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The Velvet Ants (Hymenoptera: Mutillidae): Systematics, Biology, and Biogeography of a Little-Known Family

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THE VELVET ANTS (HYMENOPTERA: MUTILLIDAE): SYSTEMATICS, BIOLOGY, AND BIOGEOGRAPHY OF A LITTLE-KNOWN FAMILY

by

George Charles Waldren

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

of

DOCTOR OF PHILOSOPHY

in

Biology

Approved:

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ABSTRACT

The Velvet Ants (Hymenoptera: Mutillidae): Systematics, Biology, and Biogeography of a Little-Known Family

by

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Utah State University, 2021

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Department: Biology

Wasps of the family Mutillidae (Hymenoptera: Aculeata), commonly known as velvet ants, are a conspicuous and yet little-known component of most tropical and temperate ecosystems. Mutillids are solitary idiobiont ectoparasitoids of several holometabolous insect orders, primarily solitary bees and apoid wasps (Hymenoptera: Apoidea). There are currently 4,603 described species among 220 genera classified into 8 subfamilies, 13 tribes, and 4 subtribes. Most species, and even some genera, are known from a single sex due to the extreme sexual dimorphism exhibited between males and females; females are always apterous, and males are usually winged. The sexes have few characters in common and have historically been challenging to associate. Molecular phylogenetics has increasingly become the method of choice for inferring relationships between species and has also been used to associate the dimorphic sexes of mutillids. Ultraconserved elements (UCEs) are highly conserved regions of the genome that are shared among distantly-related taxa and are a powerful source of data for inferring phylogenies. My dissertation was centered on understanding the diversity and relationships within Mutillidae at the family-group level as well as the species level. I tested the currently accepted higher classification of Mutillidae using UCEs, which revealed that its basal subfamily, Myrmosinae, is not a member of Mutillidae. Additionally, the analyses revealed the non-monophyletic status for all of the tribes of the two largest subfamilies, Mutillinae and Sphaeropthalminae. In light of these findings, a new higher classification for Mutillidae was proposed. I also comprehensively investigated the mating strategies known for Mutillidae, proposed new terminology that accurately describes them, and reported on a new mating strategy record for *Sphaeropthalma pensylvanica* (Lepeletier). Additionally, I performed a phylogenetic analysis for the species-rich, cosmopolitan mutillid tribe Trogaspidiini in order to discern how its members are related to one another and to determine their biogeographic history. Lastly, I revised the rare genus *Invreiella* Suárez and increased the known diversity from three to fourteen species.

(348 pages)

PUBLIC ABSTRACT

The Velvet Ants (Hymenoptera: Mutillidae):
Systematics, Biology, and Biogeography
of a Little-Known Family

George Charles Waldren

Insects are a ubiquitous and species-rich component of the biologically-diverse planet we inhabit. The majority of insects are understudied, with many species awaiting formal description and their natural history yet to be discovered. Members of the family Mutillidae, commonly known as velvet ants, are one of these little-known insect groups. Velvet ants are technically wasps, and the wingless females superficially resemble true ants of the family Formicidae. Further, they frequently have a ‘velvety’ appearance and are often brightly colored to serve as a warning to would-be predators that they have the ability to inflict a painful sting. These insects are solitary parasitoids and their young primarily feed on the immature stages of solitary bees and apoid wasps. There are currently 4,603 described species of Mutillidae and many await formal description. In order to organize the overwhelming amount of biological knowledge presently known and to accommodate for future discoveries, species are classified into a hierarchical system that was first proposed by Carl Linnaeus in 1758. Biological classifications allow us to organize, understand, and convey information about groups of species at various ranks. Further, phylogenetic analyses allow us to understand how species are related to one another and they can inform us on how to classify life on Earth. Genomic-level molecular data are becoming increasingly more accessible and have become the primary source of information for inferring phylogenetic relationships between species. To better understand the internal classification of Mutillidae, I performed several phylogenetic analyses using molecular data. I also investigated the mating strategies known for Mutillidae and reported on a new mating strategy record for *Sphaeropthalma pensylvanica* (Lepeletier). Additionally, I performed a phylogenetic analysis for the species-rich, cosmopolitan mutillid tribe Trogaspidiini in order to discern how its members are related to one another and to determine their biogeographic history. Lastly, I revised the rare genus *Invreiella* Suárez and increased the known diversity from three to fourteen species. My dissertation research sheds light on a number of understudied aspects of velvet ants and the results will aid other researchers studying these remarkable insects.

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George C. Waldren

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CHAPTER I

INTRODUCTION

An introduction to the biology of Mutillidae

Solitary wasps of the family Mutillidae (Hymenoptera: Aculeata), commonly known as velvet ants due to the superficial resemblance of the apterous females to true ants (Formicidae), are a diverse yet little-known group of insects. Many species are densely covered in brightly-colored setae and are among the most visually-striking insects in existence. Velvet ants are worldwide in distribution; however, they are notably absent in the arctic regions and oceanic island chains, such as the Hawaiian archipelago (Lo Cascio 2015). There are 4,603 described species in the family (Pagliano *et al.* 2020). Despite mutillids being a conspicuous component of tropical and temperate ecosystems, relatively little is known about them. Most species are known from a single sex (Pagliano *et al.* 2020) and the hosts for most species are unknown (Ronchetti & Polidori 2020).

Mutillids are solitary idiobiont ectoparasitoids of immature holometabolous insects, although there are a few cases of gregarious parasitoidism recorded (Brothers 1984, 1989; Brothers *et al.* 2000). The majority of host records are of solitary ground-nesting bees and apoid wasps (Brothers *et al.* 2000; Luz *et al.* 2016). Additional insect orders that have been documented as hosts include Coleoptera, Diptera, Lepidoptera, and potentially egg predators of Blattodea (Mickel 1974; Brothers 1989; Brothers *et al.* 2000; Amini *et al.* 2014). Based on the research of Cottrell (1936), Ferguson (1962), and Brothers (1972) on *Dasymutilla bioculata* (Cresson), "*Photopsis*" spp., and

Pseudomethoca frigida (Smith), respectively, adult female mutillids scour the terrain for hosts, seeking above-ground nests or subterranean burrows depending on the mutillid species' morphology and host preference (Krombein 1972; Quintero & Cambra 1996). Upon locating and gaining access into a potential host nest or burrow, the mutillid female searches for a cocoon, chews a hole in it, and examines the contents inside. If the potential host is at the desired developmental stage of prepupa or pupa, she inserts the tip of her metasoma into the opening and usually lays a single egg on the host. She may or may not sting the host to paralyze it. She then seals the cocoon opening she made with a mixture of salivary secretions and surrounding particulate matter. She moves on to parasitize other cocoons in the nest or she remains in the nest for a period of time (Cottrell 1936; Ferguson 1962; Brothers 1972). The first instar mutillid larva emerges from the egg capsule shortly thereafter, and the larva consumes the host entirely. The mature mutillid larva usually spins a cocoon inside the host cocoon or puparium, pupates, and later ecloses as an adult.

Sexual dimorphism and the difficulty in associating the sexes

A notable feature of the family is the extreme sexual dimorphism exhibited between the sexes. All known females are entirely wingless (apterous), while most males are fully winged (macropterous) (Brothers 1989). There are some cases in which males have reduced wings (brachypterous) or are entirely apterous; these features occur most commonly in male Myrmillinae (Cambra & Quintero 2007). Female aptery is a common feature observed in several other aculeate families that parasitize subterranean or concealed hosts, such as some members of the families Bethylidae, Thynnidae, and

Tiphiidae (Reid 1941). This sexual dimorphism has created considerable problems for systematists working on the family. The sexes share few characters in common and it is not usually possible to associate them based on casual examination of museum specimens. As a result of this dimorphism, the sexes are usually described as separate species, or even separate genera, until there is evidence that demonstrates they are conspecific. This dual taxonomic system has been a mainstay of the family since Linnaeus (1758), who described the male and female of *Dasylabris maura* (L.) as two separate species; they were associated well over 200 years later (Day 1979). Historically, researchers have used seven lines of evidence for reliably matching males with their respective females: 1) collecting a pair *in copula* in the field (Nonveiller 1980), 2) attracting males to a caged female in the field (Mickel 1938), 3) experimental mating trials in the laboratory (Cambra & Quintero 1993), 4) discovery of a gynandromorph (Mickel 1936), 5) rearing of both sexes from the same host nest (Taylor *et al.* 2019), 6) comparison of distributions in conjunction with species-group membership (Williams & Pitts 2013), and 7) molecular data (Pilgrim & Pitts 2006). The last two methods are the most time-efficient and most reliable way to associate the sexes and have been heavily used in recent research (Pilgrim *et al.* 2008; Pitts *et al.* 2007, 2009; Williams *et al.* 2012; Williams & Pitts 2013).

Mating biology and phoretic copulation

Some members of Mutillidae are remarkable in that they practice phoretic copulation, a type of phoresy (Linsley 1960; Evans 1969; Sheldon 1970; Nonveiller 1980; Brothers 1989; Vivallo 2020). Phoresy is defined as an interaction between two or more animals wherein one individual carries another individual(s) for purpose of travel. In phoretic copulation, a winged male will carry an apterous female by flight and/or foot, and they will either mate while in flight, or settle on a substrate to mate. Conspecific males are typically larger than females to facilitate carriage (Tormos *et al.* 2010). In addition to some subfamilies and tribes of Mutillidae, other aculeate taxa that practice phoretic copulation include certain subfamilies or genera of Bethylidae and Thynnidae (Evans 1969; Vivallo 2020). The alternative to phoretic copulation is *in situ* copulation, where the pair will mate at or near the location of their initial encounter (Linsley *et al.* 1955; Jellison 1982; Manley & Deyrup 1989).

There are two types of phoretic copulation known in Mutillidae according to Brothers (1989). The first type was described by Brothers (1989) as “true” phoretic copulation, wherein females are carried primarily by terminalic union with the male. The pair apparently mates in flight, and the male may visit flowers to feed on nectar while still paired with the female. Myrmosinae (Myrmosini) and Rhopalomutillinae are the only taxa within Mutillidae known to practice “true” phoretic copulation (Brothers 1989, 2015). As for the second type, the female is primarily carried by the male’s mandibular clasp around her pronotal collar. The pair will eventually settle on a substrate to mate (Nonveiller 1980). The only mutillid group known to practice the second type of phoretic copulation is Mutillinae (excluding Mutillini and Odontomutillini). Sex associations are more common in mutillid taxa that practice phoretic copulation as they tend to remain *in copula* longer than taxa that practice *in situ* copulation (Rothney 1903; Nonveiller 1980; Cambra *et al.* 2018).

A potential third subtype of phoretic copulation was described by O'Toole (1975) for the trogaspidiine species *Wallacidia oculata* (Fabricius): "The posture of copulation in [*W.*] *oculata* is venter to venter, with the male uppermost. The female clings to the sides of the male mesosoma, with the tarsal claws gaining purchase on the coarse sculpture of the male." This venter to venter positioning is unusual, as most other members of Trogaspidiini mate with male venter to female dorsum (Linsley 1960; Sheldon 1970; Nonveiller 1980). This observation requires further investigation.

Defenses and Müllerian mimicry

Mutillid females are well-known for their powerful sting and other defensive mechanisms (Schmidt & Blum 1977; Deyrup 1988; Manley 2000; Gall *et al.* 2018). There are at least seven defensive characteristics of mutillids that protect them from predators and from hosts defending their young: 1) females possess a long, flexible ovipositor capable of inflicting a painful sting, 2) possess a hard, smooth cuticle, 3) possess mandibular gland allomones, 4) display aposematic coloration, 5) have the ability to stridulate, 6) display erratic, rapid movement, and 7) have the ability to bite (Schmidt & Blum 1977; Fales *et al.* 1980; Tschuch & Brothers 2000; Gall *et al.* 2018; Sadler *et al.* 2018). There are no known predators that consistently feed upon female mutillids (Vitt & Cooper 1988; Manley 2000; Gall *et al.* 2018). In contrast, males are not infrequently taken as prey by robber flies (O'Neill & Seibert 1996), predatory wasps (O'Neill 2001), and other insectivorous predators.

Aposematic, or warning coloration, is an immediate indicator to a would-be predator to avoid an animal as a prey item as they may possess injurious capabilities.

Predators, in particular lizards, learn to avoid velvet ant females as prey items after previous exposure to them (Schmidt & Blum 1977; Vitt & Cooper 1988). Most diurnal velvet ant females are brightly and/or contrastingly colored, displaying setal and integumental combinations of red, orange, yellow, white, and/or black. Recent research has revealed that North American mutillids are members of the largest-known Müllerian mimicry complex (Wilson *et al.* 2012, 2013, 2015). Müllerian mimicry is a form of mimicry where two or more species capable of harm mutually benefit from sharing a common appearance, such as coloration, in order to deter predation. Wilson *et al.* (2012) identified six mimicry rings in North America that are delimited by a shared appearance and distribution: Desert, Eastern, Madrean, Texan, Tropical, and Western. Further, Wilson *et al.* (2015) identified an additional two mimicry rings: the Red-Headed *Timulla* ring and the Black-Headed *Timulla* ring. Two subfamilies and multiple genera were found to participate in these mimicry rings in North America. The Tropical ring, for example, involves at least ten genera. Lastly, participation in these mimicry rings is not limited to mutillids; several species of the spider wasp genus *Psorthaspis* (Pompilidae) were discovered to be members of five of these mutillid-based rings (Rodriguez *et al.* 2014).

Research objectives

My research is centered on investigating foundational questions regarding Mutillidae at various taxonomic levels. These questions are: 1) how many species are there, 2) how are they related to each other, 3) where do they occur, and 4) what is their natural history? With these questions as the foundation of my research, the specific objectives of my dissertation, by chapter number, were to 2) infer the phylogeny of Mutillidae using phylogenomic methods with ultraconserved elements (UCEs) to test the current higher classification hypothesis for Mutillidae, 3) report on a new observation of phoretic copulation in the sphaeropthalmine *Sphaeropthalma pensylvanica* (Lepeletier), comprehensively review all mating strategy data for Mutillidae, and propose new terminology for the several types of mating strategies known for Mutillidae, 4) infer the phylogeny of the mutilline tribe Trogaspidiini using UCEs in order to test the monophyly of the Old World and New World faunas and elucidate the tribe's biogeography at a global scale, and 5) revise the rare velvet ant genus *Invreiella* Suárez and determine its biogeographic distribution.

For my second chapter, I investigated the higher classification of Mutillidae using a phylogenomic approach. Biological classifications are foundational to organizing, understanding, and conveying the overwhelming amount of available knowledge about life. The modern higher classification of Mutillidae has been based upon two competing morphological phylogenetic hypotheses for a number of years: Brothers (1975) and Lelej & Nemkov (1997). Recently, a collaborative effort by Brothers & Lelej (2017) resulted in a newly-proposed higher classification of the family using morphology. This study represents the most morphologically-comprehensive evaluation of Mutillidae to date. However, there were few unique synapomorphies that defined many of their higher taxa, with most supported by homoplasious synapomorphies. This classification hypothesis has not yet been tested using molecular phylogenetics. Phylogenomic studies have become the standard approach for inferring relationships between taxa and have been used to answer higher-level classification questions in other insect groups, such as Phasmatodea

(Simon *et al.* 2019), auchenorrhynchan Hemiptera (Skinner *et al.* 2019), and aculeate Hymenoptera (Branstetter *et al.* 2017). One source of phylogenomic data are UCEs, which are highly conserved regions of the genome that are shared among distantly-related taxa. Each UCE is flanked by variable sites that provide phylogenetic signal, and the UCE itself, while also informative, additionally provides a series of shared character states between taxa. The goal of this study was to test the morphology-based higher classification of Mutillidae proposed by Brothers & Lelej (2017) and also the position of the controversial taxon Myrmosidae in relation to Mutillidae with a phylogenomic approach using UCEs. Additionally, the ages of Mutillidae, related families, mutillid subfamilies, and mutillid tribes were inferred in order to determine when these lineages emerged.

For my third chapter, a novel observation of phoretic copulation in the Nearctic mutillid *Sphaeropthalma pensylvanica* (Lepeletier) is described. This is the first observation of this behavior for the subfamily Sphaeropthalminae. Describing this novel observation was recognized as an opportunity to critically reevaluate all of the known mating strategies for Mutillidae. I comprehensively reviewed the literature for all records of mating strategy descriptions for mutillids to see if any patterns could be discerned for the behavior among the higher taxa of Mutillidae. Further, developing an updated terminology for each of the known mating strategies after a review of the literature was sought to allow for these behaviors to be accurately characterized. Lastly, I investigated the venter to venter mating position described by O'Toole (1975) and the disjunct distributions for several Old World sphaeropthalmine genera.

For my fourth chapter, I sought to discover the phylogenetic relationships within the mutilline tribe Trogaspidiini. This tribe is cosmopolitan in distribution and is unique among Mutillidae in this regard. Trogaspidiines practice phoretic copulation, which has, in part, been hypothesized to aid the apterous females in traversing otherwise impassable barriers such as water (Evans 1969). There has been historical controversy in the 20th century over whether the Old World and New World trogaspidiine faunas are monophyletic with respect to each other, and morphology alone has been insufficient to adequately answer this question. The New World trogaspidiines are represented solely by the morphologically-diverse genus *Timulla* Ashmead, and the Old World fauna is represented by more than forty genera based primarily on male morphology. Given that members of this tribe practice phoretic copulation, which may be a behavior amenable to long-distance dispersal, my primary question was: were there multiple trogaspidiine dispersal events between the Old World and New World? Further, were there multiple dispersal events between North and South America? Additionally, were there geologic and climactic factors that influenced the distributions of trogaspidiines that are observed today? These questions were investigated using UCEs in several phylogenetic analyses.

Lastly, for my fifth chapter, I conducted a systematic revision of the pseudomethocine genus *Invreiella* Suárez. Members of this genus are rare in natural history collections, and the discovery of several new species known from few specimens revealed the necessity of a revision. In the most recent treatment of *Invreiella*, Quintero & Cambra (2011) recognized three species, stated that the genus is mostly Nearctic in distribution, and, further, endemic to Mexico. Suárez (1966) implied the genus is Neotropical in distribution, as did several other authors. I sought to test each of these hypotheses by exhaustively searching for *Invreiella* specimens among natural history collections, critically comparing their morphology for new characters, and recording their distributions with the goal of determining the biogeographic affinities of the genus.

References

Amini, A., Lelej, A.S., Sadeghi, H., & Karimi, J. (2014) First record of the velvet ants (Hymenoptera: Mutillidae) reared from puparia of the ber fruit fly *Carpomya vesuviana* Costa (Diptera: Tephritidae) in Iran. *Zootaxa*, 3861(6): 585–590.

Branstetter, M.G., Danforth, B.N., Pitts, J.P., Faircloth, B.C., Ward, P.S., Buffington, M.L., Gates, M.W., Kula, R.R., & Brady, S.G. (2017) Phylogenomic insights into the evolution of stinging wasps and the origins of ants and bees. *Current Biology*, 27(7): 1019–1025.

Brothers, D.J. (1972) Biology and immature stages of *Pseudomethoca f. frigida*, with notes on other species (Hymenoptera: Mutillidae). *The University of Kansas Science Bulletin*, 50(1): 1–38.

Brothers, D.J. (1975) Phylogeny and classification of the aculeate Hymenoptera, with special reference to Mutillidae. *The University of Kansas Science Bulletin*, 50(11): 483–648.

Brothers, D.J. (1984) Gregarious parasitoidism in Australian Mutillidae (Hymenoptera). *Australian Journal of Entomology Magazine*, 11(1): 8–10.

Brothers, D.J. (1989) Alternative life-history styles of mutillid wasps (Insecta, Hymenoptera). In: Bruton, M.N. (Ed.), *Alternative Life-History Styles of Animals*. Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 279–291.

Brothers, D.J. (2015) Revision of the Rhopalomutillinae (Hymenoptera, Mutillidae): I, generic review with descriptions of three new genera. *Journal of Hymenoptera Research*, 46: 1–24.

Brothers, D.J. & Lelej, A.S. (2017) Phylogeny and higher classification of Mutillidae (Hymenoptera) based on morphological reanalyses. *Journal of Hymenoptera Research*, 60: 1–97.

Brothers, D.J., Tschuch, G., & Burger, F. (2000) Associations of mutillid wasps (Hymenoptera, Mutillidae) with eusocial insects. *Insectes Sociaux*, 47: 201–211.

Cambra, R.A. & Quintero A., D. (1993) Studies on *Timulla* Ashmead (Hymenoptera: Mutillidae): New distribution records and synonymies, and descriptions of previously unknown allotypes. *The Pan-Pacific Entomologist*, 69(4): 299–313.

Cambra, R.A. & Quintero A., D. (2007) *Chilemutilla*, a new genus of Mutillidae (Hymenoptera) from Chile, and the description of the first wingless mutillid male from South America. *Transactions of the American Entomological Society*, 133(1–2): 167–180.

Cambra, R.A., Quintero A., D., Waldren, G.C., Bartholomay, P.R., & Williams, K.A. (2018) Taxonomic revision of the genus *Timulla* Ashmead, 1899 of Panama (Hymenoptera: Mutillidae). *Tecnociencia*, 20(2): 23–57.

Cottrell, R.G. (1936) *The Biology of Dasymutilla bioculata (Cresson)*. Unpublished Master of Science Thesis, University of Minnesota, 42 pp., 6 plates.

Day, M.C. (1979) The species of Hymenoptera described by Linnaeus in the genera *Sphex*, *Chrysis*, *Vespa*, *Apis* and *Mutilla*. *Biological Journal of the Linnean Society*, 12(1): 45–84.

Deyrup, M. (1988) Review of adaptations of velvet ants (Hymenoptera: Mutillidae). *The Great Lakes Entomologist*, 21(1): 1–4.

Evans, H.E. (1969) Phoretic copulation in Hymenoptera. *Entomological News*, 80: 113–124.

Fales, H.M., Jaouni, T.M., Schmidt, J.O., & Blum, M.S. (1980) Mandibular gland allomones of *Dasymutilla occidentalis* and other mutillid wasps. *Journal of Chemical Ecology*, 6(5): 895–903.

Ferguson, W.E. (1962) Biological characteristics of the mutillid subgenus *Photopsis* Blake and their systematic values (Hymenoptera). *University of California Publications in Entomology*, 27(1): 1–92.

Gall, B.G., Spivey, K.L., Chapman, T.L., Delph, R.J., Brodie, Jr., E.D., & Wilson, J.S. (2018) The indestructible insect: Velvet ants from across the United States avoid predation by representatives from all major tetrapod clades. *Ecology and Evolution*, 2018: 1–11.

Jellison, W.L. (1982) Concentrations of mutillid wasps (Hymenoptera: Mutillidae). *Entomological News*, 93(1): 27–28.

Krombein, K.V. (1972) Monograph of the Madagascan Mutillidae (Hymenoptera). Part I: Myrmillini, Mutillini and Smicromyrmini. *Annales*, *Musée Royal de l'Afrique Centrale (Série in 8°)*, *Sciences Zoologiques*, 199: 1–61.

Lelej, A.S. & Nemkov, P.G. (1997) Phylogeny, evolution and classification of Mutillidae (Hymenoptera). *Far Eastern Entomologist*, 46: 1–24.

Linnaeus, C. (1758) *Systema Naturae per Regna Tria Naturae, secundum Classes, Ordines, Genera, Species, cum Characteribus, Differentiis, Synonymis, Locis*.

Tomus I. Editio Decima, Reformata. Laurentii Salvii, Holmiae (Stockholm), 823 pp.

Linsley, E.G. (1960) A fragmentary observation on the mating behavior of *Timulla* (Hymenoptera: Mutillidae). *The Pan-Pacific Entomologist*, 36(1): 36.

Linsley, E.G., MacSwain, J.W., & Smith, R.F. (1955) Observations on the mating habits of *Dasymutilla formicalia* Rohwer (Hymenoptera: Mutillidae). *The Canadian Entomologist*, 87: 411–413.

Lo Cascio, P. (2015) Worldwide checklist of the island mutillid wasps (Hymenoptera Mutillidae). *Biodiversity Journal*, 6(2): 529–592.

Luz, D.R., Waldren, G.C., & Melo, G.A.R. (2016) Bees as hosts of mutillid wasps in the Neotropical region (Hymenoptera, Apidae, Mutillidae). *Revista Brasileira de Entomologia*, 60(4): 302–307.

Manley, D.G. (2000) Defense adaptations in velvet ants (Hymenoptera: Mutillidae) and their possible selective pressures. In: Austin, A.D. & Dowton, M. (Eds), *Hymenoptera: Evolution, Biodiversity and Biological Control*. CSIRO Publishing, Collingwood, Victoria, Australia, pp. 285–289.

Manley, D.G. & Deyrup, M.A. (1989) Notes on the biology of *Dasymutilla pyrrhus* (Fox) (Hymenoptera: Mutillidae). *Journal of Entomological Science*, 24(1): 53–56.

Mickel, C.E. (1936) New species and records of Nearctic mutillid wasps of the genus *Dasymutilla* (Hymenoptera). *Annals of the Entomological Society of America*, 29: 29–60.

Mickel, C.E. (1938) Synonymical notes and new species of *Pseudomethoca* and *Dasymutilla* (Mutillidae: Hymenoptera). *Annals of the Entomological Society of America*, 31: 147–156.

Mickel, C.E. (1974) Mutillidae miscellanea: Taxonomy and distribution. *Annals of the Entomological Society of America*, 67: 461–471.

Nonveiller, G. (1980) Recherches sur les Mutillides de l'Afrique (Hymenoptera, Mutillidae). X. Bref aperçu des résultats des recherches sur la faune des Mutillides du Cameroun, effectuées au cours de la période de 1962–1975. *Memoires publies par l'Institut pour la Protection des Plantes*, *Beograd*, 14: 11–68.

O'Neill, K.M. (2001) *Solitary Wasps. Behavior and Natural History*. Comstock Publishing Associates, Ithaca and London, 406 pp.

O'Neill, K.M. & Seibert, C. (1996) Foraging behavior of the robber fly *Megaphorus willistoni* (Cole) (Diptera: Asilidae). *Journal of the Kansas Entomological Society*, 69(4): 317–325.

O'Toole, C. (1975) The systematics of *Timulla oculata* (Fabricius) (Hymenoptera, Mutillidae). *Zoologica Scripta*, 4(5–6): 229–251.

Pagliano, G., Brothers, D.J., Cambra T., R.A., Lelej, A.S., Lo Cascio, P., Matteini Palmerini, M., Scaramozzino, P.L., Williams, K.A., & Romano, M. (2020 ["2018"]) Checklist of names in Mutillidae (Hymenoptera), with illustrations of selected species. *Bollettino del Museo regionale di Scienze naturali di Torino*, 36(1–2): 5–425.

Pilgrim, E.M. & Pitts, J.P. (2006) A molecular method for associating the dimorphic sexes of velvet ants (Hymenoptera: Mutillidae). *Journal of the Kansas Entomological Society*, 79(3): 222–230.

Pilgrim, E.M., Williams, K.A., & Pitts, J.P. (2008) Sex association and synonymy in southwestern U.S. species of *Dasymutilla* (Hymenoptera: Mutillidae). *The Pan-Pacific Entomologist*, 84(1): 57–68.

Pitts, J.P., Boud, T.J., & Pilgrim, E.M. (2007) Molecular sex associations of three species of nocturnal velvet ant (Hymenoptera: Mutillidae). *Journal of the Kansas Entomological Society*, 80(2): 136–145.

Pitts, J.P., Wilson, J.S., Williams, K.A., & Boehme, N.F. (2009) Velvet ants (Hymenoptera: Mutillidae) of the Algodones sand dunes of California, USA. *Zootaxa*, 2131: 1–53.

Quintero A., D. & Cambra T., R.A. (1996) *Horcomutilla* Casal: Description of previously unknown males, new distribution records, and comments on the genus (Hymenoptera: Mutillidae). *Journal of Hymenoptera Research*, 5: 53–63.

Quintero A., D. & Cambra, R.A. (2011) Revision of the Mexican endemic genus *Invreiella* Suárez, with the description of a new species. *Transactions of the American Entomological Society*, 137(1+2): 217–224.

Reid, J.A. (1941) The thorax of the wingless and short-winged Hymenoptera. *The Transactions of the Royal Entomological Society of London*, 91(8): 367–446.

Rodriguez, J., Pitts, J.P., von Dohlen, C.D., & Wilson, J.S. (2014) Müllerian mimicry as a result of codivergence between velvet ants and spider wasps. *PLoS ONE*, 9(11): e112942.

Ronchetti, F. & Polidori, C. (2020) A sting affair: A global quantitative exploration of bee, wasp and ant hosts of velvet ants. *PLoS ONE*, 15(9): e0238888.

Rothney, G.A.J. (1903) The aculeate Hymenoptera of Barrackpore, Bengal. *Transactions of the Entomological Society of London*, 1903: 93–116.

Sadler, E.A., Pitts, J.P., & Wilson, J.S. (2018) Stinging wasps (Hymenoptera: Aculeata), which species have the longest sting? *PeerJ*, 6: e4743.

Schmidt, J.O. & Blum, M.S. (1977) Adaptations and responses of *Dasymutilla occidentalis* (Hymenoptera: Mutillidae) to predators. *Entomological Experiments and Applications*, 21: 99–111.

Sheldon, J.K. (1970) Sexual dimorphism in the head structure of Mutillidae Hymenoptera: A possible behavioral explanation. *Entomological News*, 81: 57–61.

Simon, S., Letsch, H., Bank, S., Buckley, T.R., Donath, A., Liu, S., Machida, R., Meusemann, K., Misof, B., Podsiadlowski, L., Zhou, X., Wipfler, B., & Bradler, S. (2019) Old World and New World Phasmatodea: Phylogenomics resolve the evolutionary history of stick and leaf insects. *Frontiers in Evolution and Ecology*, 7(345): 1–14.

Skinner, R.K., Dietrich, C.H., Walden, K.K.O., Gordon, E., Sweet, A.D., Podsiadlowski, L., Petersen, M., Simon, C., Takiya, D.M., & Johnson, K.P. (2019) Phylogenomics of Auchenorrhyncha (Insecta: Hemiptera) using transcriptomes: Examining controversial relationships via degeneracy coding and interrogation of gene conflict. *Systematic Entomology*, 45(1): 85–113.

Suárez, F.J. (1966 [“1965”]) Datos sobre Mutílidos Neotropicales. VI. Dos nuevos géneros de Pseudomethocini de Méjico y Brasil (Hymenoptera). *Eos*, *Revista Española de Entomología*, *Madrid*, 41(2–3): 471–482.

Tormos, J., Asís, J.D., Polidori, C., Benéitez, A., & Storino, G. (2010) The mating behaviour of the velvet ant, *Nemka viduata* (Hymenoptera: Mutillidae). *Journal of Insect Behavior*, 23: 117–127.

Tschuch, G. & Brothers, D.J. (2000) Stridulatory organs in solitary aculeate Hymenoptera (Insecta). *Zoologischer Anzeiger*, 239: 319–327.

Vitt, L.J. & Cooper, Jr., W.E. (1988) Feeding responses of skinks (*Eumeces laticeps*) to velvet ants (*Dasymutilla occidentalis*). *Journal of Herpetology*, 22(4): 485–488.

Vivallo, F. (2020 [“2021”]) Phoretic copulation in Aculeata (Insecta: Hymenoptera): A review. *Zoological Journal of the Linnean Society*, 191(3): 627–636.

Williams, K.A., Manley, D.G., Deyrup, M., von Dohlen, C., & Pitts, J.P. (2012) Systematic review of the *Dasymutilla monticola* species-group (Hymenoptera: Mutillidae): Using phylogenetics to address species-group placement and sex associations. *Zootaxa*, 3554: 1–29.

Williams, K.A. & Pitts, J.P. (2013) Caribbean and Mexican additions to the *Dasymutilla bioculata* species-group. *Annals of the Entomological Society of America*, 106(4): 429–436.

Wilson, J.S., Williams, K.A., Forister, M.L., von Dohlen, C.D., & Pitts, J.P. (2012) Repeated evolution in overlapping mimicry rings among North American velvet ants. *Nature Communications*, 3: 1272.

Wilson, J.S., Jahner, J.P., Williams, K.A., & Forister, M.L. (2013) Ecological and evolutionary processes drive the origin and maintenance of imperfect mimicry. *PLoS ONE*, 8(4): e61610.

Wilson, J.S., Jahner, J.P., Forister, M.L., Sheehan, E.S., Williams, K.A., & Pitts, J.P. (2015) North American velvet ants form one of the world's largest known Müllerian mimicry complexes. *Current Biology*, 25(16): R704–R706.

CHAPTER II

PHYLOGENOMIC INFERENCE OF THE HIGHER CLASSIFICATION OF VELVET ANTS (HYMENOPTERA: MUTILLIDAE)[1]

Abstract

A phylogenomic study of the aculeate wasp family Mutillidae (Hymenoptera) was conducted using ultraconserved elements (UCEs). All currently recognized subfamilies and tribes of Mutillidae were represented using 134 ingroup taxa. The maximum-likelihood criterion (ML) and the maximum-parsimony criterion (MP) were used to infer the phylogenetic relationships within the family and related taxa using an aligned data set of 238,764 characters; the topologies of these respective analyses were largely congruent. The modern higher classification of Mutillidae, based on morphology, is largely congruent with the phylogenomic results of this study at the subfamily level, whereas the tribal classification is poorly supported. The subfamily Myrmosinae was recovered as sister-group to Sapygidae in the ML analysis and sister-group to Sapygidae + Pompilidae in the MP analysis. Myrmosinae is consequently raised to the family level, Myrmosidae, **stat. nov.** The two constituent tribes of Myrmosidae are raised to the subfamily level, Kudakrumiinae, **stat. nov.** and Myrmosinae, **stat nov.** All four recognized tribes of Mutillinae were found to be non-monophyletic; three additional mutilline clades were recovered in addition to Ctenotillini, Mutillini, Smicromyrmini, and Trogaspidiini *sensu stricto*. These three clades are described as new tribes: Pristomutillini, **trib. nov.**,

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[1] This chapter has been formatted for submission to *Systematic Entomology*.

Psammothermini, **trib. nov.**, and Zeugomutillini, **trib. nov.** All three recognized tribes of Sphaeropthalminae were found to be non-monophyletic; six additional sphaeropthalmine clades were recovered in addition to Dasymutillini, Pseudomethocini, and Sphaeropthalmini *sensu stricto*. The subtribe Ephutina of Mutillinae: Mutillini was found to be polyphyletic, with the *Ephuta* genus-group recovered within basal Sphaeropthalminae and the *Odontomutilla* genus-group recovered as sister-group to Myrmillinae + Mutillinae. Consequently, the subtribe Ephutina is transferred from Mutillinae: Mutillini and is raised to a tribe within Sphaeropthalminae, Ephutini, **stat. nov.** and Odontomutillinae, **stat. nov.**, is raised from a synonym of Ephutina to the subfamily level. The sphaeropthalmine tribe Pseudomethocini was found to be polyphyletic, with the subtribe Euspinoliina recovered as a basal clade in Sphaeropthalminae; consequently, Euspinoliini, **stat. nov.**, is raised to a tribe in Sphaeropthalminae. The dasylabrine tribe Apteromutillini was recovered within Dasylabrini and is proposed as a **new synonym** of Dasylabrini. Dating analyses were conducted to infer the ages of the Pompiloidea families (Mutillidae, Myrmosidae, Pompilidae, and Sapygidae) and the ages of the Mutillidae subfamilies and tribes. Pompiloidea is significantly older than previously thought, with an inferred age of 154.11/144.27 Ma. Mutillidae was inferred to have emerged 123.06/105.28 Ma, and further, its two most species-rich subfamilies, Mutillinae and Sphaeropthalminae, were inferred to have emerged 40.22/34.7 Ma and 74.25/63.64 Ma, respectively.

Introduction

Wasps of the family Mutillidae (Hymenoptera: Aculeata), commonly known as velvet ants, are a conspicuous yet little-known component of the world's tropical and temperate ecosystems. Velvet ants are primarily solitary ectoparasitoids of ground-nesting bees and apoid wasps (Hymenoptera: Apoidea) with a few records known for Coleoptera, Diptera, and Lepidoptera (Brothers *et al.*, 2000; Luz *et al.*, 2016). These insects exhibit extreme sexual dimorphism, with females always apterous and males typically fully winged. The sexes have few shared characters, with most species and even many genera being known from only a single sex. Past researchers included within Mutillidae unrelated taxa that are now considered distinct families, or non-mutillid subfamilies, in part due to the superficial similarity of the apterous females; such taxa include Bradynobaenidae, Chyphotidae, Tiphiidae: Brachycistidinae, Thynnidae: Methochinae, and Myrmosidae. The phylogenetic position of Myrmosidae, in particular, has been controversial for much of its taxonomic history.

History of Mutillidae classification

The higher classification of Mutillidae has been subject to a number of iterations over the past 200 years. For much of its early taxonomic history, the family in the strict sense was primarily represented by the nominal genus *Mutilla* Linnaeus and a few other small genera. The rudiments of today's classification began with the work of Klug (1821), who proposed an informal division of the Brazilian species of "*Mutilla*" based on the shape of the male's compound eyes. Burmeister (1854, 1875), Blake (1871), and Gerstaecker (1874) proposed similar divisions of the New World "*Mutilla*" fauna, relying on the same eye shape character in the males and additionally whether the first metasomal segment was petiolate or sessile with the second segment. Blake (1871) formalized the importance of eye shape with his description of the genus *Sphaeropthalma* for the round-eyed mutillid species (*sphaer* = round; *ophthalma* = eye). These two genera were used in practice as catch-all "subfamilies" with emphasis placed on the male compound eye shape: ovate and emarginate in *Mutilla* and spherical in *Sphaeropthalma* (Blake, 1871, 1886; Cameron, 1894–1896). Further, Blake (1871) organized members of these genera into divisions primarily based on the first metasomal segment being petiolate or sessile with the second segment. These divisions mostly correspond to the tribes that are recognized today within Sphaeropthalminae (*i.e.*, Dasymutillini, Pseudomethocini, and Sphaeropthalmini).

The concept of Mutillidae was relatively broad throughout the nineteenth century. No valid higher mutillid taxa were proposed until Fox (1894), and the reason is here speculated upon. A recurring theme in the taxonomic history of Mutillidae has been the inclusion of ultimately non-mutillid taxa in the family, such as *Apterogyna* + *Bradynobaenus* (Bradynobaenidae), *Brachycistis* (Tiphiidae: Brachycistidinae), *Chyphotes* (Chyphotidae), *Fedtschenkia* (Sapygidae: Fedtschenkiinae), *Methocha* (Thynnidae: Methochinae), *Myrmosa* (Mymosidae: Myrmosinae), and even *Sclerodermus* (Bethylidae: Scleroderminae). Such highly-inclusive concepts of Mutillidae effectively minimized the differences within "*Mutilla*" and past researchers were likely unable to grapple with how to subdivide Mutillidae proper. The splitting of *Mutilla* into additional genera was discouraged as late as the early twentieth century.

The first valid classification for Mutillidae was proposed by Fox (1894) who included two subfamilies therein (although with tribal-group suffixes): Mutillini and

Myrmosini. Shortly after, Fox (1899) updated his previous classification to include two subfamilies, Mutillinae and Thynninae, with Myrmosini reduced to a tribe of Thynninae. Ashmead (1899, 1900–1904), a contemporary hymenopterist, proposed his own classification for Mutillidae. He elevated Myrmosini and Thynninae to the family level and posited that Mutillidae was composed of two subfamilies each with two tribes: Ephutinae (Ephutini and Sphaerophthalmini [sic]) and Mutillinae (Mutillini and Photopsidini). André (1899–1903, 1902, 1904) critiqued Ashmead's classification and considered Myrmosidae to be a subfamily of Mutillidae and all of Mutillidae proper to belong to the subfamily Mutillinae. André's classification was far more inclusive than that of Ashmead, as André included Apterogyninae, Fedtschenkiinae, Methochinae, and Myrmosidae as subfamilies of Mutillidae (André, 1902, 1904). In his revision of the Afrotropical Mutillidae, Bischoff (1920–1921) included Apterogyninae and Myrmosidae as mutillid subfamilies, along with the nominal Mutillinae. Further, Bischoff erected four new mutilline tribes in a key: Myrmillini, Pseudophotopsini [sic], Smicromyrmini, and Trogaspidiini. The first classification to take the world fauna into consideration was attempted by Schuster (1947, 1949). He included Apterogyninae as a mutillid subfamily and proposed Eotillinae, Rhopalomutillinae, and Typhoctinae as new subfamilies (Schuster, 1949); however, no mention was made of Myrmosidae. Additional subfamilies and tribes were subsequently proposed: Cystomutillinae by Invrea (1964), Dasylabrinae by Invrea (1964), Kudakrumiinae by Krombein (1979), Odontomutillinae by Lelej (1983), Petersenidiina (Mutillinae: Trogaspidiini) by Lelej (1996), Smicromyrmillini (Ticoplinae) by Argaman (1988), and Ticoplinae by Nagy (1970).

The modern foundation for mutillid classification is based on Brothers (1975), who conducted the first cladistic study of Aculeata with an emphasis on Mutillidae at a global scale. He classified the family into seven subfamilies, four tribes, and four subtribes. Further, Brothers (1975) moved several subfamilies out of Mutillidae and into their own family, Bradynobaenidae (Apterogyninae, Bradynobaeninae, Chyphotinae, and Typhoctinae (including Eotillini)). Brothers & Carpenter (1993) and Brothers (1999) expanded upon the study of Aculeata by Brothers (1975) using a cladistic approach and reached similar results to the latter. The mutillid classification proposed by Brothers (1975) remained the sole hypothesis for more than two decades until Lelej & Nemkov (1997) conducted a cladistic analysis that resulted in a slightly different classification. In order to resolve the differences between these two competing classifications, a joint cladistic study of Mutillidae based on morphology was undertaken by Brothers & Lelej (2017). This study is the most morphologically-comprehensive analysis of the family to date. Brothers & Lelej (2017) coded their terminals at the genus level and used 230 characters for their cladistic analysis. The higher taxa they proposed were delimited by synapomorphies discovered through their cladistic analyses and their final classification for Mutillidae is visually summarized in figs 2.1A and 2.1B. Three new tribes and a subtribe were erected: Apteromutillini (Dasylabrinae), Ctenotillini (Mutillinae), Dasymutillini (Sphaeropthalminae), and Euspinoliina (Sphaeropthalminae: Pseudomethocini). Mutillidae currently includes 4,603 valid species among 220 genera classified into 8 subfamilies, 13 tribes, and 4 subtribes (Brothers & Lelej, 2017; Pagliano *et al.*, 2020).

The phylogenetic position of the controversial taxon Myrmosidae

The phylogenetic placement of Myrmosidae has historically been controversial, having been considered its own family (Ashmead, 1899; Skorikov, 1935; Suárez, 1988; Pilgrim *et al.*, 2008; Branstetter *et al.*, 2017a), a subfamily or tribe of Mutillidae (Fox, 1894; André, 1902; Bischoff, 1920; Brothers, 1975; Lelej & Nemkov, 1997; Brothers & Lelej, 2017), or a subfamily of Tiphiidae (Krombein, 1940). Modern cladistic analyses using morphology have placed Myrmosidae as the sister-group subfamily to the remaining Mutillidae (Brothers, 1975; Lelej & Nemkov, 1997; Brothers & Lelej, 2017) while molecular phylogenetic analyses have supported it having family-level status (Pilgrim *et al.*, 2008; Debevec *et al.*, 2012; Branstetter *et al.*, 2017a). Pilgrim *et al.* (2008) and Debevec *et al.* (2012) found Myrmosidae to be sister-group to Sapygidae, and Branstetter *et al.* (2017a) found Myrmosidae to be sister-group to Mutillidae. These studies, however, used a limited number of mutillid samples, as the focus was on Aculeata as a whole.

Considering the historical controversy regarding the placement of Myrmosidae and a lack of consensus among recent phylogenetic studies using different types of data and methodologies, a phylogenomic approach to resolve these differences is desirable. Phylogenomic studies have been used to infer family-group interrelationships in other insect groups, such as Phasmatodea (Simon *et al.*, 2019), auchenorrhynchan Hemiptera (Skinner *et al.*, 2019), and aculeate Hymenoptera (Branstetter *et al.*, 2017a). Further, a phylogenomic study testing the relationships of the higher taxa of Mutillidae is also desirable, as all previous classifications have been based on morphology. The only published molecular phylogenetic analysis dedicated to a supra-generic group of mutillids is that of Pitts *et al.* (2010), who investigated the Nearctic nocturnal Sphaeropthalminae using two ribosomal DNA internal transcribed spacer regions, ITS1 and ITS2.

Critique of the Brothers & Lelej (2017) study

Despite the valuable contribution of Brothers & Lelej (2017) towards better understanding the higher-level relationships within Mutillidae, there are several problems with the approaches that were followed in the study. First, their taxa were treated at the genus level, rather than the species level. Some mutillid genera are known to be heterogeneous assemblages that serve as placeholder taxa until their constituent species can be critically studied. The monophyly of these placeholder genera has yet to be investigated through phylogenetic inference and several genera in the Brothers & Lelej (2017) study fall into this category, including *Mutilla* Linnaeus, *Pseudomethoca* Ashmead, and *Timulla* Ashmead. Some placeholder genera, such as *Myrmilla* Wesmael and *Sphaeropthalma* Blake, were explicitly noted to be considered in the strict sense; however, the former three placeholder genera were not. Second, the proposed higher-level taxa in their study were neither described nor explicitly diagnosed, but were rather supported based on lists of primarily homoplasious synapomorphies. The synapomorphies provided for each higher taxon were based on the results of their maximum-parsimony analyses, or from post-analysis rearrangements that were made to their preferred most-parsimonious cladogram. Finally, some branches in the preferred most-parsimonious cladogram of Brothers & Lelej (2017) were intentionally rearranged post-analysis in order to retrofit their new results to match the previous higher classification hypotheses of Brothers (1975) and Lelej & Nemkov (1997), and homoplasious synapomorphies were then provided as support for these retrofitted higher taxa. An example of this involves the genera *Atillum* André, *Euspinolia* Ashmead, and *Hoplocrates* Mickel. These three genera have historically been considered members of the sphaeropthalmine tribe Pseudomethocini (Brothers, 1975; Lelej & Nemkov, 1997), which is broadly characterized by females possessing 1) a large, quadrate head that is sometimes armed with processes, 2) a pear-shaped mesosoma that is more or less constricted at the propodeal spiracles in dorsal view, and 3) the first metasomal segment is sessile with the second segment. Males also have the first metasomal segment sessile with the second segment, and they often have a large head as well. In the preferred most-parsimonious cladogram and several other cladograms of Brothers & Lelej (2017), *Atillum*, *Euspinolia*, and *Hoplocrates* were not recovered as members of Sphaeropthalminae, but rather as sister-group to Sphaeropthalminae + (Dasylabrinae + (Myrmillinae + Mutillinae)). Further, these three genera were not closely associated with Pseudomethocini *sensu stricto* in any of their results. Despite a complete lack of support for membership of *Atillum*, *Euspinolia*, and *Hoplocrates* to Pseudomethocini, and even as a member of Sphaeropthalminae in many cases, these three genera were placed in Pseudomethocini as members of a new subtribe, Euspinoliina, with the following rationale:

> “The two components [(Euspinoliina and Pseudomethocina)] of this grouping [(Pseudomethocini)] are not closely associated in any of the analyses, but they are placed together here on the basis of their consistent positions in the current classifications, and the fact that this arrangement adds only five steps when compared with that in the preferred tree (Fig. 5; and see above). Because the two components are consistently shown as monophyletic in almost all of the analyses, and acknowledging the uncertainties about their true relationships to each other, however, we propose that they be recognized as distinct subtribes.”

Additional cases in which new results were retrofitted to older subfamilial and tribal concepts in Brothers & Lelej (2017) involve the following genera and groupings: *Allotilla* Schuster + *Photomorphus* Viereck + *Tallium* André, *Ancistrotilla* Brothers + *Bothriomutilla* Ashmead + *Eurymutilla* Ashmead + *Odontomyrme* Lelej, *Apteromutilla* Ashmead, *Brachymutilla* André, *Dolichomutilla* Ashmead, *Liotilla* Bischoff, *Pristomutilla* Ashmead, *Promecilla* André, *Protophotopsis* Schuster, and *Pseudocephalotilla* Bischoff.

Phylogeny of Mutillidae using ultraconserved elements

This contribution represents the first molecular phylogenetic analysis of Mutillidae at the family level. The goal of this study is to test the morphology-based higher classification of Mutillidae proposed by Brothers & Lelej (2017) and also the position of Myrmosidae in relation to Mutillidae with a phylogenomic approach using ultraconserved elements (UCEs). UCEs are highly conserved regions of the genome that are shared among distantly-related taxa. Each UCE is flanked by variable sites that provide phylogenetic signal, and the UCE itself, while also informative, additionally provides a series of shared character states between taxa. The function of UCEs in the genome is unknown, but there is evidence that they are involved in gene regulation (Pennachio *et al.*, 2006) and development (Sandelin *et al.*, 2004; Woolfe *et al.*, 2004). UCEs have recently been used to infer the phylogeny of Aculeata (Branstetter *et al.*, 2017a) and have become a popular choice for inferring phylogenies across many different taxa.

Materials and Methods

Taxon sampling

Specimens representing 192 taxa were chosen for the study with 134 ingroup taxa (Mutillidae) and 58 outgroup taxa (including Myrmosidae). All Mutillidae subfamilies and tribes recognized by Brothers & Lelej (2017) were represented, and terminals were treated at the species level rather than the genus level in contrast to Brothers & Lelej (2017). All samples were dried, pinned museum specimens of various ages collected within the last 50 years, with the oldest specimen collected in 1974. Each specimen was assigned a unique specimen identifier (USI) with the prefix MUT, TIM, EX, PS, or U depending on the taxon and the location where the laboratory work was conducted (Table 2.1). Outgroup data for 50 taxa were sourced from Branstetter *et al.* (2017a) representing most families of Aculeata and data for three taxa were sourced from an ongoing study of Pompilidae (Pitts unpub.). An additional 17 ingroup taxa were sourced from Sadler (2018). Voucher specimens from which new molecular data were acquired for this study are deposited at the Entomological Museum of Utah State University (EMUS) (Logan, Utah, USA).

Molecular data acquisition

DNA extraction was performed using a High Pure PCR Template Preparation Kit (Roche Diagnostics, Indianapolis, IN, USA). Entire specimens were primarily used for extraction except for rare species in which a single mid leg and hind leg were removed and partly crushed. The entry point for extraction material into the specimens was typically the resulting pin hole in the mesosoma after removing the pin. Specimens were remounted after extraction to allow for future study. Extracted DNA was quantified using a Qubit

Table 2.1. Voucher data for specimens used in the Mutillidae higher classification study.

Family	Subfamily: Tribe	Species	ID
AMPULICIDAE	N/A	*Ampulex compressa*	BND1382
ANDRENIDAE	Andreninae	*Andrena asteris*	HPG17
APIDAE	Apinae: Bombini	*Bombus pensylvanicus*	HPG16
BETHYLIDAE	Bethylinae	*Goniozus* sp.	EX555
BETHYLIDAE	Epyrinae	*Epyris* sp.	EX562
BETHYLIDAE	Pristocerinae	*Pristocera* sp.	EX552
BRADYNOBAENIDAE	Apterogyninae	*Apterogyna* sp.	EX572
CHRYSIDIDAE	Chrysidinae: Chrysidini	*Argochrysis armilla*	EX434
CHRYSIDIDAE	Chrysidinae: Chrysidini	*Chrysis* sp.	EX561
CHRYSIDIDAE	Loboscelidiinae	*Loboscelidia* sp.	EX554
CHYPHOTIDAE	Chyphotinae	*Chyphotes mellipes*	HPG7
CHYPHOTIDAE	Chyphotinae	*Chyphotes* sp.	EX553
COLLETIDAE	Colletinae	*Colletes dimidiatus*	BND283
CRABRONIDAE	Bembicinae: Bembicini	*Bembix americana*	EX446
DRYINIDAE	Anteoninae	*Deinodryinus atriventris*	EX475
EMBOLEMIDAE	N/A	Gen. sp.	EX483
FORMICIDAE	Myrmicinae: Pogonomyrmecini	*Pogonomyrmex occidentalis*	EX377
HETEROGYNAIDAE	N/A	*Heterogyna nocticola*	BND1290
ICHNEUMONIDAE	Ichneumoninae	Gen. sp.	EX481
MEGACHILIDAE	Megachilinae: Megachilini	*Megachile exilis*	EX393
MUTILLIDAE	Dasylabrinae: Apteromutillini	*Brachymutilla scabrosa*	EX1805
MUTILLIDAE	Dasylabrinae: Dasylabrini	*Chrestomutilla* sp.	MUT008
MUTILLIDAE	Dasylabrinae: Dasylabrini	*Dasylabris maura*	EX1804
MUTILLIDAE	Dasylabrinae: Dasylabrini	*Dasylabroides* sp.	MUT009
MUTILLIDAE	Dasylabrinae: Dasylabrini	*Orientilla* sp.	MUT051
MUTILLIDAE	Dasylabrinae: Dasylabrini	*Stenomutilla argentata*	MUT010
MUTILLIDAE	Dasylabrinae: Dasylabrini	*Tricholabioides* sp.	MUT011
MUTILLIDAE	Mutillinae: Ctenotillini	*Cephalotilla* sp.	EX1788
MUTILLIDAE	Mutillinae: Ctenotillini	*Cephalotilla* sp.	MUT019
MUTILLIDAE	Mutillinae: Ctenotillini	*Chaetomutilla fornasiini*	MUT020
MUTILLIDAE	Mutillinae: Ctenotillini	*Ctenotilla guangdongensis*	EX1787
MUTILLIDAE	Mutillinae: Ctenotillini	*Mimecomutilla renominanda*	MUT021
MUTILLIDAE	Mutillinae: Ctenotillini	*Pristomutilla* sp.	MUT022
MUTILLIDAE	Mutillinae: Ctenotillini	*Strangulotilla* sp.	MUT023
MUTILLIDAE	Mutillinae: Ctenotillini	*Zeugomutilla pycnopyga*	MUT024
MUTILLIDAE	Mutillinae: Mutillini: Ephutina	*Cockerellidia sohmi*	MUT026
MUTILLIDAE	Mutillinae: Mutillini: Ephutina	*Ephuamelia gabrielae*	MUT057
MUTILLIDAE	Mutillinae: Mutillini: Ephutina	*Ephuchaya pombera*	MUT058

MUTILLIDAE	Mutillinae: Mutillini: Ephutina	*Ephuta* sp.	EX1808
MUTILLIDAE	Mutillinae: Mutillini: Ephutina	*Ephuta trifida*	EX1807
MUTILLIDAE	Mutillinae: Mutillini: Ephutina	*Odontomutilla familiaris*	MUT025
MUTILLIDAE	Mutillinae: Mutillini: Ephutina	*Odontomutilla ovata*	MUT028
MUTILLIDAE	Mutillinae: Mutillini: Ephutina	*Odontomutilla* sp.	EX1792
MUTILLIDAE	Mutillinae: Mutillini: Ephutina	*Onoretilla merida*	MUT056
MUTILLIDAE	Mutillinae: Mutillini: Ephutina	*Yamanetilla* sp.	MUT027
MUTILLIDAE	Mutillinae: Mutillini: Mutillina	*Mutilla europaea*	EX1791
MUTILLIDAE	Mutillinae: Mutillini: Mutillina	*Mutilla marginata*	MUT031
MUTILLIDAE	Mutillinae: Mutillini: Mutillina	*Mutilla harmandi*	MUT032
MUTILLIDAE	Mutillinae: Mutillini: Mutillina	*Ronisia brutia*	MUT029
MUTILLIDAE	Mutillinae: Mutillini: Mutillina	*Tropidotilla litoralis*	MUT030
MUTILLIDAE	Mutillinae: Smicromyrmini	*Antennotilla phoebe*	MUT033
MUTILLIDAE	Mutillinae: Smicromyrmini	*Corytilla* sp.	MUT044
MUTILLIDAE	Mutillinae: Smicromyrmini	*Dentilla* sp.	MUT042
MUTILLIDAE	Mutillinae: Smicromyrmini	*Ephucilla* sp.	MUT034
MUTILLIDAE	Mutillinae: Smicromyrmini	*Mickelomyrme* sp.	MUT036
MUTILLIDAE	Mutillinae: Smicromyrmini	*Nemka viduata*	MUT035
MUTILLIDAE	Mutillinae: Smicromyrmini	*Physetopoda scutellaris*	EX1793
MUTILLIDAE	Mutillinae: Smicromyrmini	*Promecilla* sp.	MUT037
MUTILLIDAE	Mutillinae: Smicromyrmini	*Psammotherma cyanochroa*	MUT038
MUTILLIDAE	Mutillinae: Smicromyrmini	*Pseudocephalotilla* sp.	MUT039
MUTILLIDAE	Mutillinae: Smicromyrmini	*Smicromyrme rufipes*	MUT040
MUTILLIDAE	Mutillinae: Smicromyrmini	*Smicromyrme strangulatus*	MUT041
MUTILLIDAE	Mutillinae: Trogaspidiini	*Artiotilla biguttata*	TIM060
MUTILLIDAE	Mutillinae: Trogaspidiini	*Aureotilla madecassa*	TIM082
MUTILLIDAE	Mutillinae: Trogaspidiini	*Dolichomutilla* sp.	EX1796
MUTILLIDAE	Mutillinae: Trogaspidiini	*Glossotilla suavis*	EX1795
MUTILLIDAE	Mutillinae: Trogaspidiini	*Pagdenidia* sp.	EX1802
MUTILLIDAE	Mutillinae: Trogaspidiini	*Timulla dubitata*	TIM010
MUTILLIDAE	Mutillinae: Trogaspidiini	*Timulla rufogastra*	TIM047
MUTILLIDAE	Mutillinae: Trogaspidiini	*Timulla vagans*	TIM029
MUTILLIDAE	Mutillinae: Trogaspidiini	*Trogaspidia heideri*	TIM122
MUTILLIDAE	Mutillinae: Trogaspidiini	*Wallacidia oculata*	TIM091
MUTILLIDAE	Mutillinae: Trogaspidiini	*Zavatilla* sp.	TIM106
MUTILLIDAE	Myrmillinae	*Bidecoloratilla chiesi*	MUT053
MUTILLIDAE	Myrmillinae	*Bidecoloratilla leopoldina*	MUT012
MUTILLIDAE	Myrmillinae	*Bischoffitilla* sp.	MUT013
MUTILLIDAE	Myrmillinae	*Ceratotilla septemmaculata*	EX1789
MUTILLIDAE	Myrmillinae	*Labidomilla* sp. MAD	MUT045
MUTILLIDAE	Myrmillinae	*Myrmilla calva*	MUT018

