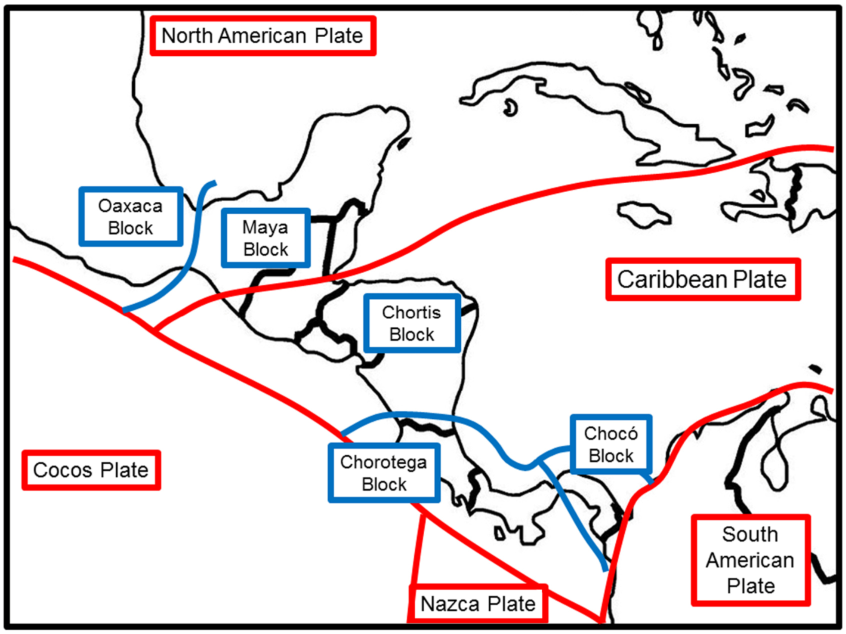

Fig. 2.2. Map of plates Central American blocks and plates. Red lines indicate fault lines separating tectonic plates and blue lines separate blocks within those plates. The Chocó and Chorotega blocks together represent Lower Central America (Area B, Fig. 1), while the Chortis, Maya, and Oaxaca blocks represent northern Neotropical North America (Area C, Fig. 2.1).

3. Results

3.1 Phylognetic relationships

A majority rules consensus tree from the mrBayes analysis (Fig. 2.3; Table 2.3) reveals 10 dasymutilline clades roughly corresponding to modern genera. The largest of these clades is further subdivided (Fig. 2.3; Table 2.3). Three dasymutilline genera, *Dasymutilla*, *Tobantilla*, and *Traumatomutilla*, were recovered as paraphyletic. The type species of *Dasymutilla*, *D. gorgon* (Blake) was recovered in Clade 10. The putative male for the type species of *Tobantilla* (Williams et al., 2011b) comprises Clade 1. The holotype of *Traumatomutilla*, *T. indica* (Linnaeus), is morphologically similar to members of Clade 9. Therefore, we apply the name *Dasymutilla* to Clade 10, *Tobantilla* to Clade 1, and *Traumatomutilla* to Clade 9 for the remainder of this study. Species from Clades 2, 3, 7, and 8 will be transferred to new genera in future morphological studies.

3.2 Molecular dates

The r8s (Fig. 2.4) and BEAST analyses (Fig. 2.5) present similar dates for the same nodes. In general, BEAST recovers older dates for the more ancient South American clades and younger dates for the more recent speciation events in North America. The dates from both r8s analyses, using 15 Ma and 20 Ma as fossil calibration dates were similar (Fig. 2.5); each divergence date from these analyses falls within the 95% credibility estimate of the BEAST analysis (Fig. 2.4). Dasymutilline ancestors began diversifying in the Oligocene (Fig. 2.5) or Eocene (Fig. 2.4), depending on the analytical method. The largest dasymutilline genera, *Dasymutilla* and *Traumatomutilla*, each originated in the early Miocene. Using the criteria that yielded the highest proportion of

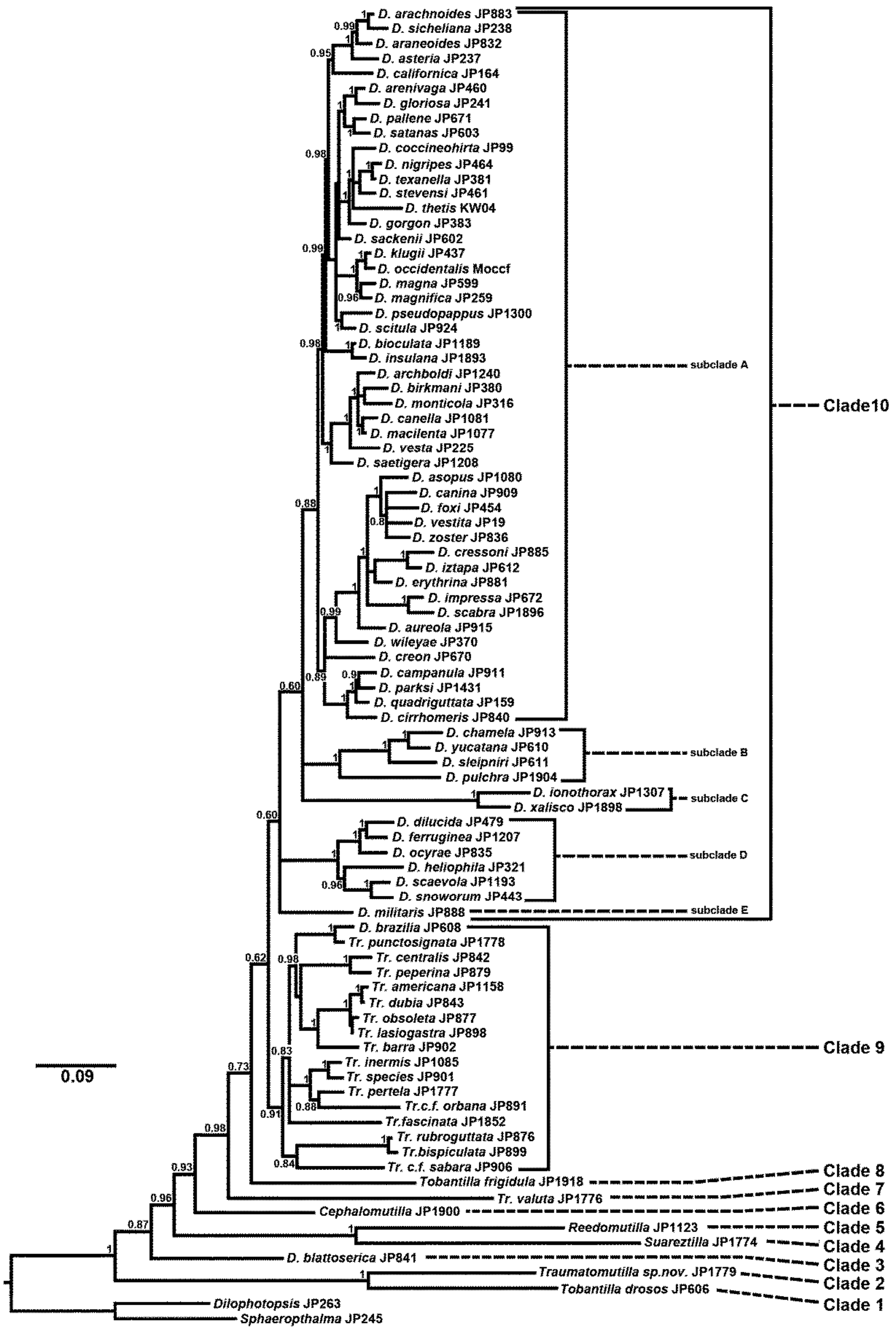

Fig. 2.3. Bayesian phylogenetic tree. 50% majority rules consensus tree from mrBayes based on concatenated sequences, indel characters, and morphology characters. Posterior probabilities are given for each node. Numbered clades represent putative genus-level groups; lettered subclades represent putative subgenera or species-groups.

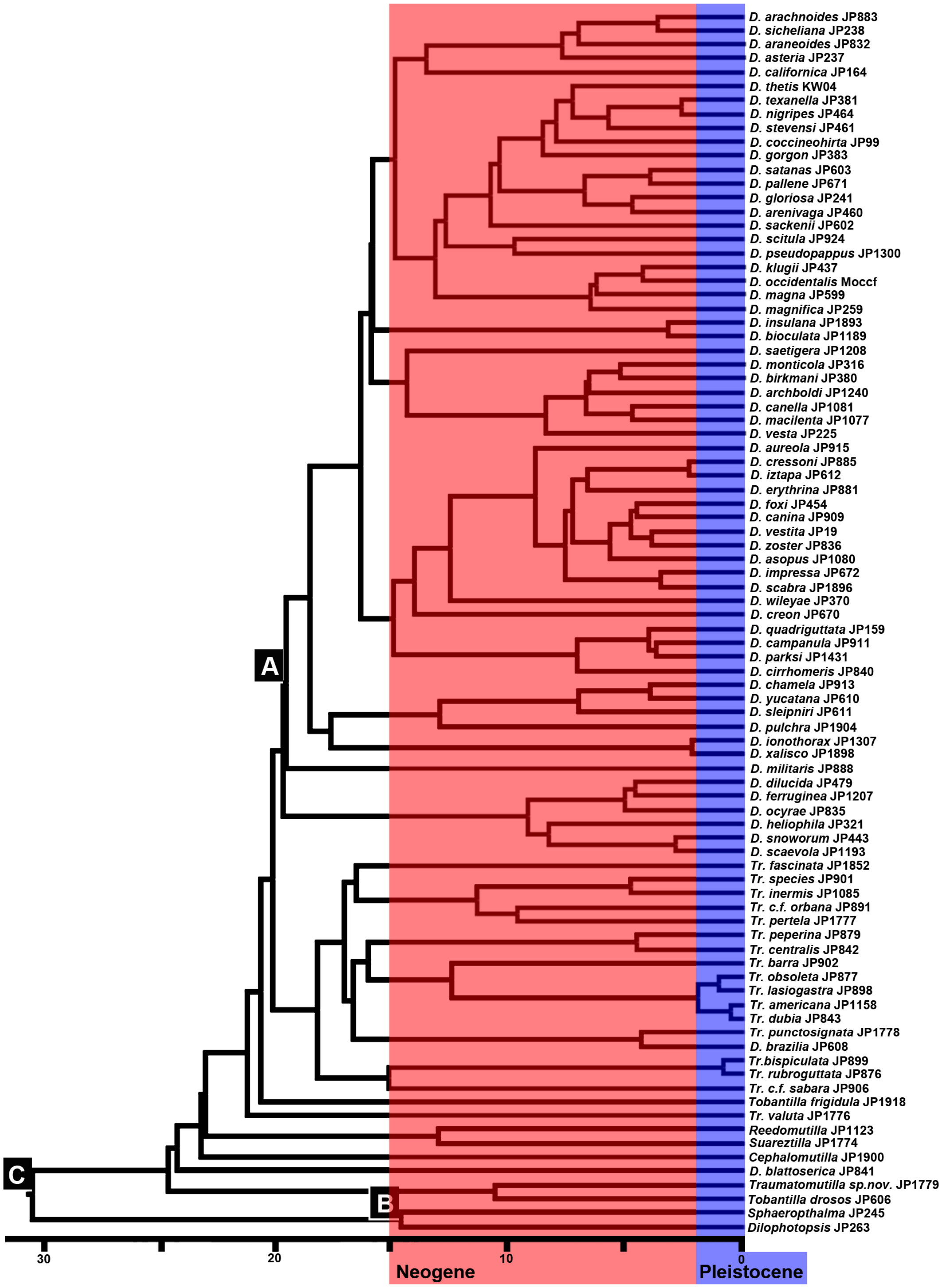

Fig. 2.4. r8s chronogram. Selected post-burnin tree from mrBayes analysis. Labeled nodes represent calibration points for the relaxed clock: Dominican amber fossil (A) set at a minimum of 20 Ma; ancestor of two outgroup species (B) set at 15 Ma; root age (C) set at 31 Ma. Highligthed ages based on hypothesized speciation events (Pitts et al., 2010), not actual epoch limits.

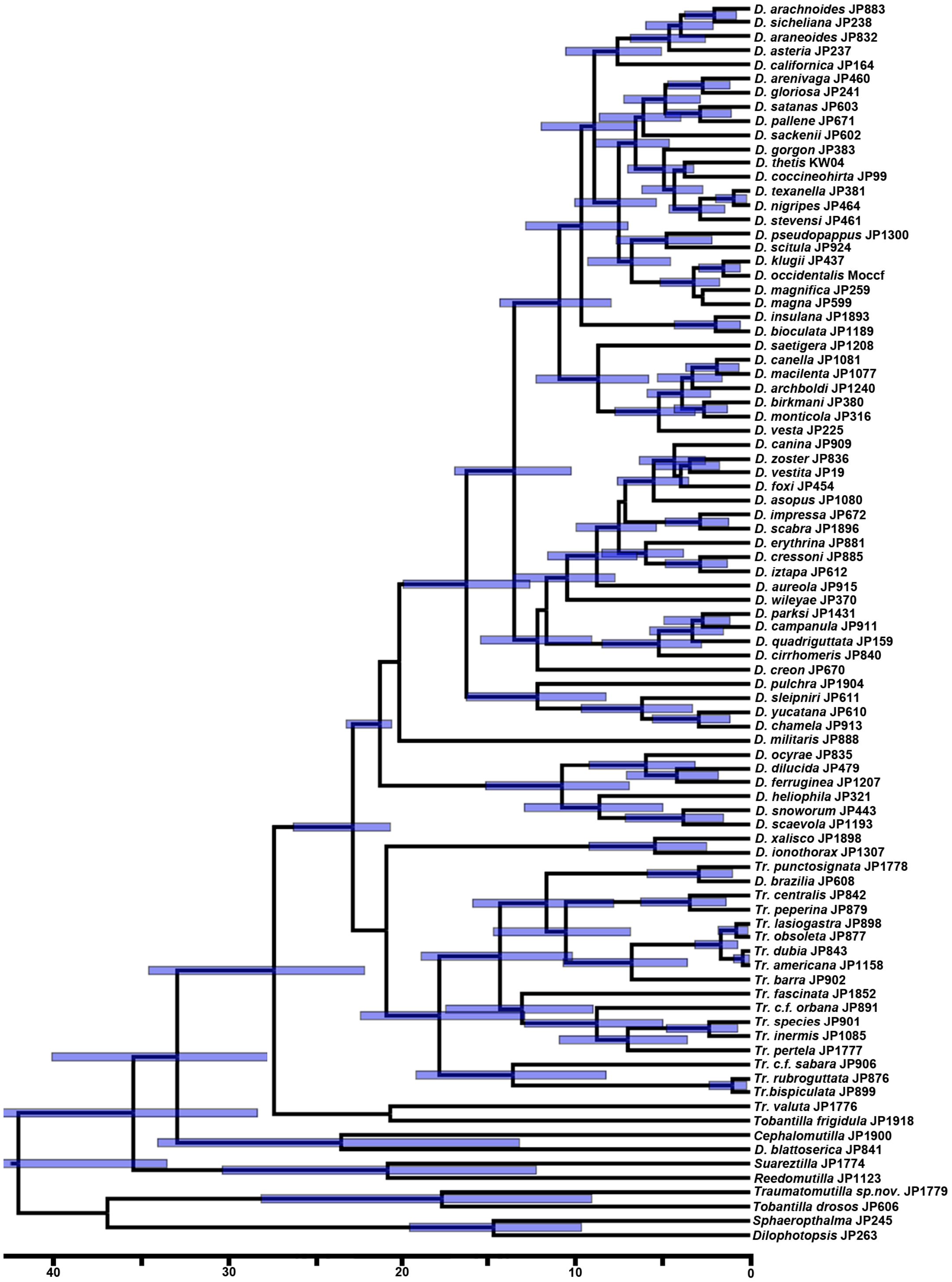

Fig. 2.5. BEAST chronogram. Post-burnin uncollapsed tree from the BEAST analysis. Blue bars at each node represent 95% credibility intervals for dates.

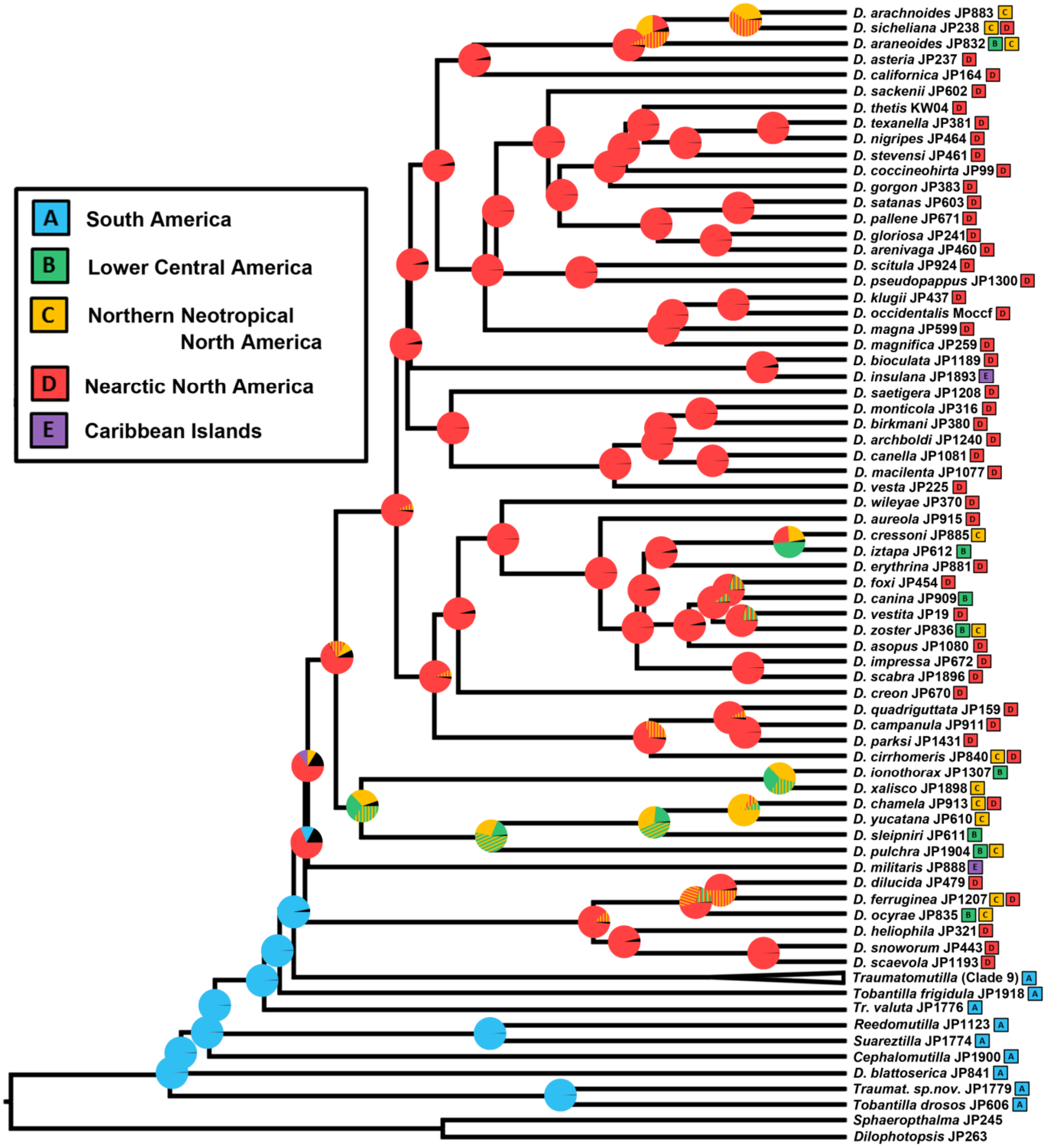

Fig. 2.6. RASP analysis. Condensed phylogeny from 10000 post burnin trees from mrBayes analysis. Pie charts at each node represent probabilities of alternative ancestral ranges. Color key matches areas from Fig. 1; multicolored portions represent reconstructions in multiple areas; black represents sum of minority ancestral ranges.

Pleistocene speciation events in dating analyses of nocturnal sphaeropthalmines (r8s, fossil > 20 Ma; Pitts et al., 2010), only three Pleistocene speciation events were recovered in the dasymutilline mutillids (Fig. 5).

3.3 Ancestral Area Reconstructions

The condensed tree with ancestral reconstructions from RASP indicates that all of the oldest ancestral nodes were recovered as South American (Fig. 2.6), implying that dasymutillines originated in South America. The single North American clade, *Dasymutilla*, has an ambiguous reconstruction for its three deepest nodes, but in each of these nodes, Nearctic North America was recovered as the ancestral area in over 60% of the replications. Some of the *Dasymutilla* subclades reveal clearer ancestral reconstructions; clades 10A and 10D had Nearctic North American ancestors. Although a single area was not recovered for the ancestors of Clades 10B and 10C, each of their most likely ancestral areas is in Neotropical North America. The Caribbean region was not recovered as ancestral to any dasymutilline species; the two included Caribbean species have Nearctic or ambiguous ancestral areas. In clades 10A and 10D, Neotropical North American species were recovered with Nearctic ancestors.

4. Discussion

4.1 Intercontinental dispersal in dasymutillines

Our study reveals that dasymutilline genera originated in South America. This is correlated with higher genus-level diversity on that continent (Table 2.3). A similar pattern was recovered in the Nocturnal sphaeropthalmine mutillids (Pitts et al., 2010). These findings are consistent with the assertion of Brothers (1975) that sphaeropthalmine mutillids had a Gondwanan origin. Two of the largest New World mutillid lineages, Ephutini and Pseudomethocina, reveal a similar pattern (Krombein, 1979; Nonveiller, 1990). Ephutini includes five South American genera, but only one genus in Nearctic North America; Pseudomethocina includes over 20 South American genera, but only three Nearctic genera. We hypothesize that these lineages also originated in South America.

The North American dasymutillines (*Dasymutilla*) are monophyletic (Clade 10; Fig. 2.3), suggesting that a single ancestral lineage colonized North America. This clade is poorly supported, but was recovered in our other phylogenetic reconstructions as well. This lineage began diversifying in the early Miocene (Figs. 2.4, 2.5), indicating that dasymutilline velvet ants dispersed between continents prior to the Pliocene GABI. This biogeographic scenario is not unprecedented, as Miocene dispersal into North America was also discovered in multiple amphibian groups (Pinto-Sánchez et al., 2011; Weigt et al., 2005), euglossine bees (Ramirez et al., 2010), beetles and pseudoscorpions (Zeh et al., 2003) and other groups (reviewed by Cody et al., 2010).

The ancestral area reconstructions do not clearly indicate which corridor dasymutillines used to enter North America. Most of the oldest nodes have ambiguous ancestral area reconstructions, although Nearctic North America itself is considered the most likely ancestral area. We hypothesize that Central America was the corridor used by dasymutillines for three reasons. First, this corridor is supported as the dispersal corridor for the other groups that entered North America during the Miocene (Cody et al., 2010). Second, the GAARlandia formation was only open from 35-33 Ma (Iturralde-Vincent & MacPhee, 1999), implying that it was closed even before ancestral South American dasymutillines began to diversify. Third, no dasymutilline species have Caribbean ancestors (Fig. 2.6) and no species are distributed in both the Caribbean and mainland, as might be expected if they had dispersed through GAARlandia land masses. Conversely, Nearctic populations of *D. chamela* (Manley & Pitts) are clearly derived from Central American ancestors (Fig. 2.6) and several Miocene species are widespread in Neotropical North America or in both Nearctic and Neotropical North America, suggesting that dasymutillines were diversifying in Central America shortly after arrival from South America.

The exact mechanisms for dasymutilline entry to North America are uncertain. Oceanic dispersal by rafting or flotation has been established in groups with limited dispersal capabilities (de Queiroz, 2004). We hypothesize that a dasymutilline ancestor arrived on the Chocó block (Fig. 2.2) from northwestern South America in the early Miocene, when only a narrow straight separated these land masses (Montes et al., 2012). At this time, the Chocó and Chorotega blocks were apparently connected until 19 Ma (Kirby et al., 2008). After division of the Choco and Chorotega blocks, the Chorotega block apparently formed a peninsula that was intermittently open until the Pliocene (Kirby et al., 2008). In addition to allowing northward expansion, these temporally intermittent land bridges may have also facilitated vicariant speciation in Miocene dasymutillines.

4.2 Assembly of Antillean fauna

Nine Antillean dasymutilline species are recognized (Table S1). Three of these are known only from Trinidad. Each of these *Traumatomutilla* species is also found in mainland northern South America (Nonveiller, 1990). Although molecular sequences were not available, morphology suggests these are members of Clade 9. Unlike the Trinidad species, the six Greater Antilles species are island endemics. Genaro (1997) divided the Greater Antillean *Dasymutilla* into two species-groups that were diagnosed by differences in male coloration. We were able to include one species from each of his groups and they are not closely related (Fig. 6). *Dasymutilla insulana* Mickel was recovered as the sister to the Nearctic species, *D. bioculata* (Cresson). These species share a Nearctic North American ancestor and resulted from Pliocene speciation. *Dasymutilla bioculata* occurs throughout the eastern United States; *D. insulana* could have entered the Greater Antilles by overwater dispersal from Florida. *Dasymutilla militaris* (Smith) was recovered on a long branch in a polytomy with the remaining *Dasymutilla* clades (10A-D). The phylogenetic uncertainty of this group, coupled with its broad geographic distribution, lead to an ambiguous ancestral area reconstruction for this node. As such, the geographic origin of *D. militaris* ancestors is unknown. Morphology of the Dominican Amber fossils, however, suggests that ancestors of *D. militaris* reached the Greater Antilles during or before the early Miocene.

4.3 Continental North American assemblages

Three continental North American assemblages were studied: southern Central America, Neotropical Mexico to northern Nicaragua, and Nearctic North America. Nearctic North America was populated mostly by members of clade 10A and 10D, although *D. chamela* (Clade 10C) has its mainly Neotropical range extending into the Madrean Archipelago. The ancestor of both clades 10A and 10D were reconstructed to have a Nearctic distribution. We hypothesize that colonization of the drier habitats of Nearctic North America, in either the Chihuahuan or Sonoran Deserts, permitted the ancestors of these clades to radiate throughout the Nearctic region. *In situ* diversification in the temperate Nearctic throughout the Neogene led to one of the most diverse lineages in all Mutillidae.

The remaining two clades of *Dasymutilla* are mostly restricted to Neotropical habitats (10B & 10C). Reconstructions for both of these clades reveal a Lower Central America, northern Neotropical, or widespread Central American ancestral distribution. Many of the speciation events in these clades occurred during the late Miocene and Pliocene. Opening and closing of seaways dividing the Chocó, Chorotega, Chortis, Mayan and Oaxaca blocks could have caused vicariant speciation (Fig. 2.2).

Members of clades 10A and 10D have also colonized the Neotropical North American regions. Neotropical species from these clades have Nearctic ancestors (Fig. 2.6), suggesting these lineages have re-invaded Central America. Many species found in Lower Central America are widespread throughout Neotropical North America and those that are restricted to Lower Central America resulted from speciation events in the late Pliocene. We hypothesize that species from Clades 10A and 10D that currently thrive on the Isthmus of Panama did not colonize that region until after its formation, 3Ma.

Traumatomutilla dictynna (Cameron) is widespread in southern Central America. Although molecular data was not available, we hypothesize that it entered southern Central America after the Chocó block accreted with the South American plate in the middle Miocene because its morphology suggests it is nested within Clade 9. It potentially only reached Costa Rica and western Panama after the Isthmus of Panama was fully formed. Only one member of the North American Clade 10 has re-invaded South America. Specimens of the Central American *D. icaris* (Cameron), putatively belonging to clade 10C, were recently examined from the Tolima Department in Colombia. Although the Great American Biotic Interchange allowed cross-continental dispersal for numerous chordate species (Simpson, 1980; Weir et al., 2009), *D. icaris* and *T. dictynna* are the only hypothesized dasymutillines to have participated in this event.

4.4 Dates of speciation events

In the Nearctic nocturnal mutillid fauna, many extant species resulted from Pleistocene speciation events (Pitts et al., 2010). Our study, however, revealed only three Pleistocene speciation events (Fig. 2.5), all occurring in the South American Clade 9. These ”speciation” events, however, could reflect conspecific divergence; each of these pairs includes one male and one female, possibly revealing new sex associations rather than sister species. Neogene orogeny events involving formation of the Madrean, Rocky, and Sierra mountain ranges, occurring between 1.8 and 15 Ma (Pitts et al., 2010), are apparently responsible for most speciation events in Nearctic North American clades (10A and 10D). Speciation occurred during the Neogene in other regions as well, implicating intermittent island and peninsula formation in Central America (Kirby et al., 2008) and habitat shifts involving Andean uplift in South America (Hoorn et al., 2010).

4.5. Summary and Conclusions

Phylogenetic reconstruction of dasymutilline Mutillidae suggests existence of 10 or more genus-level groups, rendering *Dasymutilla*, *Tobantilla*, and *Traumatomutilla* paraphyletic. Almost all of the genus-level diversity is South American; a single lineage colonized North America. We hypothesize that this lineage colonized Central America in the early Miocene by rafting. From Lower Central America, ancestors of *Dasymutilla* used temporally intermittent land bridges to expand their range northward.

Fauna of North American regions were assembled from different ancestral areas, times, and dispersal events. The southern Central American fauna, for example, were assembled from five clades and four ancestral areas. The diverse Nearctic North American fauna were almost exclusively recovered within two *Dasymutilla* clades.

Most extant dasymutilline species originated from speciation events during the middle Miocene to Pliocene (15 to 1.8 Ma). Mountain building and its effect on the surrounding climate likely caused speciation in North and South America, while opening and closing of waterways was a driving force in Central America.

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CHAPTER 3

PHYLOGENETIC EVIDENCE FOR MÜLLERIAN MIMICRY RINGS IN *DASYMUTILLA* ASHMEAD (MUTILLIDAE)[2]

INTRODUCTION

Müllerian mimicry is a mutualism in which two or more species with effective secondary defenses share a similar appearance for mutual benefit (Müller 1879). This is a well-known, yet relatively uncommon natural phenomenon. While Müllerian mimicry rings – groups of species that benefit from shared aposematic signals – have been documented from a variety of taxa including reptiles, amphibians, and arthropods (reviewed by Ruxton et al. 2004), the most well known examples are Lepidopteran (butterflies and moths) (e.g. Ritland & Brower 1991; Niehuis et al. 2007) with the Neotropical butterfly genus *Heliconius* among the most celebrated model systems (Brower 1996). Although Müllerian mimicry has been recognized in some Nearctic species (Marek & Bond 2009), species-rich mimicry rings are more often documented in tropical taxa. The apparent higher incidence of mimicry in the tropics has led to the postulation that mimicry may evolve more easily in these habitats due to higher predator and prey diversities (Merrill & Jiggins 2009), but more studies on non-Lepidopteran groups (Ruxton et al. 2004) and from non-tropical taxa are needed to better understand this phenomenon.

Velvet ants (Hymenoptera: Mutillidae) are a common, yet often overlooked, component of many North American habitats and are becoming a model system for historical biogeography in North American deserts (Wilson & Pitts 2010; Wilson et al.

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2011). Among velvet ants, the wingless females of the diurnal genus *Dasymutilla* are aposematically colored and are protected by a suite of secondary defenses, which often include stridulation (auditory aposematism), pungent exudate secretions, a strong slippery cuticle, and a painful sting (Manley 2000). Although velvet ants primarily parasitize the nests of potentially dangerous hosts, solitary bees and wasps, their well-defended nature likely evolved in response to predation pressures (Vitt & Cooper 1988; Manley 2000; Manley & Sherbrooke 2001). Many velvet ants are abundant in xeric habitats and active at the same time as most diurnal terrestrial lizards, yet lizards rarely feed on velvet ants (Vitt & Cooper 1988). Feeding trials revealed that some lizards will consume slender ant-like mutillids, but most species either avoid larger velvet ants (Vitt & Cooper 1988; Manley 2000; Manley & Sherbrooke 2001), or are quickly trained to recognize and avoid them (Schmidt & Blum 1977; Vitt & Cooper 1988).

The effectiveness of velvet ant defenses has likely been influential in the evolution of various Batesian mimics, harmless species imitating the warning signals of harmful species (Bates 1862). Velvet ant Batesian mimics include various beetles (Acorn 1988; Mawdsley 1994; Lanteri & Del Rio 2005), spiders (Edwards 1984; Nentwig 1985), and antlion larvae (Brach 1978). Several authors have noted the phenotypic similarity among unrelated velvet ant species (Ferguson 1966; Pilgrim & Pitts 2006) and even documented high amounts of color polymorphism within a widespread species (Williams et al. 2011), yet mimicry within velvet ants has only been reported from selected species that are thought to mimic ants (Yanega 1994; Manley 2000).

Velvet ants, particularly the species rich genera like *Dasymutilla*, are ideal subjects for studying aposematism and mimicry for several reasons. First, distinct color forms are present within and among *Dasymutilla* species, which permit population- and species-level studies of mimicry. Second, *Dasymutilla* mimicry rings span the North American continent, with each ring containing several species in multiple microhabitats; mimicry systems can be studied in a range of scales. Furthermore, velvet ants are sexually dimorphic, the harmless winged males often do not resemble their conspecific females; Batesian mimicry could also be studied. Aposematic color syndromes can be found on nearly every continent (KA Williams, unpubl. data), which can lead to localized mimicry ring research by scientists worldwide.

Here, we report on the largest Müllerian mimicry complex yet to be identified, with at least 65 North American *Dasymutilla* species involved in six distinct mimicry rings (Table 3.1). While structural morphological characters can often be used to distinguish each of these species, multiple geographically concordant color forms are present within and among species (Fig. 3.1). Müllerian mimicry has been documented in several taxa, yet multiple analyses have found that some of the similarities in supposed mimetic traits can be best explained by phylogenetic relatedness rather than through independent evolutionary events (Brower 1995; Dumbacher & Fleisher 2001; Marek & Bond 2009). The goals of this paper are twofold: first, to describe morphological variation among female *Dasymutilla* velvet ants that potentially comprise multiple, overlapping mimicry rings; and second, to investigate the phylogenetic context in which mimetic phenotypes have evolved. The latter objective allows us to ask if morphologies involved in Müllerian mimicry have evolved multiple times or whether different phenotypes represent single evolutionary origins.

TABLE 3.1. Collection locality for each species included in the analysis and mimicry ring designations.

species	Specimen voucher	Sex	Genbank ITS1	Genbank ITS2	Genbank wg	Mimicry Ring	Locality
1. *D. ocyrae*	JP835	female	JQ945794	JX002816	JQ945872	Tropical	NICARAGUA: Granada
2. *D. dilucida*	JP479	female	JQ945783	JX002817	JQ945851	Madrean	USA: AZ: Cochise Co.
3. *D. ferruginea*	JP667	female	JX002836	JX002820	n/a	Madrean	MEXICO: Sonora
4. *D. scaevola*	JP1196	female	JQ945802	JX002815	JQ945887	Eastern	USA: FL: Alachua Co.
5. *D. chalcocephala*	JP1146	female	JX002837	JX002821	n/a	Tropical	C.R.: Guanacaste
6. *D. creon*	JP670	male	JQ945781	JX002799	JQ945849	Eastern	USA: TX: Harris Co.
7. *D. pulchra*	JP1904	male	JQ945798	JX002809	JQ945874	Tropical	MEXICO: Jalisco
8. *D. spilota*	JP833	female	JX002838	JX002822	JX002747	Tropical	NICARAGUA: Granada
9. *D. citromaculosa*	JP913	male	n/a	JX002812	JQ945848	Madrean	MEXICO: Sonora
10. *D. phya*	JP611	male	JQ945805	JX002810	JQ945890	Tropical	PANAMA: B.C. Island
11. *D. cirrhomeris*	JP840	male	JQ945785	JX002796	JQ945858	Madrean	MEXICO: Sonora
12. *D. klugiodes*	JP910	female	JX002851	n/a	n/a	Texan	USA: NM: Lea Co.
13. *D. campanula*	JP911	female	JQ945776	JX002797	JQ945844	Western	USA: CO: Weld Co.
14. *D. quadriguttata*	JP159	male	HQ317245	HQ317267	JQ945910	Eastern	USA: AR: Pulaski Co.
15. *D. wileyae*	JP370	female	EU627572	EU627572	JQ945896	Texan	USA: TX: LaSalle Co.
16. *D. aureola*	JP915	female	JQ945773	JX002835	JQ945839	Western	USA: OR: Lake Co.
17. *D. monstrosa*	JP672	male	JQ945786	JX002800	JQ945862	Madrean	MEXICO: Sonora
18. *D. eminentia*	Demi01	female	JX002839	JX002823	n/a	Desert	USA: AZ: Cochise Co.
19. *D. furina*	Dfur01	female	JX002840	JX002824	n/a	Western	USA: AZ: Cochise Co.
20. *D. erythrina*	JP881	male	JQ945784	JX002802	JQ945911	Western	MEXICO: Michoacan
21. *D. cressoni*	JP885	female	JQ945782	JX002803	JQ945850	Tropical	MEXICO: Guerrero
22. *D. munifica*	JP612	male	JQ945789	JX002804	JQ945865	Tropical	C.R.: Guanacaste
23. *D. asopus*	JP1080	male	JQ945771	JX002808	JQ945837	Eastern	USA: FL: Orange Co.
24. *D. nigricauda*	JP440	female	JX002841	JX002825	n/a	Western	USA: TX: Randall Co.
25. *D. waco*	JP914	female	JX002852	n/a	n/a	Texan	USA: TX: Walker Co.
26. *D. canina*	JP909	female	JQ945778	JX002807	JQ945847	Tropical	NICARAGUA: Granada
27. *D. zoster*	JP836	female	JQ945811	JX002805	JQ945899	Tropical	NICARAGUA: Granada
28. *D. foxi*	JP493	female	EF433451	n/a	n/a	Western	USA: AZ: Cochise Co.
29. *D. vestita*	JP19	male	JQ945808	JX002806	JQ945895	Western	USA: UT: Cache Co.
30. *D. zelaya*	JP450	female	JX002842	JX002826	n/a	Texan	USA: TX: Val Verde Co.
31. *D. saetigera*	JP1208	male	JQ945797	JX002790	JQ945883	Madrean	MEXICO: Sonora
32. *D. vesta*	JP1233	male	JX002870	JX002887	JQ945894	Eastern	USA: FL: Polk Co.
33. *D. archboldi*	JP1240	female	JQ945769	JX002794	n/a	Eastern	USA: FL: Polk Co.
34. *D. arenneronea*	JP163	male	EU627569	EU627569	JQ945836	Eastern	USA: FL: Sarasota Co.
35. *D. canella*	JP1081	female	JQ945777	JX002793	JQ945846	Eastern	USA: MA: Plymouth Co.
36. *D. macilenta*	JP1077	male	JQ945791	JX002792	JQ945867	Eastern	USA: FL: Orange Co.
37. *D. chiron ursula*	JP256	male	HQ317257	HQ317281	EU367350	Western	USA: UT: Cache Co.
38. *D. melanippe*	JP368	female	HQ317254	HQ317276	n/a	Texan	USA: TX: LaSalle Co.
39. *D. lepeletierii*	Mlepf2	female	HQ317255	HQ317270	JX002746	Eastern	USA: SC: Florence Co.
40. *D. californica*	JP164	female	EU627568	EU627568	JQ945843	Western	USA: CA: San Diego Co.
41. *D. arachnoides*	JP1203	female	JX002843	JX002827	JX002745	Tropical	MEXICO: Guerrero
42. *D. araneoides*	JP832	female	JQ945768	JX002785	JQ945834	Tropical	NICARAGUA: Granada
43. *D. sicheliana*	JP238	female	JQ945804	JX002786	JQ945889	Madrean	USA: AZ: Cochise Co.
44. *D. asteria*	Dast01	female	JX002844	JX002828	n/a	Madrean	USA: AZ: Santa Rita Co.
45. *D. pseudopappus*	JP1300	female	JX002845	JX002829	n/a	Desert	USA: CA: Riverside Co.
46. *D. scitula*	JP924	male	JQ945803	JX002789	JQ945888	Western	USA: UT: Garfield Co.
47. *D. magna*	JP599	female	JQ945792	JX002781	JQ945868	Desert	USA: AZ: Santa Rita Co.

species	Specimen voucher	Sex	Genbank ITS1	Genbank ITS2	Genbank wg	Mimicry Ring	Locality
48. *D. magnifica*	JP259	female	DQ408497	DQ408497	JQ945869	Texan	USA: AZ: Cochise Co.
49. *D. occidentalis*	MoccF	female	HQ317246	HQ317268	JQ945909	Eastern	USA: SC: Florence Co.
50. *D. calorata*	JP438	female	JX002846	JX002830	n/a	Western	USA: TX: Randall Co.
51. *D. klugii*	JP437	male	JQ945790	JX002782	JQ945866	Texan	USA: TX: LaSalle Co.
52. *D. sackenii*	JP602	male	JQ945799	JX002783	JQ945884	Western	USA: CA: Lassen Co.
53. *D. gloriosa*	JP241	female	HQ317244	HQ317288	JQ945859	Desert	USA: AZ: Maricopa Co.
54. *D. arenivaga*	JP460	female	JQ945770	JX002775	JQ945835	Western	USA: AZ: Maricopa Co.
55. *D. nocturna*	JP266	female	DQ408502	DQ408502	n/a	Desert	USA: CA: Imperial Co.
56. *D. satanas*	JP603	female	JQ945800	JX002775	JQ945885	Western	USA: UT: Washington Co.
57. *D. pallene*	JP671	female	JQ945780	JX002780	JQ945873	Desert	MEXICO: Sonora
58. *D. nogalensis*	Dpha01	male	JX002847	JX002831	n/a	Texan	USA: AZ: Cochise Co.
59. *D. gorgon*	JP383	female	HQ317247	HQ317289	JQ945860	Texan	USA: TX: LaSalle Co.
60. *D. leda*	JP384	female	JX002848	JX002832	n/a	Western	USA: TX: Randall Co.
61. *D. coccineohirta*	JP99	female	JQ945779	JX002777	JX002743	Western	USA: CA: Riverside Co.
62. *D. thetis*	KW05	female	EF433452	EF433452	JQ945845	Desert	USA: AZ: Cochise Co.
63. *D. nigripes*	JP463	female	EU627570	EU627570	JQ945871	Eastern	USA: TX: Randall Co.
64. *D. texanella*	JP381	female	JX002853	JX002776	JQ945893	Texan	USA: TX: LaSalle Co.
65. *D. atricauda*	JP487	female	JX002849	JX002833	n/a	Western	USA: CA: Inyo Co.
66. *D. nupera*	JP449	female	JX002850	JX002834	n/a	Texan	USA: TX: LaSalle Co.
67. *D. stevensi*	JP461	male	JQ945807	JX002776	JQ945892	Western	USA: TX: Presidio Co.
Traumatomutilla inermis	JP1085	female	JQ945818	JX002765	JQ945877	n/a	BRAZIL: Espiritu Santo

MATERIALS AND METHODS

Sampling and Study Organism

Specimens were collected from sites across North America from 2002 to 2011 primarily using malaise traps, and by hand. All specimens were placed directly into 95% ethanol, and those used for molecular examination have been labeled as voucher specimens and deposited in the Department of Biology Insect Collection, Utah State University, Logan, UT (EMUS). Currently, 129 of the 178 *Dasymutilla* species are known from the female sex; ten of these species are known only from South America or the Caribbean Islands leaving 119 North American species known from females. Because

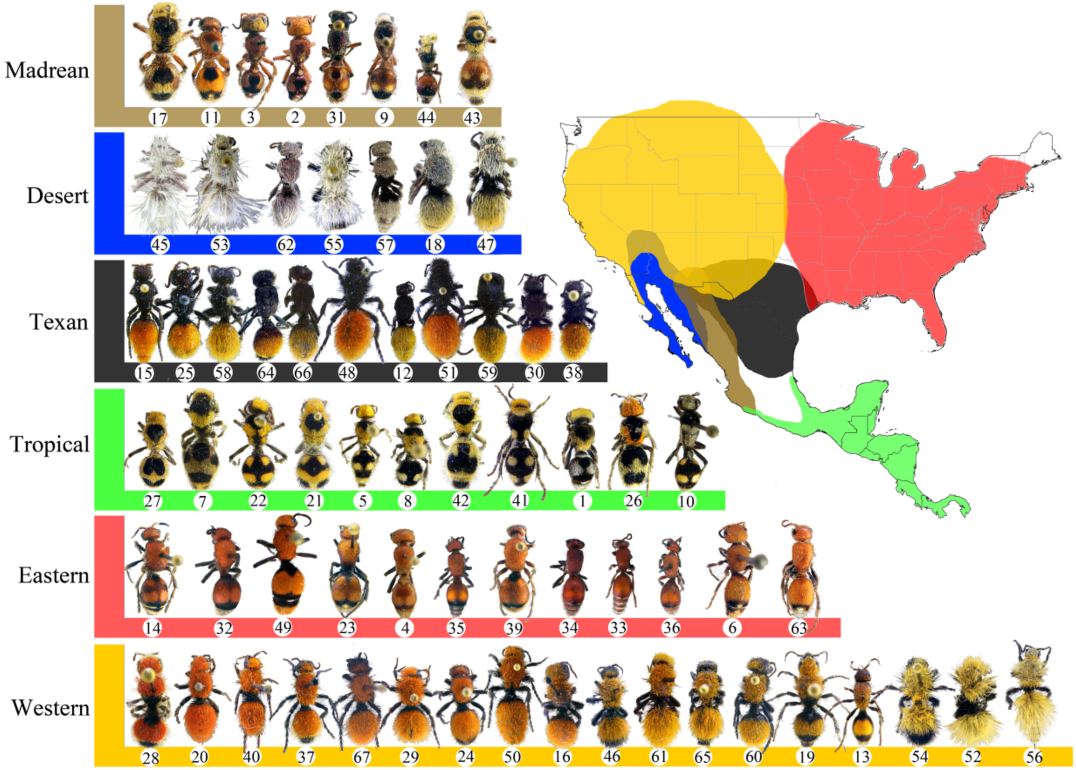

FIGURE 3.1. Velvet ant mimicry rings. 67 *Dasymutilla* species are placed into six mimicry rings that are defined by shared color form and geographic location. Numbers under each species correspond to Fig. 3.3 and Table 3.1.

recent molecular systematic treatments of this genus have resulted in numerous synonymies (e.g., Pilgrim et al. 2008a, 2009), it is likely that future taxonomic investigations will revise species estimations in this group. We sampled 67 of these *Dasymutilla* females from across North America.

Molecular Markers

Molecular methods including DNA extraction, PCR, and sequencing followed the protocol outlined in Pilgrim and Pitts (Pilgrim & Pitts 2006, Pilgrim et al. 2008b).

Because recent analyses of *Heliconius* mimicry rings have shown that phylogenetic hypotheses based on coding and non-coding genes give different results (Hines et al. 2011), we targeted a protein-coding marker, wingless (wg), and two non-coding markers, the internal transcribed spacer regions 1 and 2 (ITS1 and ITS2). The following primers were used to amplify the ITS1 and ITS2 regions of the nuclear genome. The primers 5′-GATTACGTCCCTGCCCTTTG-3′ (forward-18S) and 5′-CGATGATCAAGTGTCCTG CA-3′ (reverse-5.8S) (both from Pilgrim et al., 2002) were used for the ITS1 locus and 5′-GGCTCGTGGAATCGATGAAGAACG-3′ (forward 5.8S) and 5′-GCTTATTAATAT GCTTAAATTCAGCGG-3′ (reverse -28S) (Wilson & Pitts 2010) were used for ITS2. The primers 5′-GARTGYAARTGYCAYGGYATGTCTGG-3′ (LepWg1 for) and 5′-GC WGTRACTCACAGYATCGC-3′ (Wg290F) were used to amplify wg (Pilgrim et al. 2008b).

PCR amplification took place under the following general conditions: 20 μL total volume, with concentrations of 3 mm $MgCl_2$, 200 pm dNTPs, 2 units of *Taq* polymerase, 1 mm of each primer, standard PCR buffer and *c.* 20 ng of template DNA. The PCR program included an initial step of 94 °C for 150 s, followed by 35 cycles of 94 °C for 30 s, 46–60 °C for 60 s and 72 °C for 60 s, with a final step of 72 °C for 10 min. PCR products were visualized using standard agarose gel electrophoresis, and successful amplifications were cleaned for DNA sequencing reactions using isopropanol purification.

Sequences were analyzed with an ABI Prism 377, 3100, or 3730 Genetic Analyzer. Gel electrophoresis of each gene yielded a single band for each individual wasp and the resulting DNA was sequenced cleanly. PCR products were sequenced in both directions and were combined in SEQUENCHER, version 4.1 (Gene Code Corp.).

Phylogenetic Analyses

The non-coding (ITS1 and ITS2) and coding (wg) markers were analyzed separately with Bayesian methods using MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). The ITS regions were analyzed as a combined data set with each gene partitioned separately. The GTR+Γ model was applied to ITS1 and GTR+Γ+I applied to ITS2 as determined in MrModeltest version 2.3 (Nylander 2004). The protein-coding wg locus was analyzed under the SYM+I+Γ model. Bayesian analyses included four independent runs with three heated chains and one cold chain in each run. The MCMC chains were set for 10,000,000 generations and sampled every 100 generations; chains were run until the average standard deviation of the split frequencies dropped below 0.01. A 10% burn-in was removed after graphical determination of stationarity using Tracer v1.5 (Rambaut & Drummond 2007). A *Traumatomutilla* species was included as an outgroup. All sequences have been submitted to GenBank (Table 3.1).

Morphological Analyses

We quantified seven color characters and one setal character using digital images of each species (Fig. 3.1) using ImageJ (http://rsb.info.nih.gov/ij/). Color characters were measured from digital images of each species and included the percent black of the mesosoma, percent black of the metasoma, pattern on metasoma, integument color, and non-black metasomal color measured in red, green, and blue (RGB). Setal length was also recorded as a categorical variable. Morphological characters were analyzed using resemblance matrices, nonmetric multidimensional scaling (NMDS) based on a Bray-Curtis distance matrix, and analysis of similarity (ANOSIM) with Primer-E (Clarke & Gorley 2006). Permutation was used to test for significant clustering among species.

Forty-seven of the individuals included in this analysis were females. Of the remaining 20 taxa, 14 are recognized from both sexes in the literature (Krombein et al. 1979; Manley & Pitts, 2007; Pilgrim et al. 2008b). The remaining six species were associated with females based on shared morphology and distribution. The putative female of *D. impressa* Manley & Pitts is *D. monstrosa* Manley & Pitts; the putative female of *D. iztapa* (Blake) is *D. munifica* (Smith); the putative female of *D. chamela* Manley & Pitts is *D. citromaculosa* Manley & Pitts; the putative female of *D. sliepniri* Manley & Pitts is *D. phya* (Cameron); the putative female of *D. polia* Mickel is *D. saetigera* Mickel, an undescribed male is putatively associated with *D. cirrhomeris* Manley & Pitts. These taxa, which are represented in the phylogeny by male specimens, are designated by their putative female names in the figures and discussion for ease of comparison and the official associations will be made in future publications.

Convergent Evolution

In order to establish whether shared color patterns are truly randomly distributed on each phylogeny, or if they might be attributed to phylogenetic relationships (i.e. common descent) we used a Bayesian approach to compare our observed distribution of color forms on each phylogeny to a null, random distribution using program BaTS (Bayesian Tip-association Significance testing: Parker et al. 2008). BaTS tests for significant phylogeny-trait correlations and accounts for phylogenetic uncertainty by integrating over the entire collection of post burn-in trees generated by the MrBayes analysis of each dataset. We used 1,000 state-randomizations to create the null distribution in order to test the significance of our observed data.

RESULTS

Description of Mimicry Complex

Velvet ants in the genus *Dasymutilla* are found throughout North America, ranging from Peru to Canada. One hundred twenty-nine of the 178 *Dasymutilla* species are known from the female sex; ten of these species are known only from South America or the Caribbean Islands. We obtained molecular data for females of 67 *Dasymutilla* species (Table 3.1) from across North America, representing six geographically based mimicry rings (Fig. 3.1). These mimicry rings in *Dasymutilla* are based on shared color patterns and geographic location. Permuted analysis of similarity (ANOSIM) results indicate that each of the six mimicry rings is morphologically distinct ($_{\text{ANOSIM}}R = 0.35$-0.96, P = 0.001: Table 3.2, Fig. 3.2). Although phylogenetic and ANOSIM results are available for only 67 of the 119 continental North American females, Table 3.3 summarizes the putative mimicry complex designation for each species. Rather than name each mimicry ring based on color characteristics, as has often been done in butterfly mimicry rings (Mallet & Gilbert 1995), we categorize each mimicry ring based on the geographic location of the ring.

The **Madrean** mimicry ring gets its name from the Sierra Madre Occidental Mountains and the Madrean Archipelago (Omernik 1987) where it most commonly occurs. Species involved in the Madrean mimicry ring have reddish integumental coloration that is sharply marked with contrasting black, white, silver, gold, or yellow setal and integumental patterns (Fig. 3.1). Many of the species in this mimicry ring also have silver or golden setae on their head and white or yellow markings on the second metasomal tergite (T2). Only eight of the 19 putative members of the Madrean mimicry ring are included in the analysis.

The **Desert** mimicry ring primarily occurs in the warm deserts of North America (Mojave, Sonoran, Peninsular, and Chihuahuan) with most of the species concentrated in the Sonoran and Peninsular deserts. These species all share a common color pattern of white setae on the head and mesosoma, with a white to pale yellow metasoma (Fig. 3.1).

TABLE 3.2. Pairwise comparisons of ANOSIM results among the six mimicry rings included in this study.

	Desert	Eastern	Madrean	Tropical	Texan	Western
Desert		0.839	0.78	0.589	0.957	0.721
Eastern			0.511	0.943	0.892	0.346
Madrean				0.616	0.794	0.708
Tropical					0.827	0.756
Texan						0.651
Western						

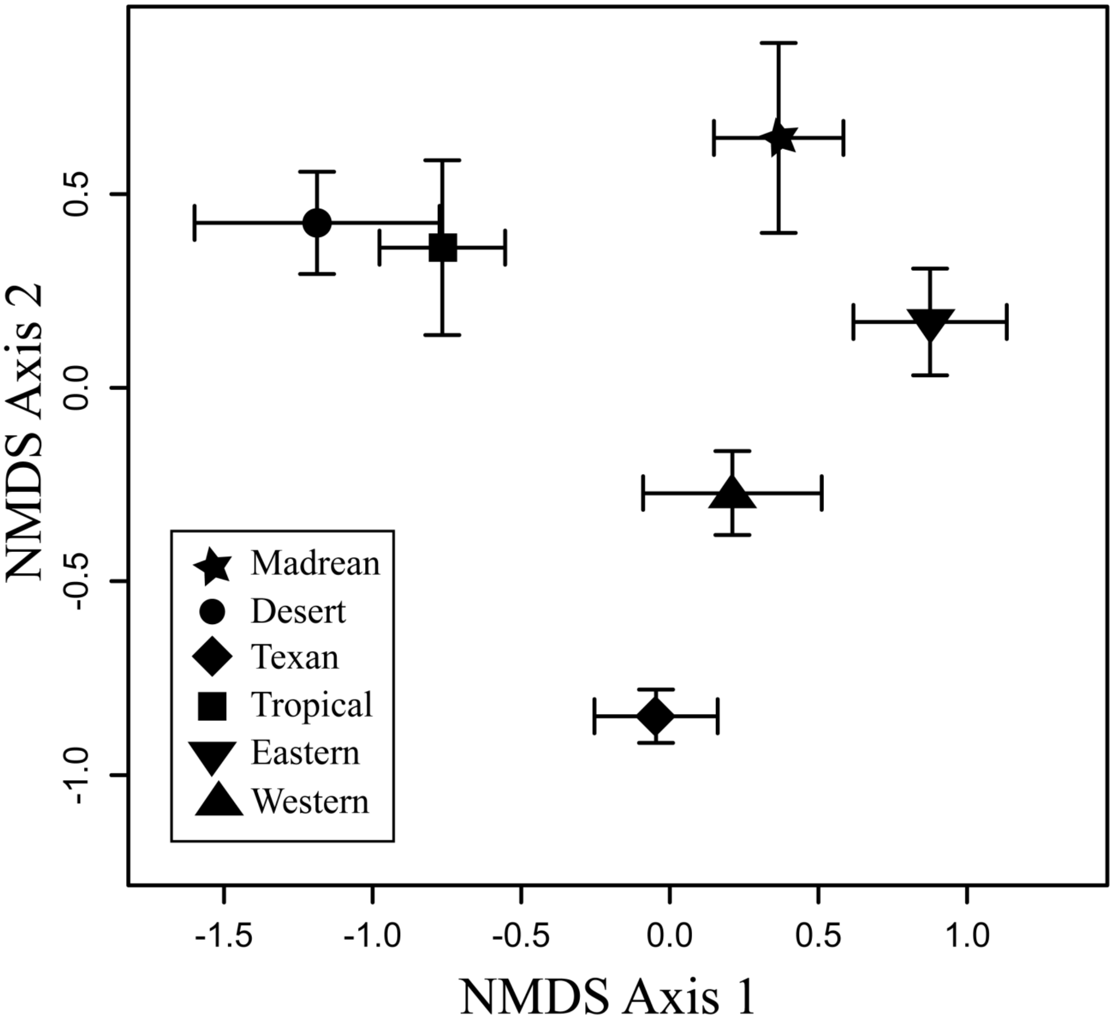

FIGURE 3.2. Ordination plot of morphological characters. NMDS plot of means and standard deviations of morphological characters from each of the six mimicry rings.

TABLE 3.3. List of continental North American females in *Dasymutilla*.

species	Clade	Western	Eastern	Tropical	Texan	Desert	Madrean	Uncertain
D. albiceris	5*	0	0	0	0	1	0	0
D. altamira	6*	0	0	0	0	0	0	1
D. angulata	5*	0	1	0	0	0	0	0
D. arachnoides	8	0	0	1	0	0	0	0
D. araneoides	8	0	0	1	0	0	0	0
D. archboldi	6	0	1	0	0	0	0	0
D. arenivaga	9	1	0	0	0	0	0	0
D. arenneronea	6*	0	1	0	0	0	0	0
D. argentea	1*	0	0	1	0	0	0	0
D. asopus	5	0	1	0	0	0	0	0
D. asteria	8	0	0	0	0	0	1	0
D. atricauda	9	1	0	0	0	1	0	0
D. aureola	5	1	0	0	0	0	0	0
D. batui	9*	0	0	0	0	0	0	1
D. bioculata	7*	1	1	0	0	0	0	0
D. birkmani	6*	0	0	0	0	0	0	1
D. bonita	6*	0	0	0	0	0	1	0
D. californica	8	1	0	0	0	0	0	0
D. calorata	9	1	0	0	0	0	0	0
D. campanula	5	1	0	0	0	0	0	0
D. canella	6	0	1	0	0	0	0	0
D. canina	5	0	0	1	0	0	0	0
D. cardinalis	5*	1	0	0	0	0	0	0
D. chalcocephala	2	0	0	1	0	0	0	0
D. chionothrix	7*	0	0	0	0	1	0	0
D. chiron ursula	7	1	0	0	0	0	0	0
D. cirrhomeris	5	0	0	0	0	0	1	0
D. citromaculosa	4	0	0	0	0	0	1	0
D. clotho	9*	0	0	0	1	0	0	0
D. coccineohirta	9	1	0	0	0	0	0	0
D. combusta	1*	0	0	0	0	0	1	0
D. creon	3	0	1	0	0	0	0	0
D. cressoni	5	0	0	1	0	0	0	0
D. creusa	7*	1	0	0	0	0	0	0
D. c. bellona	7*	1	0	0	0	0	0	0
D. dammersi	1*	0	0	0	0	0	0	1
D. dawsoni	9*	0	1	0	0	0	0	0
D. dilucida	1	0	0	0	0	0	1	0
D. dionysia	9*	0	0	0	0	0	1	0
D. eminentia	5	1	0	0	0	1	0	0
D. erythrina	5	1	0	0	0	0	0	0
D. eurynome	6*	0	0	0	0	0	0	1
D. ferruginea	1	0	0	0	0	0	1	0
D. flammifera	9*	1	0	0	0	0	0	0
D. formosa	1*	0	0	0	0	0	1	0
D. foxi	5	1	0	0	0	1	0	0
D. furina	5	1	0	0	0	0	0	0
D. gibbosa	5*	0	1	0	0	0	0	0

species	Clade	Western	Eastern	Tropical	Texan	Desert	Madrean	Uncertain
D. gloriosa	**9**	0	0	0	0	1	0	0
D. gorgon	**9**	0	0	0	1	0	0	0
D. griswoldi	**6***	0	0	0	0	0	1	0
D. heliophila	**1***	0	0	0	0	0	0	1
D. hispidaria	**9***	1	0	0	0	0	0	0
D. holotricha	**9***	0	0	0	0	0	0	1
D. homole	**5***	1	0	0	0	0	0	0
D. icaris	**4***	0	0	0	0	0	0	1
D. jalisco	**5***	0	0	1	0	0	0	0
D. klugii	**9**	0	0	0	1	0	0	0
D. klugiodes	**5**	0	0	0	1	0	0	0
D. lachesis	**9***	0	0	0	0	1	0	0
D. latebalteata	**7***	0	0	0	0	0	0	1
D. leda	**9**	1	0	0	0	0	0	0
D. lepeletierii	**7**	0	1	0	0	0	0	0
D. leve	**9***	0	0	0	0	0	1	0
D. macilenta	**6**	0	1	0	0	0	0	0
D. magna	**9**	0	0	0	0	1	0	0
D. magnifica	**9**	0	0	0	1	0	0	0
D. melanargyrea	**9***	0	0	0	0	1	0	0
D. melanippe	**7**	0	0	0	1	0	0	0
D. m. conformis	**7***	1	0	0	0	0	0	0
D. mirabilis	**5***	0	0	1	0	0	0	0
D. monstrosa	**5**	0	0	0	0	0	1	0
D. monticola	**6**	0	0	0	0	0	0	1
D. munifica	**5**	0	0	1	0	0	0	0
D. montivagoides	**5***	1	0	0	0	0	0	0
D. nigripes	**9**	0	1	0	0	0	0	0
D. niphopilis	**5***	0	0	0	0	1	0	0
D. nocticaro	**7***	0	0	0	0	0	1	0
D. nocturna	**9**	0	0	0	0	1	0	0
D. nupera	**9**	0	0	0	1	0	0	0
D. occidentalis	**9**	1	1	0	0	0	0	0
D. ocyrae	**1**	0	0	1	0	0	0	0
D. parkerorum	**4***	0	0	1	0	0	0	0
D. parksi	**5***	0	0	0	0	0	0	1
D. phya	**4**	0	0	1	0	0	0	0
D. proclea	**5***	0	0	1	0	0	0	0
D. pseudopappus	**9**	0	0	0	0	1	0	0
D. pulchra	**4**	0	0	1	0	1	0	0
D. quadriguttata	**5**	0	1	0	0	0	0	0
D. radkei	**6***	1	0	0	0	0	0	0
D. rubicunda	**5***	0	1	0	0	0	0	0
D. rufopilis	**5***	0	0	0	0	0	1	0
D. sackenii	**9**	1	0	0	0	0	0	0
D. saetigera	**6**	0	0	0	0	0	1	0
D. satanas	**9**	1	0	0	0	1	0	0
D. scabra	**5***	0	0	0	0	1	0	0
D. scaevola	**1**	0	1	0	0	0	0	0
D. schumanni	**4***	0	0	0	0	0	0	1

species	Clade	Western	Eastern	Tropical	Texan	Desert	Madrean	Uncertain
D. scitula	**9**	1	0	0	0	0	0	0
D. sicheliana	**8**	0	0	0	0	0	1	0
D. snoworum	**1***	0	0	0	0	0	0	1
D. spilota	**4**	0	0	1	0	0	0	0
D. stevensi	**9**	1	0	0	0	0	0	0
D. texanella	**9**	0	0	0	1	0	0	0
D. thetis	**9**	0	0	0	0	1	0	0
D. toluca	**5***	0	0	1	0	0	0	0
D. tomasi	**1***	0	0	0	0	0	0	1
D. twegeni	**4***	0	0	1	0	0	0	0
D. ulkei	**6***	0	0	0	0	0	0	1
D. uniguttata	**5***	0	0	0	0	0	0	1
D. vesta	**6**	0	1	0	0	0	0	0
D. vestita	**5**	1	0	0	0	0	0	0
D. vulpina	**5***	1	0	0	0	0	0	0
D. waco	**5**	0	0	0	1	0	0	0
D. whartoni	**9***	0	0	0	0	0	1	0
D. wileyae	**5**	0	0	0	1	0	0	0
D. xalisco	**4***	0	0	0	0	0	1	0
D. zelaya	**5**	0	0	0	1	0	0	0
D. zoster	**5**	0	0	1	0	0	1	0

Many species in this mimicry ring have black legs, petiole, and venter, yet others are completely covered with white setae. Only seven of the 16 members of the Desert mimicry ring are included in the analysis.

The **Texan** mimicry ring can be found throughout Texas, southern New Mexico, and northeastern Mexico. Members of this mimicry ring are characterized by having a black head and mesosoma with a reddish to orange metasoma (Fig. 3.1). All but one of the Texan species was included in the phylogeny.

The **Tropical** mimicry ring can be found in the tropical regions of North America, from Panama through southern Mexico. Members of this mimicry ring share a pattern consisting of black integument with contrasting patterns of white or yellow setal markings (Fig. 3.1). Frequently, there are yellow integumental spots on T2. Eleven of the 18 putative members of the Tropical mimicry ring were included in the phylogeny.

The **Eastern** mimicry ring is found throughout eastern North America, with species involved ranging from the Atlantic coast to the central Great Plains. Members of this mimicry ring have orange to red integument with sparse dorsal setae on the head, mesosoma, and anterior metasomal segments and contrasting patterns of silver, black or orange setae near the apex of the metasoma (Fig. 3.1). Twelve of 16 members of the Eastern mimicry ring were studied.

The **Western** mimicry ring is found throughout western North America from the Pacific coast to the Great Plains, with some members occurring south into central Mexico. Members of this mimicry ring are all covered with dense, often shaggy yellow to red dorsal setae. Some species also have contrasting black setae on the legs, petiole, and apex of the metasoma (Fig. 3.1). Eighteen of the 26 members of the Western mimicry ring were included in the analysis.

Phylogenetic Reconstructions

Both Bayesian phylogenetic analyses (using Neutral and protein-coding markers) resulted in phylogenies showing distinct color forms spread across the Bayesian tree with multiple color forms present in well-supported clades (Fig. 3.3). Nine clades that represent putative species- groups were recovered (Fig. 3.4). Clade One is equivalent to Mickel's *D. obscura* species-group (Mickel 1928). Clade Two includes only *D. chalcocephala*, which has never been placed in a species-group (Manley & Pitts 2007). Clade Three is equivalent to Mickel's *D. creon* species-group (Mickel 1928; Pilgrim et al. 2008). Clade Four includes four Neotropical species that were never placed into a species-group (Manley & Pitts 2007). Clade Five includes the entirety of Mickel's *D.*

asopus, D. fulvohirta, D. scabra, and *D. foxi* species-groups, one member each from Mickel's *D. occidentalis* and *D. zelaya* species-groups, four unplaced species, and all but one species from the *D. quadriguttata* species-group sensu Pilgrim at al. (Mickel 1928; Manley & Pitts 2007; Pilgrim et al. 2009). Clade Six includes members of the *D. monticola* species-group. Clade Seven is equivalent to Mickel's *D. bioculata* species-group (Mickel 1928). Clade Eight includes one species from the *D. quadriguttata* species-group, two from the *D. occidentalis* species-group, and two unplaced species (Mickel 1928; Manley & Pitts 2007). Clade Nine includes members of numerous species-groups, including the *D. arenivaga, D. nogalensis, D. occidentalis, D. sparsa, D. subhyalina, D. thetis,* and *D. zelaya* species-groups (Mickel 1928). Synapomorphies for these clades are not yet defined because so few species are known from both sexes. These clades will likely provide the basis for new species-group definitions in future research. Clade placement for each species, including putative clade placement for species not included in the analysis, is summarized in Table 3.3.

Phylogenetic Distribution of Mimicry Rings

Bayesian Tip-association Significance analyses were used to determine whether color patterns were phylogenetically driven (Table 3.4). Five of the color patterns are randomly distributed in the tree. The Eastern color pattern, however, was significantly correlated with phylogenetic relationships (Table 3.4). This is mostly due to Clade Six, in which five of the six sampled species participate in the Eastern mimicry ring.

Although five of the six mimicry rings are randomly distributed in the entire tree (Table 3.4), but within the individual clades, mimicry rings could indicate biogeographic

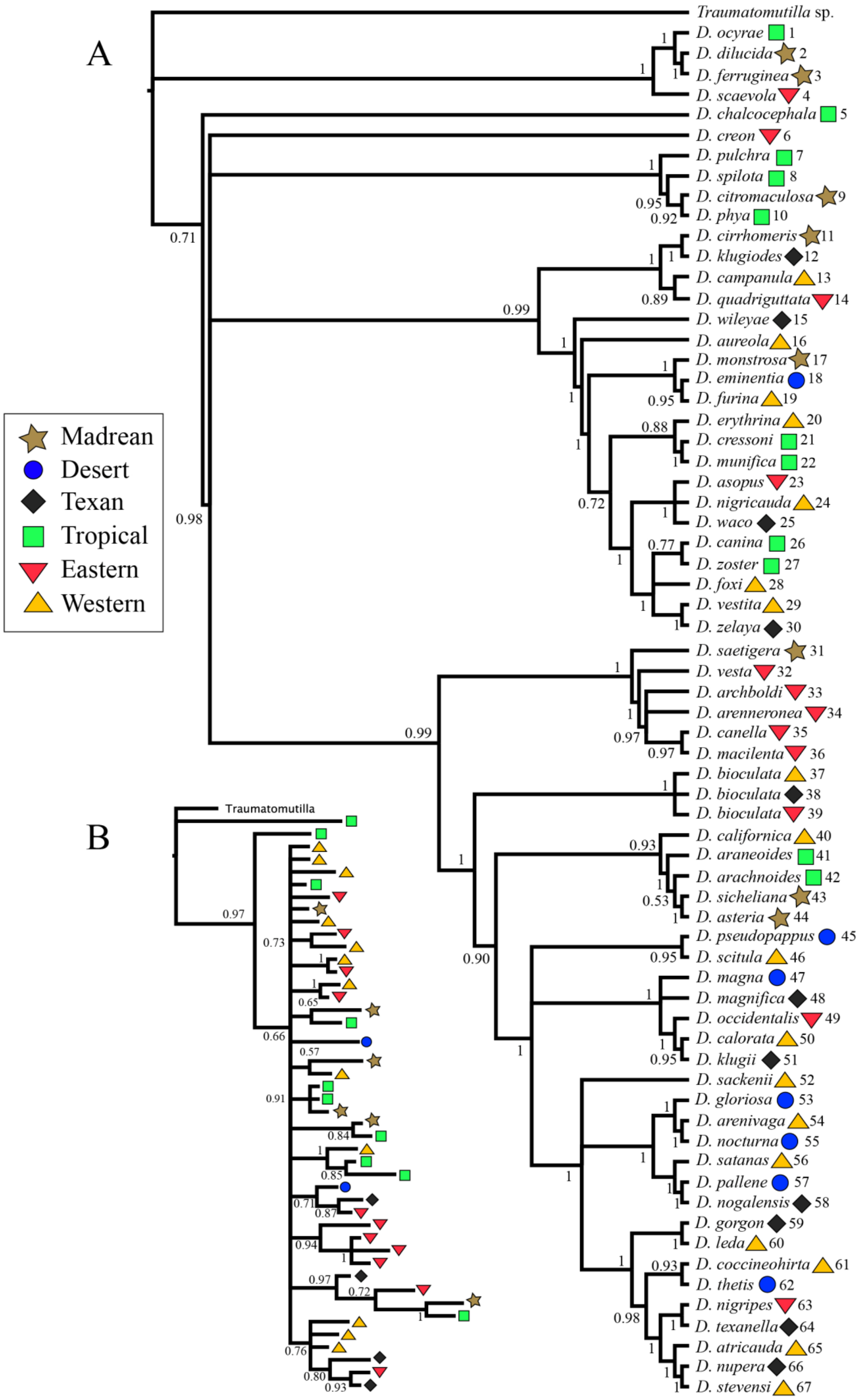

FIGURE 3.3. Bayesian phylogenetic trees. 50% majority rules consensus trees based on neutral genetic loci (a) and protein coding loci (b) with species coded with colored circles representing the mimicry ring to which they belong (Brown: Madrean; Blue: Desert; Black: Texan; Green: Tropical; Red: Eastern; Yellow: Western). Numbers following each species correspond to Fig. 1 and Table S1. Posterior probabilities are given for each node.

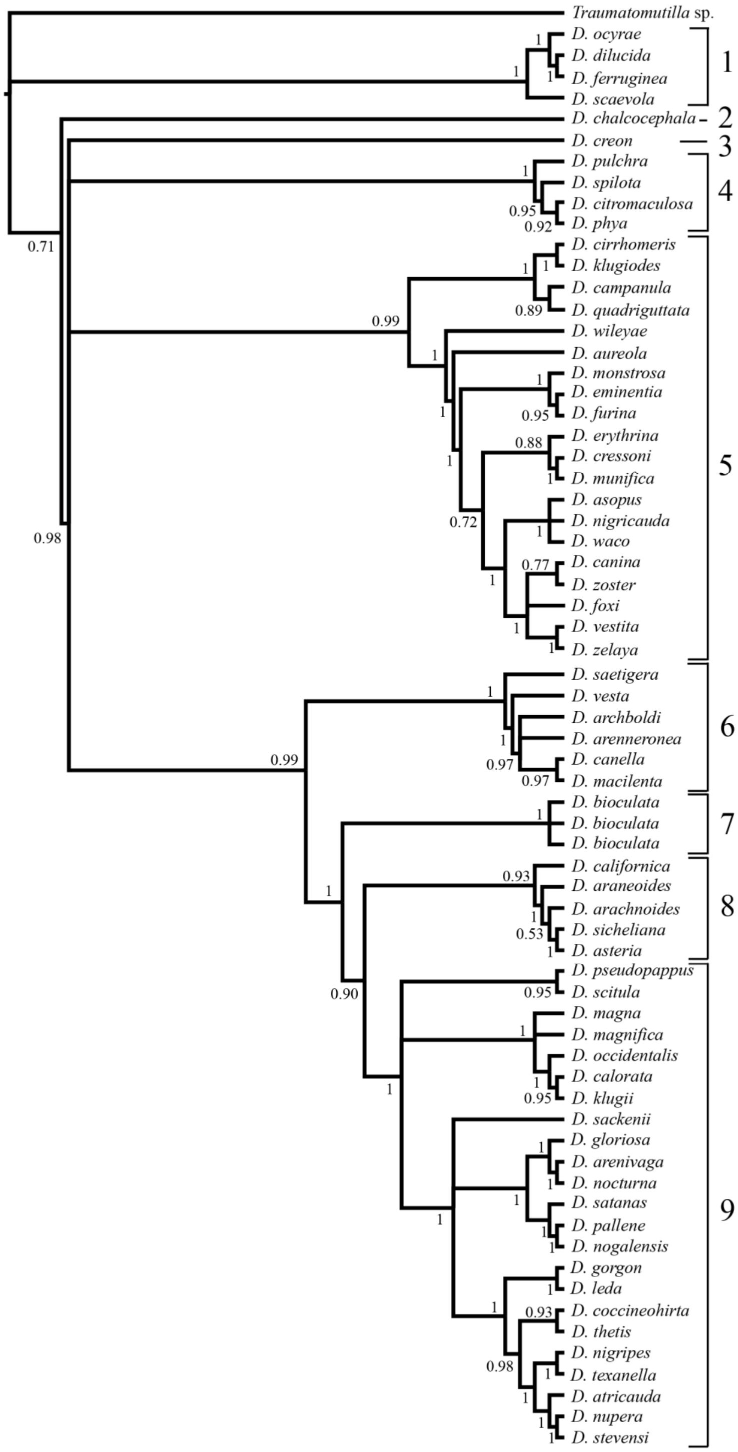

FIGURE 3.4. Bayesian phylogenetic tree. 50% majority rules consensus tree based on neutral genetic loci with nine putative species-group level clades designated. Former species-group designations are included for each terminal taxon. Posterior probabilities are given for each node.

TABLE 3.4. Results of the Bayesian Tip-association Significance testing (BaTS) for each mimicry ring. *P* values greater than 0.05 indicate random distributions across the phylogeny.

	ITS1 & ITS2			Wingless		
Mimicry Ring	Observed mean	Null mean	*P*	Observed mean	Null mean	*P*
Madrean	2	1.27	0.16	1	1.33	1
Desert	1	1.26	1	1	1.02	1
Texan	1	1.48	1	1.03	1.09	1
Tropical	2.02	1.47	0.27	1.9	1.53	0.17
Eastern	5	1.54	0.001	3.95	1.52	0.02
Western	1	1.91	1	1.74	1.92	0.43

patterns. Clades One, Two, Four, and Eight are dominated by Madrean and Tropical species. These color forms are rare in the remaining clades and completely absent from Clades Seven and Nine. Clade Nine is dominated by western Nearctic clades (Western, Texan, Desert). Clade Six is dominated by members of the Eastern mimicry ring. Clade Five is the most diverse clade, including members of all six mimicry rings.

DISCUSSION

Uniqueness of Dasymutilla Mimicry Complex

Müllerian mimicry rings have been described from a variety of North American Taxa including millipedes (7 species: Marek & Bond 2009), eastern bumble bees (8 species: Ruxton et al. 2004), and the monarch and viceroy butterflies (2 species: Ruxton et al. 2004), yet these mimetic systems often contain relatively few species. Even the genus *Heliconius*, which has become a model Müllerian system, only contains 40 species (Hines et al. 2011). To our knowledge, the 65 *Dasymutilla* species studied here represent the largest Müllerian mimicry complex yet to be identified; additional species are likely to be added to the complex (Table 3.3). Furthermore, the high degree of repeated evolution of aposematic coloration in a species-rich group like *Dasymutilla* is unique among mimetic systems, particularly in temperate areas.

While *Heliconius* mimicry rings have become the most widely known mimetic systems due to the variety of shared color forms found in apparently distantly related populations (Brower 1996; Ruxton et al. 2004), recent analyses suggest that some phenotypes share a common origin (Hines et al. 2011). Although *Heliconius* remains a valuable system to study mimicry, these recent findings indicate that mimetic races within *Heliconius* species may be driven largely by shared phylogenetic history rather than convergent evolution. Alternatively, while some species with multiple color forms (races) are present in *Dasymutilla*, most of the diversity in *Dasymutilla* mimicry rings is species-level diversity, with convergent coloration found in distantly related species (Figs. 3.1, 3.3). Furthermore, shared color patterns within *Dasymutilla* mimicry rings are often achieved through convergence of different morphological features. For example, color patterns can be based on setal color and/or integumental pigmentation (e.g., *D. furina* and *D. campanula*: Figs. 3.1, 3.3). In addition to mimicry among *Dasymutilla* species, other velvet ant taxa share similar color patterns with *Dasymutilla*. For example, members of the genus *Pseudomethoca* share color patterns with members of each of the six *Dasymutilla* mimicry rings. Furthermore, as many as eight genera are apparent participants in the Tropical mimicry ring in Central America (KAW, pers. obs.).

Ecological characteristics also make *Dasymutilla* unique among known mimetic complexes. *Dasymutilla* females, like all female velvet ants, are wingless. Velvet ants spend much of their adult lives searching for nests of their hosts in open soil or other exposed microhabitats. While birds are typically implicated as the predator driving the evolution of shared color patterns in mimicry systems (Merrill & Jiggins 2009), little evidence exists that birds regularly attack velvet ants. Instead, diurnal lizards are likely the primary predator in this system. Several North American lizard species regularly feed on insects with heavy exoskeletons and species armed with painful stings (Vitt & Cooper 1988). Feeding trials have shown that a velvet ant's strong, slippery cuticle increased prey manipulation time for predators, often allowing the velvet ant to sting, which generally resulted in a release of the velvet ant with no significant damage (Vitt & Cooper 1988; Manley 2000; Manley & Sherbrooke 2001). These defenses, when combined with chemical, visual, and auditory warning signals, separate velvet ant mimicry complexes from most other known mimicry systems because the wasps can train potential predators without being consumed or mortally wounded. Furthermore, when a lizard predator is stung and releases a velvet ant, it immediately is presented with the velvet ant's auditory and visual warnings. This combination of painful sting and aposematic signals apparently encourages rapid learning, with many lizards completely avoiding a velvet ant after a single attempted predation event (Vitt & Cooper 1988). The ability of velvet ants to quickly train predators without sustaining injuries may have driven rapid evolution of distinct color forms in this group.

Future Research Directions

While our analyses clearly demonstrate that mimicry rings exist among female *Dasymutilla*, several aspects of this mimicry complex remain unexplored and can potentially aid our understanding of aposematism and mimicry. Recent molecular analyses have found that several named velvet ant species actually represented different conspecific color forms (Pilgrim et al. 2008, 2009; Williams et al. 2011). Continued taxonomic investigations of *Dasymutilla* will certainly reveal more instances of multiple color forms within a single species. Evolutionary forces driving convergent color patterns could result from different factors at the population level than at the species level; this will be impossible to study without detailed taxonomic revision of each clade recovered in the study (Fig. 3.4).

Although male velvet ants are relatively harmless compared to their female counterparts, they often exhibit aposematic coloration; these males could be Batesian mimics of female mutillids. Interestingly, male velvet ants do not always fit into the same color syndromes as their conspecific females. The females of *D. gloriosa*, for example, are part of the Desert mimicry ring and are covered with white setae (Fig. 3.1 #53). The males, however, are black and orange and resemble members of the Western or Texan mimicry rings. Fewer than 30% of *Dasymutilla* species are known from both sexes (45 species known from both sexes; 127 known from only one sex). Continued use of molecular techniques to correct taxonomy (Pilgrim & Pitts 2006; Pigrim et al. 2008) will reveal additional synonymies and sex associations, which will make studies of Batesian mimicry in male *Dasymutilla* possible.

Velvet ants are easily collected and easily kept in captivity, facilitating direct observations of predator-prey interactions. Although velvet ant defense mechanisms were shown in lab settings, predator avoidance of unrelated species after exposure to velvet ants has not been established. It is possible that some color patterns (i.e., Desert mimicry ring) might be cryptic rather than aposematic (Ferguson 1966; Manley 2000). It is also possible that some color syndromes are more effective aposematic signals than others. In areas where multiple mimicry rings overlap, feeding experiments could reveal shared mimetic protection for separate mimicry rings or higher effectiveness for some aposematic signals. We encourage ecologists and natural historians to carry out observatory studies on predator interactions with these wasps.

While collections of *Dasymutilla* in the United States are fairly thorough, our knowledge of velvet ant diversity in Mexico is porous. There is no recognized dominant color form in mountainous southern central Mexico (Fig. 3.1). All but 48 of the continental North American female *Dasymutilla* were included in this study; of the unsampled species, 25 are endemic to Mexico and seven more have been collected in Mexico (Manley & Pitts 2007, KAW pers.obs.). Furthermore, putative mimicry rings exist in related velvet ant groups in South America and elsewhere. As additional collections are made and the necessary taxonomic work is completed, velvet ants can become a worldwide model system for studying aposematism and mimicry.

Conclusions

Here we document the diverse mimicry complex present among female *Dasymutilla* velvet ants and describe six distinct mimicry rings (Eastern, Western, Tropical, Desert, Texan, and Madrean). Morphometric analyses indicate that these mimicry rings are morphologically distinct and that members of each ring are more similar to each other than they are to members of other mimicry rings. Bayesian analyses indicate that most of the similarity in coloration within mimicry rings is a result of repeated evolution rather than shared ancestry. To our knowledge the *Dasymutilla* Müllerian mimicry rings represent the largest documented mimicry complex both in terms of geographic area and species richness. Future studies of this group will likely add to our understanding of Müllerian and Batesian mimicry and could lead to velvet ants becoming model organisms for the study of mimicry and aposematism.

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CHAPTER 4

SYSTEMATIC REVIEW OF THE *DASYMUTILLA MONTICOLA* SPECIES-GROUP (HYMENOPTERA: MUTILLIDAE): USING PHYLOGENETICS TO ADDRESS SPECIES-GROUP PLACEMENT AND SEX ASSOCIATIONS[3]

Introduction

Dasymutilla Ashmead 1899 is a diverse velvet ant genus, including some of the largest and smallest mutillid species (Mickel 1928, Manley & Pitts 2007). While widely generalist species can occur in small sizes, many mutillid taxa appear to have consistently diminutive stature. Of the Nearctic members of *Dasymutilla*, many of the smallest species belong to the *D. monticola* and *D. caneo* species-groups (Mickel 1928). These species appear to form a natural group based on shared morphology and are clearly members of *Dasymutilla*, but it is unclear which *Dasymutilla* taxa are their closest relatives.

When first described by Mickel (1928), the *D. monticola* species-group comprised *Dasymutilla* species known from males only, while the *D. caneo* species-group consisted of females only. Recent sex associations confirmed Mickel's initial belief that these species-groups were opposite sexes of a single species-group (Manley 2003, Pilgrim *et al.* 2008). There was not complete overlap between the two species-groups, however. Additional sex associations have matched females from the *D. quadriguttata* species-group with males from the *D. monticola* species-group (Krombein 1951, 1954). Also,

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[3] This chapter has been submitted for publication in *Zootaxa* and was coauthored by D. G. Manley, M. Deyrup, C. D. von Dohlen, and J.P. Pitts. Permission has been granted by the required coauthors for this research to be included in my dissertation (Appendix A); Magnolia Press has licenced me to reprint this material (Appendix B).

one member of the *D. subhyalina* species-group, which Mickel (1928) admitted was an unnatural group, was associated with *D. bonita* Mickel from the *D. caneo* species-group (Mickel 1974). To date, the *D. monticola* species-group (including members of the synonymous *D. caneo* species-group) includes five species known from both sexes: *D. canella* (Blake) (Figs 4.1A, 4.2F, 4.3F), *D. birkmani* (Melander) (Figs 4.2C, 4.3C), *D. bonita* (Figs 4.2D, 4.3E), *D. chattahoochei* Bradley (Figs 4.2B, 4.3B), and *D. monticola* (Cresson) (Figs 4.2K, 4.3J); two species known from females only: *D. saetigera* Mickel (Fig. 4.2M) and *D. eurynome* Mickel (Fig. 4.2I); and three species known from males only: *D. macilenta* (Blake) (Fig. 4.3I), *D. polia* Mickel (Fig. 4.3K), and *D. arcana* Mickel (Fig. 4.3D).

Subgenera are not used in *Dasymutilla*. Because of its tremendous size, however, species-groups are useful for identifying patterns of diversity within *Dasymutilla*. Sadly, however, most new *Dasymutilla* species published since Mickel's first revision (1928) were never conclusively placed into species-groups (Mickel 1936, 1938; Manley & Pitts 2007). Typically, rather than explicitly conjecturing about inter-specific relationships or species-group placement, authors have implied relations by discussing species identification and providing characters to separate new taxa from previously established species (*e.g.*, Mickel 1938, Schmidt & Mickel 1979, Manley & Radke 2006). In the Neotropical region, there appears to be a homogenous mixture of species that readily fit into Mickel's species-groups and other species that readily fit into undefined species-groups comprised of closely related Neotropical taxa (Manley & Pitts 2007). Many of the species described after Mickel (1928) [*e.g.*, *D. archboldi* Schmidt & Mickel (Fig. 4.2A), *D. fasciventris* Mickel (Fig. 4.3G), and *D. spilota* Manley & Pitts (Figs 4.1B, 4.2N)], have been associated with members of the *D. monticola* species-group either explicitly by direct statements of similarity or relation (*e.g.*, Schmidt & Mickel 1979), or implicitly by placement in keys (*e.g.*, Manley & Pitts 2007).

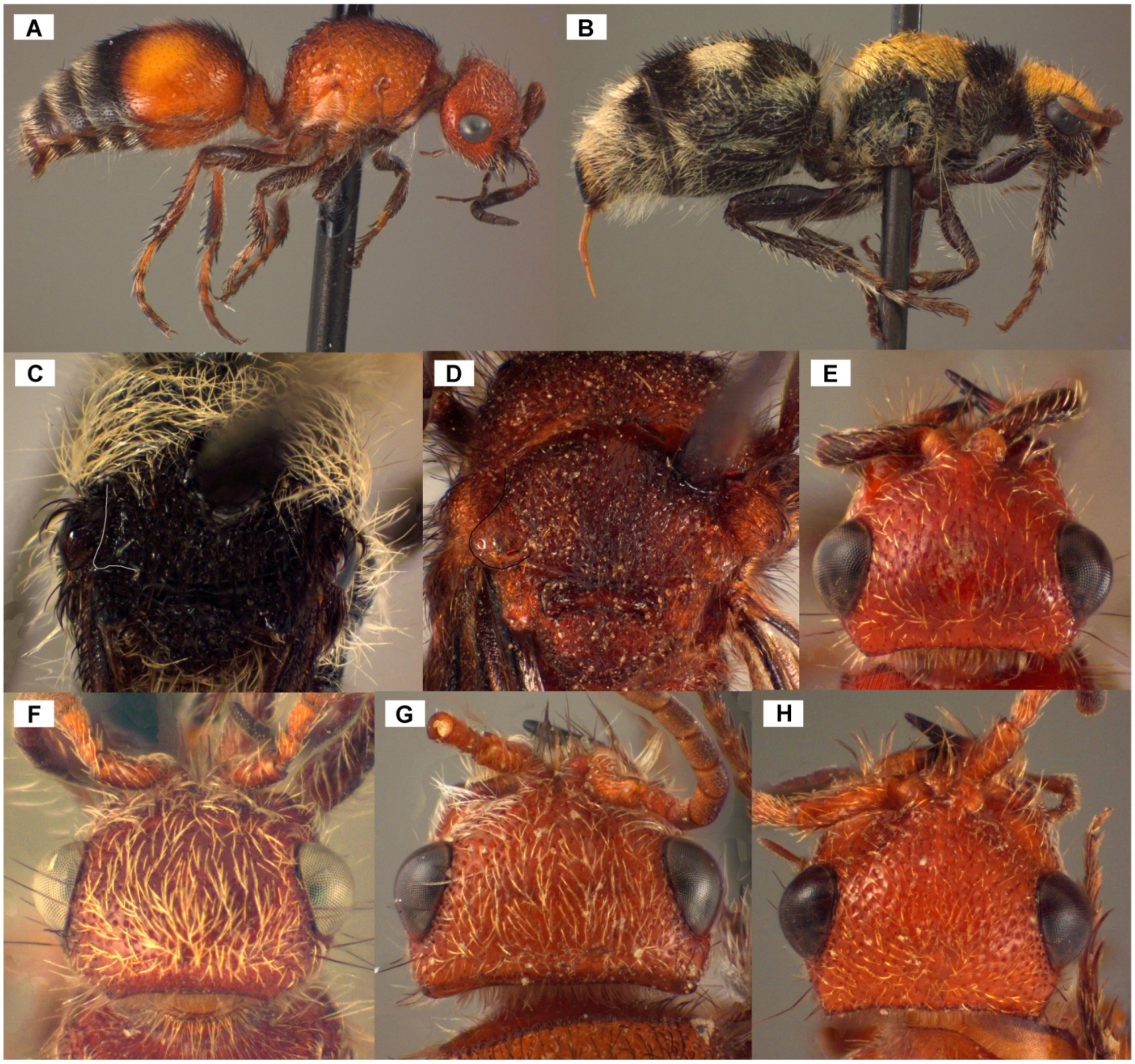

FIGURE 4.1. A,B, Habitus, lateral view, ♀. A. *Dasymutilla canella*; B. *D. spilota*. C, D, Mesosoma, dorsal view, male. C. *D. saetigera*; D. *D. vesta*. E-H, Head, dorsal view, female. E. *D. arenneronea* (*D. chattahoochei*); F. *D. birkmani*; G. *D. birkmani* (*D. corcyra*); H. *D. macilenta* (*D. chattahoochei*).

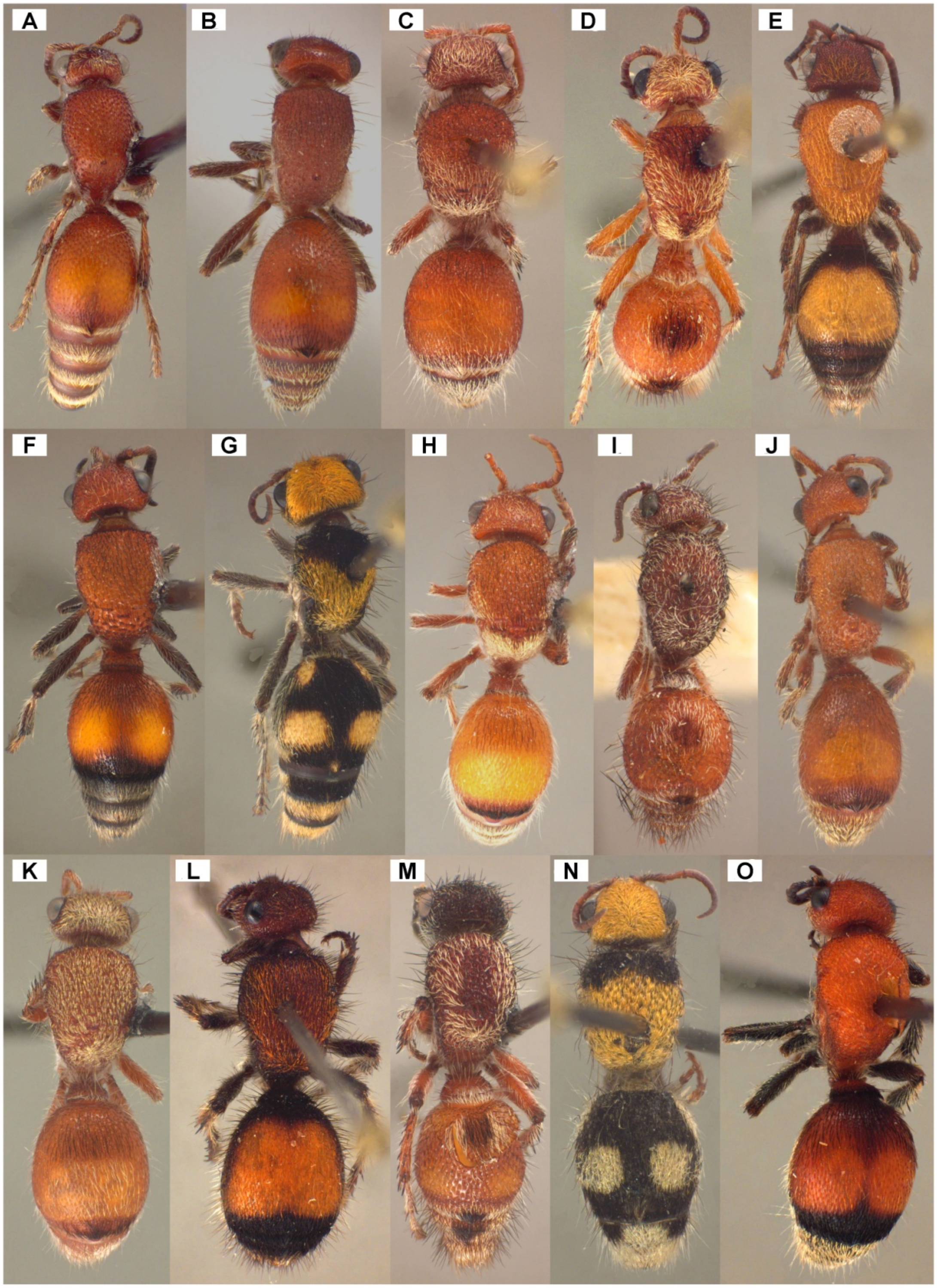

FIGURE 4.2. Habitus, dorsal view, ♀. A. *Dasymutilla archboldi*; B. *D. arenneronea* (*D. chattahoochei*); C. *D. birkmani*; D. *D. bonita*; E. *D. campanula*; F. *D. canella*; G. *D. chalcocephala*; H. *D. birkmani* (*D. corcyra*); I. *D. eurynome*; J. *D. macilenta*; K. *D. monticola*; L. *D. radkei*; M. *D. saetigera*; N. *D. spilota*; O. *D. vesta*.

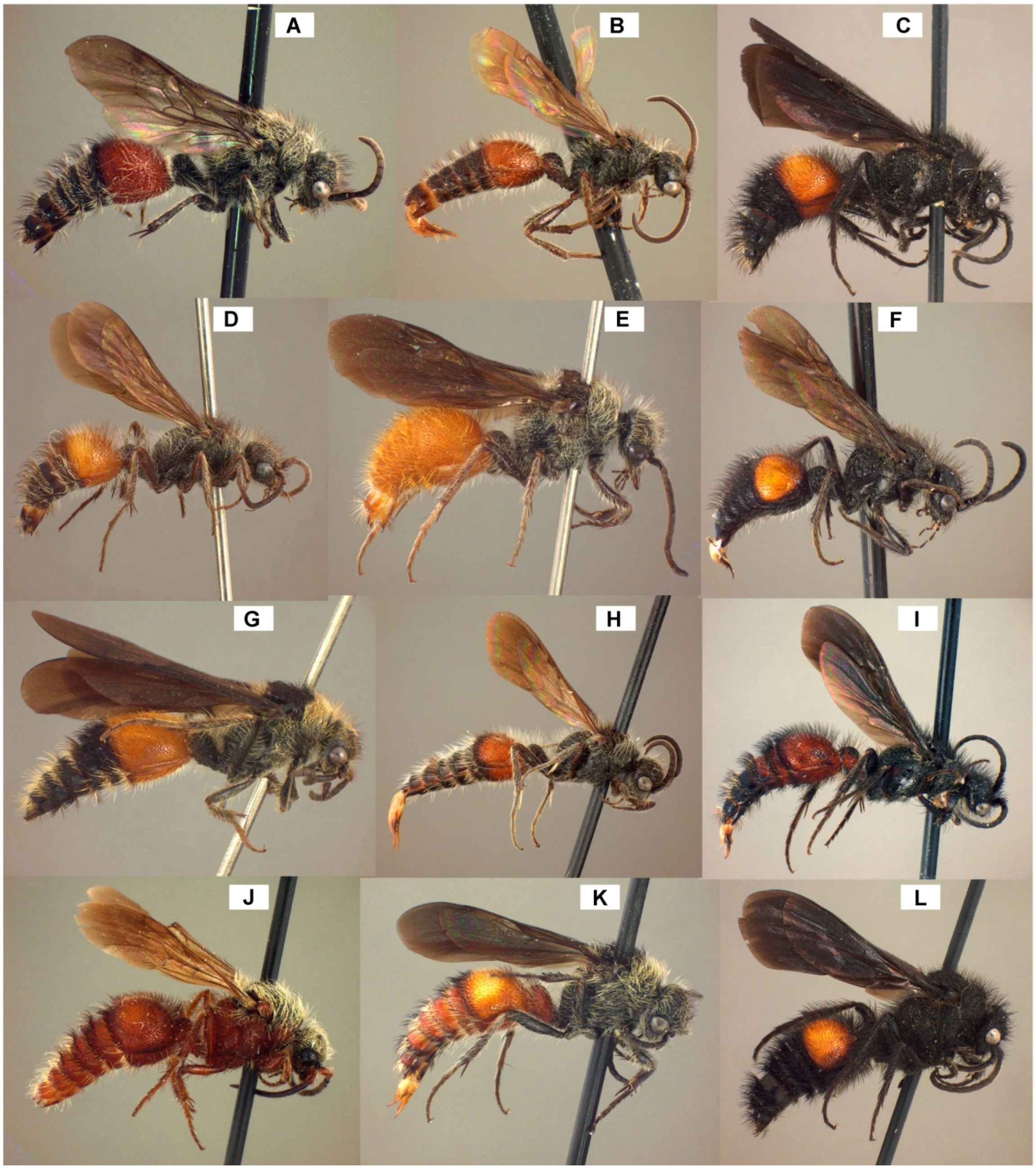

FIGURE 4.3. Habitus, lateral view, ♂. A. *Dasymutilla archboldi*; B. *D. arenneronea* (*D. chattahoochei*); C. *D. birkmani*; D. *D. birkmani* (*D. arcana*); E. *D. bonita*; F. *D. canella*; G. *D. fasciventris*; H. *D. gibbosa*; I. *D. macilenta*; J. *D. monticola*; K. *D. saetigera* (*D. polia*); L. *D. vesta*.

In this study, we use molecular data to reconstruct the phylogeny of the *D. monticola* species-group and use molecular, distributional, and morphological data to address required taxonomic changes. Specifically, we aim to determine the affiliation of multiple *Dasymutilla* species (females from other species-groups and taxa never categorized in species-groups) to the *D. monticola* species-group. We also seek to test the monophyly of the *D. monticola* species-group. Necessary taxonomic changes, including male genitalia descriptions, sex associations, synonymies, and species-group placement are addressed in light of these results.

Material and methods

Taxa chosen for this study

All taxa that were included in Mickel's *D. monticola* and *D. caneo* species-groups were examined, including: *D. arcana* Mickel (♂), *D. birkmani* (Melander) (♂♀), *D. bonita* Mickel (♂♀), *D. canella* (Blake) (♂♀), *D. chattahoochei* Bradley (♂♀), *D. eurynome* Mickel (♀), *D. macilenta* (Blake) (♂), *D. monticola* (Cresson) (♂♀), *D. polia* Mickel (♂), and *D. saetigera* Mickel (♀). Three members of the *D. quadriguttata* species-group: *D. campanula* Mickel (♀), *D. wileyae* Mickel (♀), and *D. corcyra* Mickel (♀) were also studied because females from this species-group have been associated with males from the *D. monticola* species-group in the past (Krombein 1954; Pilgrim *et al.* 2009). We also examined four species that were not formally placed into species-groups, but resemble the *D. monticola* species-group morphologically: *D. archboldi* Schmidt & Mickel (♂♀), *D. chalcocephala* Manley & Pitts (♀), *D. fasciventris* Mickel (♂), and *D. spilota* Manley & Pitts (♀). To determine which taxa are closely related to the *D. monticola* species-group, 15 *Dasymutilla* species, representing 14 of Mickel's species-groups, were also included: *D. araneoides* (Smith) (♂♀), *D. arenivaga* Mickel (♂♀), *D. asopus* (Cresson) (♂♀), *D. bioculata* (Cresson) (♂♀), *D. coccineohirta* (Blake) (♂♀), *D. pallene* (Cameron) (♂♀), *D. creon* (Blake) (♂♀), *D. foxi* (Cockerell) (♂♀), *D. nigripes* (Fabricius) (♂♀), *D. occidentalis* (Linnaeus) (♂♀), *D. quadriguttata* (Say) (♂♀), *D. thetis* (Blake) (♂♀), *D. vesta* (Cresson) (♂♀), and *D. vestita* (Lepeletier) (♂♀). One *Traumatomutilla* André species, *T. inermis* (Klug) (♀), was included as an outgroup taxon. Some species are known from a single sex only; this is indicated by the sex symbol listed after the species of interest above. Species-group identities for taxa examined in this study are summarized in Table 4.1.

Morphological methods

Pinned specimens of all species referred to in this study have been examined to compare morphological features, including the primary types. The non-type material that was examined includes 41 specimens of *D. arcana* (USA: TX, OK; MEX: Tamaulipas), over 600 male specimens of *D. archboldi* (USA: FL, GA), 120 female specimens of *D. archboldi* (USA: FL), 112 specimens of *D. birkmani* (USA: CO, NE, OK, TX), 53 specimens of *D. bonita* (USA: AZ; MEX: Guerrero, Nayarit, Sinaloa, Sonora), three specimens of *D. campanula* (USA: CO, KS), 95 specimens of *D. canella* (USA: CN, MA, MN, NY; CAN: Manitoba), 26 specimens of *D. chalcocephala* (Costa Rica, Honduras), 58 female specimens of *D. chattahoochei* (USA: FL, GA, SC, AL), 14 male specimens of *D. chattahoochei* (USA: FL), six specimens of *D. corcyra* (USA: CO, OK, TX), two specimens of *D. eurynome* (USA: TX), 21 specimens of *D. fasciventris* (USA: AZ; MEX: Jalisco, Sonora), 142 specimens of *D. macilenta* (USA: FL, GA, SC), 217

TABLE 4.1. Voucher specimens used in phylogenetic analysis.

species	previous species-group	current species-group	voucher	sex	Genbank ITS1	Genbank ITS2	Locality
D. arcana	*D. monticola*	*D. monticola*	Darc01	♂	JX002855	JX002872	USA: OK: Grady Co.
D. arcana	*D. monticola*	*D. monticola*	JP1883	♂	JX002856	JX002873	USA: TX: Brazos Co.
D. birkmani	*D. monticola*	*D. monticola*	JP377	♀	EF433440	EF433440	USA: TX: Randall Co.
D. birkmani	*D. monticola*	*D. monticola*	JP380	♂	EF433443	EF433443	USA: TX: Potter Co.
D. birkmani	*D. monticola*	*D. monticola*	JP379	♀	EF433442	EF433442	USA: TX: Ward Co.
D. birkmani	*D. monticola*	*D. monticola*	JP372	♀	EF433441	EF433441	USA: TX: LaSalle Co.
D. birkmani	*D. monticola*	*D. monticola*	JP374	♀	JX002858	JX002875	USA: TX: LaSalle Co.
D. birkmani	*D. monticola*	*D. monticola*	JP375	♀	JX002859	JX002876	USA: TX: LaSalle Co.
D. birkmani	*D. monticola*	*D. monticola*	JP1885	♀	JX002857	JX002874	USA: TX: Leon Co.
D. canella	*D. monticola*	*D. monticola*	JP1081	♀	JQ945777	JX002793	USA: MA: Plymouth Co.
D. chattahoochei	*D. monticola*	*D. monticola*	JP163	♂	EU627569	EU627569	USA: FL: Sarasota Co.
D. chattahoochei	*D. monticola*	*D. monticola*	JP1080	♀	JX002862	JX002879	USA: FL: Orange Co.
D. macilenta	*D. monticola*	*D. monticola*	JP1077	♂	JQ945791	JX002792	USA: FL: Orange Co.
D. monticola	*D. monticola*	*D. monticola*	Deur01	♀	JX002864	JX002881	USA: NE: Morrill Co.
D. monticola	*D. monticola*	*D. monticola*	JP373	♀	JX002860	JX002877	USA: NM: Roosevelt Co.
D. monticola	*D. monticola*	*D. monticola*	JP376	♀	JX002861	JX002878	USA: TX: Jeff Davis Co.
D. monticola	*D. monticola*	*D. monticola*	JP371	♂	EF433447	EF433447	USA: TX: LaSalle Co.
D. monticola	*D. monticola*	*D. monticola*	JP319	♀	EF433449	EF433449	USA: AZ: Cochise Co.
D. monticola	*D. monticola*	*D. monticola*	JP317	♂	JX002866	JX002883	USA: CA: San Bernardino Co.
D. monticola	*D. monticola*	*D. monticola*	JP316	♂	EF433448	EF433448	USA: CA: San Bernardino Co.
D. monticola	*D. monticola*	*D. monticola*	JP318	♀	JX002867	JX002884	USA: UT: Cache Co.
D. monticola	*D. monticola*	*D. monticola*	JP315	♂	EF433446	EF433446	USA: AZ: Cochise Co.
D. polia	*D. monticola*	*D. monticola*	JP1208	♂	JQ945797	JX002790	MEXICO: Sonora
D. saetigera	*D. monticola*	*D. monticola*	Dsae01	♀	JX002868	JX002885	USA: AZ: Santa Rita Co.
D. saetigera	*D. monticola*	*D. monticola*	Dsae02	♀	JX002869	JX002886	USA: AZ: Santa Rita Co.

species	previous species-group	current species-group	voucher	sex	Genbank ITS1	Genbank ITS2	Locality
D. arenivaga	*D. arenivaga*	*D. arenivaga*	JP460	♀	JQ945770	JX002775	USA: AZ: Maricopa Co.
D. asopus	*D. asopus*	*D. asopus*	JP1080	♂	JQ945771	JX002808	USA: FL: Orange Co.
D. bioculata	*D. bioculata*	*D. bioculata*	JP1189	♂	HQ317263	HQ317277	USA: NE: Morrill Co.
D. creon	*D. creon*	*D. creon*	JP670	♂	JQ945781	JX002799	USA: TX: Harris Co.
D. foxi	*D. foxi*	*D. foxi*	JP454	♀	EF433450	EF433450	USA: TX: Jeff Davis Co.
D. vestita	*D. fulvohirta*	*D. fulvohirta*	JP19	♀	JQ945808	JX002806	USA: UT: Cache Co.
D. pallene	*D. nogalensis*	*D. nogalensis*	JP671	♀	JQ945780	JX002780	MEXICO: Sonora
D. scaevola	*D. obscura*	*D. obscura*	JP1196	♀	JQ945802	JX002815	USA: FL: Alachua Co.
D. araneoides	*D. occidentalis*	*D. occidentalis*	JP832	♀	JQ945768	JX002785	NICARAGUA: Granada
D. occidentalis	*D. occidentalis*	*D. occidentalis*	moccf	♀	HQ317246	HQ317268	USA: FL: Highlands Co.
D. campanula	*D. quadriguttata*	*D. quadriguttata*	JP911	♀	JQ945776	JX002797	USA: CO: Weld Co.
D. corcyra	*D. quadriguttata*	*D. quadriguttata*	JP918	♀	JX002863	JX002880	USA: TX: Madison Co.
D. quadriguttata	*D. quadriguttata*	*D. quadriguttata*	JP159	♂	HQ317245	HQ317267	USA: AR: Pulaski Co.
D. wileyae	*D. quadriguttata*	*D. quadriguttata*	JP370	♀	EU627572	EU627572	USA: TX: LaSalle Co.
D. nigripes	*D. sparsa*	*D. sparsa*	JP463	♀	EU627570	EU627570	USA: TX: Randall Co.
D. thetis	*D. thetis*	*D. thetis*	KW05	♀	EF433452	EF433452	USA: AZ: Cochise Co.
D. vesta	*D. vesta*	*D. vesta*	JP1233	♂	JX002870	JX002887	USA: FL: Polk Co.
D. coccineohirta	*D. zelaya*	*D. zelaya*	JP99	♀	JQ945779	JX002777	USA: CA: Riverside Co.
D. archboldi	unassigned	*D. monticola*	JP1240	♀	JQ945769	JX002794	USA: FL: Polk Co.
D. archboldi	unassigned	*D. monticola*	JP1241	♂	JX002871	its2Seq19	USA: FL: Polk Co.
D. chalcocephala	unassigned	unassigned	JP1146	♀	JX002837	JX002821	COSTA RICA: Guanacaste
D. fasciventris	unassigned	*D. quadriguttata*	JP840	♂	JQ945785	JX002888	MEXICO: Sonora
D. spilota	unassigned	unassigned	JP833	♀	JX002838	JX002822	NICARAGUA: Granada
unknown male	unassigned	*D. monticola*	JP1236	♂	JX002865	JX002882	USA: FL: Polk Co.
T. inermis	**n/a**	**n/a**	JP1158	♀	JQ945818	JX002765	BRASIL: Espirito Santo

specimens of *D. monticola* (USA: AZ, CA, CO, ID, MT, ND, NE, NM, NV, OR, TX, UT, WA, WY; MEX: Sinaloa, Sonora; CAN: Alberta, British Columbia, Saskatchewan), 24 specimens of *D. polia* (USA: AZ; MEX: Sonora), 17 specimens of *D. radkei* (USA: NM, TX); 16 specimens of *D. saetigera* (USA: AZ; MEX: Nayarit), 32 specimens of *D. spilota* (Costa Rica, Honduras, Nicaragua, Panama), over 1500 specimens of *D. vesta* (USA: AL, AR, AZ, CO, DC, FL, GA, ID, IN, LA, KS, MA, MD, MI, MN, MO, MS, NC, ND, NE, NJ, NM, NY, OH, SC, SD, TN, TX, UT, VA, WY; CANADA: AB, BC, ON), and 150 specimens of a previously unidentified male (USA: FL).

Individual specimen locality data are only recorded for taxa that are newly described or that could not be accurately identified with previously available keys and descriptions. In the above mentioned paragraph, this includes only the males and females of *D. chattahoochei* and an unknown male from Florida. The following codens are used for collections referenced in the material examined sections of this paper.

ABSC	Archbold Biological Station Collection of Arthropods, Lake Placid, Florida, USA.
CUIC	Cornell University Insect Collection, Department of Entomology, Ithaca, New York, USA.
DGMC	Donald G. Manley Collection, Florence, South Carolina, USA.
EMUS	Department of Biology Insect Collection, Utah State University, Logan, Utah, USA.
FSCA	Florida State Collection of Arthropods, Division of Plant Industry, Gainesville, Florida, USA.

PMAE	Provincial Museum of Alberta, Edmonton, Alberta, CANADA.
SEMC	Snow Entomological Museum, University of Kansas, Lawrence, Kansas, USA.
UCDC	The Bohart Museum of Entomology, University of California, Davis, California, USA.
UCFC	Arthropod Collection (Bug Closet), Biology Department, University of Central Florida, Orlando, Florida, USA.
UMMZ	University of Michigan, Museum of Zoology, Insect Division, Ann Arbor, Michigan, USA.
ZMUC	University of Copenhagen, Zoological Museum, Department of Entomology, Copenhagen, DENMARK.

All specimens were examined with a stereo microscope. Genitalia were dissected using minuten pins and placed in genitalia capsules with glycerin. Specimens used in the molecular study have been labeled as voucher specimens (Table 4.1) and deposited in the Department of Biology Insect Collection, Utah State University, Logan, UT (EMUS). These specimens are therefore available for further morphological analysis.

We use the acronyms: T1, T2, etc. to refer to the metasomal terga, and the abbreviations: S1, S2, etc. to refer to the metasomal sterna. Unless stated otherwise, “male metasoma having gray or silver setae on apical terga” refers to a variable trait where gray or silver setae can be found only on T6–7 (*e.g.*, Fig. 4.3C); gray or silver setae can be interspersed with black setae on T3–7, but are most conspicuous on T6–7 (*e.g.*, Fig. 4.3J); or where gray or silver setae clothe T3–7 almost entirely (*e.g.*, Fig.

4.3A). In most species having this character, there is considerable variation in the extent and distribution of gray or silver metasomal setae; this trait, however, has been historically used to diagnose the *D. monticola* species-group (Mickel 1928) and is effective for separating these taxa from species like *D. nigripes* or *D. vesta* that have the setae of T3-7 entirely black. The cuspis length relative to the free paramere length is used in this research to quantify differences in genitalic structure (Fig. 4.4). To allow comparisons with previously available illustrations (*e.g.*, Mickel 1928) and facilitate identification without dissecting the genitalic capsule, cuspis, digitus, and paramere measurements are taken from the apical margin of the dorsal basal paramere lobe to the apex of each respective structure. These are not actual measurements of segment length, but an index to compare relative lengths; all provided length ratios of genitalic structures are based on these indices.

Molecular methods

DNA was extracted from 51 individuals representing 31 species from the genera *Dasymutilla* and *Traumatomutilla* for use in the phylogenetic reconstruction (Table 4.1). DNA was unavailable for two of the species studied, *D. bonita* and *D. eurynome*, due to lack of suitable specimens. After the initial analysis with our full dataset, a second analysis that included only members of the *D. monticola* species-group and three outgroups was run.

DNA extractions were performed using either several legs from each individual or the entire individual after puncturing the side of the thorax. This left the remainder of the external anatomy of the specimen available for morphological study. Extractions were

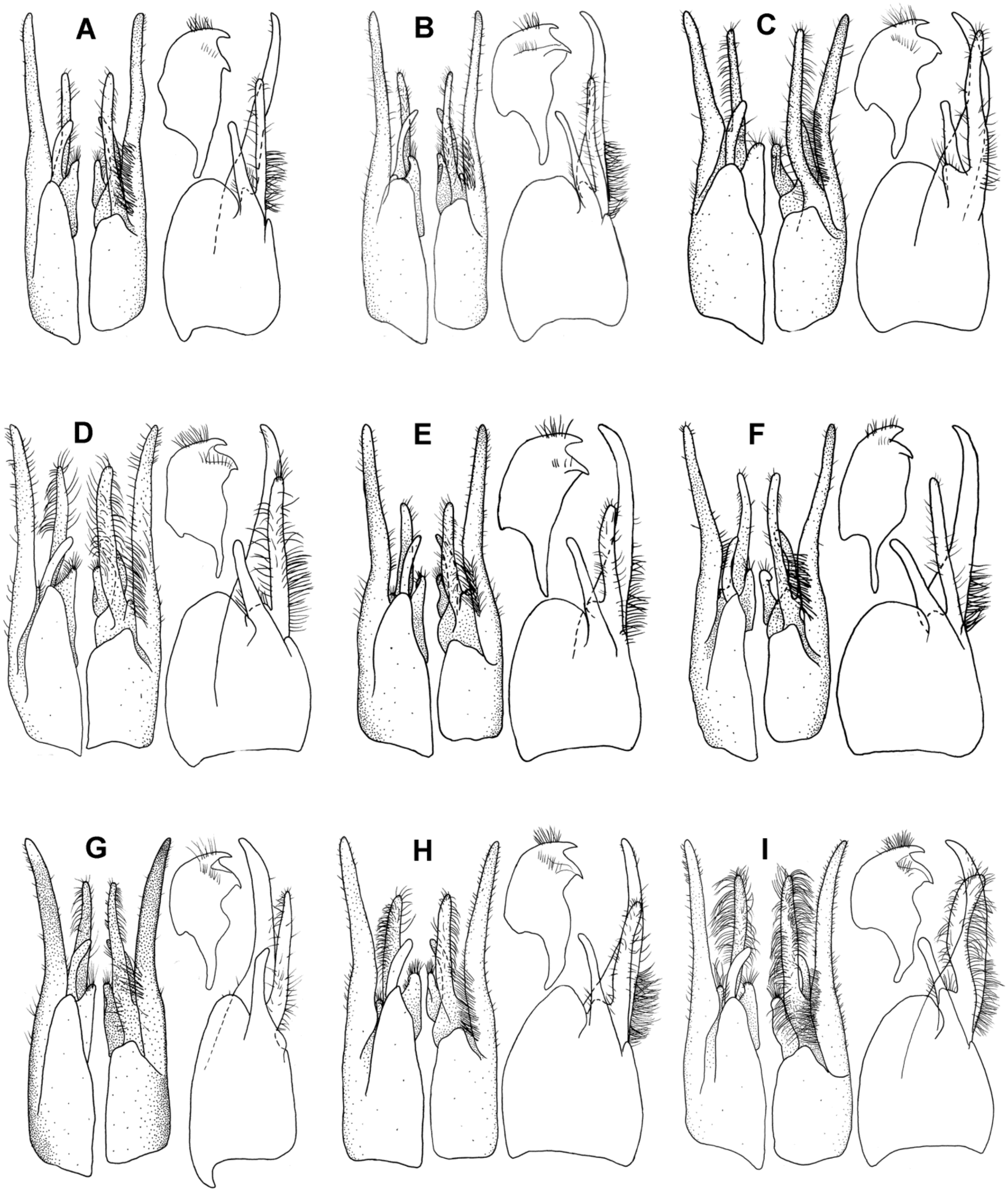

FIGURE 4.4. Male genitalia: dorsal view, ventral view, lateral view, and penial valve. A. *Dasymutilla archboldi*; B. *D. arenneronea*; C. *D. birkmani*; D. *D. bonita*; E: *D. canella*; F. *D. macilenta*; G. *D. monticola*; H. *D. saetigera*; I. *D. vesta*.

done with either the Roche High Pure PCR Template Purification Kit or the QIAgen DNeasy Tissue Kit following each manufacturer's protocol. The first and second internal transcribed spacer units of the ribosomal DNA (ITS1 and ITS2) were amplified. PCR conditions, primers, and DNA sequencing protocols were identical to those used in Pilgrim and Pitts (2006). Gel electrophoresis of each gene yielded a single band for each individual wasp and the resulting DNA was sequenced cleanly suggesting no gene heterogeneity as seen in some other organisms (Harris & Crandall 2000, Parkin & Butlin 2004, Bower *et al.* 2008).

Alignments of the ITS1 and ITS2 data sets were made using Geneious and ClustalW (Drummond *et al.* 2009, Thompson *et al.* 1994). Direct pairwise genetic distances were determined by eye using an alignment in Geneious. ITS1 and ITS2 were subjected to Bayesian analysis using MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). The analyses included four independent runs with three heated chains and one cold chain in each run. The MCMC chains were set for 3,000,000 generations and sampled every 100 generations; chains were run until the average standard deviation of the split frequencies dropped below 0.01. The burn-in period for each analysis was removed after graphical determination of stationarity. Sequences were analyzed as a combined data set, with each gene partitioned separately with all parameters unlinked across loci; gaps in the ITS1 and ITS2 sequences were coded as binary characters and treated as a third partition in the dataset. Appropriate models of nucleotide substitution for the first two partitions (direct sequences of ITS1 and ITS2) were determined in MrModeltest version 2.3 (Nylander 2004). All sequences have been deposited in GenBank (see Table 4.1).

A second Bayesian phylogenetic analysis was performed on a smaller data set based on the results of the first analysis. Because ITS1 and ITS2 are non-coding regions, they contain numerous insertions and deletions. When analyses include distant relatives with numerous indels, there is potential "noise" that can interfere with phylogenetic signal throughout the tree. This second analysis includes all individuals that were recovered within the *D. monticola* species-group in the first phylogenetic analysis and three outgroups. These taxa were chosen because they have short genetic distances from members of the *D. monticola* species-group, suggesting they possess more of the ancestral character states. The first tree is intended for species-group placement, while the second tree aids in species delimitation.

Results

Phylogenetic reconstruction

The phylogenetic reconstruction with a full dataset (Table 4.1) yields a well-supported (p.p.=1) monophyletic group that includes all members previously placed into the *D. monticola* species-group (Fig. 4.5). This clade also includes the following species: *D. vesta*, which was previously placed in the *D. vesta* species-group; *D. corcyra*, which was previously placed in the *D. quadriguttata* species-group; and *D. archboldi*, which was never assigned to a species-group. Furthermore, males and females identified as *D. chattahoochei* are found in separate clades.

Three unassociated females from the *D. quadriguttata* species-group, *D. campanula*, *D. wileyae*, and the aforementioned *D. corcyra*, were included in order to determine their appropriate species-group designation. *Dasymutilla campanula* (Fig.

4.2E), forms a sister relationship with *D. quadriguttata* and *D. wileyae* forms a sister relationship with a clade of three taxa: *D. vestita*, *D. foxi*, and *D. asopus*.

Four species that were never placed into species-groups were also included in the analysis. *Dasymutilla archboldi* males and females appear in different clades, but both are within the *D. monticola* species-group. *Dasymutilla fasciventris* forms a clade with two species from the *D. quadriguttata* species-group, *D. campanula* and *D. quadriguttata*. *Dasymutilla chalcocephala* and *D. spilota* form a clade that is widely separated from the *D. monticola* species-group.

The first and second analyses recover the same relationships within the *D. monticola* species-group (Figs 4.5, 4.6), differing only in some of the support values. Eight clades that represent putative species were recovered. In addition to phylogenetic position, morphology and distribution shape our hypotheses of species-limits. These interpretations are discussed in the remarks section for each species below.