MUTILLIDAE	Myrmillinae	*Myrmilla capitata*	MUT052
MUTILLIDAE	Myrmillinae	*Myrmilla mutica*	EX1786
MUTILLIDAE	Myrmillinae	*Odontotilla bidentata*	MUT015
MUTILLIDAE	Myrmillinae	*Sigilla dorsata*	MUT016
MUTILLIDAE	Myrmillinae	*Viereckia acrisione*	MUT017
MUTILLIDAE	Myrmosinae: Kudakrumiini	*Kudakrumia malaenglek*	MUT002
MUTILLIDAE	Myrmosinae: Kudakrumiini	*Myrmosula parvula*	MUT001
MUTILLIDAE	Myrmosinae: Myrmosini	*Krombeinella thoracica*	MUT004
MUTILLIDAE	Myrmosinae: Myrmosini	*Myrmosa* sp.	EX575
MUTILLIDAE	Myrmosinae: Myrmosini	*Myrmosa unicolor*	MUT003
MUTILLIDAE	Myrmosinae: Myrmosini	*Taimyrmosa nigrofasciata*	MUT049
MUTILLIDAE	Pseudophotopsidinae	*Pseudophotopsis binghami*	EX1783
MUTILLIDAE	Pseudophotopsidinae	*Pseudophotopsis orthophthalma*	MUT054
MUTILLIDAE	Rhopalomutillinae	*Bischoffiella* sp.	MUT047
MUTILLIDAE	Rhopalomutillinae	*Pherotilla* sp.	EX1784
MUTILLIDAE	Rhopalomutillinae	*Rhopalomutilla* sp.	MUT048
MUTILLIDAE	Rhopalomutillinae	*Rimulotilla* sp.	MUT046
MUTILLIDAE	Sphaero.: Pseudomethocini: Euspinoliina	*Atillum dulce*	MUT059
MUTILLIDAE	Sphaero.: Pseudomethocini: Euspinoliina	*Atillum jucundum*	EX1809
MUTILLIDAE	Sphaero.: Pseudomethocini: Euspinoliina	*Euspinolia albicoma*	MUT061
MUTILLIDAE	Sphaero.: Pseudomethocini: Euspinoliina	*Euspinolia clypeata*	EX1806
MUTILLIDAE	Sphaero.: Pseudomethocini: Euspinoliina	*Hoplocrates voluptuosa*	MUT060
MUTILLIDAE	Sphaero.: Pseudomethocini: Pseudomethocina	*Darditilla debilis*	EX1810
MUTILLIDAE	Sphaero.: Pseudomethocini: Pseudomethocina	*Dimorphomutilla reedi*	EX1811
MUTILLIDAE	Sphaero.: Pseudomethocini: Pseudomethocina	Gen. nov. Q sp.	EX1804
MUTILLIDAE	Sphaero.: Pseudomethocini: Pseudomethocina	*Hoplognathoca* sp. nr *robinsoni*	EX1813
MUTILLIDAE	Sphaero.: Pseudomethocini: Pseudomethocina	*Horcomutilla piala*	EX1814
MUTILLIDAE	Sphaero.: Pseudomethocini: Pseudomethocina	*Invreiella cephalargia*	PS113
MUTILLIDAE	Sphaero.: Pseudomethocini: Pseudomethocina	*Lophostigma* sp.	EX1816
MUTILLIDAE	Sphaero.: Pseudomethocini: Pseudomethocina	*Myrmilloides grandiceps*	EX1817
MUTILLIDAE	Sphaero.: Pseudomethocini: Pseudomethocina	*Pappaognatha myrmiciformis*	EX1818
MUTILLIDAE	Sphaero.: Pseudomethocini: Pseudomethocina	*Patquiatilla argentinensis*	MUT055
MUTILLIDAE	Sphaero.: Pseudomethocini: Pseudomethocina	*Pertyella martinezi*	EX1819
MUTILLIDAE	Sphaero.: Pseudomethocini: Pseudomethocina	*Pseudomethoca ajattara*	MUT062
MUTILLIDAE	Sphaero.: Pseudomethocini: Pseudomethocina	*Pseudomethoca frigida*	EX1820
MUTILLIDAE	Sphaero.: Pseudomethocini: Pseudomethocina	*Pseudomethoca sanbornii*	EX1821
MUTILLIDAE	Sphaero.: Pseudomethocini: Pseudomethocina	*Pseudomethoca* sp. INV	EX1815
MUTILLIDAE	Sphaero.: Pseudomethocini: Pseudomethocina	*Pseudomethoca vanduzei*	EX1822
MUTILLIDAE	Sphaero.: Pseudomethocini: Pseudomethocina	*Silvorientilla sinenomine*	EX1825
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Cephalomutilla haematodes*	U100J1900
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Dasymutilla bioculata*	U92J1189

MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Dasymutilla creon*	U87J670
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Dasymutilla heliophila*	U84J322
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Dasymutilla insulana*	U99J1893
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Dasymutilla monticola*	U85J371
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Dasymutilla occidentalis*	U93J1235
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Dasymutilla pseudopappus*	U94J1300
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Dasymutilla quadriguttata*	U83J313
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Dasymutilla scaevola*	EX1841
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Ephutomorpha* sp. AGM07	EX1837
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Ephutomorpha* sp. AGM5 B	EX1836
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	Gen. nr *Suareztilla* sp.	U86J606
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	Gen. sp.	U98J1779
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Lomachaeta* sp.	EX1847
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Neomutilla patagonica*	EX1844
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Protophotopsis venenaria*	EX1846
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Quwitilla blattoserica*	U88J841
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Reedomutilla gayi*	EX1839
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Suareztilla clypeata*	U95J1774
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Traumatomutilla graphica*	EX1838
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Traumatomutilla* sp.	U90J887
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Traumatomutilla* sp. TF055	U89J876
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Traumatomutilla* sp. TF139	U97J1778
MUTILLIDAE	Sphaeropthalminae: Dasymutillini	*Traumatomutilla valuta*	U96J1776
MUTILLIDAE	Sphaeropthalminae: Sphaeropthalmini	*Acanthophotopsis falciformis*	EX1826
MUTILLIDAE	Sphaeropthalminae: Sphaeropthalmini	*Acrophotopsis dirce*	EX1827
MUTILLIDAE	Sphaeropthalminae: Sphaeropthalmini	*Allotilla gibbosa*	EX1845
MUTILLIDAE	Sphaeropthalminae: Sphaeropthalmini	*Cystomutilla ruficeps*	EX1803
MUTILLIDAE	Sphaeropthalminae: Sphaeropthalmini	*Dilophotopsis paron*	EX1828
MUTILLIDAE	Sphaeropthalminae: Sphaeropthalmini	*Limaytilla pehuenche*	EX1834
MUTILLIDAE	Sphaeropthalminae: Sphaeropthalmini	*Odontophotopsis inconspicua*	EX1829
MUTILLIDAE	Sphaeropthalminae: Sphaeropthalmini	*Photomorphus cobabi*	EX1830
MUTILLIDAE	Sphaeropthalminae: Sphaeropthalmini	*Schusterphotopsis barghesti*	EX1831
MUTILLIDAE	Sphaeropthalminae: Sphaeropthalmini	*Sphaeropthalma mendica*	EX1832
MUTILLIDAE	Sphaeropthalminae: Sphaeropthalmini	*Sphaeropthalma tenuiventris*	EX1835
MUTILLIDAE	Sphaeropthalminae: Sphaeropthalmini	*Tallium* sp.	EX1823
MUTILLIDAE	Sphaeropthalminae: Sphaeropthalmini	*Xystromutilla* sp.	EX1833
MUTILLIDAE	Ticoplinae: Smicromyrmillini	*Eosmicromyrmilla* sp. cf *chinensis*	MUT050
MUTILLIDAE	Ticoplinae: Smicromyrmillini	*Smicromyrmilla* sp.	EX1785
MUTILLIDAE	Ticoplinae: Ticoplini	*Areotilla trifasciata*	MUT006
MUTILLIDAE	Ticoplinae: Ticoplini	*Nanomutilla* sp.	MUT007
PLUMARIIDAE	N/A	*Plumarius* sp.	EX550

POMPILIDAE	Ceropalinae	*Ceropales australensis*	U103PO228
POMPILIDAE	Ceropalinae	*Ceropales brethesdi*	U266PO844
POMPILIDAE	Pepsinae	*Auplopus* sp.	EX549
POMPILIDAE	Pepsinae	Gen. sp.	EX407
POMPILIDAE	Pepsinae	Gen. sp.	PS1423
POMPILIDAE	Pepsinae	*Pepsis grossa*	EX437
POMPILIDAE	Pompilinae: Aporini	*Aporus niger*	HPG6
RHOPALOSOMATIDAE	N/A	*Rhopalosoma nearcticum*	EX457
SAPYGIDAE	Sapyginae	*Sapyga pumila*	HPG8
SAPYGIDAE	Sapyginae	*Sapyga* sp.	EX466
SCLEROGIBBIDAE	N/A	Gen. sp.	EX566
SCOLIIDAE	Proscoliinae	*Proscolia* sp.	EX568
SCOLIIDAE	Scoliinae	Gen. sp.	EX577
SCOLIIDAE	Scoliinae	*Scolia verticalis*	HPG11
SIEROLOMORPHIDAE	N/A	*Sierolomorpha* sp.	EX471
THYNNIDAE	Methochinae	*Methocha* sp.	EX570
THYNNIDAE	Myzininae: Myzinini	*Myzinum* sp.	EX576
THYNNIDAE	Thynninae	Gen. sp.	EX565
TIPHIIDAE	Brachycistidinae	*Brachycistis petiolata*	U68B68
TIPHIIDAE	Brachycistidinae	*Brachycistis timberlakei*	EX440
TIPHIIDAE	Brachycistidinae	*Colocistis* sp.	EX558
TIPHIIDAE	Tiphiinae	*Tiphia* sp.	EX571
VESPIDAE	Eumeninae	*Pachodynerus alayoi*	EX400
VESPIDAE	Eumeninae	*Parancistrocerus bacu*	EX401
VESPIDAE	Masarinae	*Pseudomasaris vespoides*	EX442
VESPIDAE	Polistinae: Epiponini	*Metapolybia cingulata*	HPG10
VESPIDAE	Polistinae: Mischocyttarini	*Mischocyttarus flavitarsis*	EX441
VESPIDAE	Polistinae: Mischocyttarini	*Mischocyttarus flavitarsis*	HPG13
VESPIDAE	Polistinae: Mischocyttarini	*Mischocyttarus mexicanus*	EX398
VESPIDAE	Polistinae: Polistini	*Polistes poeyi*	EX399
VESPIDAE	Vespinae	*Vespa* sp.	EX578

3.0 Fluorometer. The following protocols for UCE molecular work were derived from Branstetter *et al.* (2017a) and were performed as follows. The extracted DNA was prepared for shearing to a target concentration of 50 ng/100 µL. The samples were then sheared/fragmented to a range between 400–600 base pairs using a Qsonica Q800R2. Library preparation was performed using a Kapa library preparation kit (Kapa Biosystems Inc., Wilmington, MA, USA). Following library preparation, PCR was performed with a thermal cycler set to 98°C/45 sec, 14 cycles of 98°C/15 sec, 60°C/30 sec, 72°C/60 sec, 72°C/5 min, and 4°C hold; samples were then quantified. Libraries were pooled at equimolar ratios of 10 samples and adjusted pool concentrations to 72 ng/100 µL. This resulted in 500 ng of DNA used for targeted UCE enrichments. Enrichments were performed using a custom RNA bait library developed for Hymenoptera (Hymenoptera 2.5Kv2A) composed of 9,446 baits for 2,524 conserved loci and 452 baits for 16 nuclear exons (Branstetter *et al.*, 2017b). RNA bait libraries were hybridized to sequencing libraries at 65°C for an incubation period of 24 hours. Each pool was then enriched using a standardized protocol ("Target Enrichment of Illumina Libraries" v.1.5, available from https://www.ultraconserved.org/). Enrichment success was determined via qPCR using a Bio-Rad CFX96 system. Pools were quantified using qPCR results and pooled into a single, final pool of 110 total libraries. The final pool was mailed to and sequenced at Novogene (Chula Vista, California, USA).

Molecular data assembly

The software package PHYLUCE v.1.6.6 was used for all post-sequence data processing and preparation for phylogenetic analysis. Raw data were first demultiplexed using

BBMap. Raw fastq reads were then cleaned using Illumiprocessor. The assembly program SPAdes was used to assemble contigs. Contigs were matched to probes using the bait set developed by Branstetter *et al.* (2017b). To be considered a match, minimum and maximum thresholds were set to 60-80, respectively, which was found to be the optimal setting across the data set to recover the most UCE loci. The data matrix was generated using fastas pulled from match counts and aligned using MAFFT v.7.407 (Katoh & Standley, 2013). Poorly aligned regions were cleaned and trimmed using Gblocks (Talavera & Castresana, 2007) with the reduced stringency parameters (b1:0.5, b2:0.5, b3:12, b4:7). Alignments were filtered for missing data using a PHYLUCE script requiring that each alignment include data for ≥75% of taxa; this threshold was found to be the ideal setting for this data set in order to account for several taxa with less available data. The resulting aligned, cleaned/trimmed, and filtered data set was used for phylogenomic analysis.

Phylogenomic analyses

The program IQ-TREE v.1.6.12 (Nguyen *et al.*, 2015) was used for maximum-likelihood inference (hereafter 'ML'). The data set was partitioned by UCE loci, with each partition allowed a different evolutionary speed ('-spp' option). ModelFinder (Kalyaanamoorthy *et al.*, 2017) was used to find the best-fit model of sequence evolution per partition (Chernomor *et al.*, 2016). Ultrafast bootstrap approximation (UFBoot, '-bb' option) (Hoang *et al.*, 2017) and SH-like approximate likelihood ratio test (SH-aLRT, '-alrt' option) (Guindon *et al.*, 2010) were used to evaluate branch stability with each set to 1000 replicates. Thresholds used to determine well-supported clades were ≥95% for

UFBoot and ≥80% for SH-aLRT. The resulting phylogenetic tree was visualized in FigTree v.1.4.4.

The program TNT v.1.5 (Goloboff & Catalano, 2016) was used for maximum-parsimony inference (hereafter 'MP'). Settings used include 100 cycles of Random Addition Sequence, 25 iterations of Drift, 25 iterations of Ratchet (Nixon, 1999), and branch-swapping with TBR. All characters were treated as unordered and equally weighted. Gaps were treated as missing data. Branch supports (Bremer, 1988, 1994; Brower, 2006) were calculated using 1,000 suboptimal trees up to 10,000 additional steps longer; these suboptimal trees were then treated to TBR branch-swapping. The resulting cladogram was visualized in FigTree v.1.4.4.

Dating analyses

The programs BEAST v.1.10.4 (Suchard *et al.*, 2018) and IQ-TREE v.2.1.1 (Minh *et al.*, 2020) were used to estimate dating for Pompiloidea with emphasis on Mutillidae. In order to reduce computation time, 50 loci were randomly sourced from the master alignment used in the ML and MP analyses, and this data set was treated as a single partition. Additionally, the ML tree was used as a reference tree in all analyses. Estimated ages are reported herein in a split format, with the estimated age inferred using BEAST first, and the estimated age inferred using IQ-TREE second (*e.g.*, 23.31/21.51 Ma).

For the BEAST analyses, BEAUti v.1.10.4 was used to generate the XML file. The substitution model used was GTR+G. An uncorrelated relaxed clock with a lognormal distribution was used (Drummond *et al.*, 2006). The tree prior used was Speciation: Birth-Death Process (Gernhard, 2008). The tree-generating operators were turned off (*i.e.*, subtreeSlide, narrowExchange, wideExchange, and wilsonBalding). Priors used to calibrate the tree were derived from both primary fossil data and secondary previously published dating analyses. The estimated ages of Apocrita (mean=194 Ma, SD=10, normal distribution) and Aculeata (mean=161 Ma, SD=10, normal distribution) were sourced from Branstetter *et al*. (2017a), specifically the median ages of their 50 random loci BEAST analysis. Priors derived from fossil data were dated using the median of date ranges sourced from http://fossilworks.org/; the geologic time scale used herein was also derived from this website. Fossil Mutillidae are only known from four Dominican amber specimens from the sphaeropthalmine tribes Dasymutillini and Ephutini. The clade *Dasymutilla* + *Traumatomutilla* within Dasymutillini was represented by *Dasymutilla dominica* Manley & Poinar, 1991 and the clade *Ephuamelia* + *Ephuchaya* + *Ephuta* within Ephutini was represented by *Ephuta clavigera* Brothers, 2003, both from Dominican amber dated 13.7–20.4 Ma (mean=17.1 Ma, SD=1, lognormal distribution, mean in real space). For other families of Pompiloidea, only the oldest known fossil was included. Myrmosidae: Kudakrumiinae was represented by *Protomutilla succinalis* Bischoff, 1916 from Baltic amber dated 37.2–33.9 Ma (mean=35.6 Ma, SD=1, lognormal distribution, mean in real space). Sapygidae was represented by *Cretofedtschenkia santanensis* Osten, 2007 from the Crato Formation dated 122.46–112.6 Ma (mean=117.5 Ma, SD=3, lognormal distribution, mean in real space). Pompilidae: Pepsinae was represented by *Cryptocheilus leleji* Waichert, Rapoza & Rodriguez in Waichert *et al.* (2019) from the Fur Formation dated 55.8–48.6 Ma (mean=52.2 Ma, SD=2, lognormal distribution, mean in real space). The standard deviations used account for the currently estimated lower and upper age boundaries, 5%

and 95% of their distribution, respectively. Lastly, the priors ucld.mean and ulcd.stdev were set to 0.001672 and 0.381, respectively, based on empirical analyses. Three independent Markov Chain Monte Carlo (MCMC) runs were performed with length of chain set to 300,000,000 and were logged every 2,000. The BEAST analyses were conducted using the CIPRES Science Gateway v.3.3 (Miller *et al.*, 2010). Logs of the BEAST analyses were assessed in Tracer v.1.7.1 (Rambaut *et al.*, 2018) to confirm stabilization and adequate effective sample sizes (ESSs). The independent runs were combined in LogCombiner v.1.10.4. Ten percent of trees were discarded as burn-in and states were resampled at a frequency of 30,000. A maximum clade credibility tree was generated in TreeAnnotator v.1.10.4 and visualized in FigTree v.1.4.4.

For the IQ-TREE analysis using the least-squares criterion (To *et al.*, 2016), the substitution model used was GTR+G, the root was dated as 194 Ma, the tips were dated as 0 Ma, and the outgroup of Aculeata was set as the taxon "Ichneumoninae_sp_EX481." The mean calibrations used in the BEAST analysis were also used in the IQ-TREE analysis. The resulting chronogram was visualized in FigTree v.1.4.4.

Results and Discussion

UCE loci recovered among the 192 taxa used in this study ranged from 77 to 2,009 (mean= 1,277). The final alignment used in the analyses was composed of 238,764 base pairs with 19.03% total missing data. 54,610 characters were constant, 23,327 were parsimony-uninformative, and 160,827 were parsimony-informative. ModelFinder assigned substitution models for 663 partitions for use in the ML analysis. The ML analysis resulted in a single tree with most major nodes having both 100% SH-aLRT and

UFBoot stability values (figs 2.2–2.4). The MP analysis resulted in two most parsimonious trees consisting of 1,781,022 steps (CI= 0.234; RI= 0.565); branch support values ranged from 2806 to 7215 (fig. 2.2).

The ML and MP analyses resulted in similar topologies (fig. 2.2) and are identical with respect to subfamily interrelationships and composition. The superfamily Pompiloidea, to which Mutillidae, Myrmosidae, Sapygidae, and Pompilidae belong, was found to be monophyletic in both ML and MP analyses. Myrmosidae was recovered as sister-group to Sapygidae in the ML analysis with relatively high stability (82.6 SH-aLRT/95 UFBoot) (figs 2.2 and 2.3) while for the MP analysis Myrmosidae was sister-group to Pompilidae + Sapygidae (branch support= 2,806) (fig. 2.2). The results of the ML analysis with Myrmosidae sister-group to Sapygidae are congruent with the results of Pilgrim *et al.* (2008).

The relationships between the Mutillidae subfamilies are consistent between the analyses and overall are mostly congruent with the results of Brothers & Lelej (2017) (fig. 2.1). The exception is that the *Odontomutilla* genus-group, classified in Brothers & Lelej (2017) as a member of the Mutillini subtribe Ephutina, was recovered as sister-group to Myrmillinae + Mutillinae. The eight subfamilies recognized in the current study were recovered as Pseudophotopsidinae + (Ticoplinae + (Rhopalomutillinae + (Sphaeropthalminae + (Dasylabrinae + (Odontomutillinae + (Myrmillinae + and Mutillinae)))))) (figs 2.2–2.4). Subfamilies were numbered 1–8 beginning with the basal subfamily Pseudophotopsidinae, and tribes were given alphanumeric labels according to their subfamily membership (*e.g.*, Mutillinae is clade 7; Mutillini is clade 7B). The final classification is visually summarized in figs 2.1C and 2.1D. The master reference for the

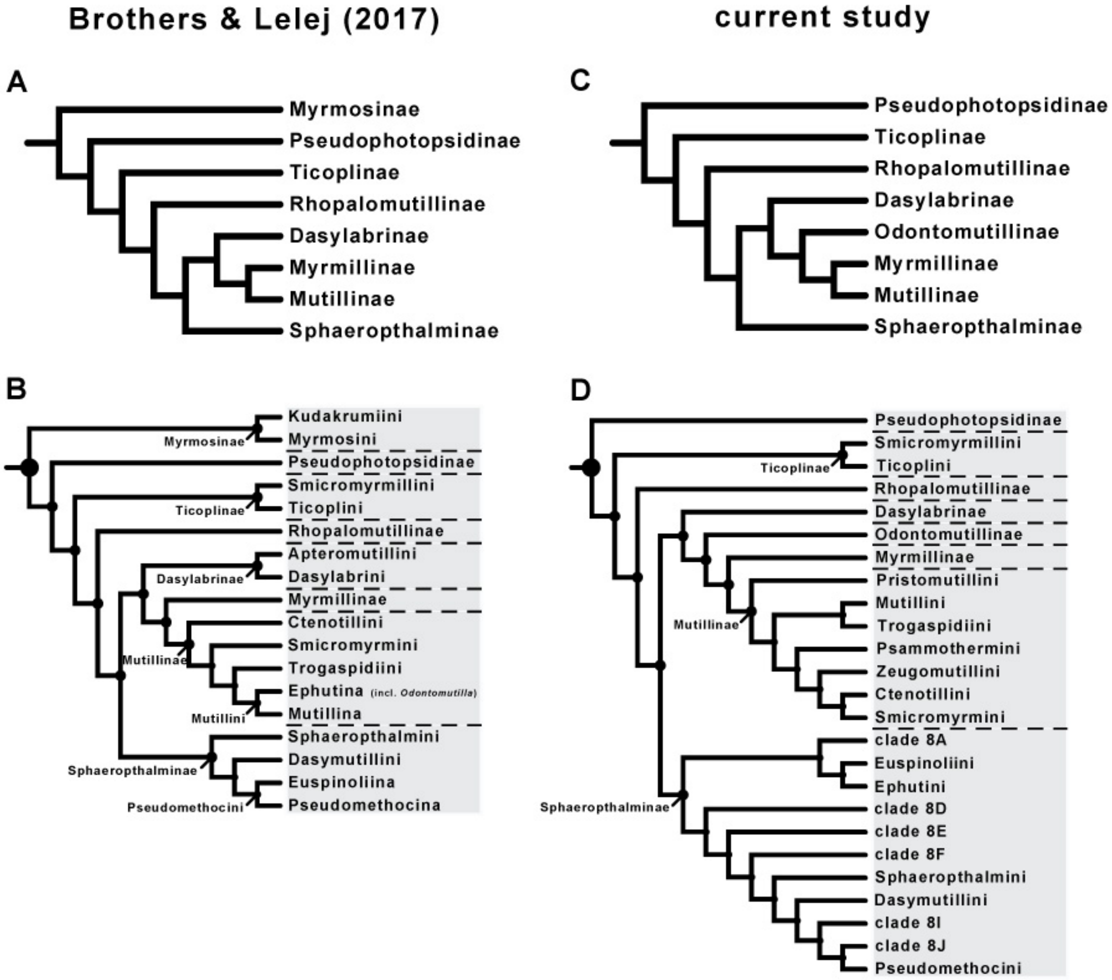

Figure. 2.1. Higher classification hypotheses for Mutillidae. (A) Mutillidae subfamily classification proposed by Brothers & Lelej, 2017; (B) Mutillidae tribal classification proposed by Brothers & Lelej, 2017; (C) Mutillidae subfamily classification newly proposed herein; (D) Mutillidae tribal classification newly proposed herein.

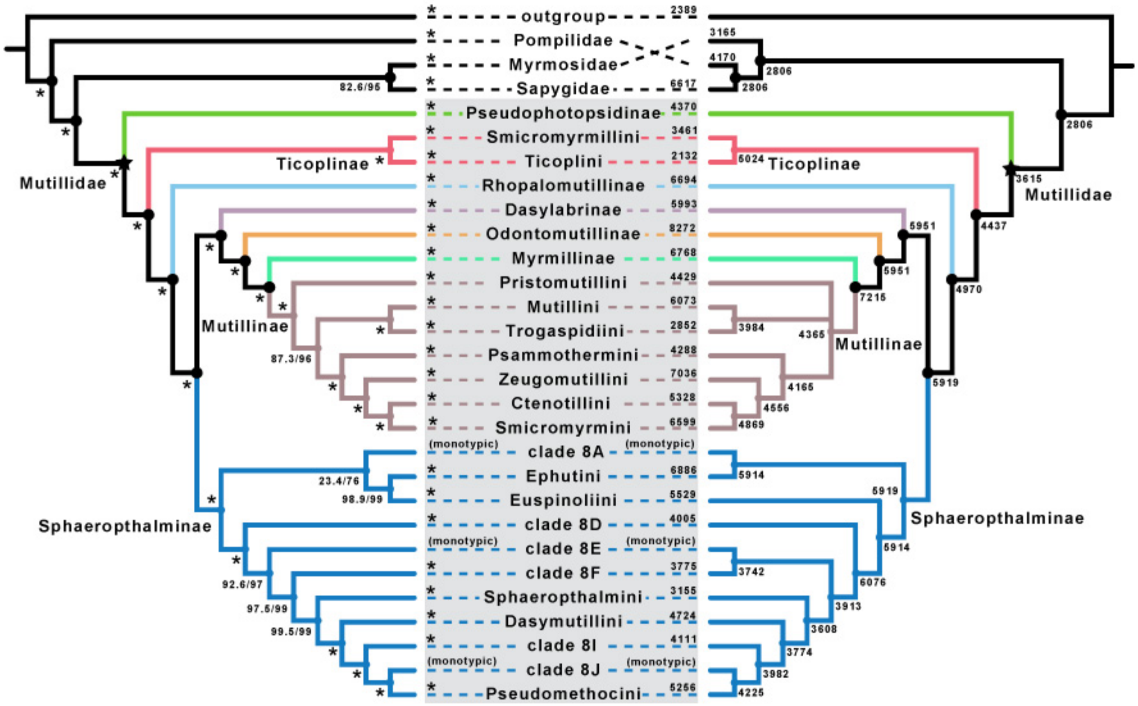

Figure 2.2. Comparison between the results of the maximum-likelihood analysis (left) and maximum-parsimony analysis (right). For the maximum-likelihood (ML) tree, the asterisks indicate SH-aLRT/UFBoot values both equal 100; if below 100, the numerical values are given in lieu of an asterisk. For the maximum-parsimony (MP) cladogram, the numerical values are branch supports and have no upper limit.

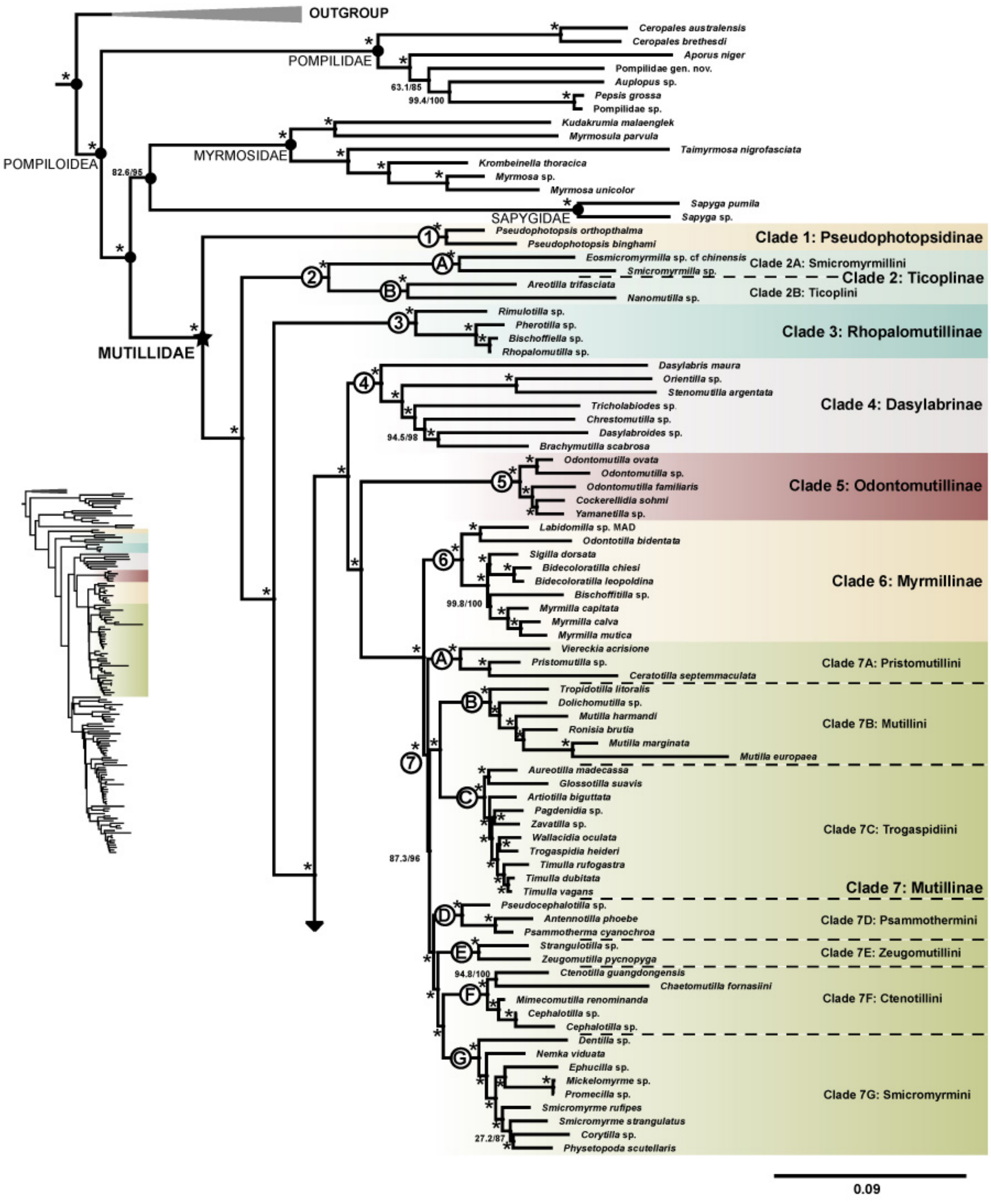

Figure 2.3. Higher classification of Mutillidae using the maximum-likelihood tree topology, part 1; for part 2 see figure 2.4.

Figure 2.4. Higher classification of Mutillidae using the maximum-likelihood tree topology, part 2; for part 1 see figure 2.3.

alphanumeric naming of clades is the ML tree (figs 2.3 and 2.4). Based on these results, the monophyletic status of the subfamilies and tribes of Mutillidae are discussed on a taxon-by-taxon basis below and are highlighted in red in their respective section of the ML tree (figs 2.5–2.13).

The BEAST and IQ-TREE analyses for ancestral dating each resulted in a single chronogram which were formatted to allow for comparisons at the family level and subfamily level (figs 2.14 and 2.15), tribe level for Mutillinae (figs 2.16 and 2.17), and tribe level for Sphaeropthalminae (figs 2.18 and 2.19).

Myrmosidae Fox, 1894, **stat. nov.**

Myrmosidae was recovered outside of Mutillidae, and depending on the analysis, as either sister-goup to Sapygidae (ML, fig. 2.2, 82.6 SH-aLRT/95 UFBoot) or sister-goup to Pompilidae + Sapygidae (MP, fig. 2.2, branch support= 2806). The various analyses of Brothers & Lelej (2017) consistently recovered Myrmosidae as sister-goup to Mutillidae and it was therein considered the basal subfamily of Mutillidae (figs 2.1A and 2.1B). In contrast to the results of Brothers & Lelej (2017), three separate studies including the current one, which analyzed different sets of molecular data, recovered Myrmosidae as sister-goup to Sapygidae and not Mutillidae: 1) Pilgrim *et al.* (2008) used MP and Bayesian approaches with four nuclear genes and a final aligned data set of 2,700 bp; 2) Debevec *et al.* (2012) used ML and Bayesian approaches and was based on an expanded data set of Pilgrim *et al.* (2008) with a final aligned data set of 4,126 bp; 3) the current study was based on UCEs with an aligned data set of 238,764 bp.

Pilgrim *et al.* (2008) used a single representative for Myrmosidae (*Myrmosula* sp. nov.) and their results differed based on the analysis and the data set. Their Bayesian analysis with the molecular-only data set recovered Myrmosidae as sister-goup to Sapygidae, while the combined molecular and morphological data set (the latter data set derived from Brothers & Carpenter 1993; Brothers 1999) resulted in Myrmosidae as sister-goup to Mutillidae. Their MP analyses for both the molecular-only and combined data sets resulted in Myrmosidae as sister-goup to Tiphiidae. Only in the Bayesian combined analysis was Myrmosidae recovered as sister-goup to Mutillidae; however, this relationship was not supported at the 0.95 PP level. Branstetter *et al.* (2017a) recovered Myrmosidae as sister-goup to Mutillidae with Sapygidae as sister-goup to Pompilidae using UCEs. However, their data set only included a single representative for Myrmosidae (*Myrmosa* sp.) and nine for Mutillidae. The UCE data set used for the current study was significantly expanded and included six myrmosid taxa and 134 mutillid taxa. Further, the data set herein includes the data from the same *Myrmosa* sp. used in Branstetter *et al.* (2017a).

Given the well-supported results of the ML and MP analyses in the current study based on six Myrmosidae taxa representing both subfamilies, an aligned data set of 238,764 characters, and the results of previous studies based on different sets of molecular data that resulted in Myrmosidae being treated as a distinct family (Pilgrim *et al.*, 2008; Debevec *et al.*, 2012), Myrmosidae, **stat. nov.**, is raised to the family level. It has two subfamilies, both of which were recovered as monophyletic: Kudakrumiinae Krombein, **stat. nov.** and Myrmosinae Fox, **stat. nov.** The relationship between these former tribes of Myrmosinae (now subfamilies of Myrmosidae) as being sister-goup taxa is congruent with Brothers & Lelej (2017).

Mutillidae Latreille, 1802

Mutillidae was recovered as monophyletic in all analyses (100 SH-aLRT/100 UFBoot; branch support= 3615) and is here considered to include eight subfamilies: Pseudophotopsidinae + (Ticoplinae + (Rhopalomutillinae + (Sphaeropthalminae + (Dasylabrinae + (Odontomutillinae, **stat. nov.** + (Myrmillinae + Mutillinae)))))) (fig. 2.1C). With the exception of Myrmosidae, **stat. nov.** and Odontomutillinae, **stat. nov.**, the ML and MP topologies recovered here are similar to those of Brothers & Lelej (2017) (*i.e.*, Myrmosinae + (Pseudophotopsidinae + (Ticoplinae + (Rhopalomutillinae + (Sphaeropthalminae + (Dasylabrinae + (Myrmillinae + Mutillinae)))))) (fig. 2.1A). The monophyly of the tribes proposed by Brothers & Lelej (2017), however, is unsupported as revealed by the molecular analyses herein (figs 2.1D, 2.5–2.13). Each subfamily and tribe is discussed below with comparisons given between the results of Brothers & Lelej (2017) and the current study.

Pseudophotopsidinae Bischoff, 1920

Clade 1

This subfamily, represented solely by the genus *Pseudophotopsis* André, is here considered the basal subfamily in Mutillidae (figs 2.1C, 2.2, 2.3).

Ticoplinae Nagy, 1970

Clade 2

Both of the currently recognized tribes of Ticoplinae, Smicromyrmillini (clade 2A) and Ticoplini (clade 2B), were found to be monophyletic and sister taxa (figs 2.1D, 2.3). These results are congruent with those of Brothers & Lelej (2017). A cladistic analysis and genus-level revision of Ticoplinae based on morphology was published by Mitchell & Brothers (2002).

Rhopalomutillinae Schuster, 1949

Clade 3

The ML and MP topologies of the four rhopalomutilline genera (*Bischoffiella* Brothers & Nonveiller, *Pherotilla* Brothers, *Rhopalomutilla* André, and *Rimulotilla* Brothers) are identical to that of Brothers & Lelej (2017) (fig. 2.3).

Dasylabrinae Invrea, 1964 (1935)

Clade 4

Dasylabrinae is currently composed of two tribes: Apteromutillini Brothers & Lelej and Dasylabrini Invrea. Apteromutillini consists of three genera: *Apteromutilla* Ashmead, *Brachymutilla* André, and *Liotilla* Bischoff. Apteromutillines are notable in that males are entirely apterous in all three component genera (Brothers & Lelej, 2017). In both ML and MP analyses, the apteromutilline *Brachymutilla scabrosa* Bischoff was found to be nested within Dasylabrini (fig. 2.5), rendering the latter tribe paraphyletic. In several analyses, and notably in their preferred tree, Brothers & Lelej (2017) recovered a paraphyletic Apteromutillini as sister-goup to Sphaeropthalminae + (Dasylabrinae +

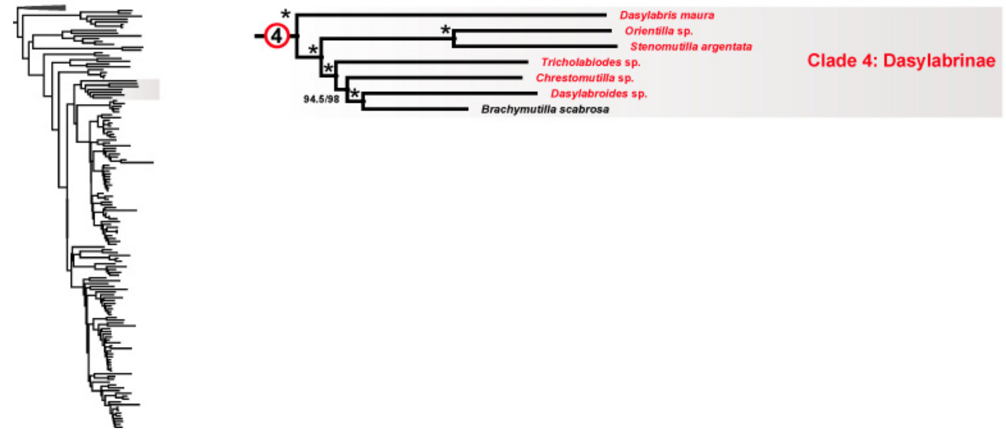

Figure 2.5. Paraphyly of Dasylabrinae: Dasylabrini (*sensu* Brothers & Lelej (2017)) (clade 4) due to inclusion of Dasylabrinae: Apteromutillini (represented by *Brachymutilla scabrosa* Bischoff).

(Myrmillinae + Mutillinae)). None of Brothers & Lelej (2017)'s analyses included Apteromutillini as a member of Dasylabrinae and the tribe was repositioned post-analysis to be a member of Dasylabrinae. The results herein demonstrate that *Brachymutilla* is a dasylabrine and *Apteromutilla* likely is as well; the position of *Liotilla* is unclear. Suitable material for molecular work was only available for *Brachymutilla* and future analyses including *Apteromutilla* and/or *Liotilla* may support the reinstatement of Apteromutillini (minus *Brachymutilla*). Apteromutillini is here synonymized under Dasylabrini, **syn. nov.**, leaving a single subfamily, Dasylabrinae, without tribal division.

Odontomutillinae Lelej, 1983, **stat. nov.**

Clade 5

The mutilline subtribe Ephutina *sensu* Brothers & Lelej (2017), with its two apparent genus-groups, the *Ephuta* genus-group and *Odontomutilla* genus-group, was recovered as polyphyletic (fig. 2.8). Neither genus-group was recovered as a member of Mutillinae or even as sister-goup taxa to each other. The *Ephuta* genus-group was nested within basal Sphaeropthalminae, and the *Odontomutilla* genus-group was recovered as sister-goup to Myrmillinae + Mutillinae. The *Odontomutilla* genus-group is herein raised to the subfamily level, Odontomutillinae, **stat. nov.** For further discussion, see the Mutillinae: Mutillini section.

Myrmillinae Bischoff, 1920

Clade 6 (= Myrmillinae *sensu stricto*), Clade 7A (= Mutillinae: Pristomutillini, **trib. nov.**)

Myrmillinae was found to be paraphyletic due to *Ceratotilla* Bischoff, *Viereckia* Ashmead, and the mutilline genus *Pristomutilla* Ashmead being sister-goup to the remaining Mutillinae (clade 7A; fig. 2.6); the relationship between these three genera was well-supported in the ML and MP analyses (100 SH-aLRT/100 UFBoot; branch support=4429). In most of the Brothers & Lelej (2017) analyses, *Ceratotilla* and *Viereckia* were recovered as sister-goup to the remaining Myrmillinae, and *Pristomutilla* was recovered as sister-goup to the remaining Mutillinae. In the Brothers & Lelej (2017) female-only analysis with additive characters and implied weighting, the single most-parsimonious tree resulted in *Ceratotilla*, *Pristomutilla*, and *Viereckia* being sister-goup to Mutillinae. These three genera are transferred to Mutillinae and are considered to form its basal lineage. A new tribe, Pristomutillini, **trib. nov.**, is erected for these genera in the Mutillinae: Ctenotillini section below.

Mutillinae Latreille, 1802

Clade 7

The topologies of the ML and MP analyses were similar (fig. 2.2), except that a polytomy was formed between three lineages in the MP strict-consensus cladogram (*i.e.*, clade 7A + (Mutillini + Trogaspidiini) + (clade 7D + (clade7E + (Ctenotillini + Smicromyrmini))). Mutillinae was rendered polyphyletic due to the subtribe Mutillini: Ephutina being recovered in two places in the topology well outside of Mutillinae. This is further discussed in the Mutillinae: Mutillini section below.

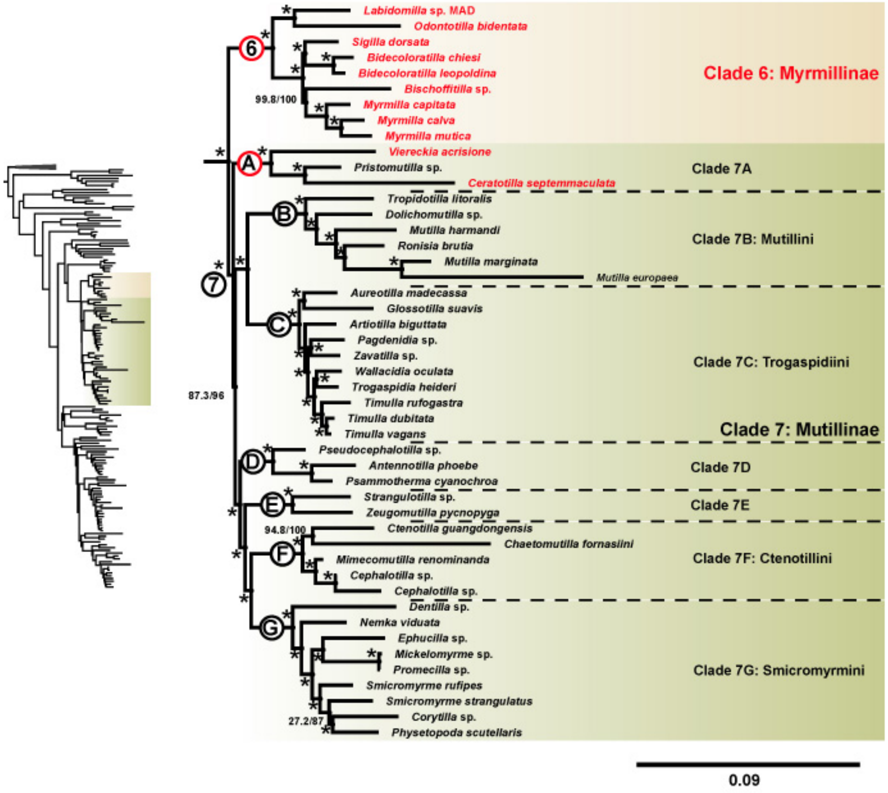

Figure 2.6. Polyphyly of Myrmillinae (*sensu* Brothers & Lelej (2017)) (clade 6) due to *Ceratotilla* and *Viereckia* being recovered in clade 7A.

Mutillinae: Ctenotillini Brothers & Lelej, 2017

Clade 7F (= Ctenotillini *sensu stricto*), Clade 7A (= Pristomutillini, **trib. nov.**), Clade 7E (= Zeugomutillini, **trib. nov.**)

Ctenotillini was found to be non-monophyletic in both ML and MP analyses, with members recovered in three separate lineages of Mutillinae (clades 7A, 7E, and 7F) (fig. 2.7). As mentioned in the Myrmillinae discussion, the ctenotilline genus *Pristomutilla* Ashmead, along with the myrmilline genera *Ceratotilla* Bischoff and *Viereckia* Ashmead, were sister-goup to the remaining Mutillinae (clade 7A; fig. 2.7). Second, the ctenotilline genera *Strangulotilla* Nonveiller and *Zeugomutilla* Chen (clade 7E) were found to be sister-goup to the remaining ctenotillines + Smicromyrmini (fig. 2.7). Clade 7F forms Ctenotillini *sensu stricto*, which includes the genera *Cephalotilla*, *Chaetomutilla*, *Lehritilla*, and *Mimecomutilla* (fig. 2.7).

Ctenotillini *sensu* Brothers & Lelej (2017) is supported only by a single unambiguously-placed homoplasious synapomorphy: the first flagellomere is less than 0.6 times the length of the second flagellomere in males. This character is shared with many Smicromyrmini and non-mutilline taxa. Further, there are four ambiguously-placed homoplasious synapomorphies supporting Ctenotillini, two of which Brothers & Lelej (2017) consider significant: 1) the prementum has a posterior dome-like tubercle in the females, and 2) the posterodorsal margin of the propodeum has more than three spines in the females. The group was relatively poorly supported in the Brothers & Lelej (2017) analyses. Both of these characters are shared with other non-ctenotilline taxa and some ctenotilline genera even lack these characters (*e.g.*, some *Pristomutilla* females lack a prementum tubercle and *Mimecomutilla* females lack propodeal spines).

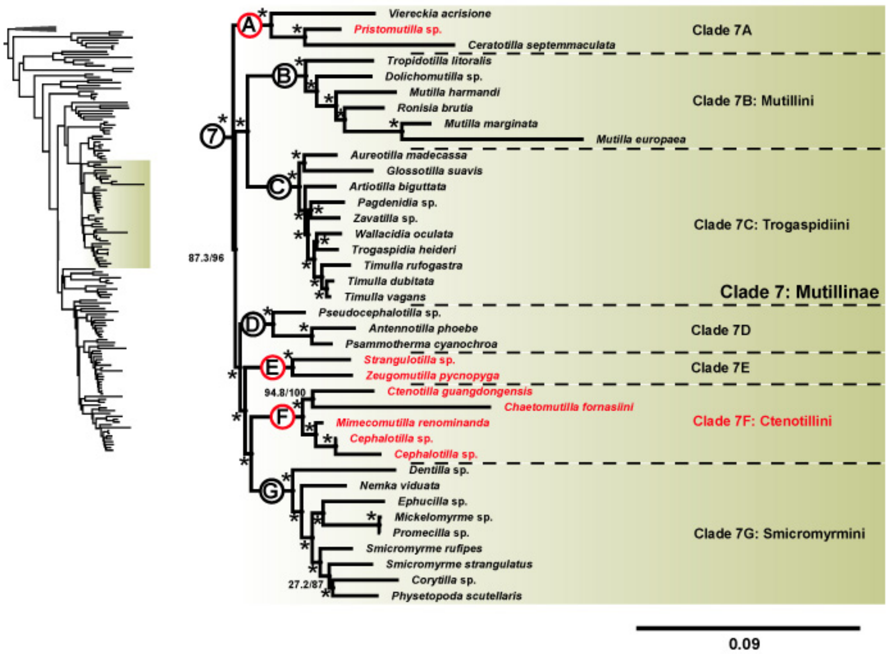

Figure 2.7. Polyphyly of Mutillinae: Ctenotillini (*sensu* Brothers & Lelej (2017)) (clade 7F) due to *Pristomutilla* sp. being recovered in clade 7A and *Strangulotilla* sp. and *Zeugomutilla pycnopyga* Chen being recovered as clade 7E.

Two tribes are here erected for the genera recovered in clades 7A and clade 7E. The tribe Pristomutillini Waldren, **trib. nov.** (type genus: *Pristomutilla* Ashmead, 1903), includes the genera *Ceratotilla* Bischoff, 1920 (♂♀), *Pristomutilla* Ashmead, 1903 (♂♀), and *Viereckia* Ashmead, 1903 (♂♀). This new tribe is diagnosed by the following combination of characters in females: 1) the presence of a short, longitudinal carina on the postgenal bridge that is perpendicularly conjoined to the hypostomal carina at its posteromedial margin (although in *Viereckia* a longitudinal, transversely-striate sulcus is present on the post-genal bridge instead of a carina), 2) the posterodorsal margin of the propodeum is lined with spine-like processes or denticles (although in *Viereckia* they are reduced to distinct tubercles), 3) the second tergum has two whitish setal spots or yellowish integumental spots, 4) the pygidium is laterally bound by a carina and the surface of the plate is longitudinally striate, 5) the mandible is apically bidentate with a small inner tooth, and 6) the absence of a scutellar scale. In contrast to the other mutilline tribes, males possess 1) a convex, short, and weakly-ovate tegula and 2) an apically-straight paramere in lateral view. Female-based characters that support these genera belonging to Mutillinae, rather than Myrmillinae, include: 1) the basal mesopleural carina anterodorsad to the mesocoxa is reduced, 2) the lateral face of the pronotum has its posterior margin distinct throughout, 3) the meso-metapleural suture terminates at the pronotal spiracle, 4) the pygidium is laterally bound by a carina and the surface of the plate is sculptured, and 5) the mandible is apically bidentate with a small inner tooth. Male-based characters that support these genera belonging to Mutillinae include: 1) the compound eye is distinctly emarginate internally, and 2) the stigma is unsclerotized, bound by veins, and is cell-like (at least for *Pristomutilla*; Brothers & Lelej (2017) coded

Viereckia males as having a sclerotized stigma). This tribe is Afrotropical and Oriental in distribution.

The tribe Zeugomutillini Waldren, **trib. nov.** (type genus: *Zeugomutilla* Chen, 1957), includes the genera *Montanomutilla* Nonveiller, 1979 (♀), *Strangulotilla* Nonveiller, 1979 (♂♀), and *Zeugomutilla* Chen, 1957 (♂♀). This new tribe is diagnosed by the following combination of characters in females: 1) the propodeum is lined with spine-like processes or denticles, 2) the pronotal humeral angle is well-developed and acute, 3) the pygidium is laterally bound by a carina and the surface of the plate is glabrous or sculptured, 4) the mandible is apically bidentate or tridentate with a small inner tooth or teeth, and 5) the absence of a scutellar scale. Males are diagnosed by their 1) symmetrical penial valves which are close in proximity and apically bispinose, 2) the cuspis is broad in lateral view and its margin lined with setae, and 3) the parameres are apically upcurved in lateral view. This tribe is Afrotropical and Oriental in distribution.

Mutillinae: Mutillini Latreille, 1802

Clade 7B (= Mutillini *sensu stricto*), Clade 5 (= Odontomutillinae, **stat. nov.**), Clade 8C (= Sphaeropthalminae: Ephutini, **stat. nov.**)

Mutillini is currently divided into two subtribes: Ephutina and Mutillina. Further, Ephutina is composed of two apparent lineages: the *Ephuta* genus-group and the *Odontomutilla* genus-group. Mutillini was rendered polyphyletic by the *Ephuta* genus-group being recovered as a member of basal Sphaeropthalminae (fig. 2.8). Additionally, the *Odontomutilla* genus-group was recovered as sister-goup to Myrmillinae + Mutillinae

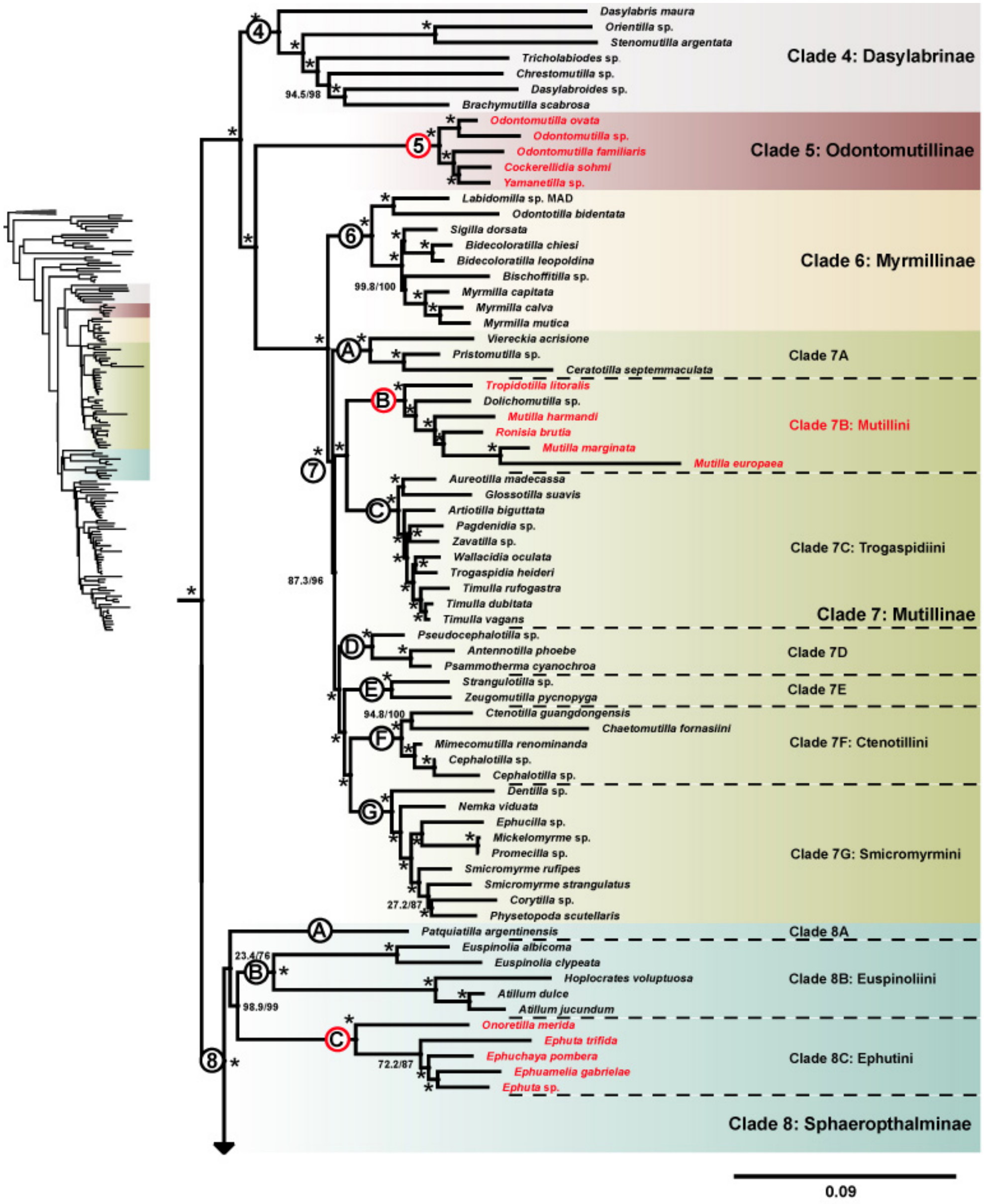

Figure 2.8. Polyphyly of Mutillinae: Mutillini (*sensu* Brothers & Lelej (2017)) (clade 7B) due to the *Odontomutilla* genus-group being recovered as clade 5 and the *Ephuta* genus-group being recovered as clade 8C.

(fig. 2.8). These results support that neither genus-group is a genuine member of Mutillinae. Ephutina *sensu* Brothers & Lelej (2017) was supported by a single unique and unambiguously-placed synapomorphy: the hypostomal carina is strong anterolaterally but is obsolete posteriorly in males. Another unique but ambiguously-placed synapomorphy is the second tergum has the felt line as a broad patch in the females. Lastly, fifteen unambiguously placed homoplasious synapomorphies are listed that are shared with a number of non-Ephutina taxa. In light of the homoplastic nature of the synapomorphies that define Ephutina *sensu* Brothers & Lelej (2017) and the results of the ML and MP analyses herein, the *Ephuta*-genus group is now considered a tribe of Sphaeropthalminae, Ephutini, **stat. nov.** and the *Odontomutilla* genus-group is now considered a subfamily, Odontomutillinae, **stat. nov.** (from synonymy with Ephutina).

The sister-goup tribe of Mutillini, Trogaspidiini (clade 7C), was rendered polyphyletic by the genus *Dolichomutilla* Ashmead being recovered within Mutillini (fig. 2.10). In Brothers & Lelej (2017)'s preferred tree, this genus is sister-goup to the remaining Mutillini *sensu* Brothers & Lelej (2017), and their male-only analysis recovered *Dolichomutilla* as a member of Mutillina. Additionally, they noted that Mutillina is not supported by any unique synapomorphies but rather three homoplasious synapomorphies: 1) the head is not broadened much but is long and rounded posteriorly in the females, 2) the mesoscutum is posterolaterally evenly rounded in winged males, and 3) the fore wing crossvein 3r-m has a bulla. All of these homoplasious synapomorphies are shared with *Dolichomutilla*. Considering the results of Brothers & Lelej (2017), as well as the results of the molecular analyses presented here, *Dolichomutilla* is transferred to Mutillini.

Mutillinae: Smicromyrmini Bischoff, 1920

Clade 7G (= Smicromyrmini *sensu stricto*), Clade 7D (= Psammothermini, **trib. nov.**)

Smicromyrmini was rendered non-monophyletic by *Antennotilla* Bischoff, *Psammotherma* Fabricius, and *Pseudocephalotilla* Bischoff (clade 7D) being sister-goup to clade 7E + Ctenotillini (clade 7F) + Smicromyrmini *sensu stricto* (clade 7G) (fig. 2.9). Brothers & Lelej (2017) noted the tribe was defined by a single unique synapomorphy: the volsella has a basal ventral lamellate expansion. Further, there are two homoplasious synapomorphies that define the group: 1) the pleurostomal carina is distinct, and together with hypostomal carina, forms a straight ridge that ends at the outer mandibular articulation, and 2) the second tergum has unpaired (odd-numbered) discal markings in the females.

A new tribe, Psammothermini Waldren, **trib. nov.** (type genus: *Psammotherma* Latreille, 1825), is erected for the genera of clade 7D: *Antennotilla* Bischoff, 1920 (♂), *Psammotherma* Latreille, 1825 (♂), and *Pseudocephalotilla* Bischoff, 1920 (♂). This new tribe is diagnosed by the following combination of characters in males: 1) the penial valves are dramatically enlarged apically, downcurved, and symmetrical in length, 2) the cuspis in lateral view is broad, concave, and internally covered with setae of varying density, 3) the basoventral margin of the volsella lacks a lobate expansion and associated long setae, and 4) the parameres are evenly arcuate and downcurved in lateral view. Females are undescribed for all three component genera; however, they are known for *Pseudocephalotilla* (Nonveiller, 1979; Lelej & Brothers, 2008; Brothers & Lelej, 2017). The tribe is Afrotropical in distribution. Two of the genera, *Antennotilla* and

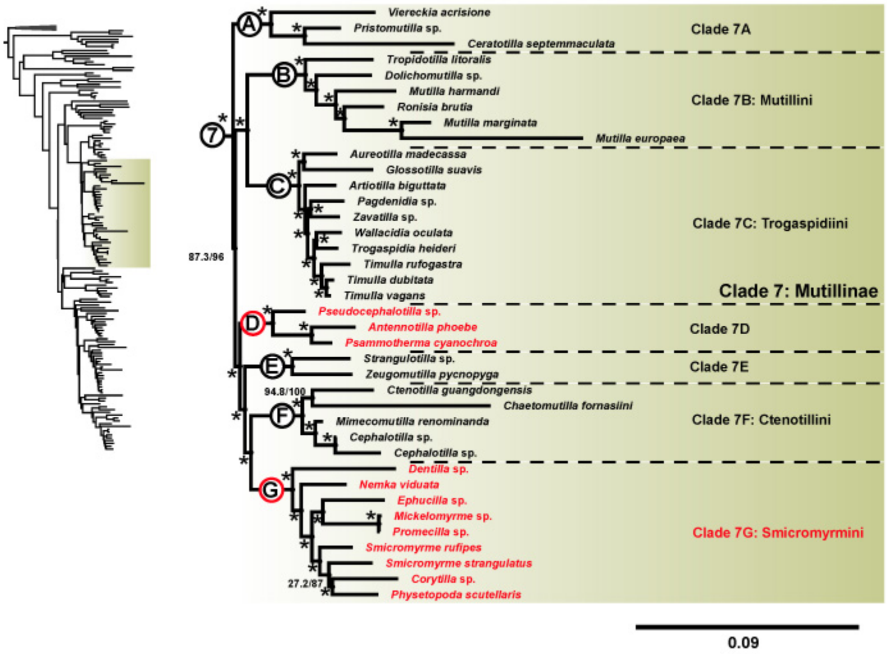

Figure 2.9. Polyphyly of Mutillinae: Smicromyrmini (*sensu* Brothers & Lelej (2017)) (clade 7G) due to *Antennotilla phoebe* (Péringuey), *Psammotherma cyanochroa* (André), and *Pseudocephalotilla* sp. being recovered as clade 7D.