***Dasymutilla monticola* species-group (Mickel, 1928)**

Diagnosis. FEMALE. The females of this species-group can not exclusively be defined by a unique combination of characters. The following features are shared by the entire group, but are not exclusive from other *Dasymutilla* species-groups. The mandible is straight, the mesosoma is longer than broad, there is a distinct scutellar scale, the pygidium has distinct striae, and the integument is not entirely black (*i.e.*, at least T2 is orange). Most species have significant modification to the propodeum, including a dense brush of silver to golden setae (Figs 4.2: C, D, H, I, K, M), broad asperations (Figs 4.1A,

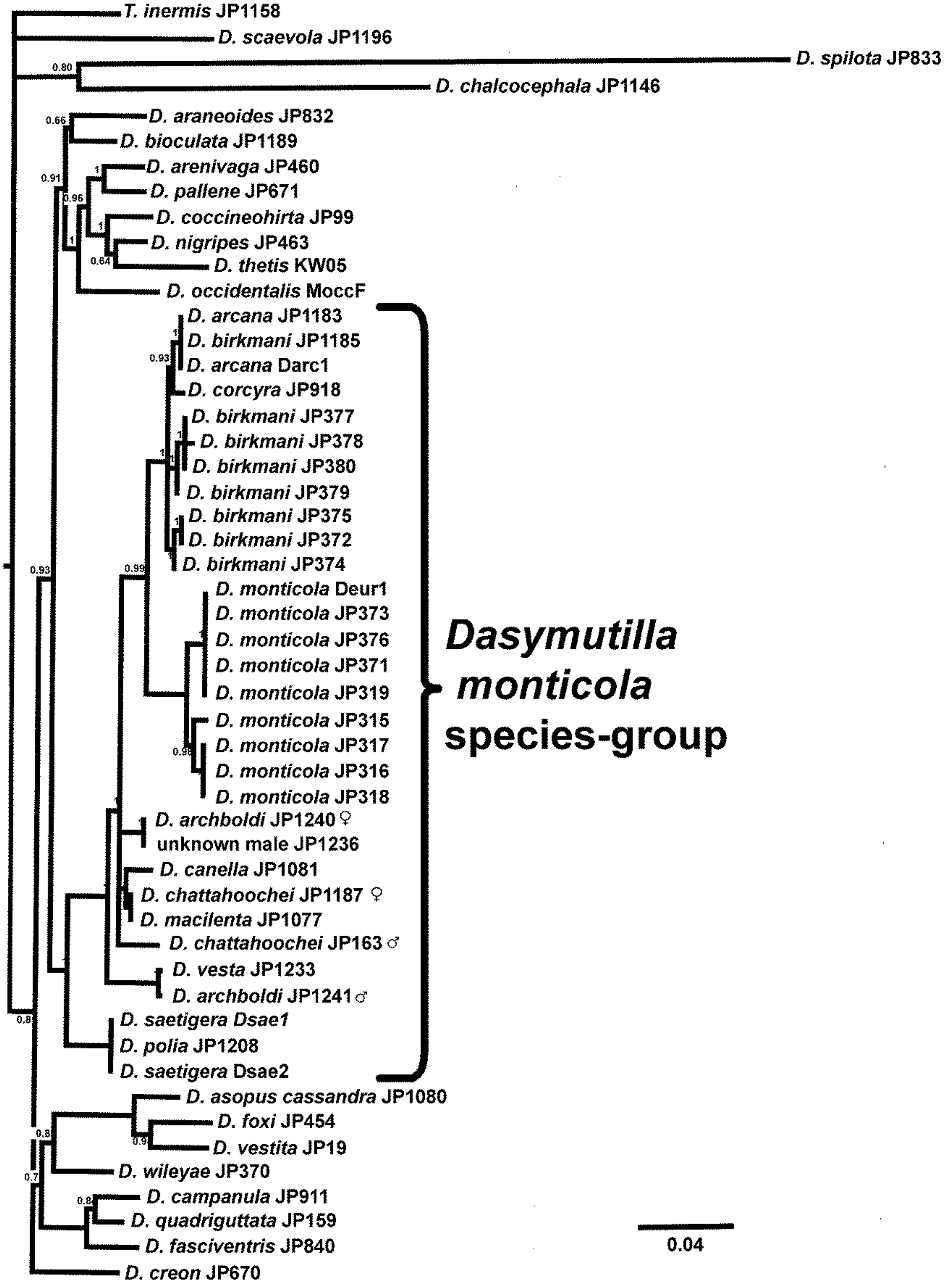

FIGURE 4.5. Bayesian phylogeny reconstructed using ITS1 and ITS2 sequences and including members of 15 *Dasymutilla* species-groups, a *Traumatomutilla* outgroup, and all available unassigned species.

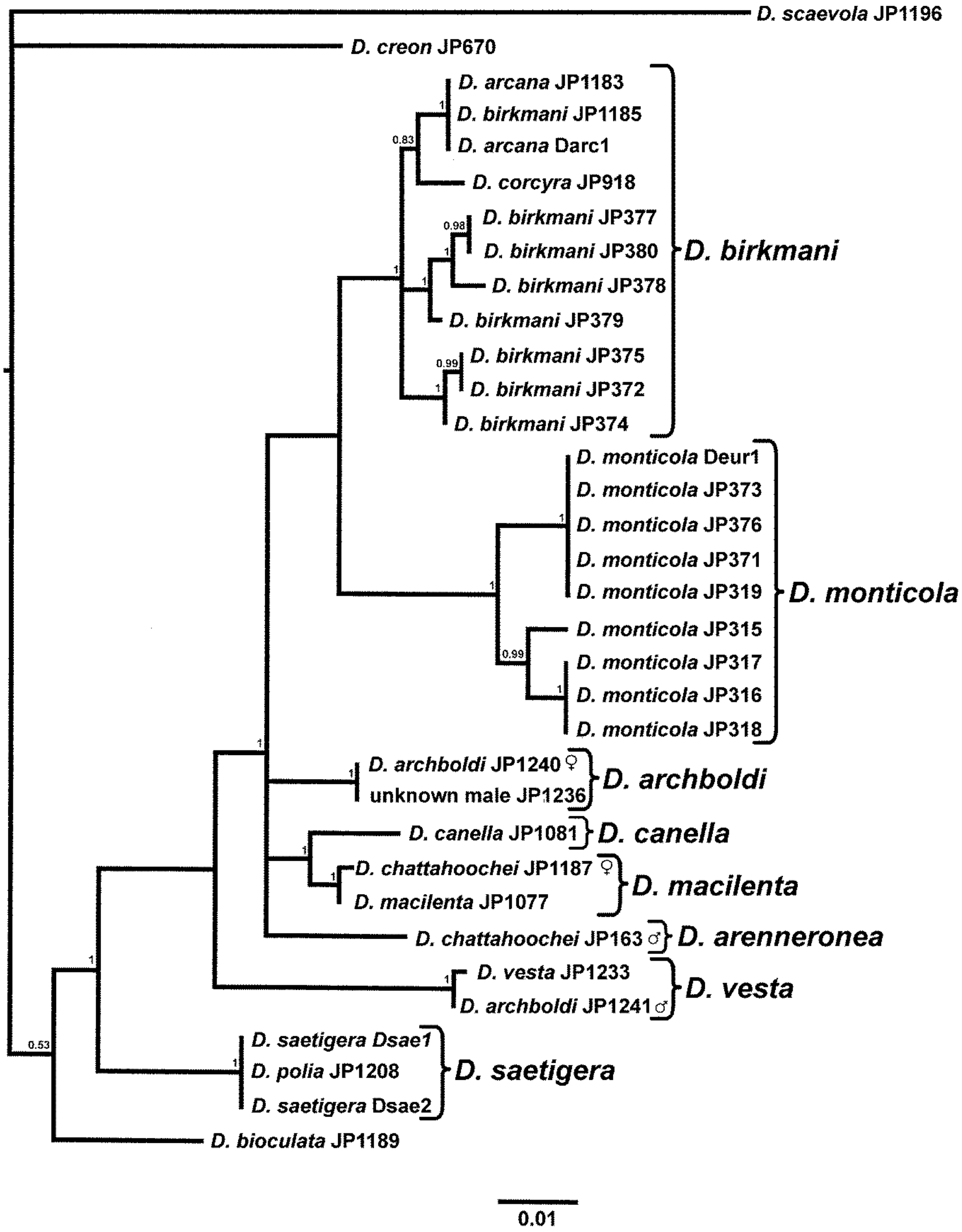

FIGURE 4.6. Bayesian phylogeny reconstructed using ITS1 and ITS2 sequences and only including members of the *Dasymutilla monticola* species-group and three outgroups from other *Dasymutilla* species-groups.

4.2F), or raised tubercles (Figs 4.2: A, B, J); in the two species that have the propodeum simply punctate, *D. vesta* (Fig. 4.2O) and *D. radkei* (Fig. 4.2L), there is no transverse carina anterior to the scutellar scale.

MALE. The males of this species-group are recognized by the following unique combination of characters: the anterior margin of the pronotum is emarginate medially; the axillae are truncate posteriorly; the metasomal integument is partially orange to red; there are gray or silver setae on the apical terga (*e.g.*, Figs 4.3I-K), except in *D. vesta* (Fig. 4.3L); and the free length of the paramere is at least as long as the basal lobe and is kinked basally (*e.g.*, Figs 4.4A-C), except in *D. bonita* (Fig. 4.4D) and *D. vesta* (Fig. 4.4I). Most of the included species also have the tegulae punctate and the posterior margin of the mesoscutum swollen laterally, especially evident in *D. vesta* (Fig. 4.1D).

Included taxa. This species-group now includes 11 Nearctic species: *D. archboldi*, *D. arenerronea*, *D. birkmani*, *D. bonita*, *D. canella*, *D. eurynome*, *D. macilenta*, *D. monticola*, *D. radkei*, *D. saetigera*, and *D. vesta*.

Remarks. Four species are newly transferred to the *D. monticola* species-group based on the phylogenetic results discussed above (Fig. 4.5). *Dasymutilla vesta* and *D. radkei* are transferred from the *D. vesta* species-group; *D. corcyra* is transferred from the *D. quadriguttata* species-group; and *D. archboldi,* which has not been previously placed in a species-group, is also placed in the *D. monticola* species-group.

Finding synapomorphies for females in this species-group has proven difficult. The mandibular shape is consistent, but the same characteristic shape can be seen in other *Dasymutilla* species-groups (*e.g., D. arenivaga* species-group). All females, except for *D. vesta* and *D. radkei*, have the propodeum modified with either a dense brush of silver or golden setae, deep asperations, or raised tubercles. Additionally, all females have extensive orange to red integument, at the very least, T2 is yellow or ferruginous; like the few other consistent characters in this group, lightened integumental coloration is common in other species-groups.

Males are almost as difficult to diagnose. Most of these species have the apical metasomal segments clothed with silver setae (*e.g.*, Figs 4.3I-K), the mesoscutum posterolaterally swollen (*e.g.*, Fig. 4.1D), and the parameres kinked basally and divergent apically (*e.g.*, Figs 4.4A-C). Although absent in some members of the *D. monticola* species-group, mesoscutal swelling and kinked parameres are apparently uniquely found in members of this species-group.

Key to species of the *Dasymutilla monticola* species-group in the USA

This key will serve to separate members of the *D. monticola* species-group occurring in the United States. To determine whether a species can be identified using this key, please consult the diagnosis provided above for this species-group. We have also included males of *D. gibbosa* (Say) in this key because forms with red integumental coloration were recently discovered that are easily misidentified as *D. arenneronea.*

Females

1. Propodeum either coarsely asperated or having numerous raised tubercles, sparsely clothed with setae … 2

\- Propodeum simply punctate or reticulate, lacking raised tubercles or asperations, if appearing tuberculate, then clothed with conspicuous silver or golden setae … 5

2. Mesonotum having pair of transverse carinae directly antero-lateral to scutellar scale; propodeal reticulations widely spaced, carinae between raised tubercles usually distinct (Fig. 4.2F) … *D. canella* (Blake)

\- Mesonotum lacking transverse carinae, usually having a few scattered tubercles posteriorly; propodeal tubercles more closely spaced, usually not connected by obvious carinae (southeastern USA: Figs 4.2: A, B, J) … 3

3. Posterior margin of head evenly rounded … *D. archboldi* Schmidt & Mickel

\- Posterior margin of head angulate laterally … 4

4. Lateral margin of head directly behind eye virtually straight leading into postero-lateral tubercle (Fig. 4.1H); medial black setae on fringe of T2 broad, covering at least 0.25 × of the fringe (Fig. 4.2J); lateral face of propodeum sparsely clothed with silver setae … *D. macilenta* (Blake)

\- Lateral margin of head directly behind eye curving inwardly leading into postero-lateral tubercle (Fig. 4.1G); medial black spot on fringe of T2 narrow (Fig. 4.2B); lateral face of propodeum densely clothed with silver setae … *D. arenerronea* Bradley

5. First flagellar segment elongate, about twice as long as apical width; T2 having dense medial patch of black setae in basal half, punctures closely spaced on black patch, but deep and widely spaced on remainder of disc (Figs 4.2: D, I, M) … 6.

\- First flagellar segment only about 1.5 × as long as apical width; T2 lacking specific setal pattern, punctures moderate to small, similar in size and distribution across disc of T2 (Figs 4.2: C, H, K, O) … 8

6. Pronotum clothed with erect and appressed black setae dorsally; integument of head and mesosoma pale ferruginous, concolorous with T2 (Fig. 4.2D) … *D. bonita* Mickel

\- Pronotum covered with appressed silver setae and erect black setae; integument of head and mesosoma dark ferruginous to black, clearly darker than T2 … 7

7. Appressed setae of vertex and mesonotum entirely silver (Fig. 4.2I) … *D. eurynome* Mickel

\- Appressed setae of vertex and medial portion of mesonotum black (Fig. 4.2M) … *D. saetigera* Mickel

8. Mesonotum lacking carinae anterior to scutellar scale; propodeal setae sparse (Figs 4.2: L, O) … 9

\- Mesonotum having transverse carinae anterior or antero-lateral to scutellar scale, propodeum clothed with dense silver or golden setae (Figs 4.2: C, H, K) … 10

9. Integument of head and mesosoma dark ferruginous to black, significantly darker than medial orange patch on T2 (Fig. 4.2L) … *D. radkei* Manley

\- Integument of head, mesosoma, and metasoma concolorous pale orange to red (Fig. 4.2O) … *D. vesta* (Cresson)

10. Head and mesosoma clothed entirely with golden setae ***and*** apical fringe of T2 broadly clothed with black setae medially (Fig. 4.2K) … *D. monticola* (Cresson)

\- Anterior region of mesosoma usually clothed with black setae dorsally (Figs 4.2: C, H), ***if*** mesosoma clothed entirely with silver or golden setae, ***then,*** apical fringe of T2 only narrowly interrupted with black setae, almost entirely clothed with golden setae (as in Figs 4.2: A-C) … *D. birkmani* (Melander)

Males (unknown in *D. eurynome* and *D. radkei*)

1. Second metasomal sternite having seta-filled pit, pit often reduced and similar in size to a single puncture … 2

- Second metasomal sternite simple, lacking seta-filled pit … 7

2. Metasoma clothed with pale orange setae, except T5–7 having some silver setae (Fig. 4.3E) … *D. bonita* Mickel

- Metasomal terga 3–7 clothed with black and silver setae only … 3

3. Vertex and pronotum clothed with bright silver or golden setae, sometimes with a few scattered erect black setae (Figs 4.3: A, J, K) … 4.

- Vertex and pronotum clothed with interspersed black and gray setae (Figs 4.3: F, I) … 6

4. Tegulae smooth, lacking punctures or setae laterally or posteriorly (Fig. 4.1C); mesoscutum clothed with black setae (Fig. 4.3K) … *D. saetigera* Mickel

- Tegulae punctate, clothed with setae (as in Fig. 4.1D); mesonotum usually clothed with silver setae, concolorous with those of vertex and pronotum (Figs 4.3: A, J) … 5

5. Propodeum having lateral patches of dense appressed setae obscuring punctation; free length of paramere weakly kinked basally and slightly recurved inwardly, making paramere apices only slightly divergent (Fig. 4.4A) … *D. archboldi* Schmidt & Mickel

- Setae of propodeum erect throughout, propodeal reticulations unobscured; free length of paramere strongly kinked basally, making paramere apices obviously divergent (Fig. 4.4.G) … *D. monticola* (Cresson)

6. Propodeal reticulations deep, surfaces between carinae convex; S2 usually black, significantly darker than T2; cuspis short, roughly half the free paramere length (Fig. 4.4E) … *D. canella* (Blake)

- Propodeal reticulations shallow, surfaces between carinae more or less flat; S2 usually red, concolorous with T2; cuspis longer, at least 0.65 × free paramere length (Fig. 4.4F) … *D. macilenta* (Blake)

7. Tibial spurs white … *D. gibbosa* (Say) [*D. quadriguttata* species-group]

- Tibial spurs black … 8

8. Metasomal terga 3–6 entirely clothed with black setae (Fig. 4.3L) … *D. vesta* (Cresson)

- Metasoma having gray or silver setae on apical terga (Figs 4.3: B-D) … 9

9. Tegula punctate on anterior third only , apical half smooth; cuspis less than 0.65 × free paramere length (Fig. 4.4B) … *D. arenerronea* Bradley

- Tegulae variable, but usually punctate throughout; cuspis long, greater than 0.8 × free paramere length (Fig. 4.4C) … *D. birkmani* (Melander)

Dasymutilla archboldi Schmidt & Mickel 1979

(Figs 4.2A, 4.3A, 4.4A)

Dasymutilla archboldi Schmidt & Mickel 1979. Proc. Ent. Soc. Wash. 81: 576. Holotype, ♀: USA: FL: Highlands Co., Archbold Biological Station, 24–25.March.1978, J.O. Schmidt & A. Hook (USNM Type No. 75944).

Diagnosis. FEMALE. The female of this species can be separated from other *Dasymutilla* species by the following combination of characters: the posterior margin of the head is evenly rounded, the scutellar scale is narrow and lacks transverse carinae anterior to it, and the propodeum has numerous raised tubercles.

MALE. The male of this species can be separated from other *Dasymutilla* by the following combination of characters: the pronotum and mesonotum are clothed with silver setae, T3–7 are clothed with silver setae, the dorsal propodeal surface is clothed with dense appressed silver setae laterally, the tegulae are punctate, and there is a large seta-filled pit on S2.

Description of male (hitherto unknown). *Coloration.* Head and mesosoma dorsally and anteriorly black. Mandible, antenna, legs, propodeum and apical segments of metasoma dark reddish brown. Basal metasomal segments red. Tibial spurs concolorous with legs, dark reddish brown. Wings weakly infuscated, veins brown, darker basally. Head and mesosoma clothed throughout with erect and appressed silver setae; sparse erect brown setae interspersed with silver setae on vertex, pronotum, mesoscutum and scutellum. T1 and S1–2 with erect silver setae; T2 with erect black setae on basal half and thick fringe of black setae apically; T3–7 and S4–8 having interspersed black and silver erect setae. *Head***:** Rounded posteriorly. Front, vertex and gena with dense coarse punctures. Mandible tridentate apically, unarmed ventrally. Antennal scrobe ecarinate. Gena ecarinate. Ocelli minuscule; ocellocular distance >5 × length of lateral ocellus, interocellar distance 3 × lateral ocellar length. Flagellomere I 1.5 × pedicel length; flagellomere II 2.0 × pedicel length. *Mesosoma***:** Pronotum with coarse dense punctures. Tegula punctate and clothed with setae throughout, convex. Mesonotum with coarse dense punctures. Mesopleuron with coarse dense punctures posteriorly and small sparse punctures anteriorly. Metapleuron with a few small punctures. Scutellum nearly flat, slightly convex, with coarse, confluent punctures. Propodeum reticulate dorsally and posterolaterally, anterolaterally with sparse small punctures. *Metasoma***:** T1 nodose, with dense confluent punctures. T2 and S2 with deep punctures that are separated by slightly less than each puncture diameter. S2 with ovate medial pit, densely filled with white setae, 0.18 × S2 length, 1.4 × longer than wide. T3–6 dense small punctures and interspersed erect and appressed setae; S3–6 with dense small punctures and erect setae only. Pygidium shagreened with punctures that are separated by slightly more than each puncture diameter, lacking apical fringe of setae. Hypopygium punctate, apical margin impunctate and shining with medial tooth. *Genitalia* (Fig. 4.4A): Free length of paramere dorsally curved apically, with ventral brush of long dense setae basally, remainder having scattered short sparse setae; paramere laterally kinked in basal 0.2 and slightly recurved in apical 0.6, apices almost imperceptibly divergent. Cuspis roughly cylindrical, ~0.6 × free-length of paramere, having long, sparse setae throughout; outer row of setae anteriorly directed, inner row posteriorly directed. Densely setose basal lobe present. Digitus laterally curved internally, tapering slightly at apex, asetose, ~0.35 × free-length of paramere. Penial valve emarginated anterodorsally, ventral margin bidentate apically, teeth separated, unidentate medially; having longitudinal row of setae at apex and subapically on external margin.

Length. Females: 4–8 mm; males: 3.5–8 mm.

Host. Hosts of *D. archboldi* are unknown. This species appears to be the commonest small mutillid in open Florida scrub habitat on the Archbold Biological Station, where the most abundant potential hosts by far are wasps in the genera *Tachysphex* and *Tachytes* (Crabronidae), of which there are at least 22 resident species.

These wasps and their enemies are difficult to study at the Station because their burrows are seldom aggregated and fill in quickly with loose silica sand.

Distribution. This species is found only in the scrub habitat of southern Florida, specifically in Highlands, Okeechobee, Orange, and Polk Counties.

Material examined. USA: Florida**:** *Highlands Co.*, Archbold Biological Station, various dates & collectors: 34♂ (PMAE, EMUS), 87♀ 10♂ (FSCA), 5♀ 35♂ (EMUS, ABSC), 2♂ (UCDC); Carter Creek North, 2♂, 25.VI.2009, M. Deyrup (EMUS); Carter Creek South, 5♂, 16.IX.2009, M. Deyrup (EMUS, ABSC); FWC Henscratch Pres., 1♂, 17.VI.2009, M. Deyrup (EMUS); Gould Road Preserve, 3♂, 13–19.V.2009, M. Deyrup (EMUS, ABSC); Lake June State Park, 2♂, 3.VI.2009, M. Deyrup (ABSC); Saddle Blanket Lakes, 2♂, 1–6.V.2009, M. Deyrup (EMUS, ABSC); *Okeechobee Co.*, Archbold Biological Station, 4♂, 21–27.VII.1987, D.B. Wahl (PMAE, EMUS); *Orange Co.*, Walt Disney World, 1♂, 30.VI–7.VII.1996, Z. Prusak & S.M. Fullerton (UCFC); *Polk Co.*, FWC Sunray Preserve: 3♂, 15–20.V.2009; 3♂, 22–29.VII.2009, M. Deyrup (EMUS, ABSC); Lake Wales Ridge State Forest: 14♂, 1–6.V.2009; 10♂, 20.VI.2009, M. Deyrup (EMUS, ABSC); Saddle Blanket Lakes, 1♂, 1–6.V.2009, M. Deyrup (EMUS, ABSC); Tiger Creek Preserve, Babson Park, 2♂, 5.V.2007, B. Pace-Aldana & A. Peterson (UCFC).

Remarks. Although they did not directly place it in a species-group, the authors suggested this species could be a member of the *D. caneo* species-group (now included in the *D. monticola* species-group). Manley (1983) recognized the female's close affiliation to *D. vesta* and, based on this similarity, hypothesized that the male should be similar to *D. vesta*. Examination of *D. vesta* series in collections yielded males that had unique red mesosomal coloration (Manley 1983) and distinctly shaped tegulae (Manley 1984). These males were described as the opposite sex of *D. archboldi* (Manley 1983).

Morphological and distributional data led us to doubt this sex association. The males attributed to *D. archboldi* by Manley are much larger than the females, which is a common occurrence in species of other genera (*Sphaeropthalma pensylvanica*: Pitts *et al.*, 2010a, *Timulla* and *Ephuta* spp.: Deyrup & Manley 1986, Pitts *et al.* 2010a), but had not been observed in other *Dasymutilla* species (Deyrup & Manley 1986). Additionally, these *D. archboldi* males were difficult to separate from the more common *D. vesta*, necessitating a publication meant to facilitate identification by a difference in tegula shape (Manley 1984) that has shown to be inconsistent (KAW & MD, pers. obs.). Finally, while *D. archboldi* females were known only from Polk and Highlands Counties in Florida, these males were found throughout the state and even in southern Georgia.

Our phylogenetic results (Fig. 4.6) further suggest that males attributed to *D. archboldi* have been misidentified, as males and females fall in separate clades. The male attributed to *D. archboldi* is sister to *D. vesta* and has similar ITS sequences to that species (98.1% identical in ITS1 and 100% identical in ITS2). The female of *D. archboldi* is recovered in a clade with a yet-unidentified male, with which it shares nearly identical ITS sequences (100% identical in ITS1 and 99.8% identical in ITS2). Therefore, we determine that the male previously attributed to *D. archboldi* is conspecific with *D. vesta* and we associate the female of *D. archboldi* with a previously undescribed male.

The true male of *D. archboldi* (Fig. 4.3A) is described above; it superficially resembles the males of *D. arenerronea* (Fig. 4.3B) and *D. macilenta* (Fig. 4.3I). This newly associated male is approximately the same size as female *D. archboldi* specimens, can be reliably separated from other Floridian *Dasymutilla*, and is found only in the scrub habitat of southern Florida like the female.

Dasymutilla archboldi appears to have a distribution restricted to the Lake Wales Ridge and part of the smaller, nearby Hundred Foot Ridge. It probably does not occur north and east of this area, based on extensive sampling by S.M. Fullerton in suitable habitat at the University of Central Florida and other areas. The Lake Wales Ridge is known to have a variety of species of plants and arthropods that are not found elsewhere, primarily due to the isolation, antiquity, size and position of this ridge (Deyrup 1990). [For a description of major Florida sand ridges, see White (1970)]. Over 85% of the xeric uplands on the Lake Wales Ridge have been converted to agricultural, industrial and residential uses (Weekly *et al.* 2008). A recent study, however, shows that *D. archboldi* occurs on at least 18 Florida scrub habitat preserves, and probably does not qualify to be considered a species of conservation concern (Deyrup, unpublished data).

Dasymutilla arenerronea **Bradley 1916, stat. resurr.**

(Figs 4.1E, 4.2B, 4.3B, 4.4B)

Dasymutilla (*Dasymutilla*) *arenerronea* Bradley, 1916. Trans. Amer. Ent. Soc. 42: 324. Holotype, ♀: St. Petersburg, Florida, August 12, 1910, J. C. Bradley (CUIC Type No. 115.1) .

Dasymutilla chattahoochei Bradley: Mickel 1928. U. S. Nat. Mus. Bull. 143: 186, ♀.

Dasymutilla chattahoochei Bradley: Krombein 1954. Trans. Amer. Ent. Soc. 80: 9, ♂ ♀.

Diagnosis. FEMALE. The female of this species can be separated from other *Dasymutilla* by the following combination of characters: the lateral margin of the head behind the eye gradually curves inward, leading into tubercles on the postero-lateral margin of the head (Fig. 4.1E); there are no transverse carinae anterior to the scutellar scale; the propodeum is covered with numerous small tubercles dorsally; the lateral face of the propodeum has dense silver setae that obscure the punctation; and the apical fringe of T2 is mostly silver, being interrupted by a small medial spot of black setae (Fig. 4.2B).

MALE. The male of this species can be separated from other *Dasymutilla* by the following combination of characters: there are silver setae on the apical terga, there is no seta-filled pit on S2, and the cuspis is less than 0.7x the free paramere length (Fig. 4.4B).

Description of male genitalia (Fig. 4.4B). Free length of paramere dorsally curved apically, with ventral brush of long dense setae basally, remainder having scattered short sparse setae; paramere laterally kinked in basal 0.3, apices slightly divergent. Cuspis roughly cylindrical, ~0.6 × free-length of paramere, having sparse short setae throughout. Densely setose basal lobe present. Digitus laterally curved internally, tapering slightly at apex, asetose, ~0.4 × free-length of paramere. Penial valve emarginated anterodorsally, ventral margin bidentate apically, teeth separated, unidentate medially; having longitudinal row of setae at apex and subapically on external margin.

Length. Females: 4.5–9.5 mm; males: 5–8 mm.

Host. Unknown.

Distribution. This species is currently known only from Florida, but because specimens have been found in the northeastern Clay and Bradford Counties, this species potentially could occur north into Georgia and South Carolina.

Material examined. USA: Florida: *Bradford Co.*, unknown locality, 1♂, 1.V.1959, H.V. Weems (FSCA); *Citrus Co.*, 12 mi NW Brooksville, 3♂1♀, 11.V.2002, M. Deyrup & J. Mosely (ABSC); *Clay Co.*: Keystone Heights, 2♀, 16.VI.1991, H.K.

Dozier (FSCA); unknown locality, 1♂, 14.V.1960, H.V. Weems (FSCA); *DeSoto Co.*, Arcadia, 3♀ 1♂, 2–3.IV.1953, K.V. Krombein (ABSC); *Highlands Co.*: Archbold Biological Station, Lake Placid, 1♀, 7.VI.1984, D. Richman (ABSC); Highlands Hammock State Park, 1♀, 2.VIII.1969, Heinrich (ZMUC); *Hillsborough Co.*, Tampa Rud, 1♀, 6.VI.1949, D.J. Downs (FSCA); *Orange Co.*, Orlando: 1♀, 4.IV.1953, K.V. Krombein (ABSC); 1♂, 3.IV.1954, K.V. Krombein (ABSC); University of Central Florida campus: 1♀, 30.VII.1990, S.M. Fullerton (UCFC); 2♂, 17.IV.1991, S.M. Fullerton (UCFC); Walt Disney World, 1♂, 5–12.V.1996, Z. Prusak & S.M. Fullerton (UCFC); *Osceola Co.*, Kissimmee St. Cloud, 1♂, 12.VI.2001, TNC staff (UCFC); *Palm Beach Co.*, Lion Country Safari: 1♂, 1.VI.1989, M. Deyrup (ABSC); *Sarasota Co.*, MCC – Venice Campus: 1♂, 17.III.1997, K.J. Maharay & S.M. Fullerton (UCFC); 1♂, 28.IV.1997, K.J. Maharay & S.M. Fullerton (EMUS); *County unknown*, 1♀, 16.V.1956, E. Suenson (ZMUC).

Remarks. Mickel (1928) synonymized this species with *D. chattahoochei*, because he considered the holotypes of the two species to be identical. He stated that the difference in head shape (Figs 4.1: E, H) was inadequate to support species-distinction, and species identification using this character would differ based on specimen orientation. Although the distinction in head shape is subtle and difficult to recognize, we found it to be consistent for separating these species. Two additional characters separate *D. arenneronea* from *D. chattahoochei,* the lateral propodeal setae and the apical fringe of T2, but these are also difficult to use. Even so, we resurrect *D. arenerronea,* because male morphology and DNA sequences (Fig. 4.6) clearly differentiate these insects from *D. macilenta* (which now includes *D. chattahoochei*).

Males of *D. arenerronea* were initially associated with *D. chattahoochei* by Krombein (1954) based on misidentified specimens. Krombein (1954) provided a detailed description for these males, except the genitalia, which are described above.

This species is clearly a member of the *D. monticola* species-group based on morphology and phylogenetic position (Fig. 4.5), even though the female was initially placed into the *D. quadriguttata* species-group. Krombein (1954) cited the *D. arenneronea* sex association as the second example of the combination of these two species-groups, after *D. canella* from the *D. monticola* species-group was associated with *D. rugulosa* from the *D. quadriguttata* species-group (Krombein, 1951).

***Dasymutilla birkmani* (Melander 1903)**

(Figs 4.1: F, G; 4.2: C, H; 4.3: C, D; 4.4C)

Mutilla Birkmani Melander 1903. Trans. Amer. Ent. Soc. 29: 313. Syntypes, ♀, Fedor, Lee County, Tex[as], G. Birkman (MCZ).

Dasymutilla corcyra Mickel, 1928. U. S. Nat. Mus. Bull. 143: 180. Holotype, ♀, Rock Island, Tex[as], Skull Creek, July 25, 1922, Grace O. Wiley (UMSP)., **syn. nov.**

Dasymutilla arcana Mickel, 1928. U. S. Nat. Mus. Bull. 143: 217. Holotype, ♂, Lee County, Texas, June 8, 1906, J. Bequaert (SEMC), **syn. nov.**

Dasymutilla reclusa Mickel, 1928. U. S. Nat. Mus. Bull. 143: 219. Holotype, ♂, Halsey, Nebr., August 29, 1924, R. W. Dawson (UMSP).

Dasymutilla birkmani Melander: Pilgrim *et al.* 2008. Pan-Pacific Ent., 84: 64, ♂ ♀,

Diagnosis. FEMALE. This species varies greatly and can be difficult to separate from *D. monticola*. In all examined specimens, the first flagellar segment is only slightly longer than the second and the propodeum has a thick brush of silver or golden setae

(Figs 4.2: C, H). Most specimens have the anterior portion of the mesosoma clothed with dark brown or black setae dorsally (Figs 4.2: C, H); these differ from *D. monticola*, which has the entire mesosoma clothed with golden setae (Fig. 4.2J). In the few specimens of *D. birkmani* that have the mesosoma clothed entirely with golden setae, the apical fringe of T2 is broadly silver with only a slight spot of black setae medially (as in Figs 4.2: A–C); *D. monticola* has the fringe of T2 clothed mostly with black setae medially (Fig. 4.2J).

MALE. The male of this species can be separated from other *Dasymutilla* by the following combination of characters: there is no seta-filled pit on S2, there are scattered silver or gray setae on the apical terga, and the cuspis is more than 0.8x the free paramere length (Fig. 4.4C).

Description of male genitalia (Fig. 4.4C). Free length of paramere dorsally curved apically, with ventral brush of long dense setae basally, remainder having scattered short sparse setae; paramere laterally kinked in basal 0.2, apices noticeably divergent. Cuspis slightly laterally compressed, tapering apically, ~0.85 × free-length of paramere, having sparse short setae throughout, except outer surface having longer denser setae; outer row of setae anteriorly directed, inner row posteriorly directed. Densely setose basal lobe present. Digitus laterally curved internally, tapering slightly at apex, asetose, ~0.4 × free-length of paramere. Penial valve emarginated anterodorsally, ventral margin bidentate apically, teeth separated, unidentate medially; having longitudinal row of setae at apex and subapically on external margin.

Length. Females: 3.5–9 mm; males: 5.5–10 mm.

Host. Unknown.

Distribution. This species is widespread in the central and western United States.

Remarks. Molecular sequence data from ITS1 and ITS2 were used previously to associate *D. birkmani* females with males (formerly *D. reclusa*; Pilgrim *et al*. 2008). Our study has found two more species that appear to be conspecific with *D. birkmani*. Specimens identified as *Dasymutilla arcana* and *D. corcyra* are recovered within a clade that includes seven females and one male identified as *D. birkmani* (Fig. 4.6). The relationships between *D. corcyra*, *D. arcana*, and two clades of *D. birkmani* are unresolved or poorly supported, but both male specimens of *D. arcana* have identical ITS1 and ITS2 sequences to one of the *D. birkmani* females (JP1885).

The only diagnostic character separating *D. arcana* from males of *D. birkmani* is the metasomal coloration, where *D. arcana* has S2 red and *D. birkmani* has S2 black. Multiple *Dasymutilla* species, including *D. quadriguttata* (Pilgrim *et al.* 2009), *D. bioculata* (Williams *et al.* 2011), and *D. vesta* (Mickel, 1928), are dimorphic for this character. Males of *D. arcana* and *D. birkmani* also have identical genitalia (Fig. 4.4C).

Mickel separated *D. corcyra* (Fig. 4.1G) from *D. birkmani* females by the presence of tubercles on the posterolateral head margins (Mickel, 1928). All female specimens of *D. birkmani*, however, have variably angulate posterolateral head margins and the posterior head margin virtually flat or concave (Fig. 4.1F). Specimens of *D. corcyra* are typically larger (7.5–9 mm, mean=8.3, n=6) than those of *D. birkmani* (3.5-8.5 mm, mean=5.4, n=40); hypothetically, the magnitude of head tubercles may be correlated with body size and not species divergence. Although not to this extent, intraspecific variation in head tubercle structure has also been observed in *D. quadriguttata* (Pilgrim *et al.* 2009).

Dasymutilla arcana, *D. birkmani*, and *D. corcyra* also have broadly overlapping geographic ranges in the central USA. Based on phylogenetic affinities, morphological similarities, and overlapping distributions, we place *D. arcana* and *D. corcyra* as **junior synonyms** of *D. birkmani*.

Dasymutilla bonita **Mickel 1928**

(Figs 4.2D, 4.3E, 4.4D)

Dasymutilla bonita Mickel, 1928. U. S. Nat. Mus. Bull. 143:208. Holotype, ♀, Bonita, Graham County, Arizona, July 12, 1917, J. Bequaert (UMSP).

Dasymutilla poliothrix Mickel, 1928. U. S. Nat. Mus. Bull. 143:285. Holotype, ♂, Nogales, Arizona, July 24, 1903, Oslar (CUIC).

Diagnosis. FEMALE. The female of this species (Fig. 4.2D) can be separated from other *Dasymutilla* by the following combination of characters: the first flagellomere is twice as long as its maximal width, the head and mesosoma are concolorous with the metasomal integument, the mesosoma is covered with black setae antero-medially, the propodeum has a brush of silver setae, and there is a dense medial patch of appressed black setae basally on T2.

MALE. The male of this species can be separated from other *Dasymutilla* by the following combination of characters: there is a seta-filled pit on S2, there are pale silver setae on T5–7, and T2–4 are clothed with pale orange setae (Fig. 4.3E).

Description of male genitalia (Fig. 4.4D). Free length of paramere dorsally curved apically, with ventral brush of long dense setae basally, remainder having scattered short sparse setae; paramere not kinked laterally. Cuspis laterally compressed, tapering apically, ~0.7 × free-length of paramere, having long setae throughout; outer row of setae anteriorly directed, inner row posteriorly directed. Densely setose basal lobe present. Digitus laterally curved internally, tapering slightly at apex, asetose, ~0.3 × free-length of paramere. Penial valve emarginated anterodorsally, ventral margin bidentate apically, teeth separated, unidentate medially; having longitudinal row of setae at apex and subapically on external margin.

Length. Females: 6–8 mm; males: 7–10.5 mm.

Host. Unknown.

Distribution. USA (Arizona); Mexico (Guerrero, Jalisco, Nayarit, Sinaloa, Sonora).

Remarks. The silver apical metasomal setae of these males (Fig. 4.3E) are not as obvious as in other members of the *D. monticola* species-group (as in Figs. 4.3: J, K), because they do not contrast strikingly against the orange setae of T2-5. Setal color, coupled with glabrous tegulae, probably prevented Mickel from affiliating these males (as *D. poliothrix* Mickel in the *D. subhyalina* species-group) with the *D. monticola* species-group. Females of *D. bonita* have the first flagellar segment elongate and males have glabrous tegulae, suggesting close relation to *D. saetigera.*

***Dasymutilla canella* (Blake 1871)**

(Figs 4.1A, 4.2F, 4.3F, 4.4E)

Mutilla (*Sphaeropthalma*) *canella* Blake, 1871. Trans. Amer. Ent. Soc. 3: 239. Holotype ♂: Texas (ANSP).

Mutilla rugulosa Fox, 1899. Trans. Amer. Ent. Soc. 25: 240. Syntypes, ♀: Southern New Jersey, September (ANSP).

Mutilla infensa Melander & Brues, 1903. Biol. Bull. 5: 24. Syntypes, ♀: Woods Hole, Massachusetts (Washington State College, Pullman, WA).

Diagnosis. FEMALE. The female of this species can be separated from other *Dasymutilla* by the head shape and mesosomal sculpture. The head is slightly angulate posterolaterally (Fig. 4.2F), the mesonotum has transverse carinae situated antero-laterally to the scutellar scale, and the propodeum is strongly asperated dorsally and posterolaterally.

MALE. The male of this species can be separated from other *Dasymutilla* by the exceptionally large and deep propodeal reticulations. The following characters are also helpful for identification: the head and mesosoma are covered entirely with black setae, there is a seta-filled pit on S2, there are silver setae on T5–7, and the cuspis is roughly 0.5x the free paramere length (Fig. 4.4E).

Description of male genitalia (Fig. 4.4E). Free length of paramere dorsally curved apically, with ventral brush of long dense setae basally, remainder having scattered short sparse setae; paramere laterally kinked in basal 0.2, apices noticeably divergent. Cuspis slightly laterally compressed, tapering apically, ~0.55 × free-length of paramere, having a few scattered long setae, those on inner surface posteriorly directed. Densely setose basal lobe present. Digitus laterally curved internally, tapering slightly at apex, asetose, ~0.35 × free-length of paramere. Penial valve emarginated anterodorsally, ventral margin bidentate apically, teeth separated, unidentate medially; having longitudinal row of setae at apex and subapically on external margin.

Length. Females: 3.5–7 mm; males: 4–7.5 mm.

Host. Unknown.

Distribution. This species is found in the northeastern USA and southeastern Canada.

Remarks. The phylogenetic results show a close affiliation between this species and *D. macilenta*. We believe these are distinct taxa, however, because of morphological differences (propodeal sculpture) seen in both sexes, differences in male genitalia, and geographic ranges.

The female of this species [as *D. rugulosa* (Fox)] was initially placed in the *D. quadriguttata* species-group by Mickel (1928), because of the angulate postero-lateral head angles.

***Dasymutilla eurynome* Mickel 1928**

(Fig. 4.2I)

Dasymutilla eurynome Mickel, 1928. U. S. Nat. Mus. Bull. 143:209. Holotype, ♀, Valentine, Presidio County, Texas, July 8, 1917, J. Bequaert (UMSP).

Diagnosis. FEMALE. The female of this species can be separated from other *Dasymutilla* by the following combination of characters: the first flagellomere is elongate; the head and mesosoma are entirely covered with appressed silver setae and erect black setae, except a medial patch of appressed black setae that extends from directly anterior to the scutellar scale to the medial portion of the posterior propodeal face (Fig. 4.2I); and there is a dense medial patch of appressed black setae in the basal half of T2 (Fig. 4.2I).

MALE. Unknown.

Length. Females: 7–8 mm.

Host. Unknown.

Distribution. This rare species has only been recorded from western Texas.

Remarks. This species is morphologically similar to *D. saetigera*, differing only in the setal coloration of the mesonotum and scutellar area. Differences in setal pattern have proven inadequate for species delimitation in other *Dasymutilla* (Williams *et al*. 2011). We support Mickel's hypothesis that this species is distinct from *D. saetigera*, however, because the species are geographically isolated from one another by the Rocky Mountain and Sierra Madre ranges and no intermediate forms are known. The male is unknown, but is likely similar to that of *D. saetigera*.

The photograph included in Manley & Pitts (2007) of this species was misidentified from the collection of KAW and is actually *D. monticola*. This error was only recognized after KAW had an opportunity to examine the paratype of *D. eurynome* (Fig. 4.2I) from the Museum of Comparative Zoology (Harvard University).

Manley & Pitts (2007) recorded this species from Nayarit, Mexico. KAW examined this specimen in the Bohart Museum of Entomology (University of California, Davis) and determined the specimen to be *D. saetigera* as there are clearly appressed black setae on the mesonotum and vertex. Four additional specimens of *D. saetigera* were examined from the same site in Nayarit (10 mi. east of Acaponeta). *Dasymutilla eurynome* appears to be restricted to the mountainous regions of western Texas and is unlikely to occur outside the Chihuahuan ecological region.

***Dasymutilla macilenta* (Blake 1871)**

(Figs 4.1H, 4.2J, 4.3I, 4.4F)

Mutilla (*Sphaeropthalma*) *macilenta* Blake, 1871. Trans. Amer. Ent. Soc. 3: 239. Holotype, ♂: Texas. (ANSP).

Dasymutilla (*Dasymutilla*) *chattahoochei* Bradley, 1916. Trans. Amer. Ent. Soc. 42: 324. Holotype, ♂: Bainbridge, Florida, July 15–27, 1909, J. C. Bradley (CUIC Type No. 114.1), **syn. nov.**

Diagnosis. FEMALE. The female of this species can be separated from other *Dasymutilla* by the following combination of characters: the lateral margin of the head behind the eye is virtually straight, leading into sharp tubercles on the postero-lateral margin of the head (Fig. 4.1H); there are no transverse carinae anterior to the scutellar scale; the propodeum is covered with numerous small tubercles dorsally; the lateral face of the propodeum has sparse silver setae; and the apical fringe of T2 has a broad patch of black setae medially, occupying at least 0.25 × the tergal width. MALE. The male of this species can be separated from other *Dasymutilla* by the following combination of characters: there are silver setae on the apical terga, the propodeum is clothed with interspersed black and gray setae, there is a small seta-filled pit (ranging from subequal in size to a single puncture to three times larger than the typical surrounding punctures) on S2, the propodeum is moderately reticulate, and the cuspis is at least 0.65 × the free paramere length (Fig. 4.4F).

Description of male genitalia (Fig. 4.4F). Free length of paramere dorsally curved apically, with ventral brush of long dense setae basally, remainder having scattered short sparse setae; paramere laterally kinked in basal 0.3, apices noticeably divergent. Cuspis roughly cylindrical, ~0.7 × free-length of paramere, laterally curving with apices slightly divergent, having sparse short setae throughout. Densely setose basal lobe present. Digitus laterally curved internally, tapering slightly at apex, asetose, ~0.35

× free-length of paramere. Penial valve emarginated anterodorsally, ventral margin bidentate apically, teeth separated, unidentate medially; having longitudinal row of setae at apex and subapically on external margin.

Length. Females: 4–8 mm; males: 4–8 mm.

Host. Unknown.

Distribution. This species is widespread in the southeastern USA.

Material examined. USA: Alabama: *Escambia Co.*, Escambia: 1♀, 22.VII.1994 (EMUS); 1♀, 8.VIII.1994 (EMUS). *Russel Co.*, 8 mi NW Seale, 3♀, 13.VI–24.X.1994, P. Kovarik (FSCA). Florida**:** *Alachua Co.*: Gainesville, 1♀, 28.VIII.1960, P.N. Marsh (UCDC); locality unknown: 2♀, 4.VII.1955, H.V. Weems (FSCA); 1♀, 16.VIII.1955, H.V. Weems (FSCA); 1♀, 15.V.1955, H.V. Weems (FSCA); 1♀, 8.VI.1954, H.V. Weems (EMUS); *Brevard Co.*, Cocoa, 1♀, VIII.1914, R.M. Bohart (UCDC); *Clay Co.*: Camp Crystal, 1♀, 20.V.1961, H.V. Weems (FSCA); Gold Head Branch State Park, 1♀, 26.IV.1971, Heinrich (ZMUC); *Gadsen Co.*, Quincy, 1♀, 20.V.1972, J.C. Reid (FSCA); *Highlands Co.*, Highlands Hammock State Park, 1♀, 16.IV.1966, H.V. Weems (FSCA); *Hillsborough Co.*, Tampa, 1♀, 12.VIII.1949, D.J. Downes (FSCA); *Lafayette Co.*, unknown city, 1♀, 22.VII.1977, L. Davis (FSCA); *Leon Co.*, Tall Timbers Research Station: 1♀, 14-21.VIII.1972, D.L. Harris (FSCA); 1♀, 4.VIII.1971, T.M. Neal (FSCA); *Levy Co.*, 4 mi S Archer, 1♀, 9–25.V.2000, P. Skelly (FSCA); unknown locality, 1♀, VIII.1976 (FSCA); *Okaloosa Co.*, 2 mi N Holt, 1♀, 28.X.1983, L.A. Stange (FSCA); *Orange Co.*, University of Central Florida campus, 4♀1♂, 17.VI.2009, K.A. Williams & S. Kelly (EMUS); Walt Disney World, 1♀, 18.VII.1995, Z. Prusak (FSCA); *Putnam Co.*, Weems property, near Red Water Lake, 4♀, 4.VII.1969, Heinrich (ZMUC). Georgia:

Decatur Co., Spring Creek, 1♀, 20.VIII.1946, P.W. Fattig (FSCA); *Dougherty Co.*, Albany, 1♀, 23.VII.1938, P.W. Fattig (FSCA); *Ware Co.*, E of Waycross, Walker State Park, 1♀, 17.V.1993, B.K. Davies (FSCA). <u>South Carolina</u>**:** *Horry Co.*, Myrtle Beach, 3♀, 29.VII–6.IX.1949, Shappiro (UMMZ); *Pickens Co.*, Dovehaven, 7 mi NE Pickens: 1♀, 13.VI.1979; 1♀, 28.VI.1979; 1♀, 14.VI.1980; 1♀, 13.VII.1980; 1♀, 29.VII.1981; A. & H.L. Dozier (FSCA).

Remarks. A male specimen of *D. macilenta* and a female of *D. chattahoochei*, both from the University of Central Florida campus are 100% identical in ITS1 and 99.8% identical in ITS2. These genetic distances, coupled with overlapping distribution clearly show that *D. chattahoochei* is the female and a **junior synonym** of *D. macilenta*.

Because of similarities in female morphology, Krombein (1954) associated the male of *D. arenerronea* with *D. macilenta* (as *D. chattahoochei*). These species were recovered from separate clades (Fig. 4.6). Additionally, the males of *D. macilenta* are easily separated from *D. arenerronea* by structural differences in S2 and the genitalia. Females are much more difficult to separate, but differ in the head shape, lateral propodeal face, and fringe of T2.

The label on the holotype of *D. macilenta* simply reads “Tex.”. Mickel (1928) suggested that the type might be mislabeled, because *D. macilenta* was known exclusively from the extreme southeastern USA (AL, FL, GA). The distribution in Alabama, however, suggests that this species may occur throughout the Gulf States (AL, LA, MS, TX). We believe the holotype is not necessarily, but could potentially be, mislabeled.

Dasymutilla monticola (Cresson 1865)

(Figs 4.2K, 4.3J, 4.4G)

Mutilla monticola Cresson, 1865. Proc. Ent. Soc. Phil. 4: 430. Syntypes, ♂, Colorado Territory (ANSP).

Mutilla caneo Blake 1879. Trans. Amer. Ent. Soc. 7: 250. Holotype, ♀, Texas. (ANSP)

Mutilla mixtura Blake 1879. Trans. Amer. Ent. Soc. 7: 251. Holotype, ♀, Texas. (ANSP)

Mutilla eximia Blake, 1886. Trans. Amer. Ent. Soc. 13: 200. Holotype, ♂, Arizona (ANSP).

Mutilla myrrha Fox, 1899. Trans. Amer. Ent. Soc. 25: 258. Holotype, ♀, Fort Collins, Colorado, Gillette (ANSP).

Ephuta boulderensis Rohwer, 1909. Trans. Amer. Ent. Soc. 35: 133. Holotype, ♂, Boulder, Colorado, August 4, 1908, S. A. Rohwer (USNM).

Dasymutilla paenulata Mickel, 1928. U. S. Nat. Mus. Bull. 143: 206. Holotype, ♀, Phoenix, Arizona, August 3, 1917.(CUIC).

Dasymutilla monticola Cresson: Pilgrim *et al.*, 2008. Pan-Pacific Ent., 84: 62, ♀♂.

Diagnosis. FEMALE. The female of this species can be separated from other *Dasymutilla* by the following combination of characters: the first flagellar segment is only slightly longer than the second, the mesosoma is clothed entirely with silver or golden setae (Fig. 4.2K), and the apical fringe of T2 is widely black medially (Fig. 4.2K).

MALE. The male of this species can be separated from other *Dasymutilla* by the following combination of characters: the pronotum and mesonotum are clothed with dense silver or golden setae (Fig. 4.3J); the apical terga are clothed with silver setae (Fig. 4.3J); the tegulae are punctate; the propodeal setae are erect, not obscuring propodeal reticulations; and there is a seta-filled pit on S2.

Description of male genitalia (Fig. 4.4G). Free length of paramere dorsally curved apically, with ventral brush of long dense setae basally, remainder having scattered short sparse setae; paramere laterally kinked in basal 0.2, apices noticeably divergent. Cuspis slightly laterally compressed, tapering apically, ~0.7 × free-length of paramere, having short sparse setae throughout, except external apical 0.5 having dense long anteriorly directed setae. Densely setose basal lobe present. Digitus laterally curved internally, tapering slightly at apex, asetose, ~0.3 × free-length of paramere. Penial valve emarginated anterodorsally, ventral margin bidentate apically, teeth separated, unidentate medially; having longitudinal row of setae at apex and subapically on external margin.

Length. Females: 3.5–7.5 mm; males: 5–9.5 mm.

Host. Unknown.

Distribution. This species is widespread in the western and central Nearctic region, being found from Tamaulipas, Mexico north to Minnesota and west to California and British Columbia, Canada.

Remarks. Molecular sequences were used previously to associate *D. paenulata* and *D. caneo* with *D. monticola* (Pigrim *et al.* 2008). Our phylogenetic results did not yield any additional synonyms of *D. monticola* and do not necessitate any additional taxonomic changes (Fig. 4.6). This is the second most widespread member of the *D. monticola* species-group, after *D. vesta*. There is variation in punctation of the tegulae and coloration, but this variation is no more extensive than what is seen in other *Dasymutilla*.

Dasymutilla radkei **Manley in Manley & Radke 2006**

(Fig. 4.2L)

Dasymutilla radkei Manley in Manley & Radke 2006. Southwest. Nat. 51: 539. Holotype, ♀: USA,New Mexico, Chaves Co., Bitter Lake National Wildlife Refuge, XI-16-96, W.R. Radke (EMUS).

Diagnosis. FEMALE. The female of this species (Fig. 4.2L) can be separated from other *Dasymutilla* by the following combination of characters: the head and mesosoma are dark orange or black and clothed with sparse orange setae, there is a yellow-orange integumental patch covering most of T2, the propodeum is simply punctate, and there is a small scutellar scale without any transverse carinae anteriorly.

MALE. Unknown.

Length. Females: 8–11 mm.

Host. Unknown.

Distribution. This species is known from eastern New Mexico and western Texas.

Remarks. This species is similar to *D. vesta*, being separated only by coloration. It was initially known only from eastern New Mexico, but 13 specimens were discovered in the BYU insect collection from Monahans Sand Hills State Park in Ward County, Texas. From this locality, males that are similar to *D. vesta* were also found. These males have the dorsal portions of the head and mesosoma dark red, rather than black as in most specimens of *D. vesta*. These are likely to be the males of *D. radkei*, but we refrain from associating them without molecular evidence or a greater understanding of intraspecific variation.

***Dasymutilla saetigera* Mickel 1928**

(Figs 4.1C, 4.2M, 4.3K, 4.4H

Dasymutilla saetigera Mickel, 1928. U. S. Nat. Mus. Bull. 143: 211. Holotype, ♀, Baboquivari Mountains, Arizona, F. H. Snow (SEMC).

Dasymutilla polia Mickel, 1928. U. S. Nat. Mus. Bull. 143: 215. Holotype, ♂, Nogales, Arizona, July 20, 1903, Oslar(CUIC), **syn. nov.**

Diagnosis. FEMALE. The female of this species can be separated from other *Dasymutilla* by the following combination of characters: the first flagellomere is elongate, the pronotum and propodeum are clothed with appressed silver setae, the mesonotum mesally and the scutellar area are clothed with black setae (Fig. 4.2M), and there is a dense medial patch of appressed setae basally on T2 (Fig. 4.2M).

MALE. The male of this species can be separated from other *Dasymutilla* by the following combination of characters: the pronotum is clothed with dense silver setae (Fig. 4.3K), the apical terga are clothed with silver setae (Fig. 4.3K); the tegulae are asetose and smooth medially and posteriorly (Fig. 4.1C), and there is a seta-filled pit on S2.

Description of male genitalia (Fig. 4.4H). Free length of paramere dorsally curved apically, with ventral brush of long dense setae basally, remainder having scattered short sparse setae; paramere laterally curved in basal 0.2, apices slightly divergent. Cuspis slightly laterally compressed, tapering apically, ~0.6 × free-length of paramere, having sparse long setae throughout, except outer surface having dense long anteriorly directed setae. Densely setose basal lobe present. Digitus laterally curved internally, tapering slightly at apex, asetose, ~0.3 × free-length of paramere. Penial valve emarginated anterodorsally, ventral margin bidentate apically, teeth separated, unidentate medially; having longitudinal row of setae at apex and subapically on external margin.

Length. Females: 7–12 mm; males: 5.5–10 mm.

Host. Unknown.

Distribution. USA (Arizona); Mexico (Sinaloa, Sonora).

Remarks. Based on genetic distances (100% identical in both ITS1 and ITS2) and shared distribution, the male of *D. saetigera* is *D. polia*, **syn. nov.** Although females of *D. saetigera* had been recorded in northern Mexico, and were included in the treatment of Neotropical *Dasymutilla* (Manley & Pitts 2007), males of *D. polia* were not known from Mexico, and not included in that treatment. Mexican males of *D. saetigera* will key to couplet 49 (Manley & Pitts 2007), where they can be separated from *D. boharti* Manley & Pitts and *D. bonita* by setal coloration of the apical terga; *D. saetigera* males have interspersed black and silver setae on T3–7 (Fig. 4.3K).

Dasymutilla vesta **(Cresson 1865)**

(Figs 4.1D, 4.2O, 4.3L, 4.4I)

Mutilla Vesta Cresson, 1865. Proc. Ent. Soc. Phil. 4: 436. Syntypes, ♀, Colorado Territory (ANSP).

Scolia unicincta Provancher, 1882. Nat. Canad. 13: 6. Lectotype, ♂ (designated by Gahan & Rohwer 1918: 196) (Provancher collection, No. 751) [ULQC].

Mutilla monozona Dalla Torre, 1897. Cat. Hym. 8: 64. N. name for *Scolia unicincta* Provancher, not *Mutilla unicincta* Lucas.

Mutilla sappho Fox, 1899. Trans. Amer. Ent. Soc. 25: 239. Syntypes, ♀, Georgia; Florida; Capron, Lake Worth, March, Mrs. Slosson coll. (ANSP).

Mutilla agenor Fox, 1899. Trans. Amer. Ent. Soc. 25: 245. Syntypes, ♂, Algonquin, Illinois, August 14, 1916 Nason coll.; British Columbia (ANSP).

Mutilla zella Rohwer, 1910. Proc. Ent. Soc. Wash. 12: 50. Holotype, ♀, Wenonah, New Jersey, July 28, F. Haimbach coll. (USNM Type No. 12914).

Pycnomutilla harmoniiformis Rohwer, 1912. Proc. U. S. Nat. Mus. 41: 455. Holotype, ♂, Lyme, Connecticut, July 31, 1910, A. B. Champlain coll. (USNM Type No. 14123).

Dasymutilla errans Rohwer, 1912. Proc. U. S. Nat. Mus. 41: 457. Holotype, ♀, Brownsville, Texas, August 30, 1896, Townsend coll. (USNM Type No. 14127).

Dasymutilla bosquensis Rohwer, 1912. Proc. U. S. Nat. Mus. 41: 457. Holotype, ♀, Waco, Bosque County, Texas (USNM No. 14128).

Dasymutilla ferrugatella Rohwer, 1912. Proc. U. S. Nat. Mus. 41: 458. Holotype, ♀, Pennsylvania, Baker coll. (USNM Type No. 14130).

Dasymutilla coloradella Rohwer, 1912. Proc. U. S. Nat. Mus. 41: 458. Holotype, ♀, Colorado, May 3, 1909, S. A. Rohwer coll. (USNM Type No. 14131).

Dasymutilla coloradella virginica Rohwer, 1912. Proc. U. S. Nat. Mus. 41: 459. Holotype, ♀, Woodstock, Virginia, June 9, 1898, F. C. Pratt coll. (USNM Type No. 14132).

Dasymutilla coloradella kamloopsensis Rohwer, 1912. Proc. U. S. Nat. Mus. 41: 459. Holotype, ♀, Kamloops, British Columbia, Wickham coll. (USNM Type No. 14133).

Dasymutilla texensis Rohwer, 1912. Proc. U. S. Nat. Mus. 41: 460. Holotype, ♀, Texas (USNM Type No. 14137).

Dasymutilla mesillae Rohwer, 1912. Proc. U. S. Nat. Mus. 41: 461. Holotype, ♀, Mesilla, New Mexico, June 17, T.D. Cockerell coll. (USNM Type No. 14138) [USNM].

Dasymutilla carolina Rohwer, 1912. Proc. U. S. Nat. Mus. 41: 462. Holotype, ♀, Columbia, South Carolina, G. F. Atkins (USNM Type No. 14141).

Dasymutilla columbiana Mickel, 1928. U. S. Nat. Mus. Bull. 143: 119. Holotype, ♂, Nicola, British Columbia, August 3, 1923, E. R. Buckell (CNCI Type No. 2817).

Dasymutilla archboldi Schmidt & Mickel: Manley 1983: *J. Georgia Ent. Soc.* 18: 252, ♂ nec ♀; 1984: 228.

Diagnosis. FEMALE. The female of this species (Fig. 4.2O) can be separated from other *Dasymutilla* by the following combination of characters: the integument of the head and mesosoma is pale orange to red, there is a narrow scutellar scale, there are no transverse carinae anterior to the scutellar scale, and the propodeum is punctate or weakly reticulate.

MALE. The male of this species can be separated from other *Dasymutilla* by the following combination of characters: the tegulae are coarsely punctate (Fig. 4.1D), the mesonotum is raised and expanded laterally (Fig. 4.1D), and T3–7 are clothed entirely with black setae (Fig. 4.3L).

Description of male genitalia (Fig. 4.4I). Free length of paramere dorsally curved apically, with ventral brush of long dense setae basally, remainder having scattered short sparse setae; paramere not kinked laterally. Cuspis laterally compressed, ~0.75 × free-length of paramere, having long setae throughout, setae of outer surface longer and denser; outer row of setae anteriorly directed, inner row posteriorly directed. Densely setose basal lobe present. Digitus laterally curved internally, tapering slightly at apex, asetose, ~0.4 × free-length of paramere. Penial valve emarginated anterodorsally, ventral margin bidentate apically, teeth separated, unidentate medially; having longitudinal row of setae at apex and subapically on external margin.

Length. Females: 5.5–13.5 mm; males: 7.5–14.5 mm.

Host. *Bembix cinerea* Handlirsch (Hymenoptera: Crabronidae) (Krombein 1958); *Trypoxylon politum* (Say) (= *albitarse* Fabricius) (Hymenoptera: Crabronidae) (Fattig 1943); and *Nomia melanderi melanderi* Cockerell (Hymenoptera: Halictidae) (Krombein 1958).

Distribution. This is a widespread species, being found from Florida north to Massachusetts west to Arizona and British Columbia, Canada. The species is also recorded from Tamaulipas, Mexico.

Remarks. Our phylogenetic results recover *D. vesta* nested within a clade that includes the entirety of the *D. monticola* species-group (Fig. 4.5, 4.6). As such, we consider *D. vesta* a member of the *D. monticola* species-group, making it the most widespread and commonly collected species in this group. There is significant variation in coloration, most readily noticed in the mesosomal and metasomal integument of males and in the tergal setae of females.

Prior to this publication, no inferences were made concerning the relation of this widespread and polymorphic species to other species-groups. The absence of gray setae on the apical terga of males and absence of propodeal modifications of females prohibited past authors from associating this species with the *D. monticola* species-group. There are similarities between *D. vesta* and selected members of the *D. monticola* species-group, though. In females, there is a well-defined scutellar scale without any transverse carinae situated anterior to the scale; this trait is shared by *D. vesta, D. archboldi, D. arenneronea, D. macilenta,* and *D. radkei*. In males, the mesoscutum is posterolaterally swollen and the tegulae are densely punctate, both traits shared by *D. vesta, D. birkmani,* and *D. monticola*.

Additional results

When Mickel (1938) described *D. fasciventris* (Fig. 4.3G), he refrained from placing it in a species-group. The presence of silver setae on the apical terga was only known in members of the *D. creon*, *D. monticola* and *D. obscura* species-groups prior to discovery of this species. Our phylogenetic results show an initially surprising close affiliation with *D. quadriguttata* and *D. campanula* from the *D. quadriguttata* species-group (Fig. 4.5). This species does, however, bear morphological similarity to *D.*

quadriguttata in the clypeus, axillae, and genitalia. Similarities in female morphology would also be expected, but within the distribution of *D. fasciventris,* none of the known *Dasymutilla* females have linear glabrous tubercles on the posterior head margin, as seen in the known *D. quadriguttata* species-group females. The homoplastic nature of head tubercles in *Dasymutilla,* however, suggests that the unknown female of *D. fasciventris* may lack this trait. We transfer *D. fasciventris* into the *D. quadriguttata* species-group, expanding the range of this species-group to include Arizona and western Mexico.