Psammotherma, have males with pectinate antennae. The only other mutillid genus with pectinate antennae, the monotypic genus *Ctenoceraea* Nonveiller, 1993 (♂), might also be a member of Psammothermini, **trib. nov.**, based off of the original description and associated illustrations (Nonveiller, 1993). The form of the antennae and shape of the cuspis in lateral view support membership of *Ctenoceraea* to this tribe; however, the penial valves are not apically enlarged and downcurved as in *Antennotilla*, *Psammotherma*, and *Pseudocephalotilla*. Due to lacking this latter character, *Ctenoceraea* is maintained in Smicromyrmini pending further study.

Mutillinae: Trogaspidiini Bischoff, 1920

Clade 7C (= Trogaspidiini *sensu stricto*), Clade 7B (= Mutillini)

Trogaspidiini (clade 7C) was rendered polyphyletic by *Dolichomutilla* Ashmead being recovered in Mutillini (clade 7B) (fig. 2.10). This genus lacks many of the diagnostic characters for Trogaspidiini and the general habitus alone is suggestive of Mutillini. This genus is herein considered a member of Mutillini and is discussed more in detail in the Mutillinae: Mutillini section.

The tribe Petersenidiini, which was found to be non-monophyletic by Brothers & Lelej (2017) and was synonymized with Trogaspidiini, will be investigated in another UCE-based study dedicated to Trogaspidiini. The comparatively smaller number of trogaspidiines included in this study does not allow for an informative assessment here.

Brothers & Lelej (2017) noted that Trogaspidiini possess a single unique synapomorphy: the first flagellomere is weakly flattened ventrally in the males (but is strongly flattened in a few). Additionally, there are five homoplasious synapomorphies,

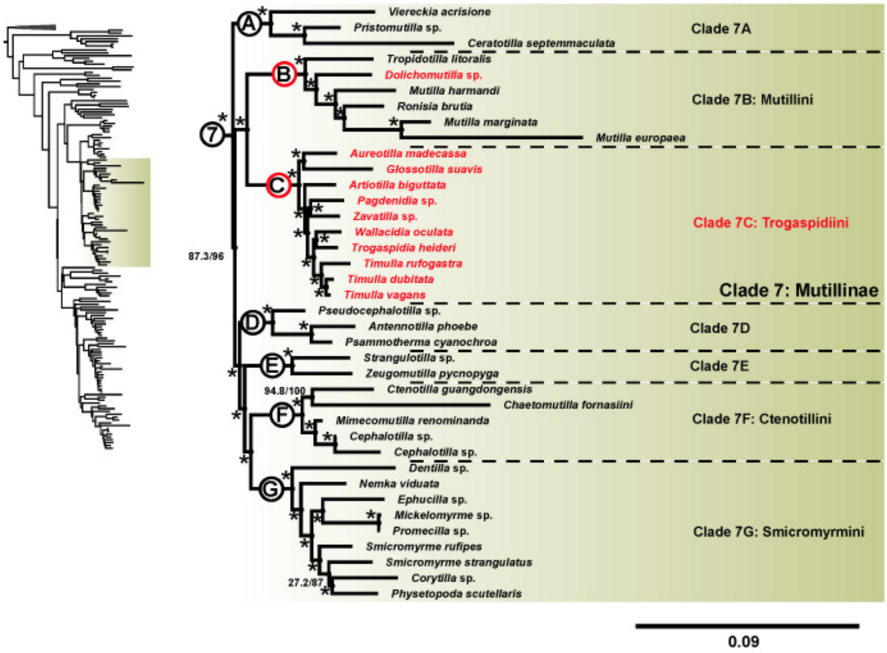

Figure 2.10. Polyphyly of Mutillinae: Trogaspidiini (*sensu* Brothers & Lelej (2017)) (clade 7C) due to *Dolichomutilla* sp. being recovered in Mutillinae: Mutillini (clade 7B).

including: 1) the propodeum has the dorsolateral margin carinate in the winged males, and 2) the first flagellomeres is much longer than wide in the males.

Sphaeropthalminae Schuster, 1949 (1903)

Clade 8

Sphaeropthalminae was recovered as monophyletic in both ML and MP analyses (fig. 2.2), whereas Brothers & Lelej (2017) did not recover Sphaeropthalminae as monophyletic due to Euspinoliini being variously placed throughout their trees depending on the analysis used. The only change to membership in Sphaeropthalminae is the unexpected addition of Ephutini, **stat. nov.**, from Mutillinae: Mutillini: Ephutina (fig. 2.8). The three sphaeropthalmine tribes recognized by Brothers & Lelej (2017), Dasymutillini, Pseudomethocini, and Sphaeropthalmini, were all found to be non-monophyletic and a relatively major overhaul of the tribal composition of the subfamily is warranted given the results herein. Brothers & Lelej (2017) noted that Sphaeropthalminae possess a single unique synapomorphy: the first tergum and/or propodeum has plumose pubescence in the females and the males. Further, the subfamily is supported by three homoplasious synapomorphies: 1) the mesopleural ridge is strong and is joined to the mesonotal tubercle, 2) the head has plumose pubescence in the males, and 3) the male gonostylus is apically upcurved in lateral view.

Several differences in tribal relationships among the basal sphaeropthalmine lineages were found between the ML and MP topologies (fig. 2.2). In the ML analysis, clade 8A (*Patquiatilla*) + (Ephutini + Euspinoliini) were sister-goup to the remaining Sphaeropthalminae (fig. 2.2); in the MP analysis only clade 8A (*Patquiatilla*) and

Ephutini were sister-goup to the remaining Sphaeropthalminae (the latter including Euspinoliini as its basal lineage) (fig. 2.2). The SH-aLRT/UFBoot values for the ML analysis were relatively low at 23.4 and 76, respectively. However, for the MP analysis, branch support for the clade 8A + Ephutini relationship was relatively high at 5,914. Another topological difference is that clade 8E (*Tallium* sp.) was sister-goup to clade 8F (*Limaytilla pehuench*e + *Protophotopsis venenaria*) and the remaining Sphaeropthalminae in the ML analysis (fig. 2.2) while in the MP analysis clade 8E (*Tallium* sp.) and clade 8F (*Limaytilla pehuench*e + *Protophotopsis venenaria*) were sister-goup to each other (fig. 2.2). The former relationship had stability values of 92.6 SH-aLRT and 97 UFBoot, while the latter had a branch support value of 3,742.

There is a notable biogeographic trend in that most of the early-branching clades of Sphaeropthalminae are restricted to South America: clade 8A (*Patquiatilla* sp., South America, hereafter "SA"), clade 8B (Euspinoliini, SA), clade 8C (Ephutini, North America+SA), clade 8D (*Allotilla gibbosa* + *Sphaeropthalma tenuiventris*, SA), clade 8E (*Tallium* sp., SA), and clade 8F (*Limaytilla pehuenche* + *Protophotopsis venenaria*, NA+SA). Further, most of the early-branching clades of Dasymutillini and Pseudomethocini are exclusively or partly South American, respectively.

Sphaeropthalminae: Dasymutillini Brothers & Lelej, 2017

Clade 8H (= Dasymutillini *sensu stricto*), Clades 8F, 8I, 8J

Dasymutillini was rendered polyphyletic due to *Protophotopsis* being recovered as an unrelated lineage of Sphaeropthalminae (clade 8F) (fig. 2.11). Further, *Lomachaeta* Mickel and two "*Ephutomorpha*" André species were sister-goup to *Neomutilla* (8J) +

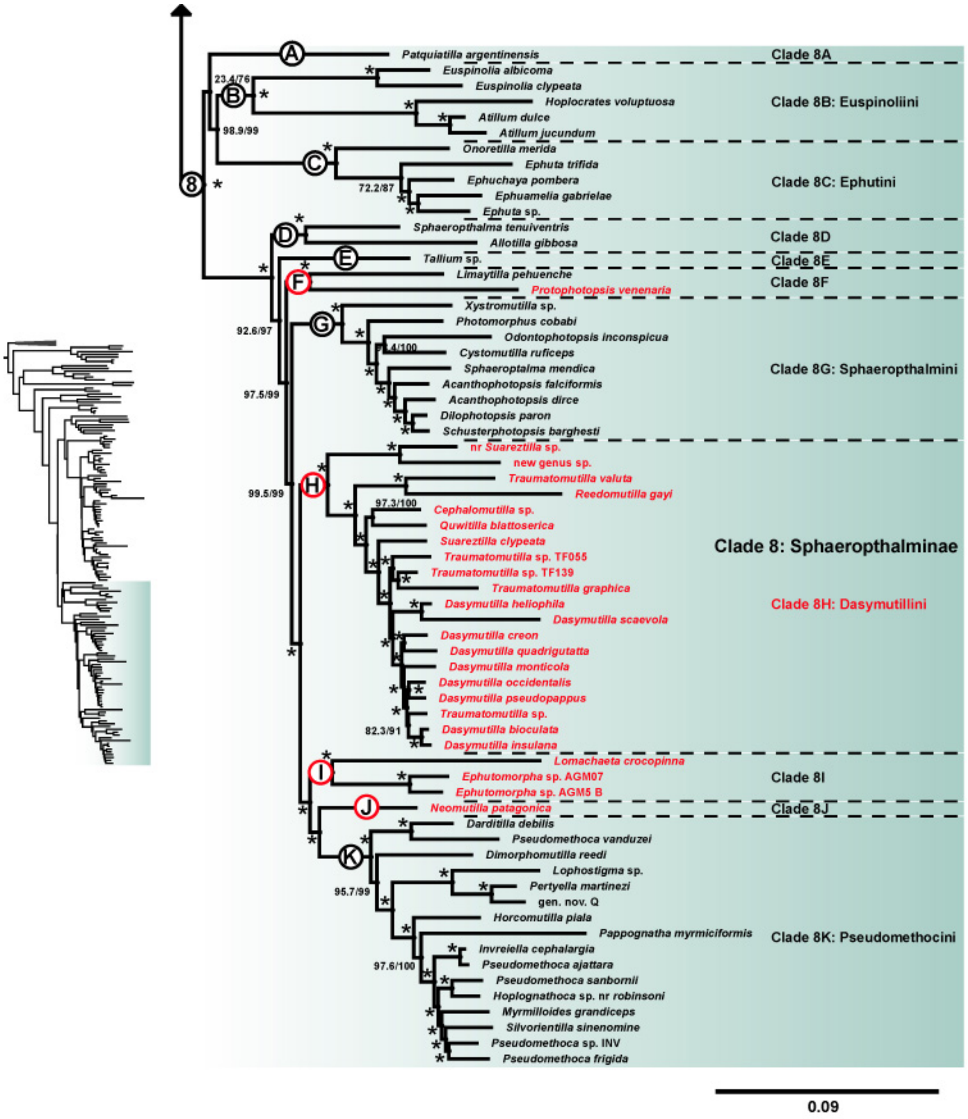

Figure 2.11. Polyphyly of Sphaeropthalminae: Dasymutillini (*sensu* Brothers & Lelej (2017)) (clade 8H) due to *Protophotopsis venenaria* (Melander) being recovered in clade 8F, *Lomachaeta crocopinna* Pitts & Manley + two "*Ephutomorpha*" spp. being recovered as clade 8I, and *Neomutilla patagonica* (Fritz & Martinez) being recovered as clade 8J.

Pseudomethocini (8K) (fig. 2.11). The Australasian sphaeropthalmine fauna, with most species placed in the catch-all genus *Ephutomorpha*, was undersampled in this study; it appears that much of that fauna is closely related to the New World genus *Lomachaeta*. Brothers & Lelej (2017) recovered Dasymutillini as paraphyletic in most analyses, except for the male-only analysis which was monophyletic. Further, the tribe was not supported by any unique synapomorphies, but rather a single homoplasious synapomorphy: the eye is strongly convex in the females. The results herein support that Dasymutillini *sensu stricto* is restricted to clade 8H.

Sphaeropthalminae: Ephutini Ashmead, 1903, **stat. nov.**

Clade 8C

This tribe is formally transferred to Sphaeropthalminae from Mutillinae: Mutillini: Mutillina. The position of this tribe in the results was unexpected given the morphology of the males, which possess emarginated compound eyes and elongate tegulae; these characters are diagnostic for male mutillines and are in contrast with the hemispherical eyes and rounded tegulae of male sphaeropthalminaes. The strongly-petiolate first metasomal segment for both sexes, however, was unique among Mutillinae and is extremely prevalent in Sphaeropthalminae. A detailed morphological study is warranted in the context of the relationship of ephutines with other basal Sphaeropthalminae; seeing the taxon in a new light as a sphaeropthalmine will likely reveal noteworthy characters. Refer to the discussion for Mutillinae: Mutillini—the tribe Ephutini was transferred from—for further comments.

Sphaeropthalminae: Euspinoliini Brothers & Lelej, 2017, **stat. nov.**

Clade 8B

The pseudomethocine subtribe Euspinoliina was recovered as a basal lineage of Sphaeropthalminae in both ML and MP analyses. Euspinoliina is composed of the genera *Atillum* André, *Euspinolia* Ashmead, and *Hoplocrates* Mickel and these genera formed a clade in agreement with Brothers & Lelej (2017)'s results. Brothers & Lelej (2017) noted the group is defined by a single unique synapomorphy: the fore tibia has an obliquely-elongate outer secretory pore in the males. Additionally, there are 13 homoplasious synapomorphies. The group is here raised to the tribe level, Euspinoliini, **stat. nov.** For further discussion on this tribe, refer to the Sphaeropthalminae: Pseudomethocini section.

Sphaeropthalminae: Pseudomethocini Brothers, 1975

Clade 8K (= Pseudomethocini *sensu stricto*), Clade 8A, Clade 8B (= Euspinoliini, **stat. nov.**)

Pseudomethocini Brothers was rendered polyphyletic due the subtribe Euspinoliina and *Patquiatilla* forming a clade with Ephutini that is sister-goup to the remaining Sphaeropthalminae (fig. 2.12). The genera comprising Euspinoliina were often recovered outside of Sphaeropthalminae in the results of Brothers & Lelej (2017) and were never recovered as being closely related to Pseudomethocina. Despite this, their preferred most-parsimonious cladogram was intentionally rearranged to retrofit the genera of Euspinoliina (*Atillum*, *Euspinolia*, and *Hoplocrates*) to reflect the older concepts of Pseudomethocini proposed by Brothers (1975) and Lelej & Nemkov (1997). Pseudomethocini *sensu* Brothers & Lelej (2017) was not supported by any unique

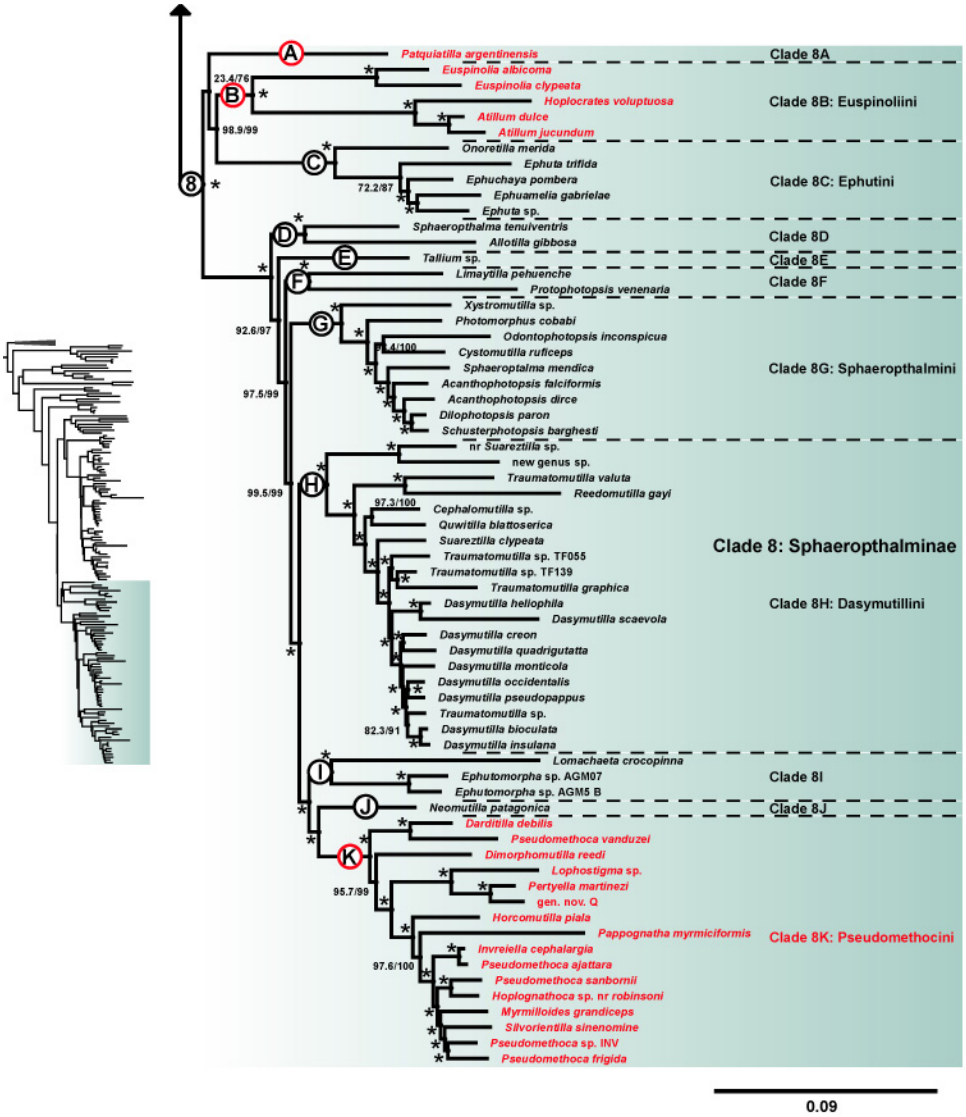

Figure 2.12. Polyphyly of Sphaeropthalminae: Pseudomethocini (*sensu* Brothers & Lelej (2017)) (clade 8K) due to *Patquiatilla argentinensis* André being recovered as clade 8A and Pseudomethocini: Euspinoliina being recovered as clade 8B.

synapomorphies, but rather by six homoplasious synapomorphies. The results of Brothers & Lelej (2017) and of the analyses herein reveal that the membership of Euspinoliina to Pseudomethocini is based on homoplasy and the former deserves tribal status: Euspinoliini, **stat. nov.** Pseudomethocini *sensu stricto* is restricted herein to clade 8K.

Sphaeropthalminae: Sphaeropthalmini Schuster, 1949 (1903)

Clade 8G (= Sphaeropthalmini *sensu stricto*), Clades 8D, 8E, 8F

Sphaeropthalmini was rendered non-monophyletic due to component taxa being recovered in four separate sphaeropthalmine lineages (fig. 2.13). *Allotilla gibbosa* Schuster and *Sphaeropthalma* ("*Photopsis*") *tenuiventris* (Spinola) were recovered as clade 8D, *Tallium* sp. was recovered as clade 8E, and *Limaytilla pehuenche* Casal was recovered in clade 8F, while the remaining Sphaeropthalmini (*sensu stricto*) were recovered as clade 8G. As previously discussed in the Sphaeropthalminae section, the topologies of the ML and MP analyses differed regarding clade 8E (*Tallium* sp.) and clade 8F (*Limaytilla pehuenche* + *Protophotopsis venenaria*) (fig. 2.2). Sphaeropthalmini *sensu* Brothers & Lelej (2017) was not known from any unique synapomorphies, but rather two homoplasious synapomorphies: 1) the hypostomal carina is simple in the males, and 2) the second sternum has a lateral felt line in males. In several of Brothers & Lelej (2017)'s analyses, *Allotilla* and *Tallium* were recovered in basal Sphaeropthalminae, which is supported by the results herein.

Sphaeropthalmini *sensu stricto* is herein restricted to clade 8G and is primarily composed of the species-rich Nearctic nocturnal fauna. The Neotropical genus

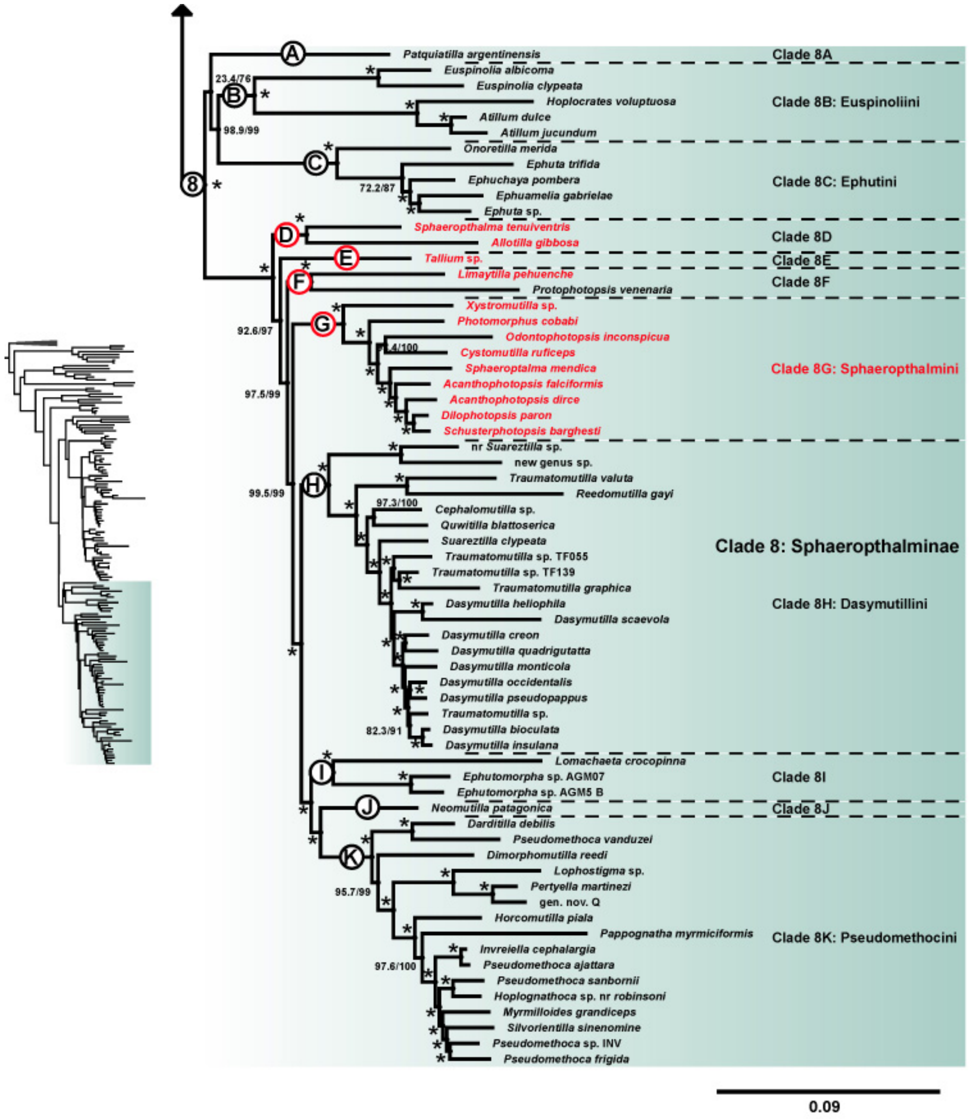

Figure 2.13. Polyphyly of Sphaeropthalminae: Sphaeropthalmini (*sensu* Brothers & Lelej (2017)) (clade 8G) due to *Allotilla* sp. and *Sphaeropthalma* (“*Photopsis*”) *tenuiventris* (Spinola) being recovered as clade 8D, *Tallium* sp. being recovered as clade 8E, and *Limaytilla pehuenche* Casal being recovered in clade 8F.

Xystromutilla André is sister-goup to this primarily Nearctic lineage. Additionally, *Cystomutilla* André was confirmed as a member of Sphaeropthalminae: Sphaeropthalmini, and along with *Hemutilla* Lelej, Tu & Chen, are the only representatives of this tribe in the Old World.

Divergence time estimates

The superfamily Pompiloidea, composed of the families Mutillidae, Myrmosidae, Pompilidae, and Sapygidae, was confirmed herein as monophyletic. Pompiloidea is estimated to have emerged in the late Jurassic or in the early Cretaceous at an inferred age of 154.11/144.27 Ma (figs 2.14 and 2.15). This estimation pushes the age of this superfamily back significantly. For perspective, the age Aculeata was inferred by Branstetter *et al.* (2017a) to be 161 Ma. Other researchers inferred the age of Pompiloidea to be 116 Ma (Wilson *et al.* 2012) and 114 Ma (Branstetter *et al.* 2017a) using a Bayesian approach. These age estimations, however, are apparently too young for the superfamily due to the fossil sapygid species *Cretofedtschenkia santanensis* Osten from the Crato Formation dated 122.46–112.6 Ma (median= 117.5 Ma). This species was placed by Osten (2007) in the enigmatic sapygid subfamily Fedtschenkiinae, and it was not used as a calibration reference in Wilson (2012) nor in Branstetter *et al.* (2017a). Placement of *Cretofedtschenkia santanensis* in Fedtschenkiinae is herein supported after comparison of the original description, photograph, and illustrations with specimens of the extant fedtschenkiine species *Fedtschenkia anthracina* (Ashmead). This fossil is the oldest specimen known among Pompiloidea.

The age of Pompilidae was inferred as 72.01/67.96 Ma. This origin date is shortly before the Cretaceous-Paleogene (K-Pg) boundary at 66 Ma. Other researchers inferred the age of Pompilidae to be 85 Ma (Wilson *et al.* 2012), 43.3 Ma (Waichert *et al.*, 2015), and 44 Ma (Branstetter *et al.* 2017a). The oldest known pompilid fossil is the recently-described species *Cryptocheilus leleji* Waichert, Rapoza & Rodriguez in Waichert *et al.* (2019). This fossil originated from the Fur Formation dated 55.8–48.6 Ma (median= 52.2 Ma) and the median age of *Cryptocheilus leleji* was used to calibrate Pepsinae.

The age of Sapygidae was inferred as 112.6/117.5 Ma in the early Cretaceous. UCE data for only two species of *Sapyga* (Sapyginae) were available and none for Fedtschenkiinae, the subfamily from which the fossil calibration point was derived. The least-squares method will not estimate a date for a fossil-calibrated clade beyond that of the number provided; this was the case here as Sapygidae was calibrated using the median age of *Cretofedtschenkia santanensis* Osten from the Crato Formation dated 122.46–112.6 Ma (median= 117.5 Ma). Other age estimations for Sapygidae include 47 Ma (Wilson *et al.*, 2012) and 23 Ma (Branstetter *et al.*, 2017a), both of which are too young considering the discovery of *Cretofedtschenkia santanensis*. Two other fossils are known for Sapygidae: a *Sapyga* sp. from Baltic amber (Brischke, 1886) and *Cretosapyga resinicola* Bennett & Engel, 2005. The latter species was described from a Burmese amber inclusion dated 99.7–94.3 Ma and was placed in its own subfamily, Cretosapyginae. It is dubiously assigned to Sapygidae.

The age of Myrmosidae was inferred as 66.18/65.15 Ma, with the group arising shortly before or after the K-Pg boundary. Myrmosidae are represented in the fossil record by the kudakrumiine genus *Protomutilla* Bischoff; a number of species are

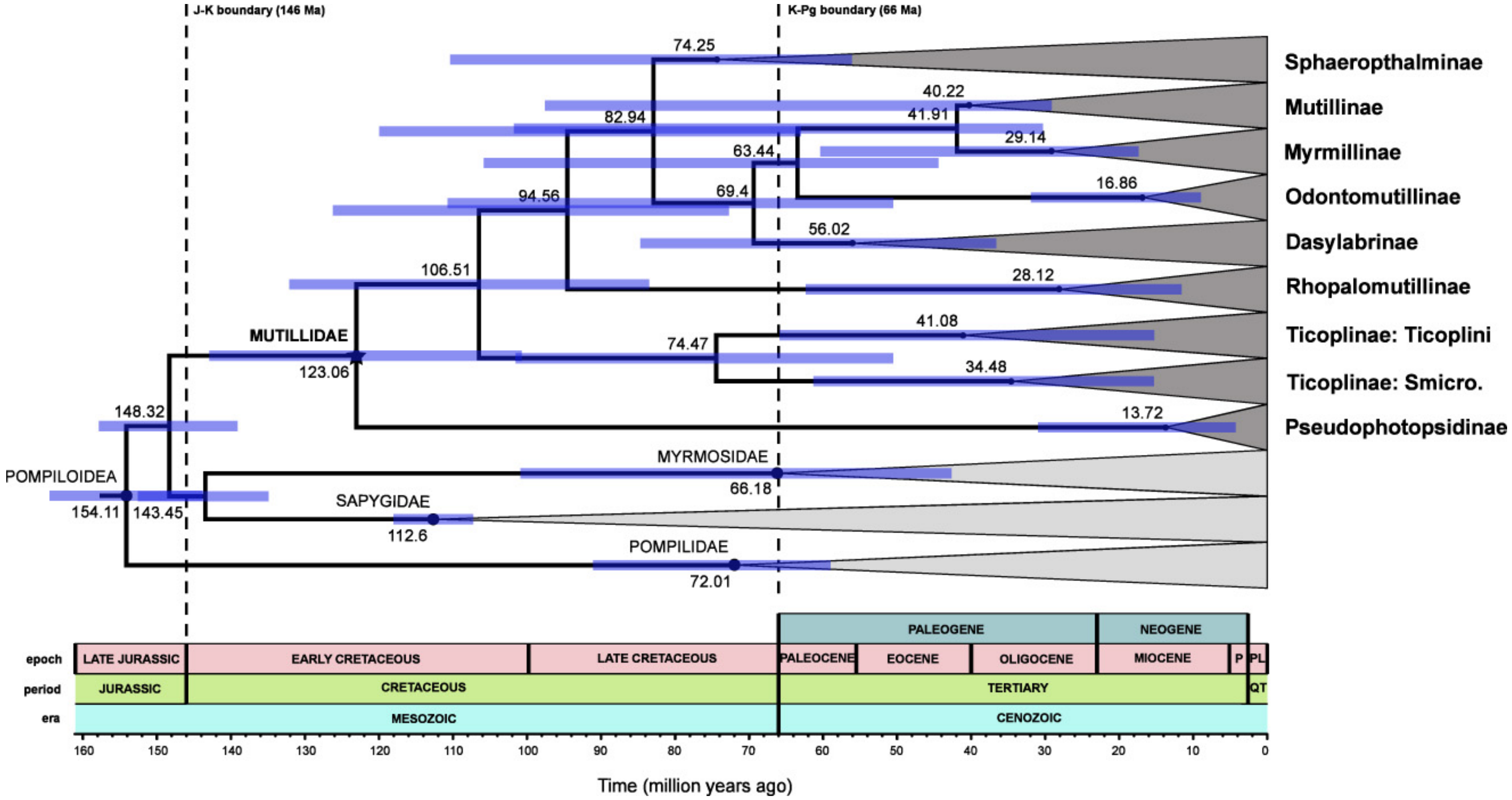

Figure 2.14. Chronogram of Pompiloidea families and Mutillidae subfamilies using BEAST v.1.10.4. The purple bars represent 95% HPD intervals.

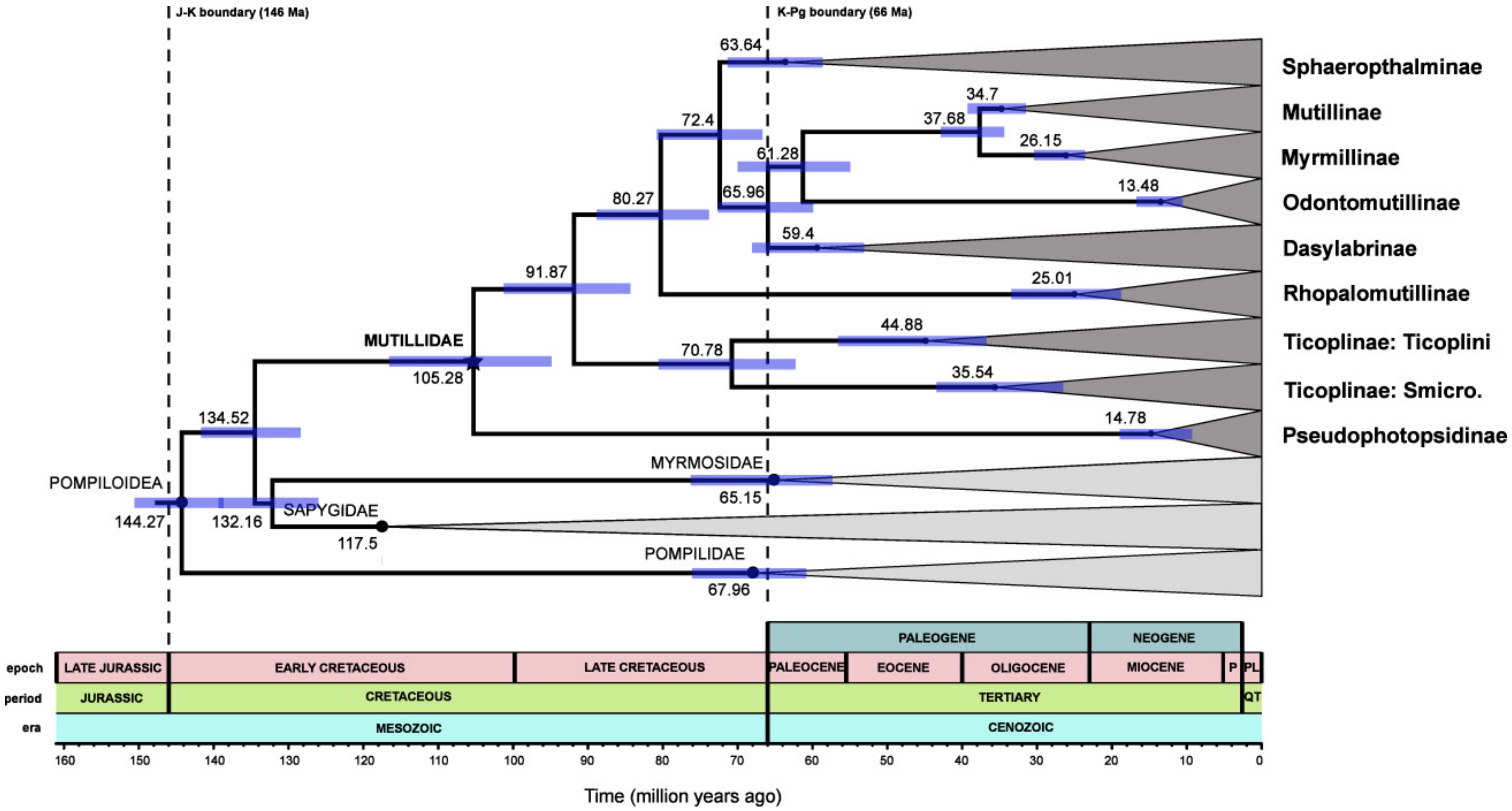

Figure 2.15. Chronogram of Pompiloidea families and Mutillidae subfamilies using IQ-TREE v.2.1.1. The purple bars represent confidence intervals. Sapygidae lacks a confidence interval due to how IQ-TREE treats calibrations.

known from Baltic amber inclusions dated 37.2–33.9 Ma (median= 35.6 Ma). The median age was used to calibrate Kudakrumiinae. Other age estimations include 47 Ma (Wilson *et al.* 2012) and 97 Ma (Branstetter *et al.*, 2017a, albeit for the clade Myrmosidae + Mutillidae).

The age of Mutillidae (*sensu stricto*) was inferred as 123.06/105.28 Ma in the early Cretaceous. Other age estimations include 85 Ma (Wilson *et al.*, 2012) and 82 Ma (Branstetter *et al.*, 2017a). According to the age estimation results, Ticoplinae is the oldest extant mutillid subfamily and is dated at 74.47/70.78 Ma in the late Cretaceous (figs 2.14 and 2.15). Two of the other early-branching subfamilies of Mutillidae, Pseudophotopsidinae and Rhopalomutillinae, were estimated to have arisen 13.72/14.78 Ma (Miocene) and 28.12/25.01 Ma (Oligocene), respectively. Several clades are broadly associated with the K-Pg boundary (66 Ma), with Dasylabrinae dated at 56.02/59.4 Ma and Sphaeropthalminae dated at 74.25/63.64 Ma. Further, Dasylabrinae + (Odontomutillinae + (Myrmillinae + Mutillinae)) was dated at 69.4/65.96 Ma (figs 2.14 and 2.15).

The fossil record for Mutillidae *sensu stricto* is relatively scant and is represented by two sphaeropthalmine tribes known from Dominican amber: a single female specimen of Ephutini (*Ephuta clavigera* Brothers, 2003) and three male specimens of Dasymutillini (*Dasymutilla dominica* Manley & Poinar, 1991, *D. albifasciatus* Manley & Poinar, 1999, and an undescribed *Dasymutilla* species (Manley & Poinar, 2003)). Dominican amber is dated from 13.7–20.4 Ma (median= 17.1 Ma). Additionally, the fossil-based taxon *Cretavus* Sharov is dubiously assigned to Mutillidae and was not taken into consideration in this study. These fossil species of *Dasymutilla*, including *D. dominica*, are apparently

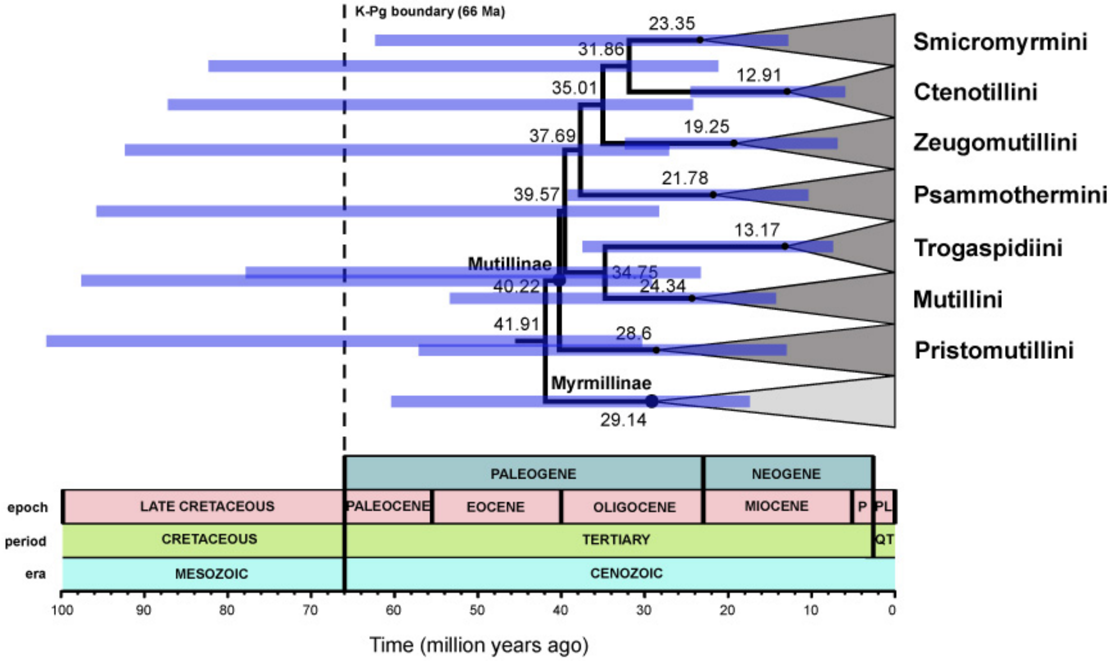

Figure 2.16. Chronogram of Mutillinae tribes using BEAST v.1.10.4. The purple bars represent 95% HPD intervals.

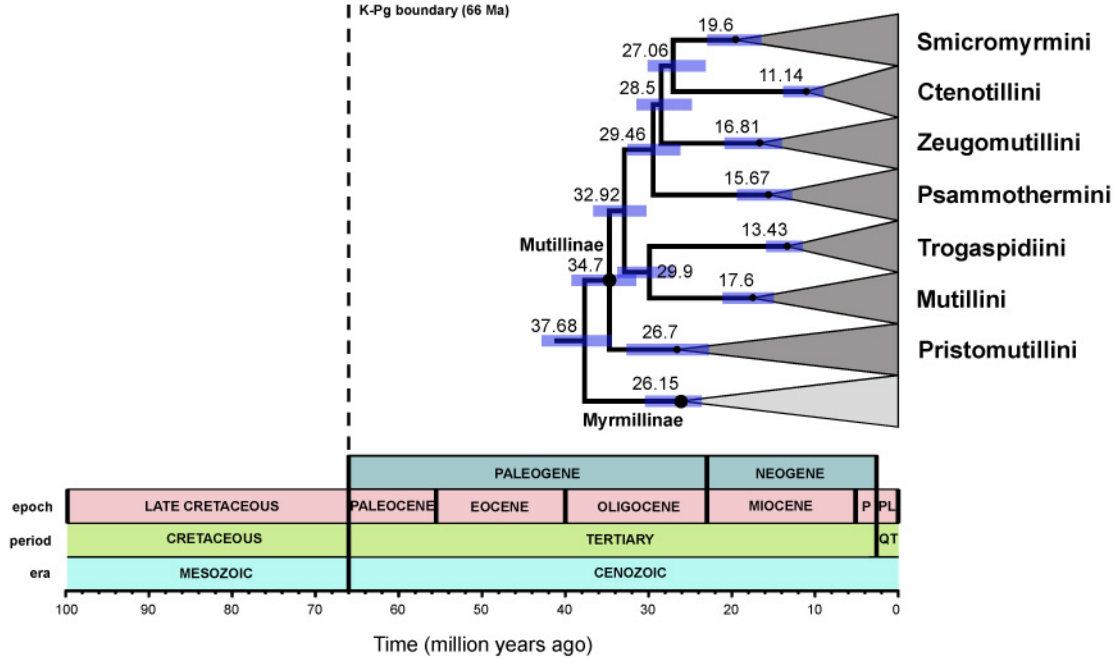

Figure 2.17. Chronogram of Mutillinae tribes using IQ-TREE v.2.1.1. The purple bars represent confidence intervals.

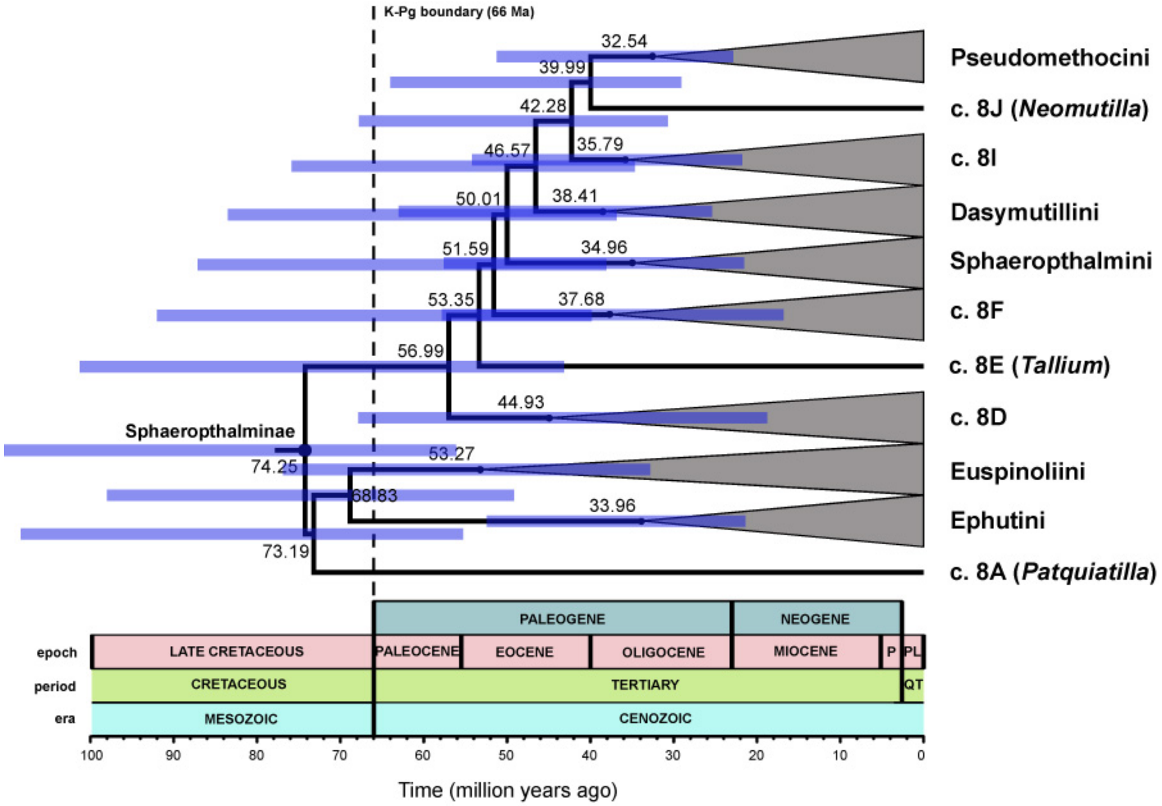

Figure 2.18. Chronogram of Sphaeropthalminae tribes using BEAST v.1.10.4. The purple bars represent 95% HPD intervals.

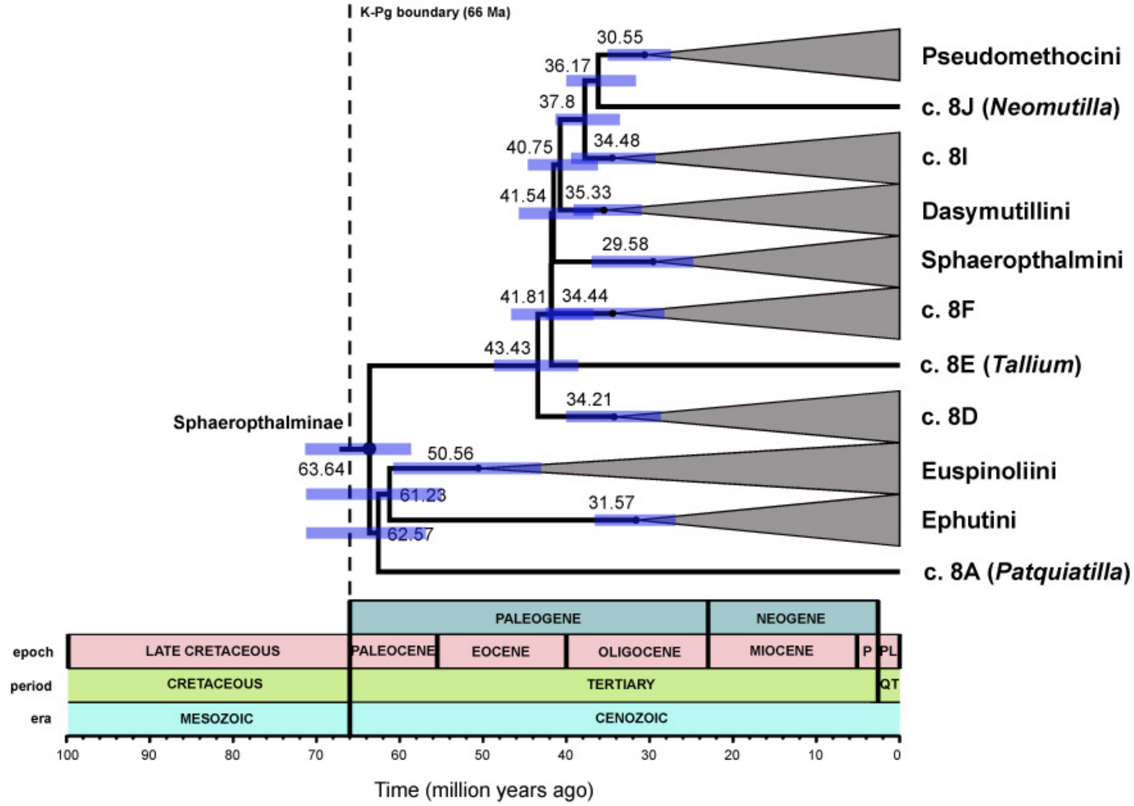

Figure 2.19. Chronogram of Sphaeropthalminae tribes using IQ-TREE v.2.1.1. The purple bars represent confidence intervals.

related to *Dasymutilla militaris*, which was inferred to be a species sister to the majority of *Dasymutilla* and *Traumatomutilla* species by Williams (2012). The median Dominican amber age of 17.1 Ma was used to calibrate the clade *Dasymutilla* + *Traumatomutilla* using the Dasymutillini topology of Williams (2012) as a guide. Regarding Ephutini, *Onoretilla* Pagliano was found to be sister-goup to the remaining members of the tribe in the ML and MP analyses. The suspected female of this genus is unusual compared to other ephutines, and the female-based fossil species *Ephuta clavigera* appears to be more closely related to the remaining taxa used in the analysis (*Ephuamelia* Casal, *Ephuchaya* Casal, and *Ephuta* Say). Consequently, the age of 17.1 Ma was assigned to the clade containing *Ephuamelia*, *Ephuchaya*, and *Ephuta*.

The tribes of the two most species-rich mutillid subfamilies, Mutillinae and Sphaeropthalminae, were estimated to have arisen during significantly different time periods (figs 2.14–2.19). The primarily Afrotropical, Oriental, and Palaearctic subfamily Mutillinae was dated at 40.22/34.7 Ma (Oligocene), with its component tribes arising in the late Oligocene to Miocene (figs 2.16 and 2.17). The primarily New World and Australasian subfamily Sphaeropthalminae was estimated to have emerged 74.25/63.64 Ma around the K-Pg boundary, with its component tribes primarily arising in the Eocene to Oligocene (figs 2.18 and 2.19).

Conclusion

The family Mutillidae has been redefined with Myrmosidae considered to be a separate family. Eight subfamilies are now recognized for Mutillidae, and the age of the family was inferred at 123.06/105.28 Ma in the early Cretaceous. As revealed by the analysis of

UCE data, most of the tribal concepts proposed by Brothers & Lelej (2017) are untenable and need reevaluation. Given the few unique synapomorphies for the subfamilies and tribes proposed by Brothers & Lelej (2017), a diagnostic approach using unique combinations of character states for each of the higher taxa is warranted. Homoplasy is widespread across the family and is particularly illustrated through the surprising addition of Ephutini to Sphaeropthalminae. The analyses herein have provided novel insights into the relationships between the higher taxa of Mutillidae that may not have been recognized through morphology alone. For example, considering Ephutini as a sphaeropthalmine rather than a mutilline may yield new, previously overlooked synapomorphies. It is hoped that these higher taxa can be approached with a new perspective and this study will galvanize additional research on their delimitation.

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References

André, E. (1899–1903) *Monographie des Mutillides d'Europe et d'Algérie. Species de Hyménoptères d'Europe & d'Algérie. Tome Huitième*. Librairie Scientifique A. Hermann, Paris.

André, E. (1902 ["1903"]) Fam. Mutillidae. *Genera Insectorum*, Fascicle 11 (ed. by P. Wytsman), pp. 1–77, plates 1–3. V. Verteneuil & L. Desmet, Bruxelles.

André, E. (1904) Examen critique d'uen nouvelle classification proposée par M. le Dr. W. H. Ashmead pour la famille des Mutillidae. *Revue d'Entomologie*, **23**(1), 27–41.

Argaman, Q. (1988) Description of the female of *Ticopla*, with biological and taxonomic notes (Hymenoptera, Mutillidae). *Fragmenta Balcanica*, **14**(5), 33–45.

Ashmead, W.H. (1899) Superfamilies in the Hymenoptera and generic synopses of the families Thynnidae, Myrmosidae, and Mutillidae. *Journal of the New York Entomological Society*, **7**, 45–60.

Ashmead, W.H. (1900–1904) Classification of the fossorial, predaceous and parasitic wasps, or the superfamily Vespoidea. *The Canadian Entomologist*, (1900) **32**, 145–155, 185–188, 295–296; (1902) **34**, 79–88, 131–137, 163–166, 203–210, 219–231, 268–273, 287–291; (1903) **35**, 3–8, 39–44, 95–107, 155–158, 199–205, 303–310, 323–332; (1904) **36**, 5–9.

Bennett, D.J. & Engel, M.S. (2005) A primitive sapygid wasp in Burmese amber (Hymenoptera: Sapygidae). *Acta zoologica cracoviensia*, **48B**(1–2), 1–9.

Bischoff, H. (1916 ["1915"]) Bernsteinhymenopteren. *Schriften der Physikalisch-ökonomischen Gesellschaft zu Königsberg in Preussen*, **56**, 139–144, figures 1–4.

Bischoff, H. (1920–1921) Monographie der Mutilliden Afrikas. *Archiv für Naturgeschichte*, (1920), **86A**(1/3), 1–480; (1921), **86A**(4), 481–830, plates 1–7.

Blake, C.A. (1871) Synopsis of the Mutillidae of North America. *Transactions of the American Entomological Society*, **3**, 217–265, 358.

Blake, C.A. (1886) Monograph of the Mutillidae of North America. *Transactions of the American Entomological Society*, **13**, 179–286.

Branstetter, M.G., Danforth, B.N., Pitts, J.P., Faircloth, B.C., Ward, P.S., Buffington, M.L., Gates, M.W., Kula, R.R. & Brady, S.G. (2017a) Phylogenomic insights into the evolution of stinging wasps and the origins of ants and bees. *Current Biology*, **27**(7), 1019–1025.

Branstetter, M.G., Longino, J.T., Ward, P.S. & Faircloth, B.C. (2017b) Enriching the ant tree of life: Enhanced UCE bait set for genome-scale phylogenomics of ants and other Hymenoptera. *Methods in Ecology and Evolution*, **8**(6), 768–776.

Bremer, K. (1988) The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution*, **42**(4), 795–803.

Bremer, K. (1994) Branch support and tree stability. *Cladistics*, **10**(3), 295–304.

Brischke, C.G.A. (1886) Die Hymenopteren des Bernsteins. *Schriften der Naturforschenden Gesellschaft in Danzig*, **6**(3–4), 278–279.

Brothers, D.J. (1975) Phylogeny and classification of the aculeate Hymenoptera, with special reference to Mutillidae. *The University of Kansas Science Bulletin*, **50**(11), 483–648.

Brothers, D.J. (1999) Phylogeny and evolution of wasps, ants and bees (Hymenoptera, Chrysidoidea, Vespoidea and Apoidea). *Zoologica Scripta*, **28**(1–2), 233–249.

Brothers, D.J. (2003) The first fossil Ephutini (Hymenoptera: Mutillidae), a new species of *Ephuta* Say from Dominican amber. *Acta zoologica cracoviensia*, **46**, 101–107.

Brothers, D.J. & Carpenter, J.M. (1993) Phylogeny of Aculeata: Chrysidoidea and Vespoidea (Hymenoptera). *Journal of Hymenoptera Research*, **2**(1), 227–304.

Brothers, D.J., Tschuch, G. & Burger, F. (2000) Associations of mutillid wasps (Hymenoptera, Mutillidae) with eusocial insects. *Insectes Sociaux*, **47**, 201–211.

Brothers, D.J. & Lelej, A.S. (2017) Phylogeny and higher classification of Mutillidae (Hymenoptera) based on morphological reanalyses. *Journal of Hymenoptera Research*, **60**, 1–97.

Brower, A.V.Z. (2006) The how and why of branch support and partitioned branch support, with a new index to assess partition incongruence. *Cladistics*, **22**(4), 378–386.

Burmeister, H. (1854) Uebersicht der brasilianischen Mutillen. *Abhandlungen der Naturforschenden Gesellschaft zu Halle*, **2**, 19–29.

Burmeister, H. (1875 ["1874"]) Mutillae Argentinae. *Boletín de la Academia Nacional de Ciencias Exactas existente en la Universidad de Cordova*, **1**, 461–502, figs 1–5.

Cameron, P. (1894–1900) Fam. Mutillidae. *Biologia Centrali-Americana. Insecta. Hymenoptera. (Fossores.)*, Vol. II. (ed. by F.D. Godman & D. Salvin), (1894) pp. 259–328; (1895) pp. 329–376; (1896) pp. 377–395; (1899) p. 404; (1900) pp. iii, v, x–xi, 405–413, plates 13–14. R.H. Porter, London.

Chernomor, O., von Haeseler, A. & Minh, B.Q. (2016) Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology*, **65**(6), 997–1008.

Debevec, A.H., Cardinal, S. & Danforth, B.N. (2012) Identifying the sister-goup to the bees: A molecular phylogeny of Aculeata with an emphasis on the superfamily Apoidea. *Zoologica Scripta*, **41**(5), 527–535.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut, A. (2006) Relaxed phylogenetics and dating with confidence. *PLoS Biology*, **4**(5), e88.

Faircloth, B.C., Branstetter, M.G., White, N.D. & Brady, S.G. (2015) Target enrichment of ultraconserved elements from arthropods provides a genomic perspective on relationships among Hymenoptera. *Molecular Ecology Resources*, **15**(3), 489–501.

Fox, W.J. (1894) A proposed classification of the fossorial Hymenoptera of North America. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **46**, 292–307.

Fox, W.J. (1899) The North American Mutillidae. *Transactions of the American Entomological Society*, **25**, 219–292.

Gernhard, T. (2008) The conditioned reconstructed process. *Journal of Theoretical Biology*, **253**(4), 769–778.

Gerstaecker, A. (1874) Mutillarum Americae meridionalis indigenarum synopsis systematica et synonymica. *Archiv für Naturgeschichte*, **40**, 41–77, 299–328.

Goloboff, P.A. & Catalano, S.A. (2016) TNT version 1.5, including a full implementation of phylogenetic morphometrics. *Cladistics*, **32**(3), 221–238.

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology*, **59**(3), 307–321.

Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q. & Vinh, L.S. (2017) UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, **35**(2), 518–522.

Invrea, F. (1964) *Mutillidae-Myrmosidae. Fauna d'Italia 5*. Edizioni Calderini, Bologna.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A. & Jermiin, L.S. (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods*, **14**, 587–589.

Katoh, K. & Standley, D.M. (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, **30**(4), 772–780.

Klug, J.C.F. (1821) Entomologiae Brasilianae specimen. *Nova acta physico-medica Academiae Caesareae Leopoldino-Carolinae Naturae Curiosorum*, **10**(2), 305–324, plates 21–23.

Krombein, K.V. (1940 ["1939"]) Studes in the Tiphiidae (Hymenoptera Aculeata). IV. A revision of the Myrmosinae of the New World with a discussion of the Old World species. *Transactions of the American Entomological Society*, **65**(4), 415–465, plate 24.

Krombein, K.V. (1979) Biosystematic studies of Ceylonese wasps, IV. Kudakrumiinae, a new subfamily of primitive wasps (Hymenoptera, Mutillidae). *Transactions of the American Entomological Society*, **105**, 67–83.

Lelej, A.S. (1983) On the velvet ants (Hymenoptera, Mutillidae) of Australia [in Russian with English summary]. *Entomologicheskoe obozrenie*, **62**(3), 612–619.

Lelej, A.S. (1996) To the knowledge of the East Asian species of the tribe Trogaspidiini Bischoff, 1920 (Hymenoptera, Mutillidae) with description of eight new genera and two new species. *Far Eastern Entomologist*, **30**, 1–24.

Lelej, A.S. & Brothers, D.J. (2008) The genus-group names of Mutillidae (Hymenoptera) and their type species, with a new genus, new name, new synonymies, new combinations and lectotypifications. *Zootaxa*, **1889**, 1–79.

Lelej, A.S. & Nemkov, P.G. (1997) Phylogeny, evolution and classification of Mutillidae (Hymenoptera). *Far Eastern Entomologist*, **46**, 1–24.

Luz, D.R., Waldren, G.C. & Melo, G.A.R. (2016) Bees as hosts of mutillid wasps in the Neotropical region (Hymenoptera, Apidae, Mutillidae). *Revista Brasileira de Entomologia*, **60**(4), 302–307.

Manley, D.G. & Poinar, Jr., G.O. (1991) A new species of fossil *Dasymutilla* (Hymenoptera: Mutillidae) from Dominican amber. *The Pan-Pacific Entomologist*, **67**(3), 200–205.

Manley, D.G. & Poinar, Jr., G.O. (1999) A second species of fossil *Dasymutilla* (Hymenoptera: Mutillidae) from Dominican amber. *The Pan-Pacific Entomologist*, **75**(1), 48–51.

Manley, D.G. & Poinar, Jr., G.O. (2003) A new specimen of fossil Mutillidae (Hymenoptera) from Dominican amber. *Proceedings of the Entomological Society of Washington*, **105**(4), 1069–1071.

Mickel, C.E. (1938) The Neotropical mutillid wasps of the genus *Timulla* Ashmead (Hymenoptera: Mutillidae). *Transactions of the Royal Entomological Society of London*, **87**(22), 529–679.

Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 1–8.

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A. & Lanfear, R. (2020) IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, **37**(5), 1530–1534.

Mitchell, A. & Brothers, D.J. (2002) Phylogeny of the genera of Ticoplinae (Hymenoptera: Mutillidae). *Journal of Hymenoptera Research*, **11**(2), 312–325.

Nagy, C.G. (1970) Further investigations on the heterogynoid wasps. *Entomologische Mitteilungen aus dem Zoologischen Museum Hamburg*, **4**(69), 83–86.

Nixon, K.C. (1999) The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics*, **15**(4), 407–414.

Nguyen, L.-T., Schmidt, H.A., von Haeseler, A. & Minh, B.Q. (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, **32**(1), 268–274.

Nonveiller, G. (1979 ["1978"]) Recherches sur les Mutillidés de l'Afrique (Hymenoptera, Mutillidae). VIII. Révision des genres *Ctenotilla*, *Cephalotilla* et *Pseudocephalotilla sensu* Bischoff. *Memoires publies par l'Institut pour la Protection des Plantes*, *Beograd*, **8**, 1–184.

Nonveiller, G. (1993) Studies on African Mutillidae. XIV. *Ctenoceraea*, a new genus with pectinate antennae and description of the type-species *C. pectinella* sp. n. (Hymenoptera). *Revue Française d'Entomologie (N.S.)*, **15**(1), 15–18.

Osten, T. (2007) Hymenoptera: Bees, wasps and ants. *The Crato Fossil Beds of Brazil: Window into an Ancient World* (ed. by D.M. Martill, G. Bechly & R.F. Loveridge), pp. 350–365. Cambridge University Press, Cambridge.

Pagliano, G., Brothers, D.J., Cambra, R., Lelej, A.S., Lo Cascio, P., Palmerini, M.M., Scaramozzino, P.L., Williams, K.A. & Romano, M. (2020) Checklist of names in Mutillidae (Hymenoptera), with illustrations of selected species. *Bollettino del Museo Regionale di Scienze Naturali*, **36**(1–2), 1–425.

Pennacchio, L.A., Ahituv, N., Moses, A.M., Prabhakar, S., Nobgrega, M.A., Shoukry, M., Minovitsky, S., Dubchak, I., Holt, A., Lewis, K.D., Plajzer-Frick, I., Akiyama, J., De Val, S., Afzal, V., Black, B.L., Couronne, O., Eisen, M.B., Visel, A. & Rubin, E.M. (2006) *In vivo* enhancer analysis of human conserved non-coding sequences. *Nature*, **444**, 499–502.

Pilgrim, E.M., von Dohlen, C.D. & Pitts, J.P. (2008) Molecular phylogenetics of Vespoidea indicate paraphyly of the superfamily and novel relationships of its component families and subfamilies. *Zoologica Scripta*, **37**(5), 539–560.

Pitts, J.P., Wilson, J.S. & von Dohlen, C.D. (2010) Evolution of the nocturnal Nearctic Sphaeropthalminae velvet ants (Hymenoptera: Mutillidae) driven by Neogene orogeny and Pleistocene glaciation. *Molecular Phylogenetics and Evolution*, **56**, 134–145.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, **67**(5), 901–904.

Sadler, E.A. (2018) *Historical biogeography and natural history of nocturnal wasps in the southwestern deserts with special emphasis on the genus Chyphotes (Hymenoptera: Chyphotidae).* Dissertation Thesis: Utah State University, Logan.

Sandelin, A., Bailey, P., Bruce, S., Engström, P.G., Klos, J.M., Wasserman, W.W., Ericson, J. & Lenhard. B. (2004) Arrays of ultraconserved non-coding regions span the loci of key developmental genes in vertebrate genomes. *BMC Genomics*, **5**, 99.

Schuster, R.M. (1947 ["1946"]) A revision of the sphaerophthalmine [sic] Mutillidae of America north of Mexico. *Annals of the Entomological Society of America*, **39**, 692–703.

Schuster, R.M. (1949) Contributions toward a monograph of the Mutillidae of the Neotropical region. III. A key to the subfamilies represented and descriptions of several new genera (Hymenoptera). *Entomologica Americana*, **29**(3–4), 59–140.

Simon, S., Letsch, H., Bank, S., Buckley, T.R., Donath, A., Liu, S., Machida, R., Meusemann, K., Misof, B., Podsiadlowski, L., Zhou, X., Wipfler, B. & Bradler, S. (2019) Old World and New World Phasmatodea: Phylogenomics resolve the evolutionary history of stick and leaf Insects. *Frontiers in Evolution and Ecology*, **7**, 345.

Skinner, R.K., Dietrich, C.H., Walden, K.K.O., Gordon, E., Sweet, A.D., Podsiadlowski, L., Petersen, M., Simon, C., Takiya, D.M. & Johnson, K.P. (2019) Phylogenomics of Auchenorrhyncha (Insecta: Hemiptera) using transcriptomes: Examining controversial relationships via degeneracy coding and interrogation of gene conflict. *Systematic Entomology*, **45**(1), 85–113.

Skorikov, A.S. (1935) Zur Mutilliden-Fauna Zentralasiens. *Trudy Tadzhikskoi Bazy, Akademiya Nauk SSSR*, **5**, 257–349.

Suárez, F.J. (1988) Mirmosidos de la Peninsula Iberica (Hymenoptera, Myrmosidae). *Graellsia*, *Revista de Entomólogos Ibéricos*, **44**, 81–158.

Suchard, M.A., Lemey, P., Baele, G., Ayres, D.L., Drummond, A.J. & Rambaut, A. (2018) Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution*, **4**(1), vey016.

Talavera, G. & Castresana, J. (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology*, **56**(4), 564–577.

To, T.-H., Jung, M., Lycett, S. & Gascuel, O. (2016) Fast dating using least-squares criteria and algorithms. *Systematic Biology*, **65**(1), 82–97.

Waichert, C., Rodriguez, J., Wasbauer, M., von Dohlen, C. & Pitts, J.P. (2015) Molecular phylogeny and systematics of spider wasps (Hymenoptera: Pompilidae): Redefining subfamily boundaries and the origin of the family. *Zoological Journal of the Linnean Society*, **175**(2), 271–287.

Waichert, C., Rodriguez, J., Rapoza, M. & Wappler, T. (2019) The oldest species of Pompilidae to date, a new fossil spider wasp (Hymenoptera: Pompilidae). *Historical Biology*. https://doi.org/10.1080/08912963.2019.1675056

Williams, K.A. (2012) *Systematics of Mutillidae (Hymenoptera) with special emphasis on Dasymutilla and their allies*. Dissertation Thesis: Utah State University, Logan.

Wilson, J.S., von Dohlen, C.D., Forister, M.L. & Pitts, J.P. (2012) Family-level divergences in the stinging wasps (Hymenoptera: Aculeata), with correlations to angiosperm diversification. *Evolutionary Biology*, **40**, 101–107.

Woolfe, A., Goodson, M., Goode, D.K., Snell, P., McEwen, G.K., Vavouri, T., Smith, S.F., North, P., Callaway, H., Kelly, K., Walter, K., Abnizova, I., Gilks, W., Edwards, Y.J.K., Cooke, J.E. & Elgar, G. (2004) Highly conserved non-coding sequences are associated with vertebrate development. *PLoS Biology*, **3**(1), e7.

CHAPTER III

PHORETIC COPULATION IN THE VELVET ANT *SPHAEROPTHALMA PENSYLVANICA* (LEPELETIER) (HYMENOPTERA, MUTILLIDAE): A NOVEL BEHAVIOR FOR SPHAEROPTHALMINAE WITH A SYNTHESIS OF MATING STRATEGIES IN MUTILLIDAE[2]

Abstract

Phoretic copulation, a form of phoresy in which a male physically transports a female by flight and/or foot from their initial site of contact before mating, is newly recorded in the Nearctic velvet ant *Sphaeropthalma pensylvanica* (Lepeletier, 1845) (Hymenoptera: Mutillidae). Further, this is the first record of the behavior in the species-rich subfamily Sphaeropthalminae. A description of the *S. pensylvanica* mating observation and photographs are provided. All published observations of copulation events in Mutillidae are critically reviewed in the context of mating strategy, and new terminology is proposed for the mating strategies currently known to occur in the family.

Keywords

Ethology, phoresy, sexual dimorphism

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Introduction

Velvet ants (Hymenoptera: Mutillidae) are ectoparasitoids of immature holometabolous insects in the orders Hymenoptera, Coleoptera, Diptera, Lepidoptera, and possibly egg predators of Blattodea (Brothers 1989; Brothers et al. 2000). Despite this wide spectrum of hosts, most host records for mutillids are from solitary bees and apoid wasps (Hymenoptera: Apoidea) (Krombein 1979; Brothers 1989; Brothers et al. 2000; Luz et al. 2016). Extreme sexual dimorphism is the general rule for the family and the sexes have little in common morphologically; males are usually macropterous and the females are always apterous. Sex associations have historically been a major challenge for researchers due to this dimorphism, and the collection of mating pairs in the field, while relatively rare, has been a reliable method for association (Mickel 1937; Nonveiller 1980; Manley and Pitts 2007). Two overarching mating strategies have been observed in Mutillidae: phoretic copulation and *in situ* copulation.

Phoresy is defined as an interaction between two or more animals in which one individual carries the other(s) for purpose of travel. The individual (or individuals) being carried is termed the phoront(s). Phoresy is particularly common with mites and pseudoscorpions wherein one or a number of individuals will simultaneously travel on a larger arthropod such as a beetle. The carrier animal rarely intentionally carries the phoront except in cases where the phoront is conspecific (or the carrier mistakes the phoront to be conspecific, a common occurrence in Thynnidae (Brown 2000)). Phoretic copulation in Hymenoptera is a form of phoresy in which a larger male physically transports a smaller conspecific female phoront by flight and/or foot from their initial site of contact before mating; the pair may settle on a substrate to mate, or mating may take place during flight (Evans 1969; Brothers 1989). The female is carried by the male primarily by either grasping her around the pronotal neck with his mandibles or by their terminalic union. Phoretic copulation has been observed in three distantly-related families of aculeate Hymenoptera with apterous females: Bethylidae, Mutillidae, and Thynnidae (Evans 1969; Clausen 1976; Brothers 1989; Gordh 1990; Osten 1999; Azevedo et al. 2016). Vivallo (2020) recently reviewed phoretic copulation in aculeate Hymenoptera as a whole with primary emphasis on Thynnidae and the biomechanical aspects of the behavior in that family. For Mutillidae, phoretic copulation has been reported in the following subfamilies and tribes: Dasylabrinae (Dasylabrini), Mutillinae (Ctenotillini, Ephutini, Smicromyrmini, and Trogaspidiini), Myrmosinae (Myrmosini), and Rhopalomutillinae (Table 3.1). The alternative strategy to phoretic copulation is *in situ* copulation, where the male does not transport the female from the initial site of contact to mate. These mating strategies in Mutillidae have, thus far, appeared to be representative of taxa at the subfamily and tribe levels. The subfamily Dasylabrinae is the exception wherein both phoretic copulation and *in situ* copulation have been observed (Table 3.1).

Sphaeropthalma pensylvanica (Lepeletier, 1845) is a widespread mutillid that occurs throughout the eastern half of the United States, extending as far west as Texas north to Kansas (Krombein 1979). It is one of the most well-studied mutillid species with respect to the parasitoid aspects of its biology (Krombein 1967; Matthews 1997; Pitts and Matthews 2000; Pitts et al. 2010a). Remarkably, there is no published information on its mating behavior. In this contribution, an observation of phoretic copulation in *S. pensylvanica* is documented and described. Additionally, the published observations of mating strategies in Mutillidae are comprehensively reviewed in order to place this mating observation into the wider behavioral context of the family. This is the first known occurrence of phoretic copulation in Sphaeropthalminae, which is the second largest subfamily of Mutillidae comprising nearly 1,500 described species (Lelej 2005).

Results

The following observation by J. Roberts of the heretofore undocumented mating behavior of *Sphaeropthalma pensylvanica* occurred on August 3, 2018 in Morgan County, Alabama, along the border of the Highland Rim and Cumberland Plateau regions (Figs 3.1–3.4). During a walk through a semi-open deciduous wooded area in late afternoon, what was at first presumed to be a solitary male *S. pensylvanica*, was observed flying from the immediate leaf littered ground to the base branches of a short cedar tree, approximately 9–10 inches (23–25 cm) above the ground. It was when the male attempted to land on these lower twigs/leaves that it was then observed that he dropped a female that he had apparently carried from the leaf litter. The female tumbled a few inches directly below the male and landed on some of the lower twigs/leaves. In an unexpected move, the male immediately descended in a quick flight-assisted scurry to retrieve the female and gripped her firmly behind the head with his mandibles. He once again briefly took flight and carried her higher up into the same small cedar tree to a height approximately 24 inches (61 cm) above the ground. A somewhat blurry, but discernible photo was captured of the moment the male began his descent to retrieve the female after he dropped her (Fig. 3.1). Once alighted on the upper twigs/leaves and quickly becoming stabilized, with the male’s mandibular grip firm on the pronotal neck of the female, they began copulation at which point it appeared the female began to

Figures 3.1–3.4. MPC-practicing pair of *Sphaeropthalma pensylvanica* (Lepeletier, 1845) in Alabama, USA; photographs by Jason D. Roberts.

extrude her stinger which facilitated the coupling of genitalia (Fig. 3.3). The entire copulative duration was just under two minutes, during which time (and immediately prior to) the male's legs were very active in rhythmic flicking motions, tapping the female on both the metasoma as well as around the gena and pronotum, while alternately tapping the top of her head with the scape of his antennae in the same rhythmic fashion, in between leg tapping. During this process the female did not remain purely passive, but kept a grip on the plant material with her mandibles, fore legs, and mid legs (Figs 3.3, 3.4). Toward the end of copulation the female used her hind legs to stroke the mid and hind legs of the male, the purpose uncertain but speculatively could be a tactile communication to the male or simply an attempt to regain footing. Once copulation was complete, the male released the female within moments and promptly flew away, while she quickly climbed downward and eventually scurried back into the leaf litter. There was no post-copula interaction observed between the pair.

Discussion

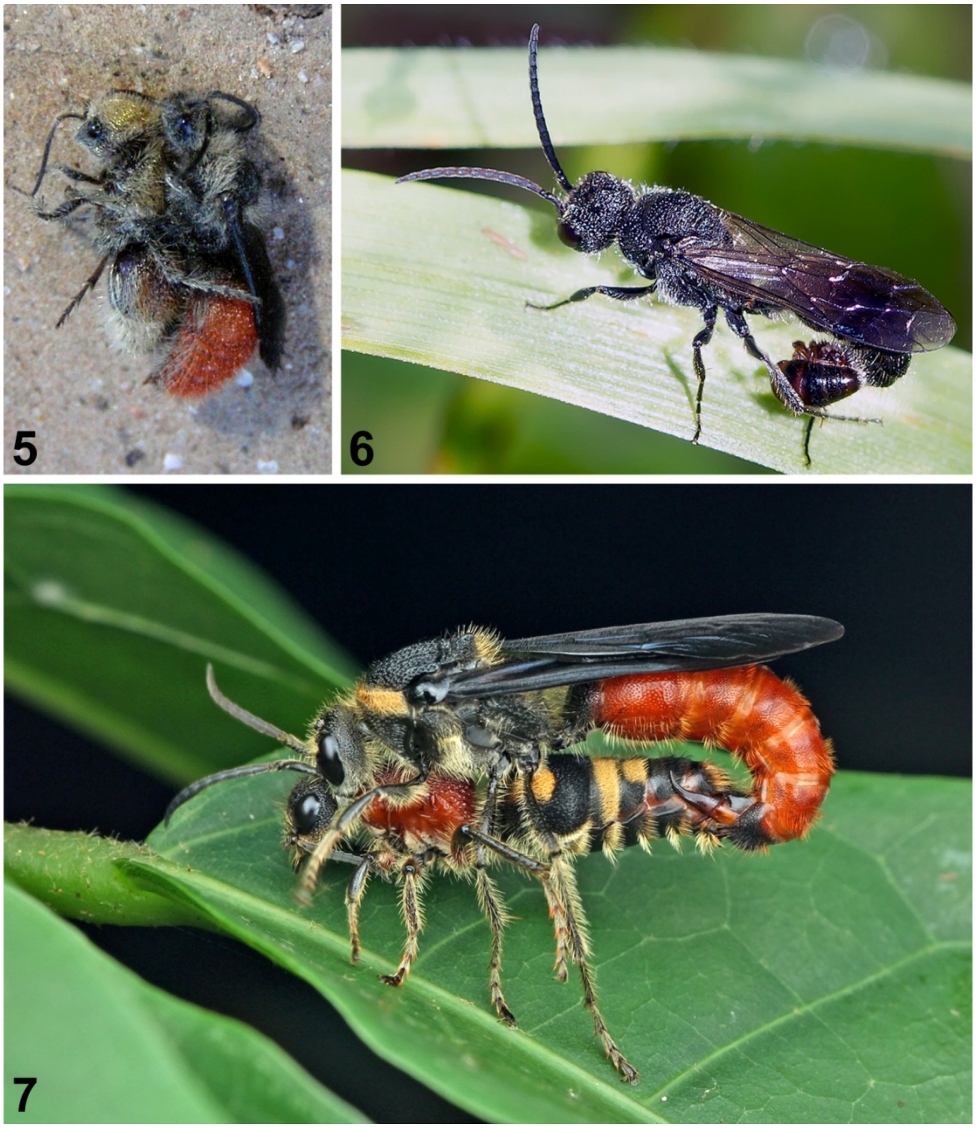
Mating strategies in Mutillidae

This new observation of phoretic copulation in *S. pensylvanica* is recognized as an opportunity to critically review the published information regarding mating strategies in Mutillidae and to develop new terminology that accurately describes them. Data on the mating strategies for 62 mutillid species are comprehensively reviewed in Table 3.1. References that merely note a pair being collected *in copula*, or copulating in captivity, were excluded. These observations are numerous in the literature and usually provide no additional information other than the sex association itself. In compiling these data, it became apparent how little is known overall on the mating behavior of the family, especially behavior documented in natural settings. Observations of mating events in captivity have been deemed problematic, as males will attempt to mate with non-conspecific and even non-congeneric females (Ferguson 1962; Manley 1977; Manley and Pitts 2007). Copulation behavior and mating time observed in the laboratory may not be congruent with behavior that would normally occur in the field. The observations cited in Table 3.1 as being conducted in captivity should be kept with this in mind. The higher classification of Mutillidae in this contribution follows Brothers and Lelej (2017), except *Dolichomutilla* Ashmead, 1899 is considered a member of Mutillini rather than Trogaspidiini, and the two apparent genus-groups that comprise the Mutillini subtribe Ephutina (the *Ephuta* genus-group and the *Odontomutilla* genus-group) are considered full tribes within Mutillinae (Ephutini and Odontomutillini, respectively). These partial modifications in classification are used here in anticipation of a molecular phylogeny of Mutillidae using Ultra-Conserved Elements (Waldren et al. in prep.).

As mentioned previously, there have been two types of mating strategies recognized in mutillids: phoretic copulation and *in situ* copulation. Two subtypes of phoretic copulation were recognized by Brothers (1989). One was termed “true phoretic copulation” wherein the male initially uses his legs to pick up a female and once terminalic union occurs, phoresy is strictly effected by the genitalia and surrounding metasomal structures; mating occurs during flight or while nectaring. Within Mutillidae, this first subtype is known to occur in the myrmosine tribe Myrmosini and the subfamily Rhopalomutillinae (Table 3.1). “True phoretic copulation” also occurs in some subfamilies of Bethylidae and Thynnidae (Evans 1969; Osten 1999; Azevedo et al. 2016).

The other subtype is known to commonly occur in the subfamily Mutillinae (excluding Mutillini and Odontomutillini) and now in Sphaeropthalminae (*S. pensylvanica*) (Table 3.1), wherein the female is primarily supported by the male's mandibular clasp around her pronotal neck, and secondarily by his legs and terminalic union. The pair travels from the initial site of contact by male flight and/or foot and eventually settle on a substrate to finish mating (Nonveiller 1980; Brothers 1989; Brothers and Finnamore 1993). However, this second subtype is technically also "true phoretic copulation," as the female is carried by the male with his mandibles throughout the mating event, even while the pair are resting on a substrate *in copula* (Nonveiller 1980; Cambra and Quintero 1993; Bartholomay et al. 2017; Cambra et al. 2018; current study). Active transport by flight while *in copula* is not required for the mating event to be considered "true phoretic copulation."

In order to accurately characterize these patterns of behavior, new terminology is proposed with respect to Mutillidae to broadly define the two types of mating strategies currently known to occur in the family. **1) Phoretic Copulation (PC)** is a form of phoresy in which a male intentionally carries a female phoront for the majority of their mating event. There are two subtypes of phoretic copulation: **1a) Terminalic Phoretic Copulation (TPC)** is phoresy primarily effected by terminalic union (*i.e.* the genitalia and surrounding structures) between a male and a female phoront for the majority of their mating event (secondarily with his legs) (Fig. 3.6). **1b) Mandibular Phoretic Copulation (MPC)** is phoresy primarily effected by a male's mandibular clasp around a female phoront's pronotal neck for the majority of their mating event (secondarily with his legs and terminalic union) (Figs 3.2–3.4, 3.7). **2) *In situ* Copulation (ISC)** is a non-



Figures 3.5–3.7. Examples of each type of mating strategy in Mutillidae **5** ISC, *Dasymutilla foxi* (Cockerell, 1894) in Arizona, USA; photograph by Mark H. Brown **6** TPC, *Myrmosa unicolor* Say, 1824 in New York, USA; photograph by A. D. Levine **7** MPC, *Wallacidia oculata* (Fabricius, 1804) in Southern District, Hong Kong; photograph by 'aabbabc.'

phoretic mating event that occurs at or near the site of initial contact between a male and a female (Fig. 3.5).

In ISC, there are some observations of males clinging to the dorsum of females during part of the mating event and even clasping their mandibles around the female's pronotal neck (Cottrell 1936; Ferguson 1962; Bayliss and Brothers 1996, 2001); these events are not considered phoretic copulation as intentional carriage by the male does not occur. This behavior in the context of ISC may play a role in courtship, recognition of conspecificity between the sexes, and/or the biomechanics of mating. Subtypes of ISC may potentially be defined at a later date once more data are available. Mating duration for species that practice PC is often considerably longer than species that practice ISC (Table 3.1); consequently, mating pairs are collected more often in PC-practicing taxa (Mickel 1937; Nonveiller 1980). The observation described herein for *S. pensylvanica* is considered MPC.

A potential third subtype of phoretic copulation was described by O'Toole (1975) for the trogaspidiine species *Wallacidia oculata* (Fabricius, 1804) and congeners. As was described: "The posture of copulation in [*W.*] *oculata* is venter to venter, with the male uppermost. The female clings to the sides of the male mesosoma, with the tarsal claws gaining purchase on the coarse sculpture of the male." This mating position is unusual, as most known mating observations in Mutillidae occur with the male venter to female dorsum (although sometimes with wide separation between the male and female's bodies except for the terminalia). In contrast to this mating posture description, O'Toole (1975) also provided evidence that MPC occurs in *W. oculata* and the now full species *Wallacidia melmora* (Cameron, 1905): "I have seen several pairs of [*W.*] *o. melmora* in museum collections in which the females are in the mandibular clasp of the males. J. Cardew (personal communication) found a male of [*W.*] *o. oculata* with a female in its mandibles, at Chang Mai, Thailand." There are two additional published records that describe a venter to venter mating position in the TPC-practicing Myrmosini species *Myrmosa atra* Panzer, 1801 and *M. unicolor* Say, 1824. As detailed in Krombein (1956), both K. V. Krombein and H. K. Townes had independently observed mating pairs of *M. unicolor* in the field that were oriented venter to venter. Additionally, Saxton (2010) observed a mating pair of *M. atra* oriented venter to venter. Prior to the pair's separation, the couple assumed an end to end mating position and Saxton (2010) determined that the male's genitalia must have rotated 180° to a facultative strophandrous position (sensu Schulmeister 2001). Male genitalic rotation is also known to occur in the TPC-practicing Thynnidae that engage in male to female feeding (Evans 1969; Vivallo 2020). In contrast to these records, Cambra et al. (2018) included a photograph of a pair of *M. unicolor* that remained *in copula* after being collected in a Malaise trap which are in a male venter to female dorsum position. An online search for photographs of mating pairs of Myrmosini revealed that females' bodies are rotated to various degrees with respect to the male. One of these photographs of a mating pair of *M. unicolor* is included here (Fig. 3.6) and shows a roughly 90° rotation of the female's body.

For Myrmosini, variable female mating position and likely male genitalic rotation are supported by observations in the field by multiple researchers. For Trogaspidiini, information on venter to venter mating is limited to O'Toole (1975). It is unknown whether this mating posture was observed with live specimens or if it was inferred from museum specimens. If the description in O'Toole (1975) was based on preserved material, the venter to venter posture of the mating pair might be an artifact of how the collector mounted the specimens (and might be how the collector envisaged the posture of the mating pair during the act if they happened to terminate copulation and separate upon being captured). Further, a photograph of a mating pair of *W. oculata* is included in this study (Fig. 3.7) and they are practicing MPC. We ultimately regard the venter to venter mating position described in O'Toole (1975) as erroneous. All known mating descriptions suggest trogaspidiines practice MPC (Table 3.1) and the available evidence supports that *Wallacidia* species are no different.

The importance of intersexual size dimorphism for phoretic copulation

Sexual dimorphism in size, with the male being larger than the female, is an important criterion for phoretic copulation to effectively occur (Nonveiller 1963; Deyrup and Manley 1986; Brothers 1989; Tormos et al. 2010; Matteini Palmerini 2013). This size dimorphism is in contrast with other parasitoid Hymenoptera wherein females are commonly larger than males (Charnov et al. 1981; O'Neill 1985; Hurlbutt 1987; van den Assem et al. 1989). In some taxa that are known to normally practice MPC, some male individuals are similar or smaller in body size to the female they are mating with and are physically unable to transport her by flight or even by foot; facultative ISC consequently occurs (Nonveiller 1963; Alicata et al. 1975; Deyrup and Manley 1986; Tormos et al. 2010; Matteini Palmerini 2013; Polidori et al. 2013). It is unknown if the reverse situation also occurs wherein a species that normally practices ISC due to similarity in male and female size might practice facultative MPC with unusually large males. In evidence against the latter situation, Cottrell (1936) observed that for *Dasymutilla bioculata*

(Cresson, 1865), a sphaeropthalmine species that practices ISC, larger males were mechanically unable to copulate with smaller females. Females are often larger than males in this species, and mating was successful when smaller males mated with larger females. Additionally, male aptery and brachyptery, which are uncommon in Mutillidae (Cambra and Quintero 2007, 2017), would limit phoretic copulation by flight but not by foot; mating behavior for species with flightless males has yet to be observed, though. The cause of adult intra- and intersexual size differences within a mutillid species is primarily predicated upon host choice.

Mutillids are generally solitary ectoparasitoids that may parasitize more than one host species. It has long been known that the size of the host determines the size of the adult mutillid, which explains the common occurrence of adult size variation (Mickel 1924; Deyrup and Manley 1986; Brothers 1989; Hennessey 2002). If a female mutillid parasitizes more than one host species that vary in size in relation to one another, her offspring will consequently vary in size. In some mutillid taxa, one sex is on average larger than the other, and the underlying mechanics for sex allocation in mutillids remained unknown until relatively recently. Of critical relevance to the new discovery of phoretic copulation in *S. pensylvanica* is an investigation into sex allocation in this species by Pitts et al. (2010a). Their results supported facultative size-dependent sex allocation in which males typically develop from larger hosts and females develop from smaller hosts. Due to the sex-determination system of haplodiploidy in Hymenoptera, female *S. pensylvanica* are able to choose whether to oviposit a fertilized or unfertilized egg onto a specific host. Unfertilized eggs, which develop into males, are more often deposited on larger hosts, such as the organ pipe mud dauber *Trypoxylon politum* (Drury,

1773) (Hymenoptera: Crabronidae); female eggs are usually deposited on smaller *Trypoxylon* species and other taxa (Matthews 1997; Pitts et al. 2010a). Pitts et al. (2010a) concluded that female *S. pensylvanica* likely use host body length and/or nest diameter as criteria for which sex of egg—male or female—to oviposit on a host rather than the criterion of host mass. The difference in size between the male and female mating pair of *S. pensylvanica* documented herein is substantial (Figs 3.2–3.4), and the size dimorphism prerequisite for phoretic copulation is clearly met. Although a rare occurrence, female *S. pensylvanica* have been reared from *T. politum* and males reared from smaller *Trypoxylon* species (Pitts et al. 2010a). More mating observations are necessary for *S. pensylvanica* to see how mating is carried out, if at all, between these smaller males and larger females. Facultative size-dependent sex allocation is likely widespread among PC-practicing mutillids due to the importance of intersexual size dimorphism.

Phoretic copulation in Sphaeropthalminae

The genus *Sphaeropthalma* Blake, 1871 is a paraphyletic assemblage of 81 described species classified into 17 species-groups (Pitts et al. 2010b; Pitts and Sadler 2015). *Sphaeropthalma pensylvanica* (Lepeletier, 1845) is currently placed in the *S. pensylvanica* species-group along with *S. auripilis* (Blake, 1871), *S. boweri* Schuster, 1944, and *S. nocticaro* Pitts, 2005 (Pitts and Sadler 2015). Given that these other members of the species-group also show the same differences in body size between the sexes, it is likely that they practice MPC as well. Unfortunately, the females of most of the remaining *Sphaeropthalma* species, as well as the related large genera *Photomorphus* Viereck, 1903 and *Odontophotopsis* Viereck, 1903, are unknown. The known females are closer in size to the males and there seem to be no other likely candidates for MPC in *Sphaeropthalma* outside of the *S. pensylvanica* species-group or the related genera *Photomorphus* and *Odontophotopsis*.

There are a few unusual distributions in Sphaeropthalminae that might be due to dispersal via PC. Sphaeropthalmines primarily occur in the Nearctic, Neotropical, and Australasian regions, with two small genera occurring in the Palaearctic (Europe, China, Japan, Republic of Korea) and Oriental (China, Taiwan) regions. These latter two genera, *Cystomutilla* André, 1896 and *Hemutilla* Lelej, Tu, & Chen, 2014 were recently reviewed by Tu et al. (2014). Molecular data has revealed that *Cystomutilla* is closely related to the nocturnal Nearctic Sphaeropthalminae (Waldren et al. in prep.). The practice of phoretic copulation, which has, in part, been hypothesized to aid the apterous females in traversing physical barriers such as water (Evans 1969), is not out of the realm of possibility in *Cystomutilla* and *Hemutilla* in light of the behavior being discovered in *S. pensylvanica*. Another genus in which PC may have played a role in dispersal is the primarily Australian genus *Ancistrotilla* Brothers, 2012. Several species are known to occur in New Caledonia and one in Vanuatu, an archipelago of volcanic origin (Brothers 2012; Lo Cascio 2015). The only species known so far from both sexes, *Ancistrotilla azurea* Brothers, 2012, which occurs in Vanuatu, meets the size prerequisite for phoretic copulation with males being larger than females. Additionally, the single known female was apparently collected in the same Malaise trap as fifteen males and could potentially have been carried into the trap by a male.

Table 3.1. Review of published mating strategy data for Mutillidae.

Taxon	Mating strategy	Size dimorphism	Time in copula	Conditions	Reference	Additional notes
Dasylabrinae: Apteromutillini						
—	—	—	—	—	—	—
Dasylabrinae: Dasylabrini						
Chrestomutilla glossinae (Turner)	MPC	—	—	in the field and in captivity	Lamborn (1916)	—
Tricholabiodes livida (André)	ISC	♂ > ♀	—	in captivity	Bayliss and Brothers (1996)	
Tricholabiodes thisbe (Péringuey)	ISC	♂ = ♀	"10–15 seconds"	in captivity	Bayliss and Brothers (1996)	
Mutillinae: Ctenotillini						
Ctenotilla caeca (Radoszkowski)†	PC	♂ > ♀	—	in the field	Nonveiller (1963)	—
Mutillinae: Ephutini						
Ephuta floridana Schuster	PC	♂ > ♀	—	in the field	Deyrup and Manley (1986)	
Ephuta sabaliana Schuster	PC	♂ > ♀	—	in the field	Deyrup and Manley (1986)	
Ephuta slossonae slossonae (Fox)	MPC	—	—	in the field	Krombein and Norden (1996)	
Mutillinae: Mutillini						
Dolichomutilla sycorax (Smith)	ISC	♂ = ♀	"60–100 seconds"	in captivity	Bayliss and Brothers (2001)	
Mutilla europaea Linnaeus	ISC	—	a few minutes	in captivity	Drewsen (1847)	—
Mutilla europaea Linnaeus	ISC?	—	—	in captivity	Hoffer (1886)	—
Mutilla europaea Linnaeus	ISC?	—	—	in captivity	Su et al. (2019)	—
Mutillinae: Odontomutillini						
—	—	—	—	—	—	—
Mutillinae: Smicromyrmini						
Nemka viduata (Pallas)	MPC	—	45 minutes (field)	in the field and in captivity	Alicata et al. (1975)	—
Nemka viduata (Pallas)	PC	—	—	in the field	Matteini Palmerini (1992)	—
Nemka viduata (Pallas)	MPC	sizes variable	"more than 2 hours"; 45 minutes	in the field and in captivity	Matteini Palmerini (2013)	—
Nemka viduata (Pallas)	PC	♂ > ♀	—	in the field	Nonveiller (1963)	—
Nemka viduata (Pallas)	PC	sizes variable	—	in the field and in captivity	Polidori et al. (2013)	Mating balls

Nemka viduata (Pallas)	MPC	sizes variable	"2 h–2 h 15 min" (captivity); "2 h 20 min"; "3 h 7 min"; "2 h 13 min"; "2 h 10 min" (field)	in the field and in captivity	Tormos et al. (2010)	—
Physetopoda halensis (Fabricius)‡	MPC	♂ > ♀	25 minutes	mating pair collected in the field and observed in captivity	Bertkau (1884)	—
Promecilla decora (Smith)	MPC	—	"1 hour 22 minutes"	mating pair collected in the field and observed in captivity	Pagden (1934)	—
Smicromyrme benefactrix (Turner)	ISC/PC	—	—	in the field and in captivity	Lamborn (1916)	Males attempted female carriage with his mandibles around her pedicel
Smicromyrme jovanovici Nonveiller§	ISC	♂ = ♀	—	in the field	Nonveiller (1963)	—
Smicromyrme rufipes (Fabricius)	MPC	—	56 minutes (field); 1 hour 3 minutes (field); 1 hour 10 minutes (captivity)	in the field and in captivity	Crèvecoeur (1930)	—
Sulcotilla sp.	MPC	—	—	museum specimens	Brothers (1975)	—
Mutillinae: Trogaspidiini						
Karlissaidia sexmaculata (Swederus)	MPC	—	"hours"	in the field	Rothney (1903)	—
Karlissaidia sp. nr *sexmaculata* (Swederus)	PC	—	—	museum specimens	O'Toole (1975)	—
Timulla cordillera Mickel	MPC	—	"approx. 16 hours"	in captivity	Cambra et al. (2018)	—
Timulla dubitata (Smith)	MPC	♂ > ♀	—	mating pair collected in the field and observed in captivity	Sheldon (1970)	—
Timulla floridensis (Blake)	PC	♂ > ♀	—	in the field	Deyrup and Manley (1986)	
Timulla nisa Mickel	MPC	♂ = ♀	—	in captivity	Cambra and Quintero (1993)	Information gleaned from photographs

Timulla oajaca (Blake)	PC	♂ > ♀	—	mating pair collected in the field	Linsley (1960)	Female was supported by male's legs and genitalic union
Timulla oajaca (Blake)	PC	—	—	in the field	Hennessey and West (2018)	
Timulla rufogastra (Lepeletier)	MPC	♂ > ♀	—	in the field	Bartholomay et al. (2017)	Mixed-species mating aggregation
Timulla runata Mickel	MPC	—	"about 20 hours"	in captivity	Cambra et al. (2018)	—
Timulla suspensa (Gerstaecker)	MPC	♂ > ♀	—	museum specimens	Bartholomay et al. (2017)	—
Timulla suspensa (Gerstaecker)	PC	—	—	in the field	Hennessey and West (2018)	
Timulla vagans (Fabricius)|	—	—	—	in the field	Fattig (1936)	Mating ball
Timulla vagans (Fabricius)	—	—	"several minutes"	in the field	Shappirio (1947b)	—
Timulla vagans (Fabricius)	MPC	♂ > ♀	—	museum specimens	Sheldon (1970)	Information gleaned from illustration
Trogaspidia fedtschenkoi (Radoszkowski)	MPC	♂ > ♀	—	museum specimens	Skorikov (1935)	Information gleaned from illustration
Trogaspidia (*Acutitropidia*) *aurata* (Bischoff)	MPC	♂ > ♀	—	in the field	Nonveiller (1980)	Information gleaned from photograph
Trogaspidia (*Acutitropidia*) *bugalana* (Bischoff)	MPC	♂ > ♀	—	museum specimens	Brothers (1989)	Information gleaned from photograph
Wallacidia melmora (Cameron)	MPC	—	—	museum specimens	O'Toole (1975)	—
Wallacidia oculata (Fabricius)	PC	—	—	museum specimens	O'Toole (1975)	Venter to venter position
Wallacidia oculata (Fabricius)	MPC	—	—	in the field	O'Toole (1975)	—
Wallacidia oculata (Fabricius)	MPC	♂ > ♀	—	in the field	current study (Fig. 7)	—
Myrmillinae						
Myrmilla calva (Villers)¶	ISC	—	5 to 15 minutes	in captivity	Monastra (1989)	—
Myrmilla erythrocephala (Latreille)#	ISC	—	just over 20 minutes; roughly for 17 to 19 minutes	in captivity	Monastra (1989)	—
Myrmosinae: Kudakrumiini						
Myrmosula parvula (Fox)	ISC	—	"14 seconds"	in captivity	Brothers (1978)	—
Myrmosinae: Myrmosini						

Myrmosa atra Panzer	TPC	♂ > ♀	"9 minutes"; "47 minutes 26 seconds"	in the field	Saxton (2010)	Venter to venter position
Myrmosa bradleyi Roberts	PC	—	—	mating pair collected in the field	Linsley (1960)	—
Myrmosa unicolor Say	TPC	♂ > ♀	—	mating pair collected in the field	Krombein (1956)	Venter to venter position
Myrmosa unicolor Say	TPC	♂ > ♀	—	museum specimens	Cambra et al. (2018)	—
Myrmosa unicolor Say	TPC	♂ > ♀	—	in the field	current study (Fig. 6)	—
Myrmosa sp.	PC	—	—	mating pair collected in the field	Pate (1947)	—
Pseudophotopsidinae						
—	—	—	—	—	—	—
Rhopalomutillinae						
Bischoffiella cristata (Bingham)	TPC	♂ > ♀	—	museum specimens	Brothers (1989, 2015)	Information gleaned from photograph
Pherotilla oceanica (Mickel)††	PC	—	—	in the field?	Pagden (1938)	—
Pherotilla rufitincta (Hammer)	TPC	♂ > ♀	—	museum specimens	Brothers (2015)	Information gleaned from photograph
Rhopalomutilla anguliceps (André)	TPC	♂ > ♀	—	mating pair collected in the field	Brothers (1989)	Mating aggregation
Rhopalomutilla clavicornis (André)	TPC	—	—	mating pair collected in the field	Bridwell (1917)	—
Sphaeropthalminae: Dasymutillini						
Dasymutilla araneoides (Smith)‡‡	—	—	—	in the field	Manley and Pitts (2007)	Mating ball
Dasymutilla araneoides (Smith)	—	—	—	in the field	Quintero and Cambra (2001)	Mating ball
Dasymutilla bioculata (Cresson)	ISC	♂ < ♀	"about twenty seconds"	in captivity	Cottrell (1936)	—
Dasymutilla bioculata (Cresson)§§	ISC	—	"less than five seconds"	in the field	Manley and Deyrup (1989)	
Dasymutilla coccineohirta (Blake)	ISC	—	"a few seconds"	in captivity while in the field	Hurd (1951)	—
Dasymutilla coccineohirta (Blake)‖	ISC	—	"2 seconds"	in the field	Manley (1977)	—
Dasymutilla erythrina (Say)¶¶	ISC	—	"five seconds"	in the field	Linsley et al. (1955)	—
Dasymutilla foxi (Cockerell)	ISC	—	"over one min on one occasion"	in the field and in captivity	Spangler and Manley (1978)	
Dasymutilla foxi (Cockerell)	ISC	♂ = ♀	—	in the field	current study (Fig. 5)	—

Dasymutilla nigripes (Fabricius)	—	—	“less than 10 seconds”	—	Shappirio (1947b)	—
Dasymutilla nigripes (Fabricius)	—	—	“a very short period”	—	Shappirio (1947b)	—
Dasymutilla occidentalis (Linnaeus)	ISC	—	"2 to 5 seconds"	in the field	Tomberlin (1997)	—
Dasymutilla quadriguttata (Say)	ISC	—	"approximately three seconds"	in captivity while in the field	Remington (1944)	—
Dasymutilla sp.	—	—	“about 30 seconds”	—	Shappirio (1947b)	—
Sphaeropthalminae: Pseudomethocini: Euspinoliina						
—	—	—	—	—	—	—
Sphaeropthalminae: Pseudomethocini: Pseudomethocina						
Calomutilla panamensis Cambra, Brothers, and Quintero	ISC	—	"35 seconds"	in captivity	Contreras 1993; Cambra et al. (2020)	
Lophomutilla corupa Casal	ISC	—	"a minimum of 1 minute 48 seconds and the maximum recorded time was 2 minutes 25 seconds; mean copulation time was 2 minutes"	in captivity	Bergamaschi et al. (2010)	—
Lynchiatilla parana Cambra in: Bergamaschi et al. (2012)	ISC	—	"83 seconds and 70 seconds"	in captivity	Bergamaschi et al. (2012)	—
Pseudomethoca frigida (Smith)	ISC	—	"about 15 seconds"	in captivity	Brothers (1972)	—
Pseudomethoca frigida (Smith)	—	—	“about fifteen seconds”	in the field	Shappirio (1947a,b)	—
Pseudomethoca propinqua (Cresson)	—	—	"mating was frequent but brief"	in the field	Jellison (1982)	Mating balls
Pseudomethoca pumila (Burmeister)	ISC	—	"less than one minute, with the maximum time recorded of 58 seconds"	in captivity	Bergamaschi et al. (2011)	—
Pseudomethoca simillima (Smith)	—	—	“about fifteen seconds”	in the field	Shappirio (1947a,b)	—
Sphaeropthalminae: Sphaeropthalmini						
Sphaeropthalma blakeii (Fox)	ISC	—	“ten to twenty seconds”	in captivity	Ferguson (1962)	—
Sphaeropthalma orestes (Fox)##	ISC	♂ > ♀	"a few seconds"	in the field	Mickel (1938)	—
Sphaeropthalma pensylvanica (Lepeletier)	MPC	♂ > ♀	“just under 2 minutes”	in the field	current study (Figs 1–4)	—
Ticoplinae: Smicromyrmillini						

—	—	—	—	—	—	—
Ticoplinae: Ticoplini						
—	—	—	—	—	—	—

‡ as *Mutilla ephippium* Fabricius
§ *nomen nudum*
| as *Mutilla* (*Timulla*) *briaxus* Blake
¶ as *Myrmilla calva distincta* (Lepeletier)
# as *Myrmilla erythrocephala bison* (Costa)
†† as *Rhopalomutilla javana* Pagden
‡‡ as *Dasymutilla deyrollesi* Mickel
§§ as *Dasymutilla pyrrhus* (Fox)
|| as *Dasymutilla clytemnestra* (Fox)
¶¶ as *Dasymutilla formicalia* Rohwer
as *Photopsis salmani* Mickel

Conclusion

Based on prior knowledge, it was thought that mating strategies in Mutillidae were confined to the family-group levels of subfamily, tribe, or subtribe (Table 3.1). Members of the subfamily Sphaeropthalminae were previously known to only practice ISC. With the discovery of MPC in *S. pensylvanica*, it is revealed that membership to a higher taxon is not always reliable for predicting a species' mating strategy. Ironically, *S. pensylvanica* is the type species of *Sphaeropthalma* Blake, the genus from which the subfamily name Sphaeropthalminae is derived. As this is the only known mating observation for this species and species-group, more information is needed to determine the consistency of this behavior especially with respect to intersexual size variation. Additional fieldwork is also necessary to get a better idea of how prevalent PC is in Sphaeropthalminae. Respecting the historical challenge of discovering mating mutillid pairs in the field, male morphology combined with consistent interspecific size differences in a species could be used as preliminary lines of evidence for the practice of phoretic copulation.

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References

Alicata P, Caruso D, Costa G, Motta S (1975 [“1974”]) Richerche eco-etologiche sulla fauna delle dune costiere di Porto Palo (Siracusa). I. *Smicromyrme viduata* (Pall.) (Hymenoptera, Mutillidae): Ritmi di attivita migrazioni e accoppiamento. Animalia 1(1/3): 89–108.

Azevedo CO, Colombo WD, Alencar IDCC, de Brito CD, Waichert C (2016) Couples in phoretic copulation, a tool for male-female association in highly dimorphic insects of the wasp genus *Dissomphalus* Ashmead (Hymenoptera: Bethylidae). Zoologia 33(6): e20160076. https://doi.org/10.1590/s1984-4689zool-20160076

Bartholomay PR, Waldren GC, de Oliveira ML (2017) Observation of a mixed-sex, mixed-species aggregation of velvet ants, genus *Timulla* Ashmead, 1899 (Hymenoptera: Mutillidae) in the Brazilian Amazon, Roraima, with a new synonymy. Zootaxa 4362(1): 135–140. https://doi.org/10.11646/zootaxa.4362.1.8

Bayliss PS, Brothers DJ (1996) Biology of *Tricholabiodes* Radoszkowski in southern Africa, with a new synonymy and review of recent biological literature (Hymenoptera: Mutillidae). Journal of Hymenoptera Research 5: 249–258. https://www.biodiversitylibrary.org/page/4491417#page/257/mode/1up

Bayliss PS, Brothers DJ (2001) Behaviour and host relationships of *Dolichomutilla sycorax* (Smith) (Hymenoptera: Mutillidae, Sphecidae). Journal of Hymenoptera Research 10(1): 1–9. https://biodiversitylibrary.org/page/2858630

Bergamaschi ACB, Cambra RA, Brothers DJ, Melo GAR (2012) *Lynchiatilla* Casal, 1963 (Hymenoptera: Mutillidae): A new species from Brazil associated with

Paroxystoglossa spiloptera Moure (Hymenoptera: Apidae: Halictinae), and notes on other species. Zootaxa 3548: 55–64. https://doi.org/10.11646/zootaxa.3548.1.3

Bergamaschi ACB, Cambra RA, Melo GAR (2010) Male description and host record for *Lophomutilla corupa* Casal, 1968 (Hymenoptera: Mutillidae), with behavioural notes on mating behaviour and host nest attacks. Journal of Natural History 44(43–44): 2597– 2607. https://doi.org/10.1080/00222933.2010.499574

Bergamaschi ACB, Cambra RA, Melo GAR (2011) New combinations, sex association, behavioural notes and potential host record for two Neotropical species of *Pseudomethoca* Ashmead, 1896 (Hymenoptera: Mutillidae). Zootaxa 3062: 55–63. https://doi.org/10.11646/zootaxa3062.1.6

Bertkau P (1884) Die begattung von *Mutilla ephippium*. Biologisches Centralblatt 3: 722–724. https://biodiversitylibrary.org/page/5324348

Bridwell JC (1917) Untitled [Exhibition of *Rhopalomutilla clavicornis*]. Proceedings of the Hawaiian Entomological Society 3(4): 1–260. https://biodiversitylibrary.org/page/25416358

Brothers DJ (1972) Biology and immature stages of *Pseudomethoca f. frigida*, with notes on other species (Hymenoptera: Mutillidae). The University of Kansas Science Bulletin 50(1): 1–38. https://biodiversitylibrary.org/page/3278313

Brothers DJ (1975) Phylogeny and classification of the aculeate Hymenoptera, with special reference to Mutillidae. The University of Kansas Science Bulletin 50(11): 483–648. https://biodiversitylibrary.org/page/4386602

Brothers DJ (1978) Biology and immature stages of *Myrmosula parvula* (Hymenoptera: Mutillidae). Journal of the Kansas Entomological Society 51(4): 698–710. https://www.jstor.org/stable/25083861

Brothers DJ (1989) Alternative life-history styles of mutillid wasps (Insecta, Hymenoptera). In: Bruton MN (Ed.) Alternative life-history styles of animals. Kluwer Academic Publishers (Dordrecht): 279–291. https://doi.org/10.1007/978-94-009-2605-9_14

Brothers DJ (2012) The new genus *Ancistrotilla* n. gen., with new species from Vanuatu and New Caledonia (Hymenoptera, Mutillidae). Zoosystema 34(2): 223–251. https://doi.org/10.5252/z2012n2a2

Brothers DJ (2015) Revision of the Rhopalomutillinae (Hymenoptera, Mutillidae): I, generic review with descriptions of three new genera. Journal of Hymenoptera Research 46: 1–24. https://doi.org/10.3897/JHR.46.5733

Brothers DJ, Finnamore AT (1993) Superfamily Vespoidea. In: Goulet H, Huber JT (Eds) Hymenoptera of the world: An identification guide to families. Agriculture Canada Publication (Ottawa): 161–278. https://cfs.nrcan.gc.ca/publications?id=35617

Brothers DJ, Lelej AS (2017) Phylogeny and higher classification of Mutillidae (Hymenoptera) based on morphological reanalyses. Journal of Hymenoptera Research 60: 1–97. https://doi.org/10.3897/jhr.60.20091

Brothers DJ, Tschuch G, Burger F (2000) Associations of mutillid wasps (Hymenoptera, Mutillidae) with eusocial insects. Insectes Sociaux 47: 201–211. https://doi.org/10.1007/PL00001704

Brown GR (2000) Some problems with Australian tiphiid wasps, with special reference to coupling mechanisms. In: Austin AD, Dowton M (Eds) Hymenoptera: Evolution, biodiversity and biological control. CSIRO Publishing (Collingwood, Victoria, Australia): 210–217.

Cambra RA, Brothers DJ, Quintero D (2020) Review of *Calomutilla* Mickel, 1952, a new species, and comparison with *Pertyella* Mickel, 1952 (Hymenoptera: Mutillidae). Zootaxa 4789(2): 466–480. https://doi.org/10.11646/zootaxa.4789.2.6

Cambra RA, Quintero D (1993) Studies on *Timulla* Ashmead (Hymenoptera: Mutillidae): New distribution records and synonymies, and descriptions of previously unknown allotypes. The Pan-Pacific Entomologist 69(4): 299–313. https://biodiversitylibrary.org/page/56147381

Cambra RA, Quintero D (2007) *Chilemutilla*, a new genus of Mutillidae (Hymenoptera) from Chile, and the description of the first wingless mutillid male from South America. Transactions of the American Entomological Society 133(1–2): 167–180. https://doi.org/10.3157/0002-8320(2007)133[167:CANGOM]2.0.CO;2

Cambra RA, Quintero D (2017) *Sphaeropthalma pinedai* sp. nov.: Primer registro de un mutílido braquíptero para América del Sur (Hymenoptera: Mutillidae). Boletín Científico del Centro de Museos de la Universidad de Caldas 21(2): 220–230.

Cambra RA, Quintero D, Waldren GC, Bartholomay PR, Williams KA (2018) Taxonomic revision of the genus *Timulla* Ashmead, 1899 of Panama (Hymenoptera: Mutillidae). Tecnociencia 20(2): 23–57.

Charnov EL, Los-den Hartogh RL, Jones WT, van den Assem J (1981) Sex ratio evolution in a variable environment. Nature 289(1/8): 27–33. https://doi.org/10.1038/289027a0

Clausen CP (1976) Phoresy among entomophagous insects. Annual Review of Entomology 21: 343–367. https://doi.org/10.1146/annurev.en.21.010176.002015

Contreras R (1993) Captura y cría de los machos desconocidos de cinco géneros de mutílidas de Panamá (Hymenoptera: Mutillidae) mediante técnicas experimentales. Tesis de Licenciatura, Universidad de Panamá, Panamá, 63 pp.

Cottrell RG (1936) The biology of *Dasymutilla bioculata* (Cresson). MS Thesis, University of Minnesota, St. Paul, 42 pp.

Crèvecoeur A (1930) Recherches biologiques sur *Smicromyrme* (*Mutilla*) *rufipes* F. (Hym., Mutillidae). Bulletin and Annales de la Société Entomologique de Belgique 70: 271–284.

Deyrup M, Manley DG (1986) Sex-biased size variation in velvet ants (Hymenoptera: Mutillidae). Florida Entomologist 69(2): 327–335. https://doi.org/10.2307/3494937

Drewsen C (1847) *Mutilla europaea* Linné. Stettiner Entomologische Zeitung 8: 210–211. https://biodiversitylibrary.org/page/33704591

Evans HE (1969) Phoretic copulation in Hymenoptera. Entomological News 80: 113–124. https://biodiversitylibrary.org/page/2740524

Fattig PW (1936) An unusual mating of velvet ants (Hymen.: Mutillidae). Entomological News 47: 51–52. https://biodiversitylibrary.org/page/2597089

Ferguson WE (1962) Biological characteristics of the mutillid subgenus *Photopsis* Blake and their systematic values (Hymenoptera). University of California Publications in Entomology 27(1): 1–92.

Gordh G (1990) *Apenesia evansi* sp.n. (Hymenoptera: Bethylidae) from Australia with comments on phoretic copulation in bethylids. Journal of the Australian Entomological Society 29: 167–170. https://doi.org/10.1111/j.1440-6055.1990.tb00341.x

Hennessey RD (2002) Population-level characteristics of *Dasymutilla nigripes*, *D. vesta*, and *Timulla vagans* (Hymenoptera: Mutillidae). Florida Entomologist 85(1): 245–253. https://doi.org/10.1653/0015-4040(2002)085[0245:PLCODN]2.0.CO;2

Hennessey RD, West SA (2018) Reproductive strategies of diurnal mutillid wasps (Hymenoptera: Mutillidae). Contributions in Science 526: 181–188. http://zoo-web02.zoo.ox.ac.uk/group/west/pdf/Hennessey&West_2018.pdf

Hoffer E (1886) Zur biologie der *Mutilla europaea* L. Zoologische Jahrbücher. Zeitschrift für Anatomie und Biologie der Thiere 1: 679–686. https://biodiversitylibrary.org/page/9960310

Hurd Jr PD (1951) The California velvet ants of the genus *Dasymutilla* Ashmead (Hymenoptera: Mutillidae). Bulletin of the California Insect Survey 1(4): 89–118. [plate 10] https://essig.berkeley.edu/documents/cis/cis01_4.pdf

Hurlbutt B (1987) Sexual size dimorphism in parasitoid wasps. Biological Journal of the Linnean Society 30: 63–89. https://doi.org/10.1111/j.1095-8312.1987.tb00290.x

Jellison WL (1982) Concentrations of mutillid wasps (Hymenoptera: Mutillidae). Entomological News 93(1): 27–28. https://www.biodiversitylibrary.org/item/20655#page/271/mode/1up

Krombein KV (1956) Biological and taxonomic notes on the wasps of Lost River State Park, West Virginia, with additions to the faunal list (Hymenoptera, Aculeata). Proceedings of the Entomological Society of Washington 58(3): 153–161. https://biodiversitylibrary.org/page/16196792

Krombein KV (1967) Trap-Nesting Bees and Wasps: Life Histories, Nests, and Associates. Smithsonian Institution Press, Washington, 570 pp. https://doi.org/10.5962/bhl.title.46295

Krombein KV (1979) Family Mutillidae. In: Krombein KV, Hurd Jr PD, Smith DR, Burks BD (Eds) Catalog of Hymenoptera in America north of Mexico. Smithsonian Institution Press, Washington, 1276–1314. https://biodiversitylibrary.org/page/4575397

Krombein KV, Norden BB (1996) Behavior of nesting *Episyron conterminus posterus* (Fox) and its cleptoparasite *Ephuta s. slossonae* (Fox) (Hymenoptera: Pompilidae, Mutillidae). Proceedings of the Entomological Society of Washington 98(2): 188–194. https://biodiversitylibrary.org/page/28254367

Lamborn WA (1916) Third report on *Glossina* investigations in Nyasaland. Bulletin of Entomological Research 7(1): 29–50. https://doi.org/10.1017/S0007485300017053

Lelej AS (2005) Catalogue of the Mutillidae (Hymenoptera) of the Oriental Region. Dalnauka, Vladivostok, 252 pp.

https://archive.org/details/LelejA.S.CatalogueOfTheMutillidaehymenopteraOfTheOrientalRegion

Linsley EG (1960) A fragmentary observation on the mating behavior of *Timulla* (Hymenoptera: Mutillidae). The Pan-Pacific Entomologist 36(1): 1–36. https://biodiversitylibrary.org/page/53497042

Linsley EG, MacSwain JW, Smith RF (1955) Observations on the mating habits of *Dasymutilla formicalia* Rohwer (Hymenoptera: Mutillidae). The Canadian Entomologist 87: 411–413. https://doi.org/10.4039/Ent87411-9

Lo Cascio P (2015) Worldwide checklist of the island mutillid wasps (Hymenoptera Mutillidae). Biodiversity Journal 6(2): 529–592. http://www.biodiversityjournal.com/contents2015_2.html#monograph3

Luz DR, Waldren GC, Melo GAR (2016) Bees as hosts of mutillid wasps in the Neotropical region (Hymenoptera, Apidae, Mutillidae). Revista Brasileira de Entomologia 60(4): 302–307. https://doi.org/10.1016/j.rbe.2016.06.001

Manley DG (1977) Notes on the courtship and mating of *Dasymutilla* Ashmead (Hymenoptera: Mutillidae) in California. The Southwestern Naturalist 21(4): 552–554. https://www.jstor.org/stable/30054044

Manley DG, Deyrup MA (1989) Notes on the biology of *Dasymutilla pyrrhus* (Fox) (Hymenoptera: Mutillidae). Journal of Entomological Science 24(1): 53–56. https://doi.org/10.18474/0749-8004-24.1.53

Manley DG, Pitts JP (2007) Tropical and subtropical velvet ants of the genus *Dasymutilla* Ashmead (Hymenoptera: Mutillidae) with descriptions of 45 new species. Zootaxa 1487: 1–128. https://doi.org/10.11646/zootaxa.1487.1.1

Matteini Palmerini M (1992 [“1989”]) Mutillidae del Museo Civico di Storia Naturale di Verona (Hymenoptera). Bollettino del Museo Civico di Storia Naturale di Verona 16: 187–226.