Two Neotropical species, *D. chalcocephala* (Fig. 4.2G) and *D. spilota* (Figs 4.1B, 4.2N), were included in the analysis because of morphological similarity to members of the *D. monticola* species-group. Detailed morphological analysis and phylogenetic results clearly show, however, that these two taxa are not closely related to *D. monticola,* and are potentially not congeneric with the Nearctic *Dasymutilla* (Fig. 4.5). Although recovered as sisters, *D. chalcocephala* and *D. spilota* are dissimilar morphologically (mandible straight and scutellar scale distinct in *D. spilota*; mandible broadly curving and scutellar scale absent in *D. chalcocephala*). Additionally, the sister relationship shared by these taxa is weakly supported (posterior probability = 0.80) and may be caused by long branch attraction. *Dasymutilla spilota* is apparently a close relative of *D. xalisco* (Blake) and *D. twegeni* Manley & Pitts based on a previously overlooked metasomal modification; the anterior portion of T2 is swollen and extends over the posterior margin of T1 (Fig. 4.1B). Relationships between *D. chalcocephala* and other Neotropical species are not apparent. Generic and species-group treatment of these and other Neotropical *Dasymutilla* cannot proceed without additional sampling and sex associations.

Discussion

Molecular data have proven to be invaluable in the advancement of mutillid taxonomy. In many instances, ITS1 and ITS2 have provided concrete answers to taxonomic questions. For example, males and females have been associated based on overlapping distributions coupled with identical DNA sequences, as in *D. heliophila* and *D. thetis* (Pilgrim *et al*. 2008). Identical ITS sequences in taxa that differed only in coloration have also supported synonymy, as in *D. foxi* (Pilgrim *et al*. 2008). This study, however, espouses the necessity of additional information in making taxonomic changes. In some instances (*e.g*., *D. canella* and *D. macilenta*), nearly identical genetic distances were recovered from comparisons between morphologically discrete species, while in other cases (*e.g.*, *D. birkmani*) relatively high genetic distances were recovered in intraspecific comparisons. Careful examination of morphology and distribution, specifically within large series, is often more valuable than genetic distances.

Extreme over-splitting has often been observed in *Dasymutilla*, necessitating 10 names to be synonymized under *D. quadriguttata* (Pilgrim *et al*. 2009) and 14 names to be synonymized under *D. bioculata* (Williams *et al.* 2011). In the female of *D. macilenta,* we see the first example of molecular data bringing light to inaccurate lumping in *Dasymutilla*. *Dasymutilla arenneronea*was previously considered a synonym of *D. macilenta* (under the synonymous name *D. chattahoochei*; Mickel 1928). Taxonomic revision is desperately needed in almost all of the species-groups; it will be important, however, to avoid a predisposition for synonymy.

With the addition of *D. vesta* and *D. archboldi*, the *D. monticola* species-group is monophyletic (Fig. 4.5). One particular clade includes six species that all exhibit small body size (*D. archboldi, D. arenneronea, D. birkmani, D. canella, D. macilenta,* & *D. monticola*). This clade forms a sister relationship with the widespread and size variable *D. vesta*. Although few host associations are known for *D. vesta*, its tremendous size polymorphism and broad geographic distribution strongly suggest a wide range of host species. We hypothesize that these six species with small body size evolved from an ancestor with a wide range of hosts, and subsequently specialized on a range of host species with small body size. We hope this taxonomic research will facilitate future studies on small body size evolution that would support or refute the aforementioned hypothesis.

Mountain-building events in the western Nearctic Region have been implicated as a cause of speciation in other mutillid genera (Pitts *et al.* 2010b, Wilson & Pitts 2010). Of the six species in the clade sister to *D. vesta*, four are restricted to the eastern USA. All of the individuals found in the mountainous west are members of a single species, *D. monticola*. It is noteworthy that three morphologically and molecularly distinct species of the *monticola* lineage occur in Florida. In Florida and parts of adjacent states there appears to have been a series of minor radiations in arthropod lineages associated with sandy uplands. Such radiations occur in the genera *Dorymyrmex* Mayr (Formicidae) (Trager 1988), *Melanoplus* Stål (Acrididae) (Hubbell 1932), *Phyllophaga* Harris (Scarabaeidae) (Woodruff & Beck 1989), *Geopsammodius* Gordon & Pittino (Scarabaeidae) (Skelley 2006), and possibly *Photomorphus* Viereck (Mutillidae) (MD, pers. obs.). These radiations might be associated with periodic expansions and contractions of xeric upland habitat during the Pleistocene (Webb 1990).

The conclusion that presence of tubercles on the head is not a homologous character in *Dasymutilla* (Pilgrim *et al.* 2009) was supported by this study. There are four separate lineages with head tubercles (Fig. 4.5) represented in the phylogeny and in one species (*D. birkmani*) the tubercles are so variable in shape that some individuals were treated as lacking tubercles (Mickel 1928). While this character is valuable in *Dasymutilla*, it cannot be treated as a discrete presence/absence character. Rather, the specific shape of these tubercles must be analyzed and intermediate forms must be accounted for.

There are still over 75 *Dasymutilla* species that have not been assigned to species-groups, including many that appear closely related to the insects studied here. Although this research treats only the Nearctic members of the *D. monticola* species-group, newly obtained information concerning species-group characters, species limits, and sex associations will provide valuable insight for studying putative members of the *D. monticola* species-group from the Neotropical Region. Phylogenetic studies similar to this one will be invaluable for research on the remaining *Dasymutilla* species-groups.

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11(1879), 2–13, 33–43, 65–76, 119–125, 129–143, 109–122, 141–150, 173–185, 205–233, 248–266, 269–281; 12(1880), 4–22, 33–48, 65–81, 97–102, 130–147, 161–180; 12(1881), 193–207, 225–241, 257–269, 289–304, 321–333, 352–362; 13(1882), 4–15, 33–51, 65–81, 97–110, 129–144, 161–175, 193–209, 225–242, 257–269.

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CHAPTER 5

PHYLOGENY AND TAXONOMY INFORM DUAL SEX-LIMITED MIMICRY HYPOTHESES IN THISTLEDOWN VELVET ANTS (HYMENOPTERA: MUTILLIDAE)[4]

Introduction

Dasymutilla gloriosa (de Saussure, 1868) is one of the most conspicuous North American velvet ant species because of its remarkable female coloration (Fig. 5.1A). The species has been given the common name Thistledown Velvet Ant, because females are entirely clothed with long white setae that give them an almost seed-like appearance. Specifically, females of *D. gloriosa* closely resemble the fruit of Creosote Bush, *Larrea tridentata* (Sesse and Moc. *ex* DC) Coville, with which they share an overlapping geographic range (Fig. 5.2). Some authors have suggested these species use a form of crypsis by camouflaging themselves as Creosote fruit (Ferguson 1967; Williams & Manley 2006). Two additional *Dasymutilla* Ashmead, 1899 species, and *D. pseudopappus* (Cockerell, 1895) and *D. thetis* (Blake, 1886), display identical female coloration to *D. gloriosa* (Figs. 5.1: C, E). These three species are collectively referred to as thistledown velvet ants throughout this study.

Males for two of these species, *D. gloriosa* and *D. thetis*, have been recognized (Mickel 1936; Pilgrim et al. 2008). Unlike their conspecific females, the males of these species have the legs and much of the venter clothed with black setae (Figs. 5.1: B, D). Males of *D. gloriosa* are dramatically different from females in coloration, because the

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[4] This chapter is formatted for submission to *Invertebrate Systematics*.

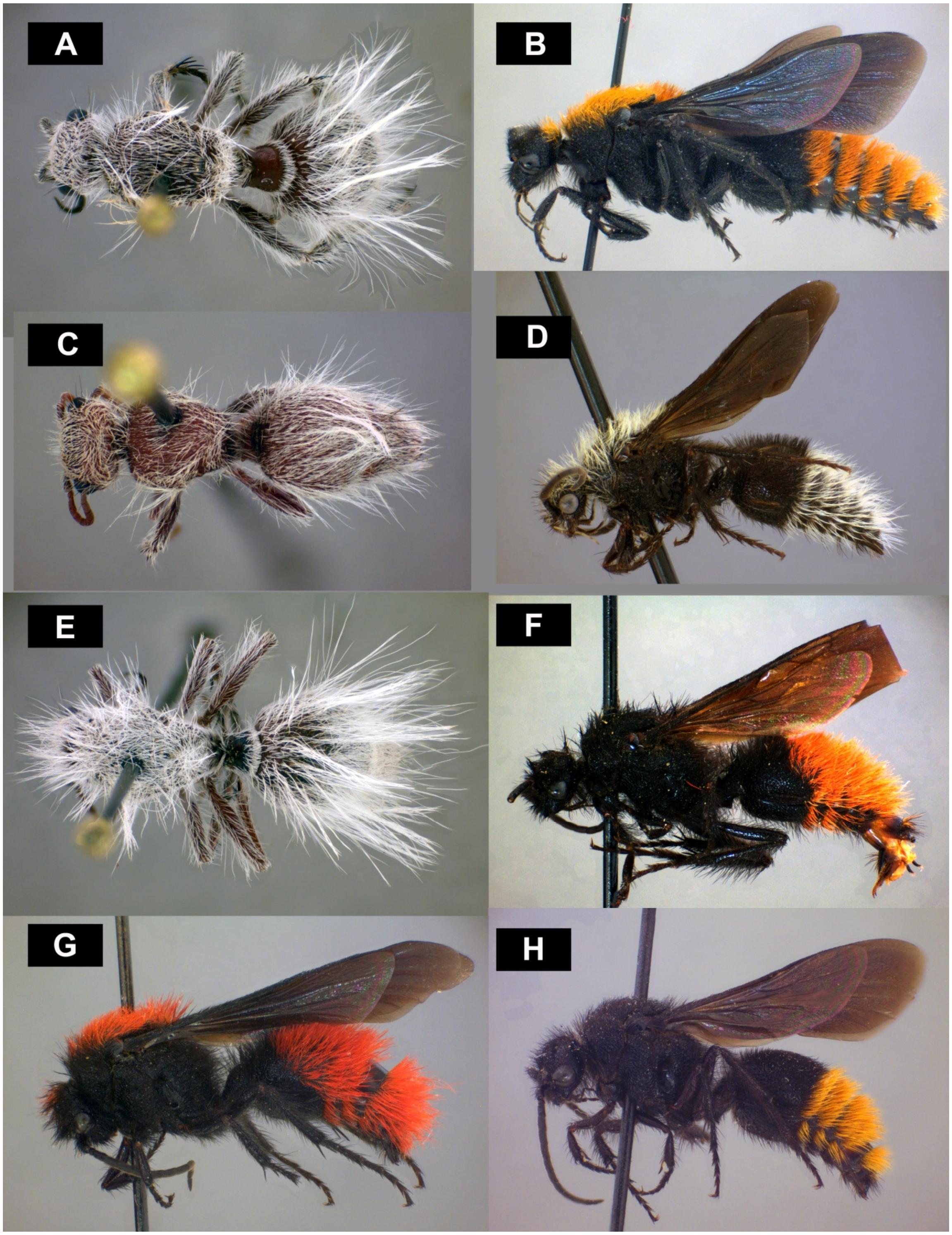

Fig. 5.1. Habitus. (A) *D. gloriosa,* female; (B) *D. gloriosa,* male; (C) *D. thetis,* female; (D) *D. thetis,* male; (E) *D. pseudopappus,* female; (F) *D. phaon phaon,* male; (G) *D. phaon fimbrialis,* male; (H) *D. chisos,* male.

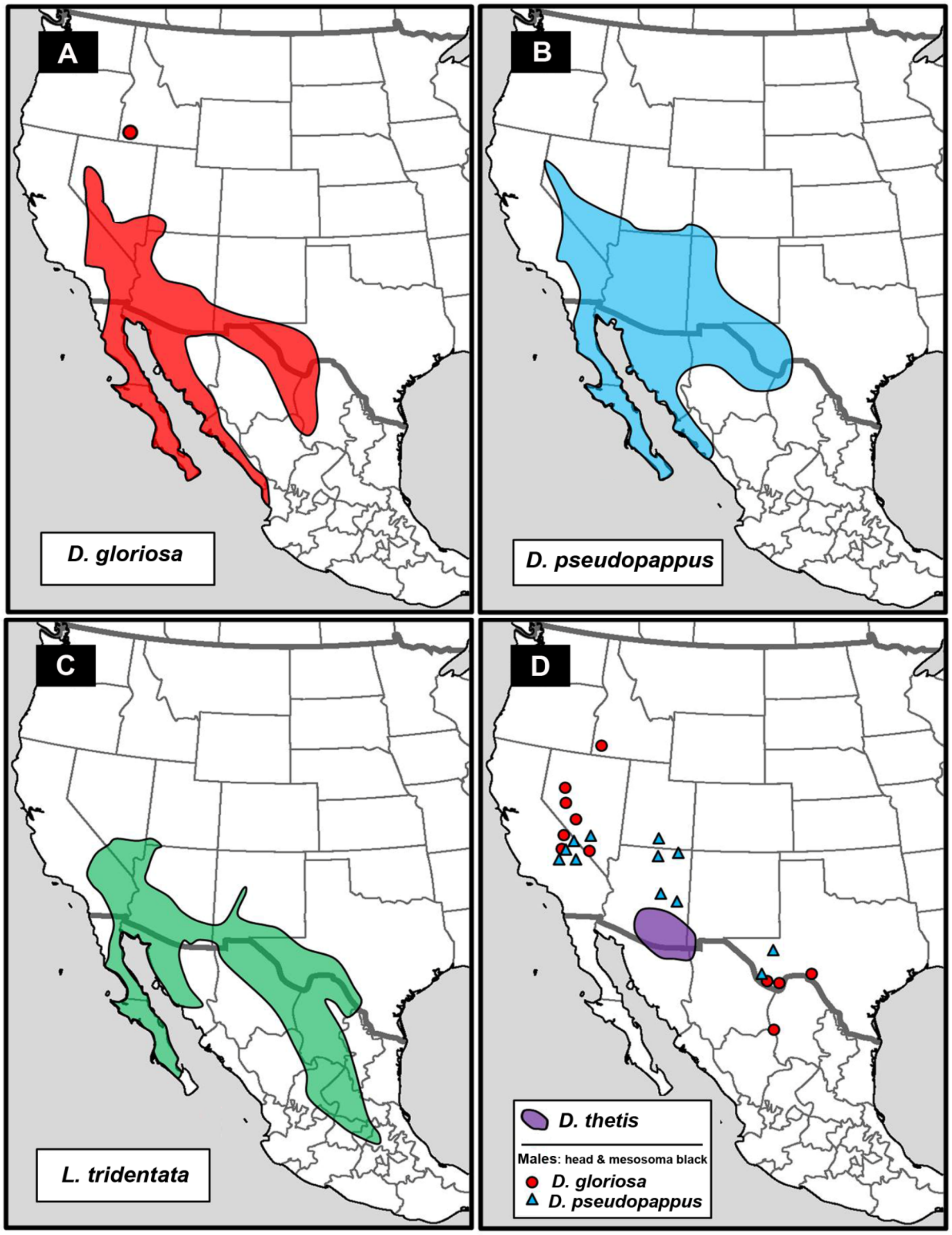

Fig. 5.2: Distribution maps based on specimens examined in this study. (A) *D. gloriosa*, including synonymous forms; (B) *D. pseudopappus*, including synonymous forms; (C) *Larrea tridentata*, recreated based on USGS distribution map; (D) D. thetis, both sexes; "Males: Head and mesosoma black" includes all localities from the literature and this study for males with the head and mesosoma clothed entirely with black setae, for *D. gloriosa* this includes specimens formerly treated as *D. chisos* and for *D. pseudopappus* this includes specimens treated as *D. phaon phaon*.

dorsal setae are typically orange or red. Because the sexes display different aposematic signals, we consider this an example of dual sex-limited mimicry. Dual sex-limited mimicry was first described in Hymenoptera by Evans (1968, 1969) based on studies of Neotropical Pompilidae. Evans found that pompilid females from different genera formed a Müllerian mimicry complex, while their respective males were Batesian mimics of species from other wasp families. Evans hypothesized that dual sex-limited mimicry arose from selection pressure on male coloration.

In this paper, we use phylogenetic methods to examine the phenomenon of dual sex-linked mimicry in Thistledown Velvet Ants. We first determine whether these species obtained their female color pattern through shared ancestry or convergence. Based on distribution and color patterns, we identify the mimicry system and potential models for each sex. Additionally, we examine whether dual sex-limited mimicry was driven by selection pressure on males or females. Based on phylogenetic and morphological data we determine the species-limits of *D. gloriosa*, *D. pseudopappus,* and *D. thetis*; necessary changes to taxonomy are made and new diagnostic characters are proposed.

Materials and methods

Material studied

Three Thistledown Velvet Ant species, *D. gloriosa* (♀♂)*, D. pseudopappus* (♀), and *D. thetis* (♂♀), were examined molecularly and morphologically. In a study of female color patterns, these three species were recovered within a single clade (Dissertation Chapter 3, Fig. 3.4, Clade Nine). All previously sampled members of that clade were included in this analysis. Five members of Mickel's *D. subhyalina* species-group have been synonymized with members of the ingroup clade (Mickel 1936; Manley 1999; Manley & Yanega 2005; Manley & Pitts 2007; Pilgrim et al. 2008). Therefore, the following four taxa from the *D. subhyalina* species-group were also studied: *D. chisos* Mickel, 1928 (♂); *D. glycera* Mickel, 1928 (♂); *D. phaon phaon* (Fox, 1899) (♂); and *D. phaon fimbrialis* Mickel, 1928 (♂). For outgroups, we included members of six other clades from Chapter Three of this dissertation, the Caribbean species *D. militaris* (Smith, 1855) (♂♀), one species from each of the genera *Sphaeropthalma* Blake, 1886 and *Traumatomutilla* André, 1901. The sex symbols listed after each species indicate whether the mentioned species is known from males only, females only, or both sexes. An overview of species examined in the molecular study, including species-group placement and clade placement based on Chapter Three of this dissertation, is provided in Table 5.1.

Specimens are housed in the following collections: Frank M. Hasbrouch Insect Collection, Department of Zoology, Arizona State University, Tempe, Arizona, USA (ASUT); Department of Entomology, California Academy of Sciences, San Francisco, California, USA (CASC); Essig Museum of Entomology, Department of Entomological Sciences, University of California, Berkeley, California, USA (CISC); Estacion del Biologia "Chamela", Universidad Nacional Autonoma de Mexico, San Patricio, Jalisco, Mexico (EBCC); Department of Biology Insect Collection, Utah State University, Logan, Utah, USA (EMUS); Insect Collection, Los Angeles County Museum of Natural History, Los Angeles, California, USA (LACM); Nevada State Department of Agriculture, Reno, Nevada, USA (NVDA); Division of Entomology, Peabody Museum of Natural History, Yale University, New Haven, Connecticut, USA (PMNH); The Bohart Museum of Entomology, University of California, Davis, California, USA (UCDC).

Table 5.1. Voucher specimens used in molecular study.

Species	Specimen voucher	Genbank ITS1	Genbank ITS2	Sex	Locality
D. arenivaga	JP460	JQ945770	JX002775	♀	USA: AZ: Maricopa Co.
D. atricauda	JP487	JX002849	JX002833	♀	USA: CA: Inyo Co.
D. bioculata	JP1189	HQ317263	HQ317277	♂	USA: NE: Morrill Co.
D. californica	JP164	EU627568	EU627568	♀	USA: CA: San Diego Co.
D. calorata	JP438	JX002846	JX002830	♀	USA: TX: Randall Co.
D. chisos	Dsp05	JX002889	JX002896	♂	USA: TX: Presidio Co.
D. chisos	JP1780	JX002890	JX002897	♂	USA: NV: Churchill Co.
D. coccineohirta	JP99	JQ945779	JX002777	♀	USA: CA: Riverside Co.
D. creon	JP670	JQ945781	JX002799	♂	USA: TX: Harris Co.
D. gloriosa	JP241	HQ317244	HQ317288	♀	USA: AZ: Cochise Co.
D. gloriosa	JP1858	JX002891	JX002898	♂	USA: AZ: Maricopa Co.
D. gloriosa	JP1919	JX002892	JX002899	♀	USA: ID: Owyhee Co.
D. gloriosa	JP1923	JX002893	JX002900	♀	MEXICO: Baja California Sur
D. gorgon	JP383	HQ317247	HQ317289	♀	USA: TX: La Salle Co.
D. glycera	Dper01	JX002894	JX002901	♂	USA: AZ: Cochise Co.
D. klugii	JP437	JQ945790	JX002782	♂	USA: TX: La Salle Co.
D. leda	JP384	JX002848	JX002832	♀	USA: TX: Randall Co.
D. magna	JP599	JQ945792	JX002781	♀	USA: AZ: Santa Rita Co.
D. magnifica	JP259	DQ408497	DQ408497	♀	USA: AZ: Cochise Co.
D. militaris	JP888	JQ945793	JX002819	♀	REP. DOMINICANA: Punta Cana
D. nigripes	JP463	EU627570	EU627570	♀	USA:TX: Randall Co.
D. nocturna	JP266	DQ408502	DQ408502	♀	USA: CA: Imperial Co.
D. nogalensis	Dpha01	JX002847	JX002831	♂	USA: AZ: Cochise Co.
D. nupera	JP449	JX002850	JX002834	♀	USA: TX: LaSalle Co.
D. occidentalis	MoccF	HQ317246	HQ317268	♀	USA: SC: Florence Co.
D. pallene	JP671	JQ945780	JX002780	♀	MEXICO: Sonora
D. phaon	JP675	JX002895	JX002902	♂	USA: CA: Kern Co.
D. phaon fimbrialis	JP1246	JQ945796	JX002788	♂	USA: NV: Clark Co.
D. pseudopappus	JP1300	JX002845	JX002829	♀	USA: CA: Riverside Co.
D. quadriguttata	JP159	HQ317245	HQ317267	♂	USA: AR: Pulaski Co.
D. sackenii	JP602	JQ945799	JX002783	♂	USA: CA: Siskiyou Co.
D. saetigera	JP1208	JQ945797	JX002790	♂	MEXICO: Sonora
D. satanas	JP603	JQ945800	JX002778	♀	USA: UT: Washington Co.
D. scaevola	JP1196	JQ945802	JX002815	♀	USA: FL: Alachua Co.
D. scitula	JP924	JQ945803	JX002789	♀	USA: UT: Garfield Co.
D. stevensi	JP461	JQ945807	JX002779	♂	USA: TX: Presidio Co.
D. texanella	JP381	JX002853	JX002776	♀	USA: TX: LaSalle Co.
D. thetis	KW04	EF433453	EF433453	♂	USA: AZ: Cochise Co.
Traumatomutilla	JP1085	JQ945818	JX002765	♀	BRAZIL: Espiritu Santo

Morphological methods

Pinned specimens of all species referred to in this study have been examined to compare morphological features. All specimens were examined with a stereo microscope. Genitalia were dissected using minuten pins and placed in genitalia capsules with glycerin. Specimens used in the molecular study were deposited in the Department of Biology Insect Collection, Utah State University, Logan, UT (EMUS); allowing morphological examination of these specimens.

We use the acronyms: T1, T2, etc. to refer to the metasomal terga, and the abbreviations: S1, S2, etc. to refer to the metasomal sterna. The cuspis length relative to the free paramere length is used frequently to quantify differences in genitalic structure. The cuspis measurements are taken from the apical margin of the basal paramere lobe to the cuspis apex; the free paramere length measurements are taken from the apical margin of the basal paramere lobe to the apex of the free paramere length.

Molecular methods

DNA was extracted from 39 individuals representing 35 species from the genera *Dasymutilla*, *Sphaeropthalma*, and *Traumatomutilla* for use in the phylogenetic reconstruction (Table 5.1). DNA extractions were performed using the entire individual after puncturing the side of the thorax or removing it from its pin mounting. This left the remainder of the external anatomy available for morphological study. Extractions were done with either the Roche High Pure PCR Template Purification Kit or the QIAgen DNeasy Tissue Kit following each manufacturer’s protocol, respectively. PCR conditions, primers, and DNA sequencing protocols were identical to those used in Pilgrim and Pitts (2006). Gel electrophoresis of each gene yielded a single band for each individual wasp and the resulting DNA was sequenced cleanly suggesting no gene heterogeneity as seen in some other organisms (e.g., Harris and Crandall 2000; Parkin and Butlin 2004; Bower *et al.* 2008).

Alignments of the ITS1 and ITS2 data sets were made using Geneious and ClustalW (Drummond *et al.* 2009; Thompson *et al.* 1994). The data sets for ITS1 and ITS2 were analyzed with MrModeltest v2.2 (Nylander 2004) to find appropriate models of molecular evolution. The data sets of the two genes were combined and subjected to Bayesian analysis with all parameters unlinked across loci. All Bayesian analyses were run with MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) and included four independent runs with three heated chains and one cold chain in each run. The MCMC chains were set for 5,000,000 generations and sampled every 100 generations. A majority consensus tree was obtained from the tree data after removing the burnin period. All sequences have been deposited in GenBank (see table 5.1).

Molecular clock approaches to calculate divergence dates have not been used in Mutillidae because of rate heterogeneity among lineages (Pitts et al. 2009). Instead, we determined divergence dates using penalized likelihood (PL) with r8s 1.71 (Sanderson, 2002), and a Bayesian MCMC averaging approach to rate smoothing using the program BEAST v1.4.8 (Drummond and Rambaut 2007) with a relaxed clock. Very few mutillid fossils are known, but there are two fossil species within the genus *Dasymutilla*, *D. dominicana* Manley & Poinar, 1991 and *D. albofasciata* Manley & Poinar, 1999. Williams et al. (2011) recognize these fossils as relatives of the extant Caribbean species *D. militaris*. These fossils, represented by the node that represents the most recent ancestor of *D. militaris* in the phylogeny, are used to calibrate our dating analyses.

Because the age of Dominican Amber is somewhat controversial, we ran our analyses using two different ages, 15Ma and 20Ma, the range found by Iturralde-Vincent and MacPhee (1996). A maximum age of 22 Ma was used for the root based on a dating analysis of the common ancestor of *Dasymutilla* and *Traumatomutilla* in a biogeographic study of *Dasymutilla* and their relatives.

Results and Discussion

Phylogenetic analysis

Most relationships in the phylogeny are highly supported (p.p. > 0.95). The ingroup for this analysis (Clade Nine in Chapter 3) was recovered as monophyletic with high support (Fig. 5.3). Within the ingroup, thistledown velvet ants were recovered in separate clades. *Dasymutilla thetis* was recovered as the sister to *D. coccineohirta* (Blake, 1871).

Four included specimens of *D. gloriosa* were recovered in a clade with two specimens of *D. chisos* (Fig. 5.3). Additionally, the *D. chisos* specimen from Nevada was recovered as the sister to a *D. gloriosa* specimen from Idaho; in fact, these specimens have identical ITS1 and ITS2 sequences. The specimen from southeastern Arizona was recovered as the sister to all the remaining *D. gloriosa* and *D. chisos* specimens.

These relationships firmly show that *D. chisos* is conspecific with *D. gloriosa*; specifically, males of *D. gloriosa* from the extreme eastern and north western ranges have the head and mesosoma clothed with black setae. The shared ancestor of all the *D. gloriosa* specimens was dated to 1.2 Ma (Fig. 5.4). *Dasymutilla gloriosa* was recovered as the sister to a clade that includes *D. arenivaga* Mickel, 1928 and *D. nocturna* Mickel, 1928. The hypothetical ancestor of these taxa was dated to 5.4 Ma (Fig. 5.4).

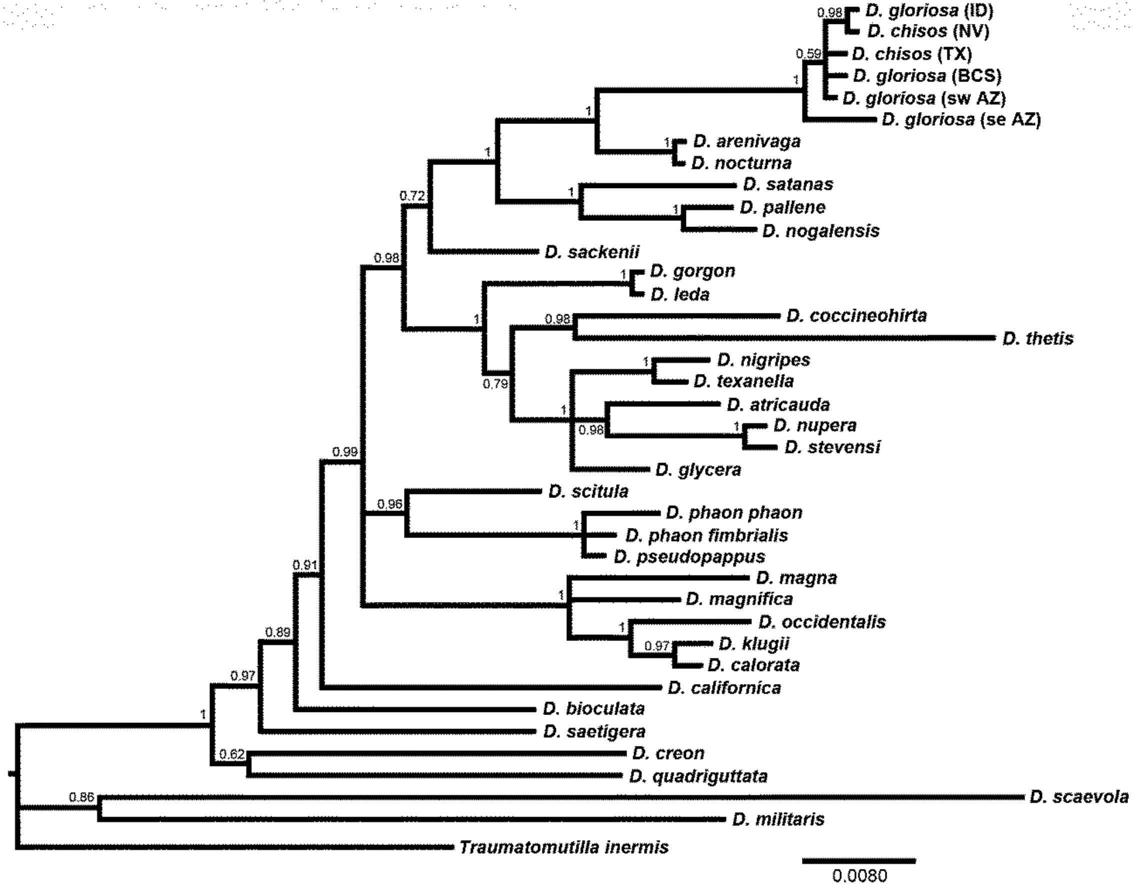

Fig. 5.3. 50% majority rules consensus Bayesian phylogenetic tree from mrBayes. Posterior probabilities are given for each node. Scale in substitutions per site.

Dasymutilla pseudopappus was recovered in a polytomy with *D. phaon* and *D. phaon fimbrialis*; these three taxa are separated by short branches (Fig. 5.3). Phylogenetic results suggest these are conspecific forms. Like *D. gloriosa*, males of *D. pseudopappus* vary considerably in setal coloration. The hypothetical ancestor of this clade was dated to 2.1 Ma (Fig. 5.4). *Dasymutilla scitula* Mickel, 1928 was recovered as the sister to *D. pseudopappus*; the node connecting these taxa was dated to 10.0 Ma (Fig. 5.4). Although molecular data was unavailable from *D. flammifera* Mickel, 1928, morphological

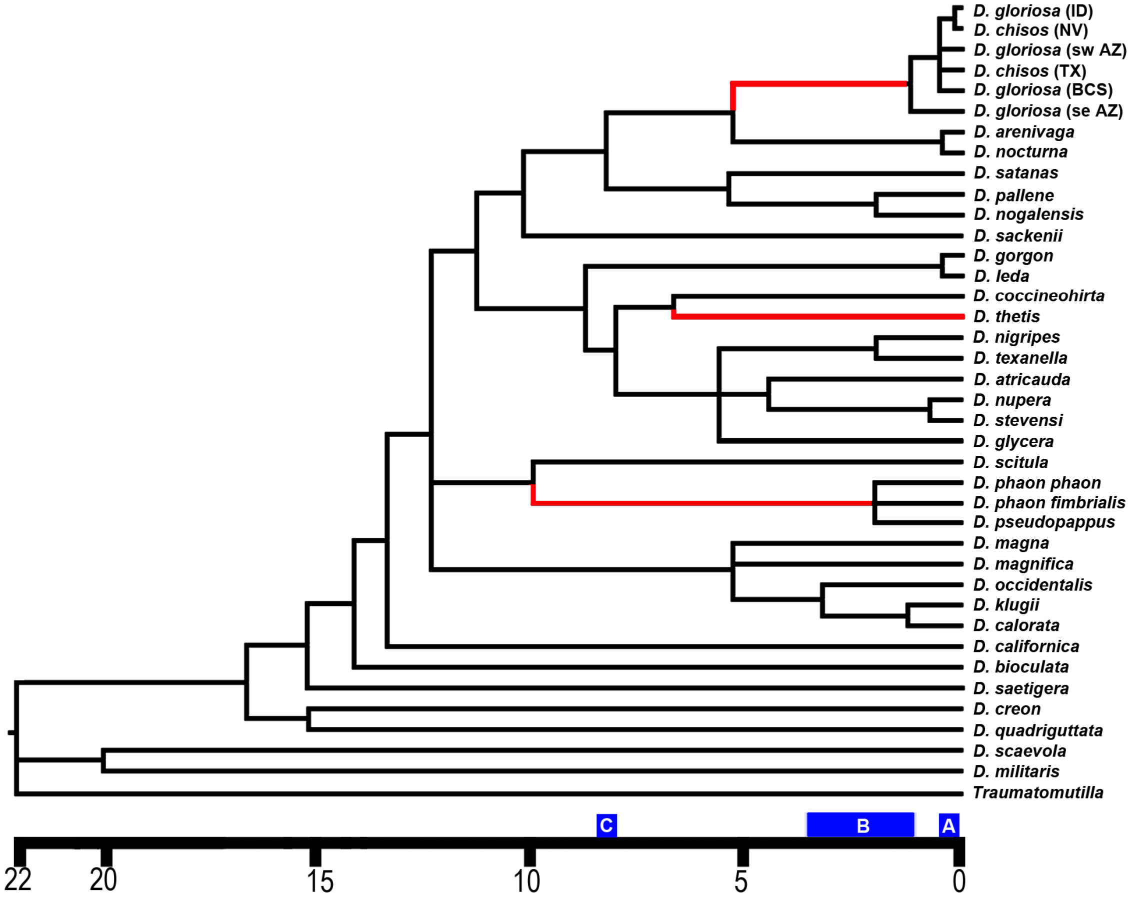

Fig. 5.4. Chronogram from r8s, constructed using penalized likelihood relaxed molecular clock with tree topology from Fig. 5.3. Evolution of white female coloration occurred along red highlighted branches for each thistledown velvet ant. Letters on timescale represent the following: (A) oldest known fossil of *L. tridentata*, 18.7 Ka (Van Devender, 1990); (B) range for Creosote introduction to North America based on pairwise divergences, 0.42–3.36 Ma (Laport et al., unpublished); (C) oldest suggested date for Creosote introduction, 8.4 Ma (Lia et al. 2001).

characters clearly support a sister relationship with *D. pseudopappus* (see Remarks in *D. pseudopappus* taxonomy section).

Dasymutilla glycera, a male from the *D. subhyalina* species-group, was recovered in a polytomy with members of Mickel's *D. sparsa* species-group (Fig. 5.3). *Dasymutilla stevensi* Mickel, 1928 is the only female of the *D. sparsa* species-group that occurs in southeastern Arizona and has similar coloration to *D. glycera*. This could mean that *D. stevensi* actually includes multiple valid species or that the members of the *D. sparsa* species-group are all conspecific forms of a widespread, variable species. Detailed comparison of morphological and molecular features is necessary to determine the species-limits in this group. This clade is closely related to *D. thetis* and further discussed in the Remarks section below.

Mimicry complexes

The stark white coloration displayed by thistledown velvet ant females has evolved three separate times (Fig. 5.3). These insects have similar coloration to the fruit of Creosote bush, *Larrea tridentata*, and two of the thistledown velvet ants, *D. gloriosa, D. pseudopappus,* are found throughout the range of Creosote (Fig. 5.2). *Larrea tridentata* is the sole North American species in a South American genus and was introduced to North America by migratory birds (Lia et al. 2001). There is some uncertainty about the date of this introduction. Molecular data suggest that Creosote entered North America 1.2 Ma (Cortes & Hunziker 1997) or 4.2-8.4 Ma (Lia et al. 2001), but the oldest known fossil of Creosote is only 18700 years old (Van Devender 1990). Recent unpublished dating analyses found dates that varied based on which sequences were used, but consistently dated Creosote's entrance to North America during the Late Pliocene to middle Pleistocene (Laport et al. 2012, in press). Our dating analyses suggest that *D. gloriosa* and *D. pseudopappus* diverged from their closest relatives 5.4 and 10.0 Ma, respectively; intraspecific sequence divergence is dated to 1.2 and 2.1 Ma, respectively (Fig. 5.4). Creosote was almost certainly present in North America when thistledown velvet ants speciated, but this does not conclusively support the camouflage hypothesis. Each of the thistledown velvet ants have sister species from California, suggesting that they evolved in the western warm deserts (Mojave and Sonoran). *Larrea tridentata*, however, theoretically originated in southern Mexico and colonized the remaining warm deserts during aridification events in the Pleistocene (Duran et al. 2005). The absence of Creosote from the fossil record until 18700 years ago further suggests that it was not a dominant warm desert plant when thistledown velvet ants speciated. Although present on the North American continent concurrently, Creosote and thistledown velvet ant ancestors may not have actually occurred sympatrically until after the insect’s had developed their unique setal pattern. We cannot conclusively support or refute a camouflage hypothesis based on phylogenetic results alone. Participation in Müllerian mimicry complexes or thermoregulatory benefits could be equally viable hypotheses for the stark white color pattern exhibited by females of *D. gloriosa, D. pseudopappus*, and *D. thetis*.

The males of both *D. gloriosa* and *D. pseudopappus* vary in setal coloration (Fig. 5.1). In both species, color pattern is associated with distribution (Fig. 5.2D). Interestingly, each male color form occurs sympatrically with multiple species of similarly colored *Dasymutilla* females that were designated as Mullerian mimicry complexes. In the eastern extent of their range, males of *D. gloriosa* (Fig. 5.1H) and *D. pseudopappus* (Fig. 5.1F) resemble members of the Texan mimicry ring, such as *D. klugii* (Gray, 1832). Throughout the warm deserts, excluding the eastern Chihuahuan, males (Figs. 5.1: B, G) resemble females from the Western mimicry ring, like *D. arenivaga*. Outside of the warm deserts (Fig. 5.2D), males of *D. pseudopappus* and *D. gloriosa* have similar coloration to specimens from Texas; members of the Texan mimicry ring are rare, but present, in these regions. With their white dorsal setae, males of *D. thetis* are all similar in appearance to members of the Desert mimicry ring (Fig. 5.1D). We hypothesize that thistledown velvet ant males are Batesian mimics that use females of other *Dasymutilla* species as unpalatable models.

Male Thistledown Velvet Ants are more similar in appearance to both sexes of their relatives than the females are. *Dasymutilla gloriosa* males from the warm deserts (Fig. 5.1B) are concolorous with both the males and females of *D. arenivaga*; most males of *D. pseudopappus* (Fig. 5.1G) and both sexes of *D. flammifera* have dense scarlet dorsal setae and black setae covering the legs and venter; males of *D. thetis* (Fig. 5.1D) are concolorous with females of *D. clytemnestra* (Fox, 1899), which is structurally identical and probably a sub-arid variant of *D. coccineohirta* (see Mickel 1928). Nearly all species from the ingroup have the legs and venter more or less clothed with black setae that contrast with lighter colored dorsal setae in both sexes; female thistledown velvet ants are the only members of the ingroup that are uniformly clothed with concolorous setae. Male coloration appears to be of an ancestral type, while females display derived coloration. This suggests that dual sex-limited mimicry in Thistledown Velvet Ants arose from selection pressure on female coloration. Increased exposure to both predators and climatic variables faced by wingless females could explain the unbalanced selection pressure observed in these wasps.

Taxonomy

***Dasymutilla gloriosa* (de Saussure)**

(Figs. 5.1: A, B, H; 5.2: A, D, 5.5: B–F)

Mutilla gloriosa de Saussure, 1868 (1867): 359; Lucas, 1867: 92, 93; Dalla Torre, 1897: 44; Melander, 1903: 296, Figs. 5, 45.

Mutilla tecta Cresson, 1875: 119.

Sphaerophthalma (!) tecta Blake, 1886: 237.

Sphaeropthalma (!) gloriosa Cameron, 1894: 359; Fox, 1895: 261.

Ephuta (Ephuta) gloriosa André, 1903: 60.

Dasymutilla chisos Mickel, 1928: 55, 284, Fig. 32; Mickel 1936, 38. **new synonym**.

Dasymutilla reperticia Mickel, 1928: 56, 287, Fig. 34.

Dasymutilla gloriosa Mickel, 1928: 46, 78, 242, 243, 244; Mickel, 1936: 21, 39, 56, 57; Williams & Manley, 2006: 103, 104; Manley & Pitts, 2007: 6, 11, 21, 54, 55, Pl. C4.

Material examined

***Dasymutilla chisos*. U.S.A.**: <u>California</u>: *Inyo Co.*: Deep Springs, 1♂, leg. J.W. MacSwain, 11.vii.1953 (EMUS); Antelope Springs, 8 mi. [12.8 km] SW Deep Springs: 3♂, leg. J.W. MacSwain, 17.vii.1953 (EMUS); 1♂, leg. J. Powell, 1.vii.1961 (EMUS); 1♂, 29.vi.1968 (EMUS); 1♂, leg. W.A. Foster, 10.vii.1962 (EMUS); 1♂, leg. G.H. Nelson, 16.vii.1983 (EMUS); <u>Idaho</u>: *Owyhee Co.*, Bruneau Dunes State Park, 1♂, leg. K.A. Williams, 6.viii.2011 (EMUS); <u>Nevada</u>: *Churchill Co.*: Blow Sand Mountains: 1♂, leg. R.C. Bechtel, 28.vi.1979 (NVDA); 1♂, leg. R.C. Bechtel, 2.vii.1979 (NVDA); Mud Springs, Dixie Valley, 1♂, leg. R.C. Bechtel, 29.vi.1970 (NVDA); *Esmerelda Co.*: 5 mi. N Dyer, 1♂, leg. R.C. Bechtel, 14.viii.1958 (NVDA); 7 mi.[11.2 km] N Dyer, leg. R.C. Bechtel, 12.vii.1958 (NVDA); *Lincoln Co.*, Caliente, 1♂, leg. R.C. Bechtel, 21.vii.1958

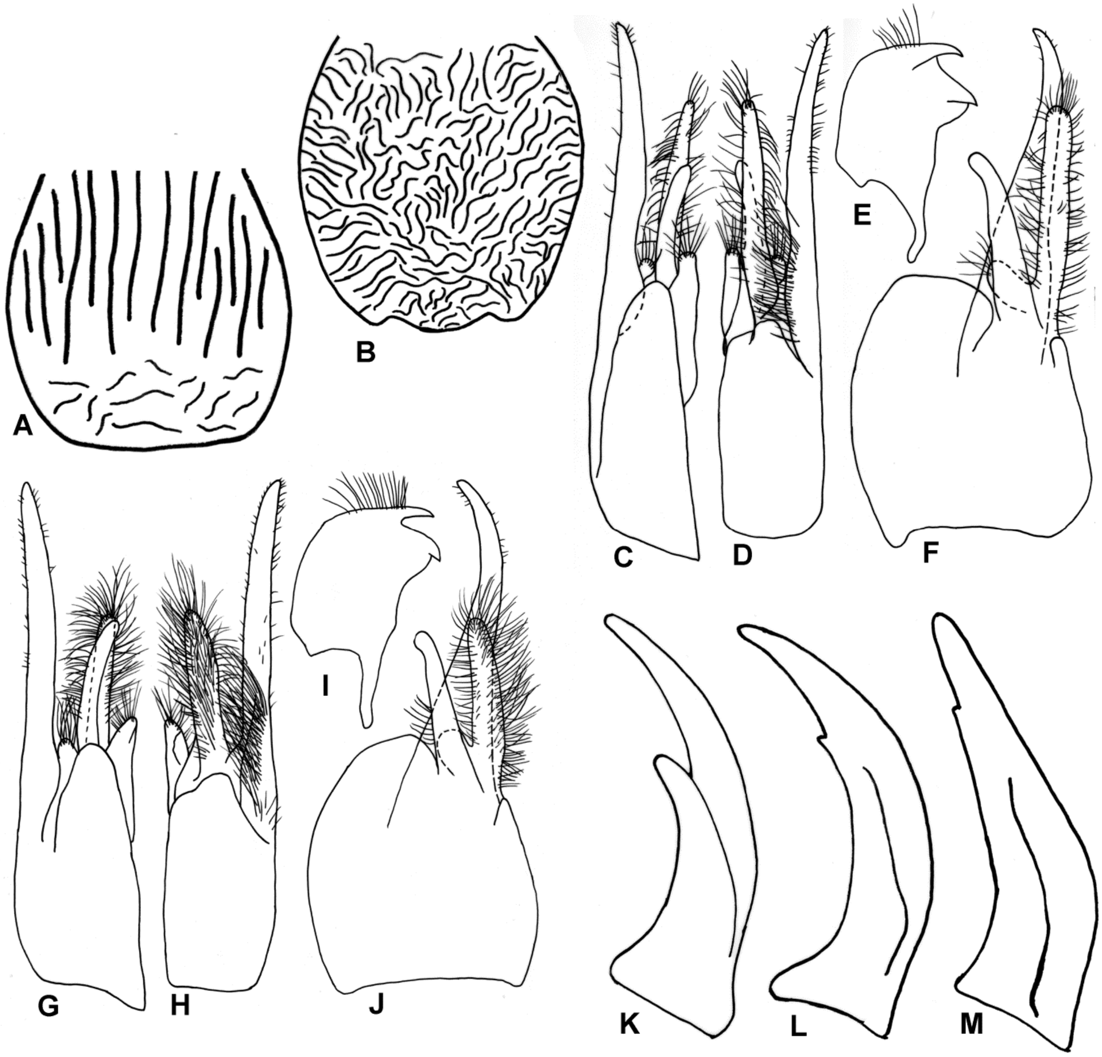

Fig. 5.5. (A) *D. pseudopappus*, pygidium, female; (B) *D. gloriosa*, pygidium, female; (C-F) *D. gloriosa*, male genitalia: dorsal view, ventral view, penis valve laterl view, internal lateral view with penis valve removed; (G-J) *D. pseudopappus*, male genitalia: dorsal view, ventral view, penis valve laterl view, internal lateral view with penis valve removed; (K), *D. thetis*, right mandible in dorsal view, female; (L), *D. nigripes*, right mandible in dorsal view, female; (M), *D. nocturna*, right mandible in dorsal view, female.

(NVDA); *Mineral Co.,* 3 mi. [4.8 km] S Marriett, 6♂, leg. F.D. Parker, 16.viii.1998 (EMUS); *Nye Co.*, Ash Meadows National Wildlife Refuge, 1♂, leg. N. Boehme, 4.viii.2009 (EMUS); <u>Texas</u>: *Brewster Co.*: Big Bend National Park, Rio Grand Village, 4♂, leg. M.A. Cazier, 23.vi.1970 (ASUT); Big Bend Ranch State Park, 1♂, leg. J.S.

Wilson, 16.v.2006 (EMUS); Rio Grande, 23.5 mi. [37.6 km] E Presidio, 2♂, leg. J.S. Wilson, 17.v.2006 (EMUS). Seventy–three additional males from California and Texas (CASC, CISC, LACM) were examined. ***Dasymutilla gloriosa*****. MEXICO:** Baja California Sur: Playa Los Cerritos, 4 km SW El Pescadero, 23°20'N 110°11'W, 1♀, leg. J. Rodriguez and K.A. Williams, 4.v.2011 (EMUS); 3 km SE Todos Santos, 475', 23°26'N 110°11'W, 3♀, leg. J. Rodriguez and K.A. Williams, 7.v.2011 (EMUS); Playa San Pedro, 6 km S Todos Santos, 23°23'N 110°12'W, 1♀, leg. J. Rodriguez and K.A. Williams, 6.v.2011 (EMUS); Durango, Reserva de la Bioserfa Mapimì, 1♀, leg. R. Ayala, 23.viii.1991 (EBCC); Nayarit, Matanchen Beach, 3♀, 1♂, leg. L.G. Bezark, 23 June 1983 (UCDC); Sinaloa, Barra de Piaxtla, 23°40'N 106°48'W, 2♀, leg. F.A. Noguera and A. Rodriguez (EBCC); Sonora, 2 km S el Empalme, 27°57'N 110°49'W, 1♀, leg. R. Ayala, 22.vii.1995 (EBCC); **U.S.A.**: Arizona: *Cochise Co.*: Ramsey Canyon, Huachuca Mts., 1♀, 27.viii.1964, R.F. Sternitzky (PMNH); Tombstone, 1♀, vii.1933, A. Petrunkevitch (PMNH); *Maricopa Co.*, Gila Basin, 10 km S Arlington, 33°13.30'N 112°45.53'W, 55♂, leg. F.D. Parker and M.E. Irwin, 18.v.–21.viii.2010 (EMUS); California: *Kern Co.*, Johannesburg, 1♀, 3.ix.1956 (PMNH); Idaho: *Owyhee Co.*, Bruneau Dunes State Park, 10♀, leg. K.A. Williams, 6.viii.2011 (EMUS); Nevada: *Clark Co.*, Corn Creek, 1♂, 2♀, vi.1965 (PMNH); *Mineral Co.*, 3 mi. [4.8 km] S Marriett, 1♀, leg. F.D. Parker, 16.viii.1998 (EMUS); *Nye Co.*, Ash Meadows National Wildlife Refuge: Non–dune site 4: 1♀, 24.vi.2009; 1♀, leg. N.F. Boehme, 4–6.ix.2009; Sand dune site 1: 1♀, leg. N.F. and S.D. Boehme, 30.x.2009; 1♀, leg. N.F. Boehme, 20.xi.2009; Sand dune site 2: 1♀, leg. N.F. Boehme, 2–3.ix.2008; Sand dune site 5: 1♀, leg. N.F. Boehme, D.A. Tanner and J.P. Pitts, 24.vi.2008; 1♀, leg. N.F. and S.D. Boehme, 17–

18.x.2008; Non–dune site 5: 1♀, leg. N.F. and S.D. Boehme, 17–18.x.2008; 1♀, 4.viii.2009; 1♀, leg. NF Boehme, 4–6.ix.2009; Copeland site: 1♀, leg. N.F. and S.D. Boehme, 17–18.x.2008; Spring meadows site: 1♀, leg. N.F. Boehme, 2–3.ix.2008; Mesquite site 1: 2♀, leg. N.F. Boehme, 2–3.ix.2008; 3♀, leg. N.F. and S.D. Boehme, 17–18.x.2008; Mesquite site 3: 1♀, leg. N.F. Boehme, 2–3.ix.2008; 1♀, leg. N.F. and S.D. Boehme, 17–18.x.2008 (EMUS); Utah, *Washington Co.*, Warner Valley, near St. George, 1♀, leg. J.S. Wilson, 25-30.viii.2010 (EMUS). Seven hundred additional females and three hundred twenty-eight males were examined from throughout the range of this species (ASUT, CASC, CISC, EMUS, NVDA, LACM, UCDC).

Diagnosis

Female: clothed entirely with white setae (Fig. 5.1A); mesosoma longer than broad and having thickened transverse carina anterior to scutellar scale; and pygidium rugose or rugo-striate, lacking raised and separated striae basally (Fig. 5.5B).

Male: integument entirely black; black setae covering legs and venter (Fig. 5.1: B, H); anterior pronotal margin emarginate dorsomedially; axillae truncate posteriorly; S2 having medially situated, longitudinally ovate, seta-filled pit; and genitalic cuspis straight, laterally compressed, and 0.7x the free paramere length (Figs. 5.5: C–F).

Geographic distribution

This species is widespread in the warm desert regions of North America (Chihuahuan, Mojave, Peninsular, and Sonoran) and Madrean Archipelago. It has also been collected in various parts of the Great Basin in California, Nevada, and Idaho and in the Sinaloa Coastal Plain south to Nayarit (Fig. 5.2A).

Remarks

The males formerly known as *D. chisos* are identical to other *D. gloriosa* males, except in setal coloration (Figs. 5.1: B, H). Multiple specimens have been recovered with interspersed black and orange setae on the head and mesosoma, revealing that setal coloration is not discrete in this case. Females from geographic areas dominated by different male color forms are indistinguishable. This information, coupled with phylogenetic position, reveals that *D. chisos* is conspecific with *D. gloriosa*.

Males of *D. gloriosa* display different color patterns throughout their range (Fig. 5.2D). Those from the eastern extent of their range have the head and mesosoma black, but the metasomal setae orange. Those from the warm deserts, excluding the eastern Chihuahuan, have concolorous orange to red setae dorsally on the head, mesosoma, and metasoma. Males from the eastern Mojave Desert and Great Basin are similar to those from Texas. Males from the southernmost range of *D. gloriosa* often have pale yellow to white dorsal setae (Williams & Manley, 2006). No males have yet been examined from the Peninsular Desert in Baja California. Because *D. gloriosa* varies so much in color, and so many *Dasymutilla* morphospecies are diagnosed by setal color, additional synonyms of *D. gloriosa* may still be found. For example, *D. imperialis* Manley & Pitts, 2004 is similar to *D. gloriosa*, but clothed entirely with black setae and it is one of the only species from Baja California that is known only from males; this species could eventually contribute to the synonymy list of *D. gloriosa*.

Dasymutilla pseudopappus (Cockerell)

(Figs. 5.1: E, F, G; 5.2: B, D, 5.5: A, G–J)

Sphaerophthalma [sic.] *gloriosa* var. *pseudopappus* Cockerell, 1895: 6.

Ephuta gloriosa pseudopappus Cockerell, 1898: 140.

Mutilla pseudopappus Fox, 1899: 237; Melander, 1903: 296, Figs. 6, 44.

Mutilla phaon Fox, 1899: 243; **new synonym**.

Ephuta (Ephuta) gloriosa var. pseudopappus André, 1903: 60.

Ephuta (Ephuta) phaon André, 1903: 62.

Dasymutilla pseudopappus Mickel, 1928: 46, 243; Mickel, 1936: 30.

Dasymutilla phaon phaon Mickel, 1928: 53, 236, 301, 302; Mickel, 1936: 37, 60; Manley & Pitts, 2007: 17, 81, Pl. C7.

Dasymutilla phaon fimbrialis Mickel, 1928: 53, 302; Mickel, 1936: 37; **new synonym**.

Material examined

***Dasymutilla phaon*. U.S.A.:** Arizona: *Coconino Co*., Grand Canyon National Park, Phantom Ranch, 1♂, leg. J.E. Slansky, 2.vi.1968 (UCDC); *Navajo Co.*, 15 mi. [24 km] NW Kayenta, 1♂, leg. H.N. Hultgren, 24.vi.1933 (CASC); California: *Inyo Co.*: Antelope Springs, 8 mi. [12.8 km] SW Deep Springs: 1♂, leg. W.A. Foster, 10.vii.1962 (EMUS); 1♂, leg. H.K. Court, 2.vi.1968 (UCDC); 7 mi. [11.2 km] E Big Pine, 10♂, leg. W.E. Ferguson, 19.vii.1953 (CASC); Indian Springs, Westgard Pass, 1♂, leg. W.D. McLelllan, 12.vii.1983 (UCDC); Mazourka Canyon, 2♂, leg. D.D. Linsdale, 2.vii.1953 (EMUS); Nevada: *Esmerelda Co.*, Lida Summit, 6♂, leg. R.C. Bechtel, 1.viii.1963 (NVDA); Texas, *Reeves Co.*, Pecos, 1♂, leg. A.T. McClay, 18.vi.1947 (UCDC). Twenty-six additional males from Inyo Co., California were examined (CISC). ***Dasymutilla phaon fimbrialis*. Mexico**: Baja California, 0.5 mi. [0.8 km] N Hamilton Ranch Airfield, 1♂, leg. H.B. Leach, 28.iv.1963 (CASC); Sinaloa, Guaymas, 1♂, leg. E.P. van Duzee, 10.iv.1921 (CASC); **U.S.A.**: Arizona: *Cochise Co.*: Hereford, 1♂, leg. P.R. Luter, 7.vi.1958 (UCDC); Huachuca Mts, 0.5 mi. [0.8 km] W Hwy. 92; leg. MacFarland, 1.vi.1994 (EMUS); *Graham Co.*, Graham Mts., Marijilda Canyon, 1♂, leg. M.A. Cazier, 21.vi.1966 (ASUT); *Maricopa Co.*, Gila Basin, 10 km S Arlington, 33°13.30'N 112°45.53'W, 6♂, leg. F.D. Parker and M.E. Irwin, 25.v.–13.vi.2010 (EMUS); *Mojave Co.*, Cattail Cove, 9 mi. [14.4 km] N Parker Dam, leg. B. Apperson, 7.iv.1972 (EMUS); *Yuma Co.*, Alamo Lake State Park, 1♂, leg. M.A. Cazier, 2.v.1970 (ASUT); 2 mi [3.2 km] NW Bouse, leg. M.A. Cazier, 7.v.1966 (ASUT); California: *Mono Co.*, Round Valley, 1♂, leg. G. Cushner, 10.vii.1958 (CASC); *Riverside Co.*: Blythe, 1♂, leg. C.D. MacNeill, 27.iv.1949 (CASC); Joshua Tree National Monument, Pleasant Valley, 1♂, leg. E.L. Sleeper, 25. v.1968 (CASC); Palm Springs, 1♂, leg. E.R. Leach, 6.v.1917 (CASC); *San Bernardino Co.*, Clipper Valley, 1♂, leg. T. Griswold, 29.v.1983 (EMUS); *San Diego Co.*: Borrego Valley, Palm Canyon, leg. R.M. Bohart, 19.iv.1957 (UCDC); Nevada: *Clark Co.*: Bunkerville, 1♂, leg. R.C. Bechtel, 13.vi.1965 (NVDA); 2 mi. [3.2 km] NW Cedar Basin, leg. R.C. Bechtel, 3.vi.1981 (NVDA); 5 mi. [8 km] SW Glendale, 1♂, leg. P.F. Torchio, 2.vi.1973 (EMUS); Juanita Springs, 1♂, leg. R.C. Bechtel, 12.v.1984 (NVDA); Juanita Springs Ranch, S of Riverside, 5♂, leg. F.D. Parker (EMUS); Las Vegas, 1♂, leg. E. Van Dyke, 24.v.1935 (CASC); Valley of Fire, 1♂, leg. G. Bohart, 27.iv.1972 (EMUS); *Mineral Co.*, Finger Rock Wash, 1♂, leg. I. Scudder, 20.vi.1974 (CASC); *Washoe Co.*, Pyramid Lake: 1♂, leg. D.S. Chandler, 18.vii.1971 (UCDC); 1♂, leg. R.M. Bohart, 25.vii.1973 (UCDC); 1♂, leg. R.D. Moon, 20.vi.1974 (UCDC); Utah: *Washington Co.*: Paradise Canyon, 1♂, leg. D. Beck, 17.vi.1983

(EMUS); Snow Canyon, 1♂, leg. W.J. Hanson, 15.vi.1983 (EMUS). Twenty-one additional males from California and Arizona were examined (EMUS, CASC, CISC, LACM). ***Dasymutilla pseudopappus*.** **Mexico**: Baja California, 9 mi. [14.4 km] S San Matias Ps., 1♀, leg. E.L. Sleeper, 6.vi.1961 (CASC); Baja California Sur: 7 mi. [11.2 km] N Arrasitras, 1♀, leg. E.L. Sleeper, 8.vi.1962 (CASC); 7.5 mi [12 km] W El Triunfo, 2♀, leg. E.L. Sleeper, 11.x.1968 (CASC); Sinaloa, Choix, 1♀, leg. T.A. Sears, 24.vii.1969 (UCDC); Sonora: Alamos, 1♀, 15.ix.1933 (UCDC); 22.5 mi. [36 km] S San Louis, 3♀, 4-5.vi.1949 (CASC); **U.S.A.**: Arizona: *Cochise Co.*: Sierra Vista, 1♀, leg. R. Wielgus, 13.vi.1984 (ASUT); Ramsey Canyon, Huachuca Mts., 2♀, leg. R.F. Sternitzky, 11-15.iii.1964 (PMNH); Tombstone, 1♀, leg. A. Petrunkevitch, vii.1933 (PMNH); *Coconino Co.*, Hooserock, 1♀, leg. J. Carned, 6.ix.1964 (EMUS); *Maricopa Co.*, Brushy Basin, 1♀, leg. D. Gavin, 1.x.1978 (ASUT); *Pima Co.*, Baboquivari Mountains, 3♀, leg. O.C. Poling, 1.vii-18.viii.1924 (CASC); Tortolita Mt., Owl's Head, 1♀, leg. L. Koenia, 1.xi.1950 (PMNH); *Yavapai Co.*, Mayer, 2♀, leg. E.R. Leach, 19.vii-12.viii.1938 (CASC); *Yuma Co.*, Yuma, 1♀, leg. J.D. Carlson, ii.1910 (CASC); California: *Inyo Co.*: Inyo Mts., Lead Canyon, 1♀, leg. D. Howell, viii.1980 (CASC); Westgard Pass, 1♀, leg. C.D. MacNeill, 26.vii.1962 (CASC); *Riverside Co.*: near Blythe, 1♀, leg. H.W. Beavis, 28.iv.1939 (CASC); 7 mi. [11.2 km] NE Blythe, Mayflower Park, 1♀, leg. K.A. Williams, 24.vi.2004 (EMUS); Joshua Tree National Monument: Lower Covington Flat, 1♀, leg. E.L. Sleeper, 20.ix.1970 (CASC); Pleasant Valley: leg. E.L. Sleeper: 1♀, 15.v.1966; 1♀, 12.vi.1966; 1♀, 24.vi.1967; 1♀, 21.x.1967 (CASC); Squaw Track, 1♀, leg. E.L. Sleeper, 20.v.1962 (CASC); Thousand Palms Canyon, 2♀, leg. R.M. Bohart, 9.iv.1964; *San Bernardino Co.*, Victorville, 1♀, leg. H. Hansen, 1.vi.1952 (EMUS); *San*

Diego Co.: 6 mi. [9.6 km] E Banner, leg. L.H. Pojogen, 26.vi.1963 (UCDC); 8 mi. [12.8 km] N Borrego Spring, 1♀, leg. E.L. Sleeper, 9.v.1969 (CASC); Colorado, *Dolores Co.*, Dove Creek, 1♀, leg. O. Fleming, 30.vii.2009 (EMUS); Nevada: *Nye Co.*, Ash Meadows National Wildlife Refuge: Non-dune site 3: 1♀, leg. N.F. Boehme, 3.x.2009; Sand dune site 1: 3♀, leg. N.F. Boehme and D.A. Tanner, 10.vi.2009; Sand dune site 2: 1♀, leg. N.F. Boehme, 24.vi.2009; Sand dune site 4: 1♀, leg. N.F. Boehme and D.A. Tanner, 10.vi.2009; Sand dune site 5: 1♀, leg. N.F. Boehme, D.A. Tanner and J.P. Pitts, 26.vi.2008; 1♀, leg. N.F. Boehme, 28.v.2009 (EMUS); Berlin-Ichthyosaur State Park, 1♀, 19.ix.1964 (CASC); New Mexico, *Rio Arriba Co.*, Puye Cliff Ruin, 1♀, leg. C.L. Remington, 17.viii.1961 (PMNH); Texas: *Brewster Co.*, Big Bend National Park, Chisos Mountains, 1♀, leg. C.R. Nelson, 21.v.1993 (EMUS); Utah: *San Juan Co.*, Hovenweep National Monument, 1♀, leg. T. Rado, vii.1974 (LACM); *Washington Co.*: Middleton, 1♀, leg. G.F. Knowlton, 26.vi.1933 (EMUS); Paradise, Snow Canyon, 1♀, leg. D. Beck, 1.ix.1963 (EMUS); Warner Valley, near St. George, 1♀, leg. J.S. Wilson, 25-30.viii.2010 (EMUS).