Matteini Palmerini M (2013) Remarks on the behaviour of the velvet ant *Nemka viduata* (Pallas, 1773) (Insecta Hymenoptera Mutillidae). Quaderno di Studi e Notizie di Storia Naturale della Romagna 37: 237–260. http://www.ssnr.it/37-18.pdf

Matthews RW (1997) Unusual sex allocation in a solitary parasitoid wasp, *Sphaeropthalma pensylvanica* (Hymenoptera: Mutillidae). The Great Lakes Entomologist 30: 51–54. https://scholar.valpo.edu/tgle/vol30/iss1/5

Mickel CE (1924) An analysis of a bimodal variation in size of the parasite *Dasymutilla bioculata* Cresson (Hymen.: Mutillidae). Entomological News 35: 236–242. https://www.biodiversitylibrary.org/item/20269#page/292/mode/1up

Mickel CE (1937) The mutillid wasps of the genus *Timulla* which occur in North America north of Mexico. Entomologica Americana 37(1): 1–56. [37(2): 57–119.] https://www.biodiversitylibrary.org/item/205663#page/13/mode/1up

Mickel CE (1938) Photopsoid mutillids collected by Dr. K. A. Salman at Eagle Lake, California. The Pan-Pacific Entomologist 14(4): 178–185. https://biodiversitylibrary.org/page/53412159

Monastra A (1989) Nuovi dati sui Mutillidi et Mirmosidi italiani. 1. Mutillidae Myrmillinae Bisch. (Hymenoptera). Bollettino della Società Entomologica Italiana 121: 204–212.

Nonveiller G (1963) Quelle est la cause de la rareté des Mutillides? Résultats de l’etudede certains de leur caractéres biologiques et écologiques (Hymenoptera, Mutillidae). Memorie della Società Entomologica Italiana 42: 24–57.

Nonveiller G (1980) Recherches sur les Mutillides de l’Afrique (Hymenoptera, Mutillidae). X. Bref aperçu des résultats des recherches sur la faune des Mutillides du Cameroun, effectuées au cours de la période de 1962–1975. Memoires publies par l’Institut pour la Protection des Plantes, Beograd 14: 11–68.

O’Neill KM (1985) Egg size, prey size, and sexual size dimorphism in digger wasps (Hymenoptera: Sphecidae). Canadian Journal of Zoology 63: 2187–2193. https://doi.org/10.1139/z85-323

Osten T (1999) The phoretic copulation of Thynninae in an ecological and evolutionary perspective. Linzer biologische Beiträge 31(2): 755–762. https://www.zobodat.at/publikation_articles.php?id=787

O’Toole C (1975) The systematics of *Timulla oculata* (Fabricius) (Hymenoptera, Mutillidae). Zoologica Scripta 4(5–6): 229–251. https://doi.org/10.1111/j.1463-6409.1975. tb00733.x

Pagden HT (1934) Biological notes on some Malayan aculeate Hymenoptera I. (Sphecoidea and Vespoidea). Journal of the Federated Malay States Museums 17: 458–466.

Pagden HT (1938) On a new species of *Rhopalomutilla* (Hym. Mutillidae) from Java. Journal of the Federated Malay States Museums 18(2): 213–217.

Pate VSL (1947) A conspectus of the Tiphiidae, with particular reference to the Nearctic forms (Hymenoptera, Aculeata). Journal of the New York Entomological Society 55(2): 115– 145. [plates VI–VII] https://www.jstor.org/stable/25005210

Pitts JP, Matthews RW (2000) Description of the larva of *Sphaeropthalma pensylvanica* (Lepeletier) (Hymenoptera: Mutillidae: Sphaeropthalminae). Journal of Entomological Science 35(3): 334–337. https://doi.org/10.18474/0749-8004-35.3.334

Pitts JP, Sadler EA (2015) Description of a new species and species-group of *Sphaeropthalma* Blake (Hymenoptera: Mutillidae) with an updated classification of the genus. Zootaxa 3947(2): 282–288. https://doi.org/10.11646/zootaxa.3947.2.10

Pitts JP, Tanner DA, Waldren GC, Parker FD (2010a) Facultative size-dependent sex allocation in *Sphaeropthalma pensylvanica* Lepeletier (Hymenoptera: Mutillidae) with further host records. Journal of the Kansas Entomological Society 83(1): 68–75. https://doi.org/10.2317/JKES0812.15.1

Pitts JP, Wilson JS, von Dohlen CD (2010b) Evolution of the nocturnal Nearctic Sphaeropthalminae velvet ants (Hymenoptera: Mutillidae) driven by Neogene orogeny and Pleistocene glaciations. Molecular Phylogenetics and Evolution 56: 134–145. https://doi.org/10.1016/j.ympev.2010.03.033

Polidori C, Beneitez A, Asís JD, Tormos J (2013) Scramble competition by males of the velvet ant *Nemka viduata* (Hymenoptera: Mutillidae). Behaviour 150: 23–37. https://doi.org/10.1163/1568539X-00003035

Quintero D, Cambra RA (2001) On the identity of *Scaptopoda* F. Lynch Arribalzaga, new taxonomic changes and new distribution records for Neotropical Mutillidae (Hymenoptera), with notes on their biology. Transactions of the American Entomological Society 127(3): 291–304. https://www.jstor.org/stable/25078749

Remington CL (1944) The relationship of *Dasymutilla permista* Mickel to *Dasymutilla quadriguttata* (Say) and behavior notes on the species. Annals of the Entomological Society of America 37(2): 198–200. https://doi.org/10.1093/aesa/37.2.198

Rothney GAJ (1903) The aculeate Hymenoptera of Barrackpore, Bengal. Transactions of the Entomological Society of London 1903: 93–116. https://doi.org/10.1111/j.1365-2311.1903. tb01128.x

Saxton SM (2010) Mating behaviour of *Myrmosa atra* (Hymenoptera: Mutillidae) with a consideration of the adaptive significance of long copulation duration. British Journal of Entomology and Natural History 23: 33–37.

Schulmeister S (2001) Functional morphology of the male genitalia and copulation in lower Hymenoptera, with special emphasis on the Tenthredinoidea s. str. (Insecta, Hymenoptera, ‘Symphyta’). Acta Zoologica 82: 331–349. https://doi.org/10.1046/j.1463-6395.2001.00094.x

Shappirio DG (1947a) Observations on wasps. The Scientific Monthly 64(4): 348–350. https://www.jstor.org/stable/19377

Shappirio DG (1947b) Observations on the biology of some mutillid wasps (Hymenoptera, Mutillidae). Bulletin of the Brooklyn Entomological Society 42(5): 162–163. https://biodiversitylibrary.org/page/50582603

Sheldon JK (1970) Sexual dimorphism in the head structure of Mutillidae Hymenoptera: A possible behavioral explanation. Entomological News 81(3): 57–61. https://biodiversitylibrary.org/page/2740852

Skorikov AS (1935) Zur Mutilliden-fauna Zentralasiens. Trudy Tadzhikskoi Bazy, Akademiya Nauk SSSR 5: 257–349.

Spangler HG, Manley DG (1978) Sounds associated with the mating behavior of a mutillid wasp. Annals of the Entomological Society of America 71(3): 389–392. https://doi.org/10.1093/aesa/71.3.389

Su W, Liang C, Ding G, Jiang Y, Huang J, Wu J (2019) First record of the velvet ant *Mutilla europaea* (Hymenoptera: Mutillidae) parasitizing the bumblebee *Bombus breviceps* (Hymenoptera: Apidae). Insects 10: 1–9. https://doi.org/10.3390/insects10040104

Tomberlin JK (1997) Mating behavior of *Dasymutilla occidentalis* (Hymenoptera: Mutillidae). Entomological News 108(4): 310–317. https://biodiversitylibrary.org/page/16343807

Tormos J, Asís JD, Polidori C, Benéitez A, Storino G (2010) The mating behaviour of the velvet ant, *Nemka viduata* (Hymenoptera: Mutillidae). Journal of Insect Behavior 23: 117–127. https://doi.org/10.1007/s10905-009-9200-5

Tu BB, Lelej AS, Chen XX (2014) Review of the genus *Cystomutilla* André, 1896 (Hymenoptera: Mutillidae: Sphaeropthalminae: Sphaeropthalmini), with description of the new genus *Hemutilla* gen. nov. and four new species from China. Zootaxa 3889(1): 71–91. https://doi.org/10.11646/zootaxa.3889.1.4

van den Assem J, van Iersel JJA, Los-den Hartogh RL (1989) Is being large more important for female than for male parasitic wasps? Behaviour 108(1–2): 160–195. https://doi.org/10.1163/156853989X00114

Vivallo F (2020) Phoretic copulation in Aculeata (Insecta: Hymenoptera): A review. Zoological Journal of the Linnean Society, zlaa069. https://doi.org/10.1093/zoolinnean/zlaa069

CHAPTER IV

PHYLOGENOMICS AND BIOGEOGRAPHY OF THE
COSMOPOLITAN VELVET ANT TRIBE
TROGASPIDIINI
(HYMENOPTERA: MUTILLIDAE: MUTILLINAE)[3]

Abstract

A phylogenomic and biogeographic study of the velvet ant tribe Trogaspidiini (Hymenoptera: Mutillidae: Mutillinae) was conducted using ultraconserved elements (UCEs). Thirty-six of the forty-four described trogaspidiine genera and subgenera were represented using 95 ingroup taxa. An additional 13 putative genera were recognized during the course of this study and were included in the analyses. The monophyly of the exclusively New World genus *Timulla* Ashmead with respect to the Old World trogaspidiine fauna was tested using 40 *Timulla* exemplars. The maximum-likelihood criterion (ML) and the maximum-parsimony criterion (MP) were used to infer the phylogeny of the tribe using an aligned data set of 1,148,582 characters; the topologies of these respective analyses were largely congruent but differed in several key areas. Trogaspidiini was recovered as monophyletic, and *Dolichomutilla* is further confirmed as a member of Mutillini. Members of Petersenidiini were recovered throughout Trogaspidiini; Petersenidiini is consequently confirmed as a synonym of Trogaspidiini. *Timulla* was recovered as monophyletic and sister-goup to a clade of Afrotropical and

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[3] This chapter has been formatted for submission to *Cladistics*.

Oriental trogaspidiines. *Trogaspidia* Ashmead (*sensu stricto*) was recovered as polyphyletic; consequently, four subgenera of Afrotropical *Trogaspidia* recognized by Nonveiller (1995b) are raised to the genus level: *Arcuatotropidia* Nonveiller, **stat. nov.**, *Chilotropidia* Nonveiller, **stat. nov.**, *Inflatispidia* Nonveiller, **stat. nov.**, and *Lobotropidia* Nonveiller, **stat. nov.** Further, *Trogaspidia* (*Acutitropidia* Nonveiller) is maintained as a subgenus of *Trogaspidia* (*sensu stricto*) pending further study. Lastly, the ancestral areas for Trogaspidiini and *Timulla* were inferred using the Bayesian Binary MCMC criterion (BBM) in order to gain insight into their biogeographic history. The Trogaspidiini were inferred to be Afrotropical in ancestry, with multiple dispersal events between the Afrotropical and Oriental regions. *Timulla* was inferred to be Afrotropical in ancestry based on the ML tree and Oriental in ancestry based on the MP cladogram. *Timulla* was inferred to have emerged 7.65/6.01 Ma. The climate of Beringia at this time was likely not amenable for dispersal for members of this primarily tropical tribe, which suggests that jump dispersal was the method of arrival into the New World.

Introduction

Trogaspidiini (Hymenoptera: Mutillidae: Mutillinae) is a species-rich tribe of velvet ants composed of 39 genera, 5 subgenera, and 928 valid species (Table 4.1) (Brothers and Lelej, 2017; Lelej, 2020; Pagliano et al., 2020; Okayasu et al., 2021). Members of this tribe represent 20.4% of all described species of Mutillidae (928 of 4,551 species, excluding Myrmosidae) (Lelej, 2020; Pagliano et al., 2020; Waldren et al., 2020b; Bartholomay et al., 2021; Okayasu et al., 2021). Further, trogaspidiines are the only cosmopolitan group of mutillids and occur in all six biogeographical regions (Pagliano et al., 2020). The tribe is remarkable in that they are one of several mutillid lineages that practice phoretic copulation—specifically mandibular phoretic copulation—wherein a larger male will intentionally carry a smaller conspecific female by flight and/or foot primarily by grasping her by the pronotal collar with his mandibles before settling on a substrate to mate (Waldren et al., 2020a). It has been hypothesized that the behavior of phoretic copulation may allow the apterous females to traverse otherwise impassable physical barriers, such as bodies of water (Evans, 1969). This behavior may have played a significant role in the worldwide dispersal of Trogaspidiini, whose members are apparently obligate practitioners of mandibular phoretic copulation (Waldren et al., 2020a). Due to the cosmopolitan distribution of this tribe and relative morphological similarity between its members, there has been debate over whether the New World and Old world faunas are monophyletic with respect to each other.

The Timulla/Trogaspidia controversy

The generic classification of trogaspidiines has been contentious throughout much of its taxonomic history since its first component genera, *Timulla* and *Trogaspidia*, were described by Ashmead (1899). Due to the cosmopolitan distribution of Trogaspidiini, these genera have more or less served as geographic delimiters for the trogaspidiine faunas of the Eastern and Western Hemispheres: *Trogaspidia* are the Old World trogaspidiines, and *Timulla* are the New World trogaspidiines.

A historical review of the controversy involving these genera was provided by Nonveiller (1995b), which is reiterated and expanded upon here. Shortly after Ashmead (1899) described *Timulla* and *Trogaspidia*, as well as proposing his classification for

Mutillidae (Ashmead, 1900–1904), André (1899–1903, 1902, 1904) critiqued the classification proposed by Ashmead (1899). Further, André rejected a number of Ashmead's new genera, including *Timulla* and *Trogaspidia*. André synonymized these latter two genera with the artificially large, catch-all genus *Mutilla* Linnaeus. However, André's views were not followed by other researchers. In his monographic revision of the Afrotropical mutillids, Bischoff (1920–1921) erected Trogaspidiini, treated *Trogaspidia* as a genus, and described seven additional trogaspidiine genera (*Aureotilla*, *Chrysotilla*, *Glossotilla*, *Lobotilla*, *Lophotilla*, *Spinulotilla*, and *Trispilotilla*). Shortly after Bischoff's revision, Bradley and Bequaert (1923, 1928) expanded on Bischoff's study of the Afrotropical mutillid fauna and treated *Trogaspidia* as a subgenus of *Smicromyrme* Thomson. This action was rejected by Mickel (1933), who noted the significant differences in male morphology that distinguish *Smicromyrme* and *Trogaspidia*. Further, Mickel's (1933) view of the relationship between *Timulla* and *Trogaspidia* had been a matter of contention that lasted for decades:

> "Although there are good structural grounds for maintaining *Trogaspidia* distinct from *Smicromyrme*, I have been unable to find any for distinguishing *Trogaspidia* from the New World genus *Timulla*. […] I am inclined to view that *Timulla* (that name having priority) is the only mutillid genus having a world-wide distribution, the Palearctic, Ethiopian, Oriental and Australian representatives having formerly been regarded as belonging to *Trogaspidia*. For convenience sake I still retain *Trogaspidia* here as of subgeneric rank, but purely on geographical grounds."

Mickel's concepts of *Smicromyrme* and *Timulla*/*Trogaspidia*, however, were broad by today's standards; the characters he used to separate these genera are now regarded as tribal-level differences for Smicromyrmini and Trogaspidiini, respectively (Lelej, 1996, 2002, 2005). The context in which Mickel viewed *Timulla* and *Trogaspidia* must be kept in mind. At the time, these two genera together essentially constituted the majority of the tribe Trogaspidiini and Mickel's concept of *Timulla* (*sensu lato*) was synonymous with Trogaspidiini. Several researchers followed Mickel's stance by using *Timulla* for Old World trogaspidiine species (Pagden, 1949; Krombein, 1971, 1972; Brothers, 1975). Others treated *Trogaspidia* as its own genus (Invrea, 1953, 1964; Chen, 1957; Suárez, 1969; Nonveiller, 1995b; Lelej, 1996). Invrea (1953) boldly claimed that the two genera are readily distinguishable. The close relationship of *Timulla* and *Trogaspidia* (*sensu lato*) is apparent; however, the monophyly of these genera has never been tested through phylogenetic inference. In the comprehensive cladistic study of the higher classification of Mutillidae by Brothers and Lelej (2017), the authors treated their terminals at the genus level, which precluded the ability to test if *Timulla* is monophyletic. Given that members of this tribe are obligate practitioners of mandibular phoretic copulation (Waldren et al., 2020a), and there may have been multiple dispersal events between the hemispheres, it is unclear based on morphology whether the Old World and New World trogaspidiine faunas are reciprocally monophyletic. A phylogenetic analysis using molecular data to answer this question is, therefore, desirable.

The current state of Trogaspidiini taxonomy

Trogaspidia was split into numerous genera in the mid-1990s based on male morphology. Nonveiller (1995b, 1996) treated the Afrotropical *Trogaspidia* and allocated 150 species into twelve genera and subgenera; sixteen species remained unclassified. Lelej (1996, 2002, 2005) treated the Palaearctic and Oriental *Trogaspidia* and allocated numerous species into seventeen genera; however, over 123 species remained in *Trogaspidia* (*sensu lato*) (Lelej, 2002, 2005). The New World fauna, represented solely by *Timulla*, has yet

Table 4.1. Data for the genera of Trogaspidiini

Genus	Biogeographic Region	In Study	Sexes Known	Species Described
Acanthomutilla Nonveiller, 1995a	AFR		♂♀	1
Allotropidia Nonveiller, 1996	AFR	X	♂	1
Amblotropidia Nonveiller, 1995b	AFR	X	♂♀	21
Artiotilla Invrea, 1950	PAL	X	♂♀	3
Aureotilla Bischoff, 1920	AFR: M	X	♂♀	6
Carinotilla Nonveiller, 1973	AFR	X	♂♀	16
Chrysotilla Bischoff, 1920	AFR: M	X	♂♀	17
Curvitropidia Nonveiller, 1995b	AFR, AUS		♂♀	4
Dentotilla Nonveiller, 1977	AFR	X	♂♀	15
Diacanthotilla Nonveiller, 1995a	AFR		♀	1
Eotrogaspidia Lelej, 1996	ORI, PAL	X	♂♀	10
Glossotilla Bischoff, 1920	AFR	X	♂♀	68
Hildbrandetia Özdikmen, 2005	AFR: M		♀	1
Karlissaidia Lelej, 2005	ORI	X	♂♀	4
Krombeinidia Lelej, 1996	ORI	X	♂♀	27
Lobotilla Bischoff, 1920	AFR, PAL	X	♂♀	7
Lophotilla Bischoff, 1920	AFR	X	♂	9
Neotrogaspidia Lelej, 1996	ORI, PAL	X	♂♀	4
Nonveilleridia Lelej, 1996	AFR:M, ORI	X	♂	1
Orientidia Lelej, 1996	ORI, PAL	X	♂♀	10
Pagdenidia Lelej, 1996	ORI	X	♂♀	7
Petersenidia Lelej *in*: Lelej & Yamane, 1992	ORI	X	♂♀	44
Promecidia Lelej, 1996	ORI	X	♂♀	11
Protrogaspidia Lelej, 1996	ORI		♂	2
Pseudolophotilla Nonveiller & Ćetković, 1995	AFR: M	X	♂♀	3
Radoszkowskitilla Lelej, 2005	ORI		♂♀	3
Serendibiella Lelej, 2005	ORI	X	♂	1
Seriatospidia Nonveiller & Ćetković, 1996	AFR		♀	5
Spinulomutilla Nonveiller, 1994	AFR, PAL	X	♂♀	24
Spinulotilla Bischoff, 1920	AFR	X	♂♀	7
Sylvotilla Viette, 1978	AFR: M		♀	4
Taiwanomyrme Tsuneki, 1993	ORI, PAL	X	♂♀	6
Timulla Ashmead, 1899	NEA, NEO	X	♂♀	180
Trispilotilla Bischoff, 1920	AFR, AFR: M, ORI	X	♂♀	22

Trogaspidia (*Acutitropidia*) Nonveiller, 1995b	AFR	X	♂♀	16
Trogaspidia (*Arcuatotropidia*) Nonveiller, 1995b	AFR	X	♂	1
Trogaspidia (*Chilotropidia*) Nonveiller, 1995b	AFR	X	♂♀	4
Trogaspidia (*Inflatispidia*) Nonveiller, 1995b	AFR	X	♂♀	4
Trogaspidia (*Lobotropidia*) Nonveiller, 1995b	AFR	X	♂♀	3
Trogaspidia (*Trogaspidia*) Ashmead, 1899	AFR	X	♂♀	25
Trogaspidia (=Trogaspidiini *incertae sedis*)	MISC	X	♂♀	292
Tuberocoxotilla Nonveiller, 1980	AFR	X	♂	2
Vanhartenidia Lelej *in*: Lelej & van Harten, 2006	AFR, ORI, PAL		♂♀	10
Wallacidia Lelej & Brothers, 2008	AUS, ORI, PAL	X	♂♀	22
Zavatilla Tsuneki, 1993	ORI	X	♂♀	4

TOTAL:	**928**

to be critically investigated as a whole since the monographic revisions of Mickel (1937, 1938).

Both *Timulla* and *Trogaspidia* (*sensu lato*) are principally diagnosed by a number of male primary and secondary sexual characteristics. The primary sexual characteristics include the frequent presence of a paracuspis on the volsella, as well as asymmetrical penial valves. The secondary sexual characteristics include the presence of a basoventral mandibular tooth, clypeal modifications, occasional expansion of the antennal scape and basal flagellomeres, "mesosternal" protuberances, mesocoxal tubercles, a pygidial process, and protuberances on the apical metasomal sterna (Mickel, 1933, 1937, 1938; Chen, 1957; Nonveiller, 1995b). There exists, however, a group of eight trogaspidiine genera with males that largely lack these primary and secondary sexual modifications. The subtribe Petersenidiina was erected by Lelej (1996) to account for these relatively unmodified males, with Trogaspidiina composed of males with the aforementioned modifications. Petersenidiina was eventually raised to the tribe level, Petersenidiini, alongside Trogaspidiini (Lelej, 2002, 2005). The cladistic analyses of Brothers and Lelej (2017) found Petersenidiini to be nested within Trogaspidiini and was consequently synonymized with the latter.

Historical biogeography

The cosmopolitan distribution of Trogaspidiini makes it an ideal taxon for investigation of its historical biogeography. The age of Trogaspidiini was inferred as 13.17/13.43 Ma (mid-Miocene) in a study of the higher classification of Mutillidae (Waldren et al., in prep.). The continents at this time were more or less configured as they are today and this relatively young age inferred for a species-rich taxon suggests that dispersal played an important role in the current distribution of the tribe. Oceanic dispersal via flight and/or wind carriage is known to occur in insects and other arthropods (Holzapfel and Harrell 1968). The occurrence of endemic aculeate Hymenoptera on remote volcanic islands demonstrates that long-distance dispersal and subsequent colonization is possible for Aculeata. The Hawaiian Islands form an archipelago of volcanic origin located roughly 3,700 km from the western coast of North America, its nearest continent. These islands are home to seven endemic lineages of solitary aculeate Hymenoptera: *Deinomimesa* Perkins (Crabronidae), *Ectemnius* Dahlbom (Crabronidae), *Euodynerus* Dalla Torre (Vespidae), *Hylaeus* (*Nesoprosopis* Perkins) (Colletidae), *Nesodynerus* Perkins (Vespidae), *Nesomimesa* Malloch (Crabronidae), and *Sierola* Cameron (Bethylidae) (Yoshimoto, 1959, 1960; Magnacca and Danforth, 2006; Carpenter, 2008; Azevedo, et al. 2018). Notably, there are no endemic ant species (Formicidae) despite their worldwide ubiquity. Other than the arrival of these insects via phoresy, which is defined as an interaction between two or more animals in which one individual carries the other(s) for purpose of travel, the presence of these endemic aculeates on volcanic islands would have to be due to jump dispersal. Taxa for which there is evidence of long-distance colonization in the Pacific Ocean generally originate from the east Pacific to the west in congruence with the direction of trade winds and storms (Gillespie et al., 2012). In apparent evidence that west to east Pacific colonization can occur, Magnacca and Danforth (2006) recovered two Japanese species of *Hylaeus* (*Nesoprosopis*) as sister-goup to all of the Hawaiian species in a phylogenetic study of the subgenus. These findings have relevant implications for the biogeographical history of Trogaspidiini.

The travel distance ability for mutillids has not yet been determined through quantitative testing; however, several examples are known for which mutillids have traversed water barriers. A modern example of mutillid dispersal capability is illustrated through the trogaspidiine *Wallacidia melmora* (Cameron), which was one of the first mutillid species recorded from the islands of Krakatau after the cataclysmic volcanic eruption of 1883 (Dammerman, 1948; O'Toole, 1975). Additionally, the occurrence of *Timulla trimaculosa* Mickel in Jamaica, a species likely synonymous with the Panamanian and Colombian species *Timulla centroamericana* (Dalla Torre) (Waldren, pers. obs.), demonstrates the ability of trogaspidiines to travel remarkable distances.

Another possible dispersal avenue that would have been available to trogaspidiines during the mid-Miocene was an intercontinental corridor between the Old and New World. The Bering land bridge (= Beringia) connected eastern Asia to western North America from the late Cretaceous to the late Pliocene (100–3.5 Ma), with several subsequent reunions during the Pleistocene due to the lowering of sea levels through glaciation (Sanmartín et al., 2001). This land bridge was responsible for the movement of a number of different terrestrial taxa between the hemispheres, such as mammals and amphibians (Repenning, 1967; Li et al., 2015). A group of aculeate wasps of relatively similar age to Trogaspidiini and also a member of Pompiloidea—the pompilid tribe Aporini—were recently inferred to have dispersed from western North America to eastern Asia via the Bering land bridge (Rodriguez et al., 2014). Another corridor, the North Atlantic land bridge, connected western Europe to eastern North America up until the early Eocene (50 Ma) (Tiffney, 1985; Sanmartín et al., 2001). However, the age of the North Atlantic land bridge (≥50 Ma) significantly predates the estimated age of

Trogaspidiini (13.17/13.43 Ma) and thus could not have been used as a dispersal route. An additional land bridge relevant to the biogeography of Trogaspidiini—specifically the exclusively New World genus *Timulla*—is the Isthmus of Panama. The closure of the Isthmus of Panama, which resulted in a unified North and South America, has been estimated to have closed as late as 3.1 Ma (Keigwin, 1978) to as early as 23–25 Ma (Farris et al., 2011).

Phylogeny and biogeography of Trogaspidiini

The purpose of this study is to test the monophyly of the New World trogaspidiine genus *Timulla* in relation to the Old World trogaspidiine fauna. Additionally, we test the relationships between the numerous Old World genera of Trogaspidiini. Further, the validity of Petersenidiini is tested, given the notably conservative nature of their morphology in contrast to Trogaspidiini. Lastly, ancestral areas were inferred for Trogaspidiini to gain insight in the tribe's historical biogeography to answer the following questions: were there multiple dispersal events into the New World from the Old World? Additionally, were there multiple dispersal events between North America and South America? A phylogenomic approach using ultraconserved elements (UCEs) was used to answer these questions. UCEs are highly-conserved regions of the genome that are shared among distantly-related taxa. Each UCE is flanked by variable sites that provide phylogenetic signal, and the UCE itself, while also informative, additionally provides a series of shared character states between taxa. The function of UCEs in the genome is unknown, but there is evidence that they are involved in gene regulation (Pennachio et al., 2006) and development (Sandelin et al., 2004; Woolfe et al., 2004).

UCEs have recently been used to infer the phylogeny of Aculeata (Branstetter et al., 2017a) and have become a popular choice for inferring phylogenies across many different taxa.

Materials and Methods

Taxon sampling

Specimens representing 113 taxa were chosen for the study with 95 ingroup taxa (Trogaspidiini) and 18 outgroup taxa (other Mutillidae). Thirty-six of the forty-four described trogaspidiine genera and subgenera were represented using an exemplar for each genus. *Timulla* was represented by 40 species that encompassed the species-group level diversity of the genus (Table 4.2). Suitable specimens were not available for nine genera: *Acanthomutilla* Nonveiller, *Curvitropidia* Nonveiller, *Diacanthotilla* Nonveiller, *Karlissaidia* Lelej, *Protrogaspidia* Lelej, *Radoszkowskitilla* Lelej, *Seriatospidia* Nonveiller and Ćetković, *Sylvotilla* Viette, and *Vanhartenidia* Lelej (Tables 4.1 and 4.2). Specimens were identified to genus and/or species primarily using Mickel (1937, 1938), Lelej (2002, 2005), and Nonveiller (1995b). Trogaspidiini was reevaluated as a whole at the genus level with emphasis on males; thirteen putative genera from the Old World were consequently discovered. These putative genera are denoted with a prefix indicative of their biogeographical region (AFR–Afrotropical; MAD–Malagasy; ORI–Oriental) followed by a unique number (e.g., ‘AFR_gen_1’). Outgroup data were sourced for one taxon from Sadler (2018). An additional 22 ingroup and outgroup taxa were sourced from a study of the higher classification of Mutillidae (Waldren et al., in prep.). All samples used were dried, pinned museum specimens of various ages collected within the last 50 years, with the oldest specimen collected in 1973. Each specimen was assigned a unique specimen identifier (USI) with the prefix TIM, MUT, EX, or U depending on the taxon and the location where the lab work was conducted (Table 4.2). Specimens from which new molecular data were acquired for this study are deposited at the Entomological Museum of Utah State University (EMUS) (Logan, Utah, USA).

Molecular data acquisition

DNA extraction was performed using a High Pure PCR Template Preparation Kit (Roche Diagnostics, Indianapolis, IN, USA). Entire specimens were primarily used for extraction, except for rare species in which a single mid leg and hind leg were removed and partly crushed. The entry point for extraction material into the specimens was typically the resulting pin hole in the mesosoma after removing the pin. Specimens were remounted after extraction to allow for future study. Extracted DNA was quantified using a Qubit 3.0 Fluorometer. The following protocols for UCE molecular work were derived from Branstetter et al. (2017a) and were performed as follows. The extracted DNA was prepared for shearing to a target concentration of 50 ng/100 µL. The samples were then sheared/fragmented to a range between 400–600 base pairs using a Qsonica Q800R2. Library preparation was performed using a Kapa library preparation kit (Kapa Biosystems Inc., Wilmington, MA, USA). Following library prepation, PCR was performed with a thermal cycler set to 98°C/45 sec, 14 cycles of 98°C/15 sec, 60°C/30 sec, 72°C/60 sec, 72°C/5 min, and 4°C hold; samples were then quantified. Libraries were pooled at equimolar ratios of 10 samples and adjusted pool concentrations to 72 ng/100 µL. This resulted in 500 ng of DNA used for targeted UCE enrichments.

Table 4.2. Voucher data for specimens used in the Trogaspidiini study

Mutillidae subfamily: tribe	**Species**	**ID**
Dasylabrinae	*Stenomutilla argentata*	MUT010
Mutillinae: Ctenotillini	*Ctenotilla guangdongensis*	EX1787
Mutillinae: Ctenotillini	*Mimecomutilla renominanda*	MUT021
Mutillinae: Mutillini	*Dolichomutilla* sp.	EX1796
Mutillinae: Mutillini	*Ronisia brutia*	MUT029
Mutillinae: Mutillini	*Tropidotilla litoralis*	MUT030
Mutillinae: Psammothermini	*Antennotilla phoebe*	MUT033
Mutillinae: Psammothermini	*Psammotherma cyanochroa*	MUT038
Mutillinae: Psammothermini	*Pseudocephalotilla* sp.	MUT039
Mutillinae: Smicromyrmini	*Mickelomyrme* sp.	MUT036
Mutillinae: Smicromyrmini	*Nemka viduata*	MUT035
Mutillinae: Smicromyrmini	*Physetopoda scutellaris*	EX1793
Mutillinae: Smicromyrmini	*Smicromyrme rufipes*	MUT040
Mutillinae: Zeugomutillini	*Zeugomutilla pycnopyga*	MUT024
Mutillinae: Trogaspidiini	AFR gen. 1 sp.	TIM078
Mutillinae: Trogaspidiini	AFR gen. 3 sp.	TIM080
Mutillinae: Trogaspidiini	AFR gen. 4 sp.	TIM081
Mutillinae: Trogaspidiini	AFR gen. 5 sp.	TIM068
Mutillinae: Trogaspidiini	*Allotropidia acuticarinata*	TIM061
Mutillinae: Trogaspidiini	*Amblotropidia* sp.	TIM062
Mutillinae: Trogaspidiini	*Artiotilla biguttata*	TIM090
Mutillinae: Trogaspidiini	*Aureotilla madecassa*	TIM082
Mutillinae: Trogaspidiini	*Carinotilla* sp.	TIM064
Mutillinae: Trogaspidiini	*Chrysotilla* sp.	TIM083
Mutillinae: Trogaspidiini	*Dentotilla* sp.	TIM067
Mutillinae: Trogaspidiini	*Eotrogaspidia auroguttata*	TIM096
Mutillinae: Trogaspidiini	*Glossotilla suavis*	EX1795
Mutillinae: Trogaspidiini	*Karlissaidia* sp.	TIM093
Mutillinae: Trogaspidiini	*Krombeinidia* sp.	EX1801
Mutillinae: Trogaspidiini	*Lobotilla charaxus*	TIM070
Mutillinae: Trogaspidiini	*Lophotilla* sp.	TIM072
Mutillinae: Trogaspidiini	MAD gen. 1 sp.	TIM086
Mutillinae: Trogaspidiini	MAD gen. 2 sp.	TIM087
Mutillinae: Trogaspidiini	MAD gen. 3 sp.	TIM088
Mutillinae: Trogaspidiini	MAD gen. 4 sp.	TIM089
Mutillinae: Trogaspidiini	MAD gen. 5 sp.	TIM084
Mutillinae: Trogaspidiini	*Neotrogaspidia pustulata*	TIM103
Mutillinae: Trogaspidiini	*Nonveilleridia* sp.	TIM098

Mutillinae: Trogaspidiini	ORI gen. 1 sp.	TIM109
Mutillinae: Trogaspidiini	ORI gen. 2 sp.	TIM110
Mutillinae: Trogaspidiini	ORI gen. 3 sp.	TIM111
Mutillinae: Trogaspidiini	ORI gen. 5 sp.	TIM094
Mutillinae: Trogaspidiini	*Orientidia* sp.	TIM104
Mutillinae: Trogaspidiini	*Orientidia* sp.	TIM113
Mutillinae: Trogaspidiini	*Pagdenidia* sp.	EX1802
Mutillinae: Trogaspidiini	*Petersenidia hylonome*	TIM105
Mutillinae: Trogaspidiini	*Promecidia* sp.	TIM102
Mutillinae: Trogaspidiini	*Pseudolophotilla* sp.	TIM085
Mutillinae: Trogaspidiini	*Serendibiella* sp.	TIM092
Mutillinae: Trogaspidiini	*Spinulomutilla* sp.	TIM073
Mutillinae: Trogaspidiini	*Spinulotilla* sp.	TIM074
Mutillinae: Trogaspidiini	*Taiwanomyrme friekae*	TIM107
Mutillinae: Trogaspidiini	*Timulla absentia*	TIM030
Mutillinae: Trogaspidiini	*Timulla adrastis*	TIM031
Mutillinae: Trogaspidiini	*Timulla barbata*	TIM028
Mutillinae: Trogaspidiini	*Timulla baucis*	TIM045
Mutillinae: Trogaspidiini	*Timulla belti*	TIM033
Mutillinae: Trogaspidiini	*Timulla coxalis*	TIM032
Mutillinae: Trogaspidiini	*Timulla cryptica*	TIM022
Mutillinae: Trogaspidiini	*Timulla cyllene*	TIM023
Mutillinae: Trogaspidiini	*Timulla diversita*	TIM035
Mutillinae: Trogaspidiini	*Timulla dubitata*	TIM010
Mutillinae: Trogaspidiini	*Timulla duodecimmaculata*	TIM046
Mutillinae: Trogaspidiini	*Timulla ferrugata*	TIM004
Mutillinae: Trogaspidiini	*Timulla flavofasciata*	TIM048
Mutillinae: Trogaspidiini	*Timulla floridensis*	TIM008
Mutillinae: Trogaspidiini	*Timulla grotei*	TIM013
Mutillinae: Trogaspidiini	*Timulla leona*	TIM005
Mutillinae: Trogaspidiini	*Timulla manni*	TIM038
Mutillinae: Trogaspidiini	*Timulla mediata*	TIM039
Mutillinae: Trogaspidiini	*Timulla navasota*	TIM006
Mutillinae: Trogaspidiini	*Timulla neobule*	TIM042
Mutillinae: Trogaspidiini	*Timulla ocellaria*	TIM025
Mutillinae: Trogaspidiini	*Timulla ordinaria*	TIM044
Mutillinae: Trogaspidiini	*Timulla ornatipennis*	TIM115
Mutillinae: Trogaspidiini	*Timulla osberti*	TIM036
Mutillinae: Trogaspidiini	*Timulla rufogastra*	TIM047
Mutillinae: Trogaspidiini	*Timulla rufogastra*	TIM040
Mutillinae: Trogaspidiini	*Timulla rufosignata*	TIM116

Mutillinae: Trogaspidiini	*Timulla* sp. 1	TIM057
Mutillinae: Trogaspidiini	*Timulla* sp. CA01	TIM052
Mutillinae: Trogaspidiini	*Timulla* sp. CA14	TIM054
Mutillinae: Trogaspidiini	*Timulla* sp. CA16	TIM055
Mutillinae: Trogaspidiini	*Timulla* sp. CA22	TIM056
Mutillinae: Trogaspidiini	*Timulla* sp. MX09	TIM058
Mutillinae: Trogaspidiini	*Timulla spoliatrix*	TIM051
Mutillinae: Trogaspidiini	*Timulla subrobusta*	TIM037
Mutillinae: Trogaspidiini	*Timulla suspensa*	TIM027
Mutillinae: Trogaspidiini	*Timulla talus*	TIM053
Mutillinae: Trogaspidiini	*Timulla taygete*	TIM114
Mutillinae: Trogaspidiini	*Timulla tumidula*	TIM043
Mutillinae: Trogaspidiini	*Timulla vagans*	TIM029
Mutillinae: Trogaspidiini	*Trispilotilla melanocephala*	EX1798
Mutillinae: Trogaspidiini	*Trogaspidia* (*Acutitropidia*) sp.	TIM060
Mutillinae: Trogaspidiini	*Trogaspidia* (*Arcuatotropidia*) *vetustata*	TIM063
Mutillinae: Trogaspidiini	*Trogaspidia* (*Chilotropidia*) sp.	TIM066
Mutillinae: Trogaspidiini	*Trogaspidia* (*Inflatispidia*) sp.	TIM069
Mutillinae: Trogaspidiini	*Trogaspidia* (*Lobotropidia*) sp.	TIM071
Mutillinae: Trogaspidiini	*Trogaspidia* (*Trogaspidia*) *heideri*	TIM122
Mutillinae: Trogaspidiini	*Trogaspidia* (*s.l.*) *cooki*	TIM100
Mutillinae: Trogaspidiini	*Trogaspidia* (*s.l.*) *doricha*	TIM112
Mutillinae: Trogaspidiini	*Trogaspidia* (*s.l.*) *mackieae*	TIM097
Mutillinae: Trogaspidiini	*Trogaspidia* (*s.l.*) sp.	EX1799
Mutillinae: Trogaspidiini	*Trogaspidia* (*s.l.*) sp. THAI	TIM099
Mutillinae: Trogaspidiini	*Trogaspidia* (*s.l.*) sp. 2 TAJ	TIM095
Mutillinae: Trogaspidiini	*Trogaspidia* (*s.l.*) sp. nr *nallinia*	TIM108
Mutillinae: Trogaspidiini	*Tuberocoxotilla lingulata*	TIM077
Mutillinae: Trogaspidiini	*Wallacidia oculata*	TIM091
Mutillinae: Trogaspidiini	*Zavatilla* sp.	TIM106
Myrmillinae	*Myrmilla capitata*	MUT052
Odontomutillinae	*Odontomutilla familiaris*	MUT025
Odontomutillinae	*Odontomutilla ovata*	MUT028
Sphaeropthalminae: Dasymutillini	*Dasymutilla bioculata*	U92J1189

Enrichments were performed using a custom RNA bait library developed for Hymenoptera (Hymenoptera 2.5Kv2A) composed of 9,446 baits for 2,524 conserved loci and 452 baits for 16 nuclear exons (Branstetter et al., 2017b). RNA bait libraries were hybridized to sequencing libraries at 65°C for an incubation period of 24 hours. Each pool was then enriched using a standardized protocol ("Target Enrichment of Illumina Libaries" v.1.5, available from https://www.ultraconserved.org/). Enrichment success was determined via qPCR using a Bio-Rad CFX96 system. Pools were quantified using qPCR results and pooled into a single, final pool of 110 total libraries. The final pool was mailed to and sequenced at Novogene (Chula Vista, California, USA).

Molecular data assembly

The software package PHYLUCE v.1.6.6 was used for all post-sequence data processing and preparation for phylogenetic analysis. Raw data were first demultiplexed using BBMap. Raw fastq reads were then cleaned using Illumiprocessor. The assembly program SPAdes was used to assemble contigs. Contigs were matched to probes using the bait set developed by Branstetter et al. (2017b). To be considered a match, minimum and maximum thresholds were set to 60-80, respectively, which was found to be the optimal setting across the data set to recover the most UCE loci. The data matrix was generated using fastas pulled from match counts and aligned using MAFFT v.7.407 (Katoh and Standley, 2013). Poorly aligned regions were cleaned and trimmed using Gblocks (Talavera and Castresana, 2007) with the reduced stringency parameters (b1:0.5, b2:0.5, b3:12, b4:7). Alignments were filtered for missing data using a PHYLUCE script requiring that each alignment include data for ≥75% of taxa; this threshold was found to be the ideal setting for this data set in order to account for several taxa with less available data. The resulting aligned, cleaned/trimmed, and filtered data set was used for phylogenomic analyses.

Phylogenomic analyses

The program IQ-TREE v.1.6.12 (Nguyen et al., 2015) was used for maximum-likelihood inference (hereafter 'ML'). The data set was partitioned by UCE loci with each partition allowed a different evolutionary speed ('-spp' option). ModelFinder (Kalyaanamoorthy et al., 2017) was used to find the best-fit model of sequence evolution per partition (Chernomor et al., 2016). Ultrafast bootstrap approximation (UFBoot, '-bb' option) (Hoang et al., 2017) and SH-like approximate likelihood ratio test (SH-aLRT, '-alrt' option) (Guindon et al., 2010) were used to evaluate branch stability with each set to 1000 replicates. Thresholds used to determine well-supported clades are $\geq$95% for UFBoot and $\geq$80% for SH-aLRT. The resulting phylogenetic tree was visualized in FigTree v.1.4.4.

The program TNT v.1.5 (Goloboff and Catalano, 2016) was used for maximum-parsimony inference (hereafter 'MP'). Settings used include 100 cycles of Random Addition Sequence, 25 iterations of Drift, 25 iterations of Ratchet (Nixon, 1999), and branch-swapping with TBR. All characters were treated as unordered and equally weighted. Gaps were treated as missing data. Branch supports (Bremer, 1988, 1994; Brower, 2006) were calculated using 2,000 suboptimal trees up to 25,000 additional steps longer; these suboptimal trees were then treated to TBR branch-swapping. The resulting cladogram was visualized in FigTree v.1.4.4.

Dating analyses

The programs BEAST v.1.10.4 (Suchard et al., 2018) and IQ-TREE v.2.1.1 (Minh et al., 2020) were used to estimate dating for Pompiloidea with emphasis on Mutillidae. In order to reduce computation time, 50 loci were randomly sourced from the master alignment used in the ML and MP analyses and this data set was treated as a single partition. Additionally, the ML tree was used as a reference tree in all analyses. Estimated ages are reported herein in a split format, with the estimated age inferred using BEAST first, and the estimated age inferred using IQ-TREE second (e.g., 23.31/21.51 Ma). Fossil Mutillidae are only known from four Dominican amber specimens from the sphaeropthalmine tribes Dasymutillini and Ephutini and none are known for Mutillinae; consequently, primary fossil data were not used for this study and dates were sourced from a study of the higher classification of Mutillidae using UCEs (Waldren et al., in prep). The geologic time scale used herein was derived from the date ranges provided by http://fossilworks.org/.

For the BEAST analyses, BEAUti v.1.10.4 was used to generate the XML file. The substitution model used was GTR+G. An uncorrelated relaxed clock with a lognormal distribution was used (Drummond et al., 2006). The tree prior used was Speciation: Birth-Death Process (Gernhard, 2008). The tree-generating operators were turned off (i.e., subtreeSlide, narrowExchange, wideExchange, and wilsonBalding). The root was dated as 82.94 Ma (SD=10, normal distribution). The clade Dasylabrinae + (Odontomutillinae + (Myrmillinae + Mutillinae)) was dated as 69.4 Ma (SD=10, normal distribution) and Trogaspidiini was dated as 13.17 Ma (SD=1, normal distribution). The three calibration dates used (82.94 Ma, 69.4 Ma, and 13.17 Ma) were derived from a BEAST analysis in a study of the higher classification of Mutillidae using UCEs (Waldren et al., in prep.). Lastly, the priors ucld.mean and ulcd.stdev were set to 0.001 and 0.333, respectively, based on empirical analyses. Three independent Markov Chain Monte Carlo (MCMC) runs were performed with the length of chain set to 300,000,000 and were logged every 2,000. The BEAST analyses were conducted using the CIPRES Science Gateway v.3.3 (Miller et al., 2010). Logs of the BEAST analyses were assessed in Tracer v.1.7.1 (Rambaut et al., 2018) to confirm stabilization and adequate effective sample sizes (ESSs). The independent runs were combined in LogCombiner v.1.10.4. Ten percent of trees were discarded as burn-in and states were resampled at a frequency of 30,000. A maximum clade credibility tree was generated in TreeAnnotator v.1.10.4 and visualized in FigTree v.1.4.4.

For the IQ-TREE analysis using the least-squares criterion (To et al., 2016), the substitution model used was GTR+G, the root was dated as 72.4 Ma, the tips were dated as 0 Ma, and the outgroup was set as the taxon "Dasymutilla_bioculata_U92J1189." The clade Dasylabrinae + (Odontomutillinae + (Myrmillinae + Mutillinae)) was dated as 65.96 Ma and Trogaspidiini was dated as 13.43 Ma. The three calibration dates used (72.4 Ma, 65.96 Ma, and 13.43 Ma) were derived from an IQ-TREE analysis in a study of the higher classification of Mutillidae using UCEs (Waldren et al., in prep). The resulting chronogram was visualized in FigTree v.1.4.4.

Ancestral area analyses

The program RASP v.4.2 (Yu et al., 2019) was used to infer ancestral areas with the Bayesian Binary MCMC (BBM) criterion (Ronquist and Huelsenbeck, 2003; Ali et al., 2012; Yu et al., 2019). Two separate analyses were performed: one to determine the ancestral areas for Trogaspidiini worldwide and another to determine the ancestral areas and intercontinental dispersal for *Timulla* in the New World. For the first analysis (Trogaspidiini), taxa were coded according to the biogeographic region the species belongs to: A) Afrotropical, M) Afrotropical (Malagasy), N) Nearctic + Neotropical, O) Oriental, P) Palaearctic, and U) Australasian. For the second analysis (*Timulla*), taxa were coded according to the New World biogeographic region the species belongs to: A) Nearctic North America, B) Neotropical North America, and C) Neotropical South America. These New World biogeographic regions correspond with those proposed by Morrone (2014), except that the Neotropical region was artificially split into North America and South America to infer the ancestral continent for *Timulla* and ancestral dispersal events between the continents. For the consensus tree, both the ML tree and MP cladogram were used in separate analyses for Trogaspidiini, while the ML tree was used for the *Timulla* analysis as the topology was identical with the MP cladogram. The number of cycles used was 1,000,000 sampled every 100. State frequencies were set to 'Fixed (JC)' and the among-site variation set to 'Gamma (+G)' (i.e., model= JC+G). The resulting ancestral area trees were visualized in RASP v.4.2.

Results and Discussion

UCE loci recovered among the 113 taxa used in this study ranged from 149 to 2,014 (mean= 1,750). The final alignment used in the analyses was composed of 1,148,582 base pairs with 6.89% total missing data. 370,581 characters were constant, 228,365 were parsimony-uninformative, and 549,636 were parsimony-informative. ModelFinder assigned substitution models for 1,110 partitions for use in the ML analysis. The ML analysis resulted in a single tree with most major nodes having both 100% SH-aLRT and UFBoot stability values (fig. 4.1). The MP analysis resulted in a single most parsimonious tree consisting of 3,018,319 steps (CI= 0.448; RI= 0.509); branch support values ranged from 13 to 25,000 (fig. 4.2) across the total data set, while for the ingroup (Trogaspidiini) ranged from 13 to 7,451.

The ML and MP analyses resulted in somewhat similar topologies with several important distinctions between them (figs 4.1 and 4.2). Nine major Trogaspidiini clades were recovered in the analyses (figs 4.1 and 4.2). The tribe Petersenidiini was recovered as polyphyletic (fig. 4.3, with current member taxa highlighted in red). *Trogaspidia* (*sensu* Nonveiller, 1995b) was recovered as polyphyletic with the subgenera recovered in three separate lineages (fig. 4.4, with current member taxa highlighted in red). *Timulla* was recovered as monophyletic and is sister-goup to a clade composed of Afrotropical and Oriental trogaspidiines (figs 4.1 and 4.2).

The BEAST and IQ-TREE analyses for ancestral dating each resulted in a single chronogram (figs 4.5 and 4.6). The BBM analyses resulted in three ancestral area trees (figs 4.7–4.9). The ancestor of Trogaspidiini was inferred to be Afrotropical (figs 4.7 and 4.8), and the ancestor of *Timulla* (= clades 8 + 9) was inferred to be Afrotropical in the ML BBM analysis (fig. 4.7) and Oriental in the MP BBM analysis (fig. 4.8). Lastly, the ancestor of *Timulla* was inferred to have originated in Neotropical North America (fig. 4.9).

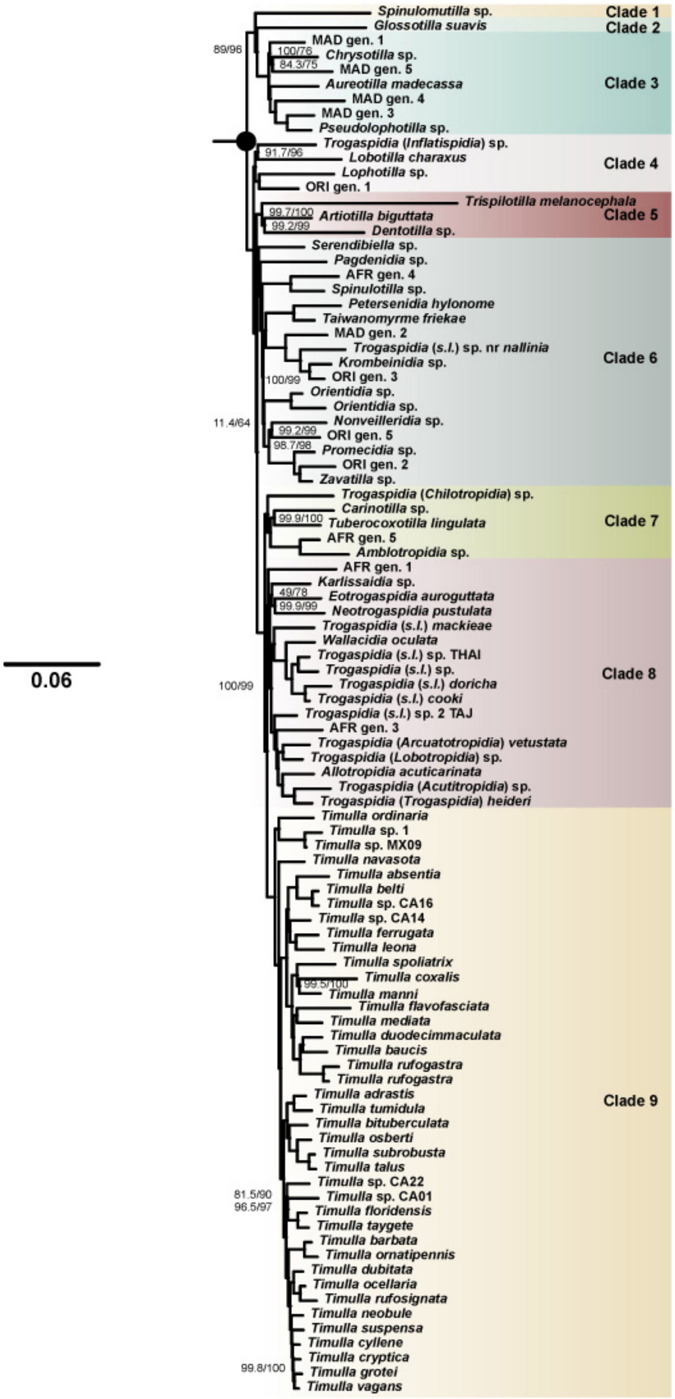

Figure 4.1. Maximum-likelihood (ML) phylogenetic tree of Trogaspidiini. Unmarked nodes indicate the SH-aLRT/UFBoot values both equal 100; if below 100, the numerical values are provided.

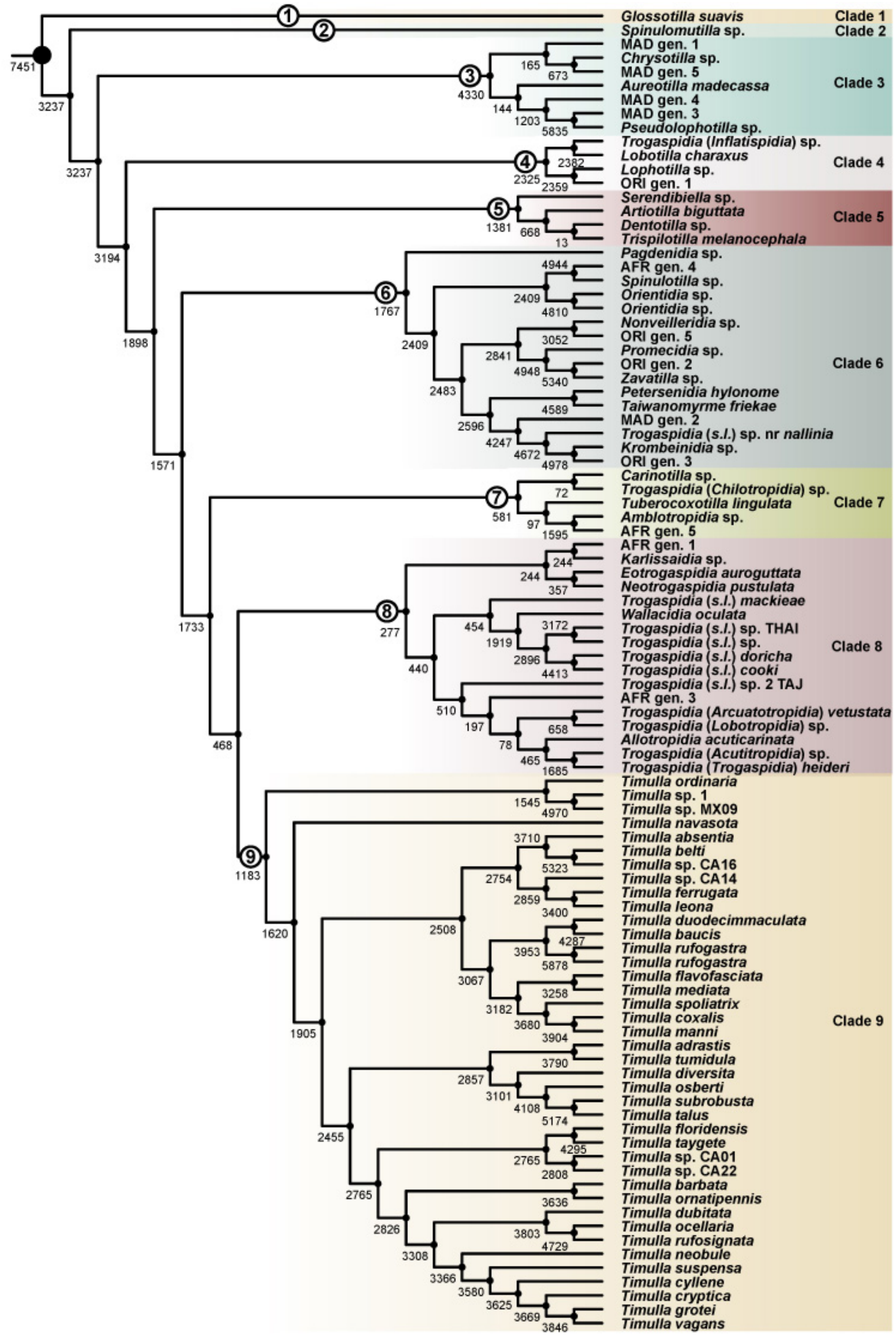

Figure 4.2. Maximum-parsimony (MP) cladogram of Trogaspidiini. Major clades are numbered 1–9. The remaining numerical values are branch supports and have no upper limit.

The monophyletic status of Trogaspidiini and the validity of Petersenidiini

In a study of the higher classification of Mutillidae (Waldren et al., in prep.), Trogaspidiini was rendered polyphyletic by *Dolichomutilla* being recovered as a member of the mutilline tribe Mutillini; this genus was consequently transferred to the latter tribe. In the present study, *Dolichomutilla* was also recovered as a member of Mutillini. This genus lacks many of the characters that are diagnostic for trogaspidiines and the general habitus is suggestive of Mutillini. Additionally, Brothers and Lelej (2017) recovered *Dolichomutilla* as a member of Mutillini: Mutillina in their male-only analysis and it possesses all of the homoplasious synapomorphies that they considered diagnostic for Mutillini. Considering the results of these studies, *Dolichomutilla* is maintained as a member of Mutillini.

Trogaspidiini was recovered as having nine major component clades. The clades were numbered and named using the MP cladogram (fig. 4.2), starting with the basal lineage first, as follows: 1) *Glossotilla* genus-group, 2) *Spinulomutilla* genus-group, 3) *Aureotilla* genus-group, 4) *Lobotilla* genus-group, 5) *Trispilotilla* genus-group, 6) *Petersenidia* genus-group, 7) *Carinotilla* genus-group, 8) *Trogaspidia* genus-group, and 9) *Timulla* genus-group (figs 4.1 and 4.2). There are several important discrepancies between the MP and ML topologies. First, the positions of *Glossotilla suavis* (Gerstaecker) and *Spinulomutilla* sp. differ dramatically between the analyses. In the ML tree, *Glossotilla suavis* and *Spinulomutilla* form a clade with the Malagasy trogaspidiines (= clades 1 + 2 + 3), with *Spinulomutilla* being the basal taxon in clade 1 (fig. 4.1) and *Glossotilla suavis* (clade 2) sister-goup to the Malagasy taxa. In the MP cladogram, *Glossotilla suavis* is the basal taxon in clade 1 and is sister-goup to the remaining

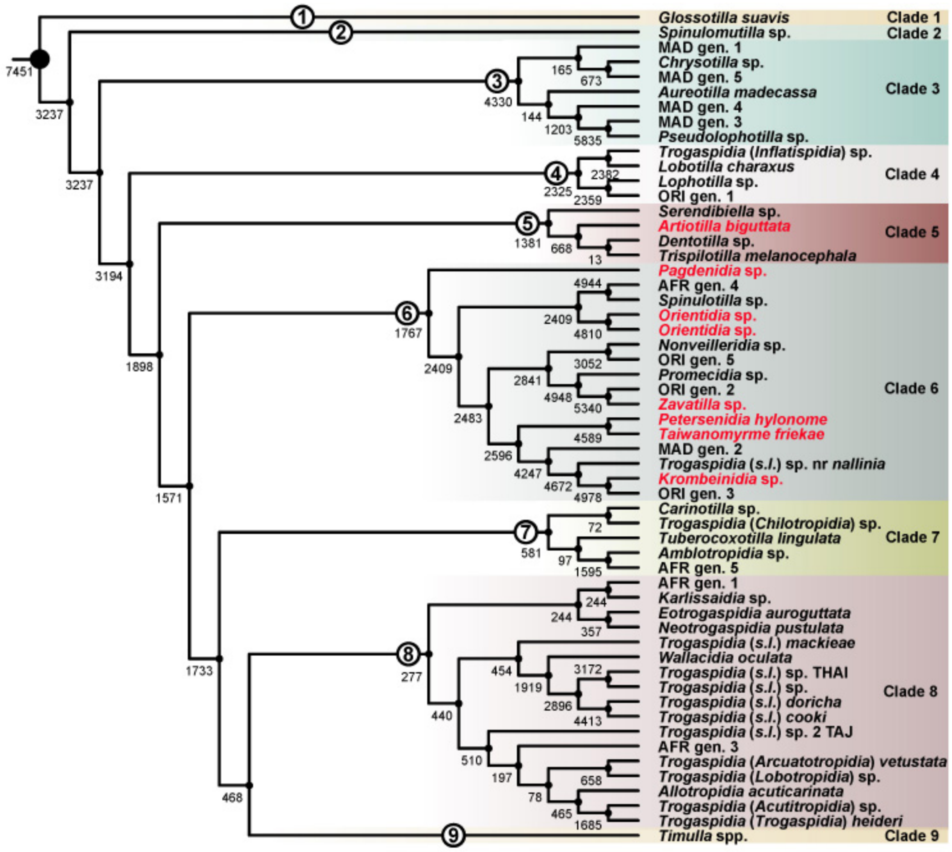

Figure 4.3. Polyphyly of Petersenidiini (*sensu* Lelej 2002, 2005).

Trogaspidiini (= clade 1 + (2–9)); further, the clade *Spinulomutilla* sp. + Trogaspidiini is sister-goup to *Glossotilla suavis* (= clade 2 + (3–9)) (fig. 4.2). A few additional discrepancies include *Serendibiella* sp. being a member of clade 6 in the ML analysis (fig. 4.1) and a member of clade 5 in the MP analysis (fig. 4.2). Also, 'AFR gen. 1' was sister-goup to the remaining members of clade 8 in the ML analysis (fig. 4.1), in contrast to being nested within clade 8 in the MP analysis (fig. 4.2). This latter result has important biogeographic implications for *Timulla* in the BBM analyses and is further discussed below. Several relationships within clades 6 and 7 also differ between the analyses.

The tribe Petersenidiini was recovered as polyphyletic, and its members were mostly confined to clade 6 (fig. 4.3). Seven of the eight total petersenidiine genera were included in this study: *Artiotilla* Invrea, *Krombeinidia* Lelej, *Orientidia* Lelej, *Pagdenidia* Lelej, *Petersenidia* Lelej, *Taiwanomyrme* Tsuneki, and *Zavatilla* Tsuneki; the only genus for which material wasn't available for study was *Radoszkowskitilla* Lelej. Most members of Petersenidiini were recovered in clade 6, with *Artiotilla* recovered in clade 5 (fig. 4.3). Petersenidiines are primarily characterized by having symmetrical penial valves and generally lack most secondary sexual characteristics that most other trogaspidiines possess. This clade overall aligns with this diagnosis with a few exceptions. *Nonveilleridia* Lelej and *Promecidia* Lelej have asymmetrical penial valves, and a few other taxa have a basoventral mandibular tooth and metasomal sterna protuberances. Petersenidiini is here confirmed as a synonym of Trogaspidiini and is referred to as the *Petersenidia* genus-group (= clade 6).

The phylogenetic relationship between Timulla and Trogaspidia (sensu lato)

The New World genus *Timulla* was recovered as monophyletic and derived from the Old World trogaspidiines (figs 4.1 and 4.2). The Afrotropical and Oriental trogaspidiine faunas are highly polyphyletic, indicating multiple dispersal events between these biogeographical regions. Eight of the nine clades contain Afrotropical taxa and four of the nine clades contain Oriental taxa using the MP BBM analysis as a reference (fig. 4.8). Two Palaearctic taxa (i.e., *Artiotilla biguttata* (Costa) and *Trogaspidia* (*s.l.*) sp. 2 TAJ) are members of two independent lineages of Afrotropical trogaspidiines (clade 5 and clade 8 (in part)), while *Taiwanomyrme friekae* (Zavattari) is nested within the predominantly Oriental clade 6. *Trogaspidia* (*sensu* Nonveiller 1995b) was recovered as polyphyletic, with the six subgenera recovered in three separate lineages (fig. 4.4). The genus *Allotropidia*, described by Nonveiller (1996) but was not classified in his subgeneric concept of *Trogaspidia*, was nested within a clade containing the subgenera *Acutitropidia*, *Arcuatotropidia*, *Lobotropidia*, and *Trogaspidia* (fig. 4.4, with current member taxa highlighted in red). Additionally, the remaining subgenera *Chilotropidia* and *Inflatispidia* were recovered well outside of *Trogaspidia* (*sensu* Nonveiller, 1995b). Consequently, four subgenera of Afrotropical *Trogaspidia* recognized by Nonveiller (1995b) are raised to the genus level: *Arcuatotropidia* Nonveiller, **stat. nov.**, *Chilotropidia* Nonveiller, **stat. nov.**, *Inflatispidia* Nonveiller, **stat. nov.**, and *Lobotropidia* Nonveiller, **stat. nov.** Further, *Trogaspidia* (*Acutitropidia* Nonveiller) is maintained as a subgenus of *Trogaspidia* (*sensu stricto*) pending further study. The genus *Trogaspidia* (*sensu stricto*) is Afrotropical in distribution and the Oriental species placed in *Trogaspidia* are misclassified.

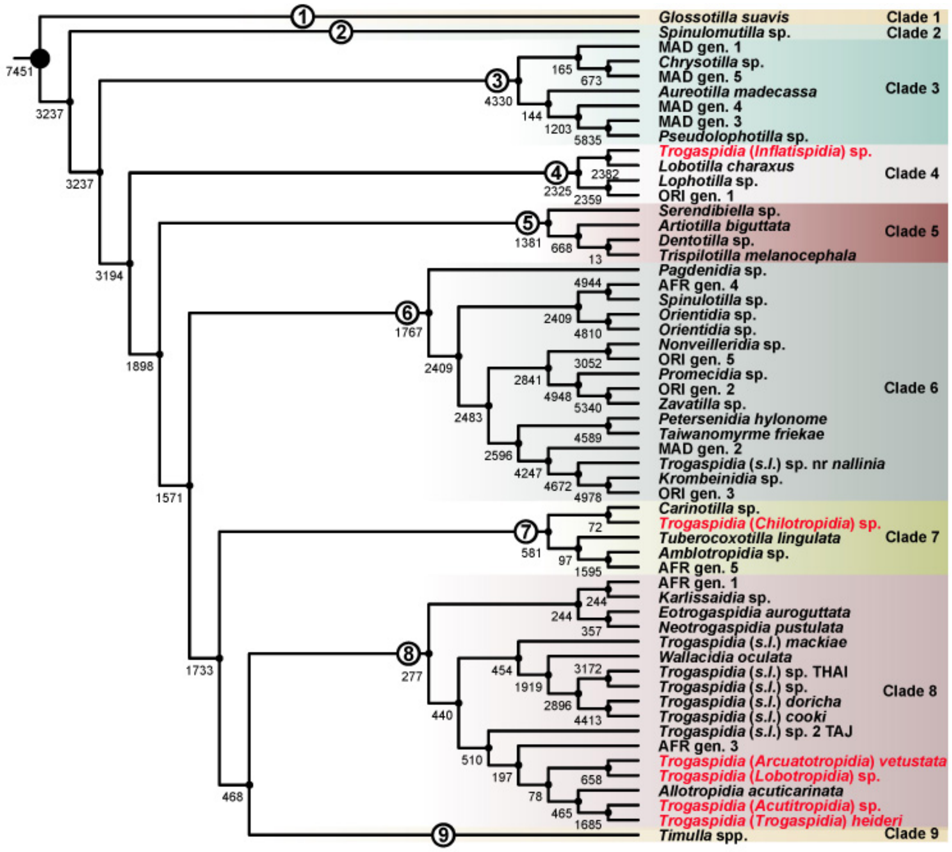

Figure 4.4. Polyphyly of *Trogaspidia* (*sensu* Nonveiller 1995b) subgenera.

Biogeography of the Old World trogaspidiines

Trogaspidiines are Afrotropical in ancestry as inferred with the BBM analyses (figs 4.7 and 4.8). Eight of the nine clades contain Afrotropical trogaspidiines and four of the nine clades contain Oriental taxa. In the BBM analysis using the ML tree, all of the backbone ancestral nodes were inferred to be Afrotropical in distribution (fig. 4.7). For the MP cladogram, the four basal nodes were inferred to be Afrotropical, and among the remaining nodes, three were Oriental and one was mixed Afrotropical + Oriental (fig. 4.8). These faunas are both highly polyphyletic, with multiple introductions and reintroductions between these biogeographical regions. The Madagascan trogaspidiine fauna is primarily derived from a single introduction early in the history of the tribe (clade 3, 8.04/7.75 Ma) with an additional introduction from the Oriental region ('MAD gen. 2' in clade 6). Further, at least one Malagasy species is related to the Afrotropical genus *Trispilotilla* Bischoff (clade 5); it was not included in this study. In total, there were at least three independent colonization events into Madagascar by trogaspidiines and no subsequent dispersals out of the island. The close relationship of the Malagasy fauna and flora to both the Afrotropical and Oriental regions is well documented (Yoder and Nowak, 2006; Warren et al., 2010). Clade 6 represents the first major radiation of trogaspidiines in the Oriental region, with a subsequent radiation represented within clade 8. Clade 4 and clade 5 (ML analysis only via *Serendibiella* sp.) contain Oriental taxa originating from Afrotropical stock. Reintroductions back into the Afrotropical region from the Oriental region are represented in clade 6 by *Spinulotilla* species. Clade 8 contains two additional Afrotropical reintroductions from the Oriental region ('AFR gen. 1' and an internal clade containing *Trogaspidia* (*sensu stricto*), the

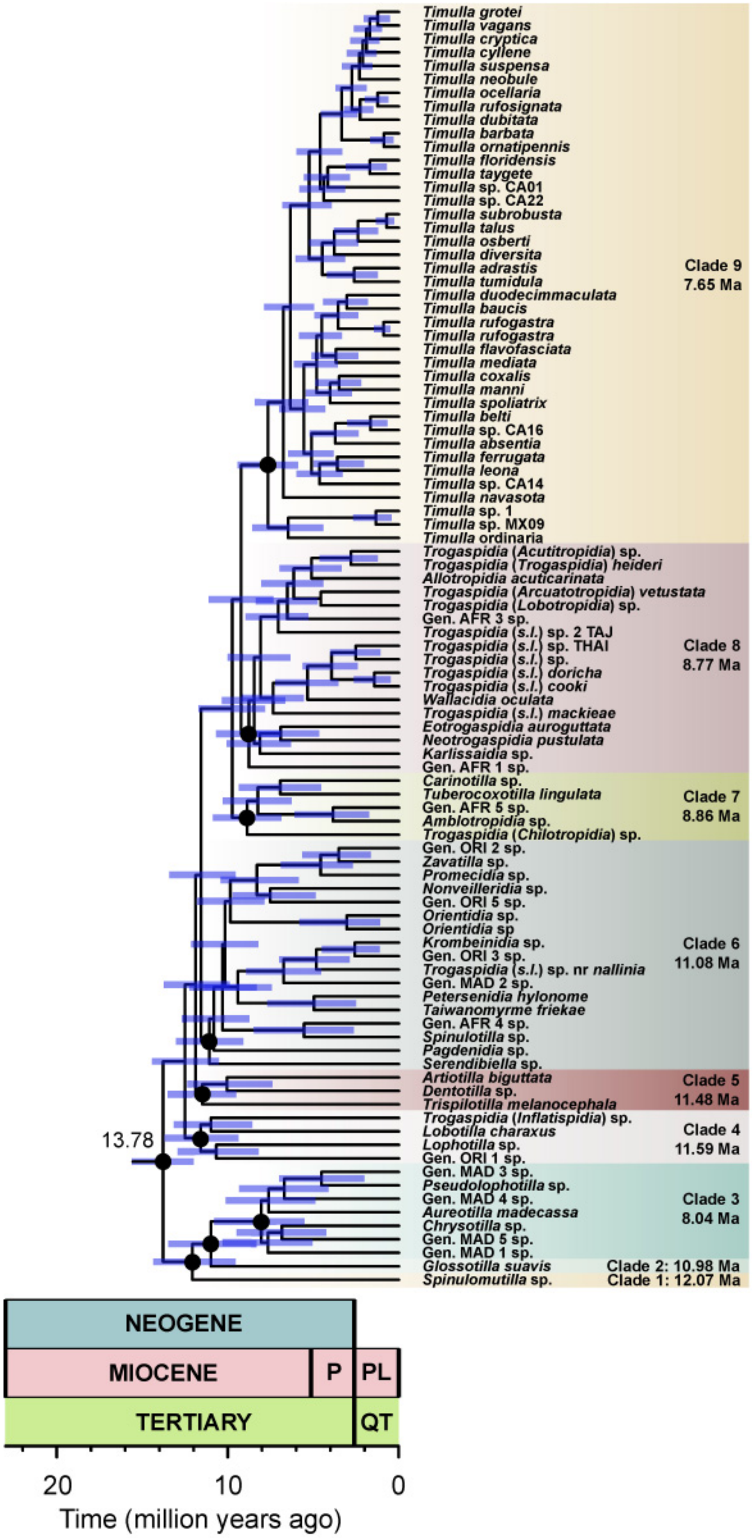

Figure 4.5. Chronogram of Trogaspidiini using BEAST v.1.10.4. The purple bars represent 95% HPD intervals.

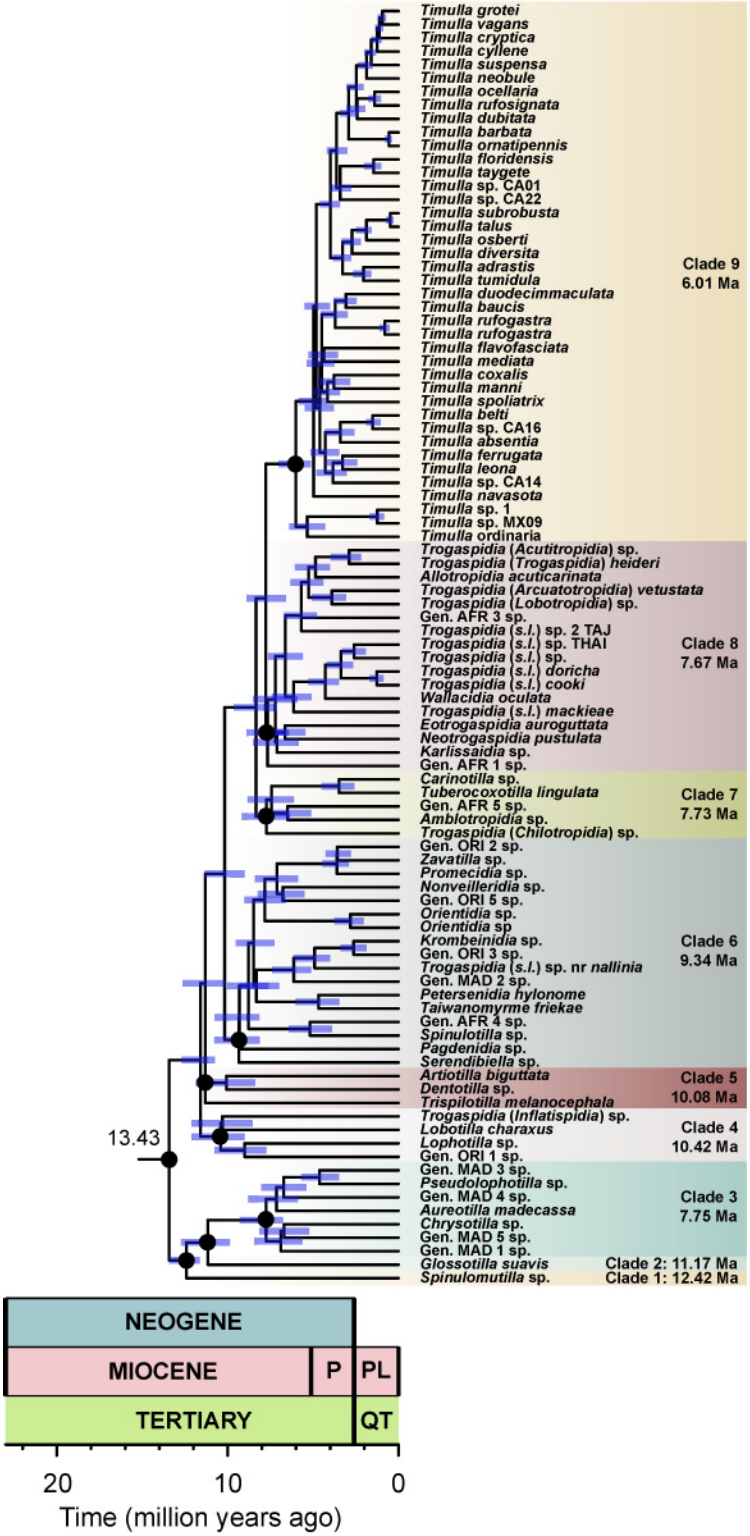

Figure 4.6. Chronogram of Trogaspidiini using IQ-TREE v.2.1.1. The purple bars represent confidence intervals.

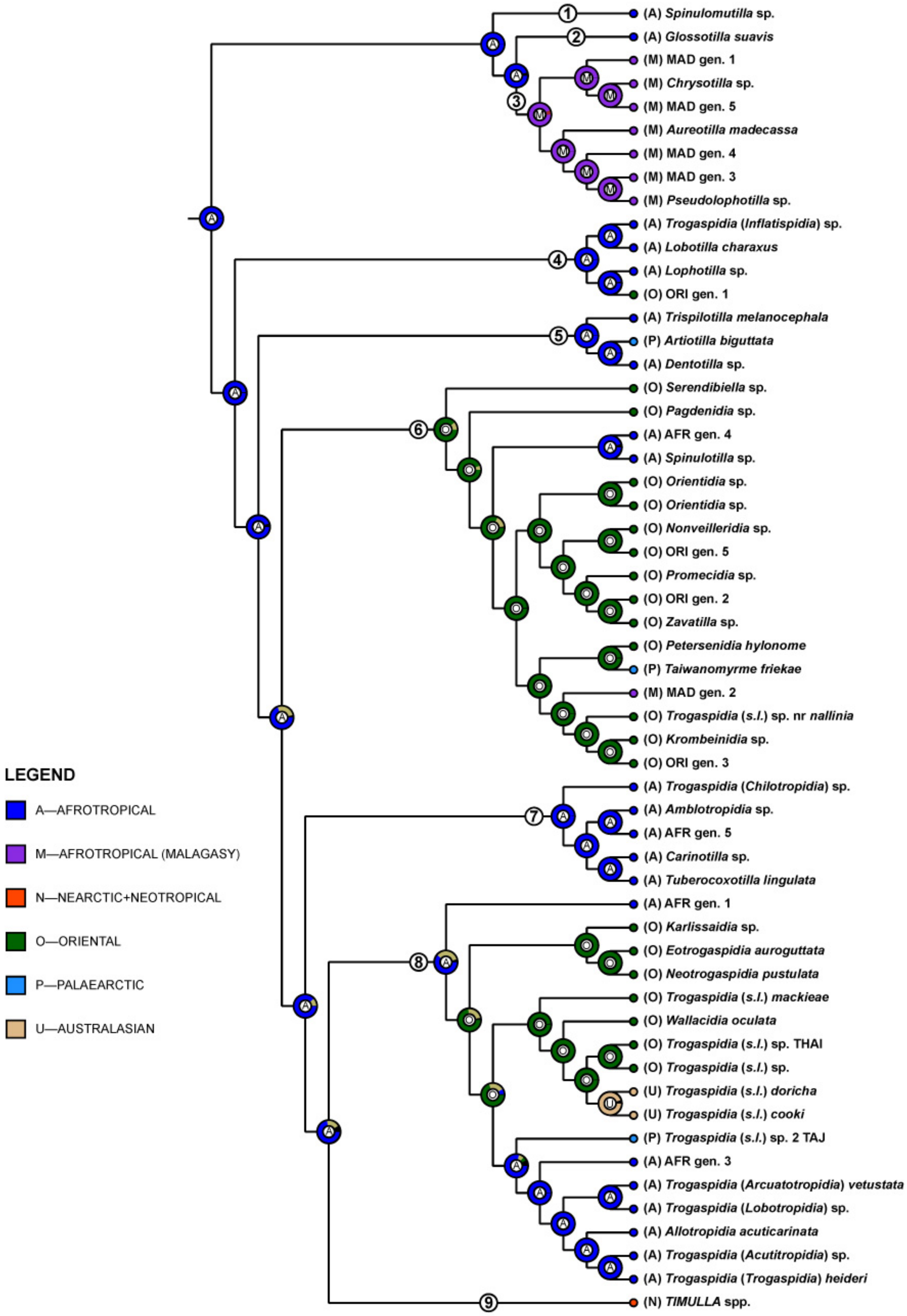

Figure 4.7. Ancestral area inference for Trogaspidiini using the ML tree.

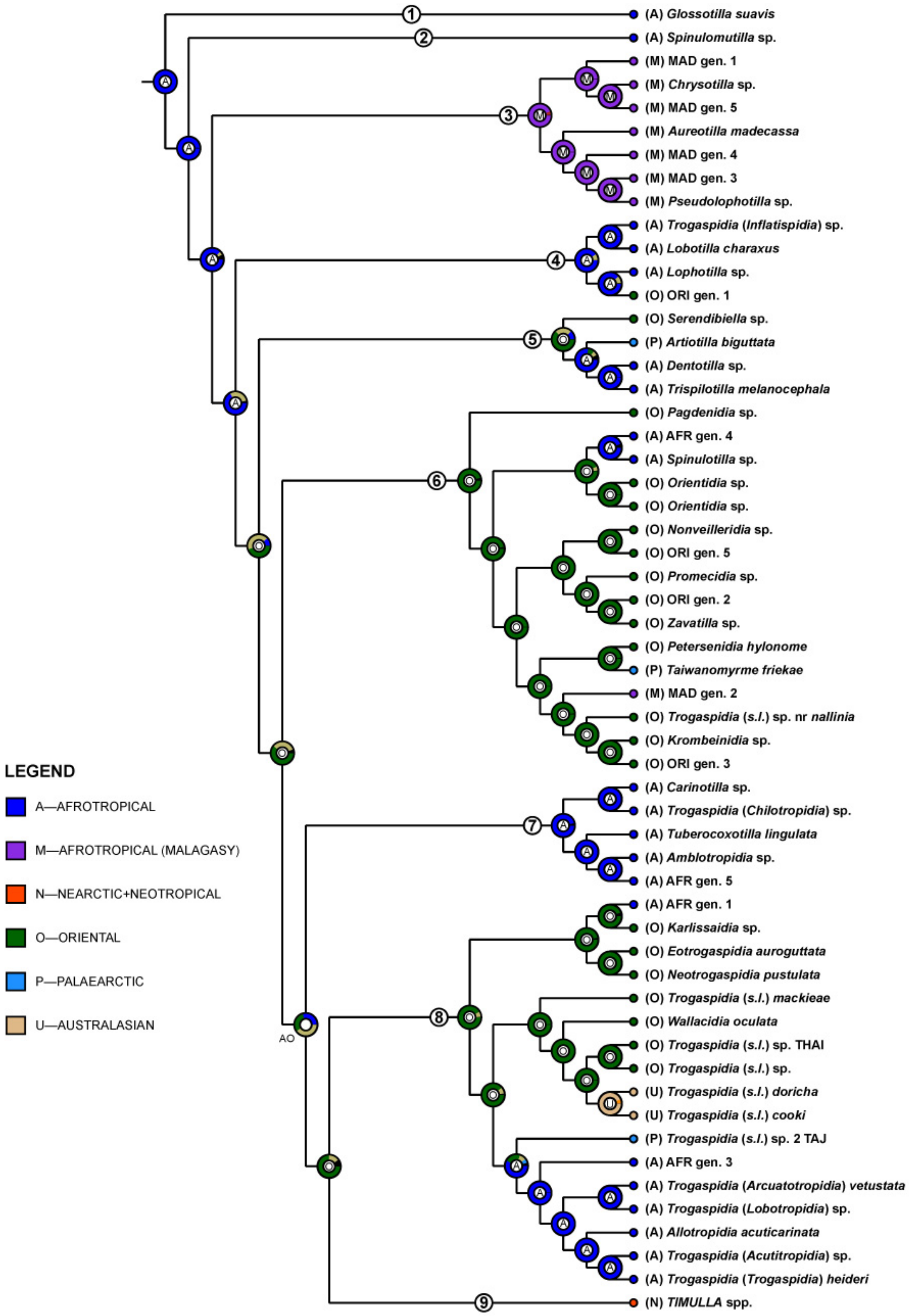

Figure 4.8. Ancestral area inference for Trogaspidiini using the MP cladogram.

latter being a species-rich radiation). Additionally, clade 8 also contains an introduction into the Australasian region. Lastly, there were at least three independent introductions into the Palaearctic region: *Artiotilla biguttata* in the Western Palaearctic from an Afrotropical ancestor (clade 5), '*Trogaspidia* (*s.l.*) sp. TAJ' in the Eastern Palaearctic from an Afrotropical ancestor (clade 8), and *Taiwanomyrme friekae* in the Eastern Palaearctic from an Oriental ancestor (clade 6).

At 13.17/13.43 Ma when Trogaspidiini were estimated to have arisen, the continents were configured more or less as they are today. Dispersal is thus hypothesized to have been a major factor in the disjunct distributions observed herein. Taking the timing into consideration, the options for the mode(s) of dispersal that occurred between the Afrotropical and Oriental regions are considerably more restricted. There are a few dispersal avenues that may have resulted in these distribution patterns. First, numerous islands in the Indian Ocean between India and Madagascar/East Africa were exposed due to fluctuating sea levels at least over the past 5 Ma (Miller et al., 2005; Warren et al., 2010). In addition to the exposure of islands that are underwater today, the further exposure of the Maldives, Mascarenes, and Seychelles archipelagoes was significant (Warren et al., 2010). An over-water, stepping-stone method of dispersal for trogaspidiines may have occurred with these islands between the Afrotropical and Oriental regions. Similarly, the primarily South American *Timulla mediata* species-group may have dispersed in a stepping-stone manner from South America throughout the Lesser Antilles and to Jamaica. Second, wind-aided dispersal via the Indian monsoons may have played a role, with the summer monsoon winds directed northeast toward India and the winter monsoon winds directed southwest toward Africa (Goswami and

Rajagopal, 2003; Wang et al., 2003). These regional climactic cycles, particularly if concomitant with low sea level-derived island exposure, may have aided in the long-distance dispersal of a trogaspidiine pair practicing phoretic copulation.

Biogeography of the New World trogaspidiines

The New World was inferred to have been colonized by a single introduction of trogaspidiines in the late Miocene (figs 4.7–4.9). *Timulla* (clade 9) was recovered as monophyletic and is sister-goup to clade 8 that is composed of Afrotropical and Oriental trogaspidiines (figs 4.1 and 4.2). The ancestral area of clades 8 + 9 is Afrotropical in the BBM analysis using the ML tree (fig. 4.7), while the ancestral area is Oriental using the MP cladogram (fig. 4.8). This significant discrepancy is due to the taxon 'AFR gen. 1' being recovered as sister-goup to the remaining members of clade 8 in the ML analysis (fig. 4.1) while being nested within clade 8 in the MP analysis (fig. 4.2). The basal lineage of *Timulla* containing *Timulla ordinaria* (Smith) is notable in that the males possess an unmodified, convex pygidium, an apparently plesiomorphic character shared with many of the Oriental trogaspidiines in clade 8; all remaining *Timulla* have a variable process on the pygidium. Further, an Oriental ancestral area for clades 8 + 9 would support a Bering land bridge dispersal route, while an Afrotropical ancestral area would not. Trogaspidiini is primarily a tropical tribe with comparatively fewer representatives in the Holarctic (Table 4.1). The common ancestor of clades 8 + 9 was inferred as age 9.22/7.77 Ma (figs 4.5 and 4.6). The climate of Beringia was potentially not amenable for trogaspidiine dispersal at this time, as summer temperatures were in the process of decreasing until they reached their present day levels during the Pliocene around 5–6 Ma

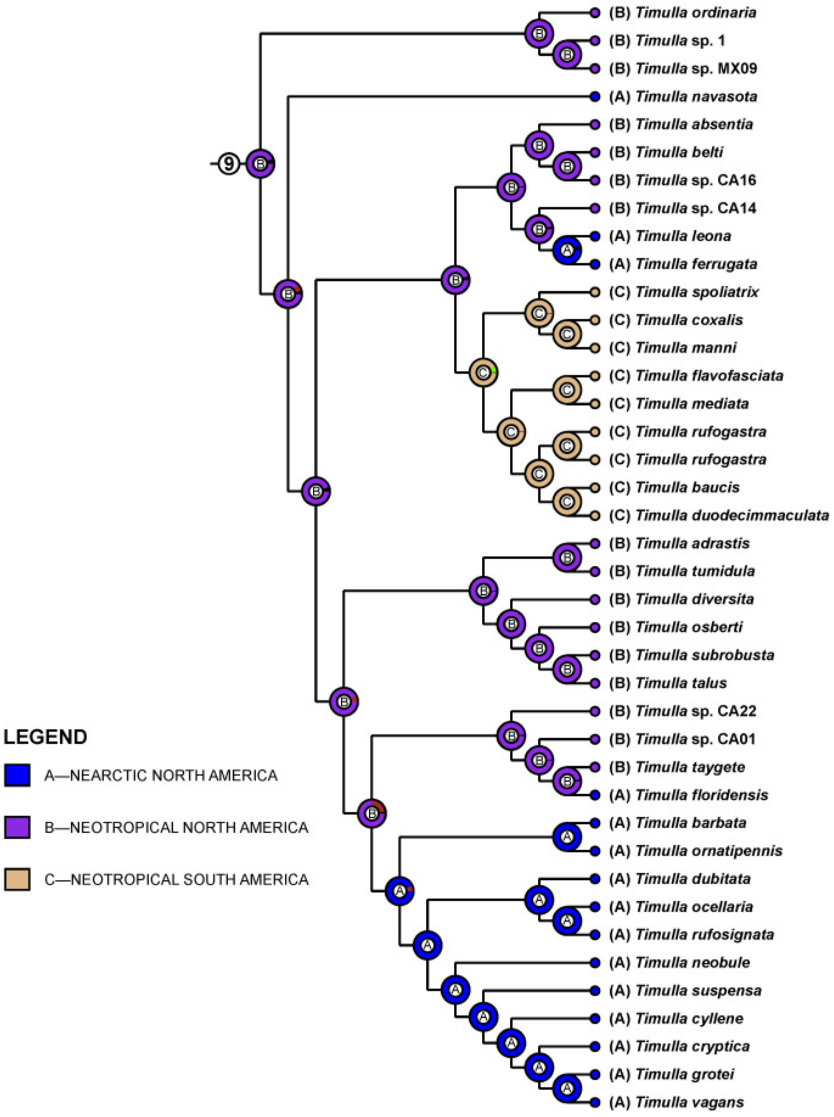

Figure 4.9. Ancestral area inference for *Timulla*.

(Wolfe, 1994); additionally, the dominant biome in Beringia at this time was boreal forest (Wolfe and Tanai, 1980). However, subsequent glacial cycles during the Pleistocene likely influenced trogaspidiine distributions that are observed today and the reduced Holarctic representation may be a reflection of this. Ruling Beringia out due to unfavorable climate and habitat, long distance, oceanic dispersal of ancestral *Timulla* into the New World likely occurred.

Mutillinae were a relatively new arrival to the New World, as *Timulla* was estimated to have emerged 7.65/6.01 Ma. This is in contrast to Sphaeropthalminae, the other subfamily occurring in the New World, the age of which was inferred as 74.25/63.64 Ma. The ancestral area of *Timulla* within the New World was inferred as Neotropical North America (fig. 4.9, identical for ML and MP topologies). Nearctic North America was colonized by four independent *Timulla* lineages, notably by species related to *Timulla dubitata* (Smith) which represent most of the fauna in the region. South America was primarily colonized by a lineage that dominates the continent today (fig. 4.9). A few additional introductions of North American taxa into South America presumably also occurred, although they were not included in this study. These few South American species with apparent North American origins are related to *Timulla adrastis* Mickel, *T. leona* (Blake), *T. ordinaria* (Smith), and *T. tumidula* Mickel. The *T. leona* species-group is notable in that it is the only group within *Timulla* to occur in all three biogeographic regions analyzed herein. A few reintroductions of South American lineages into North America also occurred, although these taxa were not included in this study. The primarily South American *T. mediata* species-group is represented by *Timulla centroamericana* (Dalla Torre) in Panama, and the primarily South American *T.*

rufogastra species-group is represented by several species in Costa Rica and Panama. Further, the T. *mediata* species-group occurs throughout the Lesser Antilles and Jamaica. The Isthmus of Panama has historically been hypothesized to have fully closed by the late Pliocene at 3.1 Ma (Keigwin, 1978). However, more recent research demonstrates that the collision of the Caribbean Plate including Panama and the South American Plate began as early as 23–25 Ma (Farris et al., 2011). This latter estimate significantly predates the age of the species-rich South American *Timulla* clade at 4.8/4.5 Ma, meaning this dispersal route from North to South America would have been available to this lineage. The Darién Gap in southern Panama may act as a filter barrier for mutillids between North and South America, as there are few species that occur in both Panama and Colombia (Cambra et al., 2018a, 2018b; Bartholomay et al., 2019).

Conclusion

Trogaspidines underwent a remarkable worldwide radiation since the emergence of the tribe at an estimated 13.17/13.43 Ma, resulting in 44 described genera and subgenera with 928 described species. The phylogenomic approach that was used allowed insight into the systematic relationships and biogeography of a taxonomically-challenging tribe that would likely not have been achievable if morphology were solely relied upon. With this approach, the relationships between a number of taxa were resolved, including the exclusion of *Dolichomutilla* from Trogaspidiini, the confirmation of Petersenidiini as a synonym of Trogaspidiini, the discovery of the polyphyly of the *Trogaspidia* (*sensu stricto*) subgenera and the raising of four of them to genus level, the discovery of nine genus-groups in Trogaspidiini, the monophyly of *Timulla*, and the biogeographic relationships between members of Trogaspidiini. The New World fauna was inferred to be related to either the Afrotropical fauna or Oriental fauna, depending on the analyis. The results presented herein will be a foundational resource for much-needed revisionary work and biogeographic research on trogaspidiines.

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References

Ali, S.S., Yu, Y., Pfosser, M. and Wetschnig, W. (2012) Inferences of biogeographical histories within subfamily Hyacinthoideae using S-DIVA and Bayesian binary MCMC analysis implemented in RASP (Reconstruct Ancestral State in Phylogenies). Ann. Bot., 109: 95–107.

André, E. (1899–1903) Monographie des Mutillides d'Europe et d'Algérie. Species de Hyménoptères d'Europe and d'Algérie. Tome Huitième. Paris: Librairie Scientifique A. Hermann, 480 pp., 15 plates.

André, E. (1902 ["1903"]) Fam. Mutillidae. In: Wytsman, P. (Ed.) Genera Insectorum, Fascicle 11. Bruxelles: V. Verteneuil & L. Desmet, pp. 1–77, plates 1–3.

André, E. (1904) Examen critique d'uen nouvelle classification proposée par M. le Dr. W. H. Ashmead pour la famille des Mutillidae. Rev. Entomol., 23(1): 27–41.

Ashmead, W.H. (1899) Superfamilies in the Hymenoptera and generic synopses of the families Thynnidae, Myrmosidae, and Mutillidae. J. New York Entomol. S., 7: 45–60.

Ashmead, W.H. (1900–1904) Classification of the fossorial, predaceous and parasitic wasps, or the superfamily Vespoidea. Can. Entomol., (1900) 32: 145–155, 185–188, 295–296; (1902) 34: 79–88, 131–137, 163–166, 203–210, 219–231, 268–273, 287–291; (1903) 35: 3–8, 39–44, 95–107, 155–158, 199–205, 303–310, 323–332; (1904) 36: 5–9.

Azevedo, C.O., Alencar, I.D.C.C., Ramos, M.S., Barbosa, D.N., Colombo, W.D., Vargas, R.J.M. and Lim, J. (2018) Global guide of the flat wasps (Hymenoptera, Bethylidae). Zootaxa, 4489(1): 1–294.

Bartholomay, P.R., Williams, K.A., Cambra, R.A. and Oliveira, M.L. (2019) Does the genus *Dasymutilla* Ashmead occur in South America? The new genus *Quwitilla*, new combinations, and new distribution records from Neotropical velvet ants (Hymenoptera: Mutillidae). Zootaxa, 4623(2): 261–282.

Bartholomay, P.R., Williams, K.A., Cambra, R.A. and Oliveira, M.L. (2021) Revision of the *Traumatomutilla gemella* species-group (Hymenoptera, Mutillidae) with the description of its hitherto unknown males. Zoosystema, 43(1): 1–28.

Bischoff, H. (1920–1921) Monographie der Mutilliden Afrikas. Arch. Naturgesch., (1920) 86A(1/3): 1–480; (1921) 86A(4): 481–830, plates 1–7.

Bradley, J.C. and Bequaert, J. (1923) Studies in African Mutillidae. Rev. Zool. Afr., 11(3): 211–258.

Bradley, J.C. and Bequaert, J. (1928) A synopsis of the Mutillidae of the Belgian Congo. Bull. Am. Mus. Nat. Hist., 58(2): 63–122, plate I.

Branstetter, M.G., Danforth, B.N., Pitts, J.P., Faircloth, B.C., Ward, P.S., Buffington, M.L., Gates, M.W., Kula, R.R. and Brady, S.G. (2017a) Phylogenomic insights into the evolution of stinging wasps and the origins of ants and bees. Curr. Biol., 27(7): 1019–1025.

Branstetter, M.G., Longino, J.T., Ward, P.S. and Faircloth, B.C. (2017b) Enriching the ant tree of life: Enhanced UCE bait set for genome-scale phylogenomics of ants and other Hymenoptera. Meth. Ecol. Evol., 8(6): 768–776.

Bremer, K. (1988) The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evol., 42(4): 795–803.

Bremer, K. (1994) Branch support and tree stability. Cladistics, 10(3): 295–304.

Brothers, D.J. (1975) Phylogeny and classification of the aculeate Hymenoptera, with special reference to Mutillidae. Univ. Kansas Sci. Bull., 50(11): 483–648.

Brothers, D.J., Lelej, A.S.. and Williams, K.A. (2019) Genus-group names of Mutillidae (Hymenoptera): Corrections and updates since 2008. Zootaxa, 4651(3): 578–588.

Brothers, D.J. and Lelej, A.S. (2017) Phylogeny and higher classification of Mutillidae (Hymenoptera) based on morphological reanalyses. J. Hymenopt. Res., 60: 1–97.

Brower, A.V.Z. (2006) The how and why of branch support and partitioned branch support, with a new index to assess partition incongruence. Cladistics, 22(4): 378–386.

Cambra, R.A., Williams, K.A., Quintero A., D., Windsor, D.M., Pickering, J. and Saavedra, D. (2018a) *Dasymutilla* Ashmead (Hymenoptera, Mutillidae) in Panama: New species, sex associations and seasonal flight activity. Insecta Mundi, 0608: 1–17.

Cambra, R.A., Quintero, D., Waldren, G.C., Bartholomay, P.R. and Williams, K.A. (2018b) Taxonomic revision of the genus *Timulla* Ashmead, 1899 of Panama (Hymenoptera: Mutillidae). Tecnociencia, 20(2): 23–57.

Carpenter, J.M. (2008) Review of the Hawaiian Vespidae (Hymenoptera). Bishop Mus. Occ. Pap., 99: 1–18.

Chen, C. (1957) A revision of the velvety ants or Mutillidae of China. Quart. J. Taiwan Mus., 10(3–4): 135–224, plates 1–6.

Chernomor, O., von Haeseler, A. and Minh, B.Q. (2016) Terrace aware data structure for phylogenomic inference from supermatrices. Syst. Biol., 65(6): 997–1008.

Dammerman, K.W. (1948) The fauna of Krakatau, 1883–1933. Amsterdam: N.V. Noord-Hollandsche Uitgevers Mij., xii, 594 pp., 11 plates.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J. and Rambaut, A. (2006) Relaxed phylogenetics and dating with confidence. PLoS Biol., 4(5): e88.

Evans, H.E. (1969) Phoretic copulation in Hymenoptera. Entomol. News, 80: 113–124.

Faircloth, B.C., Branstetter, M.G., White, N.D. and Brady, S.G. (2015) Target enrichment of ultraconserved elements from arthropods provides a genomic perspective on relationships among Hymenoptera. Mol. Ecol. Resour., 15(3): 489–501.

Farris, D.W., Jaramillo, C., Bayona, G., Restrepo-Moreno, S.A., Montes, C., Cardona, A., Mora, A., Speakman, R.J., Glascock, M.D. and Valencia, V. (2011) Fracturing of the Panamanian Isthmus during initial collision with South America. Geol., 39(11): 1007–1010.

Gernhard, T. (2008) The conditioned reconstructed process. J. Theor. Biol., 253(4): 769–778.

Gillespie, R.G., Baldwin, B.G., Waters, J.M., Fraser, C.I., Nikula, R. and Roderick, G.K. (2012) Long-distance dispersal: A framework for hypothesis testing. Trends Ecol. Evol., 27(1): 47–56.

Goloboff, P.A. and Catalano, S.A. (2016) TNT version 1.5, including a full implementation of phylogenetic morphometrics. Cladistics, 32(3): 221–238.

Goswami, B.N. and Rajagopal, E.N. (2003) Indian Ocean surface winds from NCMRWF analysis as compared to QuikSCAT and moored buoy winds. J. Earth Syst. Sci., 112(1): 61–77.

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W. and Gascuel, O. (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. Syst. Biol., 59(3): 307–321.

Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q. and Vinh, L.S. (2017) UFBoot2: Improving the ultrafast bootstrap approximation. Mol. Biol. Evol., 35(2): 518–522.

Holzapfel, E.P. and Harrell, J.C. (1968) Transoceanic dispersal studies of insects. Pacific Insects, 10(1): 115–153.

Invrea, F. (1950) Mutillidi nuovi o notevoli del Bacino Mediterraneo (Hymenoptera, Mutillidae). Mem. Soc. Entomol. Ital., 29: 19–27.

Invrea, F. (1953) Mutillidi della Dalmazia e del litorale Montene grino. Nota Prima. Mem. Soc. Entomol. Ital., 32: 143–159.

Invrea, F. (1964) Mutillidae-Myrmosidae. Fauna d'Italia 5. Bologna: Edizioni Calderini, xii, 303 pp.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A. and Jermiin, L.S. (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Meth., 14: 587–589.

Katoh, K. and Standley, D.M. (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in performance and usability. Mol. Biol. Evol., 30(4): 772–780.

Keigwin, Jr. L.D. (1978) Pliocene closing of the Isthmus of Panama, based on biostratigraphic evidence from nearby Pacific Ocean and Caribbean Sea cores. Geol., 6(10): 630–634.

Krombein, K.V. (1971) A monograph of the Mutillidae of New Guinea, Bismarck Archipelago and Solomon Islands, part I: Mutillinae (Hymenoptera, Aculeata). In: Asahinas, L., Gressitt, J.L., Hikada, Z., Nishida, T., and Nomura, K. (Eds) Entomological Essays to Commemorate the Retirement of Professor K. Yasumatsu. Tokyo: Hokuryukan Publishing Co., pp. 25–60.

Krombein, K.V. (1972) Monograph of the Madagascan Mutillidae (Hymenoptera). Part I: Myrmillini, Mutillini and Smicromyrmini. Ann., Mus. R. Afr. Cent. (Ser. 8°), Sci. Zool., 199: 1–61.

Lelej, A.S. (1996) To the knowledge of the East Asian species of the tribe Trogaspidiini Bischoff, 1920 (Hymenoptera, Mutillidae) with description of eight new genera and two new species. Far East. Entomol., 30: 1–24.

Lelej, A.S. (2002) Catalogue of the Mutillidae (Hymenoptera) of the Palaearctic Region. Vladivostok: Dalnauka, 172 pp.

Lelej, A.S. (2005) Catalogue of the Mutillidae (Hymenoptera) of the Oriental Region. Vladivostok: Dalnauka, 252 pp.

Lelej, A.S. (2020) To the knowledge of velvet ants of the genera *Artiotilla* Invrea, *Radoszkowskitilla* Lelej and *Taiwanomyrme* Tsuneki (Hymenoptera: Mutillidae). Far East. Entomol., 410: 1–10.

Lelej, A.S. and Yamane, S. (1992) Interesting velvet ants (Hymenoptera, Mutillidae) from Japan and Korea. Jap. J. Entomol., 60(3): 625–632.

Lelej, A.S. and Nemkov, P.G. (1997) Phylogeny, evolution and classification of Mutillidae (Hymenoptera). Far East. Entomol., 46: 1–24.

Lelej, A.S. and van Harten, A. (2006) A review of the Mutillidae (Hymenoptera) of Yemen. Zootaxa, 1226: 1–50.

Lelej, A.S. and Brothers, D.J. (2008) The genus-group names of Mutillidae (Hymenoptera) and their type species, with a new genus, new name, new synonymies, new combinations and lectotypifications. Zootaxa, 1889: 1–79.

Li, J.-T., Wang, J.-S., Nian, H.-H., Litvinchuk, S.N., Wang, J., Li, Y., Rao, D.-Q. and Klaus, S. (2015) Amphibians crossing the Bering land bridge: Evidence from Holarctic treefrogs (*Hyla*, Hylidae, Anura). Mol. Phylogenet. Evol., 87: 80–90.

Magnacca, K.N. and Danforth, B.N. (2006) Evolution and biogeography of native Hawaiian *Hylaeus* bees (Hymenoptera: Colletidae). Cladistics, 22: 393–411.

Mickel, C.E. (1933) The Mutillidae of Formosa. Ann. Entomol. Soc. Am., 26: 381–423.

Mickel, C.E. (1937) The mutillid wasps of the genus *Timulla* which occur in North America north of Mexico. Entomol. Am., 37(1): 1–56; 37(2): 57–119.

Mickel, C.E. (1938) The Neotropical mutillid wasps of the genus *Timulla* Ashmead (Hymenoptera: Mutillidae). Trans. R. Entomol. Soc. Lond., 87(22): 529–679.

Miller, K.G., Kominz, M.A., Browning, J.V., Wright, J.D., Mountain, G.S., Katz, M.E., Sugarman, P.J., Cramer, B.S., Christie-Blick, N. and Pekar, S.F. (2005) The Phanerozoic record of global sea-level change. Science, 310: 1293–1298.

Miller, M.A., Pfeiffer, W. and Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proc. Gateway Comp. Environ. Workshop (GCE): 1–8.

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A. and Lanfear, R. (2020) IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. Mol. Biol. Evol., 37(5): 1530–1534.

Morrone, J.J. (2014) Biogeographical regionalisation of the Neotropical region. Zootaxa, 3782 (1): 1–110.

Nixon, K.C. (1999) The parsimony ratchet, a new method for rapid parsimony analysis. Cladistics, 15(4): 407–414.

Nguyen, L.-T., Schmidt, H.A., von Haeseler, A., and Minh, B.Q. (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol., 32(1): 268–274.

Nonveiller, G. (1973) Recherches sur les Mutillides de l'Afrique. Hymenoptera, Mutillidae. II. *Carinotilla* gen. nov. (♂♀). Ann. Fac. Sci. Cameroun, 14: 47–102.

Nonveiller, G. (1977) Recherches sur les Mutillides de l'Afrique – VII. *Dentotilla* gen. nov. ♂♀ (Hymenoptera: Mutillidae). Beitr. Entomol., 27(2): 255–300.

Nonveiller, G. (1980) Recherches sur les Mutillides de l'Afrique (Hymenoptera, Mutillidae). XI. Révision des mâles du genre *Trogaspidia* Ashmead, 1899 (♀, ♂) apparentés ou semblables a *medon* (Smith) 1855 (♂) avec la description du sous-genre nouveau *Tuberocoxotilla* (♂♂). Mem. Inst. Prot. Plantes, Beograd, 14: 69–108.

Nonveiller, G. (1994) Description du nouveau genre Afrotropical *Spinulomutilla* et de onze espècies nouvelles (Hymenoptera : Mutillidae). Ann. Soc. Entomol. Fr. (N.S.), 30(3): 329–344; 30(4): 367–389.

Nonveiller, G. (1995a) Recherches sur les Mutillides de l'Afrique XVII. Note pour servir a la Connaissance du genre *Pristomutilla* Ashmead, 1903 (♂♀) avec description du mâle encore inconnu du genre, d'espècies nouvelles du genre et des nouveaux sous-genres *Diacanthotilla* (♀♀) et *Acanthomutilla* (♀♀) (Hymenoptera, Mutillidae). Entomofauna, Z. Entomol., 16(5): 29–120.

Nonveiller, G. (1995b) A preliminary approach to a revision of the Afrotropical representatives of the genus *Trogaspidia* Ashmead, 1899 (*sensu* Bischoff, 1920) (Hymenoptera, Mutillidae). Studies on African Mutillidae XXI. (Third contribution to the knowledge of the Afrotropical *Trogaspidia*). Ann. Soc. Ent. Fr. (N.S.), 31(4): 349–368.

Nonveiller, G. (1996) *Allotropidia acuticarinata*, nouveau genre et nouvelle espècie de l'Afrique (Hymenoptera, Mutillidae). Bull. Inst. R. Sci. Nat. Belg., Entomol., 66: 75–78.

Nonveiller, G. and Ćetković, A. (1995) Recherches sur les Mutillides de l'Afrique (Hymenoptera, Mutillidae). XIX. Révision du genre *Lophotilla* Bischoff, 1920. Étude des mâles. Nouv. Rev. Entomol. (N.S.), 12(2–3): 161–185.

Nonveiller, G. and Ćetković, A. (1996) *Seriatospidia* n. gen. with a description of three new species (Hymenoptera Mutillidae). Trop. Zool., 9: 305–314.

Okayasu, J., Lelej, A.S. and Williams, K.A. (2021) Review of *Eotrogaspidia* Lelej (Hymenoptera: Mutillidae: Trogaspidiini). Zootaxa, 4920(1): 56–90.

O'Toole, C. (1975) The systematics of *Timulla oculata* (Fabricius) (Hymenoptera, Mutillidae). Zool. Scr., 4(5–6): 229–251.

Özdikmen, H. (2005) Five new substitute names for Hymenoptera (Insecta) genera. J. Entomol. Sci., 40(2): 237–239.

Pagden, H.T. (1949) Description and records of Austro-Malaysian Methocidae and Mutillidae (Hymenoptera). Trans. R. Ent. Soc. Lond., 100(8): 191–231.

Pagliano, G. (2019) Nuove specie di *Curvitropidia* dell'Australia e dell'Africa (Hymenoptera, Mutillidae). Boll. Mus. Reg. Sci. Nat. Torino, 35(1–2): 85–92.

Pagliano, G., Brothers, D.J., Cambra, R., Lelej, A.S., Lo Cascio, P., Palmerini, M.M., Scaramozzino, P.L., Williams, K.A., and Romano, M. (2020) Checklist of names in Mutillidae (Hymenoptera), with illustrations of selected species. Boll. Mus. Reg. Sci. Nat. Torino, 36(1–2): 1–425.

Pennacchio, L.A., Ahituv, N., Moses, A.M., Prabhakar, S., Nobgrega, M.A., Shoukry, M., Minovitsky, S., Dubchak, I., Holt, A., Lewis, K.D., Plajzer-Frick, I., Akiyama, J., De Val, S., Afzal, V., Black, B.L., Couronne, O., Eisen, M.B., Visel, A. and Rubin, E.M. (2006) *In vivo* enhancer analysis of human conserved non-coding sequences. Nature, 444: 499–502.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G. and Suchard, M.A. (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Syst. Biol., 67(5): 901–904.

Repenning, C.A. (1967) Palearctic-Nearctic Mammalian dispersal in the late Cenozoic. In: Hopkins, D.M. (Ed.) The Bering Land Bridge. Stanford: Stanford University Press, pp. 288–311.

Rodriguez, J., Pitts, J.P. and von Dohlen, C.D. (2014) Historical biogeography of the widespread spider wasp tribe Aporini (Hymenoptera: Pompilidae). J. Biogeogr., 42(3): 495–506.

Ronquist, F. and Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetics inference under mixed models. Bioinformatics, 19(12): 1572–1574.

Sadler, E.A. (2018) Historical biogeography and natural history of nocturnal wasps in the southwestern deserts with special emphasis on the genus *Chyphotes* (Hymenoptera: Chyphotidae). Utah State University at Logan, Ph.D.

Sandelin, A., Bailey, P., Bruce, S., Engström, P.G., Klos, J.M., Wasserman, W.W., Ericson, J. and Lenhard, B. (2004) Arrays of ultraconserved non-coding regions span the loci of key developmental genes in vertebrate genomes. BMC Genomics, 5: 99.

Sanmartín, I., Enghoff, H. and Ronquist, F. (2001) Patterns of animal dispersal, vicariance and diversification in the Holarctic. Biol. J. Linn. Soc., 73: 345–390.

Suárez, F.J. (1969) Contribution à la faune du Congo (Brazzaville). Mission A. Villiers et A. Descarpentries. LXXXIV. Hyménoptères Apterogynidae et Mutillidae. Bull. Inst. Afr. Noire, Dakar. Ser. A: Sci. Nat., 31(1): 102–137.

Suchard, M.A., Lemey, P., Baele, G., Ayres, D.L., Drummond, A.J. and Rambaut, A. (2018) Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus Evol., 4(1): vey016.

Talavera, G. and Castresana, J. (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst. Biol., 56(4): 564–577.

Tiffney, B.H. (1985) The Eocene North Atlantic land bridge: Its importance in Tertiary and modern phytogeography of the Northern Hemisphere. J. Arnold Arbor., 66: 243–273.

To, T.-H., Jung, M., Lycett, S. and Gascuel, O. (2016) Fast dating using least-squares criteria and algorithms. Syst. Biol., 65(1): 82–97.

Tsuneki, K. (1993) On some Taiwanese Mutillidae collected in 1976 by Mr. T. Murota, with descriptions of new taxa (Hymenoptera). Spec. Pub. Jap. Hymenopt. Assoc., 41: 39–50.

Viette, P. (1978 [“1977”]) Les types des Mutilles malgaches décrites par G. Olsoufieff (Hym. Mutillidae). Bull. Soc. Entomol. Fr., 82: 224–228.

Waldren, G.C., Roberts, J.D. and Pitts, J.P. (2020a) Phoretic copulation in the velvet ant *Sphaeropthalma pensylvanica* (Lepeletier) (Hymenoptera, Mutillidae): A novel behavior for Sphaeropthalminae with a synthesis of mating strategies in Mutillidae. J. Hymenopt. Res., 78: 69–89.

Waldren, G.C., Williams, K.A., Cambra, R.A. and Pitts, J.P. (2020b) Systematic revision of the North American velvet ant genus *Invreiella* Suárez (Hymenoptera: Mutillidae) with description of eleven new species. Zootaxa, 4894(2): 151–205.

Wang, B., Clemens, S.C. and Liu, P. (2003) Contrasting the Indian and East Asian monsoons: Impacts on geologic timescales. Mar. Geol., 201: 5–21.

Warren, B.H., Strasberg, D., Bruggemann, J.H., Prys-Jones, R.P. and Thébaud, C. (2010) Why does the biota of the Madagascar region have such a strong Asiatic flavor? Cladistics, 26: 526–538.

Wolfe, J.A. (1994) An analysis of Neogene climates in Beringia. Palaeogeogr., Palaeoclimatol., Palaeoecol., 108: 207–216.

Wolfe, J.A. and Tanai, T. (1980) The Miocene Seldovia Point flora from the Kenai Group, Alaska. Geol. Surv. Prof. Pap., 1105: 1–52.

Woolfe, A., Goodson, M., Goode, D.K., Snell, P., McEwen, G.K., Vavouri, T., Smith, S.F., North, P., Callaway, H., Kelly, K., Walter, K., Abnizova, I., Gilks, W., Edwards, Y.J.K., Cooke, J.E. and Elgar, G. (2004) Highly conserved non-coding sequences are associated with vertebrate development. PLoS Biol., 3(1): e7.

Yoder, A.D. and Nowak, M.D. (2006) Has vicariance or dispersal been the predominant biogeographic force in Madagascar? Only time will tell. Ann. Rev. Ecol. Syst., 37: 405–301.

Yoshimoto, C.M. (1959) Revision of the Hawaiian Pemphredoninae (Hymenoptera: Sphecidae). Proc. Hawaii Entomol. Soc., 17(1): 128–149.

Yoshimoto, C.M. (1960) Revision of Hawaiian Crabroninae with synopsis of Hawaiian Sphecidae (Hym.). Pacific Insects, 2(3): 301–337.

Yu, Y., Blair, C. and He, X. (2019) RASP 4: Ancestral state reconstruction tool for multiple genes and characters. Mol. Biol. Evol., 37(2): 604–606.

CHAPTER V

SYSTEMATIC REVISION OF THE NORTH AMERICAN VELVET ANT GENUS *INVREIELLA* SUÁREZ (HYMENOPTERA: MUTILLIDAE) WITH DESCRIPTION OF ELEVEN NEW SPECIES[4]

Abstract

The velvet ant genus *Invreiella* Suárez, 1966 is redescribed and revised. Females are grouped into five species-groups, with eleven new species described based on females: *I. acuminata* Waldren, **sp. nov.**, *I. australis* Waldren, **sp. nov.**, *I. bimaculata* Waldren, **sp. nov.**, *I. breviclypeata* Waldren, **sp. nov.**, *I. chihuahuensis* Waldren, **sp. nov.**, *I. cuernavaca* Waldren, **sp. nov.**, *I. erythrocephala* Waldren, **sp. nov.**, *I. manleyi* Waldren, **sp. nov.**, *I. mesomexicana* Waldren, **sp. nov.**, *I. suarezi* Waldren, **sp. nov.**, and *I. tequila* Waldren, **sp. nov.** One new combination is included, *I. cephalargia* (Mickel, 1924), **comb. nov.**, formerly placed in *Pseudomethoca* Ashmead, 1896. Additionally, *I. curoei* Quintero & Cambra, 2011 is synonymized with *I. cardinalis* (Gerstaecker, 1874), **syn. nov.**, and *I. megacantha* (Cockerell & Casad, 1894), **stat. resurr.**, is treated as a *nomen dubium*. The lectotype of *Mutilla satrapa* Gerstaecker, 1874 originally designated by C. Mickel is here validated. An illustrated key to species is included.

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Key words: Aculeata, biogeography, Mexican transition zone, Müllerian mimicry, mutillid wasps, Nearctic, Neotropical, *Pseudomethoca*, Sphaeropthalminae

Introduction

The genus *Invreiella* Suárez, 1966 is a little-known group of velvet ants that occur in Mexico and the southwestern United States. There have been relatively few mentions of this genus in the literature since its description, which are primarily limited to catalogs (Nonveiller 1990; Fernández 2001; Pagliano 2005; Lelej & Brothers 2008; Pagliano *et al.* 2020) and phylogenetic studies (Brothers 1975; Lelej & Nemkov 1997; Pitts *et al*. 2010; Brothers & Lelej 2017). Additionally, the clypeal morphology of *I. satrapa* (Gerstaecker, 1874) was briefly discussed in Cambra *et al.* (2014). The genus was not included in the two most recent keys to the New World mutillid genera (Manley & Pitts 2002; Brothers 2006a), nor in an overview of the Neotropical mutillid fauna (Brothers 2006b). A revision of *Invreiella* was recently published by Quintero & Cambra (2011), which represents the only taxonomic treatise on the genus since Suárez (1966). The authors misapplied the name *I. cardinalis* (Gerstaecker, 1874) to an undescribed taxon or taxa and redescribed *I. cardinalis* as a new species, *I. curoei* Quintero & Cambra, 2011. The aim of this contribution is to resolve this taxonomic issue and to further describe the diversity and distribution of *Invreiella*. The genus is uncommon in natural history collections and most species are known from fewer than five specimens. This may be, in part, an artifact of limited collecting where these insects occur. The males are presently unknown.