Diagnosis

Female: clothed entirely with white setae (Fig. 5.1E); mesosoma longer than broad; transverse carina anterior to scutellar scale weak or absent; pygidium with raised, separated, longitudinally parallel striae basally (Fig. 5.5A).

Male: integument entirely black; black setae clothing legs and venter (Figs. 5.1: F, G); axillae truncate posteriorly; S2 lacking seta-filled pit and simply convex; and genitalic cuspis ~0.5x the free paramere length (Figs. 5.5: G–J).

Geographic distribution

This species is widespread in the warm desert regions of North America (Chihuahuan, Mojave, Peninsular, and Sonoran) and Madrean Archipelago. It also occurs in various parts of the Great Basin in California and Nevada; the Colorado Plateau in Arizona, New Mexico, and Colorado; and in the Sinaloa Coastal Plain in Sonora and Sinaloa (Fig. 5.2A)

Remarks

Females of *D. pseudopappus* are commonly misidentified as *D. gloriosa*. Mickel (1928, 1936) used three characters to separate *D. pseudopappus* from *D. gloriosa* in his keys. *Dasymutilla pseudopappus* was stated to have: the pygidium prominently longitudinally striate, the body and leg color blackish, and the gena bounded by an obscure carina. *Dasymutilla gloriosa*, on the other hand, was stated to have: the pygidium irregularly rugose, the body and legs red, and the gena rounded posteriorly. The pygidium of *D. gloriosa* varies from being irregularly rugose to rugo-striate (many of the rugae are parallel and longitudinally oriented). Both *D. pseudopappus* and *D. gloriosa* have the integumental color varying from pale red to black. The genal carina of *D. pseudopappus* is typically obscured by dense white setae; also, this carina can be so weakly produced that it is interrupted by the surrounding genal punctures.

After observing over 700 females of *D. gloriosa* and over 50 *D. pseudopappus* females, the following traits appear to consistently separate these taxa. First, the pygidium of *D. pseudopappus* has separated and highly raised parallel striae in the basal half at least (Fig. 5.5A); *D. gloriosa* has the pygidial striations or rugae tightly spaced and equally raised throughout the pygidial plate (Fig. 5.5B). Second, *D. pseudopappus* has the mesosoma armed with a narrow, raised scutellar scale, if any transverse carinae are present anterior to the scale, they are obscured by setae and much lower than the scutellar scale (Fig. 5.1E); *D. gloriosa* has a broader scutellar scale that is accompanied by one transverse carina directly anterior to the scale and two transverse carinae situated anterolaterally of the scale, these transverse carinae are equal in height to the scutellar scale and typically visible through the mesosomal setae (Fig. 5.1A).

In addition to the molecular and phylogenetic data, Morphological similarity to *D. flammifera* assisted us in associating the sexes of *D. pseudopappus*. Females of these species have similar scutellar and pygidial morphology, while males are seemingly identical except for genitalic differences (Manley, unpublished). Although we were unable to obtain molecular data from *D. flammifera*, morphological similarities reveal these as sister-taxa.

The newly associated males of *D. pseudopappus* are nearly as variable in color as those of *D. gloriosa* (Figs. 5.1: E, F). These males are usually easy to identify, but some can be confused with *D. zelaya*. Males of *D. pseudopappus* have S2 evenly convex and the genitalic cuspis roughly 0.5x the free paramere length, while *D. zelaya* has S2 flattened medially and its cuspis over 0.7x the free paramere length.

Dasymutilla thetis **(Blake)**

(Figs. 5.1: C, D; 5.2D; 5.5K)

Sphaerophthalma (!) *thetis* Blake 1886: 214.

Mutilla thetis Dalla Torre, 1897: 91; Fox, 1899: 246.

Ephuta (Ephuta) thetis Andre, 1903: 64.

Dasymutilla thetis Mickel, 1928: 45, 78; Mickel, 1936: 29, 42; Pilgrim et al., 2008: 58, Figs. 1-4.

Dasymutilla candida Mickel, 1928: 53, 296, Fig. 39; Mickel, 1936: 36.

Material examined

U.S.A.: Arizona: *Cochise Co.*: Dos Cabezas Mts., 2 km SW Apache Pass, 1♀, leg. F.D. Parker, 3.ix.1986 (EMUS); Portal, 1♀, leg. M.A. Cazier, 1.vii.1963 (ASUT); 6 km N Sonoita, 1♂, leg. F Parker, 6.ix.1986 (EMUS); 2 mi. S Willcox, 12♂ 25♀, leg. J.P. Pitts, J.O. Schmidt, E.M. Pilgrim, and K.A. Williams, 19–26.vii.2005 (EMUS); 5 mi. [8 km] SE Willcox, 1♀, leg. F Parker, 2.xi.1986 (EMUS); *Graham Co.* 2.4 mi. W Hwy 366 from Hwy 191, 1♂, leg. Hara, 14–26.viii.1993 (EMUS); *Maricopa Co.*, Gila River, 10 km S. Arlington, 33°13.30'N 112°45.53'W, 2♀, 82♂, leg. F.D. Parker and M.E. Irwin, 25.v.–21.viii.2010 (EMUS); *Santa Cruz Co.*: 10 mi. [16 km] SW Patagonia, 1♀, leg. P.D. Hurd, 13.ix.1958 (EMUS); Santa Cruz River Wash, 8 km E Nogales, 2♀, leg. E.E. and K.A. Williams, 3.VIII.2003 (EMUS); New Mexico, *Hidalgo Co.*, Rodeo, 1♀, leg. C.G. Moore, 3.viii.1958 (UCDC).

Diagnosis

Female: Mandible with inner tooth strongly raised and continuous with dorsal mandibular carina (Fig. 5.5K); clothed entirely with white setae; mesosoma as broad as long and lacking scutellar scale (Fig. 5.1C).

Male: dorsum of the head, mesosoma, apical fringe of T2, and T3-7 with white setae (Fig. 5.1D); pronotum not emarginated anteromedially; axillae glabrous on outer lateral margin and sharply dentate posteriorly; S2 lacking seta-filled pit; hypopygium narrow with medial tooth on apical margin; and pygidium with raised longitudinally parallel rugae.

Geographic distribution

This species has been recorded only from the Madrean Archipelago and eastern Sonoran Desert, including Pima, Maricopa, Santa Cruz, and Cochise Counties in Arizona and Hidalgo County in New Mexico (Fig. 5.2D).

Remarks

Unlike the other Thistledown Velvet Ants, *D. thetis* has a narrow geographic range (southern Arizona only) and consistent male coloration that is somewhat similar to the female color (dorsal setae concolorous white).

This species is similar morphologically to the *D. asopus, D. foxi, D. scabra* and *D. fulvohirta* species-groups because of the female's broad mesosoma and male's dentate axillae. In this and previous phylogenetic analyses, however, a close relationship has never been shown between those taxa and *D. thetis*. Our analysis recovers *D. thetis* as the sister to *D. coccineohirta*, with these species showing affinity to *D. glycera, D. gorgon, D. leda,* and members of the *D. sparsa* species-group. Traditional species-group characters have proven inadequate for grouping these taxa, but the females all have similar mandibles. All known females in this clade have long curved mandibles (e.g. Figs. 5.5: K, L), while the remaining *Dasymutilla* usually have shorter straight mandibles (e.g. Fig. 5.5M). Potential synapomorphies for males of this clade have not yet been discovered.

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CHAPTER 6

MULTI-FACETED ASSESSMENT OF SPECIES VALIDITY IN THE *DASYMUTILLA BIOCULATA* SPECIES-GROUP (HYMENOPTERA: MUTILLIDAE)[5]

Introduction

North American velvet ants in the genus *Dasymutilla* Ashmead have conspicuous integumental and setal colour patterns. These colour patterns have been used by taxonomists to differentiate species (*e.g.,* Rohwer, 1912; Mickel, 1928, 1936; Manley & Pitts, 2007). Recent studies using ecological, morphological, and molecular methods have shown that a single species can have differing colour patterns that are typically geographically dependent. This has required multiple taxa to be treated as single, widespread, variable species, rather than geographically isolated, discrete species (*e.g.,* Manley, 1980, 1999a; Pilgrim *et al*., 2008, 2009). It is now known that in many cases these species were thought of as "discrete" only because of insufficient sample size. As sample size was increased, intermediate forms were found that discredited the discreteness of the species.

Mickel (1928) revised *Dasymutilla* and described 19 species-groups based on shared morphological traits. Most of these species-groups lack unique synapomorphies, but are, rather, defined by a unique combination of structural characters, which are

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frequently polymorphic within the species-group (KAW pers. obs.). No inferences about relationships between species-groups were made by Mickel (1928). While there are misplaced taxa in some species-groups (Pilgrim *et al.*, 2009), many of the species-groups appear to be natural groups defined by phylogenetically valuable structural characters. In some cases, Mickel's species-groups are monotypic, either by original designation (*D. thetis* species-group) or by subsequent synonymy (*D. creon* and *D. foxi* species-groups). In the case of *D. foxi,* morphological and molecular studies show that the three included taxa are simply colour variants of a single, widespread species (Manley, 1980; Pilgrim *et al.,* 2008). Reduction of species-groups to monotypy appears necessary in more of Mickel's species-groups, as the *D. bioculata, D. arenivaga, D. nogalensis, D. scabra, D. vesta, D. fulvohirta,* and *D. asopus* species-groups are composed entirely of taxa that can be separated by coluoration, but are lacking consistent morphological differences (KAW pers. obs.). As such, further scrutiny of Mickel's species-groups, as seen with *D. quadriguttata,* is required given the problems associated with over-reliance on colour characters (Pilgrim *et al*., 2009).

The *D. bioculata* species-group, one of Mickel's largest, currently includes ten species and eight subspecies that are diagnosed mainly by colouration. Mickel (1928) illustrated the male genitalia of only one species, because he found all of the species to have identical genitalia. Furthermore, these species are often notoriously difficult to identify due to the existence of specimens with intermediate colouration or structural characters (DGM, KAW, Justin O. Schmidt, James Zimmermann pers. obs.). Most of the same characters used to separate these taxa are polymorphic in other *Dasymutilla* species, such as *D. vesta* (Cresson) and *D. quadriguttata* (Say) (Mickel, 1928; Pilgrim *et al.*, 2009). The tenuous nature of characters used to differentiate the species of the *D. bioculata* species-group, coupled with the identical male genitalia, suggests that some, if not all, of these species may be conspecific.

Molecular sequence data have also suggested that some of the species of the *D. bioculata* species-groups could be conspecific. Pilgrim and Pitts (2006) illustrated the usefulness of the internal transcribed spacer regions of ribosomal DNA, ITS1 and ITS2, for associating the dimorphic sexes of Mutillidae, specifically for *Dasymutilla*. In their initial study, Pilgrim and Pitts (2006) compared sequences of *D. ch. ursula* (Cresson) [subspecies not designated by Pilgrim & Pitts] and *D. lepeletierii* (Fox) and found the sequences completely identical in ITS1 and 99.6-99.7% identical in ITS2 (Pilgrim & Pitts, 2006). This suggested that these two nominal taxa were possibly conspecific, and the authors strongly advocated that additional morphological and molecular comparisons were needed in this species-group to determine the legitimacy of the species. Subsequent research on other *Dasymutilla* species has also found these loci to be useful in determining the validity of closely related species (Pilgrim *et al*., 2008, 2009).

The purpose of this study was to test the validity of the 14 taxa placed within the *D. bioculata* species-group. We evaluated these taxa using four lines of evidence: structural and colour-based morphology, field observations of mating pairs, geographical distribution, and genetic distances using ITS1 and ITS2. Using these data, we searched for inferred (molecular and ecological) or actual (morphological) diagnostic synapomorphies or combinations of characters. Under the phylogenetic species concept, *sensu* Wheeler & Platnick (in Wheeler & Meier, 2000), these taxa cannot be treated as valid species unless they can be consistently defined by a unique combination of character states.

Material and Methods

Taxa chosen for this study

Our main group of interest is the *D. bioculata* species-group *sensu* Mickel (1928) and a single species described recently by Manley & Pitts (2007). This species-group includes *D. bimaculata* Manley & Pitts (♂), *D. bioculata* (Cresson) (♀♂), *D. chiron chiron* (Blake) (♂), *D. chiron ursula* (Cresson) (♀♂), *D. creusa creusa* (Cresson) (♀), *D. creusa bellona* (Cresson) (♀), *D. lepeletierii* (Fox) (♀♂),*D. medea* (Cresson) (♀),*D. melanippe melanippe* Mickel (♀), *D. melanippe conformis* Mickel (♀), *D. praegrandis praegrandis* (Mickel) (♀♂), *D. praegrandis russata* Mickel (♂),and *D. sulcatulla* Mickel (♀♂). Some species in this group are known from a single sex only; this is indicated by the sex symbol listed after the species of interest above.

For our molecular analysis we also chose four outgroup species (based on similarities in morphology to the *D. bioculata* species-group) from the *D. quadriguttata* [*D. quadriguttata* (Say)] and *D. occidentalis* species-groups [*D. gorgon* (Blake), *D. occidentalis* (Gray), and *D. gloriosa* (Saussure)].

Morphological methods

Pinned specimens, including all primary types, of all taxa referred to in this study were examined to compare morphological features. The non-type material that was examined included 6 specimens of *D. bimaculata* (USA: AZ, NM, TX), over 180 *D. bioculata* (USA: CO, ID, IL, KS, MN, MO, ND, NE, NM, OK, OR, SD, TX, UT, WA, WI, WY; CANADA: Alberta, Manitoba, Saskatchewan; MEXICO: Chihuahua), 180 *D. ch. chiron* (USA: TX; MEXICO: Chihuahua, Coahuila), over 500 *D. ch. ursula* (USA: AZ, CO, ID, KS, MT, NM, NV, OK, OR, TX, UT, WA, WY; CANADA: British Columbia; MEXICO: Chihuahua, Durango), over 600 *D. cr. creusa* (USA: AR, CO, ID, KS, MO, NE, NM, OK, TX, WY; CANADA: British Columbia; MEXICO: Chihuahua), 50 *D. cr. bellona* (USA: CO, ID, KS, NE, NM, UT, WY; CANADA: British Columbia), over 500 *D. lepeletierii* (AR, CT, FL, GA, IA, IL, IN, KS, MA, MD, MI, MO, NC, NJ, NY, SC, TX, VA, WI), 8 *D. medea* (CO, KS, NM, TX), 150 *D. mel. melanippe* (USA: TX; MEXICO: Tamaulipas), 80 *D. mel. conformis* (OK, TX), 40 *D. pr. praegrandis* (OK, TX), 30 *D. pr. russata* (AR, NM, TX), over 300 *D. pyrrhus* (FL), and 25 *D. sulcatulla* (USA: TX; MEXICO: Durango).

All specimens were examined with a stereo microscope. Genitalia were dissected using minuten pins; the genitalia of more than 60 males were examined. Rough estimates of specimen length were made using a clear plastic 15cm (six-inch) ruler. Rough measurements were used because the mounted position of a specimen’s head and metasoma varies greatly (Fig. 6.1) and metasomal segments are often variably extended based upon the position a specimen dies in or subsequent modifications of the specimen, particularly genitalia extraction (Figs 6.2: A-C). These factors make exact body length measurements extremely difficult, even using a micrometer. Specimens used in the molecular study were labeled as voucher specimens and deposited in the Department of Biology Insect Collection, Utah State University, Logan, UT (EMUS).

We use the acronyms: T1, T2, etc. to refer to the metasomal terga, and the abbreviations: S1, S2, etc. to refer to the metasomal sterna. All characters used in past keys to differentiate these species were examined and scored for multiple specimens (Mickel, 1928, 1936; Manley & Pitts, 2007); these characters are listed in table and matrix form for ease of comparison (Tables 6.1-4).

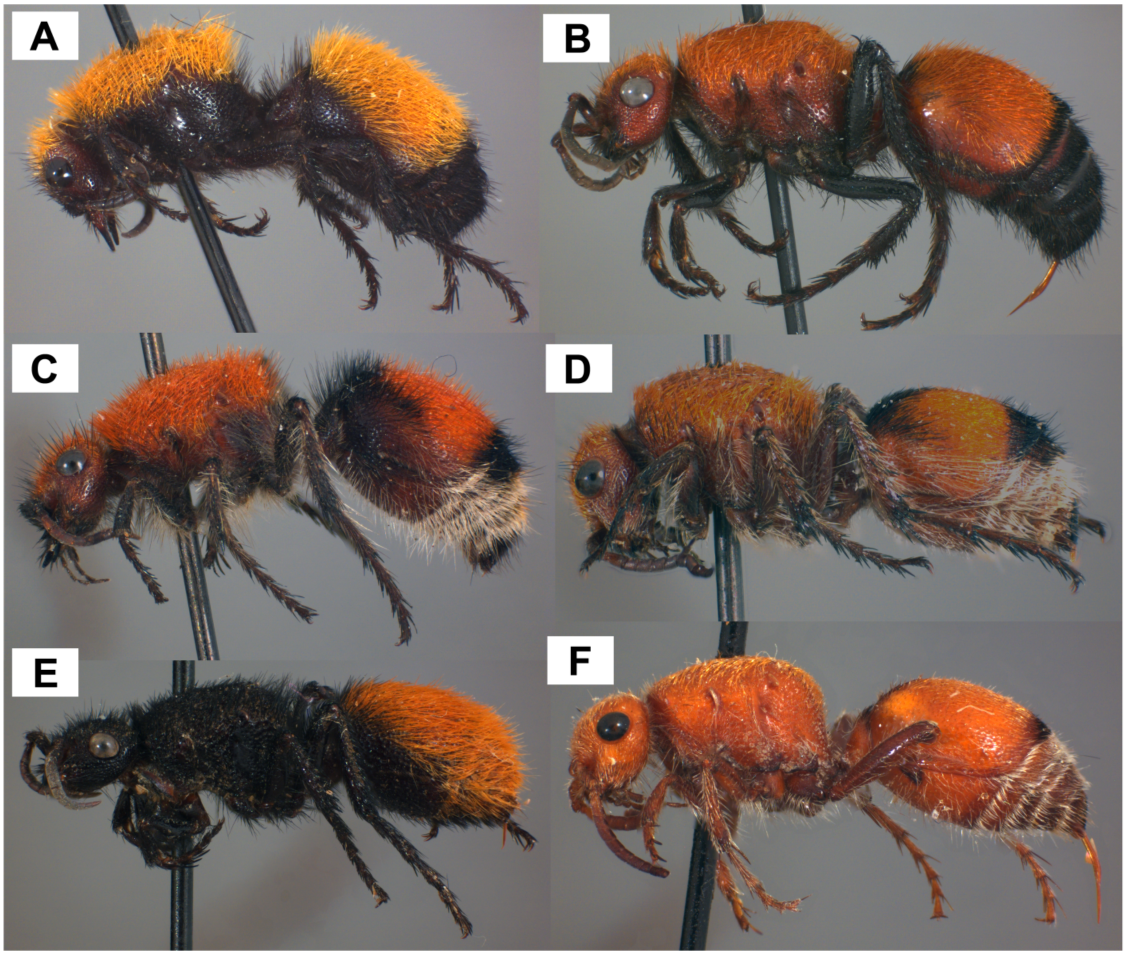

Fig. 6.1. Habitus, female. A, *Dasymutilla creusa creusa* (New Mexico); B, *D. praegrandis praegrandis* (Texas); C, *D. bioculata* (Washington); D, *D. bioculata* (Nebraska); E, *D. melanippe melanippe* (Texas); F, *D. lepeletierii* (North Carolina).

Fig. 6.2. Male apical terga, lateral view. A, *Dasymutilla bioculata*; B, intermediate form; C, *D. chiron ursula*. Male head and mesosoma, dorsal view. D, *Dasymutilla praegrandis russata;* E-F, intermediate forms; G, *D. pr. praegrandis.* Female apical terga, dorsal view. H-I, *D. ch. ursula*; J, *D. bioculata*; K-M, *D. lepeletierii*.

Table 6.1. Morphological characters used to diagnose females of the *D. bioculata* species-group. States designated by numbers; letters represent intermediate forms between two states (in. btw. = intermediate between provided states).

Characters	**States**
1. Antennal Scrobe	**0**=ecarinate; **1**=distinctly carinate; **a**= in. btw.0,1
2. Flagellomeres 3-10	**0**=not sulcate; **1**=sulcate; **a**=in. btw.0,1
3. Dorsal setal colour of head and mesosoma	**0**=yellow to red; **1**=black; **a**=in. btw.0,1
4. Setal type of head and mesosoma	**0**=sparse and appressed; **1**=moderately dense, having both appressed and erect setae; **a**=in. btw.0,1
5. Punctures of T2	**0**=moderate and circular, **1**=large and elongate
6. Apical fringes of T2-5	**0**=T3-5 concolourous with disc of T2, yellow to red; **1**=T2-5 silver; **2**=T2 silver medially and black laterally, T3-5 silver; **3**=T2-3 entirely black; T4-5 silver; **4**=T3-5 silver laterally, black medially; **5**= T3-5 entirely black; **a**=in. btw.0,1; **b**=in. btw.2,3; **c**=in. btw.3,5; **d**=in. btw.4,5
7. Setae of apical sterna	**0**=black; **1**=silver; **2**=yellow to red; **a**=in. btw.0,1

Table 6.2. Matrix designating female characters listed in Table 6.1.

	Characters							
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Length (mm)**
D. bioculata	0	0	0	0,1,a	0	3,c	1	8-15mm
D. ch. ursula	0,1,a	0	0	1,a	0,1	1,4,a,d	1,a	7-16mm
D. cr. creusa	0,a	0	0,a	1	0,1	0,5	0	6-17mm
D. cr. bellona	0	0	0	1,a	0	5	1	7-12mm
D. lepeletierii	0	0	0	0,a	0,1	2,b	1	6-17mm
D. medea	1,a	0	0	1	0	5	0,a	9-16mm
D. mel. melanippe	0,a	0	1	1	0	0	0	6-18mm
D. mel. conformis	1	0	0,a	1	0	0	0	7-19mm
D. pr. praegrandis	0	0	0	0	0,1	5	0	7-16mm
D. pyrrhus	0	0	0	0	0	2,b	1	7-16mm
D. sulcatulla	1	0,1,a	0	1	0	1,4	1,2	8-14mm

Table 6.3. Morphological characters used to diagnose males of the *D. bioculata* species-group. States designated by numbers; letters represent intermediate forms between two states (in. btw. = intermediate between provided states).

Characters	**States**
1. Antennal Scrobe	**0**=ecarinate; **1**=distinctly carinate; **a**= in. btw.0,1
2. Dorsal setal colour of head and mesosoma	**0**=yellow to red; **1**=black; **a**=in. btw.0,1
3. Second metasomal sterna	**0**=lacking seta-filled pit; **1**=having medial seta-filled pit; **a**=in. btw.0,1
4. Setae of T3-6	**0**=yellow to red, **1**=black; **a**=in. btw.0,1
5. Setae of S3-6	**0**=yellow to red, **1**=black; **a**=in. btw.0,1

Table 6.4. Matrix designating male characters listed in Table 6.3.

	Characters					
Species	**1**	**2**	**3**	**4**	**5**	**Length (mm)**
D. bimaculata	1	0	1	0,a	1	12-14mm
D. bioculata	0,a	0,a	0,a	1	1	10-18mm
D. ch. chiron	0,a	1	0,1	0	1,a	8-20mm
D. ch. ursula	a,1	0	0,a	0,a	1,a	9-19mm
D. lepeletierii	0,a,1	1	1	1	1	8-18mm
D. pr. praegrandis	0,a,1	1	1	1	1	13-15mm
D. pr. russata	0,a,1	0,a	1	1	1	10-15mm
D. pyrrhus	0,a	0,a	1	1	1	8-19mm
D. sulcatulla	a	1	0	0	0,1	8-13mm

Methods for sex associations in the field

Discovery of copulating pairs has been used extensively to associate males and females of mutillid taxa (*e.g.*, Cambra & Quintero, 1993; Manley, 1980). However, mating takes only seconds (Manley & Deyrup, 1989), and capturing mating pairs is rare. Although following a virgin female could allow discovery of conspecific males attracted to her, this is a time-consuming process that is rarely likely to result in successful mating observations. Mating in captivity can provide misleading results, because males will attempt to mate with non-conspecific females (DGM pers. obs.).

Another method for making sex associations in the field is the use of caged females. Virgin females release a pheromone in order to attract a mate (Brothers, 1972). When potential virgin females are collected, they are placed into small plastic cages, which have had the ends cut out and replaced with wire screen (Manley, 1999b). If the female is a virgin, then males approach the cage and attempt to mate with her through the screen (Manley, 1999b). These males are then captured and can be putatively associated with the female if morphological and distributional data also support the association.

The majority of mating pairs examined for this study were associated using the caged-female method, but some were collected while mating in the field. Several mating pairs were discovered in collections; we assume that they were collected mating in the field, but they could have been associated using other means.

Molecular methods

DNA was extracted from 19 individuals of eight species and four subspecies from the *D. bioculata* species-group; four additional *Dasymutilla* species were sequenced

Table 6.5. Voucher specimens used in molecular comparisons.

species	Specimen voucher	Genbank ITS1	Genbank ITS2	Sex	Collection Data
D. bimaculata	JP1242	HQ317264	HQ317275	♂	USA: TX: Bastrop Co.
D. bioculata	JP362	HQ317249	HQ317285	♂	USA: TX: Ward Co.
D. bioculata	JP363	HQ317248	HQ317274	♂	USA: TX: Ward Co.
D. bioculata	JP1189	HQ317263	HQ317277	♂	USA: NE: Morrill Co.
D. ch. chiron	JP307	HQ317262	HQ317286	♂	USA: TX: Bastrop Co.
D. ch. ursula	JP224	HQ317265	HQ317282	♀	USA: UT: Cache Co.
D. ch. ursula	JP248	HQ317250	HQ317279	♀	USA: AZ: Cochise Co.
D. ch. ursula	JP249	HQ317251	HQ317280	♀	USA: AZ: Cochise Co.
D. ch. ursula	JP257	HQ317257	HQ317281	♀	USA: UT: Cache Co.
D. cr. creusa	JP365	HQ317252	HQ317284	♀	USA: TX: Ward Co.
D. lepeletierii	mlepf1	HQ317255	HQ317270	♀	USA: SC: Florence Co.
D. lepeletierii	mlepm2	HQ317256	HQ317271	♂	USA: SC: Florence Co.
D. mel. melanippe	JP366	HQ317254	HQ317276	♀	USA: TX: Cameron Co.
D. mel. melanippe	JP369	HQ317253	HQ317283	♀	USA: TX: La Salle Co.
D. pr. praegrandis	JP1076	HQ317260	HQ317287	♀	USA: TX: Leon Co.
D. pr. praegrandis	JP1075	HQ317258	HQ317278	♂	USA: TX: Leon Co.
D. pr. russata	JP1074	HQ317266	HQ317269	♂	USA: TX: Leon Co.
D. pyrrhus	JP216	HQ317261	HQ317272	♂	USA: FL: Highlands Co.
D. pyrrhus	JP1078	HQ317259	HQ317273	♀	USA: FL: Orange Co.
D. gloriosa	JP241	HQ317244	HQ317288	♀	USA: AZ: Cochise Co.
D. gorgon	JP383	HQ317247	HQ317289	♀	USA: TX: La Salle Co.
D. occidentalis	moccf	HQ317246	HQ317268	♀	USA: SC: Florence Co.
D. quadriguttata	JP159	HQ317245	HQ317267	♂	USA: AR: Pulaski Co.

for use as outgroups (Table 6.5). Fresh specimens of *D. cr. bellona, D. medea, D. mel. conformis* and *D. sulcatulla* were not available for extraction. DNA extractions were performed using either several legs from each individual or the entire individual after puncturing the side of the thorax. This left the remainder of the external anatomy of the specimen available for morphological study. Extractions were done with either the Roche High Pure PCR Template Purification Kit or the QIAgen DNeasy Tissue Kit following each manufacturer's protocol. PCR conditions, primers, and DNA sequencing protocols were identical to those used in Pilgrim and Pitts (2006). Gel electrophoresis of each gene yielded a single band for each individual wasp and the resulting DNA was sequenced cleanly, suggesting no gene heterogeneity as seen in some other organisms (*e.g.*, Harris & Crandall, 2000; Parkin & Butlin, 2004; Bower *et al.*, 2008).

Alignments of the ITS1 and ITS2 data sets were made using Geneious and ClustalW (Drummond *et al.*, 2009; Thompson *et al.*, 1994). In order to calculate genetic distances, models of evolution for each alignment were determined using jModelTest (Posada, 2008). Genetic distances were calculated using the program MEGA4 (Tamura *et al.*, 2007). All sequences have been deposited in GenBank (accession numbers HQ317244 to HQ317289: Table 6.5).

Results

Structural and colour-based morphology

Intermediate colour forms were found. Six of the 12 characters (three from males, three from females) used to identify members of the *D. bioculata* species-group are based on setal colouration (Mickel, 1928, 1936; Manley & Pitts, 2007). For each of these colour characters, at least some taxa show an intermediate state (Tables 6.1-4). This intermediate state expresses itself as interspersed yellow to red or black setae (*e.g.*, Fig. 6.2: B, F) in an area that should be either entirely black (*e.g.*, Figs 6.2: A, G) or entirely yellow to red (*e.g.*, Figs 6.2: C, D), but not both. Setal colouration of female apical terga is the most polymorphic diagnostic character in the species-group, with six states (Table 6.1). Intermediate forms between these character states are abundant, with *D. bioculata* and *D. lepeletierii* providing excellent examples. *Dasymutilla bioculata* females have the fringes of T2-3 clothed with black setae (Fig. 6.2J), while most *D. lepeletierii* specimens have T3 entirely clothed with silver setae and T2 with a medial spot of silver setae (Fig. 6.2M). Intermediate forms of this character are common, however, with many *D. lepeletierii* specimens having the terga ranging from T2 completely black and T3 entirely silver (Fig. 6.2L) to T2 completely black and T3 mostly black with a small medial silver spot (Fig. 6.2K).

Intermediate forms were also found for the remaining six structural characters (Mickel, 1928, 1936; Manley & Pitts, 2007) (Tables 6.1- 4). For both males and females, the presence or absence of a carina on the antennal scrobe is used to separate species (Mickel, 1936). This character was already known by Mickel to vary within a taxon, because *D. ch. ursula* was placed in the key twice, once for individuals lacking this carina and again for individuals having a disticnt carina (Mickel, 1936, female couplets 66 & 88). Furthermore, three females were diagnosed as having a feeble carina (*D. sulcatulla, D. mel. melanippe,* and *D. mel. conformis*), emphasizing the variation seen in this character. Multiple species vary considerably in this trait (Tables 6.1-4). The structural character used to diagnose *D. sulcatulla* is also variable. Females of *D. sulcatulla* were initially diagnosed by the presence of a sulcate furrow on the antennal flagellomeres, although the author himself questioned the validity of this character because one specimen had the furrow present on fewer flagellar segments (Mickel, 1928). More recent studies documented further variation in this state with some specimens completely lacking a sulcation on the flagellum (Manley, 1996). In females, general setal length, or "shagginess", has also been used to separate species, like *D. cr. creusa* (Fig. 6.1A) from *D. praegrandis* (Fig. 6.1B). By its very nature, this character is subjective and can vary greatly with the condition of a specimen (KAW, DGM pers. obs.). Additionally, "shagginess" can vary within a single species, such as *D. bioculata*, where eastern forms (Fig. 6.1D: Nebraska) have shorter and sparser dorsal setae than western forms (Fig. 6.1C: Washington). As a final example, presence or absence of a seta-filled pit on the male S2 is used extensively to separate species (Mickel, 1936; Manley & Pitts, 2007). In the literature (Mickel, 1928, 1936), *D. bioculata* and *D. ch. ursula* are diagnosed as lacking this pit, but they often have a weak indentation on S2 (Table 6.4). Furthermore, some specimens of *D. ch. chiron* possess a well defined sternal pit filled with setae (Table 6.4).

Variation was also seen within populations of these taxa. On June 10, 2009, KAW and George C. Waldren collected 27 males and 23 females of *D. praegrandis* at a blown-out sand dune, five miles north of Flynn, in Leon County, Texas. This species was previously known from only a limited number of specimens (Mickel, 1928). According to Mickel, this species included only individuals with large body size; similarities in size and distribution were used to associate the male and female. In the newly collected specimens, females range from 7 to 13mm and males from 10 to 15mm. Two of the males were identified as *D. pr. praegrandis* by the entirely black dorsal setae on the mesosoma (Fig. 6.2G), while three males were identified as *D. pr. russata* by the presence of golden setae on the vertex, pronotum, and mesonotum (Fig. 6.2D). The remaining 22 specimens were intermediate, however, having the head and pronotum clothed with black setae and the mesonotum covered, entirely or in part, with golden setae (Figs. 6.2 E, F). Additionally, *D. praegrandis* supposedly differs from *D. lepeletierii* and *D. pyrrhus* by the presence of a distinct carina on the antennal scrobe (Mickel, 1928). Of the 27 males examined, only two have a distinct carina, while 14 have a feeble carina and 11 completely lack a carina. Variation in the scrobal carina does not correlate with variation in any other characters in this series, including mesosomal colouration. The males vary in no other characters than the ones discussed here and are undoubtedly all conspecific. The females, while diagnosed as lacking a carina on the antennal scrobe (Mickel, 1928), show similar variation in this trait.

Another example of variation within populations can be seen in series of *D. bioculata/ D. ch. ursula* from the Museum of Comparative Zoology, Harvard University. Twelve females and 10 males were collected from July 20-21, 1954 at Great Sand Dunes National Park, Colorado by H.E. & M.A. Evans. All of the females are *D. ch. ursula*, but there is significant variation in the setal colouration of the apical metasomal segments. Five of the females have silver setae, at least laterally, on metasomal segments 3-5 (Fig. 6.2H), as is typical for *D. ch. ursula*. The seven remaining specimens, however, have metasomal segment 5 and sometimes segment 4 completely clothed with black setae (Fig. 6.2I). Of the males at this site, two specimens are clearly *D. ch. ursula*, two specimens are clearly *D. bioculata*, and the remaining six specimens are intermediate forms. Mickel (1936) separated males of *D. bioculata* from *D. ch. ursula* by setal colouration: T3-6 are clothed entirely with black setae in *D. bioculata* (Fig. 6.2A), while yellow to red in *D. ch. ursula* (Fig. 6.2C). The intermediate specimens all have interspersed patterns of black and orange setae on the apical terga (Fig. 6.2B). The immense variation in these setal patterns in both sexes suggests that differences in apical setal colouration are not sufficient to separate species of either sex. Further confounding this situation is variation in the pit of S2. Both *D. bioculata* and *D. ch. ursula* lack a seta-filled pit on S2, although specimens of *D. bioculata* have been recorded with a slight indentation loosely filled with setae on S2. Of these ten males, six completely lack a pit on S2, while four, including the clearly identifiable *D. ch. ursula* specimens, have a weak indentation.

Finally, size variation is comparable in nearly all members of the *D. bioculata* species-group (Tables 6.2, 6.4). In all cases, species with a smaller range of lengths can be explained by the limited availability of specimens to measure.

Mating study

At Oasis State Park, Roosevelt Co., New Mexico, and Pedernales Falls State Park, Blanco Co., Texas DGM collected numerous specimens in the *D. bioculata* species-group, including mating pairs. It was noted at the time that the mating pairs did not conform to the then-accepted criteria for the species.

Numerous females of *D. cr. creusa* from Oasis State Park were observed crawling on the ground in an area of sand dunes, while males flew above the area searching for females. Seven mating pairs were collected. The male of *D. cr. creusa* was then unknown. Four of the males from the mating pairs were *D. ch. ursula*. The other three males did not match any species known at the time of collection, but were subsequently recognized as *D. bimaculata* (Manley & Pitts, 2007), which was known only from Mexico upon description.

Numerous females and occasional males of *D. mel. melanippe* and *D. mel. conformis* were observed at Pedernales Falls State Park. Four mating pairs were collected. The females of all four mating pairs were *D. mel. conformis*, for which the male was unknown. Three of the males were identified as *D. ch. ursula*, while the fourth was identified as *D. ch. chiron.*

Only seven other mating pairs from within this species-group were examined. In five of the seven, females and males represented different "species" (Table 6.6).

Geographic study

Each taxon in the *D. bioculata* species-group shares an overlapping distribution with at least one of its relatives (Fig. 6.3). The two species with the smallest geographic ranges, *D. pyrrhus* in Florida and southern Georgia, and *D. sulcatulla* in the Big Bend region of Texas and northern Mexico also share part of their range with more widespread species. *Dasymutilla pyrrhus* and *D. lepeletierii* are both found in Florida and Georgia (Fig. 6.3A); while *D. bioculata, D. cr. creusa, D. ch. chiron, D. ch. ursula,* and *D. sulcatulla* are all found in western Texas.

The distribution of the male of *D. bioculata* is far more extensive than that of the associated female (Fig. 6.3B). Males and females of *D. bioculata* are both abundant in the Great Lakes region, northern and eastern Great Plains regions, and mountainous northwestern USA and southwestern Canada. Males, however, extend beyond this range into New Mexico, Utah, southwestern Colorado, western Oklahoma, and northern and western Texas, even though no female *D. bioculata* have been found in those regions

Table 6.6. Associated sexes examined in the *D. bioculata* species-group.

Collection Data	Males	Females
USA: NM: Roosevelt Co., Oasis State Park, 30.Jun.1992, D.G.Manley coll. (DGMC)	*D. bimaculata*	*D. cr. creusa*
USA: NM: Roosevelt Co., Oasis State Park, 4.Sep.1992, D.G.Manley coll. (DGMC)	*D. bimaculata*	*D. cr. creusa*
USA: NM: Roosevelt Co., Oasis State Park, 5.Sep.1992, D.G.Manley coll. (DGMC)	*D. bimaculata*	*D. cr. creusa*
USA: WY: Fremont Co.: Riverton: 11.Jul.1955: G.E.Bohart coll. (EMUS)	*D. bioculata*	*D. ch. ursula*
USA: OK: Grayson Co.: Lake Texoma, 2 mi. E Willis, Jul.1965: R.M.Bohart coll. (UCDC)	*D. bioculata*	*D. cr. creusa*
USA: TX: Blanco Co.: Pedernales Falls St. Park, 10.Jun.1992, D.G.Manley coll. (DGMC)	*D. ch. chiron*	*D. mel. conformis*
USA: TX: Kennedy Co., Risken Ranch, 21 Jul. 1977, H.E.Fields coll. (DGMC)	*D. ch. chiron*	*D. mel. melanippe*
USA: AZ: Graham Co., Bonita, 15.Jul.1992, D.G.Manley coll. (DGMC)	*D. ch. ursula*	*D. ch. ursula*
USA: CO: Mesa Co., Colorado River, S.Loma, 10.Jul.1963, C.J.McCoy coll. (DGMC)	*D. ch. ursula*	*D. ch. ursula*
USA: UT: Garfield Co., Calf Creek, 28-29.Jul.1982, A.&K.Menke coll. (DGMC)	*D. ch. ursula*	*D. ch. ursula*
USA: TX: Blanco Co.: Pedernales Falls St. Park, 10.Jun.1992, D.G.Manley coll. (DGMC)	*D. ch. ursula*	*D. mel. conformis*
USA: TX: Blanco Co.: Pedernales Falls St. Park, 10.Jun.1992, D.G.Manley coll. (DGMC)	*D. ch. ursula*	*D. mel. conformis*
USA: NM: Roosevelt Co., Oasis State Park, 5.Sep.1992, D.G.Manley coll. (DGMC)	*D. ch. ursula*	*D. cr. creusa*
USA: NM: Roosevelt Co., Oasis State Park, 5.Sep.1992, D.G.Manley coll. (DGMC)	*D. ch. ursula*	*D. cr. creusa*
USA: NM: Roosevelt Co., Oasis State Park, 5.Sep.1992, D.G.Manley coll. (DGMC)	*D. ch. ursula*	*D. cr. creusa*
USA: NM: Roosevelt Co., Oasis State Park, 5.Sep.1992, D.G.Manley coll. (DGMC)	*D. ch. ursula*	*D. cr. creusa*
USA: TX: Blanco Co.: Pedernales Falls St. Park, 12.Sep.1992, D.G.Manley coll. (DGMC)	*D. ch. ursula*	*D. mel. conformis*
USA: Highlands Co., Archbold Biol.St., Lake Placid, 18.May.2005, D.G.Manley (DGMC)	*D. pyrrhus*	*D. pyrrhus*

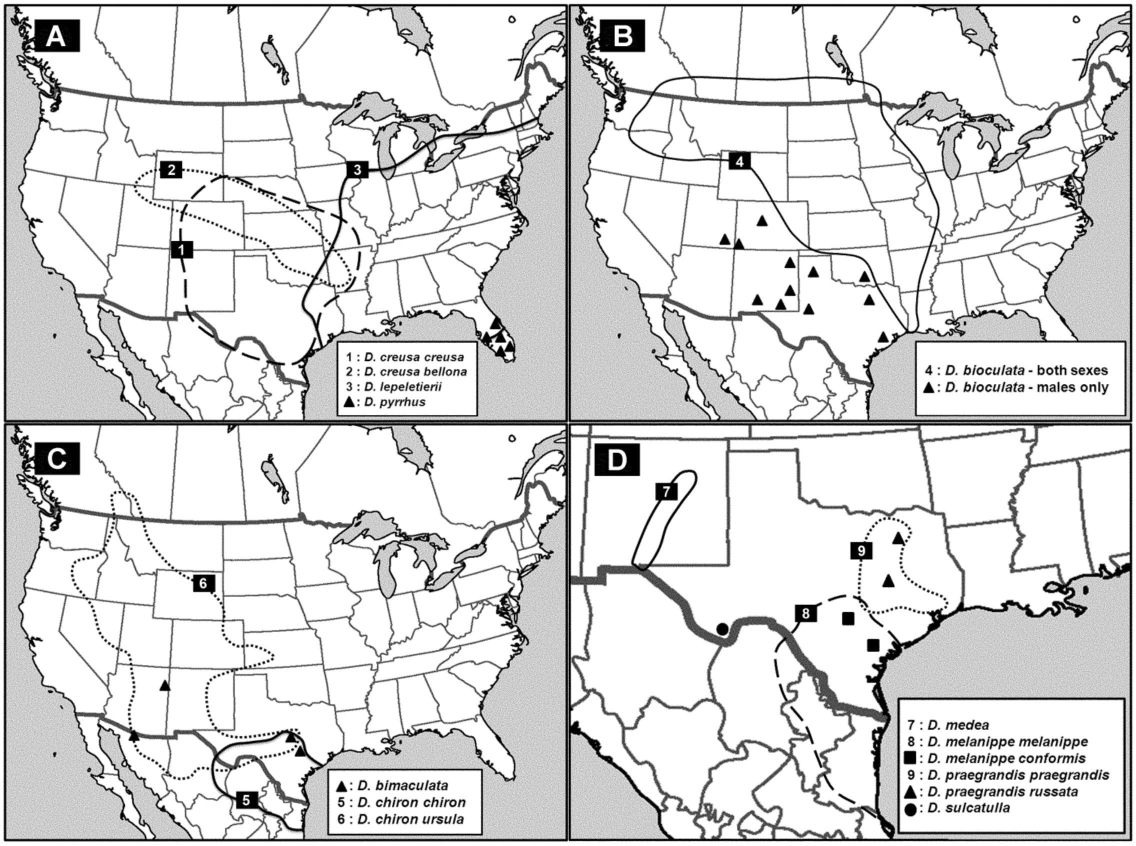

Fig. 6.3. Distribution maps.

(Fig. 6.3B). These southwestern males have been attracted to caged females of *D. ch. ursula* and *D. cr. creusa* (Table 6.6). Males from different regions, and even males that have mated with females from different nominal taxa, are morphologically indistinguishable.

Molecular study

For the 19 individuals of the *D. bioculata* species-group sequenced for the ITS1 locus, all sequences were 434 bp in length. The Kimura 2-Parameter model (Kimura, 1980) was determined to be optimal for this dataset. Genetic distances calculated under these criteria are provided in Table 6.7. The ITS1 sequences of the ingroup were polymorphic at only one of the 434 sites (0.22%). One of the three *D. bioculata* specimens (JP363) had a unique substitution at position 65; the remaining 18 specimens from the *D. bioculata* species-group had identical ITS1 sequences (Table 6.7). Mean ITS1 genetic distances between the outgroup taxa and members of the *D. bioculata* species-group ranged from 1.10-3.94% (Table 6.7). Pairwise genetic distances were also calculated by hand without the use of a model as per Pilgrim and Pitts (2006). Genetic distances recovered from ingroup comparisons were identical in both analyses (with and without a model); direct pairwise genetic distances were slightly lower in comparisons between members of the *D. bioculata* species-group and the outgroups (1.09-3.72%).

In ITS2, the sequences varied from 1016-1019 bp in length, but the alignment contained 1020 bases because of two insertions. The Kimura 2-Parameter model (Kimura, 1980) with Gamma distribution (K2P+G) was determined to be optimal for this dataset. Genetic distances calculated under these criteria are provided in Table 6.8. The ITS2 sequences of the ingroup were polymorphic at 13 of the 1020 sites (1.28%). Intraspecific variation in the *D. bioculata* species-group ranged from 0.00-0.20%, while interspecific comparisons within the *D. bioculata* species-group yielded genetic distances from 0.00-0.40% (Table 6.8). Mean ITS2 genetic distances between the outgroup taxa and members of the *D. bioculata* species-group ranged from 3.13-4.34% (Table 6.8). As seen in the ITS1 dataset, genetic distances recovered from ingroup comparisons were identical in both analyses (with and without a optimal model); direct pairwise genetic distances were slightly lower in comparisons between outgroup taxa and members of the *D. bioculata* species-group (2.89-3.98%).

Table 6.7. ITS1 ranges and means of genetic distances.

		bimaculata	*bioculata*	*chiron*	*creusa*	*lepeletierii*	*melanippe*	*praegrandis*	*pyrrhus*
bimaculata	range	n/a	0.00-0.22%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	mean	**n/a**	**0.11%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**
bioculata	range		0.00-0.22%	0.00-0.22%	0.00-0.22%	0.00-0.22%	0.00-0.22%	0.00-0.22%	0.00-0.22%
	mean		**0.11%**	**0.11%**	**0.11%**	**0.11%**	**0.11%**	**0.11%**	**0.11%**
chiron	range			0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
(*inc. ursula*)	**mean**			**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**	**0.00%**
creusa	range				n/a	0.00%	0.00%	0.00%	0.00%
	mean				**n/a**	**0.00%**	**0.00%**	**0.00%**	**0.00%**
lepeletierii	range					0.00%	0.00%	0.00%	0.00%
	mean					**0.00%**	**0.00%**	**0.00%**	**0.00%**
melanippe	range						0.00%	0.00%	0.00%
	mean						**0.00%**	**0.00%**	**0.00%**
praegrandis	range							0.00%	0.00%
(*inc. russata*)	**mean**							**0.00%**	**0.00%**
pyrrhus	range								0.00%
	mean								**0.00%**
Outgroups									
gloriosa	**mean**	**3.37%**	**3.49%**	**3.37%**	**3.37%**	**3.37%**	**3.37%**	**3.37%**	**3.37%**
gorgon	**mean**	**1.10%**	**1.22%**	**1.10%**	**1.10%**	**1.10%**	**1.10%**	**1.10%**	**1.10%**
occidentalis	**mean**	**2.23%**	**2.35%**	**2.23%**	**2.23%**	**2.23%**	**2.23%**	**2.23%**	**2.23%**
quadriguttata	**mean**	**3.82%**	**3.94%**	**3.82%**	**3.82%**	**3.82%**	**3.82%**	**3.82%**	**3.82%**

Table 6.8. ITS2 ranges and means of genetic distances.

		bimaculata	*bioculata*	*chiron*	*creusa*	*lepeletierii*	*melanippe*	*praegrandis*	*pyrrhus*
bimaculata	range	n/a	0.10%	0.10-0.20%	0.10%	0.30%	0.10%	0.10-0.30%	0.30%
	mean	**n/a**	**0.10%**	**0.15%**	**0.10%**	**0.30%**	**0.10%**	**0.20%**	**0.30%**
bioculata	range		0.00-0.20%	0.00-0.30%	0.00-0.20%	0.20-0.40%	0.00-0.20%	0.00-0.40%	0.20-0.40%
	mean		**0.10%**	**0.15%**	**0.10%**	**0.20%**	**0.10%**	**0.20%**	**0.20%**
chiron	range			0.00-0.20%	0.00-0.10%	0.20-0.30%	0.00-0.10%	0.00-0.30%	0.20-0.30%
(*inc. ursula*)	**mean**			**0.10%**	**0.05%**	**0.25%**	**0.05%**	**0.15%**	**0.25%**
creusa	range				n/a	0.20%	0.00%	0.00-0.20%	0.20%
	mean				**n/a**	**0.20%**	**0.00%**	**0.10%**	**0.20%**
lepeletierii	range					0.00%	0.20%	0.20-0.40%	0.00%
	mean					**0.00%**	**0.20%**	**0.30%**	**0.00%**
melanippe	range						0.00%	0.00-0.20%	0.20%
	mean						**0.00%**	**0.10%**	**0.20%**
praegrandis	range							0.00-0.20%	0.20-0.40%
(*inc. russata*)	**mean**							**0.10%**	**0.30%**
pyrrhus	range								0.00%
	mean								**0.00%**
Outgroups									
gloriosa	**mean**	**3.95%**	**4.02%**	**4.02%**	**3.96%**	**4.07%**	**3.96%**	**4.08%**	**4.07%**
gorgon	**mean**	**3.13%**	**3.20%**	**3.20%**	**3.14%**	**3.25%**	**3.14%**	**3.14%**	**3.25%**
occidentalis	**mean**	**4.05%**	**4.12%**	**4.12%**	**4.06%**	**4.29%**	**4.06%**	**4.06%**	**4.29%**
quadriguttata	**mean**	**4.33%**	**4.34%**	**4.28%**	**4.22%**	**4.33%**	**4.22%**	**4.22%**	**4.33%**

Discussion

Evidence for synonymy in the D. bioculata species-group

Every morphological character historically used to differentiate members of the *D. bioculata* species-group occurs in an intermediate state or is polymorphic in some taxa (Tables 6.1-4). No new morphological characters capable of consistently separating these taxa were discovered in this study. No members of the *D. bioculata* species-group can be consistently diagnosed by morphological synapomorphies or unique combinations of character states.

Males of different taxa (*e.g., D. bimaculata, D. bioculata* & *D. ch. chiron*) have been attracted to caged females of the same taxon (*e.g., D. cr. creusa*). Also, males from a single taxon (e.g., *D. bioculata*) have been attracted to caged females of multiple taxa (*e.g. D. bioculata, D. ch. ursula,* & *D. cr. creusa*). Males and females of the *D. bioculata* species-group are not exclusive in mate selection.

These taxa also overlap with one-another in distribution (Fig. 6.3) and intermediate color forms are found in transition zones. Associated sexes of nominal taxa are known to have disparate ranges (Fig. 6.3B). None of the taxa has a unique geographic range.

Previous studies in *Dasymutilla* (Pilgrim et al, 2008, 2009) and other Mutillidae (Wilson & Pitts, 2008) have used genetic distances in ITS1 and ITS2 to supplement phylogenetic or morphological data in studies of species validity. Our molecular data show extremely low genetic distances between the taxa of interest (< 0.5% in ITS1 and ITS2). These genetic distances are lower than those previously recorded in conspecific taxa (Pilgrim & Pitts, 2006; Pilgrim et al., 2008, 2009) and are much lower than genetic distances observed between members of the *D. bioculata* species-group and other *Dasymutilla* species (Tables 6.7, 6.8). Additionally, none of the included taxa had any unique base-pair substitutions that were present in each individual.

The nominal taxa studied here do not have unique combinations of morphological character states. Ecological characteristics, like distribution and sexual attraction, overlap significantly. Unique molecular sequences were not found, even though the examined regions have revealed species-level differences in other Mutillidae. Therefore, using the phylogenetic species concept, we conclude that the *D. bioculata* species-group is a single species with *Dasymutilla bioculata* having taxonomic priority. *Dasymutilla bimaculata, D. ch. chiron, D. ch. ursula, D. cr. creusa, D. cr. bellona, D. lepeletierii, D. medea, D. mel. melanippe, D. mel. conformis, D. pr. praegrandis, D. pr. russata, D. pyrrhus,* and *D. sulcatulla* are **junior synonyms** of *D. bioculata*. *Dasymutilla bioculata* can be consistently recognized in both sexes by the unique combination of characters listed in the diagnosis below, supporting this synonymy under the phylogenetic species concept.

The subtaxa cannot be consistently defined by unique combinations of characters and none of them is geographically isolated from the other *D. bioculata* populations. As such, subspecies designation cannot be supported for any synonymous forms of *D. bioculata*.

Dasymutilla bioculata (Cresson)

Mutilla fenestrata Lepeletier, 1845. Hist. Nat. Ins. Hym., 3: 627. ♂. Preocc. Klug, 1821. **syn.n.**

Mutilla bioculata Cresson, 1865.Ent. Soc. Phila., Proc. 4: 431. ♂.

Mutilla Creusa Cresson, 1865.Ent. Soc. Phila., Proc. 4: 431. ♀. **syn.n.**

Mutilla Medea Cresson, 1865.Ent. Soc. Phila., Proc. 4: 432. ♀. **syn.n.**

Mutilla Bellona Cresson, 1865.Ent. Soc. Phila., Proc. 4: 434. ♀. **syn.n.**

Mutilla Aegina Cresson, 1865.Ent. Soc. Phila., Proc. 4: 435. ♀.

Mutilla (Sphaeropthalma) Chiron Blake, 1872. Amer. Ent. Soc., Trans. 4: 72. ♂. **syn.n.**

Mutilla Ursula Cresson, 1875. Amer. Ent. Soc., Trans. 5: 120. ♂. **syn.n.**

Mutilla Ursula var. *texana* Cresson, 1875. Amer. Ent. Soc., Trans. 5: 120. ♂. **syn.n.**

Mutilla pyrrhus Fox, 1899. Amer. Ent. Soc., Trans. 25: 243. ♂. **syn.n.**

Mutilla Lepeletierii Fox, 1899. Amer. Ent. Soc., Trans. 25: 244. N. name for *M. fenestrata* Lepeletier. **syn.n.**

Mutilla chlamydata Melander, 1903. Amer. Ent. Soc., Trans. 29: 299. ♀.

Dasymutilla vierecki Rohwer, 1910. Ent. Soc. Wash., Proc. 12: 49. ♀. **syn.n.**

Dasymutilla ferrugata var. *balabetei* Rohwer, 1912. U.S. Natl. Mus., Proc. 41: 456. ♀. **syn.n.**

Dasymutilla georgiana Rohwer, 1912. U.S. Natl. Mus., Proc. 41: 456. ♀. **syn.n.**

Dasymutilla plesia Rohwer, 1912. U.S. Natl. Mus., Proc. 41: 456. ♀. **syn.n.**

Dasymutilla ferrugata var. *ballabetei* (sic) Bradley, 1916. Amer. Ent. Soc., Trans. 42: 327. ♀. **syn.n.**

Dasymutilla praegrandis Mickel, 1928. U.S. Natl. Mus., Bul. 143: 148. ♀, Male. **syn.n.**

Dasymutilla praegrandis var. *russata* Mickel, 1928. U.S. Natl. Mus., Bul. 143: 152. ♂. **syn.n.**

Dasymutilla melanippe Mickel, 1928. U.S. Natl. Mus., Bul. 143: 152. ♀. **syn.n.**

Dasymutilla melanippe var. *conformis* Mickel, 1928. U.S. Natl. Mus., Bul. 143: 153. ♀. **syn.n.**

Dasymutilla lauta Mickel, 1928. U.S. Natl. Mus., Bul. 143: 154. ♀. **syn.n.**

Dasymutilla sulcatulla Mickel, 1928. U.S. Natl. Mus., Bul. 143: 155. ♀. **syn.n.**

Dasymutilla bimaculata Manley & Pitts, 2007. Zootaxa. 1487: 30. ♂. **syn.n.**

Diagnosis of D. bioculata:

Female: Head narrower than pronotum, rounded posteriorly, and lacking tubercles on posterior margin; mesosoma longer than broad, with well-defined broad scutellar scale with transverse carina anterior to it; dorsal setae of head and mesosoma yellow, orange, red, or black; propodeal setae concolourous with those of mesonotum; pygidium longitudinally striate.

Male: Cuspis of genitalia curving downward apically, glabrous dorsally and densely setose ventrally and laterally; clypeus weakly bidentate anteriorly, with anterior teeth connected by a transverse lamella; pronotum emarginated anteromedially; axillae truncate posteriorly; tegulae glabrous posteriorly; T2 with yellow to orange integumental maculae; propodeum coarsely reticulate; pygidium with well defined apical setal fringe.

Remarks: With this taxonomic treatment, *D. bioculata* has 23 synonyms, which is more than any other mutillid species and alludes to the immense variation observed in this species.

Intraspecific variation in D. bioculata

Dasymutilla bioculata displays tremendous morphological polymorphism in both males and females. Many of these polymorphic characters are currently used to diagnose species in other *Dasymutilla* species-groups. As recorded in other *Dasymutilla* (Pilgrim et al., 2008, 2009), setal colour patterns are shown to be widely variable within a species and inadequate for diagnosis (Figs 6.1, 5.2). Polymorphism in the carinate condition of the antennal scrobe in both sexes is also revealed in *D. bioculata,* suggesting the need to scrutinize this widely used character as well. Perhaps the most surprising polymorphism in *D. bioculata* is the presence or absence of a seta-filled pit on the second metasomal sternum. In Mickel's (1936) most recent and widely used key, this character is used to separate 24 taxa without a seta-filled pit from 38 taxa that have this pit, or another setal modification of S2. In the only Neotropical key (Manley & Pitts, 2007), this character is used to separate 30 taxa that lack a seta-filled pit from 44 species that have a pit or other sternal modification. This character, while apparently useful for species identification, must be carefully scrutinized in *Dasymutilla* and the related genus *Traumatomutilla* André in light of its polymorphism within *D. bioculata*.

Identification of D. bioculata

Until a new key to the Nearctic species is published, the best method for identification is to use the most recent keys to *Dasymutilla* (Mickel, 1936; Manley & Pitts, 2007) to identify the formerly distinct species and subspecies. After a specimen is identified as a *D. bioculata* synonym, it should be checked against the diagnostic characters listed above for verification.

In some regions, *D. bioculata* is easily confused with other species. Many females from Texas (*e.g.*, Fig. 6.1E) could be misidentified as *D. klugiodes* Mickel, based on the lack of a carina on the antennal scrobe and metasomal setal colouration (Mickel, 1936). If a specimen keys out to *D. klugiodes*, the genal carina and pygidium should be carefully examined. *Dasymutilla klugiodes* has a weak genal carina, while *D. bioculata* completely lacks a genal carina. Additionally, *D. klugiodes* has slightly wavy and apically convergent striae on the pygidium, while *D. bioculata* has straight and parallel striae on the pygidium.

Eastern males of *D. bioculata* can easily be misidentified as *D. quadriguttata* (Mickel, 1936; Pilgrim *et al.,* 2009). The clypeus (Pilgrim *et al*., 2009: figs 11, 12) and genitalia (Pilgrim *et al*., 2009: figs 5-10) should be compared in any specimens that key out to *D. lepeletierii, D. hersilia* Mickel or *D. hora* Mickel using Mickel's (1936) key. Males from the western range of *D. bioculata* that have a pit on S2 will most likely be misidentified as *D. occidentalis comanche* (Blake) or *D. klugii* (Gray) based on mesosomal setal colouration. *Dasymutilla bioculata* can immediately be separated from these taxa by the presence of orange integumental spots on T2, rather than having the metasoma completely black.

Mexican specimens of *D. bioculata* will be difficult to identify using the only current key (Manley & Pitts, 2007). Some females with antennal scrobes ecarinate will terminate at couplet 36, where they can be recognized as *D. bioculata* by having the mesosoma entirely clothed with black setae (*e.g.,* Fig. 6.1E). Additional females with antennal scrobes carinate will key out at either couplet 64 with another *D. bioculata* synonym (*D. sulcatulla*) or couplet 66, where they can be separated from *D. sackenii* (Cresson) and *D. coccineohirta* (Blake) by the entirely black mesosomal setal colour (*e.g.,* Fig. 6.1E). Some females (*e.g.,* Fig. 6.1A) will terminate at couplet 42, which includes two additional *D. bioculata* synonyms. The remaining females are not problematic and will key out to *D. ch. chiron, D. ch. ursula* or *D. sulcatulla*, which are all junior synonyms of *D. bioculata*. All known males of *D. bioculata* occurring in Mexico will key out adequately to *D. bimaculata, D. bioculata, D. ch. chiron, D. ch. ursula*, or *D. sulcatulla* using Manley and Pitts (2007).

Species-Group Implications

All synonyms of *D. bioculata*, except for *D. bimaculata* (which was not placed in a species-group when it was described), were placed in the *D. bioculata* species-group (Mickel, 1928). No other extant species have been included in this species-group. Two fossil *Dasymutilla* species from Dominican amber were also placed in the *D. bioculata* species-group based on species-group characters of males recognized by Mickel (1928), and their position in Mickel’s (1936) key (Manley & Poinar, 1991, 1999). They are clearly not conspecific with *D. bioculata* because the metasoma is concolourous black in both fossil species, while all specimens of *D. bioculata* have some orange to red integumental colouration on T2 at least. Additionally, the type of *D. albifasciata* Manley & Poinar has the genitalia extruded (Manley & Poinar, 1999) revealing that they are unlike those of *D. bioculata* (KAW pers. obs.). Specifically, the cuspis of *D. albifasciata* is roughly half the free length of the parameres, straight, and setose throughout (cuspis >0.65X free length of paramere, curving downward apically, and glabrous dorsally in *D. bioculata*). The two fossil species seem to be related to modern Caribbean *Dasymutilla* species, which potentially belong to a single monophyletic unit, but have never been placed into a species-group (KAW pers. obs.).