Invreiella is a member of the Sphaeropthalminae. Historically, the genus has been further classified as either a member of Sphaeropthalmini: Pseudomethocina (Brothers 1975, 1999), Pseudomethocini (Lelej & Nemkov 1997), and most recently, Pseudomethocini: Pseudomethocina (Brothers & Lelej 2017). Female pseudomethocines are typically characterized by a large quadrate head, a pear-shaped mesosoma in dorsal view that is constricted at the propodeal spiracles, and T1 is sessile with T2. Males often also have a quadrate head and T1 is sessile with T2; additionally, their integument is often mostly black (Brothers 1975, 1995; pers. obs.). Brothers & Lelej (2017) determined that Pseudomethocina are not supported by any unique synapomorphies, but rather by three homoplasious synapomorphies: compound eye of females with faintly distinguishable ommatidia, metacoxa of females carinate mesad, and axilla of winged males with broad vertical flange.

History of *Invreiella* Suárez

The first researcher to make contributions to *Invreiella* was Gerstaecker (1874) who described *Mutilla cardinalis* and *M. satrapa*. Additional species were described shortly thereafter: *Mutilla macrocephala* Smith, 1879, *Sphaeophthalma* [sic] *jocularis* Cameron, 1894, and *S. megacantha* Cockerell & Casad, 1894. Dalla Torre (1897) synonymized all described mutillid genera with *Mutilla* Linnaeus, 1758, which created numerous species-level homonyms. Consequently, *Mutilla ganahlii* Dalla Torre, 1897 was the new name assigned to *Mutilla macrocephala* Smith, 1879, *nec* Olivier, 1811. An additional species, *Pseudomethoca cephalargia* Mickel, 1924, was also described. Most of these species languished in *Mutilla* Linnaeus, 1758 or *Sphaeropthalma* Blake, 1871, until Mickel

(1937, 1964) transferred them to *Pseudomethoca* Ashmead, 1896. Additionally, he synonymized several names: *S. jocularis* Cameron and *S. megacantha* Cockerell & Casad under *P. cardinalis* (Gerstaecker), and *M. macrocephala* Smith and *M. ganahlii* Dalla Torre under *P. satrapa* (Gerstaecker) (Mickel 1964). C. Mickel's decisions were based on his study of the type specimens more than thirty years earlier. While the placement of these species in *Pseudomethoca* was a substantially more accurate representation of their phylogenetic affinity than *Mutilla*, it was not entirely on point; *Pseudomethoca* has long been recognized as a heterogeneous assemblage of taxa (Suárez 1962; Quintero & Cambra 2011; pers. obs.). Few studies have aimed to resolve this problem likely due to the enormity and complexity of the task.

Among the few who have attempted work on the *Pseudomethoca* issue, Suárez (1962) examined specimens of *Pseudomethoca* and *Sphinctopsis* Mickel in preparation for his description of a new genus, *Hoplognathoca* Suárez. He noted genus-level differences between the type species of *Pseudomethoca* and *Sphinctopsis* (*Mutilla frigida* Smith, 1855 (figs 5.23, 5.79, 5.110) and *M. melanocephala* Perty, 1833 (=*M. spixi* Diller, 1990), respectively), as well as some species placed incorrectly in *Mutilla*, including *Mutilla jocularis* Cameron (=*I. cardinalis*). Suárez (1966), thus, described *Invreiella*, a patronym dedicated to the Italian hymenopterist Fabio Invrea. Suárez transferred two species into the new genus: *I. cardinalis* (Gerstaecker) and *I. satrapa* (Gerstaecker), along with their respective synonyms published by Mickel (1964). Suárez considered the genus to be near *Pseudomethoca* (*sensu stricto*), or possibly a subgenus thereof (Suárez 1966).

Lastly, a revision of *Invreiella* was recently published by Quintero & Cambra (2011), who redescribed *I. cardinalis* as a new species (*I. curoei* Quintero & Cambra), included a key to species, and provided distribution data for their concepts of *I. cardinalis* and *I. satrapa*.

Biogeography and biology

Invreiella occurs throughout most of the Nearctic portions of Mexico and in the extreme southwestern United States. There has been conflicting information in the past regarding whether the genus is Nearctic or Neotropical in distribution. Suárez (1966) implied the genus is Neotropical, while Quintero & Cambra (2011) stated that the genus is mostly Nearctic, and, further, endemic to Mexico. Additionally, Lelej & Brothers (2008) and Pitts *et al.* (2010) list the distribution of the genus as Neotropical. These assertions are inaccurate in light of new data documented in this revision. We report here two species from Arizona and New Mexico, *I. cephalargia* (Mickel, 1924), **comb. nov.** and *I. manleyi*, **sp. nov.**, which represent the first *Invreiella* records for the United States and are the northernmost members of the genus (fig. 5.138). Additionally, *I. australis*, **sp. nov.** was collected north of the city of Villaflores in Chiapas, Mexico; this species represents the southernmost distribution record of the genus and is the only known species found east of the Isthmus of Tehuantepec (fig. 5.131). We regard *Invreiella* as having an intergrade Nearctic-Neotropical distribution. Many specimen records are from the Mexican transition zone (fig. 5.127), wherein the faunas of the Nearctic and Neotropical regions overlap (Halffter 1976, 1987; Morrone 2014, 2015; Halffter & Morrone 2017). The genus does not significantly extend into either the Nearctic region or the Neotropical region, with its members occurring at the southern and northern limits of these regions, respectively. Further, most elevation records for *Invreiella* are relatively high. We have examined or referenced specimens that were collected at elevations ranging from 301–2743 meters:

I. australis — 1100 m
I. bimaculata — 350 m
I. breviclypeata — 1829 m
I. cardinalis — 1524 m; 1676 m; 1707 m; 1829 m; 1900 m; 1970 m; 2012 m
I. cephalargia — 301 m; 1340 m
I. chihuahuensis — 2164 m
I. erythrocephala — 1280 m
I. manleyi — 2377 m
I. satrapa — 2073 m; 2134 m; 2377 m; 2408 m; 2743 m
I. suarezi — 762 m; 1425 m
I. tequila — 1646 m

The biology of *Invreiella* is unknown. There are no known host records, although the presence of a protarsal rake and a coarsely-sculptured pygidial plate bound by lateral carinae in the females indicate that members of this genus likely parasitize ground-nesting hosts (Krombein 1972; Quintero & Cambra 1996, referencing Naumann (1991); Williams *et al*. 2011). Further, members of Pseudomethocini are thus far only known to parasitize bees (Hymenoptera: Anthophila) (Krombein 1992; Luz *et al*. 2016). One female specimen of *I. cardinalis* was collected on flowers of the Mexican kidneywood tree, *Eysenhardtia polystachya* (Ortega) Sarg., likely nectaring. Another female was collected on an *Ipomoea* sp. (morning glory). Additionally, an *I. satrapa* female was collected on *Bidens pilosa* Linnaeus (beggar-ticks/Spanish needle) and another on a pepper tree.

Materials, methods, and terminology

The Phylogenetic Species Concept *sensu* Wheeler & Platnick (2000) was used for this study. These authors define a species as "... the smallest aggregation of (sexual) populations or (asexual) lineages diagnosable by a unique combination of character states."

The abbreviations F1, F2, etc., refer to the first, second, etc., **antennal flagellomeres**. The term **antennal rim** is used for the convex section of cuticle that surrounds the antennal foramen. Tagma terminology follows Michener (1944): the **mesosoma** and **metasoma** refer to the apparent thorax and abdomen; the mesosoma includes the thorax plus the first true abdominal segment, referred to as the **propodeum**. The **scutellar area** as defined by Bartholomay *et al.* (2018) is the region apparently composed of both mesoscutellar and metascutellar tissue that is found between the propodeal spiracles on the mesosomal dorsum in females; the **scutellar scale** is a transverse carina or lamella found anteromedially in this scutellar area in females. The abbreviations T1, T2, etc., refer to the first, second, etc., **metasomal terga**, and S1, S2, etc., refer to the first, second, etc., **metasomal sterna**. The term **pygidial plate** is used for the median portion of the last metasomal tergum that is laterally delimited by a carina and its surface is often sculptured. Setal terminology follows Ferguson (1967): **simple setae** have smooth, unbarbed surfaces, **brachyplumose setae** have barbed surfaces with the lengths of the barbs equal to or less than the diameter of the hair at the point of attachment, and **plumose setae** have barbed surfaces with the lengths of the barbs greater than the diameter of the hair at the point of attachment.

Descriptions are comprehensive and not based solely on the holotype/lectotype; if present, notable variation is provided in the remarks. Specimen condition of types is noted if they are damaged. Label data for examined type material were transcribed verbatim, with transformed data provided last in brackets and parentheses; labels are separated by "//" within brackets and lines by "/." Further regarding type label data, labels are rectilinear in shape unless otherwise noted, handwritten portions of labels are italicized (while typeset portions are not), and ink is black unless otherwise noted. Label data for type material we did not examine, non-type material we examined, or referenced material were all transformed to a standardized format. Unique specimen identifiers (USIs) were assigned to specimens examined by the senior author (except for type material of species described prior to this study) and are included on holotype, paratype, and determination labels. The USI format used includes the prefix "GCW_HYM" followed by a unique number (*e.g.* GCW_HYM0000052). For each specimen and series of specimens, the following data are provided in parentheses: the number of specimens per the collecting event, sex, abbreviated USI without the prefix GCW_HYM, and collection abbreviation where the material is housed (*e.g.* (1♀–0000052–EMUS)). USIs are included in the figure legends and have been abbreviated without the prefix as well.

Localities were georeferenced primarily using Google Earth and are noted in brackets in decimal degrees for each specimen or series of specimens. Selander & Vaurie (1962) was relied upon for localities from Cameron (1894–1900). GPS coordinates that were included on specimen labels were transformed to decimal degrees if not already in this format. Geographic distribution maps were generated with SimpleMappr (Shorthouse 2010) using these coordinates. Biogeographic distributions were determined using the diagnoses of Morrone (2014) and the map in Morrone *et al.* (2017); Google Earth was used to determine the general elevation for specimens without elevation data to aid in determining biogeographic province membership. The biogeographic distribution map was generated in SimpleMappr (Shorthouse 2010) using the shapefiles from Morrone *et al.* (2017).

Measurements were recorded using an ocular reticle. Five specimens per species were measured, and if there were fewer than five known specimens, all available specimens were measured.

This revision is based on the study of 87 female specimens. Data for an additional 13 specimens were provided by several curators and collection managers which are denoted with an asterisk (*) after the collection abbreviations in the material examined sections. The material studied or referenced in this revision is deposited in the following collections (abbreviations primarily based on Evenhuis (2020)):

AMNH	American Museum of Natural History, New York, New York, USA.
ANSP	Academy of Natural Sciences at Drexel University, Philadelphia, Pennsylvania, USA.

ASUHIC	Hasbrouck Insect Collection, Arizona State University, Tempe, Arizona, USA.
CASC	California Academy of Sciences, San Francisco, California, USA.
CNC	Canadian National Collection of Insects, Ottawa, Ontario, Canada.
CSCA	California State Collection of Arthropods, California Department of Food and Agriculture, Sacramento, California, USA.
DGMC	Donald G. Manley Collection, Florence, South Carolina, USA.
DJBC	Collection of Denis J. Brothers, to be deposited in Iziko South African Museum, Cape Town, South Africa (SAMC).
EBCC	Estación de Biología "Chamela," Universidad Nacional Autónoma de México, San Patricio, Jalisco, Mexico.
EMEC	Essig Museum of Entomology, University of California, Berkeley, California, USA.
EMUS	Entomology Museum, Utah State University, Logan, Utah, USA.
FSCA	Florida State Collection of Arthropods, Gainesville, Florida, USA.
GCWC	George C. Waldren Collection, Logan, Utah, USA.
MADUG	Museo de Historia Natural Alfredo Dugès, Universidad de Guanajuato, Guanajuato, Guanajuato, Mexico.
MIUP	Museo de Invertebrados G.B. Fairchild, Universidad de Panamá, Panama City, Panama.
MNCN	Museo Nacional de Ciencias Naturales, Madrid, Spain.
MRSN	Museo Regionale di Scienze Naturali, Torino, Italy.

MSBA	Museum of Southwestern Biology, University of New Mexico, Albuquerque, New Mexico, USA.
NHMD	Statens Naturhistoriske Museum, Copenhagen, Denmark.
NHMUK	The Natural History Museum, London, England, UK.
NMNH	National Museum of Natural History, Washington, D.C., USA.
RBINS	Koninklijk Belgisch Instituut voor Natuurwetenschappen, Brussels, Belgium.
SEMC	Snow Entomological Museum Collection, University of Kansas, Lawrence, Kansas, USA.
TAMU	Department of Entomology Insect Collection, Texas A&M University, College Station, Texas, USA.
UCDC	Bohart Museum of Entomology, University of California, Davis, California, USA.
UMSP	University of Minnesota Insect Collection, St. Paul, Minnesota, USA.
UNAM	Universidad Nacional Autónoma de México, Mexico City, Mexico.
ZMHB	Museum für Naturkunde, Berlin, Germany.

Invreiella **Suárez, 1966**

Invreiella Suárez, 1966 [“1965”]: 472. Type species (♀): *Mutilla satrapa* Gerstaecker, 1874, by original designation. Brothers 1975: 590; Nonveiller 1990: 41; Lelej & Nemkov 1997: 19; Fernández 2001: 114; Lelej & Brothers 2008: 29; Pitts *et al.* 2010: 135; Quintero & Cambra 2011: 217; Brothers & Lelej 2017: 94; Pagliano *et al.* 2020: 102.

Diagnosis (female). Females are distinguished from other genera of Pseudomethocini by a combination of five characters. First, the procoxa is anteriorly dentate near the trochanter (fig. 5.63) (Suárez 1966). Second, the pronotum has a carina of variable form separating the dorsal and lateral faces of the sclerite (here termed the **pronotal carina**, figs 5.30, 5.31, 5.35, 5.36, 5.41–43, 5.46–48, 5.53, 5.54, 5.96–5.109, 5.112–5.125) (Mickel, unpublished key to Neotropical *Pseudomethoca* (*sensu lato*) species; Suárez 1966). This carina is usually visible dorsally and laterally, but is sometimes obscure or absent in some species in lateral view (see the *I. cephalargia* species-group and *I. suarezi* species-group). The pronotal carina begins at the humeral angle at the dorsolateral margin of the mesosoma and continues posteriorly, curving ventrad usually just anterior to the pronotal spiracle. This carina can accentuate the distinctness of the pronotal sclerite from the mesopleural sclerite. Third, the gena is posteriorly carinate and ventrally produced into a process of variable form (here termed the **genal process**, figs 5.28, 5.29, 5.32–5.34, 5.51, 5. 52, 5.65–5.78) (Mickel, unpub. key; Suárez 1966). Fourth, T1 is sessile with T2 and not petiolate (figs 5.9–5.22); further, T1 is short and transverse in dorsal view, with anterior and dorsal surfaces (not separated by a transverse carina), the anterior surface flattened. Finally, a pygidial plate is present and laterally defined by a carina, the sculpture of the plate being transversely rugose to rugose-granulate. **Male.** Unknown.

Description (female). Body length 7.49–12.95 mm.

Setae: Setae simple, without brachyplumose or plumose setae.

Head: Head 0.93–1.35 × as wide as mesosoma, quadrate in dorsal view. Sides of head diverging dorsally to ventrally in frontal view, with base of mandible extending beyond exterior-most protrusion of eye. Occipital carina present yet incomplete, dorsally distinct and ventrally obscure, not reaching or connected to hypostomal tooth. Weak median longitudinal impression spanning from vertex to frons present. Eye circular, protruding. Distance from posterior margin of eye to posterolateral corner of head 0.93–2.13 × maximum diameter of eye. Malar space short, with eye close to base of mandible. Antennal scrobe carinate dorsally, prominent, arcuate to straight in form, not spinose or conspicuously tuberculate at inner point of termination dorsad antennal rim, not overlapping antennal rim though sometimes close to touching it. Antennal rims well-separated, not connected basally. Scape arcuate, long, with single ventral carina and moderate scattered punctures. Antenna with 10 flagellomeres, cylindrical in cross section. F1 1.56–2.89 × as long as F2. Clypeus either flattened, rugose-granulate, and bituberculate medioapically, or with complete/medially interrupted transverse arcuate carina and lateral tubercle ventrad to carina. Mandible dorsally and ventrally carinate, with row of punctures anterior to and closely parallel to each carina, punctures each bearing long raised seta, forming row. Mandible apically tridentate, inner second tooth small, parallel with base of mandible, inner third tooth large and triangular, directed dorsally. Mandible with internal angulation midway between third apical tooth and dorsal base of mandible. Base of mandible without ventral tooth. Genal process present, either denticulate, triangular, or spinose in form, with posterior genal carina present, either convex, straight, or sinuate in form. Genal carina sometimes continuing beyond apex of process anteriorly, becoming obscure between proboscidal fossa and genal process, faintly appearing to curve across genal venter and terminate into weak to moderate hypostomal tooth. Postgenal bridge present, transversely rugose-striate, with midventral line. Proboscidal fossa triangular, separated from pleurostomal fossa by cuticular bridge. Maxillary palp with 6 palpomeres, labial palp with 4 palpomeres. Third labial palpomere transversely expanded.

Mesosoma: Mesosoma 0.99–1.42 × as wide as long, widest at pronotal carina or pronotal spiracle. Anterodorsolateral margin of pronotum usually outcurved. Humeral angle prominently carinate, terminating into tuberculate epaulet at anterodorsolateral margin of pronotum. Pronotal carina often present, separating dorsal and lateral faces of pronotum, or sometimes absent, with cluster of dense, contiguous, crenulate punctures in its place, simulating weak carina. Pronotal-mesonotal suture obscure. Metanotal-propodeal suture obscure to absent. Scutellar area with scutellar scale absent. Mesosoma constricted at propodeal spiracle in dorsal view, with dorsolateral margin of propodeum expanded and usually wider than point of constriction at propodeal spiracle in dorsal view. Posterior face of propodeum weakly concave. Dorsolateral margin of propodeum serially lined with blunt denticles, denticles sometimes conjoined. Mesopleuron without vertical carina, with variable vertical column of moderate punctures anterior to mesopleural-metapleural suture, punctures sequential at dorsal half of sclerite and becoming more separated at ventral half, punctures and suture close in proximity roughly at midpoint and diverging at dorsal and ventral sections, punctures sometimes anteriorly and/or posteriorly tuberculate. Mesopleuron posteriorly carinate at least along ventral half of mesopleural-metapleural suture, carina terminating roughly at midpoint of suture or beyond it, sometimes diverging from suture and continuing to dorsolateral margin of mesosoma. Dorsal portion of mesopleural-metapleural suture absent, obscure, or present, terminating into tubercle anterior to propodeal spiracle at dorsolateral margin. Propleural cavity at coxal insertion anteriorly bound by prominent, arcuate carina. Metasternal process present between metacoxae.

Legs: Procoxa anteriorly dentate near trochanter. Internal margin of metacoxa with longitudinal carina. Protarsal rake present. Meso- and metatibiae dorsally with two longitudinal rows of stout cylindrical spines, with internal row more prominent. Tibial spur formula 1-2-2. Tarsal claws with three stout setae on internal margin of each hook.

Metasoma: T1 sessile with T2. T1 short, transverse in dorsal view, with anterior and dorsal surfaces, without transverse carina separating surfaces, anterior surface flattened. T2 evenly convex, without longitudinal carinae or lateral protrusions, merely punctate. Felt line present on T2, absent on S2. S1 with longitudinal carina, without distinctive tubercles present on carina. S2 with weak to moderate transverse basal carina in shape of wide "V." Pygidium with pygidial plate present, laterally defined by carina, with sculpture of plate transversely rugose to rugose-granulate.

MALE. Unknown. An attempt was made to associate some male-based species of *Pseudomethoca* (*sensu lato*) with *Invreiella* females using distribution and similarity in size. The few promising candidates for *Invreiella* males were also potential males for several *Pseudomethoca* species, such as *Pseudomethoca bethae* Krombein, 1992 in the southwestern USA.

Etymology: Named after the Italian hymenopterist Dr. Fabio Invrea (1884–1968).

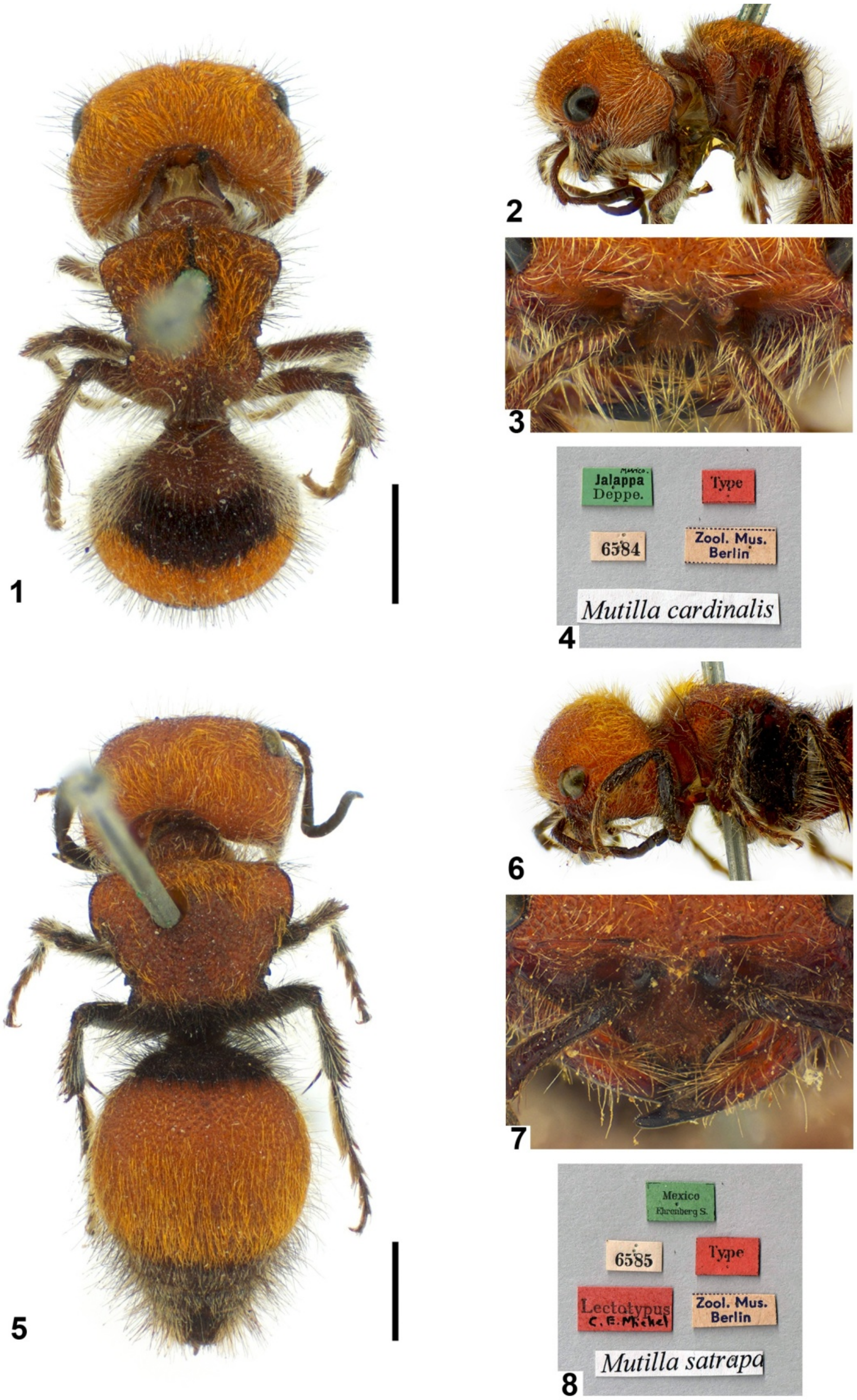
Distribution: Mexico (Chiapas, Chihuahua, Coahuila, Durango, Guanajuato, Guerrero, Hidalgo, Jalisco, Mexico, Michoacan, Morelos, Nayarit, Oaxaca, Puebla, San Luis Potosi, Sinaloa, Sonora, Veracruz, and Zacatecas); USA (Arizona and New Mexico).

Biogeography: Mexican transition zone; Nearctic region; Neotropical region.

Host(s): Unknown.

Remarks: At the time of his description of *Invreiella*, Suárez (1966) only knew of two species, *I. cardinalis* and *I. satrapa*. It seems that Suárez examined only one specimen of each species, which are housed at MNCN. With the description of eleven new female-based species and the addition of *I. cephalargia*, **comb. nov.**, generic redescription of the females became necessary to account for this increase in known diversity. Variation in the pronotal carina is especially notable: in two species-groups it is reduced or essentially absent (*I. cephalargia* and *I. suarezi* species-groups, respectively), in contrast to the *I. satrapa* species-group, where the pronotal carina is the most prominently developed in the genus. The shape of the dorsolateral margin of the mesosoma in dorsal view (figs 5.96–5.109), when coupled with the form of the pronotal carina, is valuable for delimiting species-groups and species.

Determining relationships between *Invreiella* and members of *Pseudomethoca* (*sensu lato*) is outside the scope of this study; the present catch-all nature of *Pseudomethoca* requires a comprehensive analysis of its constituent taxa. *Invreiella* is not present in either of the published keys to the New World mutillid genera (Manley & Pitts 2002; Brothers 2006a). We have provided several couplets at the beginning of the key to *Invreiella* females to help the user distinguish this genus from other members of Pseudomethocini.



FIGURES 5.1–5.8. *I. cardinalis*, holotype female: **5.1**–dorsal habitus**, 5.2**–lateral habitus, **5.3**–clypeus, **5.4**–labels. *I. satrapa*, lectotype female: **5.5**–dorsal habitus, **5.6**–lateral habitus, **5.7**–clypeus, **5.8**–labels. Scale bars = 2 mm.

9
I. australis
(0000001)

10
I. bimaculata
(0000002)

11
I. mesomexicana
(0000035)

12
I. acuminata
(0000056)

13
I. cardinalis
(0000018)

14
I. cuernavaca
(0000032)

15
I. cephalargia
(0000024)

16
I. manleyi
(0000037)

17
I. breviclypeata
(0000003)

18
I. chihuahuensis
(0000031)

19
I. satrapa
(0000049)

20
I. erythrocephala
(0000034)

21
I. suarezi
(0000051)

22
I. tequila
(0000053)

23
P. frigida

FIGURES 5.9–5.23. Female habitus, dorsal view. Scale bars = 2 mm.

Taxonomic synopsis of *Invreiella* Suárez, 1966

Invreiella australis species-group

♀ 1. *I. australis* Waldren, **sp. nov.**

♀ 2. *I. bimaculata* Waldren, **sp. nov.**

♀ 3. *I. mesomexicana* Waldren, **sp. nov.**

Invreiella cardinalis species-group

♀ 4. *I. acuminata* Waldren, **sp. nov.**

♀ 5. *I. cardinalis* (Gerstaecker, 1874)

= *Sphaerophthalma* [sic] *jocularis* Cameron, 1894

= *Invreiella curoei* Quintero & Cambra, 2011, **syn. nov.**

♀ 6. *I. cuernavaca* Waldren, **sp. nov.**

Invreiella cephalargia species-group

♀ 7. *I. cephalargia* (Mickel, 1924), **comb. nov.**

♀ 8. *I. manleyi* Waldren, **sp. nov.**

Invreiella satrapa species-group

♀ 9. *I. breviclypeata* Waldren, **sp. nov.**

♀ 10. *I. chihuahuensis* Waldren, **sp. nov.**

♀ 11. *I. satrapa* (Gerstaecker, 1874)

= *Mutilla macrocephala* Smith, 1879

= *Mutilla ganahlii* Dalla Torre, 1897

Invreiella suarezi species-group

♀ 12. *I. erythrocephala* Waldren, **sp. nov.**

♀ 13. *I. suarezi* Waldren, **sp. nov.**

♀ 14. *I. tequila* Waldren, **sp. nov.**

Nomen dubium

♀ 15. *I. megacantha* (Cockerell & Casad, 1894), **stat. resurr.**

Key to *Invreiella* females

1. Procoxa anteriorly dentate (fig. 5.63) **2**

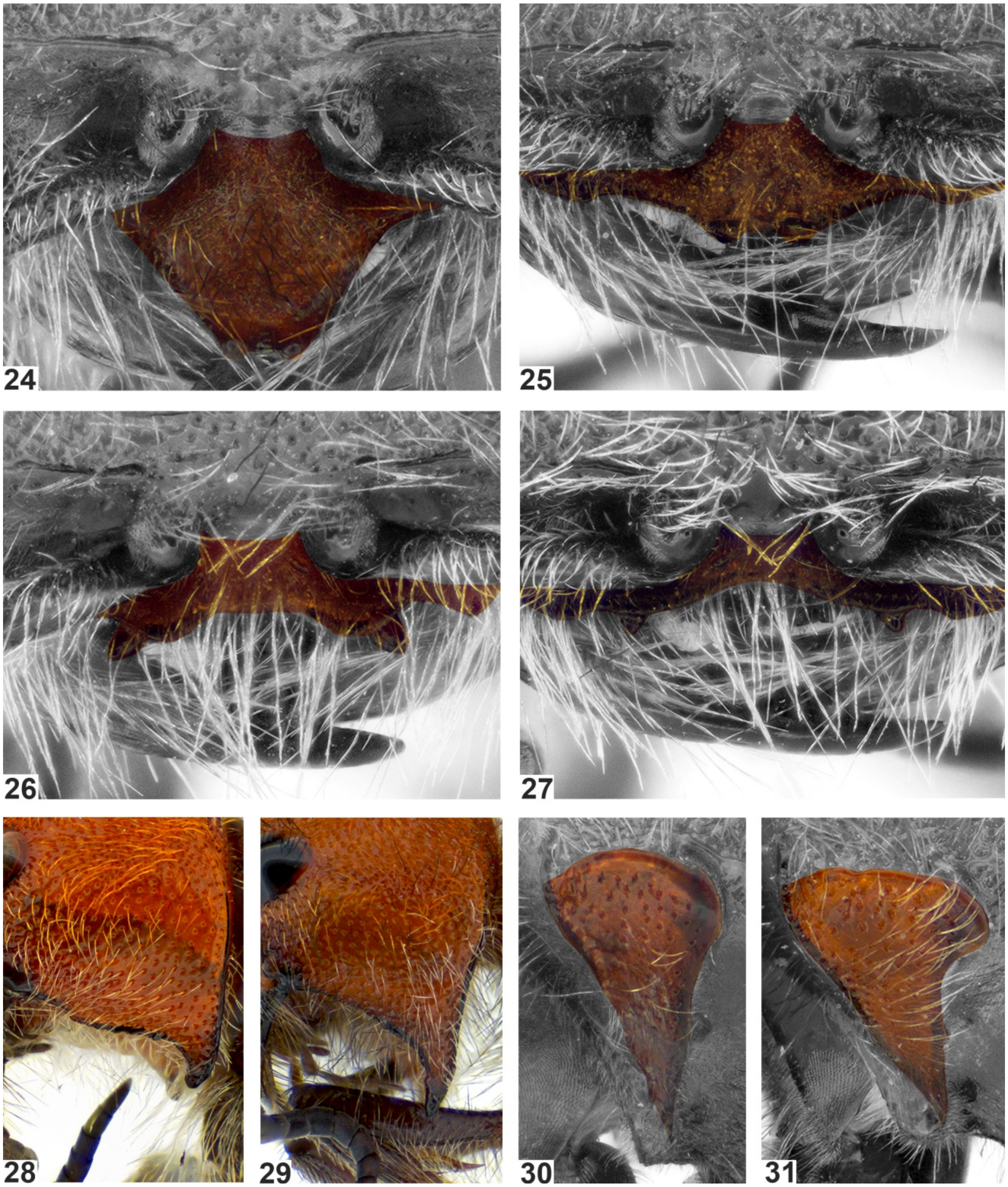
– Procoxa anteriorly edentate, merely convex .... **most Pseudomethocini**

2 (1). Genal process present and posteriorly carinate (figs 5.28, 5.29, 5.32–5.34, 5.51, 5.52, 5.65–5.78). Mesosoma widest at pronotum in dorsal view. Dorsolateral margin of mesonotum merely crenulate-carinate, sometimes with tubercle anterior to propodeal spiracle (figs 5.96–5.110) .... **3 (*Invreiella*)**

– Genal process absent, gena merely convex and carinate. Mesosoma widest at mesonotum in dorsal view. Dorsolateral margin of mesonotum anteriorly produced into curved process.. **undescribed genus**

3 (2). Clypeus produced into flattened plate-like structure, apical margin medially with two closely-spaced tubercles (figs 5.24, 5.25)........................ **4 (*I. satrapa* species-group)**

– Clypeus with complete or medially interrupted transverse arcuate carina and lateral tubercle ventrad to carina (figs 5.26, 5.27) **6**



FIGURES 5.24–5.31. Female clypeus: **5.24**–*I. chihuahuensis* (0000031), **5.25**–*I. breviclypeata* (0000003), **5.26**–*I. cardinalis* (0000018), **5.27**–*I. australis* (0000001). Female head, left lateral view: **5.28**–*I. chihuahuensis* (0000031), **5.29**–*I. satrapa* (0000049). Female pronotum, left lateral view: **5.30**–*I. chihuahuensis* (0000031), **5.31**–*I. satrapa* (0000049).

4 (3). Clypeus with apical margin medially elongate (fig. 5.24), tubercles triangular in dorsal view (sometimes worn down) and basally conjoined. Apical 1/4 of T2 with orange to red-brown integument with orange-red setae, apical fringe of T2 with median patch of black setae, remainder fringed with whitish setae (figs 5.18, 5.19).....................................**5**

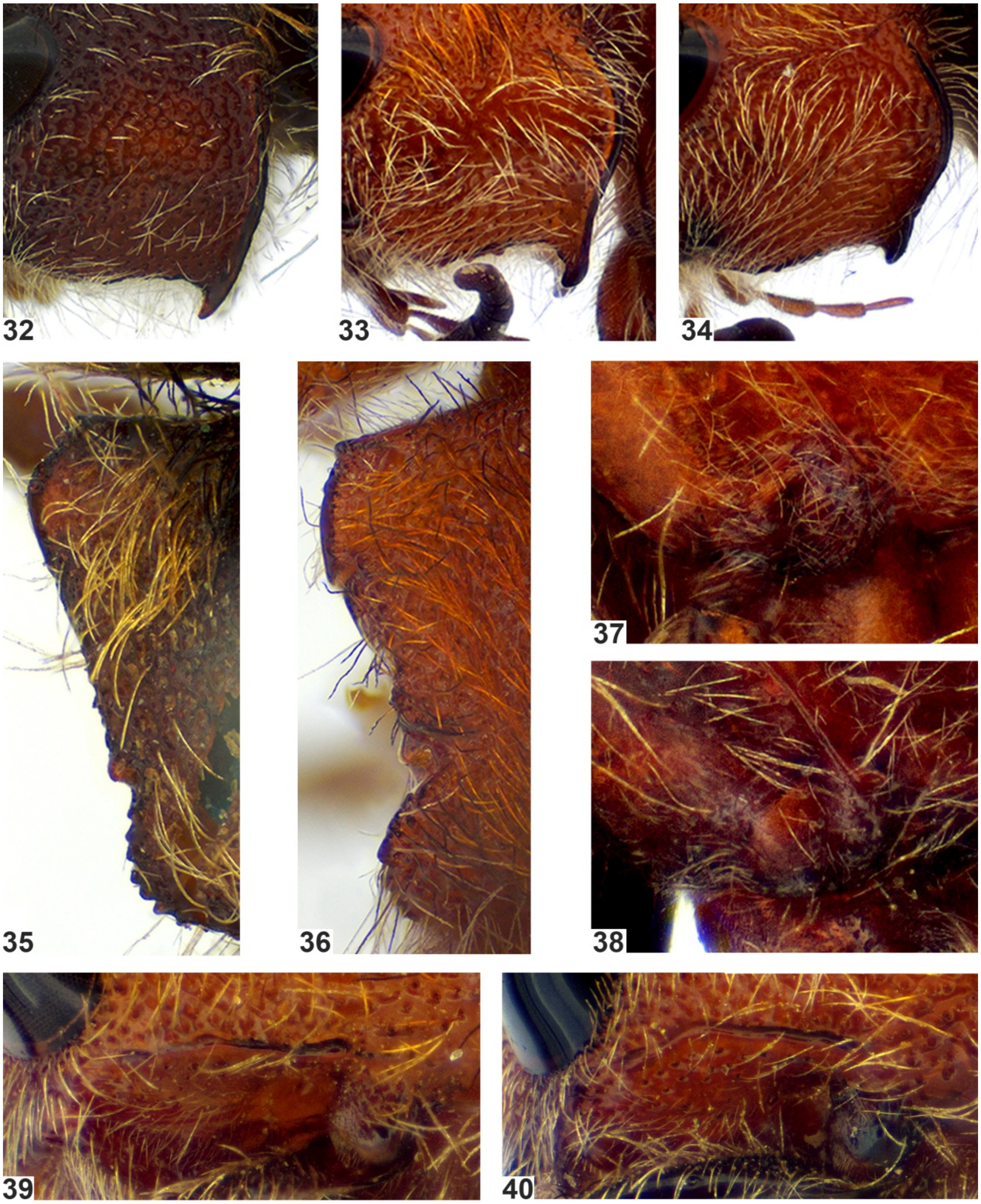
– Clypeus with apical margin only slightly longer medially than laterally (fig. 5.25), apical tubercles semi-circular in dorsal view (sometimes worn down) and basally separated. Apical 1/4 of T2 with black integument covered with black setae, apical fringe of T2 with wide median band of black setae, extreme lateral margin fringed with whitish setae (fig. 5.17) .... ***I. breviclypeata* Waldren, sp. nov.** (p. 260)

5 (4). Genal process straight (fig. 5.29). Posterior declivity of pronotal carina lobate and projecting away from mesopleuron, overlapping and obscuring dorsal portion of pronotal-mesopleural suture (figs 5.31, 5.106)...............***I. satrapa* (Gerstaecker)** (p. 268)

– Genal process curved (fig. 5.28). Posterior declivity of pronotal carina evenly rounded and flush with mesopleuron, dorsal portion of pronotal-mesopleural suture clearly visible (figs 5.30, 5.105)..***I. chihuahuensis* Waldren, sp. nov.** (p. 264)

6 (3). Clypeus with transverse arcuate carina broadly interrupted medially, area merely punctate, resulting in bilateral carinate processes each with enlarged tubercle ventrad to process (fig. 5.26) ....**7 (*I. cardinalis* species-group)**

– Clypeus with transverse arcuate carina complete, with small lateral tubercle ventrad to carina (fig. 5.27)...**9**



FIGURES 5.32–5.40. Female head, left lateral view: **5.32**–*I. acuminata* (0000056), **5.33**–*I. cardinalis* (0000018), **5.34**–*I. cuernavaca* (0000032). Female mesosoma, left dorsal view: **5.35**–*I. acuminata* (0000056), **5.36**–*I. cardinalis* (0000018). Female mesopleuron dorsad mesocoxa, left lateral view: **5.37**–*I. cardinalis* (0000018), **5.38**–*I. cuernavaca* (0000032). Female antennal scrobe, left anterior view: **5.39**–*I. cardinalis* (0000018), **5.40**–*I. cuernavaca* (0000032).

7 (6). Genal carina posteriorly sinuate in lateral view (figs 5.33, 5.34). Dorsolateral margin of mesosoma, from pronotal carina to propodeal spiracle, sinuate and undulating in outline in dorsal view (fig. 5.36). Integument of dorsum of head, mesosoma, and center of T2 orange-red (figs 5.13, 5.14)..**8**

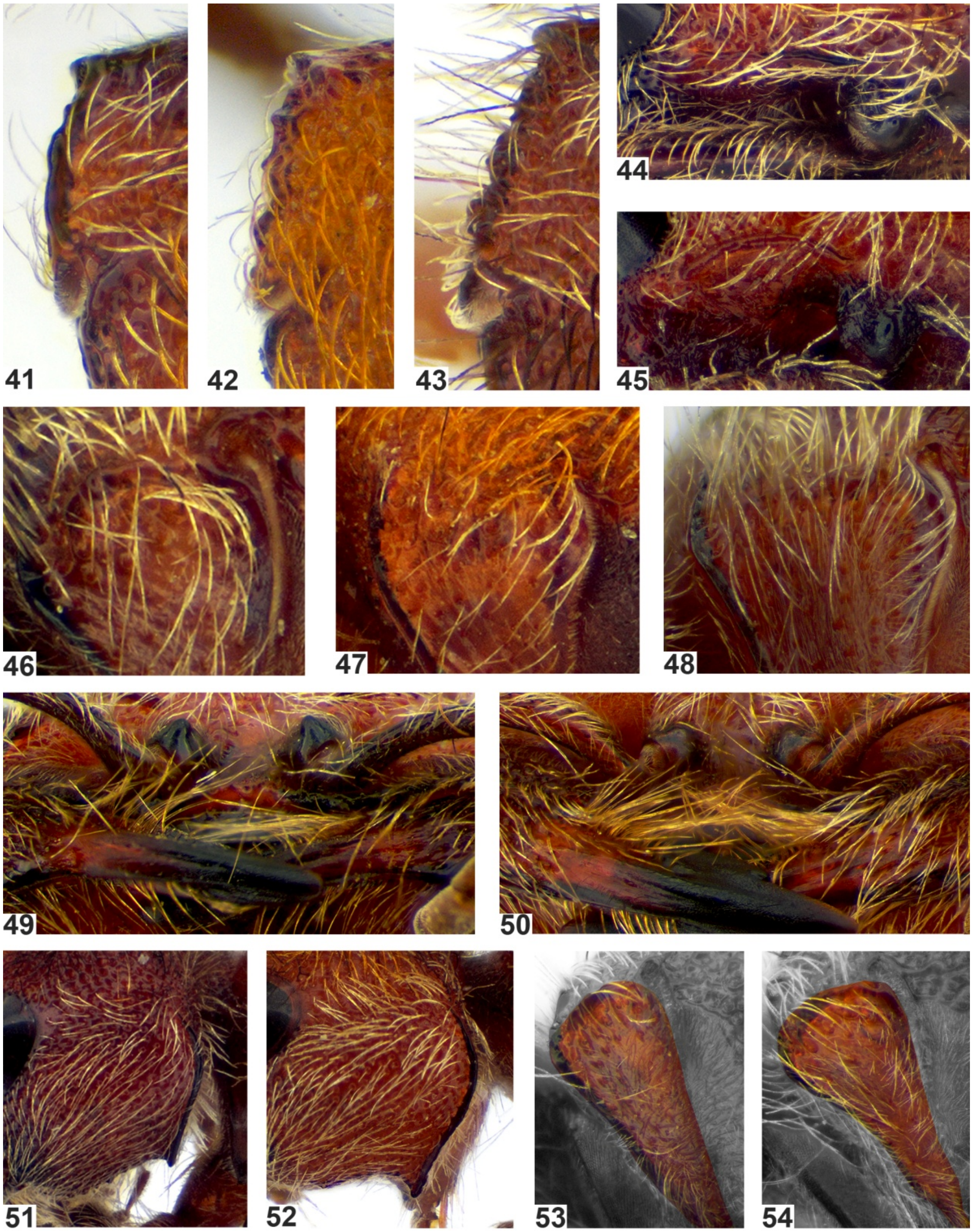
– Genal carina posteriorly convex in lateral view (fig. 5.32). Dorsolateral margin of mesosoma, from pronotal carina to propodeal spiracle, straight in outline in dorsal view (fig. 5.35). Integument of dorsum of head, mesosoma, and T2 dark brown-red (fig. 5.12).. ....***I. acuminata* Waldren, sp. nov.** (p. 231)

8 (7). Antennal scrobe carina straight (fig. 5.39). Genal process spinose and curved (fig. 5.33), carina anterior to process distinctly crenulate-tuberculate. Mesopleuron with ventral 1/3 rugose-granulate with weak rugae and few moderate punctures (fig. 5.37)........ .... ***I. cardinalis* (Gerstaecker)** (p. 235)

– Antennal scrobe carina arcuate (fig. 5.40). Genal process triangular and weakly curved (fig. 5.34), carina anterior to process obscurely crenulate. Mesopleuron with ventral 1/3 micropunctate with interpunctal space smooth (fig. 5.38).. ....***I. cuernavaca* Waldren, sp. nov.** (p. 242)

9 (6). Antennal rim tuberculate (fig. 5.49). Pronotal carina present and glabrous, often crenulate (figs 5.41, 5.46, 5.53, 5.54). T2 with two separate or coalescing light integumental spots (figs 5.9–5.11)......................................**10 (*I. australis* species-group)**

– Antennal rim rounded (fig. 5.50). Pronotal carina absent, area merely punctate to rugose-punctate, puncture edges sometimes tuberculate, simulating obscure pronotal



FIGURES 5.41–5.54. Female pronotum, left dorsal view: **5.41**–*I. australis* (0000001), **5.42**–*I. manleyi* (0000037), **5.43**–*I. tequila* (0000053). Female antennal scrobe, left anterior view: **5.44**–*I. australis* (0000001), **5.45**–*I. mesomexicana* (0000035). Female pronotum, left lateral view: **5.46**–*I. australis* (0000001), **5.47**–*I. manleyi* (0000037), **5.48**–*I. tequila* (0000053). Female antennal rims, ventral view: **5.49**–*I. bimaculata* (0000002), **5.50**–*I. tequila* (0000053). Female head, left lateral view: **5.51**–*I. bimaculata* (0000002), **5.52**–*I. mesomexicana* (0000035). Female pronotum, left lateral view: **5.53**–*I. bimaculata* (0000002), **5.54**–*I. mesomexicana* (0000035).

carina (figs 5.42, 5.43, 5.47, 5.48). T2 integument without integumental spots (figs 5.15, 5.16, 5.20), or with setal and integumental pattern in exaggerated “W” shape (figs 5.21, 5.22) **12**

10 (9). Antennal scrobe carina arcuate (fig. 5.45). Integument of head orange to dark brown-red, without patches of black integument (figs 5.10, 5.11, 5.66, 5.67)................. **11**

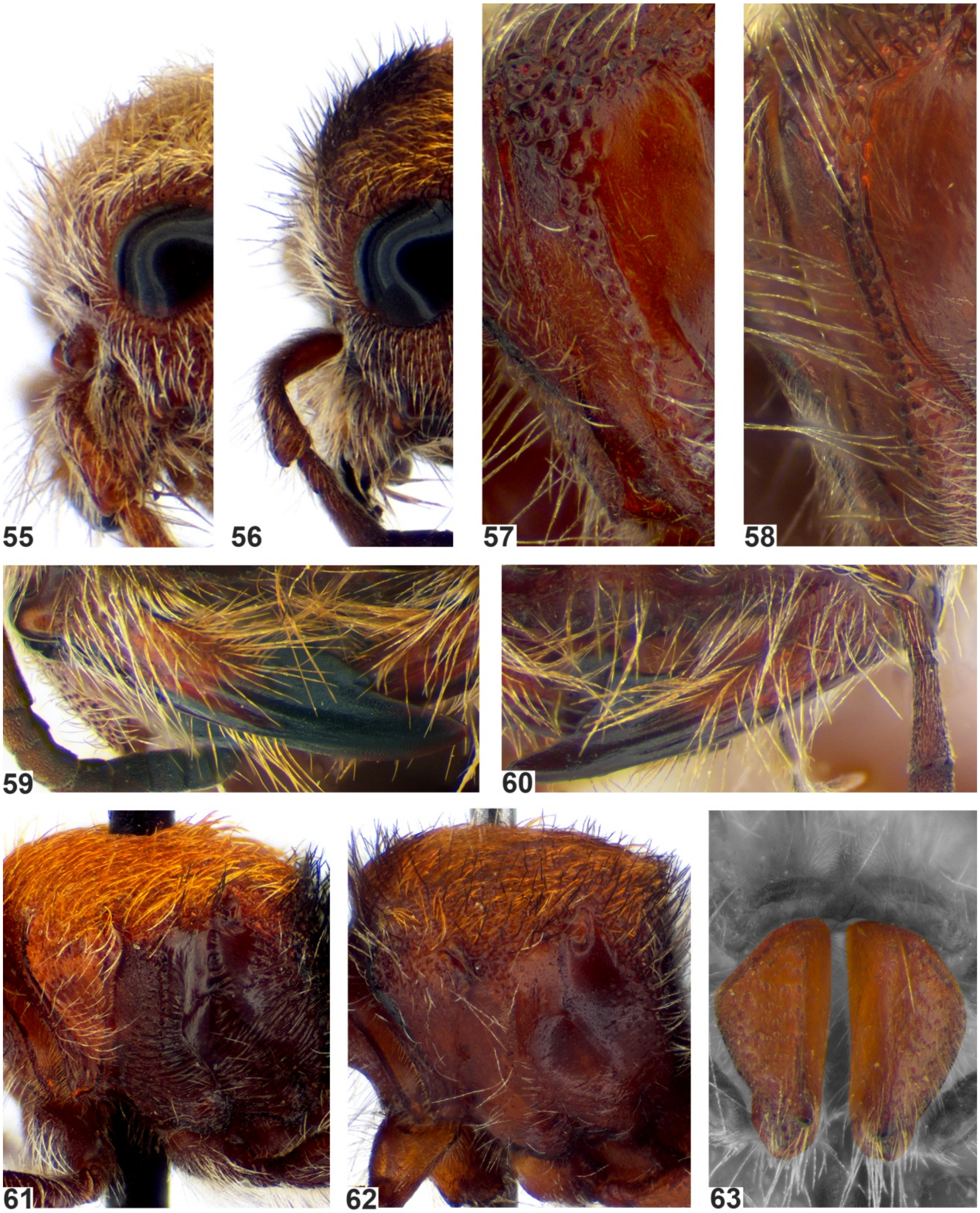
– Antennal scrobe carina straight (fig. 5.44). Integument of head black at posterolateral corners, gena, and occiput; integument of frons and part of vertex orange (figs 5.9, 5.65) . ... ***I. australis* Waldren, sp. nov.** (p. 216)

11 (10). Genal process denticulate, inconspicuous (fig. 5.51). Lateral face of pronotum mostly with moderate punctures (fig. 5.53). T2 with two separated yellow integumental spots (fig. 5.10) .... ***I. bimaculata* Waldren, sp. nov.** (p. 221)

– Genal process triangular (fig. 5.52). Lateral face of pronotum with few moderate punctures (fig. 5.54). T2 with two coalescing light orange integumental spots (fig. 5.11)... .... ***I. mesomexicana* Waldren, sp. nov.** (p. 225)

12 (9). Frons not transversely recessed and not concave below antennal scrobe carinae, antennal rims visible in lateral view (fig. 5.55). Mesopleuron and lateral face of propodeum mostly striate-rugose (fig. 5.61). Northwestern Mexico (Chihuahua) and southwestern USA (Arizona and New Mexico) .... **13 (*I. cephalargia* species-group)**

– Frons transversely recessed and concave below antennal scrobe carinae, antennal rims not visible in lateral view (fig. 5.56). Mesopleuron and lateral face of propodeum mostly



FIGURES 5.55–5.63. Female head, left lateral view: **5.55**–*I. cephalargia* (0000024), **5.56**–*I. tequila* (0000053). Female mesopleuron, left lateral view: **5.57**–*I. suarezi* (0000051), **5.58**–*I. tequila* (0000053). Female mandible, anterior view: **5.59**–*I. tequila* (0000053), **5.60**–*I. suarezi* (0000051). Female mesosoma, left lateral view: **5.61**–*I. manleyi* (0000037), **5.62**–*I. erythrocephala* (0000034). Female procoxae, anterior view: **5.63**–*I. satrapa* (0000045).

weakly sculptured, with few punctate and rugose patches (fig. 5.62). Southern Mexico (Guerrero, Jalisco, and Morelos) .... **14 (*I. suarezi* species-group)**

13 (12). Vertex with dense golden setae (fig. 5.15). Dorsum of T2 with central patch, or two median patches, of golden setae (sometimes sparse or worn off) (fig. 5.15). Antenna, pleura, lateral face of propodeum, and legs orange (fig. 5.15) .... ***I. cephalargia* (Mickel)** (p. 249)

– Vertex with orange setae and scattered black setae (fig. 5.16). Dorsum of T2 without central patch/patches of golden setae, with predominantly orange-red setae (fig. 5.16). Antenna, pleura, lateral face of propodeum, and legs dark red-brown to black (fig. 5.16)... .... ***I. manleyi* Waldren, sp. nov.** (p. 253)

14 (12). Vertex with light yellow setae (fig. 5.21) or coppery-orange setae (fig. 5.22). T2 with distinct pattern in exaggerated “W” shape (figs 5.21, 5.22) **15**

– Vertex with red-orange and few black setae (fig. 5.20). T2 concolorous orange-red, without distinct pattern (fig. 5.20) .... ***I. erythrocephala* Waldren, sp. nov.** (p. 277)

15 (14). Vertex with coppery-orange setae (fig. 5.22). T2 pattern narrow in shape (fig. 5.22). Mandible dilated sub-apically (fig. 5.59). Mesopleuron with vertical column of punctures tuberculate both anteriorly and posteriorly, forming parallel carinae bounding punctures (fig. 5.58) .... ***I. tequila* Waldren, sp. nov.** (p. 286)

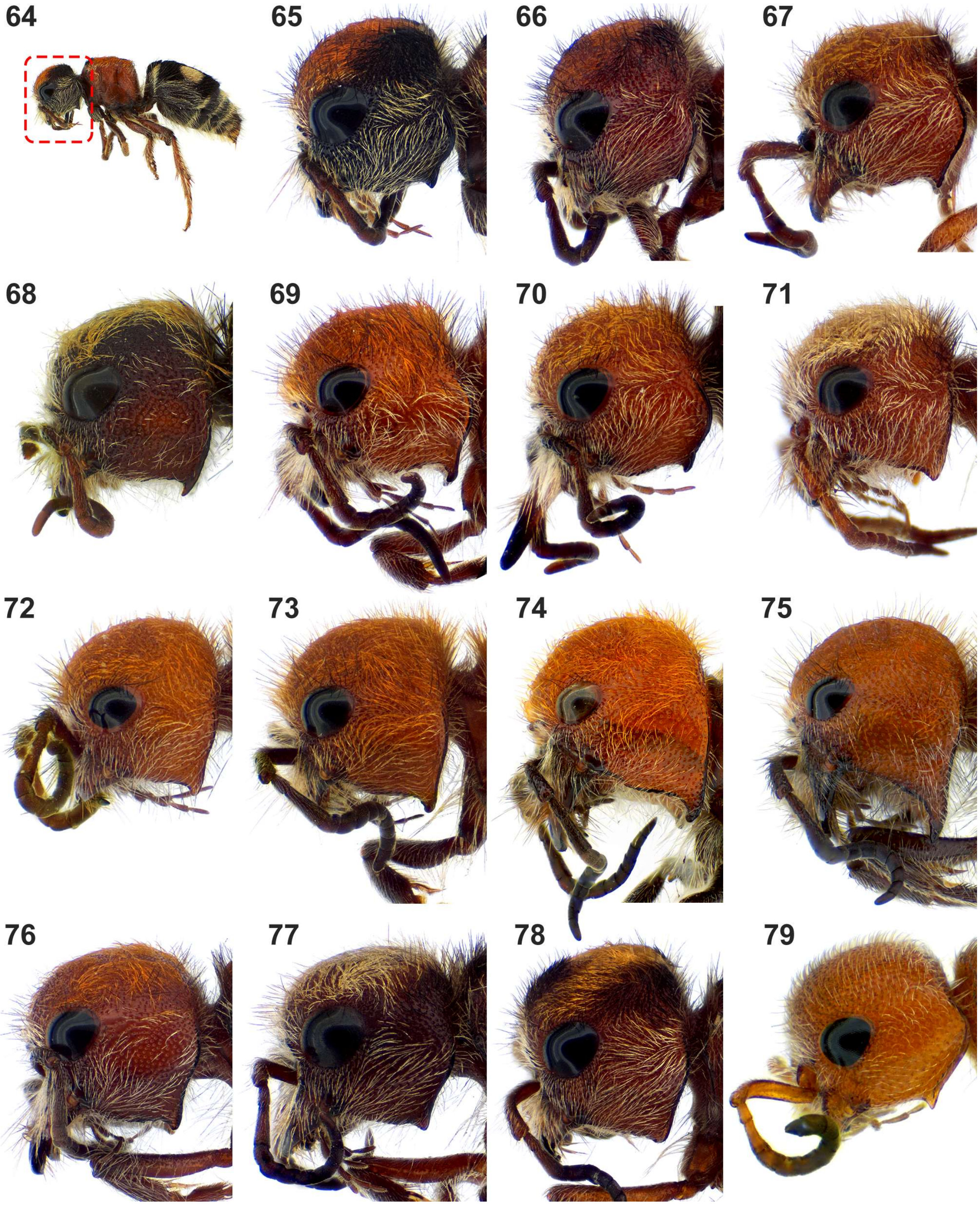
– Vertex with light yellow setae (fig. 5.21). T2 pattern broad in shape (fig. 5.21). Mandible acuminate (fig. 5.60). Mesopleuron with vertical column of punctures only tuberculate posteriorly, forming a single carina adjacent to punctures (fig. 5.57) .... ***I. suarezi*** **Waldren, sp. nov.** (p. 282)

Invreiella australis **species-group**

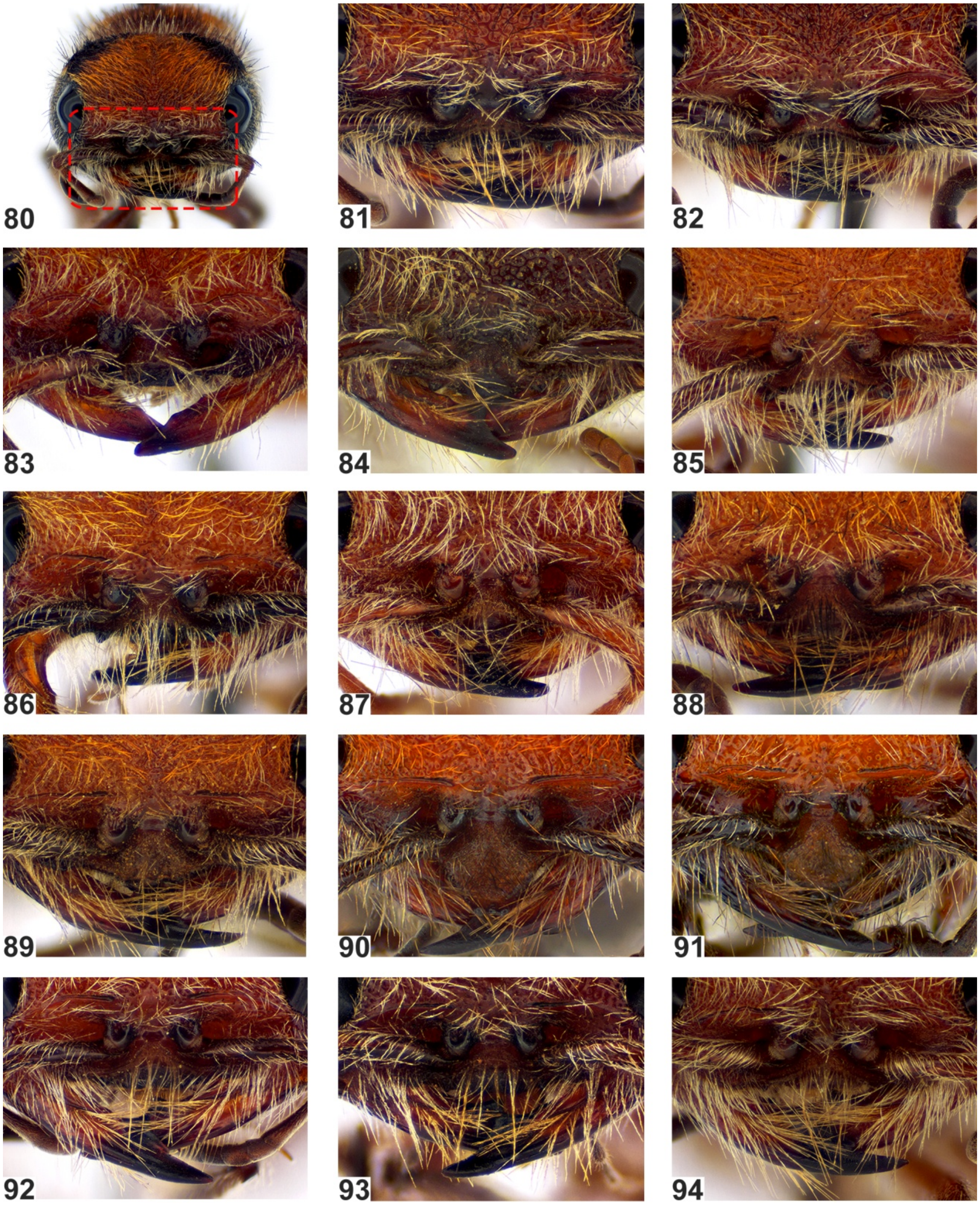
(Fig. 5.131)

Members of this female-based species-group are recognized by the following combination of character states:

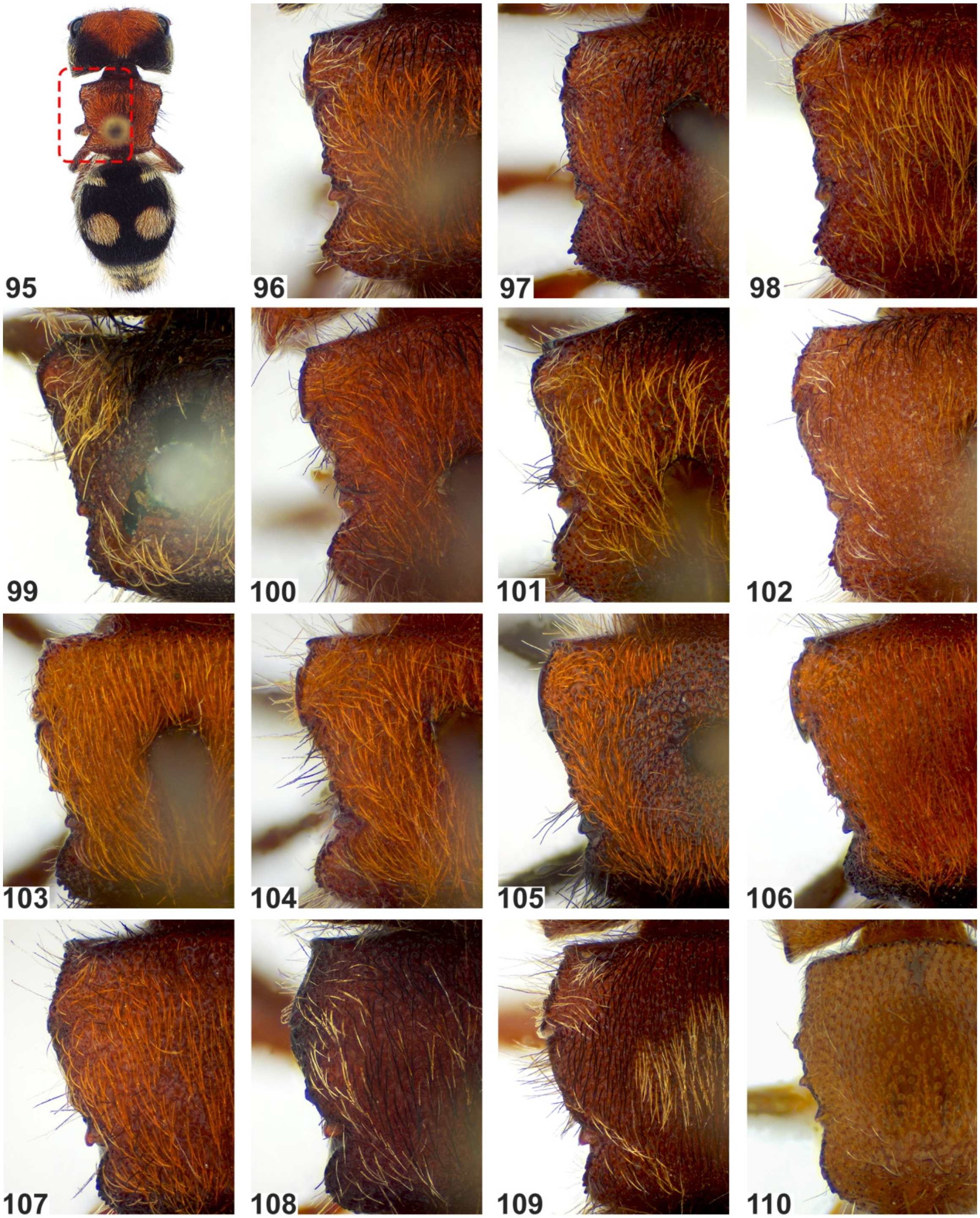
1. Antennal scrobe carina position: inner tip close to antennal rim.
2. Frons and antennal rim position: frons not transversely recessed and concave below antennal scrobe carina, antennal rim consequently not recessed and is visible when head viewed laterally.
3. Antennal rim: tuberculate and protruding.
4. Clypeus: concave, with transverse arcuate carina complete, with small lateral tubercle ventrad to carina.
5. Genal process: denticulate to triangular, posterior genal carina sinuate to straight.
6. Pronotal carina: present, glabrous, crenulate due to overlapping lateral punctures, visible both dorsally and laterally.
7. Lateral face of pronotum sculpture: little to mostly punctate throughout with moderate punctures.