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CHAPTER 7

CARIBBEAN AND MEXICAN ADDITIONS TO THE *DASYMUTILLA BIOCULATA* SPECIES-GROUP (HYMENOPTERA: MUTILLIDAE)[6]

Introduction

Recent studies have effectively used mutillid wasps to elucidate biogeographical patterns in the western Nearctic Region (Wilson and Pitts 2008, 2010a, 2010b, 2011; Pitts et al. 2010), but these studies have virtually neglected Central America. It is often difficult to get specimens from Central America and taxonomic treatments in the United States historically have preceded treatments of Mexican and other Neotropical groups. For example, in *Dasymutilla* Ashmead a monograph of American species North of Mexico was published nearly 80 years before the Neotropical taxa were revised (Mickel 1928, Manley and Pitts 2007).

This common pattern is repeated in modern studies of *Dasymutilla bioculata* (Cresson) and its relatives. Fourteen putative Nearctic species were recently synonymized with *D. bioculata* (Williams et al. 2011). These represented all known members of the *D. bioculata* species-group. The long synonymy list is correlated with significant morphological variation. In fact, the only unique synapomorphy of *D. bioculata* recognized by Williams et al. (2011) was the shape of the genitalic cuspis (Figs. 7.1: A-C). Recently, five species have been studied with similar genitalia to those of *D. bioculata*. Three of these species, *D. nocticaro* Manley & Pitts, *D. truxali* Manley

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[6] This chapter has been formatted for submission to *Annals of the Entomological Society of America*.

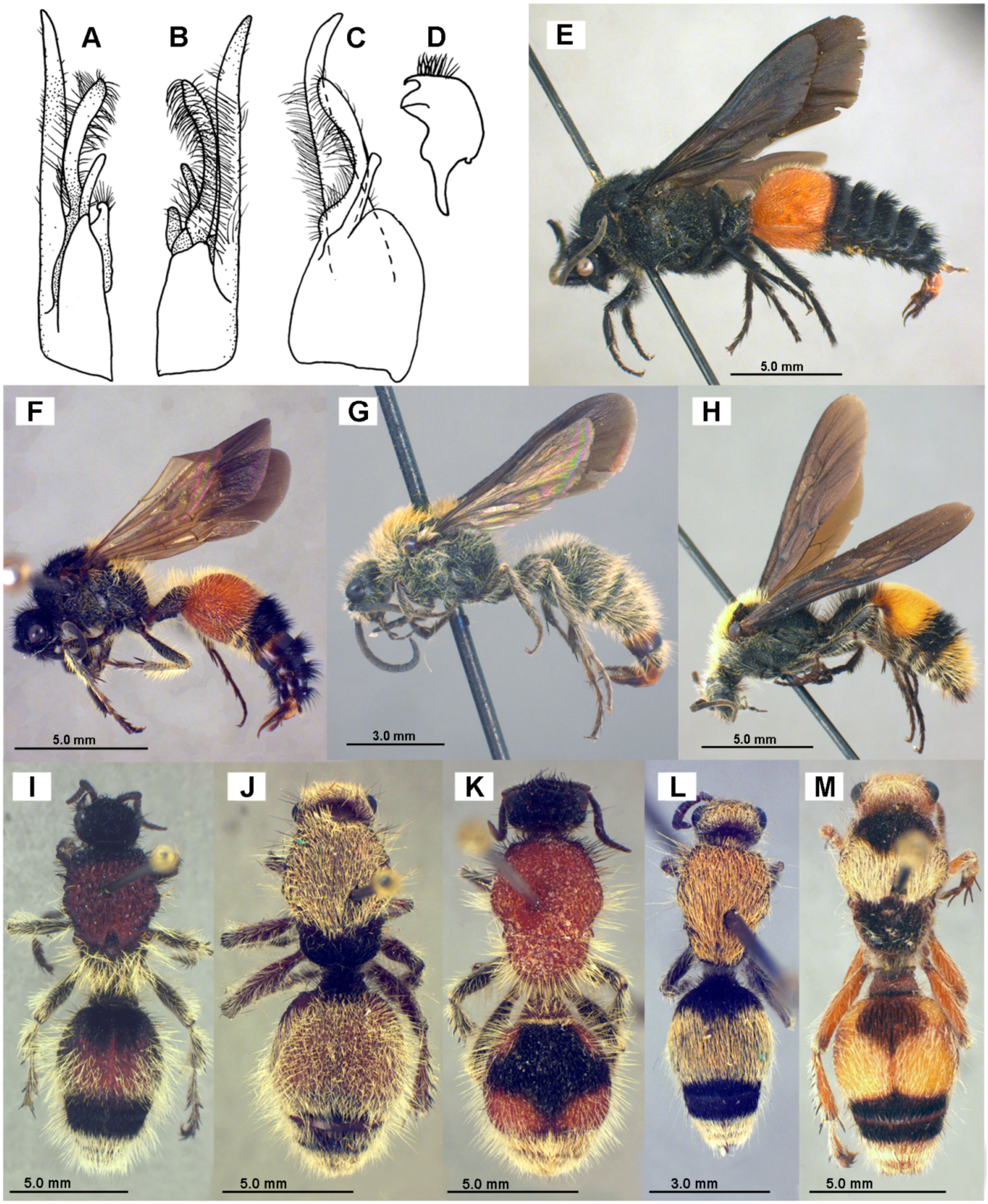

Fig. 7.1. Figures A-D, Male genitalia: dorsal view, ventral view, lateral view, and penis valve. Figs. A-D: *Dasymutilla bioculata*. Figures E-H. Habitus, lateral view, male. Fig. E: *D. bouvieri*; Fig. F: *D. insulana*; Fig. G: *D. latebalteata*; Fig. H: *D. nocticaro*. Figures I-M, Habitus, dorsal view, female. Fig. I: *D. bouvieri*; Fig. J: *D. chionothrix*; Fig. K: *D. insulana*; Fig. L: *D. latebalteata*; Fig. M: *D. nocticaro*.

& Pitts, and *D. veracruz* Manley & Pitts, are found in Mexico and are known exclusively from the male sex; these species have never been placed into a species-group. The other two taxa, *D. bouvieri* (Andre) and *D. insulana* Mickel, are found in the Caribbean and are known from both sexes; Genaro (1997) placed these species into a group he defined and called Group II based on similarities in coloration of the speices. Genaro's (1997) species-groups, however, are only applicable to the Caribbean fauna and were not compared to the species-groups of Mickel (1928). Although the males of *D. bouvieri* and *D. insulana* were recognized and placed into a key by Genaro (1997), they have never been fully described.

Based on structural similarities to *D. bioculata*, the five aforementioned species and three species known only from females (*D. chionothrix* Manley & Pitts, *D. concordia* Manley & Pitts, & *D. latebalteata* Manley & Pitts) are transferred into the *D. bioculata* species-group. Using morphological and distributional data, sex associations are made in the Mexican fauna. Detailed descriptions for the males of *D. insulana* and *D. bouvieri* are provided. These newly defined species limits and species-group placements will be vital for future studies of this widespread lineage.

Materials and Methods

Pinned specimens of all species referred to in this study have been examined to compare morphological features. We use the acronyms: T1, T2, etc. to refer to the metasomal terga, and the abbreviations: S1, S2, etc. to refer to the metasomal sterna. The following codens represent collections that provided new specimens for this research.

AMNH	American Museum of Natural History, New York, USA.

CISC	Essig Museum of Entomology, Department of Entomological Sciences, University of California, Berkeley, California, USA.
CNCI	Canadian National Collection of Insects, Ottawa, Ontario, Canada.
CUIC	Cornell University Insect Collection, Department of Entomology, Ithaca, New York, USA.
DGMC	Donald G. Manley Collection, Florence, South Carolina, USA.
EMUS	Department of Biology Insect Collection, Utah State University, Logan, Utah, USA.
LACM	Insect Collection, Los Angeles County Museum of Natural History, Los Angeles, California, USA.
MCZC	Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA.
SEMC	Snow Entomological Museum, University of Kansas, Lawrence, Kansas, USA.
UCDC	The Bohart Museum of Entomology, University of California, Davis, California, USA.
UMSP	University of Minnesota Insect Collection, St. Paul, Minnesota, USA.
USNM	United States National Museum of Natural History (Smithsonian Institution), Washington, D.C., USA.

Results

Dasymutilla bioculata species-group Mickel, 1928

Female. *Diagnosis*: These females can be diagnosed by the following combination of characters: the head is narrower than the pronotum, is rounded posteriorly, and lacks tubercles on the posterior margin; the mandible is virtually straight and narrowly bidentate apically; the lateral mesonotal margins are rounded, the mesosoma is longer than broad, and has a well-defined, broad scutellar scale with a transverse carina anterior to the scale, this transverse carina is contiguously raised.

Male. *Diagnosis*: These males can be immediately recognized by unique genitalia, wherein the cuspis curves downward apically, and is glabrous dorsally, but densely setose ventrally and laterally (Figs. 7.1: A-C). The following characters are also useful for identification: the clypeus is weakly bidentate anteriorly, with the anterior teeth connected by a transverse lamella; the axillae are truncate posteriorly; the tegulae are glabrous posteriorly; the mesopleuron is rounded, not armed with a dorsal tooth or tubercle; the propodeum is coarsely reticulate; and the pygidium has a well defined apical fringe of setae.

Included taxa. This species-group now includes six species: *D. bioculata, D. bouvieri, D. chionothrix, D. insulana, D. latebalteata,* and *D. nocticaro*.

Discussion. Males can be immediately recognized by the unique genitalia, but females cannot be diagnosed by any unique characters. The females are all similar in appearance and share the aforementioned diagnostic features. These characters, however, are found in numerous other *Dasymutilla* species. For identification of the central American species, specimens should be keyed out using Manley and Pitts (2007) and then examined for the diagnostic features listed here.

Dasymutilla bioculata **(Cresson)**

(Figs. 7.1: A-D)

Mutilla fenestrata Lepeletier, 1845. Hist. Nat. Ins. Hym., 3: 627. ♂. Preocc.
Mutilla bioculata Cresson, 1865.Ent. Soc. Phia., Prc. 4: 431. ♂.
Mutilla Creusa Cresson, 1865.Ent. Soc. Phia., Prc. 4: 431. ♀.
Mutilla Medea Cresson, 1865.Ent. Soc. Phia., Prc. 4: 432. ♀.
Mutilla Bellona Cresson, 1865.Ent. Soc. Phia., Prc. 4: 434. ♀.
Mutilla Aegina Cresson, 1865.Ent. Soc. Phia., Prc. 4: 435. ♀.

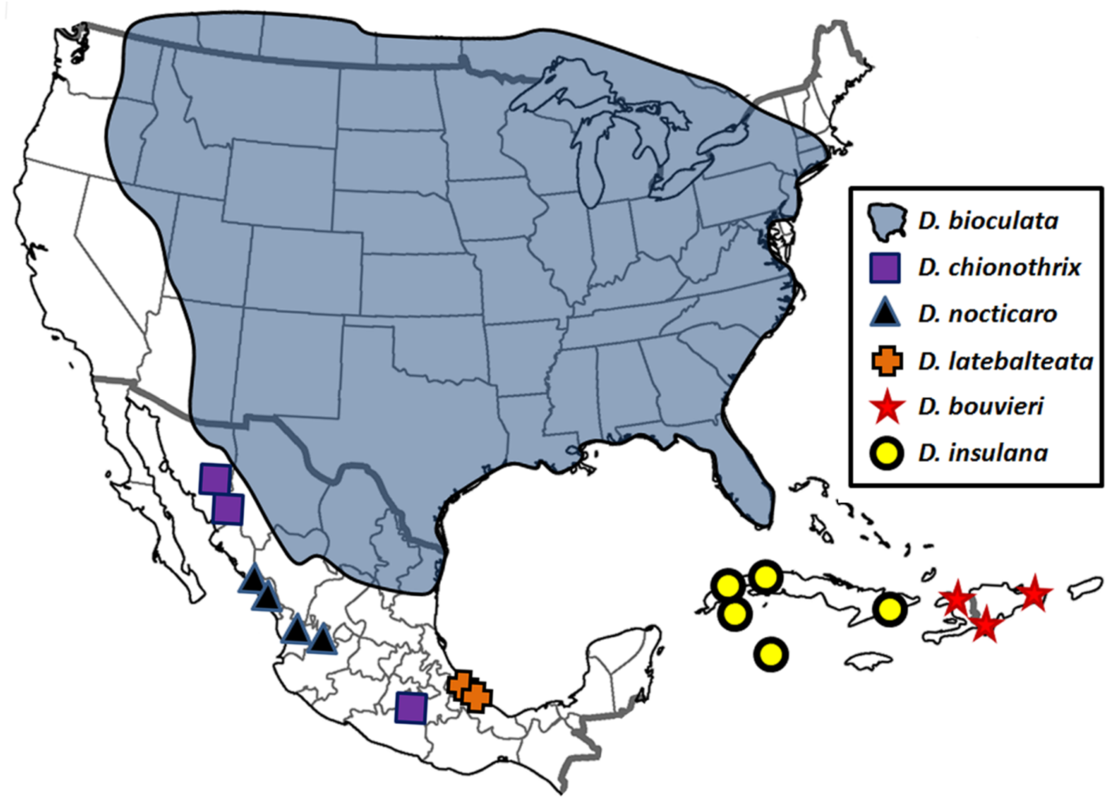

Fig. 7.2. Distribution of *Dasymutilla bioculata* species-group members.

Mutilla (Sphaeropthalma) Chiron Blake, 1872. Amer. Ent. Soc., Trans. 4: 72. ♂.

Mutilla Ursula Cresson, 1875. Amer. Ent. Soc., Trans. 5: 120. ♂.

Mutilla Ursula var. *texana* Cresson, 1875. Amer. Ent. Soc., Trans. 5: 120. ♂.

Mutilla pyrrhus Fox, 1899. Amer. Ent. Soc., Trans. 25: 243. ♂.

Mutilla Lepeletierii Fox, 1899. Amer. Ent. Soc., Trans. 25: 244. N. name for *M. fenestrata* Lepeletier.

Mutilla chlamydata Melander, 1903. Amer. Ent. Soc., Trans. 29: 299. ♀.

Dasymutilla vierecki Rohwer, 1910. Ent. Soc. Wash., Proc. 12: 49. ♀.

Dasymutilla ferrugata var. *balabetei* Rohwer, 1912. U.S. Natl. Mus., Proc. 41: 456. ♀.

Dasymutilla georgiana Rohwer, 1912. U.S. Natl. Mus., Proc. 41: 456. ♀.

Dasymutilla plesia Rohwer, 1912. U.S. Natl. Mus., Proc. 41: 456. ♀.

Dasymutilla ferrugata var. *ballabetei* (sic) Bradley, 1916. Amer. Ent. Soc., Trans. 42: 327. ♀.

Dasymutilla praegrandis Mickel, 1928. U.S. Natl. Mus., Bul. 143: 148. ♀, Male.

Dasymutilla praegrandis var. *russata* Mickel, 1928. U.S. Natl. Mus., Bul. 143: 152. ♂.

Dasymutilla melanippe Mickel, 1928. U.S. Natl. Mus., Bul. 143: 152. ♀.

Dasymutilla melanippe var. *conformis* Mickel, 1928. U.S. Natl. Mus., Bul. 143: 153. ♀.

Dasymutilla lauta Mickel, 1928. U.S. Natl. Mus., Bul. 143: 154. ♀.
Dasymutilla sulcatulla Mickel, 1928. U.S. Natl. Mus., Bul. 143: 155. ♀.
Dasymutilla bimaculata Manley & Pitts, 2007. Zootaxa. 1487: 30. ♂.

Female. *Diagnosis*. The female of this species can be separated from other *D. bioculata* species-group members by the following unique combination of characters: the head and mesosoma have concolorous integument, ranging from black to ferruginous; the dorsal setae of the mesosoma lack a specific pattern of contrasting setal coloration, the propodeal and pronotal setae are concolorous with the mesonotal setae; and the pygidium is longitudinally striate with at least 15 tightly spaced striae. 6-19 mm.

Male. *Diagnosis*. Males can be separated from other *D. bioculata* species-group members by coloration: T2 has yellow to orange integumental maculae or is entirely ferruginous; the legs, mesosomal pleurae, and propodeum are clothed with black setae; and the setae of T3-7 are concolorous, either black or orange. 8-20 mm.

Material Examined. Over 2300 specimens were examined by the authors (Williams et al. 2011).

Distribution. This is a widespread Nearctic species (Fig. 7.2) that is excluded only from northern Canada, the Pacific Region, the Mojave Desert, the Sonoran Desert and Baja California.

Biology. *Dasymutilla bioculata* is a recorded parasitoid of *Bembecinius quinquespinosus* (Say), *Bembix americana spinolae* Lepeletier, *Bembix amoena* Handlirsch, *Bembix pruinosa* Fox, *Bembix sayi* Cresson, *Bembix texana* Cresson, and *Microbembex monodonta* (Say) [Cottrell, 1936; Evans, 1966; Evans & O’Neill, 2007; Krombein, 1979; Manley & Deyrup ,1989; Mickel, 1924].

Discussion. Biology, identification, distribution, and variation of this species are discussed by Williams et al. (2011).

Dasymutilla bouvieri (André)

(Figs. 7.1: E, I)

Mutilla (Ephuta) Bouvieri André, 1898. Ent. Soc. Fra., Ann. 67:51. ♀.

Dasymutilla bouvieri (André)*;* Genaro, 1997. 33: 264. ♀♂.

Female. *Diagnosis.* This species can be separated from other members of the *D. bioculata* species-group by the scutellar armature, wherein there is a patch of dense black posteriorly directed setae covering the scutellar scale, and these setae are surrounded by the medial portion of a transverse carina anterior to the scutellar scale. Additionally, the head is black, contrasting with the dark orange mesosoma; the propodeum is clothed with erect white setae that contrast with the black mesonotal setae; T3 and the apical fringe of T2 are clothed with black setae, while T4-6 are clothed with white setae; and the pygidium has widely separated, irregular, wavy striae. Body Length: 14-17 mm.

Male. *Diagnosis*. This species can be separated from other members of the *D. bioculata* species-group by the following combination of characters: T2 and S2 are orange; the entire insect is clothed with black setae, except T2 and S2 are clothed with pale yellow setae; and S2 lacks a seta-filled pit. Body Length: 15-17 mm. *Description. Coloration:* Body entirely black except T2 and S2 are orange. Tibial spurs black. Wings fuscous, veins dark brown. T2 and S2 clothed with pale golden setae except apical fringe black, remainder of body clothed with black setae. *Head***:** Rounded posteriorly, apically produced behind ocelli. Front, vertex and gena with dense coarse punctures. Mandible tridentate apically, unarmed ventrally. Antennal scrobe feebly carinate above. Gena ecarinate. Ocelli miniscule; ocellocular distance 4.5X length of lateral ocellus, interocellar distance 1.0X lateral ocellar length. Flagellomere I 2.5X pedicel length; flagellomere II 3.0X pedicel length. *Mesosoma***:** Pronotum, mesoscutum, scutellum, dorsal face of axilla, mesopleuron and propodeum reticulate, setigerously micropunctate inside reticulae. Tegula convex, glabrous except anterior margin. Metapleuron having few small punctures. Scutellum nearly flat, slightly convex. Axilla posterolaterally produced, extreme apical margin glabrous, posterior face deeply hollowed out. *Metasoma***:** T1 nodose, with dense confluent punctures. S1 with medial longitudinal carina, raised posteriorly, angulate. T2 and S2 with small moderately spaced punctures. S2 flattened medially, without seta-filled pit. T3-6 with dense small punctures and interspersed erect and appressed setae; S3-6 with dense small punctures and erect setae only. Pygidium obscurely granulostriate, with apical fringe of setae. Hypopygidium punctate, glabrous and narrowly bidentate apically. *Genitalia*: Free length of paramere dorsally curved apically, with ventral brush of long dense setae basally, remainder having scattered short sparse setae. Cuspis roughly cylindrical, strongly downcurved apically, ~0.7x free-length of paramere, with few scattered setae dorsally and densely setose ventrally, having long, sparse setae throughout; outer row of setae anteriorly directed, inner row posteriorly directed. Densely setose basal lobe present. Digitus laterally curved internally, tapering slightly at apex, asetose, ~0.35x free-length of paramere. Penis valve emarginated bidentate apically, teeth separated, having longitudinal row of setae at apex and subapically on external margin.

Material examined. DOMINICAN REPUBLIC: Indepencia: 1♀ Lago Enriquillo, near La Descubierta, 5-XII-1991, L. Masner, CNCI; 2♀ 2♂, Los Barbolones, SW Lago Enriquillo: 30-I-1991, USNM (identified by J.A. Genaro in 1995); Barahona: 2 ♀, Barahona, 9-II-1975, W.L. & D.E. Brown, CUIC; HAITI: 3♀, Cul-de-sac Plain, 18-XII-1928, R.O. Smith, AMNH & UMSP (compared with holotype by C.E. Mickel in 1931); 1♀, Manneville, W.M. Mann, MCZC (identified by D.J. Brothers in 1976).

Distribution. This species is only recorded from the island Hispaniola (Dominican Republic, Haiti).

Biology. Hosts for *D. bouvieri* are unknown.

Discussion. The female's antennal scrobes are often feebly produced. If the antennal scrobes are treated as ecarinate, this species will key out to *D. altamira* (Blake) in Manley and Pitts (2007), but can be immediately separated from this species by coloration (Fig. 7.1I) and distribution (*D. altamira* is known only from Mexico).

The male of this species will key out to *D. nigripes* (Fabricius) in Manley & Pitts (2007). It can be immediately separated from *D. nigripes* by the axillar structure; *D. nigripes* has axillae that are rounded posteriorly and glabrous, while *D. bouvieri* has the axillae truncate, hollowed out posteriorly, and reticulate dorsally. The species-group characteristics will also serve to distinguish *D. bouvieri* from *D. nigripes*.

The only other known Antillean males of *Dasymutilla*, *D. militaris* (Smith) and *D. spiniscapula* Manley & Pitts, have the second metasomal segment black and the genitalic cuspis less than 0.4X the free paramere length. *Dasymutilla bouvieri* has the second metasomal segment pale red and the genitalic cuspis roughly 0.7X the free paramere length.

The specimens described here are from the series used by Genaro (1997). Aside from the specimens collected in 1991, we are unaware of any other curated males of *D. bouvieri*.

Dasymutilla chionothrix **Manley & Pitts**

(Fig. 7.1J)

Dasymutilla chionothrix Manley & Pitts, 2007. Zootaxa. 1487: 41. ♀.

Dasymutilla truxali Manley & Pitts, 2007. Zootaxa. 1487: 97. ♂.

Female. *Diagnosis.* This species is recognized by the following combination of characters: the integument is entirely red; the dorsal setae of the head, pronotum, and mesonotum are white or orange and contrast with the black propodeal setae; the pleural and ventral setae are gray or white; T2 is clothed with white or orange setae, except the extreme anteromedial and posteromedial margins have black setae; and the pygidial striae are tightly spaced. Body Length: 12-15 mm.

Male. *Diagnosis.* This species can be separated from other members of the *D. bioculata* species-group by the following combination of characters: the metasoma is black, except the apical half of T2 is obscurely dark ferruginous; the head, mesosoma and metasomal segments 2-7 are dorsally clothed with grayish white setae; the legs, pleurae, and propodeum are clothed with black setae; the apical fringes of S3-5 are clothed with white setae; and S2 has a small longitudinally ovate seta-filled pit situated posteromesally. Body Length: 14 mm (variation in body length could not be assessed for the males due to sample size)

Material examined. MEXICO: Morelos: 1♀, Yautepec, 13-VII-1963, F. D. Parker and L. A. Stange, DGMC(Paratype of *D. chionothrix*); Sonora: 1♀, 10 mi [16 km] SE Alamos, 29-VI-1963, F. D. Parker & L. A. Stange, UCDC (Holotype of *D. chionothrix*); 1♀, Guirocoba, 24-VIII-1933, LACM (Paratype of *D. chionothrix*); 2♀, Otates, 1-VII-1935, G. E. B[ohart], UMSP (Paratypes of *D. concordia*); 1♂ Rio Cuchuhaqui, 7 mi [11.2 km] S Alamos, 25-VII-7-VIII-1953, F.S. Truxal, LACM (Holotype of *D. truxali*).

Distribution. The only known specimens of *D. chionothrix* were collected in Morelos and Sonora, Mexico.

Biology. Hosts are unknown.

Discussion. The male of this species is similar to many males of *D. bioculata* and can only be separated from them by setal color; *D. bioculata* has black or yellow to orange dorsal setae, while *D. chionothrix* has grayish-white dorsal setae. Additionally, this species has the northernmost distribution of the Neotropical members of the *D. bioculata* species-group. Because no intermediate forms are yet known and the distributions do not overlap, we treat *D. chionothrix* as a discrete species rather than another synonym of *D. bioculata*.

Two specimens that were designated as paratypes of *D. concordia* (Manley & Pitts 2007; specimens housed in UMSP) are actually *D. chionothrix*. These specimens have orange dorsal setae, while all previously known specimens of *D. chionothrix* have gray or white dorsal setae. Unlike *D. nocticaro*, these specimens have the pronotal and mesonotal setae concolorous and, unlike *D. bioculata*, these specimens have the propodeal setae black and contrasting with the orange dorsal mesosomal setae.

Additionally, these specimens have the pleural and ventral setae gray, as seen in the other *D. chionothrix* females.

***Dasymutilla insulana* Mickel**

(Figs. 7.1: F, K)

Dasymutilla insulana Mickel, 1926. Ent. Mitt. 15:197. ♀.

Dasymutilla insulana Mickel; Genaro, 1997. 33: 264. ♀♂.

Female. *Diagnosis.* In *D. insulana*, the head is black, contrasting with the orange mesosoma; the anterior pronotal margin is clothed with black setae and there are erect white propodeal setae, the remainder of the mesosoma is clothed with appressed pale red setae; T3-6 are clothed with white setae; and the pygidium has widely separated, irregular, wavy, basally convergent striae. Body Length: 11-15 mm.

Male. *Diagnosis.* This species can be separated from other members of the *D. bioculata* species-group by the following combination of characters: T2 and S2 are orange; the legs, pleurae, and propodeum are clothed with white setae; T3-7 are clothed entirely with black setae; and S2 lacks a seta-filled pit medially. Body Length: 10-13 mm. *Description. Coloration:* Body entirely black except T2 and S2 are orange. Tibial spurs black. Forewing hyaline in basal two thirds, fuscous apically, veins dark brown. Head, pronotum, mesoscutum, axillae, anterior half of scutellum, T3-7, and S5-7 clothed with black setae; remainder of body clothed with white setae. *Head***:** Rounded posteriorly, apically produced behind ocelli. Front, vertex and gena with dense coarse punctures. Mandible tridentate apically, unarmed ventrally. Antennal scrobe feebly carinate above. Gena ecarinate. Ocelli small; ocellocular distance 6.0X length of lateral ocellus, interocellar distance 2.0X lateral ocellar length. Flagellomere I 2.0X pedicel length; flagellomere II 2.5X pedicel length. *Mesosoma***:** Pronotum, mesoscutum, mesopleuron, and scutellum reticulate, setigerously micropunctate inside reticulae. Tegula convex, glabrous except anterior margin. Metapleuron having a few small punctures. Scutellum nearly flat, slightly convex. Axilla posterolaterally produced, anterior half of dorsal face reticulate and densely setose, apical half of axilla glabrous, posterior face truncate, having posteriorly directed setae. Propodeum reticulate. *Metasoma***:** T1 nodose, with dense confluent punctures. S1 with medial longitudinal carina, posterior portion of carina weakly raised. T2 densely punctate and S2 moderately punctate. S2 flattened medially, without seta-filled pit. T3-6 and S3-6 densely punctate. Pygidium obscurely granulate, with apical fringe of setae. Hypopygidium punctate, glabrous and unidentate apically. *Genitalia*: Free length of paramere dorsally curved apically, with ventral brush of long dense setae basally, remainder having scattered short sparse setae. Cuspis roughly cylindrical, strongly downcurved apically, ~0.6x free-length of paramere, glabrous dorsally and densely setose ventrally. having long, sparse setae throughout; outer row of setae anteriorly directed, inner row posteriorly directed. Densely setose basal lobe present. Digitus laterally curved internally, tapering slightly at apex, asetose, ~0.3x free-length of paramere. Penis valve emarginated bidentate apically, teeth separated, having longitudinal row of setae at apex and subapically on external margin.

Material examined. CUBA: 1 ♀, Guantanamo Bay, UMSP (Paratype); 2♂, Guantanamo Bay, 17-21.VI.2010, S.W. Droege, USNM; 1♂, Guantanamo Bay,10.XI.2010, S.W. Droege, USNM. CAYMAN ISLANDS: 1♀, Little Cayman, 23-V-1981, J.F.G. Clarke, USNM (identified by D. Quintero in 1996).

Distribution. This species is recorded from Cuba, the Cayman Islands, and the Isle of Pines.

Biology. No hosts are known for *D. insulana*.

Discussion. Because of variation in the female antennal scrobe, this species keys out in couplets 33 and 72 in Manley & Pitts (2007).

The male of *D. insulana* will key to *D. nigripes* in couplet 17 of Manley and Pitts (2007). It can be separated from *D. nigripes* by setal coloration; *D. nigripes* has the mesosoma and legs clothed with concolorous black setae, while *D. insulana* has the mesosomal pleurae, propodeum, scutellum, and legs clothed with white setae (Fig. 7.1F).

***Dasymutilla latebalteata* (Cameron)**

(Figs. 7.1: G, L)

Sphaerophthamla (sic.) latebalteata Cameron, 1897. Ann. Mag. Nat. Hist. 19:377. ♀.

Dasymutilla veracruz Manley & Pitts, 2007. Zootaxa. 1487: 98. ♂. **syn.n.**

Female. *Diagnosis.* This species can be separated from other *D. bioculata* species-group members by its pygidial structure, there are fewer than 12 widely-spaced parallel striae. The setal coloration is also useful for identification (Fig. 7.1L). Body Length: 7-10 mm.

Male. *Diagnosis.* This species can be separated from other *D. bioculata* species-group members by coloration; the entire body is dark ferruginous to black and clothed with white to pale yellow setae (Fig. 7.1G). Body Length: 8-12 mm.

Material Examined. MEXICO: Veracruz: 4♀, El Conchal, S of Veracruz, 20-VII-1990, I. Yarom, SEMC; 4♀, 8 km S Boca del Rio Veracruz, 21-22-VII-1990, Bell, Conlon, and Minckley, ex. sanddunes, SEMC; 1♂, 8 km SE Boca del Rio, S Veracruz, 21-VII-1990, I. Yaron, SEMC; 7♂, Vera Cruz, 20-VI-1951, P. D. Hurd, CISC (Holotype and six Paratypes of *D. veracruz*); 6♀ 5♂, Veracruz, 20-VI-1951, H.E. Evans, CUIC & EMUS.

Distribution. This species has only been collected in the Mexican state of Veracruz.

Biology. Hosts are unknown.

Discussion. Manley and Pitts (2007) suggest that *D. andreniformis* (Smith) is similar in coloration and structure to the males of *D. latebalteata*. Both species are known from Veracruz, as well. Unlike *D. latebalteata*, however, *D. andreniformis* has a seta-filled pit on S2. The presence of a seta-filled pit on S2 has already shown to be polymorphic in *Dasymutilla* species, including *D. bioculata* (Williams et al. 2011). These are potentially conspecific, but because we have not seen the holotype and the genitalia of *D. andreniformis* have not been described, there is not enough evidence to synonymize *D. latebalteata* with *D. andreniformis*.

***Dasymutilla nocticaro* Manley & Pitts**

(Figs. 7.1: H, M)

Dasymutilla nocticaro Manley & Pitts, 2007. Zootaxa. 1487: 76. ♂.

Dasymutilla concordia Manley & Pitts, 2007. Zootaxa. 1487: 45. ♀. **syn.n.**

Female. *Diagnosis.* This species is unique among members of the *D. bioculata* species-group because of the mesosomal setal pattern; there is a broad arcuate transverse band of white to yellow setae on the mesonotum that contrasts with the black pronotal and propodeal setae (Fig. 7.1M). Additionally, this species has the integument entirely red and the pygidial striae tightly spaced. Body Length: 10-16 mm.

Male. *Diagnosis.* This species can be recognized by the following combination of characters: the legs and mesosomal pleurae are clothed with white setae; T3 and the apical fringe of T2 are clothed with black setae, while T4-7 are clothed with yellow to orange setae; and S2 has a medial seta-filled pit. Body Length: 13 mm.

Material Examined. MEXICO: Nayarit: 3♀, Jesus Maria, 26-VI-1955, B. Malkin, CISC (Paratypes of *D. concordia*); 10♀, Jesus Maria, 6-VII-1955, B. Malkin, CISC (Paratypes of *D. concordia*); Sinaloa: 1♀, Concordia, 4-VII-1963, F. D. Parker & L. A. Stange, UCDC (Holotype of *D. concordia*); 1♂, 8 mi. [12.8 km] SE Elota, 19-V-1962, F. D. Parker, UCDC (Holotype of *D. nocticaro*); 1♂, 8 mi. [12.8 km] SE Elota, 2-VII-1963, F. D. Parker & L. A. Stange, DGMC (Paratype of *D. nocticaro*); Zacatecas: 1♀, 10 mi. S. Jalpa, 17-IX-1970, G. E. & R. M. Bohart, EMUS (Paratype of *D. concordia*).

Distribution. This species is known from central and western Mexico, including Nayarit, Sinaloa, and Zacatecas.

Biology. No hosts are known.

Discussion. The male of this species is similar to *D. iztapa* (Smith) in coloration. It can be separated from that species by the genitalia and axillae; *D. iztapa* has the cuspis straight and the axillae dentate posteriorly, while *D. nocticaro* has the cuspis apically downcurved and the axillae truncate posteriorly.

Two previously recognized female paratypes of *D. concordia* from Sonora are actually *D. chionothrix*. The diagnostic feature for *D. nocticaro* females, proposed by

Manley & Pitts (as *D. concordia*) and supported in this paper, is the presence of black pronotal setae. The paratypes from Sonora, however, have concolorous orange pronotal and mesonotal setae. These specimens were likely misplaced because all previously known specimens of *D. chionothrix* had white dorsal setae.

A further misidentification exists with the paratypes of this species. One paratype of *D. concordia* from Jesus Maria in Nayarit, Mexico (CISC) is a misidentified specimen of *D. holotricha* (Saussure).

Discussion

The importance of designating species-groups and correctly placing species into them is illustrated by this research. When the Mexican fauna was studied, a single male and single female of the *D. bioculata* species-group were found in each of three areas (Fig. 7.2). For example, the Humid Gulf of Mexico Coastal Plains in Veracuz only have one male, *D. veracruz*, **syn.nov.**, and one female, *D. latebalteata*, that match the characters of the *D. bioculata* species-group. Additionally, these insects are similar in setal coloration (Figs. 7.1: G, L). Overlapping distributions and membership in the *Dasymutilla bioculata* species-group are sufficient to associate the sexes. Similarities in coloration of the sympatric males and females provide additional evidence for their association.

The Central American and Caribbean members of the *D. bioculata* species-group are apparently rarer than the North American species *D. bioculata*. Over 2500 specimens of *D. bioculata* have been examined by the authors, but fewer than 30 specimens are recorded for any one of the other species. Study in two of Mexico’s largest collections, the National Universidad and Chamela Research Station, failed to reveal any additional specimens in the *D. bioculata* species-group. Recent malaise trap surveys in the Alamos region of southern Sonora have recovered over 200 *Dasymutilla* specimens representing 10 species, but no members of the *D. bioculata* species-group, even though most specimens of *D. chionothrix* were collected in this region. This evidence suggests that sampling effort alone cannot explain the disparity in abundance between *D. bioculata* and the Mexican species. The cause for their rareness is unknown, but future studies may reveal limitations in habitat or host preference.

The members of the *D. bioculata* species-group are all morphologically similar. For example, *D. bioculata, D. chionothrix*, and *D. nocticaro* lack significant structural differences in either sex (although they differ in some polymorphic structural traits) and can only be separated by setal and integumental coloration. Tremendous color variation has been observed in multiple *Dasymutilla* species including *D. monticola* (Pilgrim et al. 2008), *D. quadriguttata* (Pilgrim et al. 2009), and *D. bioculata* (Williams et al. 2011). In each of these examples, large series revealed intermediate forms and overlapping ranges. It may be that some of the Mexican species studied here will be revealed as conspecific color variants of *D. bioculata*, but differences in setal color, however, have proven to be diagnostic in other Mutillidae (Wilson and Pitts 2009). As it currently stands, the species each have disparate ranges and intermediate forms have not been seen. This could easily be a factor of small sample size, though. *Dasymutilla chionothrix*, for example, is known from only one male specimen and five female specimens. Continued research and increased collecting efforts in Mexico are absolutely necessary.

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CHAPTER 8

NEW SPECIES OF *TOBANTILLA* CASAL, 1965 AND A NEW GENUS AND SPECIES, *GOGOLTILLA CHICHIKOVI* GEN. ET SP. NOV., FROM ARGENTINA (HYMENOPTERA: MUTILLIDAE)

Introduction

The genus *Tobantilla* Casal, 1965 was erected to include a single new species, *T. montonera*, which was known from females only (Casal 1965a). Casal (1969) described a second species, *T. charrasca*, which was also known only from females. In this same paper, Casal discussed males that had been collected in the same locality as *T. charrasca* and generally resembled the putative relatives of *Tobantilla*: *Traumatomutilla* André, 1901, *Reedomutilla* Mickel, 1964 and *Suareztilla* Casal, 1968. When *Tobantilla* was listed in a phylogeny of mutillid higher taxa, Brothers (1975) recorded that the genus was known from both sexes and included four species, indicating that the male was known to him and he had seen a further two undescribed species. In a recent catalog of mutillid genera (Lelej & Brothers 2008), however, the *Tobantilla* male indicated by Brothers (1975) was said to have been based on a misidentification (it is described as a new genus below).

Although they have been discussed in the literature briefly, males of *Tobantilla* remain undescribed and the genus is still recorded only from Argentina (Nonveiller

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[5] This chapter has been published in *Zootaxa*. Permission has been given for the reproduction of this work here (Appendix A); Magnolia Press has licenced me to reprint this material (Appendix B).Please use the following citation when referring to this work: Williams, K.A., D.J. Brothers, and J.P. Pitts. 2011. New species of *Tobantilla* Casal, 1965 and a new genus and species, *Gogoltilla chichikovi* gen. et sp. nov., from Argentina (Hymenoptera: Mutillidae). *Zootaxa*, 3064: 41-68.

1990). In this study, we describe seven new species of *Tobantilla*, including males, from Argentina, Colombia and Venezuela, transfer one Brazilian species into *Tobantilla* (from *Mutilla* Linnaeus), redescribe the previously known species to provide a consolidated account, and describe a new Argentinean genus and species that closely resembles *Tobantilla*.

Materials and methods

The following codens are used for institutions housing the material discussed in the current study:

AMNH	Department of Entomology Collection, American Museum of Natural History, New York, New York, USA.
CASC	Department of Entomology, California Academy of Sciences, San Francisco, California, USA.
CMNH	Carnegie Museum of Natural History, Pittsburgh, Pennsylvania, USA.
CPDC	Centro de Pesquisas do Cacau, CEPEC, CEPLAC, Divisao de Zoologia Agricola, Itabuna, Bahia, Brazil.
DJBC	Collection of Denis J. Brothers, to be deposited in Iziko South African Museum (SAM), Cape Town, South Africa.
EMUS	Department of Biology Insect Collection, Utah State University, Logan, Utah, USA.
IAvH	Instituto Alexander von Humboldt, Villa de Leyva, Colombia.
MACN	Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”, Buenos Aires, Argentina.

MCZC	Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA.
USNM	Department of Entomology, Smithsonian Institution, National Museum of Natural History, Washington, District of Colombia, USA.

We use the term “simple setae” for setae that are smooth and do not have barbed surfaces. “Brachyplumose setae” refers to setae with barbs that are less than or equal to the diameter of the shaft at the attachment of the barb. “Plumose setae” have longer barbs. We use the abbreviations T2, T3, etc., to denote the second, third, etc., metasomal terga while S2, S3, etc., denote the second, third, etc., metasomal sterna. To compare mesosomal length and width, the distance between the anteromedial pronotal margin (excluding the anterior collar) and the scutellar-scale apex is divided by the distance between the extreme posterolateral pronotal margins, the maximum mesosomal width. The digitus or cuspis length relative to the free paramere length is used here to quantify differences in genitalic structure. For ease of comparison and to facilitate identification without dissecting the genitalic capsule, the cuspis, digitus and paramere measurements are taken in dorsal view from the apical margin of the parapenial lobe to the apex of each respective structure. Using this method, all measurements can be taken from the dorsal view and a single anchor point can be used for all three measurements. These are not actual measurements of structure length, but an index to compare relative lengths; all provided length ratios of genitalic structures are based on these indices.

Results

Tobantilla Casal, 1965

(Figs 8.1–8.5)

Tobantilla Casal, 1965a. Rev. Soc. Mex. Hist. Nat. 25: 209–215.

Type species. *Tobantilla montonera* Casal, 1965, by original designation.

Diagnosis. FEMALE. Distinguished from all other New World mutillid genera by the following combination of characters: the mesosoma is dorsally flattened or concave in lateral view and has a well defined scutellar scale; the dorsal and posterior propodeal faces are more or less perpendicular and usually separated by lateral transverse carinae; the tibial spurs are white or yellow, being paler than the tibiae and tarsi; the first metasomal segment is clearly petiolate and posteriorly constricted.

MALE. Distinguished from other New World mutillid genera by the following combination of characters: the clypeus is anteriorly produced into either a medial tooth or a shallowly emarginate plate; the axillae are impunctate and asetose postero-dorsally and truncate posteriorly; the first metasomal segment is petiolate and posteriorly constricted; S2 lacks a median seta-filled pit; the pygidium is margined with posterolateral carinae; and brachyplumose setae are present, especially obvious on the head, propodeum and basal metasomal segments.

Description. FEMALE. T2 with paired pale integumental spots. *Head*. Broadly transverse, dull, vertex and gena shallowly and coarsely reticulate, mostly clothed with stout lanceolate decumbent setae and scattered slender finely brachyplumose erect setae.

Occipital carina well developed dorsally. Vertex posterolaterally with a subtriangular

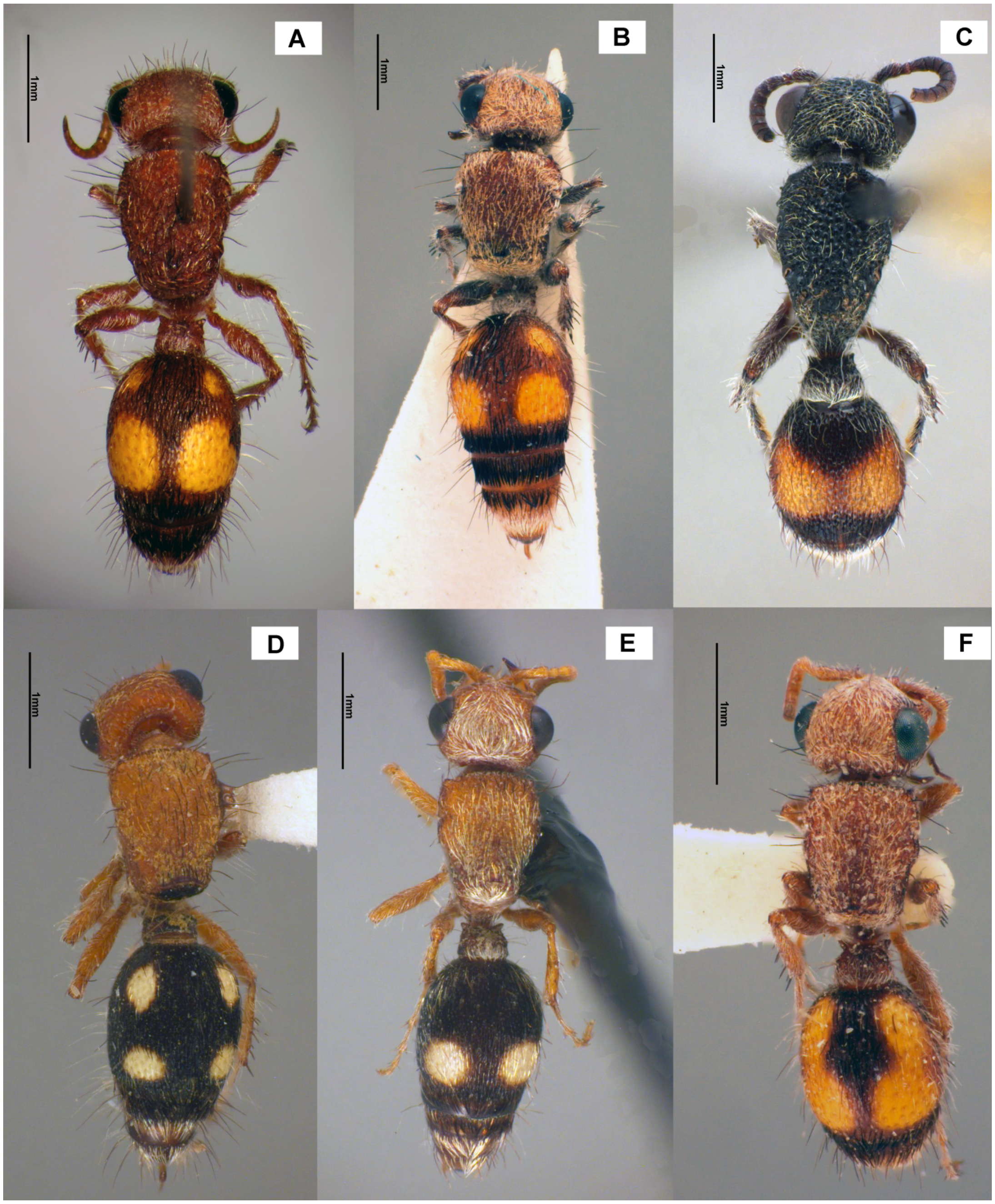

Figure 8.1. *Tobantilla* species, females, habitus, dorsal view. A, *T. aleatrix* **sp. nov.**; B, *T. charrasca* Casal; C, *T. frigidula* (Cresson); D, *T. kolasma* **sp. nov.**, holotype; E, *T. krima* **sp. nov.**, holotype; F, *T. montonera* Casal.

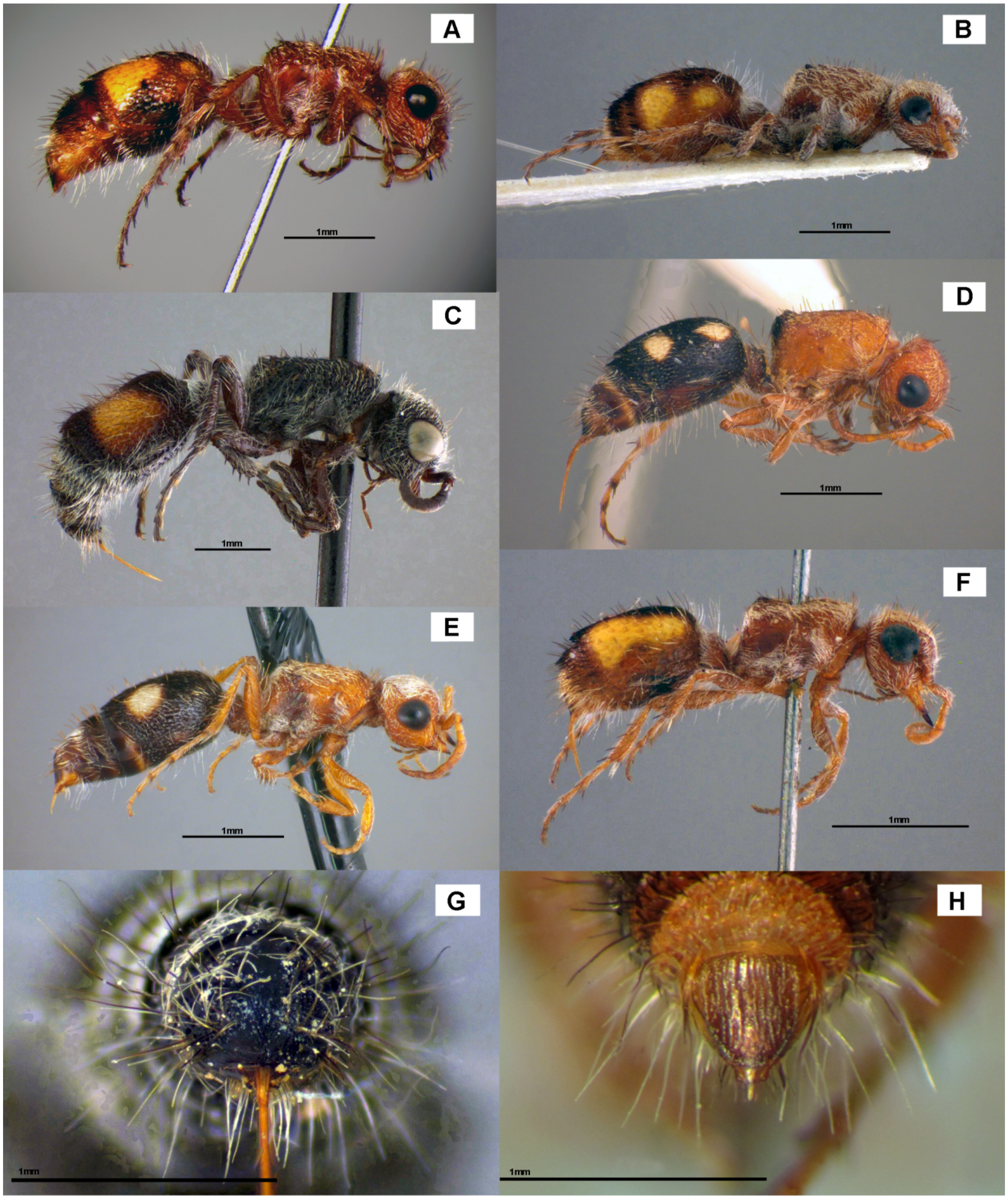

Figure 8.2. *Tobantilla* species, females. A-F, Habitus, lateral view. A, *T. aleatrix* sp. nov.; B, *T. charrasca* Casal; C, *T. frigidula* (Cresson); D, *T. kolasma* sp. nov., holotype; E, *T. krima* sp. nov., holotype; F, *T. montonera* Casal. G-H, Pygidium (T6). G, *T. frigidula*; H, *T. aleatrix*.

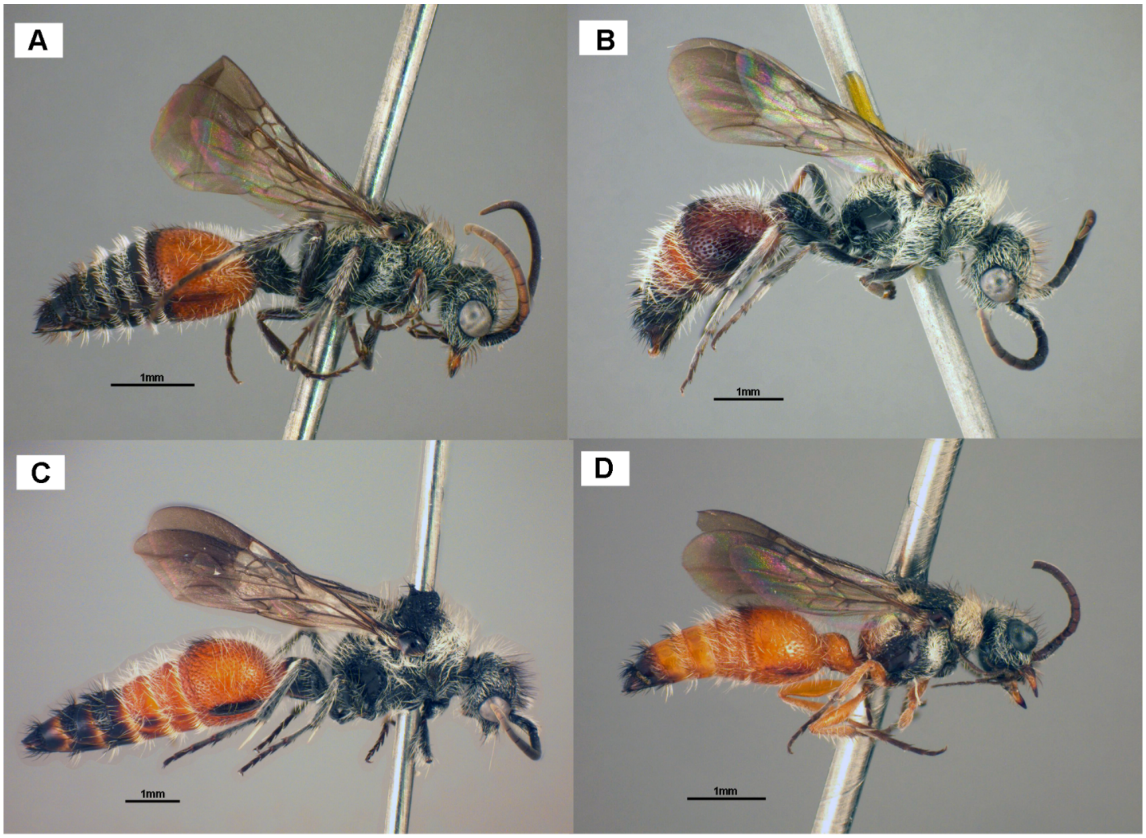

Figure 8.3. *Tobantilla* species, males, habitus, lateral view. A, *T. andrikos* sp. nov., holotype; B, *T. drosos* sp. nov., holotype; C, *T. ephemeros* sp. nov.; D, *T. xouthos* sp. nov., holotype.

glabrous tubercle merging with occipital carina. Eye circular, protruding, ommatidia distinct. Antennal tubercles smooth, basally contiguous. Antennal scrobe with lateral carina, without dorsal carina. Clypeus with complete strong setose transverse carina above short smooth transverse concave area, ventral margin straightish medially. Malar space almost as high as combined length of pedicel + flagellomere 1. Genal carina weak, irregular, ventrally reaching hypostomal carina and dorsally not reaching vertex. Hypostomal carina distinct, even, ending laterally at posterior mandibular condyle. Proboscidal and mandibular fossae narrowly confluent anteriorly; proboscidal fossa about

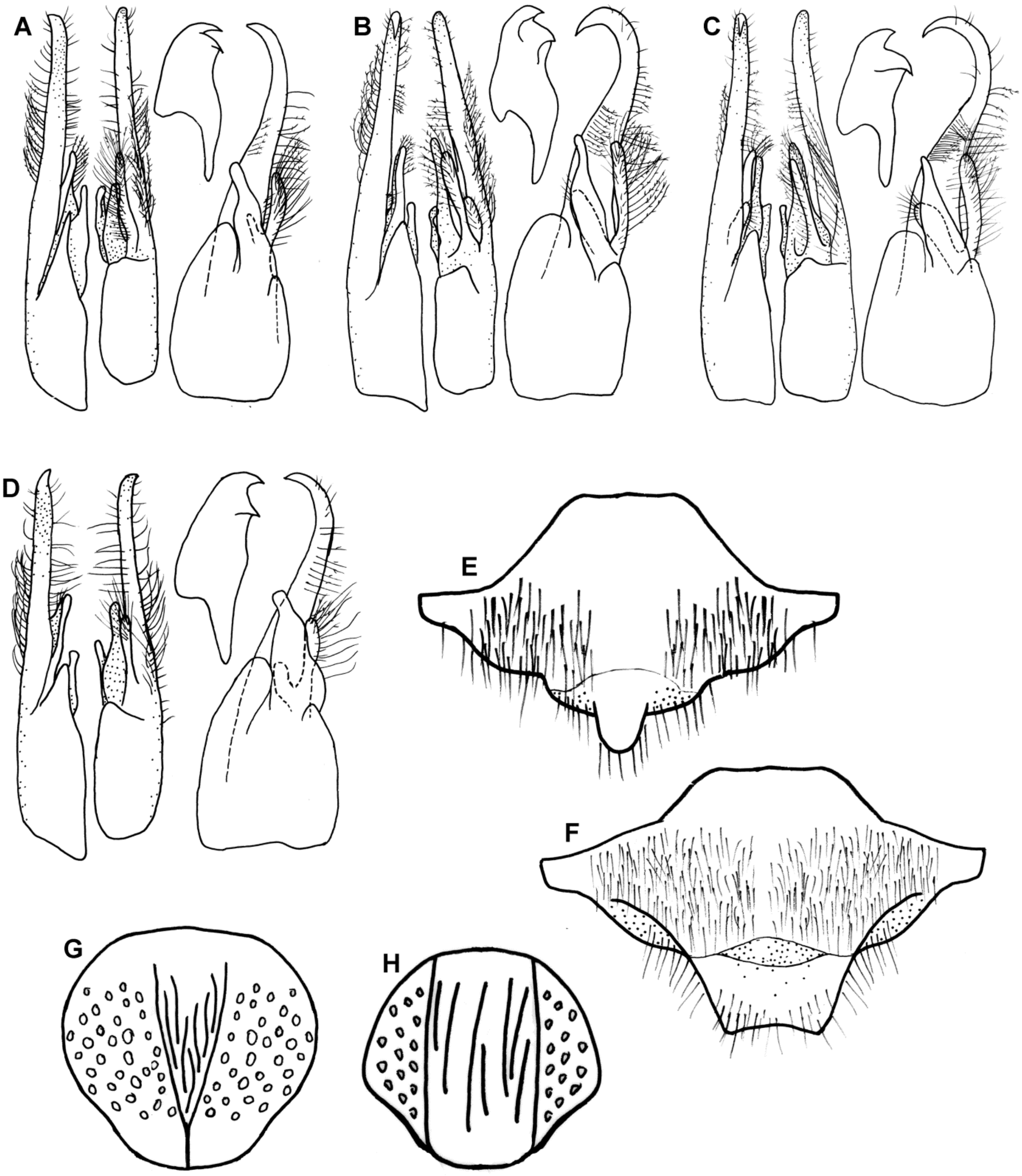

Figure 8.4. *Tobantilla* species. A–D, Male genitalia: dorsal view (left), ventral view (middle), lateral view of penis valve (above), and lateral view with penis valve removed (right). A, *T. andrikos* **sp. nov.**; B, *T. drosos* **sp. nov.**; C, *T. ephemeros* **sp. nov.**; D, *T. xouthos* **sp. nov.** E–F, Male clypeus. E. *T. andrikos* **sp. nov.**; F. *T. ephemeros* **sp. nov.** G–H, Female pygidium (T6). G, *T. frigidula* (Cresson); H, *T. kolasma* **sp. nov.**.

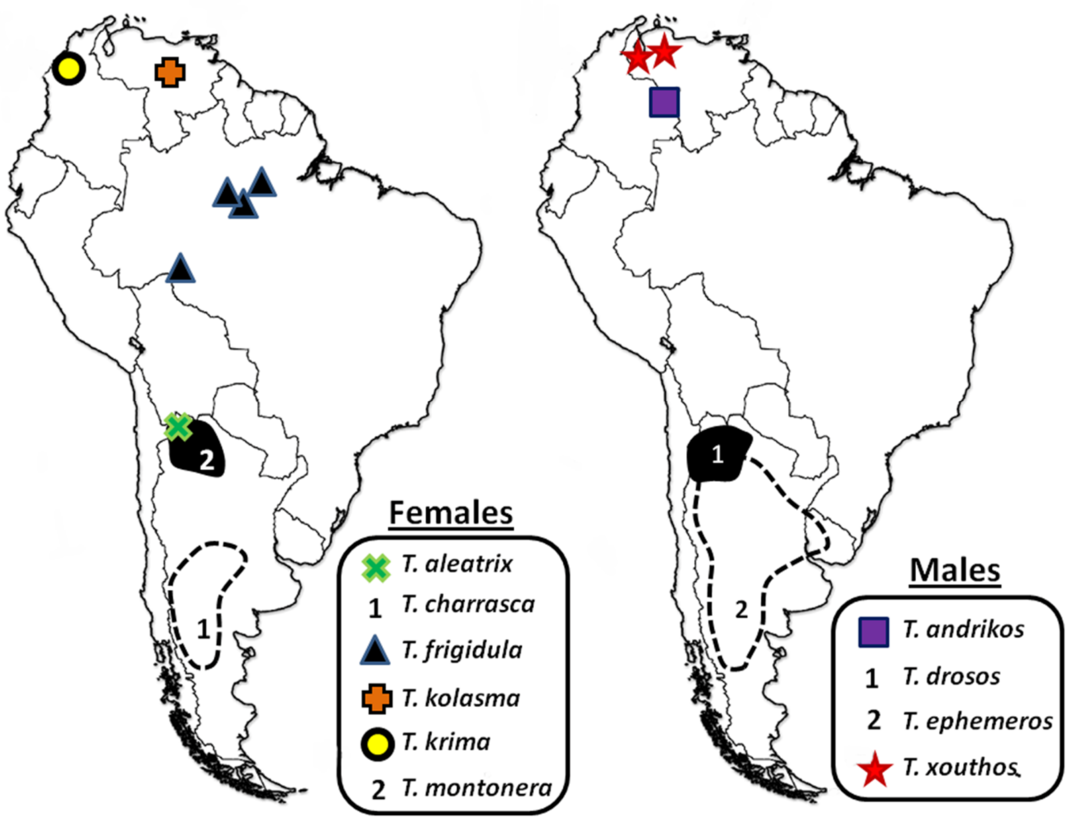

Figure 8.5. Distribution of *Tobantilla* species.

0.6 × length of ventral surface of head. Scape simple, punctate above. Pedicel slightly longer than wide, shorter than first flagellomere; first flagellomere much longer than wide, longer than second. Mandible nearly straight, tapering distad, unidentate apically, unarmed ventrally. Maxillary palp 6-segmented; labial palp 4-segmented; intermediate palpomeres slightly expanded apicad. Mentum smooth, weakly convex. *Mesosoma*. Slightly elongate, coffin-shaped, widest at pronotal spiracle. Dorsum dull, shallowly and coarsely reticulate, clothed with stout lanceolate decumbent setae and scattered slender finely brachyplumose erect setae. Pronotal dorsal face (excluding anterior collar/flange) with anterior margin almost straight, weakly carinate, epaulet dentate, humeral angle blunt; anterior face and collar longitudinally striate. Posterodorsal margin of pronotum faintly indicated by very weak ridge, specially laterally. Scutellar scale strong, protruding, basally interrupting well developed metathoracic-propodeal suture. Propodeal disk short, posteriorly carinate on each side, distinct from and perpendicular to posterior declivity. Lateral face of pronotum densely and finely punctate, clothed with fine decumbent setae; pronotal-mesopleural suture scarcely discernible. Mesopleuron anteriorly finely and sparsely punctate, posteriorly reticulate, with fine decumbent setae. Metapleuron finely punctate, clothed with fine decumbent setae. Metapleural-propodeal suture obliterated dorsal to endophragmal pit. Lateral face of propodeum almost entirely smooth and shining, with a few fine decumbent setae ventrally; posterior face reticulate dorsally, smooth ventrally, with stout lanceolate decumbent setae near dorsal margin, scattered finely brachyplumose erect setae ventrally. Metasternal process triangular, unidentate acute apically, as long as metacoxal height. *Legs*. Tibial spur formula 1-2-2. Foreleg with tarsal comb, a few long strong articulated spines on posterior/lateral margins of tarsomeres. Mid- and hind tibiae each with two rows of prominent spines, about 4 spines in each row, apical spines not inserted on any process; subapically with small inconspicuous secretory pore near base of inner spur; apical spurs broad, finely and deeply pectinate. Metacoxa with weak irregular longitudinal carina on inner/posterior margin. *Metasoma*. T1 about as wide as long, < 0.5 × width of T2, almost parallel-sided in dorsal view, oblique anterior and horizontal dorsal faces distinct, with fine decumbent pubescence and brachyplumose erect setae; anterior auricle prominent, dentate. T2 clothed with laterally flattened lanceolate setae medially between pale spots and in apical band; lateral felt line broad, about 0.3 × as long as T2 laterally. T3–5 clothed with lanceolate decumbent setae. T6 with well defined longitudinally striate pygidial area. S1 with strong simple median carina. S2 without felt line.

MALE. Without defined pubescent or integumental spots. *Head.* Broadly transverse, at least as wide as mesosoma, surface shiny, densely punctate, clothed with simple decumbent and brachyplumose erect setae. Occipital carina distinct. Vertex weakly swollen and produced behind ocelli. Eye transversely ovate, protruding, ommatidia distinct. Ocelli small, but median ocellus distinctly larger than lateral ocelli. Antennal tubercles simple, mostly smooth, contiguous. Antennal scrobe broadly concave to eye margin, with prominent complete flangelike transverse carina above. Clypeus with anterior/ventral lobelike process medially. Malar space about half basal height of mandible, about as long as pedicel. Gena ecarinate. Hypostomal carina distinct but weak, even, ending laterally at posterior mandibular condyle. Proboscidal and mandibular fossae very narrowly confluent anteriorly; proboscidal fossa about 0.6 × length of ventral surface of head. Scape strongly bicarinate anteriorly. Pedicel about as long as wide, shorter than first flagellomere, which much shorter than second flagellomere. Mandible tapering, bidentate apically, unarmed ventrally, in dorsal/anterior view with lateral margin broadly and obtusely angulate at midlength and almost straight basally and apically. Maxillary palp 6-segmented; labial palp 4-segmented; intermediate palpomeres slightly flattened and expanded apicad. Mentum smooth, with distinct median longitudinal carina.*Mesosoma.* Surface shiny, densely punctate except smooth on anterior face of pronotum, axillar tooth, metapleuron and lateral face of propodeum, and reticulate on remainder of propodeum; clothed with simple decumbent setae and brachyplumose erect setae. Pronotal dorsal face (excluding anterior collar/flange) with anterior margin almost straight, weakly carinate medially above depression on anterior face, epaulet scarcely evident, humeral angle blunt. Posterodorsal margin of pronotum broadly V-shaped. Tegula ovate, evenly convex, almost entirely smooth and glabrous. Mesoscutum with notaulus forming an elongate depression on posterior fifth at most, parapsis almost indistinguishable; posterolateral corner forming a raised lobe continuous with axilla. Scutellum pulvinate. Axilla laterally produced as a prominent obliquely projecting blunt tooth. Metanotum simple, transverse. Propodeum strongly convex, disk and declivity fairly distinct in lateral view. Metasternal process unidentate, acute, almost as long as metacoxal height.*Wings*. Forewing with moderate ovate sclerotized pterostigma; marginal cell short, truncate apically; two closed submarginal cells. *Legs*. Tibial spur formula 1-2-2. Mid- and hind tibiae each with a few strong spines and several strong slender semi-erect macrosetae dorsally, apical spines not inserted on any process; subapically with small secretory pore near base of inner spur; apical spurs almost cylindrical, densely clothed with microsetae. Metacoxa with weak irregular longitudinal carina on inner/posterior margin, there with patch of erect plumose setae.*Metasoma*. T1 petiolate, longer than wide, < 0.5 × width of T2, posteriorly weakly constricted in dorsal view, oblique anterior and horizontal dorsal faces distinct, punctate with finely brachyplumose erect setae; anterior auricle moderate, forming rounded oblique flattened lobe. T2 punctate, with brachyplumose erect and narrowly lanceolate semi-decumbent setae; felt line broad and long. T3–6 punctate, with brachyplumose to simple erect and narrowly lanceolate semi-decumbent setae. T7 punctate with simple to brachyplumose narrowly lanceolate erect setae basally, pygidial area distinct, convex, with apical marginal carina. Sterna punctate with brachyplumose to simple erect to semi-decumbent setae. S1 with weak longitudinal carina. S2 simple, evenly convex, without felt line. S7 very short, almost entirely concealed. Hypopygium long with median lobe or tooth on posterior margin. *Genitalia*. Paramere and/or cuspis with long shaggy plumose erect setae. Paramere elongate, more or less cylindrical, acute, dorsally recurved apically. Parapenial lobe well developed. Cuspis deeply divided near base, both components cylindrical; digitus at least as long as cuspis. Penis valve asetose, bidentate apicoventrally, teeth separated.

Distribution. This genus likely occurs throughout South America, except Chile. It is recorded from Argentina, Brazil, Colombia, Uruguay, and Venezuela (Fig. 8.5).

Remarks. We agree with Casal's (1969) discussion regarding the males of *Tobantilla*. The *Tobantilla* males described below were collected in countries where *Tobantilla* females occur, are similar in size to *Tobantilla* females and, like *Tobantilla* females, have brachyplumose setae on the scape, clypeus, posterior head margin, posteroventral propodeal face and base of T1–2.

Currently, none of the *Tobantilla* species is known from both sexes. When biological observations, molecular data, or large series become available, sex associations can be made and some species described below may need to be synonymized.

Many members of *Dasymutilla* Ashmead, particularly those from the *D. monticola* species-group, closely resemble this genus. They are generally small bodied, have a well developed scutellar scale and orange head and mesosomal integument in females, and have at least T2 reddish and the head and mesosoma clothed with silver and black setae in males. *Tobantilla* can be separated from *Dasymutilla* by the male genitalic structure and by the dorsally flattened mesosoma in females. Additionally, none of the *Dasymutilla* that could easily be confused with *Tobantilla* is found in South America.

Key to species of *Tobantilla*

Males (unknown for *T. aleatrix* **sp. nov.**, *T. charrasca* Casal, *T. frigidula* (Cresson), *T. kolasma* **sp. nov.**, *T. krima* **sp. nov.**, *T. montonera* Casal)

1. Anteromedial margin of clypeus flat, shallowly emarginate (Fig. 8.4F) … 2
- Anteromedial margin of clypeus with single raised tubercle (Fig. 8.4E) … 3
2. Base of T2 with patch of appressed white setae (Fig. 8.3B) … *T. drosos* **sp. nov.**
- T2 having asetose interspaces and sparse erect white setae throughout disc (Fig. 8.3D) … *T. ephemeros* **sp. nov.**
3. Metasoma entirely black except for second segment (Fig. 8.3A); legs blackish, concolorous with pleura … *T. andrikos* **sp. nov.**
- Metasoma entirely yellowish-brown except segments six and seven infuscated (Fig. 8.3D); legs yellowish-brown, much lighter than dark brown to black pleura … *T. xouthos* **sp. nov.**

Females (unknown for *T. andrikos* **sp. nov.**, *T. drosos* **sp. nov.**, *T. ephemeros* **sp. nov.**, *T. xouthos* **sp. nov.**)

1. Head and mesosoma dark red to black (Figs 8.1C, 8.2C); pygidium having single longitudinal medial carina apical to small pygidial plate (Figs 8.2G, 8.4G) … *T. frigidula* (Cresson)

- Head and mesosoma pale brown to orange (*e.g.* Fig. 8.1A); large pygidial plate well defined by lateral carinae, reaching apex of pygidium (*e.g.* Figs 8.2H, 8.4H) … 2

2\. Scutellar scale connected with carinae separating dorsal and posterior propodeal faces, forming broad continuous arcuate ridge (Fig. 8.1D); posterior face of propodeum clothed with dense black setae (Fig. 8.2D) … *T. kolasma* **sp. nov.**

- Scutellar scale relatively narrow, distinct from carinae separating dorsal and posterior propodeal faces (*e.g.* Figs 8.1B, 8.1F); posterior face of propodeum clothed with pale silver, golden, or brown setae (*e.g.* Figs 8.2B, 8.2F) … 3

3\. T2 having only one pair of yellow integumental spots (Fig. 8.1E); pronotum clothed with black setae, contrasting with silver mesonotal setae (Fig. 8.1E) … *T. krima* **sp. nov.**

- T2 having two pairs of yellow integumental spots, sometimes with anterior and posterior spots confluent (Figs 8.1: A, B, F); mesosomal dorsum clothed with concolorous pale golden setae, sometimes with scattered brown setae on posterior portion of mesonotum (Figs 8.1: A, B, F) … 4

4\. T4 clothed entirely with silver setae; head about 1.2 × width of pronotum; T2 with anterior and posterior spots confluent or scarcely separated (Fig. 8.1F) … *T. montonera* Casal

- T4 having black setae, at least basally; head about 1.1 × width of pronotum; T2 with anterior and posterior spots distinctly separated (Figs 8.1: A, B)… 5

5\. Mesosomal width slightly greater than length, mesothorax as wide as pronotum, propodeum parallel-sided and flattened posteriorly with posterolateral angle abrupt (Fig. 8.1B); femora darkened medially; flagellomere two only slightly longer than pedicel … *T. charrasca* Casal

- Mesosomal length slightly greater than width, mesothorax narrower than pronotum, propodeum narrowing posterad and weakly convex posteriorly with posterolateral angle rounded (Fig. 8.1A); femora entirely light orange brown; flagellomere two about one-third longer than pedicel … *T. aleatrix* **sp. nov.**

Tobantilla aleatrix **Williams, Brothers & Pitts, sp. nov.**

(Figs 8.1A, 8.2B, 8.2H)

Diagnosis. FEMALE. The head and mesosoma are pale orange-brown with sparse pale golden setae dorsally (Fig. 8.1A), the sculpture of the pronotum clearly evident; the mesosoma is longer than broad and evenly narrowed posterior to the pronotum; the scutellar scale is separated from the dorsal propodeal carinae; the posterior propodeal face is fairly densely clothed with pale golden setae dorsally (Fig. 8.1A); T2 is broadest posterior to its midlength, and has both an anterior and posterior pair of yellow integumental spots; and T4 is clothed with black setae.

Description. FEMALE. Body length 4.1–4.8 mm. *Coloration*. Body and appendages pale orange-brown except T5 and S5–6 paler yellowish-brown; T2 with two pairs of separated yellow spots, anterior spots circular, much smaller than posterior triangular to trapezoidal patches. Tibial spurs white. Head and mesosoma clothed with fairly sparse decumbent pale golden or silvery lanceolate setae, except medial portion of mesosomal dorsum with a few dark brown setae interspersed, area directly anterior to scutellar scale with cluster of dark brown setae, and dorsum of head and mesosoma with scattered erect long brachyplumose brown setae; posterior face of propodeum with moderately dense decumbent pale golden setae and a few dark brown setae dorsally, almost glabrous ventrally. T2 clothed with dark brown decumbent lanceolate setae between spots and along apical margin, lateral thirds and integumental spots with sparse silver decumbent and erect setae. T3–4 entirely clothed with decumbent dark brown lanceolate setae. T1, T5 and S1–5 clothed with silvery white decumbent and erect setae. T6 and S6 with erect brown setae. *Head*. Rounded posteriorly, with occipital carina stronger laterally from base of weak narrowly triangular glabrous tubercle on posterolateral margin. Head width 1.1 × pronotal width. Eye almost circular. Front, vertex and gena reticulate. Genal carina slightly produced, extending anteriorly to hypostomal carina. Mandible oblique, tapering, bidentate apically but preapical inner tooth minute and usually obliterated, unarmed ventrally. Antennal scrobe with distinct lateral vertical carina but no dorsal carina. Antennal tubercle finely and sparsely punctate basally. Scape simply punctate. Flagellomere 1 1.5 × pedicel length; flagellomere 2 1.3 × pedicel length. *Mesosoma*. Mesosomal length 1.1 × width; pronotum 1.1 × as wide as mesothorax. Mesosomal dorsum reticulate. Humeral carina well developed, extending mesally beyond strongly dentate epaulet. Lateral face of pronotum and mesopleuron micropunctate, dull, mesopleural ridge coarsely reticulate; clothed with fine recumbent setae. Metapleuron dorsal to endophragmal pit and dorsal two-thirds of lateral face of propodeum glabrous, smooth; ventral regions of metapleuron and lateral face of propodeum micropunctate and clothed with fine recumbent setae. In dorsal view, mesosoma gradually narrowed posterior to pronotum, lateral margin of mesothorax weakly sinuate anterior to propodeal spiracle. Scutellar scale narrow and highly raised, with broadly w-shaped transverse carina anterior to it. Moderate transverse carina separating dorsal and posterior propodeal faces on each side. Propodeum narrowed posterior to spiracle, posterolateral angle broadly rounded, posterior face weakly convex, vertical, reticulate and clothed with moderately dense decumbent lanceolate setae on dorsal half, smooth and almost glabrous ventrally. *Metasoma*. T1 narrow and petiolate, somewhat cylindrical, 0.4 × as wide as T2. T2 1.1 × as long as wide, with maximum width slightly posterior to midlength. Disc of T2 with moderate-sized longitudinally ovate contiguous punctures, except yellow spots sparsely punctate with interspaces wider than punctures; T3–5 densely punctate. S1 with low longitudinal carina, S1–5 moderately punctate, punctures smaller and denser on S3–5. Pygidium well defined by continuous lateral and apical carinae, posterior margin broadly convex, surface weakly convex with about 10 irregular longitudinal striae almost reaching apical margin, spaces between striae irregularly granulate.

Male. Unknown.

Type material. *Holotype*, ♀, ARGENTINA: Jujuy, Huacalera, 17 km.N. Tilcara, 2800 m., 6 January 1972, D.J.Brothers (MACN). *Paratype*, 1♀, same label data as holotype (DJBC).

Distribution. This species is known only from the type locality.

Host. Unknown.

Etymology. From the Latin, the female of *aleator*, “gambler”; noun in apposition. This species, like two others here, is named after a work by Russian author Fyodor Mikhailovich Dostoevsky, in this case the novel Игрок “The Gambler”, with an allusion to initial uncertainty about its specific status.

Remarks. This species is very similar in many respects, particularly in color, to *T. charrasca*, with which it was initially confused. However, *T. charrasca* has the mesosomal dorsal pubescence denser (*e.g.*, concealing the sculpture of the pronotum) as is that of the posterior face of the propodeum and of T1, the anterior epaulet less developed, the mesosoma broader than long, the mesothorax as wide as the pronotum, the lateral margin of the mesosoma more strongly sinuate (broadening behind the pronotum then narrowing strongly to the propodeal spiracle and then almost straight to the blunt posterolateral angle of the propodeum), the second flagellomere about 1.1 × the length of the pedicel, and T2 about as long as wide and with its greatest width slightly anterior to its midlength. In addition, *T. charrasca* has the femora distinctly darkened medially.

Tobantilla andrikos **Williams, Brothers & Pitts, sp. nov.**

(Figs 8.3A, 8.4: A, E)

Diagnosis. MALE. The clypeus has a medial tubercle anteriorly (Fig. 8.4E), while the metasoma is entirely black except for the red T2 and S2 (Fig. 8.3A) and the legs are dark ferruginous and concolorous with the pleura.

Description. MALE. Body length 5.5–7 mm. *Coloration.* Head, mesosoma and metasoma black, except basal nine tenths of T2 and S2 red. Antennae, legs, medial portion of mandibles, and tegulae brown. Tibial spurs white. Wings weakly infuscated, veins brown. Head clothed with appressed silver setae, vertex with interspersed erect silver and brown setae. Mesosoma clothed with appressed silver setae, except medial half of mesoscutum and axilla clothed with black setae. Posterior half of T2 and metasomal segments 5–6 with interspersed black and silver setae; pygidium and hypopygidium clothed with black setae, remainder of metasoma clothed with silver setae. *Head.* Rounded posteriorly, with slight posterior projection behind ocelli. Head width 1.1 × pronotal width. Eye transversely ovate, in dorsal view eye diameter 2.7 × distance from posterior eye margin to posterolateral angle of head. Ocelli small; ocellocular distance 4.0 × length of lateral ocellus, interocellar distance 1.2 × lateral ocellar length. Occipital carina weak but distinct, extending ventrally almost to level of oral fossa. Punctation on front and gena obscured by dense shaggy recumbent setae; vertex moderately punctate. Gena ecarinate. Antennal scrobe broadly concave to eye margin, with prominent complete flangelike transverse carina above. Clypeus having sharp anteromedial tubercle (Fig. 8.4E). Oral fossa 1.4 × length of hypostomal bridge. Scape bicarinate ventrally. Flagellomere 1 1.2 × pedicel length; flagellomere 2 2.0 × pedicel length. Mandible oblique, tapering, bidentate apically, unarmed ventrally. Maxilla and labium elongate. Maxillary palp 6-segmented, third and fourth segments slightly flattened and apically expanded, other segments almost cylindrical. Labial palp 4-segmented, second and third segments slightly flattened and apically expanded, other segments almost cylindrical. *Mesosoma.* Epaulets lowly produced; pronotum emarginate anteromedially. Pronotal dorsal punctation coarsely contiguous but obscured by dense shaggy recumbent mesally directed setae; anterior face densely punctate, except emarginated medial area glabrous; lateral face moderately punctate above but almost smooth ventrally. Tegula convex, glabrous except with long recumbent setae anterolaterally and posteromesally. Mesoscutal punctation coarsely reticulate, micropunctate inside reticulations; notaulus and parapsis indistinguishable; posterolateral corner forming a coarsely punctate raised lobe. Scutellum slightly convex, contiguously punctate. Axilla produced posteriorly, contiguously punctate on dorsal anterior half, glabrous on dorsal posterior half, posterior face truncate with posteriorly directed setae; anterolateral axillar margin contiguous with posterior mesonotal margin. Mesopleural punctation obscured by dense setae. Metapleuron glabrous with small scattered punctures. Propodeum strongly convex, entirely reticulate, except smooth and shining adjacent to metapleuron. *Wings*. Forewing with moderate ovate sclerotized pterostigma; marginal cell short and truncate apically; two submarginal cells. *Legs*. Mid- and hind tibiae each with a few strong spines dorsally, distinct apical secretory pore on inner surface near base of inner spur; spurs densely clothed with microsetae. *Metasoma*. T1 petiolate, 0.45 × width of T2, with moderately spaced coarse punctures. T2 moderately punctate; apex obscured by dense fringe of mesally directed setae; felt line 0.4 × lateral length of T2. T3–6 densely punctate. Pygidium margined laterally and posteriorly by a strong sharp carina; surface shallowly concave except medially raised, granulate with a few raised rugae. S1 with longitudinal carina in anterior half and contiguously punctate in posterior half. S2 moderately punctate, evenly convex. S3–6 moderately punctate. Hypopygidium moderately punctate, posteromedial margin with sharp tooth. *Genitalia* (Fig 8.4A). Free length of paramere dorsally curved apically, with ventral brush of long dense setae basally, remainder having scattered short sparse setae; apex of ventral face not visible from above. Cuspis deeply divided near base, cylindrical, ~0.25 × free-length of paramere, posterior half having long dense posteriorly directed; densely setose basal lobe present. Digitus laterally compressed, constricted at apex, asetose, ~0.3 × free-length of paramere. Parapenial lobe well developed, apically angulate. Penis valve asetose, ventral margin bidentate apically, teeth separated.

FEMALE. Unknown.

Type material. *Holotype*, ♂, COLOMBIA, Vichada, PNN Tuparro Bosque Sabana, 5°21'N 67°51'W, 100m, malaise, 22–31.I.2001, W. Villalba (IAvH). *Paratypes*, 2♂, same data as holotype (IAvH, EMUS).

Distribution. This species is known only from the type locality.

Host. Unknown.

Etymology. From the Greek *andrikos* "manly" named in honor of Donald G. Manley for his research on Mutillidae; indeclinable.

Remarks. This species and *T. krima* are the first *Tobantilla* recorded from Colombia. Insects do not obey political boundaries, however, so there is no reason to assume that *T. andrikos* and *T. krima* are conspecific without a greater understanding of their geographic ranges. In fact, these species were collected in areas over 800 km apart (Fig. 8.5).

Tobantilla charrasca Casal, 1969

(Figs 8.1B, 8.2B)

Tobantilla charrasca Casal, 1969. Physis. 29: 102. Holotype ♀, Argentina, Rio Negro, Gral. Roca (AMNH).

Diagnosis. FEMALE. The mesosoma is not longer than broad (Fig. 8.2B); the mesosomal dorsum is clothed with concolorous pale golden setae, the sculpture of the pronotum obscured; the scutellar scale is separated from the dorsal propodeal carina; T2 is broadest at about its midlength and has both an anterior and posterior pair of yellow integumental spots; and T4 is clothed with black setae, at least basally.

Description. FEMALE. Body length 3-6.5 mm. *Coloration.* Body and appendages pale brown except pleura and femora dark brown; T2 with two pairs of separated yellow spots, anterior spots circular, much smaller than posterior triangular to trapezoidal patches. Tibial spurs white. Head and mesosoma clothed with fairly sparse decumbent pale golden or silvery lanceolate setae, except dorsum of head and mesosoma with scattered erect long brachyplumose brown setae; posterior face of propodeum with moderately dense decumbent pale golden setae. T2 clothed with dark brown decumbent lanceolate setae between spots and along apical margin, lateral thirds and integumental spots with sparse silver decumbent and erect setae. T3entirely clothed with decumbent dark brown lanceolate setae. Disc of T4 clothed with dark brown setae, apical fringe of T5 clothed with pale silver setae. T1, T5 and S1–5 clothed with silvery white decumbent and erect setae. T6 and S6 with erect brown setae. ***Head*.** Rounded posteriorly, with occipital carina stronger laterally from base of weak narrowly triangular glabrous tubercle on posterolateral margin. Head width 1.1 × pronotal width. Eye almost circular. Front, vertex and gena reticulate. Genal carina produced, extending anteriorly to hypostomal carina. Mandible oblique, tapering, bidentate apically but preapical inner tooth minute and usually obliterated, unarmed ventrally. Antennal scrobe with distinct lateral vertical carina but no dorsal carina. Antennal tubercle finely and sparsely punctate basally. Scape simply punctate. Flagellomere 1 1.5 × pedicel length; flagellomere 2 1.3 × pedicel length. ***Mesosoma*.** Mesosomal length 0.9 × width; pronotal width subequal to mesothorax width. Mesosomal dorsum reticulate. Humeral carina well developed, extending mesally beyond epaulet. Lateral face of pronotum and mesopleuron micropunctate, dull, mesopleural ridge coarsely reticulate; clothed with fine recumbent setae. Metapleuron dorsal to endophragmal pit and dorsal two-thirds of lateral face of propodeum glabrous, smooth; ventral regions of metapleuron and lateral face of propodeum micropunctate and clothed with fine recumbent setae. In dorsal view, lateral margins of mesothorax virtually parallel directly behind pronotal spiracle and anterior to propodeal spiracle, strongly narrowed posterior to anterior portion of mesothorax. Scutellar scale narrow and highly raised, with broadly w-shaped transverse carina anterior to it. Strong transverse carina separating dorsal and posterior propodeal faces on each side. Dorsal propodeal face parallel-sided posterior to spiracle, posterolateral angle nearly perpendicular, posterior face virtually flat, vertical, reticulate and clothed with moderately dense decumbent lanceolate setae on dorsal half. *Metasoma***.** T1 narrow and petiolate, somewhat cylindrical, 0.4 × as wide as T2. T2 1.15 × as long as wide, with maximum width situated medially. Disc of T2 with moderate-sized longitudinally ovate contiguous punctures, except yellow spots sparsely punctate with interspaces wider than punctures; T3–5 densely punctate. S1 with low longitudinal carina, S1–5 moderately punctate, punctures smaller and denser on S3–5. Pygidium well defined by continuous lateral and apical carinae, posterior margin broadly convex, surface weakly convex with about 10 irregular longitudinal striae almost reaching apical margin, spaces between striae irregularly granulate.

MALE. Unknown.

Material examined. Type material: *Holotype*, ♀, ARGENTINA: Rio Negro, G[ene]ral. Roca, I.1962, A. Bachmann (AMNH); *Paratypes*, ARGENTINA: Mendoza, Potrerillos, 1 ♀, I.1956, Fritz (AMNH); Rio Negro, G[ene]ral Roca, 1 ♀, I.1962, A. Bachmann (AMNH); C[olo]nel Belisle, 3 ♀, 6.XII.1961, A. Bachmann (AMNH). Other material: ARGENTINA: Cordoba, Xacanto, 1♀, XI.1966, M.A. Fritz (EMUS); Chubut, 3 km N Puerto Lobos, dunes, 2♀, 14.XII.1966, E.I. Irwin & M. Irwin (CASC); Rio Negro, Choele-Choel: 1♀, XII.1997, U.&M. Fritz (EMUS); 4♀, I.1991, U. Fritz (1 DJBC, 3 EMUS); 2♀, 15.XII.1989, M.A. Fritz (EMUS).

Distribution. Argentina (Cordoba, Chubut, Mendoza, Neuquén and Rio Negro).

Host. Unknown.

Remarks. This species is more widespread than *T. montonera* and is known from more specimens (Fig. 8.5). Similarly, *T. ephemeros* is more widespread and abundant than *T. drosos*. It seems likely that the male of *T. charrasca* is *T. ephemeros* but this is purely speculation, as the males of *T. ephemeros* and *T. drosos* share an overlapping range (Fig. 8.5).

Tobantilla drosos Williams, Brothers & Pitts, sp. nov.

(Figs 8.3B, 8.4B)

Diagnosis. MALE. The clypeus forms a flattened plate that is shallowly emarginate anteriorly (as in Fig. 8.4F), while the base of T2 has a dense patch of appressed silver setae (Fig. 8.3B).

Description. MALE. Body length 5–8.5 mm. *Coloration.* Head, mesosoma and legs black. T1 black, remainder of metasoma red, felt lines and apical segments infuscated. Antennae, medial portion of mandibles, and tegulae brown. Tibial spurs white. Wings weakly infuscated, veins brown. Head clothed with appressed silver setae, vertex with dense interspersed erect silver and brown setae. Mesosoma clothed with appressed silver setae, except posterior half of mesoscutum clothed with black setae.

Posterior half of T2 and metasomal segments 5–6 with interspersed black and silver setae; pygidium and hypopygidium clothed with black setae, remainder of metasoma clothed with silver setae. ***Head*.** Rounded posteriorly, with posterior projection behind ocelli. Head width 1.1 × pronotal width. Eye transversely ovate in dorsal view eye diameter 2.1 × distance from posterior eye margin to posterolateral angle of head. Ocelli small; ocellocular distance 3.8 × length of lateral ocellus, interocellar distance 1.3 × lateral ocellar length. Occipital carina weak but distinct, extending ventrally almost to level of oral fossa. Punctation on front, gena and vertex obscured by dense setae. Gena ecarinate. Antennal scrobe broadly concave to eye margin, with prominent complete flangelike transverse carina above. Clypeus projecting and shallowly emarginate anteriorly, anterolateral margins carinate. Oral fossa 1.7 × length of hypostomal bridge. Scape bicarinate ventrally. Flagellomere I 1.2 × pedicel length; flagellomere II 2.5 × pedicel length. Mandible oblique, tapering, bidentate apically, unarmed ventrally. Maxilla and labium elongate. Maxillary palp 6-segmented, third and fourth segments slightly flattened and apically expanded, other segments almost cylindrical. Labial palp 4-segmented, second and third segments slightly flattened and apically expanded, other segments almost cylindrical. *Mesosoma*. Epaulets lowly produced; pronotum emarginate anteromedially. Pronotal dorsal punctation coarsely contiguous but obscured by dense shaggy recumbent mesally directed setae; anterior face moderately punctate, except emarginated medial area glabrous; lateral face moderately punctate above but almost smooth ventrally. Tegula convex, glabrous almost hemispherical, glabrous except with long recumbent setae anterolaterally and posteromesally. Mesoscutum with moderately-spaced coarse punctures, punctation obscured on anterior fifth by dense shaggy recumbent posteriorly directed setae; micropunctate inside reticulations; notaulus weakly produced, extending to anterior margin; parapsis indistinguishable; posterolateral corner forming a coarsely punctate raised lobe. Scutellum slightly convex, with coarse punctures. Scutellum slightly convex, contiguously punctate. Axilla produced posteriorly, contiguously punctate on dorsal anterior half, glabrous on dorsal posterior half, posterior face truncate with posteriorly directed setae; anterolateral axillar margin contiguous with posterior mesonotal margin. Metanotum surface obscured by dense shaggy mesally facing recumbent setae. Mesopleural punctation obscured by dense setae. Metapleuron glabrous with small scattered punctures. Propodeum strongly convex, broadly reticulate, except smooth and shining adjacent to metapleuron, reticulations obscured by dense setae on anterior half of dorsal face. *Wings*. Forewing with moderate ovate sclerotized pterostigma; marginal cell moderate and truncate apically; three submarginal cells. *Legs*. Mid- and hind tibiae each with a few strong spines dorsally, distinct apical secretory pore on inner surface near base of inner spur; spurs densely clothed with microsetae. *Metasoma***.** T1 petiolate, 0.45 × width of T2, with moderately spaced coarse punctures. T2 with moderately spaced coarse punctures; apex obscured by dense fringe of mesally directed setae; felt line 0.4 × lateral length of T2. T3–6 densely punctate. Pygidium margined laterally and posteriorly by a sharp carina; surface convex, glabrous except apex moderately punctate and setose. S1 with obscure longitudinal carina, having scattered large punctures. S2 moderately punctate, evenly convex. S3–6 moderately punctate. Hypopygidium moderately punctate, posteromedial margin with sharp tooth. *Genitalia* (Fig. 8.4B). Free length of paramere dorsally curved apically, with ventral brush of long dense setae basally, remainder having scattered short sparse setae; apex of ventral face visible from above. Cuspis deeply divided near base, cylindrical, ~0.3 × free-length of paramere, posterior half having long dense posteriorly directed; densely setose basal lobe present, lobe roughly 0.6 × cuspis length. Digitus laterally compressed, constricted at apex, asetose, ~0.35 × free-length of paramere. Parapenial lobe well developed, apically sharply angulate. Penis valve asetose, ventral margin bidentate apically, teeth separated.

FEMALE. Unknown.

Type material. *Holotype*, ♂, ARGENTINA: Catamarca, Coneta, 16 km S Catamarca, 28°34.07'S 65°52.74'W, 554m, 25.X–12.XI.2003, coll. M.E. Irwin & F.D. Parker (EMUS). *Paratypes*, ARGENTINA: 9♂, same data as holotype (1 DJBC, 5 EMUS, 3 MACN); Catamarca, San Pablo, 28°41.81'S 66°02.55'W, 712m, malaise trap, 5♂, 24.X–12.XI.2003, M.E. Irwin & F.D. Parker (1 DJBC, 2 EMUS, 2 AMNH); Salta, 8 km N La Viña, 25°23.77'S 65°32.67'W, 1171m, 1♂, 26.X–13.XI.2003, M.E. Irwin & F.D. Parker (EMUS); 9 km N Cafayate, 26°00.46'S 65°58.44'W, 1684m, 1♂, 26.X–13.XI.2003, M.E. Irwin & F.D. Parker (EMUS); Tucumán, 8 km S Tichucho, 1♂, 24.III.1990, J.G. Rozen & A. Roig (AMNH); 11 km W Las Cajas, 2♂, 1–16.XI.1967, C.C. Porter (identified as ?*Tobantilla* sp. by DJB in 1989, MCZC).

Distribution. Argentina (Catamarca, Salta and Tucumán).

Host. Unknown.

Etymology. From the Greek *drosos* “dew” metaphorically reflecting the probability that this species will be associated with *T. charrasca* or *T. montonera*; noun in apposition.

Remarks. This species appears to be restricted to the northwestern Argentinean provinces, but shares an overlapping range with *T. ephemeros*.

There is significant variation in the integumental coloration of T2, ranging from bright orange to dark red. There is also variation in the size of the setal patch on T2; in some specimens it covers the entire basal half of T2, while others have only the anteromedial third covered with appressed setae. T1 can vary from being entirely red to being black with only the apical margin red.

Tobantilla ephemeros Williams, Brothers & Pitts, sp. nov.

(Figs 8.3C, 8.4: C, F)

Diagnosis. MALE. The clypeus forms a flattened plate that is shallowly emarginate anteriorly (Fig. 8.4F), and the disc of T2 has the interstitial spaces glabrous without decumbent setae (Fig. 8.3C).

Description. MALE. Body length 5–9 mm. *Coloration.* Head, mesosoma and legs black. T1 black, remainder of metasoma red, felt lines and apical segments infuscated. Antennae, medial portion of mandibles, and tegulae brown. Tibial spurs white. Wings weakly infuscated, veins brown. Head clothed with appressed silver setae, front and vertex with dense erect black setae. Mesosoma clothed with appressed silver setae, except mesoscutum with glabrous interstitial spaces; pronotum, mesonotum and scutellum with erect black setae. Posterior two thirds of disc of T2 and metasomal segments five through seven entirely clothed with black setae, remainder of metasoma clothed with silver setae. *Head.* Rounded posteriorly, with strong posterior projection behind ocelli. Head width 1.1 × pronotal width. Eye transversely ovate in dorsal view eye diameter 2.3 × distance from posterior eye margin to posterolateral angle of head. Ocelli small; ocellocular distance 5.0 × length of lateral ocellus, interocellar distance 1.2 × lateral ocellar length. Occipital carina weak but distinct. Front, gena and vertex moderately setose, densely punctate. Gena ecarinate. Antennal scrobe broadly concave to eye margin, with prominent complete flangelike transverse carina above. Clypeus projecting and shallowly emarginate anteriorly, anterolateral margins carinate. Oral fossa 1.25 × length of hypostomal bridge. Scape bicarinate ventrally. Flagellomere I 1.4X pedicel length; flagellomere II 2.5 × pedicel length. Mandible oblique, tapering, bidentate apically, unarmed ventrally. Maxilla and labium elongate. Maxillary palp 6-segmented, third and fourth segments slightly flattened and apically expanded, other segments almost cylindrical. Labial palp 4-segmented, second and third segments slightly flattened and apically expanded, other segments almost cylindrical. *Mesosoma.* Epaulets lowly produced; pronotum emarginate anteromedially. Pronotal dorsal punctation coarsely contiguous but obscured by dense shaggy recumbent mesally directed setae; anterior face moderately punctate, except emarginated medial area glabrous; lateral face moderately punctate above but almost smooth ventrally. Tegula convex, glabrous almost hemispherical, glabrous except with long recumbent setae anterolaterally and posteromesally. Mesoscutum with moderately-spaced coarse punctures, punctation obscured on anterior fifth by dense shaggy recumbent posteriorly directed setae; micropunctate inside reticulations; notaulus weakly produced, extending to anterior margin; parapsis indistinguishable; posterolateral corner forming a coarsely punctate raised lobe. Scutellum slightly convex, with coarse punctures. Axilla produced posteriorly, contiguously punctate on dorsal anterior half, glabrous on dorsal posterior half, posterior face truncate with posteriorly directed setae; anterolateral axillar margin contiguous with posterior mesonotal margin. Metanotum surface obscured by dense shaggy mesally facing recumbent setae. Mesopleural punctation obscured by dense setae. Metapleuron glabrous with small scattered punctures. Propodeum strongly convex, broadly reticulate, except smooth and shining adjacent to metapleuron, reticulations obscured by dense setae on anterior half of dorsal face. *Wings*. Forewing with moderate ovate sclerotized pterostigma; marginal cell moderate and truncate apically; three submarginal cells. *Legs*. Mid- and hind tibiae each with 1–2 inconspicuous strong spines and several strong slender semi-erect macrosetae dorsally, distinct apical secretory pore on inner surface near base of inner spur; spurs densely clothed with microsetae. *Metasoma.* T1 petiolate, 0.45 × width of T2, with moderately spaced coarse punctures. T2 with moderately spaced coarse punctures, interstitial spaces glabrous; apex obscured by dense fringe of mesally directed setae; felt line 0.4 × lateral length of T2. T3–6 densely punctate. Pygidium margined laterally and posteriorly by a sharp carina; surface convex, glabrous except apex moderately punctate and setose. S1 with obscure longitudinal carina, having scattered large punctures. S2 moderately punctate, evenly convex. Hypopygidium moderately punctate, posteromedial margin with sharp tooth. *Genitalia* (Fig. 8.4C). Free length of paramere dorsally curved apically, with ventral brush of long dense setae basally, remainder having scattered short sparse setae; apex of ventral face visible from above. Cuspis deeply divided near base, cylindrical, ~0.4 × free-length of paramere, posterior half having long dense posteriorly directed; densely setose basal lobe present, lobe roughly 0.6 × cuspis length. Digitus laterally compressed, constricted at apex, asetose, ~0.4 × free-length of paramere. Parapenial lobe well developed, apically sharply angulate. Penis valve asetose, ventral margin bidentate apically, teeth separated.

FEMALE. Unknown.

Type material. *Holotype*, ♂, ARGENTINA: La Rioja, Sierra de Argañaraz, 29 km N Chepes, 31°10.81'S 66°41.26'W, 1020m, malaise trap, 2–16.IX.1999, M.E. Irwin & F.D. Parker (EMUS). *Paratypes.* ARGENTINA: Catamarca, Andalgala, 1♂, 4.XI.1972, G.E. Bohart (EMUS); 4 km W Chumbicha, 28°51.33'S 66°16.13'W, 440m, 1♂, 13–15.IX.1999, M.E. Irwin & F.D. Parker (EMUS); Coneta, 16 km S Catamarca, 28°34.07'S 65°52.74'W, 554m, 2♂, 25.X–12.XI.2003, M.E. Irwin & F.D. Parker (EMUS); Palo Labrado, 23 km S La Merced, 28°19.94'S 65°37.13'W, 734m, 7♂, 23–25.X.2003, M.E. Irwin & F.D. Parker (EMUS); 11 km S Palo Labrado, 28°25.68'S 65°38.03'W, 668m, small dry wash w/ subtropical decid[uous] veg[etation], 1♂, 23–25.X.2003, M.E. Irwin & F.D. Parker (EMUS); San Pablo, 10♂, 28°41.81'S 66°02.55'W, 712m, malaise trap, 24.X–12.XI.2003, M.E. Irwin & F.D. Parker (1 DJBC, 9 EMUS); Sierra de Ambato, 13 km NW Chumbicha, 28° 50.00'S 66°24.46'W, 700m: 1♂, 13.IX.1999, pan trap, M.E. Irwin & F.D. Parker (DJBC); 1♂, 5.IX.1999, malaise trap, M.E. Irwin & F.D. Parker (EMUS); Sierra de Ambato, HW 60 18 km NW Chumbicha, 28°48.17'S 66°23.36'W, 950m, malaise trap, 1♂, 6–13.IX.1999, M.E. Irwin & F.D. Parker (EMUS); La Rioja, Rosaro V. Penaloza, Sierra de Argañaraz, El Rocillo, 31.2066°S 66.7126°W, 2370 ft., 1♂, 11–22.X.1997, M.E. Irwin, F.D. Parker & S. Roig (EMUS); Chilecito, 5 km E Piedra Pintada, 29.3598°S 67.7857°W, 2600 ft., 3♂, 12–15.X.1997, M.E. Irwin, F.D. Parker, & S. Roig (EMUS); Sierra de Ambato, Hwy 60 26 km SE Estación Mazán, 28°46.04'S 66°22.69'W, 1050m, 2♂, 6–9.IX.1999, M.E. Irwin & F.D. Parker (EMUS); Rio Negro, Choele Choel: 1♂, XII.1997, U. & M. Fritz (identified as “*Tobantilla* undet[ermined]” in Fritz collection, AMNH); 3♂, I.1991, U. Fritz (identified as “*Tobantilla* undet[ermined]” in Fritz collection, AMNH); 2♂, XI.1991, Fritz (AMNH); Luls Beltrán, 2♂, 25.xi.1991, Fritz (AMNH); Lamarque, 2♂, U. Fritz (identified as “*Tobantilla* undet[ermined]” in Fritz collection, AMNH); Salta, 9 km N Cafayate, 26°00.46'S 65°58.44'W, 1684m, 24♂, 26.X–13.XI.2003, M.E. Irwin & F.D. Parker (1 DJBC, 13 EMUS, 5 MACN, 5 AMNH); 10 km S Cafayete, 26°09.05'S 65°57.31'W, 1644m, malaise trap, 2♂, 26.X–13.XI.2003, M.E. Irwin & F.D. Parker (EMUS); Tucumán, 8 km NW Amaicha del Valle, 26°32.35'S 65°58.37'W, 1847m, Malaise trap, 1♂, 22–25.X.2003, M.E. Irwin & F.D. Parker (EMUS); 4 km S Capitan Cáceres, 27°13.54'S 65°38.34'W, 430m, 14♂, 24.X–12.XI.2003, M.E. Irwin & F.D. Parker (1 DJBC, 13 EMUS). URUGUAY, Montevideo, So[uth] Amer[ican] Peras Lab, 1♂, 17.I.1943, Barry (USNM).

Distribution. Argentina (La Rioja, Catamarca, Rio Negro, Salta, Tucumán) and Uruguay.

Host. Unknown.

Etymology. From the Greek *ephemeros* “short-lived” in reference to the probability that this species will be associated with *T. charrasca*, or *T. montonera*; indeclinable.

Remarks. This species is the most widespread *Tobantilla*, extending east to Uruguay, south to Rio Negro Province and northwest to Salta Province. Although more widespread than *T. drosos*, this species displays less color variation: T2 is red and T1 is black anteriorly in all of the paratypes.

Tobantilla frigidula **(Cresson, 1902), comb. nov.**

(Figs 8.1C; 8.2: C, G; 8.4G)

Mutilla frigidula Cresson, 1902. Trans. Amer. Ent. Soc. 28: 40. Holotype ♀, Brazil, Santarem (CMNH).

Mutilla frigidula: Nonveiller 1990: 113, ♀ (incertae sedis).

Diagnosis. FEMALE. The pygidial plate is small, distant from the apical margin and connected to it by a single median longitudinal carina (Figs 8.2G, 8.4G); the head and mesosoma are dark red to black (Fig. 8.1C); the scutellar scale is broadly arcuate, but not reaching the lateral propodeal margins; the posterior propodeal face is clothed with silver setae; and T2 has two large, nearly confluent yellowish-orange integumental patches.

Description. FEMALE. Body length 5.5-8 mm. *Coloration.* Body and appendages dark red; T2 with one pair of large trapezoidal yellow spots. Tibial spurs white. Head and mesosoma clothed with fairly sparse decumbent pale silvery setae, except anteromedial portion of mesosomal dorsum with patch of dark brown setae, anterolateral portions of mesonotum clothed with denser patch of silver setae, area directly anterior to scutellar scale with cluster of dark brown setae, and dorsum of head and mesosoma with scattered erect long brachyplumose brown setae; posterior face of propodeum with moderately dense decumbent silver setae and a few dark brown setae dorsally. T2 clothed with dark brown erect and appressed setae between spots and along apical margin, lateral thirds and integumental spots with sparse silver erect setae. T3–6 entirely clothed with decumbent silver setae and interspersed erect dark brown and silver setae. ***Head*****.** Angulate posterolaterally, with occipital carina stronger laterally from base of weak narrowly triangular glabrous tubercle on posterolateral margin. Head width 1.1 × pronotal width. Eye almost circular. Front, vertex and gena reticulate. Genal carina produced, extending anteriorly to hypostomal carina. Mandible oblique, tapering, bidentate apically but preapical inner tooth minute and usually obliterated, unarmed ventrally. Antennal scrobe lacking carina. Antennal tubercle finely and sparsely punctate basally. Scape simply punctate. Flagellomere 1 1.6 × pedicel length; flagellomere 2 1.1 × pedicel length. ***Mesosoma*.** Mesosomal length 1.25 × width; pronotum slightly wider than mesothorax. Mesosomal dorsum reticulate. Humeral carina well developed. Lateral face of pronotum and mesopleuron micropunctate, dull, mesopleural ridge coarsely reticulate; clothed with coarse silver setae. Metapleuron dorsal to endophragmal pit and dorsal two-thirds of lateral face of propodeum clothed with fine appressed silver setae; ventral regions of metapleuron and lateral face of propodeum micropunctate and clothed with coarse silver setae. In dorsal view, mesosoma gradually narrowed posterior to pronotum, lateral margin of mesothorax weakly divergent anterior to propodeal spiracle. Scutellar scale broad and highly raised, with obscure w-shaped transverse carina anterior to it. Dorsal and posterior propodeal faces not separated by transverse carina. Dorsal propodeal face convergent posterior to spiracle, posterolateral angle rounded, posterior face virtually flat, vertical, reticulate and clothed with moderately dense decumbent lanceolate setae on dorsal half. ***Metasoma*.** T1 narrow and petiolate, somewhat cylindrical, 0.4 × as wide as T2. T2 1.2 × longer than wide, dorsomedially flattened, with maximum width situated posteriorly. Disc of T2 deeply and narrowly reticulate anteriorly and coarsely punctate posteriorly; T3–5 densely punctate. S1 with longitudinal carina, S2 with coarse moderately-spaced punctures, punctures small and dense on S3–5. Pygidium lacking lateral carinae, medially having two posteriorly convergent carinae with scattered longitudinal rugae and shagreened medially, anteriorly this “pygidial plate” 0.2 × the pygidial width; areas lateral to these carinae moderately punctate and clothed with erect setae; posterior 0.2 × pygidial area asetose and obscurely punctured, posterior margin with contiguous low transverse carina.

MALE. Unknown.

Material examined. Type material. *Holotype*, ♀, BRAZIL: Pará, Santarem, H.H. Smith (identified as *Lomachaeta frigidula* (Cresson) by D. Quintero in 1996, CMNH). Other material. BRAZIL: Amazonas, Amazon River, 50 km S Manaus, 1♀, 5.I.2004 (EMUS); Pará, Taperinha, 1♀, 8.XII.1968, on mango leaves, coll. R.L. Jeanne (MCZC); Rondônia, P.E. Guajara Mirim, 1♀, 6.II.1998, coll. J.R.M. Santos (CPDC).

Distribution. Brazil (Amazonas, Pará, Rondônia).

Host. Unknown.

Remarks. This species has not been treated since its description (Cresson 1902) and was still in the genus *Mutilla* Linnaeus, 1758 until this paper (Nonveiller 1990). The pygidial sculpture (Figs 8.2G, 8.4G), coloration (Figs 8.1C, 8.2C) and distribution in northern Brazil (Fig. 8.5) likely contributed to the difficulty in placing this species in *Tobantilla*. The two previously recognized *Tobantilla* species have a well defined pygidial plate and were only known from Argentina (Casal 1965a, 1969). Like the other *Tobantilla* females, the mesosoma is flattened dorsally, appearing virtually concave anterior to the scutellar scale. The scutellar and propodeal armature of this species is nearly identical to that of *T. kolasma*, a species that matches the traditional diagnostic features for *Tobantilla*. The petiole is apparently more disciform than other *Tobantilla*, but is weakly constricted apically and is therefore considered petiolate, like other *Tobantilla* females.

Cresson (1902) noted the presence of a “rather long longitudinal groove” on each side of the propodeum. These are simply large reticulations situated immediately posteromesal to each propodeal spiracle. In the holotype, this reticulation is apparently undivided and elongate, but there is significant variation in the punctation of this region. In the specimen from Rondônia, it bears three contiguous setigerous reticulations that are slightly less differentiated from each other than from the surrounding reticulations. This trait does not seem much different from what is seen in other *Tobantilla* females.

Tobantilla kolasma **Williams, Brothers & Pitts, sp. nov.**

(Figs 8.1D, 8.2D, 8.4H)

Diagnosis. FEMALE. The head and mesosoma are pale brown (Fig. 8.1D); the scutellar scale is contiguous with the carinae that separate the dorsal and posterior propodeal faces (Fig. 8.1D); the posterior propodeal face is clothed with dense black setae (Fig. 8.2D); and the pygidial plate is defined by lateral carinae.

Description. FEMALE. Body length 4.5–5.5 mm. *Coloration.* Head, mesosoma, legs, antennae and mandibles pale brown. Metasoma brown except T2–4 dark brown to black; T2 having two pairs of yellow circular spots, anterior pair of spots longitudinally ovate, slightly larger than posterior circular pair. Tibial spurs white. Head and mesosoma clothed with pale golden or silvery setae, except medial portion of mesosoma having interspersed black and golden setae, area directly anterior to scutellar scale clothed with black setae, posterior face of propodeum densely clothed with thick black setae, and dorsum of head and mesosoma having a few scattered erect brown setae. T2–3 clothed with black setae, except lateral margins and integumental spots clothed with silver setae. T5–6 and S1–6 clothed with silvery white setae. ***Head*.** Rounded posteriorly, with lowly produced rectangular glabrous tubercles on posterolateral margin. Head width 1.1 × pronotal width. Eye almost circular. Front, vertex and gena reticulate. Genal carina lowly produced, extending anteriorly to hypostomal carina. Mandible oblique, tapering, bidentate apically, unarmed ventrally. Antennal scrobe with weak dorsal carina. Antennal tubercle moderately punctate basally. Scape simply punctate. Flagellomere 1 1.5 × pedicel length; flagellomere 2 1.3 × pedicel length. ***Mesosoma*.** Mesosomal length 1.1 × width; pronotum slightly wider than mesothorax. Mesosomal dorsum reticulate. Humeral carina low, extending to epaulet. Lateral face of pronotum and mesopleuron micropunctate, dull, mesopleural ridge broadly and shallowly reticulate; clothed with sparse silver setae. Metapleuron dorsal to endophragmal pit and dorsal two-thirds of lateral face of propodeum shagreened; ventral regions of metapleuron and lateral face of propodeum shagreened. In dorsal view, mesosoma gradually narrowed posterior to pronotum, lateral margin of mesothorax weakly sinuate anterior to propodeal spiracle. Scutellar scale broad and highly raised, contiguous with carina separating dorsal and posterior propodeal faces, lacking transverse carinae anteriorly. Dorsal propodeal face weakly convergent posterior to spiracle, posterolateral angle rounded, posterior face virtually flat, vertical, reticulate, and clothed with dense dark brown setae. ***Metasoma*.** T1 narrow and petiolate, somewhat cylindrical, 0.4 × as wide as T2. T2 1.1 × longer than wide, with maximum width situated medially. Disc of T2 with small longitudinally ovate contiguous punctures, except yellow spots sparsely punctate; S2 sparsely punctate. T3–5 densely punctate, S2–5 moderately punctate. Pygidium well defined by lateral carinae, posterior margin truncate, having 8 widely separated striae, spaces between striae irregularly granulate.

MALE. Unknown.

Type material. *Holotype*, ♀, VENEZUELA: Guarico, Hato Masaguaral (44 km S Calabozo), 11–19.V.1985, A.S. Menke & J. Carpenter (USNM). *Paratypes*. 2♀, same data as holotype (USNM, EMUS).

Distribution. This species is known only from the type locality.

Host. Unknown.

Etymology. From the Greek *kolasma* "punishment", a noun in apposition. This species, along with *T. krima* is named after the Russian novel Преступление и наказание "Crime and Punishment" written by Fyodor Mikhailovich Dostoevsky.

Remarks. The scutellar scale of this species is similar to that of *T. frigidula*. This species also has a narrower pygidial plate than *T. krima* and the Argentinean *Tobantilla* species.

Tobantilla krima Williams, Brothers & Pitts, sp. nov.

(Figs 8.1E, 8.2E)

Diagnosis. FEMALE. The pronotum is clothed with black setae that contrast with the pale golden mesonotal setae (Fig. 8.1E); the scutellar scale is separated from the dorsal propodeal carina; and T2 has a single pair of yellow integumental spots, on the posterior half (Fig. 8.1E).

Description. FEMALE. Body length 4.5 mm. *Coloration.* Head and mesosoma pale brown, except metapleuron and lateral face of propodeum brown. Metasoma dark brown to black, except T4–6 brown and T2 having a pair of yellow circular spots in basal half. Legs, antennae and mandibles pale brown. Tibial spurs white. Head and mesosoma clothed with pale golden or silvery setae, except pronotum clothed with black setae and dorsum of head and mesosoma having a few scattered erect brown setae. T2–3 clothed with black setae, except lateral margins and integumental spots clothed with silver setae. T5–6 and S1–6 clothed with silvery white setae. *Head***.** Rounded posteriorly, with lowly produced rectangular glabrous tubercles on posterolateral margin. Head width 1.0 × pronotal width. Eye almost circular. Front and vertex coarsely contiguously punctate; gena shallowly reticulate. Genal carina lowly produced, extending anteriorly to hypostomal carina. Mandible oblique, tapering, bidentate apically, unarmed ventrally. Antennal scrobe lacking dorsal carina. Antennal tubercle moderately punctate basally. Scape simply punctate. Flagellomere 1 1.2 × pedicel length; flagellomere 2 1.2 × pedicel length. *Mesosoma***.** Mesosomal length 1.1 × width; pronotum 0.95 × as wide as mesothorax. Mesosomal dorsum reticulate. Humeral carina low, extending to epaulet. Lateral face of pronotum and mesopleuron micropunctate, dull, mesopleural ridge broadly and shallowly reticulate; clothed with sparse silver setae. Metapleuron dorsal to endophragmal pit and dorsal two-thirds of lateral face of propodeum shagreened; ventral regions of metapleuron and lateral face of propodeum shagreened. In dorsal view, mesosoma gradually narrowed posterior to pronotum, lateral margin of mesothorax weakly sinuate anterior to propodeal spiracle. Scutellar scale highly raised, having transverse w-shaped carinae anteriorly. Dorsal and posterior propodeal faces separated by transverse carina. Dorsal propodeal face convergent posterior to spiracle, posterolateral angle rounded, posterior face virtually flat, vertical, reticulate and clothed with moderately dense silver setae on dorsal half. *Metasoma***.** T1 narrow and petiolate, somewhat cylindrical, 0.5 × as wide as T2. T2 1.l5 × longer than wide, with maximum width situated medially. T2 densely punctate, punctation similar on integumental spots and disc of T2; S2 moderately punctate. T3–5 densely punctate, S2–5 moderately punctate. Pygidium well defined by lateral carinae, posterior margin rounded, having 8 widely separated striae, spaces between striae glabrous.

MALE. Unknown.

Type material. *Holotype*, ♀, COLOMBIA: Bolivar, Zambrano, Alrededores Hacienda Toleteme, 9°N 74°W, 13.XII.1994, *Sobre carretera destapada* (on dirt road), M. Ospina (AMNH).

Distribution. This species is known from the type locality only.

Host. Unknown.

Etymology. From the Greek *krima* “crime”, a noun in apposition. This species, along with *T. kolasma*, is named after the Russian novel Преступление и наказание “Crime and Punishment” written by Fyodor Mikhailovich Dostoevsky.

Remarks. This species was collected over 800 km away from the only known Colombian male, *T. andrikos*, but was collected 400 km away from the Venezuelan male, *T. xouthos* (Fig. 8.5). More field observations, collection data or molecular data are necessary to make a concrete sex association.

The scutellar armature of this species is similar to that of the Argentinean species, *T. charrasca* and *T. montonera*, and dissimilar to that of *T. frigidula* and *T. kolasma*.

Tobantilla montonera Casal, 1965

(Figs 8.1F, 8.2F)

Tobantilla montonera Casal, 1965a. Rev. Soc. Mex. Hist. Nat. 25: 211. Holotype female, Argentina, Salta, Güemes, XII-1956, M. A. Fritz (AMNH).

Diagnosis. FEMALE. The mesosoma is longer than broad (Fig. 8.1F); the mesosomal dorsum is clothed with concolorous pale golden setae, except the mesonotum has some black setae medially; the scutellar scale is separated from the dorsal propodeal carina; T2 has both an anterior and posterior pair of yellow integumental spots that may be confluent; and T4 is clothed entirely with silver setae. Body length 3–5 mm.

Description. FEMALE. Body length 3-5 mm. *Coloration.* Body and appendages pale brown; T2 with two pairs of yellow spots, anterior and posterior spots typically confluent. Tibial spurs white. Head and mesosoma clothed with fairly dense decumbent pale golden or silvery lanceolate setae, except setae sparser medially on mesosoma, dorsum of head and mesosoma with scattered erect long brachyplumose brown setae, and area directly anterior to scutellar scale with decumbent dark brown setae; posterior face of propodeum with moderately dense decumbent pale golden setae. T2 clothed with dark brown decumbent lanceolate setae between spots and along apical margin, lateral thirds and integumental spots with sparse silver decumbent and erect setae. T3entirely clothed with decumbent dark brown lanceolate setae. T1, T4-5 and S1–5 clothed with silvery white decumbent and erect setae. T6 and S6 with erect brown setae. *Head***.** Rounded posteriorly, with occipital carina stronger laterally from base of weak narrowly triangular glabrous tubercle on posterolateral margin. Head width 1.1 × pronotal width. Eye almost circular. Front, vertex and gena reticulate. Genal carina produced, extending anteriorly to hypostomal carina. Mandible oblique, tapering, bidentate apically but preapical inner tooth minute and usually obliterated, unarmed ventrally. Antennal scrobe with distinct lateral vertical carina but no dorsal carina. Antennal tubercle finely and sparsely punctate basally. Scape simply punctate. Flagellomere 1 2.0 × pedicel length; flagellomere 2 1.5 × pedicel length. *Mesosoma***.** Mesosomal length 1.15 × width; pronotum 1.05 × as wide as mesothorax. Mesosomal dorsum reticulate. Humeral carina well developed. Lateral face of pronotum and mesopleuron micropunctate, dull, mesopleural ridge coarsely reticulate; clothed with fine recumbent setae. Metapleuron dorsal to endophragmal pit and dorsal two-thirds of lateral face of propodeum glabrous, smooth; ventral regions of metapleuron and lateral face of propodeum micropunctate and clothed with fine recumbent setae. In dorsal view, mesosoma gradually narrowed posterior to pronotum, lateral margin of mesothorax weakly sinuate anterior to propodeal spiracle. Scutellar scale narrow and highly raised, with broadly w-shaped transverse carina anterior to it. Strong transverse carina separating dorsal and posterior propodeal faces on each side. Dorsal propodeal face parallel-sided posterior to spiracle, posterolateral angle nearly perpendicular, posterior face virtually flat, vertical, reticulate and clothed with moderately dense decumbent lanceolate setae on dorsal half. *Metasoma***.** T1 narrow and petiolate, somewhat cylindrical, 0.4 × as wide as T2. T2 1.1 × longer than wide, with maximum width situated posteriorly. Disc of T2 with moderate-sized longitudinally ovate contiguous punctures, except yellow spots sparsely punctate with interspaces wider than punctures; T3–5 densely punctate. S1 with low longitudinal carina, S1–5 moderately punctate, punctures smaller and denser on S3–5. Pygidium well defined by continuous lateral and apical carinae, posterior margin broadly convex, surface weakly convex with about 8 irregular longitudinal striae almost reaching apical margin, spaces between striae irregularly granulate.

MALE. Unknown.

Host. Unknown.

Material examined. Type material: *Holotype*, ♀, ARGENTINA: Salta, Güemes, XII.1956, M. A. Fritz (AMNH); *Paratype*, 1 ♀, ARGENTINA, Salta, Tartagal, 6.iii.1961, O.H. Casal (AMNH). Other material. ARGENTINA: Jujuy, San Pedro, 1♀, 21.II.1971, M.A. Fritz & A. Martinez (EMUS); Salta, Alemania, 1♀, II.1983, M.A. Fritz (EMUS); Santiago del Estero, Tintina, 2♀, 15.XII.1971 D.J. Brothers (DJBC); 50 km NE Santiago del Estero, 3♀, 16.XII.1971 D.J. Brothers (2 DJBC, 1 EMUS).

Distribution. Argentina (Jujuy, Salta, Santiago del Estero).

Remarks. This species has only been recorded from northwestern Argentina. Both Argentinean species known from males are found in Salta; one of those is likely to be conspecific with *T. montonera*.

Tobantilla xouthos Williams, Brothers & Pitts, sp. nov.

(Figs 8.3D, 8.4D)

Diagnosis. MALE. The clypeus has a medial tubercle anteriorly (as in Fig. 8.4E) and the metasoma is entirely red except that segments six and seven are darkened (Fig. 8.3D).

Description. MALE. Body length 5 mm. *Coloration.* Head and mesosoma dark red to black, except humeral angles and propodeal dorsum bright red. Legs yellow-brown, tarsi and coxae darker brown. Metasoma yellow-brown except felt lines and metasomal segment seven darkened. Antennae, tegulae and mandibles brown. Tibial spurs white. Wings weakly infuscated, veins brown. Head clothed with appressed silver setae, vertex with interspersed erect silver and brown setae. Mesosoma clothed with appressed silver setae, except posterior half of mesoscutum clothed with black setae. Posterior quarter of T2 and metasomal segments five and six with interspersed black and silver setae; pygidium and hypopygidium clothed with black setae, remainder of metasoma clothed with silver setae. *Head.* Rounded posteriorly, with posterior projection behind ocelli. Head width 1.1 × pronotal width. Eye transversely ovate in dorsal view eye diameter 2.4 × distance from posterior eye margin to posterolateral angle of head. Ocelli small; ocellocular distance 4.0 × length of lateral ocellus, interocellar distance 1.5 × lateral ocellar length. Occipital carina weak but distinct. Punctation on front obscured by dense shaggy recumbent setae; gena and vertex moderately punctate. Gena ecarinate. Antennal scrobe broadly concave to eye margin, with prominent complete flangelike transverse carina above. Clypeus dentate anteromedially. Oral fossa 1.1 × length of hypostomal bridge. Scape bicarinate ventrally. Flagellomere I 1.0 × pedicel length; flagellomere II 2.2 × pedicel length. Mandible oblique, tapering, bidentate apically, unarmed ventrally. Maxilla and labium elongate. Maxillary palp 6-segmented, third and fourth segments slightly flattened and apically expanded, other segments almost cylindrical. Labial palp 4-segmented, second and third segments slightly flattened and apically expanded, other segments almost cylindrical. *Mesosoma.* Epaulets lowly produced; pronotum emarginate anteromedially. Pronotal dorsal punctation coarsely contiguous but obscured by dense shaggy recumbent mesally directed setae; anterior face shagreened, except emarginated medial area glabrous; lateral face moderately punctate above but almost smooth ventrally. Tegula convex, glabrous almost hemispherical, glabrous except with long recumbent setae anterolaterally and posteromesally. Mesoscutal punctation coarsely reticulate, micropunctate inside reticulations; notaulus and parapsis indistinguishable; posterolateral corner forming a coarsely punctate raised lobe. Scutellum slightly convex, contiguously punctate. Scutellum slightly convex, punctation obscured by dense silver setae. Axilla produced posteriorly, contiguously punctate on dorsal anterior half, glabrous on dorsal posterior half, posterior face truncate with posteriorly directed setae; anterolateral axillar margin contiguous with posterior mesonotal margin. Metanotum surface obscured by dense shaggy mesally facing recumbent setae. Mesopleural punctation obscured by dense setae. Metapleuron glabrous with small scattered punctures. Propodeum strongly convex, broadly and shallowly reticulate, with only one to two rows of reticulations on lateral propodeal face, smooth and shining adjacent to metapleuron, reticulations obscured by dense setae on anterior half of dorsal face. *Wings*. Forewing with moderate ovate sclerotized pterostigma; marginal cell short and truncate apically; two submarginal cells. *Legs*. Mid- and hind tibiae each with a few strong spines dorsally, distinct apical secretory pore on inner surface near base of inner spur; spurs densely clothed with microsetae. *Metasoma*. T1 petiolate, 0.4 × width of T2, with moderately spaced coarse punctures. T2 moderately punctate, interstitial spaces glabrous; apex obscured by dense fringe of mesally directed setae; felt line 0.35 × lateral length of T2. T3–6 densely punctate. Pygidium margined laterally and posteriorly by a sharp carina; surface convex, glabrous except apex moderately punctate and setose. S1 with uneven sharp longitudinal carina, having scattered large punctures. S2 moderately punctate, evenly convex. S3–6 moderately punctate. Hypopygidium moderately punctate, posteromedial margin with sharp tooth. *Genitalia* (Fig. 8.4D). Free length of paramere dorsally curved apically, with ventral brush of long dense setae basally, remainder having scattered short sparse setae; apex of ventral face visible from above. Cuspis deeply divided near base, cylindrical, ~0.3 × free-length of paramere, posterior half having long dense posteriorly directed; densely setose basal lobe present. Digitus laterally compressed, constricted at apex, asetose, ~0.35 × free-length of paramere. Parapenial lobe well developed, apically angulate. Penis valve asetose, ventral margin bidentate apically, teeth separated.

FEMALE. Unknown.

Type material. *Holotype*, ♂, VENEZUELA: Zulia, 6 km W La Concepcion, 18.VI.1976, A.S. Menke & D. Vincent (USNM). *Paratype*, ♂, VENEZUELA: Zulia, 10 mi E Machiques, 14.IX.1978, R.M. Bohart (identified as *Lophostigma* sp. by D.G. Manley, UCDC).

Distribution. This species is known from the type locality only.

Host. Unknown.

Etymology. From the Greek *xouthos* "yellow-brown" in reference to the body color, indeclinable.

Remarks. This species is most easily distinguished from *T. andrikos* by metasomal coloration. In addition, it generally appears to be less densely clothed with setae and to have less obvious barbs on the brachyplumose setae. It can be immediately distinguished from the Argentinean species by the clypeal structure; *T. xouthos* has a single median tubercle anteriorly on the clypeus, while *T. drosos* and *T. ephemeros* have the clypeus flattened and truncate anteriorly.

Gogoltilla **Williams, Brothers & Pitts, gen. nov.**

(Fig. 8.6)

Type species. *Gogoltilla chichikovi* Williams, Brothers & Pitts, **sp. nov.**

Diagnosis. MALE. The mandible is broadly dilated with a bilobate dorsal lamella (Fig. 8.6F); the axilla is posterolaterally produced and truncate posteriorly; the first metasomal segment is subsessile with the second (Fig. 8.6B); the free length of the paramere is slightly dorsoventrally flattened and initially downcurved toward the apex, but the extreme apex is upcurved and anteriorly directed (Fig. 8.6E6); and the cuspis has dense posteriorly directed plumose setae (Fig 8.6E).

FEMALE. The mesosoma is constricted just anterior to the propodeum and has a weak scutellar scale; the dorsal and flattened posterior propodeal faces are at an obtuse angle and separated by weak lateral transverse carinae; the first metasomal segment is narrow but weakly campanulate and not constricted posteriorly; the second metasomal tergum is clothed with dense stout striated setae in a basal triangular patch; the pygidium is broadly oval, entirely coarsely granulate, with lateral flangelike carina.

Description. FEMALE. T2 with paired indefinite pale integumental spots. *Head*. Broadly transverse, shiny, punctate, mostly clothed with slender decumbent setae and with a few slender simple erect setae near posterior margin of eye. Occipital carina absent. Vertex posterolaterally with inconspicuous broadly triangular glabrous elevation.

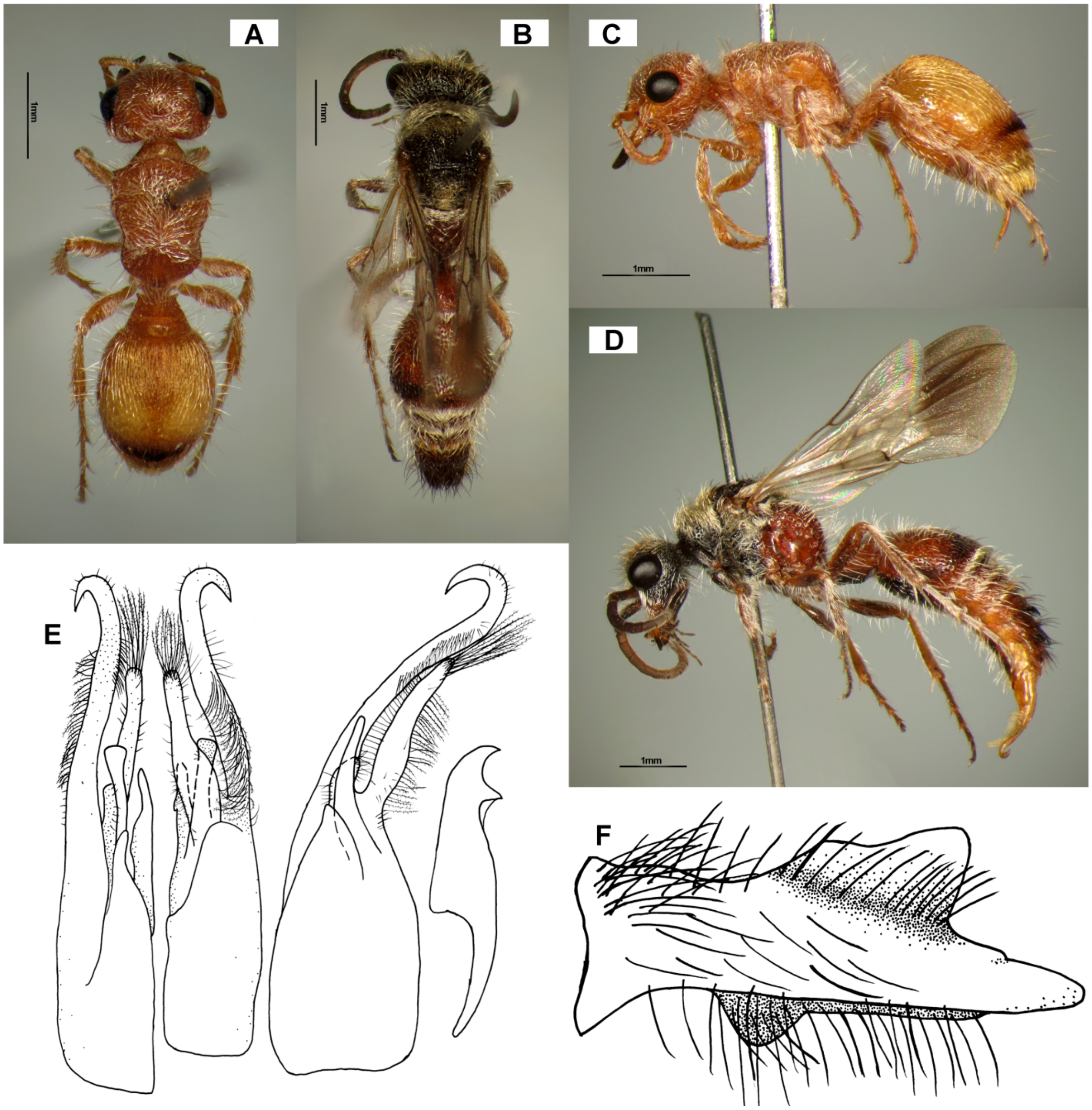

Figure 8.6. *Gogoltilla chichikovi* **gen. et sp. nov.** A. Female, habitus, dorsal view; B. Male holotype, habitus, dorsal view; C. Female, habitus, lateral view; D. Male holotype, habitus, lateral view; E, Male genitalia: dorsal view (left), ventral view (middle left), lateral view with penis valve removed (middle right), and lateral view of penis valve (right); F, Male mandible, lateral external view.

Eye circular, protruding, ommatidia distinct. Antennal tubercles with small dorso-medial tubercle, basally separated. Antennal scrobe smooth, unbounded laterally, without dorsal carina. Clypeus with complete weak setose transverse ridge above depressed transverse smooth area, ventral margin weakly emarginate medially. Malar space almost as high as combined length of pedicel + flagellomere 1. Genal carina very weak, extending about half height of gena, ending distant from hypostomal carina ventrally. Hypostomal carina distinct, even, following margin of oral fossa, ending laterally near inner margin of mandible, not reaching posterior mandibular condyle. Proboscidal and mandibular fossae separated by complete bridge, proboscidal fossa about as long as ventral surface of head. Scape simple, punctate above. Pedicel slightly longer than wide, shorter than first flagellomere; first flagellomere much longer than wide, longer than second. Mandible slender, tapering, gently arcuate, bidentate apically (subapical tooth minute, distant from apex and often obliterated through wear), unarmed ventrally. Maxillary palp 6-segmented; labial palp 4-segmented. Mentum smooth. *Mesosoma*. About as long as wide, broadened to point just posterior to pronotal spiracle, then strongly narrowed to propodeal spiracle, propodeum short and abruptly broadened posteriorly. Dorsum with surface shining, densely punctate to finely reticulate posteromedially, clothed with slender decumbent setae and with a few slender simple erect setae along lateral margin. Pronotal dorsal face (excluding anterior collar/flange) with anterior margin slightly convex, weakly carinate, epaulet inconspicuous, humeral angle acutely protruding and vertically carinate; anterior face and collar finely longitudinally striate. Posterodorsal margin of pronotum faintly indicated by very weak ridge, specially medially. Scutellar scale small and indistinct, merging with weak carina separating very short propodeal disk and oblique posterior declivity. Lateral face of pronotum densely and finely punctate, clothed with fine decumbent setae; pronotal-mesopleural suture scarcely discernible. Mesopleuron and metapleuron finely punctate, clothed with fine decumbent setae. Metapleural-propodeal suture obliterated dorsal to endophragmal pit. Lateral face of propodeum smooth and shining dorsally with isolated fine setae, micropunctate and densely setose ventrally; posterior face broadly reticulate dorsally, smooth ventrally, with slender decumbent setae near dorsal margin, scattered fine slender erect setae ventrally. Metasternal process triangular, unidentate acute apically, almost as long as metacoxal height. *Legs*. Tibial spur formula 1-2-2. Foreleg with tarsal comb, a few long strong articulated spines on posterior/lateral margins of tarsomeres. Mid- and hind tibiae each with two rows of prominent spines, 4–6 spines in each row, apical spines not inserted on any process; apical spurs narrow, finely and shallowly pectinate. Metacoxa with strong even longitudinal carina on inner margin. *Metasoma*. T1 slightly wider than long, > 0.5 × width of T2, fairly narrow and evenly expanded from base, weakly campanulate, not constricted apically, with slender simple erect setae and weak posterior fringe of slender lanceolate semi-decumbent setae; anterior auricle prominent, vertically lamellate. T2 densely clothed with thickened striated blunt setae in a broad basal triangle, apically with transverse band of laterally flattened lanceolate setae; lateral felt line broad, almost 0.5 × as long as T2 laterally. T3–5 clothed with slender lanceolate to simple decumbent setae. T6 with broad and flat pygidial area, entirely densely and coarsely granulate, well defined by lateral flangelike carina, posterior margin broadly convex. S1 with weak simple median carina. S2 without felt line.

MALE. Without defined pubescent or integumental spots. *Head.* Broadly transverse, about as wide as mesosoma, surface shiny, densely punctate, clothed with simple decumbent and erect setae (latter very rarely briefly barbed). Occipital carina distinct dorsally. Vertex evenly convex behind ocelli. Eye transversely ovate, protruding, ommatidia distinct. Ocelli small. Antennal tubercles punctate dorsally, smooth mesoventrally below a slight longitudinal ridge, contiguous. Antennal scrobe broadly concave to eye margin, with prominent complete flangelike transverse carina above. Clypeus transversely concave, with transverse anterior/ventral setose lobe on each side above emarginate margin. Malar space about equal to basal height of mandible, about as long as first flagellomere. Gena ecarinate. Hypostomal carina distinct but weak, even, following margin of oral fossa, ending laterally near inner margin of mandible, not reaching posterior mandibular condyle. Proboscidal and mandibular fossae separated by complete bridge, proboscidal fossa about as long as ventral surface of head. Scape weakly bicarinate anteriorly on apical half. Pedicel slightly wider than long, shorter than first flagellomere, which much shorter than second flagellomere. Mandible with broad elongate dorsal flange at about midlength, bidentate apically, ventral carina on inner surface expanded as a lamellate tooth near base, in dorsal/anterior view with lateral margin evenly curved. Maxillary palp 6-segmented; labial palp 4-segmented; intermediate palpomeres scarcely flattened but expanded apicad. Mentum smooth, longitudinally convex. *Mesosoma.* Surface shiny, densely punctate except smooth on anterior face of pronotum, metapleuron and lateral face of propodeum, and reticulate on remainder of propodeum; clothed with simple to narrowly lanceolate decumbent and erect setae (latter very rarely briefly barbed). Pronotal dorsal face (excluding anterior collar/flange) with anterior margin slightly convex, rounded, epaulet scarcely evident, humeral angle rounded. Posterodorsal margin of pronotum broadly concave, sigmoidal on each side. Tegula trianguloid, truncate posteriorly, evenly convex, almost entirely smooth and glabrous. Mesoscutum with notaulus deep and complete, parapsis almost indistinguishable; posterolateral corner forming a slight raised lobe discontinuous with axilla. Scutellum pulvinate. Axilla laterally produced as an obliquely projecting blunt tooth. Metanotum simple, transverse. Propodeum strongly convex, disk and declivity fairly distinct in lateral view. Metasternal process unidentate, apex blunt, shorter than metacoxal height. *Wings*. Forewing with moderate ovate sclerotized pterostigma; marginal cell fairly short, rounded apically; two closed submarginal cells. *Legs*. Tibial spur formula 1-2-2. Mid- and hind tibiae each with two rows of a few strong spines and several strong slender semi-erect macrosetae dorsally, apical spines not inserted on any process; subapically with conspicuous secretory pore near base of inner spur; apical spurs longitudinally concave dorsally, margins microserrate, densely clothed with microsetae. Metacoxa with weak longitudinal carina on inner/posterior margin, there with patch of erect simple to brachyplumose setae. *Metasoma*. T1 longer than wide, > 0.5 × width of T2, gradually broadened posteriad, sessile apically in dorsal view, oblique anterior and dorsal faces evenly merging, punctate with simple erect setae; anterior auricle moderate, forming rounded vertical flattened lobe. T2 punctate, with simple erect and narrowly lanceolate decumbent setae; felt line fairly narrow and long. T3–6 punctate, with simple erect and narrowly lanceolate decumbent setae. T7 punctate with simple narrowly lanceolate erect setae basally, pygidial area distinct, flattened, with strong lateral and apical marginal carina. Sterna punctate with simple erect to decumbent setae. S1 with weak longitudinal carina. S2 simple, evenly convex, without felt line. S7 concealed. Hypopygium with median weakly upcurved tongue-like lobe on posterior margin. *Genitalia*. Paramere and cuspis with long finely plumose erect setae. Paramere elongate, dorsoventrally flattened, acute, dorsally strongly recurved apically. Parapenial lobe well developed. Cuspis deeply divided near base, dorsal lobe (paracuspis) cylindrical, ventral lobe triangular in cross-section; digitus flattened, much shorter than cuspis. Penis valve asetose, bidentate apicoventrally, teeth separated.

Species included. Type species only.

Distribution. Argentina (Santa Fe, Santiago del Estero).

Etymology. This genus is named in honor of the Ukrainian and Russian author Nikolai Vasilievich Gogol, with a common suffix for mutillid genera. Gender feminine.

Remarks. Metasomal shape, female mesosomal shape and male genitalia closely link *Gogoltilla* to some genera in the subtribe Pseudomethocina (*sensu* Brothers 1975, 1999). First, the parameres are dorsoventrally flattened and initially downcurved (Fig. 8.6E); second, the penis valve is asetose (Fig. 8.6E); third, the first tergite is subsessile with the second (Fig. 8.6: A–D); and finally, the female mesosoma is sharply constricted anterior to the propodeal spiracle in dorsal view (Fig. 8.6A).

Some of this wasp's features, however, are not typically associated with pseudomethocine genera. There are plumose or brachyplumose setae on the genitalia, scape and antennal scrobe, which are commonly characteristic of nocturnal sphaeropthalmines. Many Pseudomethocini, including some Nearctic members of *Pseudomethoca* Ashmead, 1899, have brachyplumose setae in these same body regions, however. Also, the axillae are strongly armed, which is characteristic of *Dasymutilla* and its relatives (including *Reedomutilla*, *Suareztilla* and *Traumatomutilla*) and of *Lomachaeta* Mickel, 1936. We have observed variation in axillar armature of pseudomethocine genera in North and South America (pers. obs.), however. Also, the axillar armature of this genus differs from that of *Dasymutilla* or *Lomachaeta* by being disconnected from the posterior mesonotal margin anterolaterally. Perhaps this genus shares the most traits with *Tobantilla* (Sphaeropthalmina): the coloration is similar in both sexes, the male paramere is sharply upcurved apically, and brachyplumose setae are similarly distributed in both genera.

Gogoltilla clearly contains a mixture of traits seen in both Sphaeropthalmina and Pseudomethocina genera. When recognising the subtribes, Brothers (1975) expressed uncertainty concerning this classification and suggested that additional or fewer subtribal taxa may be necessary. *Gogoltilla*, along with *Lophomutilla* Mickel, 1952, *Tallium* André, 1902 and *Protophotopsis* Schuster, 1947, is yet another genus that cannot easily be placed into either subtribe. These genera indicate that Sphaeropthalmina and Pseudomethocina might not be strictly monophyletic, and that formal subtribes within the tribe Sphaeropthalmini may not be warranted.

Gogoltilla chichikovi **Williams, Brothers & Pitts, sp. nov.**

(Fig. 8.6)

Description. MALE. Body length 6.6 mm. *Coloration*. Head and mesosoma reddish-black to black, except posterior fifth of mesopleuron, metapleuron and propodeum reddish-brown (Figs 8.6: B, D). Legs, mandibles and tegulae yellowish-brown; antennae dark brown. Metasoma reddish-brown, T2 weakly infuscated near posterior margin on each side and along felt line. Tibial spurs white. Forewing hyaline between veins, apical third infuscated, veins brown; hindwing hyaline. Body clothed with whitish setae, except clypeus with brownish setae, posterior two thirds of mesonotum, axillae and anterior third of scutellum, posterior third of disc of T2 and T5–7 with blackish setae, S6 and S8 with reddish-brown setae. *Head*. Rounded subquadrate, face strongly concave, posterolateral angles rounded. Head width 1.2 × pronotal width. Eye transversely ovate; in dorsal view eye diameter 3.0 × distance from posterior eye margin to posterolateral angle of head. Ocelli small; ocellocular distance 5.0 × length of lateral ocellus, interocellar distance 3.1 × lateral ocellus length. Occipital carina weak but distinct, extending ventrally almost to level of oral fossa. Punctation on front obscured by dense shaggy recumbent setae; gena and vertex moderately punctate. Gena ecarinate. Antennal scrobe broadly concave to eye margin, with prominent complete flangelike transverse carina above. Clypeus strongly concave, smooth and shining, ventral margin produced as a rounded elevation on each side bearing dense erect setae. Oral fossa very large, hypostomal bridge much reduced. Scape bicarinate ventrally on apical half. Flagellomere 1 1.7 × pedicel length; flagellomere 2 2.3 × pedicel length. Mandible strongly dilated on about middle third where dorsal carina lamellate and broadly bilobate dorsally; apical tooth long, subapical tooth small; ventral carina on outer surface unarmed and gradually becoming obsolete anteriorly; ventral carina on inner surface expanded as an obliquely triangular lamellate tooth along inner margin near base. Maxilla and labium elongate. Maxillary palp 6-segmented, third segment slightly flattened and apically expanded, other segments almost cylindrical. Labial palp 4-segmented, second and third segments slightly flattened and apically expanded, other segments almost cylindrical.

Glossa produced as a narrow tongue about half length of labial palp. *Mesosoma*. Epaulets weakly produced. Pronotal dorsal punctation coarsely contiguous but obscured by dense shaggy recumbent mesally directed setae; anterior face almost smooth; lateral face moderately punctate above but almost smooth ventrally. Tegula convex, almost hemispherical, glabrous except with long recumbent setae anterolaterally and posteromesally. Mesonotal punctation coarsely contiguous, obscured on anterior third by dense shaggy recumbent posteriorly directed setae; flattened slender lanceolate setae on posterior two-thirds; notaulus broad and deep posteriorly, becoming narrower and evanescent anteriorly; parapsis short and almost indistinguishable; posterolateral corner forming a deeply punctate raised lobe. Scutellum slightly convex, punctation coarse and contiguous, obscured by dense shaggy recumbent setae on posterior two-thirds. Axilla produced posterolaterally as a toothlike flange, coarsely contiguously punctate but smooth posterolaterally, posteriorly truncate; anterolateral axillar margin and posterolateral mesonotal lobe separated by strong incision. Metanotum surface obscured by dense shaggy mesally facing recumbent setae. Propodeum strongly convex, entirely coarsely and deeply reticulate except smooth and shining adjacent to metapleuron. Mesopleural punctation coarsely contiguous dorsally becoming finer posteroventrally, mainly obscured by dense shaggy recumbent setae. Metapleuron smooth and shining, glabrous except at dorsal and ventral extremities. Wings. Forewing with moderate elongate sclerotized pterostigma; marginal cell broadly rounded and truncate apically; two submarginal cells. Legs. Mid- and hind tibiae each with a few strong spines dorsally, distinct apical secretory pore on inner surface near base of inner spur; spurs finely serrate on margins. *Metasoma*. T1 weakly campanulate, gradually broadened from base, not constricted apically although weakly discontinuous with T2, 0.5 × width of T2, sparsely and finely punctate; apex with weak obtusely triangular band of mesally directed recumbent setae. T2 with coarse to moderate punctures, interspaces smooth and narrower than punctures; apex obscured by dense fringe of mesally directed recumbent setae; felt line 0.6 × lateral length of T2. T3–4 densely and finely punctate, covered with moderately dense mesally directed recumbent setae. T5–6 moderately and finely punctate, covered with less dense recumbent and erect setae. Pygidium broadly oval, margined laterally and posteriorly by a strong sharp carina, apical margin shallowly emarginate; surface shallowly concave, densely and very finely punctate, covered with dense very fine erect setae. S1 almost entirely smooth, surfaces defined by blunt Y-shaped ridge. S2 sparsely punctate, medially flattened, surface smooth and shining. S3–6 moderately and finely punctate, with fairly sparse erect and recumbent setae. S7 entirely concealed. Hypopygium smooth, moderately and finely punctate, posteromedial margin produced as a narrowly rounded tooth. *Genitalia* (Fig. 8.6E). Free length of paramere dorsoventrally flattened, initially downcurved but with apex strongly recurved upwards and directed anteriorly, with long ventrolateral fringe of long dense erect setae at about midlength, shorter dorsomesal brush of short dense recumbent setae near apical curvature, remainder with scattered short to long setae. Cuspis deeply divided near base; basal (dorsal) lobe finger-like, almost as long as apex of penis valve, setose; ventral lobe elongate, triangular in cross section, strongly narrowed at about half length, ~0.6 × free-length of paramere, apex with ventral flattened tuft of dense long brachyplumose setae. Digitus elongate and curved, slightly flattened, weakly broadened and spatulate apically, asetose, ~0.3 × free-length of paramere. Parapenial lobe well developed, apically narrow and digitiform, obliquely flattened. Penis valve asetose, ventral margin bidentate apically, teeth separated; anterodorsal area angulate and sharply raised.

FEMALE. Body length 3.7–5.8 mm. *Coloration.* Entire body and appendages pale brown except T2 with one pair of indefinite large yellow ovate to triangular integumental spots on posterior half, spots mesally approximated but not fused. Mid and hind tibial spurs whitish. Head and mesosoma sparsely clothed with stout decumbent silvery or pale golden setae dorsally, decumbent setae finer on gena and very fine on mesosomal pleura, sparse long erect simple pale setae specially on mesosomal margins. T1 with posterior fringe of laterally flattened narrowly lanceolate orange-brown setae, anteriorly with a patch of long erect simple pale setae, a transverse row of long erect pale setae just anterior to fringe. T2 densely clothed with thickened striated blunt golden setae in a broad basal triangle produced posteriorly as a narrow band between pale integumental spots, some setae in a small patch at anterior base of triangle darker reddish-brown (sometimes extending almost to mid-length of tergum); elsewhere with sparse simpler setae, silvery on lateral surface and golden on pale spots; posteriorly with complete band of narrowly lanceolate reddish-brown setae; patch of dense silvery setae along margin posteroventral to felt line. T3 clothed with narrowly lanceolate orange-brown setae. T4–6 densely clothed with narrowly lanceolate golden setae (confined to small lateral patch on T6). S1–4 with sparse fine simple silvery setae, forming denser fringes posteriorly on S2–4. S5–6 with dense narrowly lanceolate golden setae (confined to minute lateral patch on S6). ***Head.*** Broadly transverse, posterior margin slightly concave with very weak triangular glabrous tubercle merging ventrolaterally with weak diagonal ridge on each side, occipital carina absent. Head width 1.1 × pronotal width. Eye circular, protruding, ommatidia distinct. Front, vertex and gena smooth and shining, moderately punctate, interspaces mainly wider than punctures, but punctation denser anteromesal to eye. Genal carina very weak, extending about half height of gena. Clypeus with complete almost straight setose transverse ridge above depressed transverse smooth almost glabrous area, ventral margin weakly emarginate. Mandible slender, tapering, bidentate apically (subapical tooth minute, distant from apex and usually obliterated through wear), unarmed ventrally. Antennal scrobe smooth dorsally, without carina. Antennal tubercle moderately punctate, with distinct smooth elevation anteromesally. Scape simple, moderately punctate. Flagellomere 1 1.5 × pedicel length; flagellomere 2 1.2 × pedicel length. Maxilla and labium elongate. Maxillary palp 6-segmented, third segment slightly flattened and apically expanded, other segments almost cylindrical. Labial palp 4-segmented, second and third segments slightly flattened and apically expanded, other segments almost cylindrical. Glossa produced as a narrow tongue about half length of labial palp. *Mesosoma***.** Mesosomal length 0.9 × width. Mesosomal dorsum finely shagreened, densely punctate on anterior half, more coarsely reticulate posteriorly. Humeral angle acutely protruding, vertical carina on dorsal third of pronotum lateral face moderate to strong, humeral carina weak and extending to epaulet. Meso- and metapleura shining, finely to micro-punctate, clothed with fine decumbent setae. Lateral face of propodeum smooth and shining dorsally with isolated fine setae, micropunctate and densely setose ventrally. In dorsal view, mesosoma broadened to anterior third, strongly narrowed at propodeal spiracle, propodeum short and abruptly broadened. Posterodorsal margin of pronotum faintly indicated by very weak ridge, specially medially. Scutellar scale small and indistinct, merging with weak carina separating dorsal and posterior propodeal faces. Posterior face of propodeum diagonal, weakly concave, very coarsely reticulate dorsally but almost smooth ventrally, separated from lateral face by weak lateral carina on dorsal half, with sparse fine erect setae but setae coarser and recumbent dorsally. ***Legs*.** Foreleg with a few long strong articulated spines on posterior/lateral margins of tarsomeres, one at midlength and two at apex of tarsomere 1, one at apex of tarsomere 2. Mid- and hind tibiae each with two rows of prominent spines, 4–6 spines in each row; apical spurs finely serrated laterally. Hind tibia with indistinct secretory pore on inner/posterior surface near base of inner spur. ***Metasoma*.** T1 fairly narrow and evenly expanded from base, weakly campanulate, not constricted apically, 0.5 × as wide as T2; anterodorsal face smooth but finely and densely punctate ventrally, posteriorly with band of dense small punctures. T2 with small longitudinally ovate contiguous punctures anteromesally (punctation there mostly concealed by dense setae), laterally more sparsely punctate, yellow spots very sparsely and finely punctate (interspaces about 3 × width of punctures), posterior margin densely and finely punctate; felt line broad, 0.4 × as long as T2 laterally. T3–5 densely punctate. Pygidium broad and flat, well defined by lateral flangelike carinae, posterior margin broadly convex, entirely densely and coarsely granulate. S1 coarsely reticulate, with weak simple median carina. S2 sparsely punctate. S3–5 indistinctly punctate. S6 densely and finely punctate.

Type material. *Holotype*, ♂, ARGENTINA: Santiago del Estero, 50 km NE Santiago del Estero, 16.XII.1971, D.J. Brothers, ♂ pursuing ♀ on ground (MACN). *Paratypes*, ARGENTINA: Santa Fe,1♀, Laguna Pana, III.1972, Martinez (AMNH); Santiago del Estero,1♂, Termas Rio Hondo, 20–21.II.1972, H.E. Evans (identified as

?*Tobantilla* sp. by DJB in 1989, MCZC); 12 ♀♀, same data as holotype, 1♀ pursued by ♂ on ground (6 DJBC, 3 EMUS, 3 MACN).

Distribution. This species is known from Santiago del Estero and Santa Fe Provinces in Argentina.

Host. Unknown. The specimens collected by DJB were on sandy soil. This, and the long spines and setae on the legs of the female, as well as its well developed pygidial plate, imply soil-dwelling hosts.

Etymology. This species is named after the fictional character Pavel Ivanovich Chichikov, the protagonist of Мёртвые души "Dead Souls", widely considered the greatest novel by Nikolai Vasilievich Gogol. A noun in the genitive case.

Remarks. This species superficially resembles *Tobantilla* species in coloration and genitalic structure. It can immediately be distinguished in both sexes by the shape of T1; *Tobantilla* species have T1 narrowly petiolate (*e.g.* Figs 8.1, 8.2), *G. chichikovi* has T1 subsessile with T2 (Figs 8.4: A–D).

Discussion

No host information is currently known for *Tobantilla*. The only clues we have are morphology of the pygidium and tarsal rake in females, label data from two specimens, a statement by Casal (1965a) and personal observations by DJB. Previous studies have revealed a link between pygidial shape and host preference in Mutillidae (*e.g.*, Pitts & Manley 2004). Species with a well defined pygidial plate in the female typically parasitize ground-nesting hosts while females with an undefined pygidium parasitize arboreal or twig-nesting hosts. Although *Tobantilla frigidula* technically possesses a pygidial plate, this plate is narrow and present only in the basal portion of T6 (Figs 8.2G, 8.4G). The fore-tarsal rake of *T. frigidula* is weaker than that in the other species. Additionally, one of the specimens was collected “on Mango leaves.” This evidence suggests that *T. frigidula* is parasitic on arboreal or twig-nesting hosts. The remaining five *Tobantilla* females have a well defined pygidial plate (*e.g.* Figs 8.2H, 8.4H) and long thickened fore-tarsal spines. The only specimen of *T. krima* was collected “*Sobre carretera destapada”* (on dirt road). Casal (1965a) stated that the paratype of *T. montonera* was collected “*en un camino, alrededor de las 13 horas, en días despejado y con una temperatura de 33°*” (on a roadway at about 13:00 in fine weather and a temperature of 33°C). The specimens collected by DJB were found on sandy soil under similar conditions. This evidence suggests parasitism on ground-nesting hosts.

All females of *Tobantilla* will accurately key out in the most recent key to Neotropical genera (Brothers 2006). *Tobantilla frigidula* could be easily misidentified, however, because of peculiarities in the pygidium. This species will key to *Lomachaeta* if the pygidial structure is considered undefined; it can be separated from *Lomachaeta* by the petiole shape, as *Lomachaeta* species have a short disciform petiole while *T. frigidula* has a clearly petiolate petiole. The newly described males of *Tobantilla* will key to *Dasymutilla/Traumatomutilla*. They can be separated from those genera by the anteriorly projecting clypeus that forms either a median tooth (Fig. 8.4E) or truncate plate (Fig. 8.4F) and the abundant brachyplumose setae on the head and propodeum. *Dasymutilla* and *Traumatomutilla* have the clypeus not so produced and lack obvious brachyplumose setae. Females of *Gogoltilla* will key to *Darditilla* Casal, 1965b. They can be distinguished from *Darditilla* by the campanulate condition of T1 (sessile with T2 in

Darditilla) and the presence of a dense patch of appressed thickened setae anteromesally on T2 (T2 with simple setae in *Darditilla*). Males of *Gogoltilla* terminate at couplet 69 in Brothers (2006) because they lack a felt line on S2 and have well developed notauli. They can be recognized by the genitalic structure and diagnostic features mentioned above.

Based on similarities in clypeal structure, *T. andrikos* is closely related to *T. xouthos* and *T. drosos* is closely related to *T. ephemeros*. Setal pattern or integumental coloration is used to diagnose species in these pairs, but structural differences are not obvious between these putative sisters. Over-reliance on differences in coloration, however, has necessitated synonymy in many Nearctic mutillids, including *Dasymutilla foxi* (Cockerell), *Dasymutilla quadriguttata* (Say) and *Dasymutilla bioculata* (Cresson) (Pilgrim *et al.* 2008, Pilgrim *et al.* 2009, Williams *et al*. 2011). In the Neotropical Mutillidae, as well, over-reliance on integumental color in the genus *Lomachaeta* (Williams & Pitts 2007) necessitated synonymy (Williams & Pitts 2009) after intermediate color forms were found. Wilson and Pitts (2009), however, showed that setal coloration was the only consistent morphological difference separating the phylogenetically distinct *Sphaeropthalma unicolor* (Cresson) and *S. mendica* (Blake). Unlike the *Dasymutilla* and *Lomachaeta* examples above, the color differences separating *T. andrikos* from *T. xouthos* are supported by differences in distribution and density of setae and punctures as noted in the descriptions. Therefore, we hypothesize that *T. andrikos* and *T. xouthos* are discrete species, rather than conspecific color forms. Additional sampling may fail to support this hypothesis, however. Females of *Tobantilla*, unlike these males, are all separated by clearly defined structural characters of the mesosoma and pygidium. Differences in coloration are supplementary tools for aid in species identification.

Males of *Tobantilla* and *Gogoltilla* have parameres that are upcurved apically. *Tobantilla* males from southern South America, *T. drosos* (Fig. 8.4B) and *T. ephemeros* (Fig. 8.4C), have much more obviously upcurved parameres than the northern South American species, *T. andrikos* (Fig. 8.4A) and *T. xouthos* (Fig. 8.4D). Three additional southern South American genera have strongly upcurved parameres: *Reedomutilla*, *Suareztilla* and *Chilephotopsis* Cambra & Quintero, 2006. While *Reedomutilla*, *Suareztilla* and *Tobantilla* are apparently closely related based on metasomal and axillar similarities, *Chilephotopsis* and *Gogoltilla* are apparently from other lineages. *Chilephotopsis* is a nocturnal genus similar to *Sphaeropthalma* Blake, 1871 and its relatives. *Gogoltilla* is apparently a member of subtribe Pseudomethocina (see above). If these putative relationships are accurate, then extreme upcurving of the male paramere appears to have evolved at least three separate times in southern South American Mutillidae. This is surprising because sexual traits like male genitalia are typically subject to divergent, rather than convergent, selection.

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CHAPTER 9

SUMMARY AND CONCLUSIONS

Velvet ants are diverse and charismatic wasps that have unrealized research potential and could be economically important because they attack pollinating insects. Limitations in taxonomic, natural history, and phylogenetic knowledge have impeded research on these insects. Recent molecular studies have facilitated taxonomic changes (e.g., Pilgrim et al. 2009) and made mutillids model organisms for biogeography studies (e.g., Pitts et al. 2010; Wilson & Pitts 2010, 2011). The most well-known and frequently studied mutillid wasps are members of the dasymutilline genera (e.g., Manley & Pitts 2007). The goal of this dissertation was to address phylogenetic and taxonomic hypotheses across multiple hierarchical levels in the dasymutilline genera. The early chapters examined biogeography and mimicry within and between genera using molecular phylogenetics. The middle chapters used molecular and morphological data to address phylogenetic, natural history, and taxonomic hypotheses among closely related species. The later chapters, informed by previous molecular results, addressed taxonomic hypotheses with morphological data only.

This dissertation reveals the value of phylogenetics. Species-group nomenclature is especially important when studying immensely large genera like *Dasymutilla*. In Chapter 4, I showed that limits of the *D. monticola* species-group could be determined using phylogenetic reconstruction. In this same chapter, phylogenetic results clearly revealed incorrect previous sex associations and permitted these to be corrected. In Chapter 7, sex associations were facilitated by species-group placement. Overall, molecular and morphological data permitted publication of 13 new species-group assignments, seven new sex associations, and 24 new synonyms. Taxonomic research is often slighted in the scientific community and is difficult to publish in high impact journals. Taxonomy, however, is a vital component for studying evolution, because scientific names allow previous research to be indexed and accurately replicated. Additionally, comparative studies of evolutionary patterns are impossible without accurate nomenclature; in fact, incorrect classification schemes have led to erroneous conclusions concerning the mechanisms of evolutionary phenomena.

The taxonomic and phylogenetic data in turn were useful for addressing evolutionary and natural history hypotheses. In Chapter 5, determination of species limits and association of sexes (taxonomy) was necessary to identify mimetic models for each differently colored sex (natural history). In the same chapter, phylogenetic patterns revealed that dual sex-limited mimicry was driven by selection pressure on the female sex. In Chapter 4, the only dasymutilline species for which phoretic copulation could be hypothesized (associated males are significantly larger than females) was shown to be incorrectly associated; the true male of *D. archboldi* is the same size as its females. As shown in other mutillid lineages, dasymutillines can become model organisms for evolutionary hypothesis testing. In addition to continued biogeographic studies in Chapter 2, the dasymutilline Müllerian mimicry complex discovered in this dissertation is the largest such complex ever studied.

This research serves as a starting point for many more taxonomic and evolutionary discoveries. These phylogenetic results contain valuable information concerning four new genera, 45 new sex associations, and up to 80 more synonyms. My holistic research model, implemented on the fascinating and poorly known velvet ants, proposes evolutionary hypotheses in a group with untapped potential and provides the necessary taxonomic framework for further advances all at once.

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APPENDICES

Appendix A

Coauthor Permission Letters

18 April, 2012

Kevin Williams has my permission to include the following paper, which was submitted for publication, of which I was a co-author, in his doctoral dissertation.

Williams, K.A., Manley, D.G., Pilgrim, E.M., von Dohlen, C.D. and Pitts, J.P. "Multi-faceted assessment of species validity in the *Dasymutilla bioculata* species-group (Hymenoptera: Mutillidae)."

Williams, K.A., Manley, D.G., Deyrup, M., von Dohlen, C.D. and Pitts, J.P. "Systematic review of the *Dasymutilla monticola* species-group (Hymenoptera: Mutillidae): using phylogenetics to address species-group placement and sex associations."

Donald G. Manley

20 April, 2012

Kevin Williams has my permission to include the following paper, which was submitted for publication, of which I was a co-author, in his doctoral dissertation.

Williams, K.A., Manley, D.G., Deyrup, M., von Dohlen, C.D. and Pitts, J.P. "Systematic review of the *Dasymutilla monticola* species-group (Hymenoptera: Mutillidae): using phylogenetics to address species-group placement and sex associations."

Mark Deyrup

Mark Deyrup

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Kevin Williams has my permission to include the following paper, which was submitted for publication, of which I was a co-author, in his doctoral dissertation.

Williams, K.A., Manley, D.G., Pilgrim, E.M., von Dohlen, C.D. and Pitts, J.P. "Multi-faceted assessment of species validity in the *Dasymutilla bioculata* species-group (Hymenoptera: Mutillidae)."

Erik M. Pilgrim

18 April, 2012

Kevin Williams has my permission to include the following paper, which was submitted for publication, of which I was a co-author, in his doctoral dissertation.

Williams, K.A., Brothers, D.J., and Pitts, J.P. “New species of *Tobantilla* Casal, 1965 and a new genus and species, *Gogoltilla chichikovi* gen. et sp. nov., from Argentina (Hymenoptera: Mutillidae).”

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Appendix B

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CURRICULUM VITAE

Kevin Andrew Williams

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Education

Ph.D., Biology, Insect Systematics, Utah State University, Logan, UT, USA.
(August 2005 ─ present)
Advisors: James P. Pitts and Carol D. von Dohlen

B.S., Entomology, University of California, Davis, CA, USA .
(September 2003 ─ June 2005)

A.S., General Sciences, Yuba Community College, Marysville, CA, USA.
(August 2001 ─ June 2003)

Employment

Laboratory Instructor, Utah State University, Logan, UT, USA. Part time. August – December: 2005 – 2011; January – May: 2006 – 2008 & 2010 – 2012. Courses outlined below.

Research Assistant, Utah State University, Logan, UT, USA. Part time. June – August: 2006 – 2011. In 2006 and 2007, I mainly curated specimens in the insect collection. From 2009 – 2011, I monitored Utah for the Red Imported Fire Ant (*Solenopsis invicta*) with visual inspections.

Assistant Insect Collection Manager. Utah State University, Logan, UT, USA. Voluntary. August 2005 – August 2012. I taught insect curation techniques (pinning, labeling, dissecting, identification), to all undergraduate employees and incoming graduate students. I collected specimens for the University insect collection in 16 US states and 4 foreign countries (Brazil, Colombia, Mexico, Nicaragua). I trained fellow graduate students in insect collecting techniques.

Assistant Laboratory Teaching Coordinator. Utah State University, Logan, UT, USA. Voluntary. August – December: 2005 – 2011; January – May: 2006 – 2008 & 2010 – 2012. During weekly lab meetings, I provided advice concerning possible pitfalls during the lab. I was frequently responsible for describing the lab protocols in their entirety. During my labs, other TAs were invited to observe my teaching methods. I was also responsible for helping to prepare all the implements and specimens for each weekly lab.

Assistant Molecular and Genetics Lab Manager. Utah State University, Logan, UT, USA. Voluntary. January 2011 – August 2012. I trained and oversaw undergraduate students in molecular studies, including DNA extraction, amplification, and sequencing.

Lecturer, Utah State University, Logan, UT, USA. Part time. January – May, 2009. Course outlined below.

Agricultural Assistant, California Department of Food and Agriculture, Plant Pest Diagnostics Laboratory, Sacramento, CA, USA. Part time. September 2003 – August 2005. My responsibilities included curation of specimens and preliminary screening for non-targeted potential invasive pests.

Agriculture Inspector Aide, Sacramento County Department of Agriculture, Sacramento, CA, USA. Full time. June – September: 2002 & 2003. I was responsible for monitoring Sacramento County, CA for the Glassy Winged Sharpshooter (*Homaladisca coagulata*) through visual inspections of residential and commercial properties.

Laboratory Preparation Assistant, Yuba Community College, Marysville, CA, USA. Part time. October 2002 – June 2003. I was responsible for washing laboratory equipment and preparing materials for multiple Biology labs, including General Biology, Botany, Zoology, Anatomy, and Physiology.

Agriculture Inspector Aide, United States Department of Agriculture, Marysville, CA, USA. Part time. June – August 2001. I inspected incoming peach shipments to determine their quality and value for the canneries.

Courses Taught

Biology 3500, Plagues, Pests, and People. Lecture course (21 students), Spring 2009. Course for non-majors about the impact of infectious diseases on human history, emphasizing insect-vectored diseases.

Biology 5530, Insect Systematics Laboratory. One section (8-14 students) per semester, Fall 2005, 2007, 2011. Course for Biology majors and graduate students about insect identification and classification.

Biology 1610. General Biology Laboratory. One to three sections (30 students) per semester, Fall 2005-2010. Subjects included: microscopy, cell division, Mendelian genetics, DNA fingerprinting, plant diversity.

Biology 1620, General Biology Laboratory. One to three sections (30 students) per semester, Spring 2006-2008, 2010, 2011. Subjects included: population genetics, phylogenetics, scientific method, animal diversity.

Biology 1610, 1620. Honors Biology Laboratory. One section (15-20 students) per semester, Fall 2011 ─ Spring 2012. Identical to General 1610 and 1620 labs, except students were required to summarize and present scientific publications that related to each given lab.

Peer-reviewed Publications (* indicates undergraduate coauthor)

1. Wilson, J.S., **K.A. Williams,** M.L. Forister, C.D. von Dohlen, and J.P. Pitts. Rapid Evolution in overlapping mimicry rings among North American velvet ants. *Proceedings of the National Academy of Sciences*, in review.
2. **Williams, K.A.,** D.G. Manley, M. Deyrup, C.D. von Dohlen, and J.P. Pitts. Systematic review of the *Dasymutilla monticola* species-group (Hymenoptera: Mutillidae): using phylogenetics to address species-group placement and sex associations. *Zootaxa*, in press.
3. Wilson, J.S., S.L. Clark*, **K.A. Williams,** and J.P. Pitts. 2012. Historical biogeography of the arid-adapted velvet ant *Sphaeropthalma arota* (Hymenoptera: Mutillidae) reveals cryptic species. Journal of Biogeography, 39: 336-352.
4. **Williams, K.A.,** D.J. Brothers, and J.P. Pitts. 2011. New species of *Tobantilla* Casal, 1965 and a new genus and species, *Gogoltilla chichikovi* gen. et sp. nov., from Argentina (Hymenoptera: Mutillidae) . *Zootaxa,* 3064: 41-68.
5. **Williams, K.A.,** D.G. Manley, E.M. Pilgrim, C.D. von Dohlen, and J.P. Pitts. 2011. Multi-faceted assessment of species validity in the *Dasymutilla bioculata* species-group (Hymenoptera: Mutillidae). *Systematic Entomology* 36: 180-191.
6. Wilson, J.S., **K.A. Williams**, C.F. Gunnell* and J.P. Pitts. 2010. Preliminary phylogeography of the widespread, arid-adapted antlion *Brachynemurus sackeni* Hagen (Neuroptera: Myrmeleontidae). *Psyche: A Journal of Entomology* 804709: 1-7.
7. Brabant, C., **K. A. Williams**, and J. P. Pitts. 2010. True females of the *Photomorphus* subgenus *Photomorphina* Schuster (Hymenoptera: Mutillidae). *Zootaxa*, 2559: 58–68.
8. Pitts, J.P., J.S. Wilson, **K.A. Williams,** and N. Boehme*. 2010. Nocturnal velvet ant males (Hymenoptera: Mutillidae) of Deep Canyon, California including four new species and a fifth new species from Owens Lake Valley, California. *Zootaxa,* 2553: 1–34.
9. Wilson, J.S., **K.A. Williams**, D. Tanner, and J.P. Pitts. 2010. Nectaring by nocturnal velvet ants (Hymenoptera: Mutillidae). *Southwestern Naturalist,* 55:443-444.
10. Wilson, J.S., **K.A. Williams**, and J.P. Pitts. 2010. Preliminary Assessment of velvet ant (Hymenoptera: Mutillidae) diversity in the deserts of southern California. Western North American Naturalist, 70 (2): 224-232.
11. **Williams, K.A.,** and J.P. Pitts. 2009. Eight New species of *Lomachaeta* Mickel and the Synonymy of *Smicromutilla* Mickel (Hymenoptera: Mutillidae). *Journal of Hymenoptera Research,* 18: 227-243.
12. Pilgrim, E.M., **K.A. Williams**, D.G. Manley, and J.P. Pitts. 2009. Addressing the *Dasymutilla quadriguttata* species-group and species-complex (Hymenoptera: Mutillidae): several distinct species or a single, morphologically variable species? *Journal of the Kansas Entomological Society,* 82 (3): 231-249.
13. Pitts, J.P., J.S. Wilson, **K.A. Williams**, and N.F. Boehme*. 2009. Velvet Ants (Hymenoptera: Mutillidae) of the Algodones Sand Dunes of California, USA. *Zootaxa,* 2131: 1-53.
14. **Williams, K.A.,** and J.P. Pitts. 2008. Three Species Masquerading as One: Updating the Taxonomy of *Pseudomethoca russeola* Mickel and *P. donaeanae* (Cockerell & Fox) (Hymenoptera: Mutillidae). *Journal of Hymenoptera Research,* 17: 127-133.

15. Pilgrim, E.M., **K.A. Williams**, and J.P. Pitts. 2008. Sex association and synonymy in Southwestern U.S. species of *Dasymutilla* (Hymenoptera: Mutillidae). *Pan-Pacific Entomologist*, 84: 58-69.
16. **Williams, K.A.,** and J.P. Pitts. 2007. New species of predominately temperate velvet ant genera *Lomachaeta* Mickel and *Sphaeropthalma* Blake from Central America and northern South America (Hymenoptera: Mutillidae). *Transactions of the American Entomological Society,* 133: 297-326.
17. **Williams, K.A.** and D.G. Manley. 2006. A New Color Variant of *Dasymutilla gloriosa* (Saussure) from Mexico (Hymenoptera: Mutillidae). *Pan-Pacific Entomologist,* 82:103-104.
18. Manley, D.G. and **K.A. Williams.** 2005. A New Color Variant of *Dasymutilla atricauda* Mickel from Imperial County, California (Hymenoptera: Mutillidae) *Pan-Pacific Entomologist*, 81:184-185.

Undergraduate student advising and mentoring

1. **Alex Kelley,** 2011-2012. I advised Alex in teaching and am training him in molecular techniques and insect curation. He is currently a Biology major at Utah State University.
2. **Austin Spencer,** 2012. I am training Austin in molecular techniques. He is a current Biology major at Utah State University.
3. **Stephany Perez**, 2009-2011. I trained Stephany in insect curation, scientific illustration, and molecular techniques. I also advised her in teaching and coauthored a poster with her. She is currently working on a MS in biochemistry at Utah State University.
4. **Becca Condie,** 2009-2011. I trained Becca in insect curation, dissection, and identification. She is currently serving an LDS mission.
5. **Lydia Bushman,** 2009-2011. I trained Lydia in insect curation techniques. She graduated from Utah State University as a Business major.
6. **Clayton Gunnell,** 2008-2010, I helped to train Clay in molecular techniques. I advised him in research presentation and teaching. He was awarded undergraduate researcher of the year (Utah State University) and is currently a MS student in a dental program at Creighton University, Omaha, NE.
7. **Sarah Clark,** 2008-2010, I helped to train Sarah in insect curation, collecting, and identification. I advised her on teaching. She has coauthored one publication with me. She is currently a MS student studying pollination ecology at Utah State University.
8. **Nicole Boehme,** 2006-2008, I helped to train Nicole in insect curation, collecting, identification, and scientific illustration. I advised her on teaching and writing. She has coauthored two publications with me. She is currently a MS student studying pollination ecology at Utah State University.

Presentations

Oral Presentations

1. **Williams, K.A.** The velvet ants (Hymenoptera: Mutillidae) of Trinidad: A springboard for biodiversity studies in South America. Intermountain West Graduate Student Symposium, Logan, UT, April, 2012.
2. **Williams, K.A.**, C.D. von Dohlen, & J.P. Pitts. Phylogenetic studies of *Dasymutilla* and their relatives shed light on mimicry, biodiversity, and biogeography (Hymenoptera: Mutillidae). Entomological Society of America, National Meeting, Reno, NV, November 2011.
3. **Williams, K.A.**, J.S. Wilson, C.D. von Dohlen, & J.P. Pitts. Investigations of Dual Sex-Limited Mimicry in the Thistledown Velvet Ant (Hymenoptera: Mutillidae). Intermountain West Graduate Student Symposium, Logan, UT, USA, April, 2011. **Second Place.**
4. **Williams, K.A.**, J.S. Wilson, & J.P. Pitts. Does the Thistledown Velvet Ant (Hymenoptera: Mutillidae) mimic Creosote seeds? A phylogenetic approach. Entomological Society of America, National Meeting, San Diego, CA, USA, December 2010. **Second Place.**
5. **Williams, K.A.**, C.D. von Dohlen, & J.P. Pitts. Mimicry confuses taxonomy: Lessons from the *Dasymutilla bioculata* species-group (Hymenoptera: Mutillidae). Intermountain West Graduate Student Symposium, Logan, UT, USA, March, 2010. **First Place.**
6. **Williams, K.A.** Phylogenetic investigation of mimicry complexes in *Dasymutilla* (Hymenoptera: Mutillidae). Entomological Society of America, National Meeting, Reno, NV, USA, November, 2008.

Poster Presentations (* indicates undergraduate presenter)

1. **Williams, K.A.**, A.W. Hook, & J.P. Pitts. The Mutillidae (Hymenoptera) of Trinidad: Matching sexes in limited space. Entomological Society of America, National Meeting, Reno, NV, USA, November 2011.
2. Perez, S.M.*, **K.A. Williams** & J.P. Pitts. Identification and differentiation of females in the *Odontophotopsis parva* and *O. venusta* species-groups (Hymenoptera: Mutillidae). Undergraduate Research Symposium, Utah State University, Logan, UT, USA, December 2010.
3. Gunnell, C.*, J.S. Wilson, **K.A. Williams**, and J.P. Pitts. 2010. Do phylogenetic relationships based solely on ITS1 & 2 adequately represent evolution?. Undergraduate Research Symposium, Utah State University, Logan, UT, USA, December 2010.
4. **Williams, K.A.** & J.P. Pitts. Coping with confusing coloration: molecular and morphological analysis of the *Dasymutilla occidentalis* species-complex (Hymenoptera: Mutillidae). Entomological Society of America, National meeting, San Diego, CA, USA, December, 2007.
5. **Williams, K.A.**, E.M. Pilgrim, & J.P. Pitts. Synonymies and sex associations in the velvet ant genus *Dasymutilla* (Hymenoptera: Mutillidae). Pacific Branch Entomological Society of America meeting, Portland, OR, USA, March, 2007. **First Place.**
6. Pitts, J.P., J.S. Wilson, and **K.A. Williams.** Phylogenetics of the Nearctic Nocturnal velvet ant genus *Odontophotopsis* Viereck (Hymenoptera: Mutillidae). Pacific Branch Entomological Society of America meeting, Portland, OR, USA, March, 2007.

Invited seminars

1. **Williams, K.**A. Hymenoptera Diversity in the Great Basin National Park. Presented to volunteers and park staff during the Great Basin National Park's annual Bioblitz, Baker, NV, USA. August 2011.
2. **Williams, K.A.** Phylogenetic investigation of mimicry complexes in *Dasymutilla* (Hymenoptera: Mutillidae). Presented to the Utah State University Entomology Club, Logan, UT, USA. October 2008.
3. **Williams, K.A.** and J.S. Wilson. Diversity of insects in Jackson, Wyoming. Presented to the public at the Teton Science School, Jackson, Wyoming in conjunction with a Bioblitz and visit from E. O. Wilson. October 2007.

Awards

Second Place. Graduate student oral presentations, 15 minute talks in Biology: Investigations of Dual Sex-Limited Mimicry in the Thistledown Velvet Ant (Hymenoptera: Mutillidae). Intermountain West Graduate Student Symposium, Logan, UT, USA, 5 April, 2011.

Second Place Students Competition for the President's Prize, 10 minute talks: Does the Thistledown Velvet Ant (Hymenoptera: Mutillidae) mimic Creosote seeds? A phylogenetic approach. Entomological Society of America, National Meeting, San Diego, CA, 15 Dec. 2010.

First Place Graduate student oral presentations, 15 minute talks in Biology: Mimicry confuses taxonomy: Lessons from the *Dasymutilla bioculata* species-group (Hymenoptera: Mutillidae). Intermountain West Graduate Student Symposium, Logan, UT, 31 March, 2010.

Second Place Students Competition for the President's Prize, 10 minute talks: Phylogenetic Investigation of mimicry complexes in *Dasymutilla* Ashmead (Hymenoptera: Mutillidae). Entomological Society of America, National Meeting, Reno, NV, 15 Nov. 2008.

First Place Student Poster Competition: Synonymies and sex associations in the velvet ant genus *Dasymutilla* Ashmead (Hymenoptera: Mutillidae). Entomological Society of America: Pacific Branch Meeting, Portland, OR, 15 March 2007.

Grants

2009	Theodore Roosevelt Memorial Grant from the American Museum of Natural History. Transectional Survey of Mutillidae (Hymenoptera) across the Deming Plain. **$1600**.
2007	Research Grant from the California Desert Research Fund at The Community Foundation of Riverside and San Bernardino Counties. Endemism and diversity in velvet ants (Hymenoptera: Mutillidae) of the Southern California deserts (as a coauthor). **$3,194**.
2006	Theodore Roosevelt Memorial Grant from the American Museum of Natural History. Revision of Nearctic *Pseudomethoca* Ashmead (Insecta: Hymenoptera: Mutillidae). **$1200**.

Insect Identification and Museum Curation

I have studied or worked in the following insect collections. The approximate number of specimens that I identified or borrowed for further research is provided.

Utah State University Entomological Museum, Logan, UT, USA.
30 000 specimens. Contact: James Pitts, Wilford Hanson.
California Academy of Sciences, San Francisco, CA, USA.
10 500 specimens. Contacts: Vincent Lee, Wojcech Pulawski, Bob Zuparko.
Bohart Museum of Entomology, University of California, Davis, Davis, CA, USA.
8 000 specimens. Contacts: Lynn Kimsey, Steve Heydon.
California State Collection of Arthropods, Sacramento, CA, USA.
8 000 specimens. Contact: Steve Gaimari, Chuck Bellamy.
Essig Museum of Entomology, University of California, Berkeley, Berkeley, CA, USA.
6 500 specimens. Contacts: Cheryl Barr, Bob Zuparko.
Florida State Collection of Arthropods, Gainesville, FL, USA.
6 500 specimens. Contacts: Jim Wiley, Lionel Stange.
American Museum of Natural History, New York, NY, USA.
4 500 specimens. Contact: Christine LeBeau.
“The Bug Closet”, University of Central Florida, Orlando, FL, USA.
4 000 specimens. Contacts: Stuart Fullerton, Shawn Kelly.
Museum of Zoology, University of Michigan, Ann Arbor, MI, USA.
3 500 specimens. Contact: Mark O’Brein.
Canadian National Collection of Insects, Ottawa, ON, CANADA.
3 000 specimens. Contacts: Andrew Bennett, John Huber, Gary Johnson, Lubomir Masner.
Entomological Teaching and Research Collection, University of California, Riverside, Riverside, CA, USA.
2 500 specimens. Contact: Doug Yanega.
Monte L. Bean Life Science Museum, Brigham Young University, Provo, UT, USA.
2 500 specimens. Contact: Sean Clark.
Cornell University Insect Collection, Ithaca, NY, USA.
2 000 specimens, Contacts: E.R. Hoebeke, James Liebherr.
Los Angeles County Museum, Los Angeles, CA, USA.
1 800 specimens. Contact: Brian Brown, Weiping Xie.
Field Museum of Natural History, Chicago, IL, USA.
1 800 specimens. Contact: James Boone.
United States National Entomological Collection, Smithsonian Institute, Washington, DC, USA.
1 500 specimens. Contact: Brian Harris.
Gillette Arthropod Biodiversity Museum, Colorado State University, Fort Collins, CO, USA.
1 500 specimens. Contact: Boris Kondratieff.
University of Minnesota Insect Collection, St. Paul, MN, USA.
1 400 specimens. Contact: Paul Tinerella, Philip Clausen.
Carnegie Museum of Natural History. Pittsburgh, PA, USA.
1 200 specimens. Contact: John Rawlins.
American Entomological Institute, Gainesville, FL, USA.
1 000 specimens. Contact: David Wahl.
Department of Entomology Insect Collection, Texas A&M University, College Station, TX, USA.
1 000 specimens. Contact: Ed Riley.
Snow Entomological Museum, University of Kansas, Lawrence, KS, USA.
1 000 specimens. Contact: Zachary Falin.
Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA.
800 specimens. Contacts: Phillip Perkins, Stefan Cover.
Instituto de Alexander von Humboldt, Villa de Leyva, BOY, Colombia.
800 specimens. Contact: Claudia Medina.
Museu Paraense Emilio Goeldi, Universidad Federal do Para, Belem, PA, Brazil.
700 specimens. Contact: Osvaldo Silveira.
Peabody Museum of Natural History, Yale University, New Haven, CT, USA.
700 specimens. Contact: Raymond Pupedis.
Department of Entomology Collection, University of Arizona, Tucson, AZ, USA.
500 specimens. Contacts: Carl Olson, Jim Zimmerman, Wendy Moore.
Universidad Estadual do Feira de Santana, Feira de Santana, BA, Brazil.
300 specimens. Contact: Sergio Andena.

University of Nebraska State Museum, Lincoln, NE, USA.
250 specimens. Contact: Brett Ratcliffe.
Academy of Natural Sciences, Philadelphia, PA, USA.
200 specimens. Contact: Jason Weintraub.
Centro de Pesquisas do Cacau, CEPEC, Ilheus, BA, Brazil.
200 specimens. Contact: Jaques DeLabie.
University of Colorado Museum of Natural History, Boulder, CO, USA.
200 specimens, Contact: Virginia Scott.
Universidad Federal do Espiritu Santo, Vitoria, ES, Brazil.
200 specimens. Contact: Celso Azevedo.
Coleccion del Instituto de Biologia, Universidad Nacional Autonomo de Mexico, Mexico City, DF, Mexico.
150 specimens. Contact: Alejandro Zalvidar.
Estación del Biología, Chamela, San Patricio, JL, Mexico.
150 specimens. Contact: Ricardo Ayala.
PeeDee Research Center, Clemson University, Florence, SC, USA.
100 specimens. Contact: Don Manley.
Illinois Natural History Survey Insect Collection, Champaign, IL, USA.
100 specimens. Contact: Paul Tinerella.
Tulare County Agricultural Commissioner Collection, Tulare, CA, USA
50 specimens. Contact: Dennis Haines.
West Texas A&M University Insect Collection, Canyon, TX, USA.
50 specimens. Contact: Bonnie Pendleton, David Sissom.
Iowa State University Insect Collection, Ames, IA, USA.
50 specimens. Contact: Greg Courtney.
Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, CUN, Colombia.
50 specimens. Contact: Fernando Fernandez.

I have borrowed and identified specimens for 15 additional insect collections around the world.

I have carried out numerous curatorial tasks at Utah State University, including sending and receiving loans of other insect groups, identifying specimens for the general public, and directing tours of the insect collection.

Society Memberships

International Society of Hymenopterists, 2005-2012
Entomological Society of America, 2005-2012