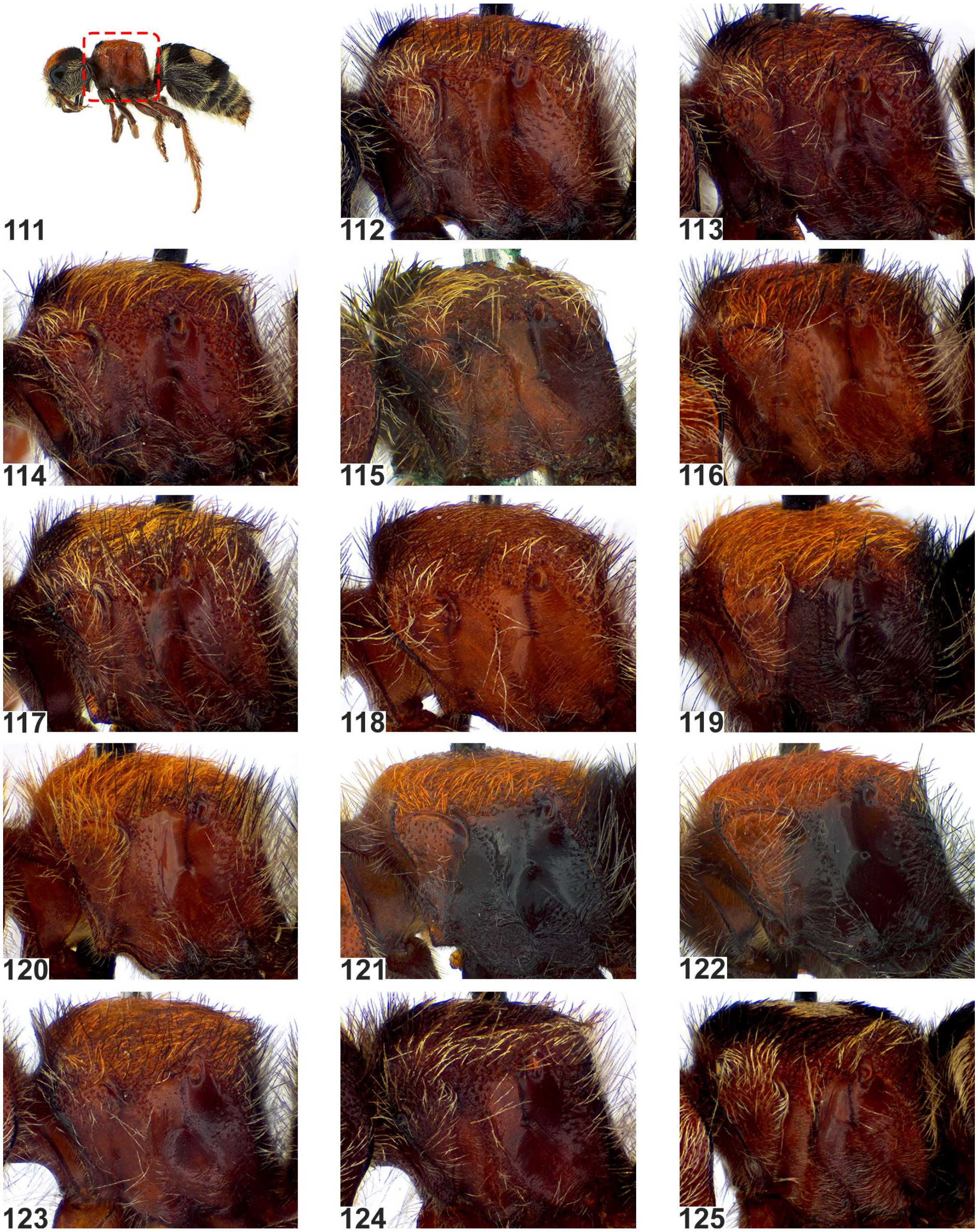
FIGURES 5.64–5.79. *I. australis* (0000001): **5.64**–female habitus, lateral view. Female head, left lateral view: **5.65**–*I. australis* (0000001), **5.66**–*I. bimaculata* (0000002), **5.67**–*I. mesomexicana* (0000035), **5.68**–*I. acuminata* (0000056), **5.69**–*I. cardinalis* (0000018), **5.70**–*I. cuernavaca* (0000032), **5.71**–*I. cephalargia* (0000024), **5.72**–*I. manleyi* (0000037), **5.73**–*I. breviclypeata* (0000003), **5.74**–*I. chihuahuensis* (0000031), **5.75**–*I. satrapa* (0000049), **5.76**–*I. erythrocephala* (0000034), **5.77**–*I. suarezi* (0000051), **5.78**–*I. tequila* (0000053), **5.79**–*Pseudomethoca frigida*.



FIGURES 5.80–5.94. *I. australis* (0000001): **5.80**–female head, anterior view. Female clypeus: **5.81**–*I. australis* (0000001), **5.82**– *I. bimaculata* (0000002), **5.83**–*I. mesomexicana* (0000035), **5.84**–*I. acuminata* (0000056), **5.85**–*I. cardinalis* (0000018), **5.86**–*I. cuernavaca* (0000032), **5.87**–*I. cephalargia* (0000024), **5.88**–*I. manleyi* (0000037), **5.89**–*I. breviclypeata* (0000003), **5.90**–*I. chihuahuensis* (0000031), **5.91**–*I. satrapa* (0000049), **5.92**–*I. erythrocephala* (0000034), **5.93**–*I. suarezi* (0000051), **5.94**–*I. tequila* (0000053).



FIGURES 5.95–5.110. *I. australis* (0000001): **5.95**–female habitus, dorsal view. Female mesosoma, left dorsal view: **5.96**–*I. australis* (0000001), **5.97**–*I. bimaculata* (0000002), **5.98**–*I. mesomexicana* (0000035), **5.99**–*I. acuminata* (0000056), **5.100**–*I. cardinalis* (0000018), **5.101**–*I. Cuernavaca* (0000032), **5.102**–*I. cephalargia* (0000024), **5.103**–*I. manleyi* (0000037), **5.104**–*I. breviclypeata* (000003), **5.105**–*I. chihuahuensis* (0000031), **5.106**–*I. satrapa* (0000049), **5.107**–*I. erythrocephala* (0000034), **5.108**–*I. suarezi* (0000051), **5.109**–*I. tequila* (0000053), **5.110**–*Pseudomethoca frigida*.



FIGURES 5.111–5.125. *I. australis* (0000001): **5.111**–female habitus, lateral view. Female mesosoma, left lateral view: **5.112**–*I. australis* (0000001), **5.113**–*I. bimaculata* (0000002), **5.114**–*I. mesomexicana* (0000035), **5.115**–*I. acuminata* (0000056), **5.116**–*I. cardinalis* (0000018), **5.117**–*I. cuernavaca* (0000032), **5.118**–*I. cephalargia* (0000024), **5.119**–*I. manleyi* (0000037), **5.120**–*I. breviclypeata* (0000003), **5.121**–*I. chihuahuensis* (0000031), **5.122**–*I. satrapa* (0000049), **5.123**–*I. erythrocephala* (0000034), **5.124**–*I. suarezi* (0000051), **5.125**–*I. tequila* (0000053).

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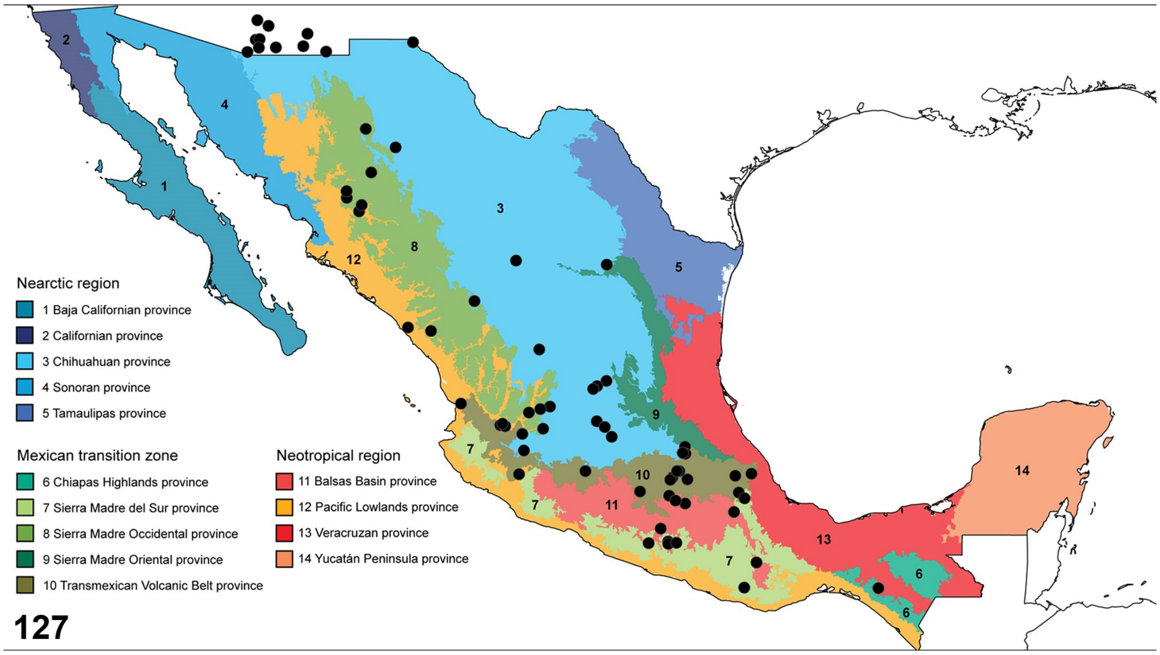

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FIGURES 5.126 and 5.127. 5.126–Geographic distribution of *I.* species. **5.127–** Biogeographic distribution of *I.* species (shapefiles from Morrone *et al.* 2017).

8. Vertical column of punctures of mesopleuron: puncture edges weakly tuberculate, not forming anterior or posterior carinae.
9. Mesopleuron, metapleuron, and lateral face of propodeum sculpture, where present: microgranulate with moderate punctures and micropunctures.

Species included: *I. australis* Waldren, **sp. nov.**, *I. bimaculata* Waldren, **sp. nov.**, and *I. mesomexicana* Waldren, **sp. nov.**

Distribution: Mexico (Chiapas, Guanajuato, Jalisco, Nayarit, Oaxaca, Sinaloa, and Zacatecas).

Biogeography: Mexican transition zone (Chiapas Highlands province, Sierra Madre del Sur province; Sierra Madre Occidental province; Transmexican Volcanic Belt province); Nearctic region (Chihuahuan Desert province); Neotropical region (Pacific Lowlands province).

1. *Invreiella australis* Waldren, sp. nov.

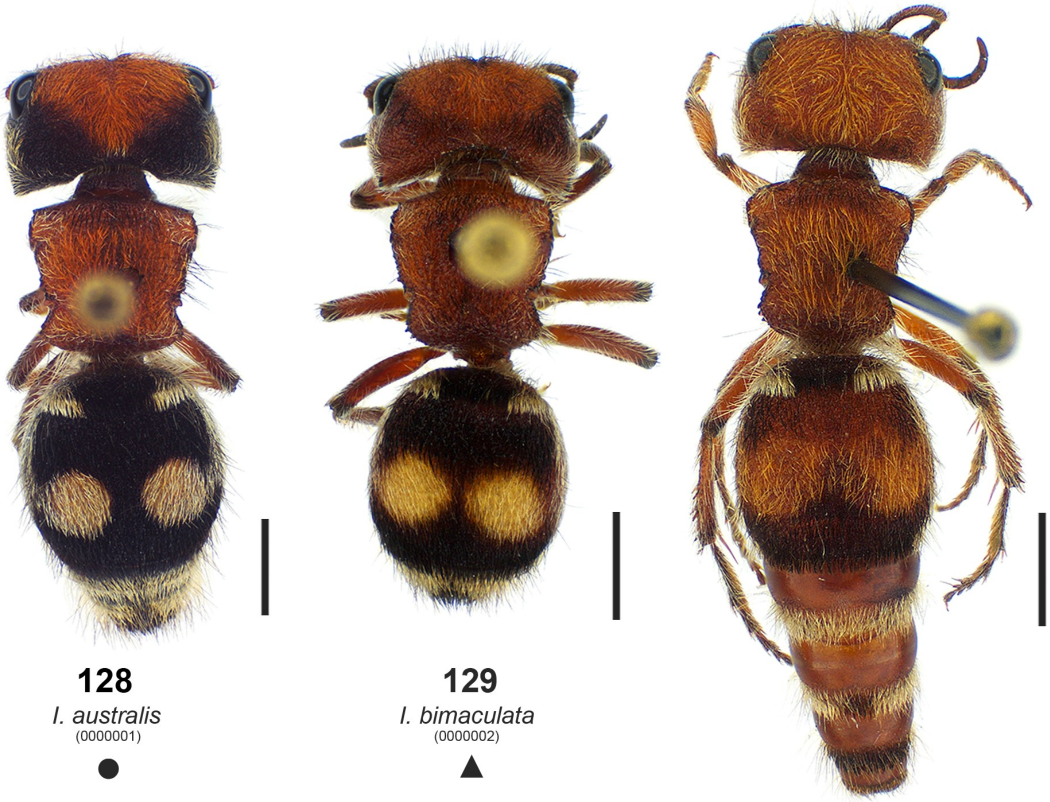
(Figs 5.9, 5.27, 5.41, 5.44, 5.46, 5.64, 5.65, 5.80, 5.81, 5.95, 5.96, 5.111, 5.112, 5.128, 5.131)

Diagnosis (female). This species is distinguished from other members of the *I. australis* species-group by the following combination of characters: antennal scrobe carina straight (figs 5.44, 5.81), genal process triangular with posterior genal carina sinuate (fig. 5.65), lateral face of pronotum with moderate punctures mostly throughout (fig. 5.112), integument of head black at posterolateral corners, gena, and occiput, with integument of frons and part of the vertex orange (figs 5.9, 5.65, 5.128), and T2 with two pale yellow integumental spots that are separate and not coalescing, with the remaining dorsal integument of T2 black (figs 5.9, 5.128).

Description (female). Body length 10.92 mm.

Head: Head 1.26 × as wide as mesosoma. Vertex and frons contiguously punctate. Antennal scrobe carina straight, with inner tip of carina close to antennal rim. Frons not transversely recessed and concave below antennal scrobe carina, antennal rim consequently not recessed and is visible when head viewed laterally. Antennal rim apically glabrous, tuberculate and protruding. F1 2.17 × as long as F2. Clypeus concave, with transverse arcuate carina complete, with small lateral tubercle ventrad to carina. Mandible acuminate. Distance from posterior margin of eye to posterolateral corner of head 1.41 × maximum diameter of eye. Genal process triangular, posterior genal carina sinuate. Gena densely punctate laterally, interpunctal space smooth. Postgena transversely rugose-striate.

Mesosoma: Mesosoma 1.19 × as wide as long. Dorsum and posterior face of mesosoma densely, contiguously punctate, surfaces coarse. Anterodorsolateral margin of pronotum outcurved. Pronotal carina prominent, glabrous, crenulate due to overlapping lateral punctures, visible both dorsally and laterally, not strongly protruding posteriorly in dorsal view and not entirely obscuring dorsal portion of pronotal-mesopleural suture. Tubercle anterior of propodeal spiracle present, prominent. Width of propodeum greater than distance between propodeal spiracles in dorsal view. Lateral face of pronotum with moderate separated punctures throughout, interpunctal space microgranulate with micropunctures, lateral face posteriorly carinate along pronotal-mesopleural suture. Mesopleuron microgranulate with micropunctures mostly throughout. Mesopleuron with



128
I. australis
(0000001)
●

129
I. bimaculata
(0000002)
▲

130
I. mesomexicana
(0000035)
■

FIGURES 5.128–5.131. Female habitus, dorsal view: *I. australis* species-group. Scale bars = 2 mm. **5.131**–Geographic distribution of the *I. australis* species-group.

vertical column of punctures weakly tuberculate, not forming anterior or posterior carinae. Mesopleuron posteriorly carinate along ventral half of mesopleural-metapleural suture, carina diverging from suture roughly at midpoint and terminating shortly after, dorsal portion of suture obscure, present as faint groove. Metapleuron with scattered micropunctures, weakly rugose-striate just dorsad metacoxa, with few moderate punctures. Metapleural-propodeal suture without overlapping striae. Lateral face of propodeum with scattered moderate punctures, especially medially and posteriorly, with few clusters of micropunctures and microstriae. Coxae coarsely sculptured, with small to moderate punctures.

Metasoma: T1–5 densely, contiguously punctate. Visible portion of pygidial plate not obscured by setal hood transversely rugose-granulate. S2 densely punctate, punctures large, interpunctal space smooth, basolateral concave area sparsely punctate, nearly glabrous between punctures. S3–6 densely punctate, coarsely microgranulate between punctures. Hypopygium with arcuate row of setae near apical margin, lateral setae longer than median setae.

Integument coloration: Frons, median triangular section of vertex, mesosoma, tibiae, and tarsi dull red-orange, with two pale yellow maculae on T2 that are separate and not coalescing, remaining dark brown-red to black: posterolateral corner of head, gena, postgena, antennal rim, antenna, apical half and basal quarter of mandible, base of clypeus, coxae, trochanters, femora, and metasoma.

Pubescence: Frons ventrally with whitish setae, medially with few fuscous setae. Frons dorsally and median triangular section of vertex covered with mostly decumbent orange setae overlapping orange integument. Posterolateral corner of head with triangular patch of mostly decumbent black setae overlapping nearly black integument. Mandible with dorsal and ventral longitudinal row of pale orange setae. Remainder of head with whitish setae. Dorsum of mesosoma mostly covered with decumbent orange setae, dorsolateral edges with sparse raised fuscous setae. Pronotum with dorsal transverse band of black setae, except area between epaulet and pronotal spiracle with whitish setae. Median patch of decumbent black setae present on dorsum of propodeum and just anterior to it. Remainder of mesosoma including legs with whitish setae (except tarsi with dense orange bristles). Anterior face of T1 with whitish setae. T1 apically fringed with moderately wide band of black setae medially, laterally fringed with whitish setae. T2 with decumbent light yellow setae overlapping two yellow integumental maculae, remainder of dorsum of T2 with black setae surrounding maculae. Felt line of T2 and surrounding lateral area with whitish setae. T2 apically fringed with wide band of black setae medially, laterally fringed with whitish setae. T3 covered and fringed with whitish setae. T4 similar to T3 except with small patch of black setae medially. T5 mostly covered and fringed with black setae medially, laterally fringed with whitish setae. T6 with light orange setae surrounding pygidial plate, partially obscuring basal half. Remainder of metasoma with whitish setae.

MALE. Unknown.

Etymology: The specific epithet is derived from the Latin *australis* in reference to this species being the southernmost member of *Invreiella*.

Distribution: Mexico (Chiapas, Oaxaca).

Biogeography: Mexican transition zone (Chiapas Highlands province, Sierra Madre del Sur province).

Host(s): Unknown.

Remarks: This species is the only member of *Invreiella* known to occur east of the Isthmus of Tehuantepec.

Material examined (*I. australis*, 2♀).

Holotype: ♀ (0000001–DGMC), [label 1 (white):] 9 mi. north / Villa Flores / Chis. MEX. / VIII 12 1963 [// label 2 (white):] F.D. Parker / L.A. Stange / Collectors [// label 3 (red):] HOLOTYPE ♀ / Invreiella australis / Waldren, 2018 / GCW_HYM0000001 [// label 4 (white):] Mutillidae: / Pseudomethoca sp. ♀ / det. D.G. Manley 19*99*. [(**MEXICO: Chiapas:** Villaflores, 9 mi. N, [16.320°N 93.341°W], 12.Aug.1963, F.D. Parker & L.A. Stange (1♀–0000001–DGMC))]

Additional specimen (non-type): MEXICO: Oaxaca: [San Pedro] Juchatengo, La Luna, [16.335°N 97.112°W], 1100 m, 05.Oct.2005, Curoe (1♀–MIUP).

2. *Invreiella bimaculata* Waldren, sp. nov.

(Figs 5.10, 5.51, 5.53, 5.66, 5.82, 5.97, 5.113, 5.129, 5.131)

Diagnosis (female). This species is distinguished from other members of the *I. australis* species-group by the following combination of characters: antennal scrobe carina arcuate (*i.e.* fig. 5.45; fig. 5.82), genal process denticulate with posterior genal carina straight (figs 5.51, 5.66), lateral face of pronotum with moderate punctures throughout except bare spot posteriorly (figs 5.53, 5.113), integument of head dark brown-red, without patches of black integument (figs 5.10, 5.66, 5.129), and T2 with two yellow integumental spots that are close but not coalescing, with the remaining dorsal integument of T2 dark brown-red (figs 5.10, 5.129).

Description (female). Body length 9.27 mm.

Head: Head 1.27 × as wide as mesosoma. Vertex and frons contiguously punctate. Antennal scrobe carina strongly arcuate, with inner tip of carina close to antennal rim. Frons not transversely recessed and concave below antennal scrobe carina, antennal rim consequently not recessed and is visible when head viewed laterally. Antennal rim apically microgranulate, tuberculate and protruding. F1 1.80 × as long as F2. Clypeus concave, with transverse arcuate carina complete, with small lateral tubercle ventrad to carina. Mandible acuminate. Distance from posterior margin of eye to posterolateral corner of head 1.27 × maximum diameter of eye. Genal process denticulate, posterior genal carina straight. Gena densely punctate laterally, punctures contiguous, interpunctal space smooth. Postgena transversely rugose-striate, with punctures between striae.

Mesosoma: Mesosoma 1.15 × as wide as long. Dorsum and posterior face of mesosoma densely, contiguously punctate, surfaces coarse. Anterodorsolateral margin of pronotum outcurved. Pronotal carina prominent, glabrous, crenulate due to overlapping lateral punctures, visible both dorsally and laterally, not strongly protruding posteriorly in dorsal view and not entirely obscuring apical portion of pronotal-mesopleural suture. Tubercle anterior of propodeal spiracle present. Width of propodeum greater than distance between propodeal spiracles in dorsal view. Lateral face of pronotum with large, close to separated punctures throughout, except for bare spot posteriorly, interpunctal space microgranulate, lateral face posteriorly carinate along pronotal-mesopleural suture. Mesopleuron mostly microgranulate with micropunctures. Mesopleuron with vertical column of punctures weakly tuberculate, not forming anterior or posterior carinae. Mesopleuron posteriorly carinate along ventral half of mesopleural-metapleural suture, carina diverging from suture roughly at midpoint and terminating shortly after, dorsal portion of suture obscure, faintly present. Metapleuron weakly rugose-striate just dorsad metacoxa, with few moderate punctures, medially with micropunctures, dorsal portion glabrous. Metapleural-propodeal suture without overlapping striae. Lateral face of propodeum mostly glabrous, patchily microgranulate, with clusters of micropunctures, posteriorly with moderate scattered punctures. Coxae coarsely sculptured, with small to moderate punctures.

Metasoma: T1–5 densely, contiguously punctate. Visible portion of pygidial plate not obscured by setal hood granulate-rugose. S2 densely punctate, punctures moderate, interpunctal space smooth, basolateral concave area sparsely punctate, nearly glabrous between punctures. S3–6 densely punctate, coarsely microgranulate between punctures. Hypopygium with near-straight transverse row of setae near apical margin, lateral setae longer than median setae.

Integument coloration: Mostly dull red-orange, with two close yellow maculae on T2 that are separate and not coalescing, remaining dark brown-red to black: antennal rim, antenna, apical third of mandible, apices of femora, apices of metatibia, metatarsus, anterior and posterior third of T2, T3–6, pygidial plate, and apical portion of hypopygium.

Pubescence: Frons ventrally with whitish setae, medially with fuscous setae. Frons dorsally and vertex with mostly decumbent orange setae, with scattered fuscous setae. Posterolateral corner of head with triangular patch of black setae. Mandible with dorsal and ventral longitudinal row of pale orange setae. Remainder of head with whitish setae. Dorsum of mesosoma mostly covered with decumbent orange setae, dorsolateral edges with sparse raised fuscous setae. Pronotum with dorsal transverse band of black setae, except area between epaulet and pronotal spiracle with whitish setae. Median patch of sparse black setae present on dorsum of propodeum, extending longitudinally nearly halfway up mesosoma. Remainder of mesosoma including legs with whitish setae (except tarsi with dense orange bristles). Anterior face of T1 with whitish setae. T1 apically fringed with moderately wide band of black setae medially, laterally fringed with whitish setae. T2 with light yellow-orange setae overlapping two yellow-orange maculae, remainder of dorsum of T2 with black setae surrounding maculae. Felt line of T2 and surrounding lateral area with whitish setae. T2 apically fringed with wide band of black setae medially, laterally fringed with whitish setae. T3 covered and fringed with whitish setae. T4 similar to T3 except with small patch of black setae medially. T5 mostly covered and fringed with black setae medially, laterally fringed with whitish setae. T6 with light orange setae surrounding pygidial plate, partially obscuring basal half. Remainder of metasoma with whitish setae.

MALE. Unknown.

Etymology: The specific epithet is derived from the Latin *bi*- and *macula* in reference to the two yellow integumental spots on T2.

Distribution: Mexico (Sinaloa).

Biogeography: Neotropical region (Pacific Lowlands province).

Host(s): Unknown.

Remarks: This species is known only from the holotype.

Material examined (*I. bimaculata*, 1♀).

Holotype: ♀ (0000002–UNAM) [metasoma glued onto specimen], [label 1 (white):] MEXICO: Sinaloa / Microondas El Indio / 28-VII-1995 Alt. 350 m / 23°39’32” N 106°33’44” O / Col. R. Ayala [// label 2 (red):] HOLOTYPE ♀ / Invreiella bimaculata / Waldren, 2018 / GCW_HYM0000002. [(**MEXICO: Sinaloa:** Microondas El Indio, 23.658889°N 106.562222°W, 350 m, 28.Jul.1995, R. Ayala (1♀–0000002–UNAM))]

3. *Invreiella mesomexicana* Waldren, sp. nov.

(Figs 5.11, 5.45, 5.52, 5.54, 5.67, 5.83, 5.98, 5.114, 5.130, 5.131)

Diagnosis (female). This species is distinguished from other members of the *I. australis* species-group by the following combination of characters: scrobe carina arcuate (figs 5.45, 5.83), genal process triangular with posterior genal carina weakly sinuate (figs 5.52, 5.67), lateral face of pronotum with few moderate punctures (figs 5.54, 5.114), integument of head orange, without patches of black integument (figs 5.11, 5.67, 5.130), and T2 with two light orange integumental spots that are slightly coalescing, with the remaining dorsal integument of T2 brown-red (figs 5.11, 5.130).

Description (female). Body length 12.57–13.34 mm (holotype metasoma extended on latter measurement (fig. 5.11)).

Head: Head 1.26–1.35 × as wide as mesosoma. Vertex and frons contiguously punctate. Antennal scrobe carina strongly arcuate, with inner tip of carina close to antennal rim. Frons not transversely recessed and concave below antennal scrobe carina, antennal rim consequently not recessed and is visible when head viewed laterally. Antennal rim apically microgranulate, tuberculate and protruding. F1 1.79–2.16 × as long as F2. Clypeus concave, with transverse arcuate carina complete, with small lateral tubercle ventrad to carina. Mandible acuminate. Distance from posterior margin of eye to posterolateral corner of head 1.39–1.43 × maximum diameter of eye. Genal process triangular, posterior genal carina weakly sinuate. Gena densely, contiguously punctate laterally, interpunctal space smooth. Postgena transversely rugose-striate.

Mesosoma: Mesosoma 0.99–1.15 × as wide as long. Dorsum and posterior face of mesosoma densely, contiguously punctate, surfaces coarse. Anterodorsolateral margin of pronotum outcurved. Pronotal carina prominent, glabrous, crenulate due to overlapping lateral punctures, visible both dorsally and laterally, not strongly protruding posteriorly in dorsal view and not entirely obscuring apical portion of pronotal-mesopleural suture. Tubercle anterior of propodeal spiracle obscure. Width of propodeum greater than distance between propodeal spiracles in dorsal view. Lateral face of pronotum mostly microgranulate with micropunctures, with cluster of moderate punctures at pronotal carina, remainder of lateral face with few scattered moderate punctures, lateral face posteriorly carinate along pronotal-mesopleural suture. Mesopleuron mostly microgranulate with micropunctures. Mesopleuron with vertical column of punctures weakly tuberculate, not forming anterior or posterior carinae. Mesopleuron posteriorly carinate along ventral half of mesopleural-metapleural suture, carina diverging from suture roughly at midpoint and terminating shortly after, dorsal portion of suture absent to obscure, present as faint groove. Metapleuron weakly rugose-striate just dorsad metacoxa, with few moderate punctures, medially with micropunctures, apically microgranulate and nearly glabrous. Metapleural-propodeal suture without overlapping striae. Lateral face of propodeum mostly glabrous, patchily microgranulate, with clusters of micropunctures, posteriorly with moderate scattered punctures. Coxae coarsely sculptured, with small to moderate punctures.

Metasoma: T1–5 densely, contiguously punctate. Visible portion of pygidial plate not obscured by setal hood granulate-rugose. S2 densely punctate, punctures large, interpunctal space smooth, basolateral concave area sparsely punctate, nearly glabrous between punctures. S3–6 densely punctate, coarsely microgranulate between punctures. Hypopygium with arcuate row of setae near apical margin, lateral setae longer than median setae.

Integument coloration: Mostly orange-brown, with two large, slightly coalescing, light orange maculae on T2, remaining brown-red to black: antennal rim, antenna, apical quarter of mandible, base of clypeus, base of coxae, trochanters, and apices of femora (latter three orange-brown in paratype), apical 1/3 and basal 1/3 of T2, and T6 including pygidial plate.

Pubescence: Frons ventrally with whitish setae, medially with few fuscous setae. Frons dorsally and vertex with mostly decumbent light orange setae, with scattered fuscous setae. Posterolateral corner of head with sparse triangular patch of fuscous setae. Mandible with dorsal and ventral longitudinal row of pale orange setae. Remainder of head with whitish setae. Pronotum dorsally with transverse band of black setae. Dorsum of mesosoma mostly covered with decumbent light orange setae, dorsolateral edges with sparse raised fuscous setae. Pronotum with dorsal transverse band of black setae, except area between epaulet and pronotal spiracle with whitish setae. Median patch of sparse black setae present apically at propodeal declivity. Remainder of mesosoma including legs with whitish setae (except tarsi with dense orange bristles). Anterior face of T1 with whitish setae. T1 apically fringed with moderately wide band of black setae medially, laterally fringed with whitish setae. T2 with decumbent light orange setae overlapping two coalescing light orange integumental maculae, remainder of dorsum of T2 with black setae surrounding maculae. Felt line of T2 and surrounding lateral area with whitish setae. T2 apically fringed with wide band of black setae medially, laterally fringed with whitish setae. T3 covered and fringed with whitish setae. T4 similar to T3 except with small patch of black setae medially. T5 mostly covered and fringed with black setae medially, laterally fringed with whitish setae. T6 with light orange setae surrounding pygidial plate, partially obscuring basal half. Remainder of metasoma with whitish setae.

MALE. Unknown.

Etymology: The specific epithet is derived from the Greek *meso* and Mexico in reference to this species occurring in middle Mexico.

Distribution: Mexico (Guanajuato, Jalisco, Nayarit, and Zacatecas).

Biogeography: Mexican transition zone (Sierra Madre Occidental province; Transmexican Volcanic Belt province); Nearctic region (Chihuahuan Desert province).

Host(s): Unknown.

Remarks: None.

Material examined (*I. mesomexicana*, 5♀).

Holotype: ♀ (0000035–EMUS) [left antenna mounted on point, with F5–10 missing], [label 1 (tan):] *ROQUE* / *GTO* [// label 2 (tan):] *Suelo* / *29.x.62* [// label 3 (tan):] *R. Padilla* [// label 4 (yellow):] LACM [// label 5 (red):] HOLOTYPE ♀ / Invreiella mesomexicana / Waldren, 2018 / GCW_HYM0000035 [// label 6 (white):] *Invreiella* / *cardinalis* / *(Gerstaecker) 1874* / Det K.A. Williams 2012. [(**MEXICO: Guanajuato:**

Roque, N of Celaya, [20.581°N 100.838°W], 29.Oct.1962, R. Padilla (1♀–0000035–EMUS))]

Paratype: MEXICO: Nayarit: San Blas/Tepic, [21.518°N 105.075°W], Oct.1894, G. Eisen & F.H. Vaslit (1♀–0000036–NMNH).

Additional specimens (non-types, examined or referenced*): MEXICO: Jalisco: Guadalajara, [20.663°N 103.350°W], 06.Jul., McClendon (1♀–MIUP); 09.Jul.1903 (1♀–ANSP). **Zacatecas:** Moyahua de Estrada, [21.265°N 103.165°W], 07.Aug.1965, A.R. Gillogly (1♀–DJBC*).

***Invreiella cardinalis* species-group**

(Fig. 5.135)

Members of this female-based species-group are recognized by the following combination of character states:

1. Antennal scrobe carina position: inner tip separated from antennal rim.
2. Frons and antennal rim position: frons not transversely recessed and concave below antennal scrobe carina, antennal rim consequently not recessed and is visible when head viewed laterally.
3. Antennal rim: weakly tuberculate to tuberculate.
4. Clypeus: concave, with transverse arcuate carina broadly interrupted medially, area merely punctate, resulting in bilateral carinate processes each with enlarged tubercle ventrad to process.

132
I. acuminata
(0000056)
("Mexico")

133
I. cardinalis
(0000018)
●

134
I. cuernavaca
(0000032)
▲

FIGURES 5.132–5.135. Female habitus, dorsal view: *I. cardinalis* species-group. Scale bars = 2 mm. **5.135**–Geographic distribution of the *I. cardinalis* species-group.

5. Genal process: triangular to spinose, posterior genal carina convex to sinuate.
6. Pronotal carina: present, glabrous, weakly crenulate, visible both dorsally and laterally.
7. Lateral face of pronotum sculpture: with moderate punctures, somewhat striate-rugose.
8. Vertical column of punctures of mesopleuron: puncture edges not tuberculate, not forming anterior or posterior carinae.
9. Mesopleuron, metapleuron, and lateral face of propodeum sculpture, where present: microgranulate with micropuntures and moderate punctures, rugose-granulate to rugose-striate in some areas.

Species included: *I. acuminata* Waldren, **sp. nov.**, *I. cardinalis* (Gerstaecker, 1874), and *I. cuernavaca* Waldren, **sp. nov.**

Distribution: Mexico (Durango, Guanajuato, Guerrero, Jalisco, Mexico, Morelos, Oaxaca, Puebla, Veracruz, and Zacatecas).

Biogeography: Nearctic region (Chihuahuan Desert province); Mexican transition zone (Sierra Madre del Sur province, Sierra Madre Occidental province, Sierra Madre Oriental province, Transmexican Volcanic Belt province); Neotropical region (Balsas Basin province).

4. *Invreiella acuminata* Waldren, sp. nov.

(Figs 5.12, 5.32, 5.35, 5.68, 5.84, 5.99, 5.115, 5.132, 5.135)

Diagnosis (female). This species is distinguished from other members of the *I. cardinalis* species-group by the following combination of characters: antennal scrobe carina straight (*i.e.* fig. 5.39; fig. 5.84), antennal rim weakly tuberculate, genal process spinose and curved with posterior genal carina convex (figs 5.32, 5.68), with carina anterior to process obscurely crenulate, anterodorsolateral margin of pronotum weakly incurved in dorsal view (figs 5.35, 5.99), dorsolateral margin of mesosoma, from pronotal carina to propodeal spiracle, straight in outline in dorsal view (figs 5.35, 5.99), width of propodeum scarcely greater than distance between propodeal spiracles in dorsal view (figs 5.35, 5.99), mesopleuron with ventral one-third coarsely sculptured, rugose-granulate with weak rugae and few moderate punctures (*i.e.* fig. 5.37; fig. 5.115), and integument of dorsum of head, mesosoma, and T2 dark brown-red (figs 5.12, 5.132).

Description (female). Body length 9.27 mm.

Head: Head 1.06 × as wide as mesosoma. Vertex and frons contiguously punctate. Antennal scrobe carina straight, with inner tip of carina separated from antennal rim. Frons not transversely recessed and concave below antennal scrobe carina, antennal rim consequently not recessed and is visible when head viewed laterally. Antennal rim apically glabrous, weakly tuberculate. F1 1.94 × as long as F2. Clypeus concave, with transverse arcuate carina broadly interrupted medially, area merely punctate, resulting in bilateral carinate processes each with enlarged tubercle ventrad to process. Mandible acuminate. Distance from posterior margin of eye to posterolateral corner of head 1.46 × maximum diameter of eye. Genal process spinose and curved, posterior genal carina convex, with carina anterior to process obscurely crenulate. Gena densely punctate laterally, punctures moderately large and nearly contiguous, interpunctal space smooth. Postgena transversely rugose-striate medially.

Mesosoma: Mesosoma 1.36 × as wide as long. Dorsum and posterior face of mesosoma densely, contiguously punctate, surfaces coarse. Anterodorsolateral margin of pronotum weakly incurved in dorsal view. Pronotal carina prominent, glabrous, weakly crenulate, visible both dorsally and laterally, not strongly protruding posteriorly in dorsal view and not entirely obscuring apical portion of pronotal-mesopleural suture. Dorsolateral margin of mesosoma, from pronotal carina to propodeal spiracle, straight in outline in dorsal view; tubercle anterior of propodeal spiracle obscure. Width of propodeum scarcely greater than distance between propodeal spiracles in dorsal view. Lateral face of pronotum rugose-striate, with moderate punctures between rugae, lateral face posteriorly carinate along pronotal-mesopleural suture. Mesopleuron with dorsal two-thirds mostly microgranulate with micropunctures, ventral one-third coarsely sculptured, rugose-granulate with weak rugae, with few moderate punctures. Mesopleuron with vertical column of punctures not tuberculate, not forming anterior and posterior carinae. Mesopleuron posteriorly carinate along ventral three-fourths of mesopleural-metapleural suture, dorsal portion of suture absent. Metapleuron rugose-striate dorsad metacoxa, with few micro- and moderate punctures, remainder of sclerite weakly microgranulate, near glabrous to glabrous. Metapleural-propodeal suture with some overlapping striae at ventral one-third. Lateral face of propodeum posteriorly and with ventral one-third rugose-punctate, with moderate punctures, dorsal two-thirds weakly microgranulate, near glabrous to glabrous. Coxae coarsely sculptured, with small to moderate deep punctures.

Metasoma: T1–5 densely, contiguously punctate, punctures coarse, T2 anteriorly with patch of punctures that are strongly anteriorly tuberculate. Visible portion of pygidial plate not obscured by setal hood rugose-granulate. S2 densely punctate, punctures near contiguous in some areas, basolateral concave area sparsely punctate, nearly glabrous between punctures. S3–6 densely punctate, coarsely microgranulate between punctures. Hypopygium with slightly arcuate transverse row of setae near apical margin, lateral setae longer than median setae.

Integument coloration: Dark brown-red, without integumental maculae on T2.

Pubescence: Frons ventrally with whitish setae. Frons dorsally and vertex mostly covered with decumbent, and some raised, light golden setae. Mandible with dorsal and ventral longitudinal row of light yellow setae. Remainder of head with whitish setae. Dorsum of mesosoma mostly covered with decumbent light golden setae. Pronotum with dorsal transverse patch of dense black setae, except area between epaulet and pronotal spiracle with light golden setae. Remainder of mesosoma including legs with whitish setae (except tarsi with orange bristles). Anterior face of T1 with whitish setae. T1 apically fringed with wide median band of black setae, laterally fringed with whitish setae. T2 with two large circular patches of decumbent light golden setae, patches close but not coalescing, anteriorly and posteriorly surrounded with black setae. Felt line of T2 and surrounding lateral area with whitish setae. T2 apically fringed with wide band of black setae medially, laterally fringed with whitish setae. T3 covered and fringed with whitish setae. T4 similar to T3 except with patch of black setae medioapically. T5 mostly covered and fringed with black setae medially, laterally fringed with whitish setae. T6 with orange setae surrounding pygidial plate, partially obscuring basal half. Remainder of metasoma with whitish setae.

MALE. Unknown.

Etymology: The specific epithet is derived from the Latin *acuminatus* in reference to the genal carina ventrally terminating into a pointed process.

Distribution: Mexico.

Biogeography: Unknown.

Host(s): Unknown.

Remarks: This distinctive species is known only from the holotype, which is worn, damaged, and lacks collection data other than "Mexico."

Material examined (*I. acuminata*, 1♀).

Holotype: ♀ (0000056–NHMUK) [pedicel and all flagellomeres of right antenna missing; mesosoma dorsally split due to pin; tarsomere #5 of left proleg missing; entire left mesoleg missing; tibia and tarsus of left metaleg missing; tarsomeres #2–5 of right proleg missing; tarsomere #5 of right mesoleg missing], [label 1 (tan):] Mexico. [// label 2 (tan):] Smith coll. / pres. by / Mrs. Farren White. / 99-303. [// label 3 (tan, red ink):] 52 [// label 4 (tan):] British / Museum. [// label 5 (red):] HOLOTYPE ♀ / Invreiella acuminata / Waldren, 2018 / GCW_HYM0000056 [//label 6 (tan):] *Pseudomethoca* / *cardinalis* / *(Gerst.)* / ♀ *Det. C.E. Mickel '55*. [(**MEXICO. Unknown.** (1♀–0000056–NHMUK))]

5. *Invreiella cardinalis* (Gerstaecker, 1874)

(Figs 5.1–5.4, 5.13, 5.26, 5.33, 5.36, 5.37, 5.39, 5.69, 5.85, 5.100, 5.116, 5.133, 5.135)

Mutilla cardinalis Gerstaecker, 1874: 64 [holotype ♀ (ZMHB)]. Dalla Torre 1897: 21.

-*Sphaerophthalma* [sic] *cardinalis*: Cameron 1895: 364.

-*Ephuta* (*Ephuta*) *cardinalis*: André 1902: 58.

-*Pseudomethoca cardinalis*: Mickel 1964: 166.

-*Invreiella cardinalis*: Suárez 1966: 475; Nonveiller 1990: 41; Quintero & Cambra 2011: 218; Pagliano *et al.* 2020: 102.

Sphaerophthalma [sic] *jocularis* Cameron, 1894: 315; Tab. 14, fig. 3 [holotype ♀ (NHMUK)]. Junior subjective synonym of *Pseudomethoca cardinalis* (Gerstaecker, 1874) according to Mickel 1964: 166. **Synonym status confirmed.**

-*Ephuta* (*Ephuta*) *jocularis*: André 1902: 61.

-?*Pseudomethoca jocularis*: Mickel 1924: 46.

-*Pseudomethoca jocularis*: Suárez 1962: 120.

Invreiella curoei Quintero & Cambra, 2011: 218 [holotype ♀ (MIUP)]. Pagliano *et al.* 2020: 102. **New synonym.**

Diagnosis (female). This species is distinguished from other members of the *I. cardinalis* species-group by the following combination of characters: antennal scrobe carina straight (figs 5.39, 5.85), antennal rim weakly tuberculate, genal process spinose and curved with posterior genal carina sinuate (figs 5.33, 5.69), with carina anterior to process crenulate-tuberculate, anterodorsolateral margin of pronotum outcurved (figs 5.36, 5.100), dorsolateral margin of mesosoma, from pronotal carina to propodeal spiracle, distinctly sinuate and undulating in outline (figs 5.36, 5.100), width of propodeum greater than distance between propodeal spiracles in dorsal view (figs 5.36, 5.100), mesopleuron with ventral one-third coarsely sculptured, rugose-granulate with weak rugae and few moderate punctures (figs 5.37, 5.116), and integument of dorsum of head, mesosoma, and center of T2 orange-red (figs 5.13, 5.133).

Redescription (female). Body length 8.51–11.94 mm.

Head: Head 1.16–1.31 × as wide as mesosoma. Vertex and frons contiguously punctate. Antennal scrobe carina straight, with inner tip of carina separated from antennal rim and slightly more prominent than rest of carina. Frons weakly transversely recessed and concave below antennal scrobe carina, antennal rim consequently not recessed and is visible when head viewed laterally. Antennal rim apically glabrous, weakly tuberculate. F1 1.83–2.89 × as long as F2. Clypeus concave, with transverse arcuate carina broadly interrupted medially, area merely punctate, resulting in bilateral carinate processes each with enlarged tubercle ventrad to process. Mandible acuminate. Distance from posterior margin of eye to posterolateral corner of head 1.43–2.05 × maximum diameter of eye. Genal process spinose and curved, posterior genal carina sinuate, carina anterior to process crenulate-tuberculate. Gena densely punctate laterally, punctures large and nearly contiguous, interpunctal space smooth; Postgena transversely rugose-striate medially.

Mesosoma: Mesosoma 1.10–1.28 × as wide as long. Dorsum and posterior face of mesosoma densely, contiguously punctate, surfaces coarse. Anterodorsolateral margin of pronotum outcurved. Pronotal carina prominent, glabrous, weakly crenulate, visible both dorsally and laterally, not strongly protruding posteriorly in dorsal view and not entirely obscuring apical portion of pronotal-mesopleural suture. Dorsolateral margin of mesosoma, from pronotal carina to propodeal spiracle, distinctly sinuate and undulating in outline in dorsal view; tubercle anterior of propodeal spiracle obscure. Width of propodeum greater than distance between propodeal spiracles in dorsal view. Lateral face of pronotum weakly to moderately rugose-striate, with moderate punctures between rugae, lateral face posteriorly carinate along pronotal-mesopleural suture. Mesopleuron with dorsal two-thirds microgranulate with micropunctures, ventral one-third coarsely sculptured, rugose-granulate with weak rugae, with few moderate punctures. Mesopleuron with vertical column of punctures not tuberculate, not forming anterior or posterior carinae. Mesopleuron posteriorly carinate along ventral half of mesopleural-metapleural suture, dorsal portion of suture absent. Metapleuron rugose-striate dorsad metacoxa, with few micro- and moderate punctures, remainder of sclerite weakly microgranulate, near glabrous to glabrous. Metapleural-propodeal suture with overlapping striae at ventral one-third. Lateral face of propodeum posteriorly and with ventral one-third to one-half rugose-striate, with moderate punctures between rugae, dorsal one-third to one-half weakly microgranulate, near glabrous to glabrous. Coxae coarsely sculptured, with small to moderate deep punctures.

Metasoma: T1–5 densely, contiguously punctate. Visible portion of pygidial plate not obscured by setal hood rugose-granulate. S2 densely punctate, punctures near contiguous in some areas, basolateral concave area sparsely punctate, nearly glabrous between punctures. S3–6 densely punctate, coarsely microgranulate between punctures. Hypopygium with slightly arcuate transverse row of setae near apical margin, lateral setae longer than median setae.

Integument coloration: Orange, except the following ranging from dark red-brown to black: antenna, antennal rim, apex and base of mandible, tip of genal process, legs including coxae and trochanters (rarely femora orange), and metasoma (excluding two conjoined orange maculae on T2 and rarely T1 orange).

Pubescence: Frons ventrally with whitish setae, medially with few fuscous setae. Frons dorsally and vertex mostly covered with decumbent, and some raised, light yellow to orange setae. Posterolateral corner of head with few scattered raised fuscous setae.

Mandible with dorsal and ventral longitudinal row of light orange setae. Remainder of head with whitish setae. Dorsum of mesosoma mostly covered with short decumbent light yellow to orange-red setae, dorsolateral edges with raised fuscous to whitish setae. Pronotum with dorsal transverse band of black setae, or only orange setae, with area between epaulet and pronotal spiracle with few whitish setae. Remainder of mesosoma including legs with whitish setae (except tarsi with orange bristles). Anterior face of T1 with whitish setae. T1 apically fringed with wide median band of black setae, laterally fringed with whitish setae. T2 with decumbent light yellow to orange setae overlapping conjoined orange integumental maculae, with maculae anteriorly and posteriorly surrounded with black setae. Felt line of T2 and surrounding lateral area with whitish setae. T2 apically fringed with wide band of black setae medially, laterally fringed with whitish setae. T3 covered and fringed with whitish setae, few scattered raised fuscous setae present. T4 similar to T3 except apically fringed with small patch of black setae medially. T5 mostly covered and fringed with black setae medially, laterally fringed with whitish setae. T6 with light orange setae surrounding pygidial plate, partially obscuring basal half. Remainder of metasoma with whitish setae.

MALE. Unknown.

Etymology: The specific epithet is likely derived from the Latin *cardinalis* in reference to the red-orange coloration of this species.

Distribution: Mexico (Durango, Guanajuato, Guerrero, Jalisco, Mexico, Morelos, Oaxaca, Puebla, Veracruz, and Zacatecas).

Biogeography: Nearctic region (Chihuahuan Desert province); Mexican transition zone (Sierra Madre del Sur province, Sierra Madre Occidental province, Sierra

Madre Oriental province, Transmexican Volcanic Belt province); Neotropical region (Balsas Basin province).

Host(s): Unknown.

Remarks: In contrast to *I. satrapa*, Gerstaecker described *I. cardinalis* based on a single specimen. There are no syntypes of *I. cardinalis* at ZMHB (Lukas Kirschey, pers. comm.). Further, Gerstaecker gives a single length as part of the description, rather than a length range as in *I. satrapa*, implying that a single specimen was examined by him.

There is some variation in setal coloration of the dorsum of the pronotum, which can range from light yellow to orange to black. One small female from Veracruz, Mexico (0000016–CASC) has the genal carina less strongly recurved and the genal process slightly reduced, unlike those of the other specimens examined. Another specimen from Durango, Mexico (0007207–NHMUK) also has the genal carina less recurved than most specimens, with there being minor asymmetry in curvature (the left carina slightly less recurved than the right carina). Further, this specimen has the integument of the femora and T1 orange, which is unusual for this species.

Label data on one specimen (0000014–EMEC) indicates that it was collected on flowers of the Mexican kidneywood tree, *Eysenhardtia polystachya* (Ortega) Sarg.; this female was likely nectaring. Another specimen was collected on an *Ipomoea* sp. (morning glory) (SEMC).

Material examined (*I. cardinalis*, 31♀).

Holotype #1 (*Mutilla cardinalis* Gerstaecker) (examined, figs 5.1–5.4): ♀ (ZMHB) [head and propleura (with attached prolegs) glued onto specimen], [label 1 (green):] *Mexico.* / Jalappa / Deppe. [// label 2 (red):] Type [// label 3 (tan):] 6584 [// label

4 (tan):] Zool. Mus. / Berlin [// label 5 (white):] Mutilla cardinalis. [(**MEXICO: Veracruz:** Xalapa, [19.543°N 96.910°W], F. Deppe (1♀–ZMHB))]

Holotype #2 (*Sphaerophthalma* [sic] *jocularis* Cameron) (examined): ♀ (NHMUK) [F7–10 of right antenna missing; both prolegs entirely missing (apparently glued back onto specimen at one point); mesosoma dorsally cracked due to pin; propodeum mostly covered with thick layer of dirt], [label 1 (tan):] Amula, / Guerrero, / 6000 ft. / Sept. H. H. Smith. [// label 2 (tan):] B.C.A.Hymen.II. // Sphaerophthalma / jocularis, / Cam. [// label 3 (tan, folded):] *Sphaerophthalma* / *jocularis* / *Cam. type* / *BCA ii 315* [// label 4 (tan):] 1903–297. [// label 5 (circular, tan center with red outline):] Type [// label 6 (tan):] B.M. TYPE / HYM. / *15.1042.* [(**MEXICO: Guerrero:** Amula village, [17.633°N 99.250°W], 1828.8 m, Sep., H.H. Smith (1♀–NHMUK))]

Holotype #3 (*Invreiella curoei* Quintero & Cambra) (examined): ♀ (MIUP). [(**MEXICO: Puebla:** Tehuacán, [18.466°N 97.400°W], 5500 ft., 06.Jul.1941, H. Seevers (1♀–MIUP))]

Additional specimens (non-types, examined or referenced*): MEXICO: Durango: Ciudad Lerdo, [25.544°N 103.526°W], H. Höge (1♀–0007207–NHMUK). **Guanajuato:** [Guanajuato], [21.019°N 101.257°W], E. Dugès (1♀–0006575–RBINS). **Guerrero:** Chichihualco, 18 mi. W, [17.587°N 99.799°W], 1970 m, 28.Aug.1977, E.I. Schlinger, EMEC 1135899 (1♀–0000015–EMEC); Tixtla, 10 mi. E, [17.569°N 99.247°W], 16.Jul.1983, J. Chemsak, A. & M. Michelbacher, EMEC 1135898 (1♀–0000013–EMEC). **Jalisco:** Magdalena, [20.910°N 103.969°W], 15.Jul.1965, W.F. Chamberlain (1♀–0000018–DGMC); Teocaltiche, Rancho La Quinta, [21.433°N 102.571°W], 5600 ft., 25.Aug.1979, B. Villegas (1♀–0006576–UCDC); Tepatitlán de

Morelos, [20.809°N 102.765°W], 2011.7 m, 20.Aug.1954, J.G. Chillcott (1♀–0000007–CNC); Tuxpan, Atenquique, [19.529°N 103.442°W], H. Höge (1♀–0000061–NHMUK). **México:** Temascaltepec, [19.043°N 100.041°W], 1931, G.D. Hinton (2♀–0000009, 0000010–EMUS). **Morelos:** Cuernavaca, [18.924°N 99.221°W], 08.Nov.–06.Dec.1987, F.D. Parker (1♀–0000008–EMUS). **Oaxaca:** Monte Albán, [17.043°N 96.767°W], 1900 m, 23.Dec.1990, L. Godinez, #652, "ex. *Ipomoea* sp." (1♀–SEMC). **Puebla:** Tehuacán, [18.466°N 97.400°W], 12.Jul.1935, A.E. Pritchard (1♀–0000005–UMSP); 23.Jun.1951, P.D. Hurd, "on flowers of *Eysenhardtia polystachya* (Ort.)," EMEC 1135783 (1♀–0000014–EMEC). **Unknown:** (2♀–0000004, 0000006–UMSP); (1♀–0000011–NHMD); (1♀–0000012–NMNH); (3♀–0000060, 0007208, 0007209–NHMUK); Sallé, MNCN_Ent 171985 (1♀–MNCN*). **Veracruz:** Citlaltépetl, Pico de Orizaba, plateau, [19.013°N 97.266°W], 1524 m, 03.Aug.1964, L.W. Swan (1♀–0000016–CASC). **Zacatecas:** Moyahua de Estrada, [21.265°N 103.165°W], 07.Aug.1965, A.R. Gillogly (3♀–DJBC*; 1♀–SEMC); Nochistlán, [21.363°N 102.843°W], 23.Aug.1970, B.L. Villegas (1♀–0000017–DGMC).

6. *Invreiella cuernavaca* Waldren, sp. nov.

(Figs 5.14, 5.34, 5.38, 5.40, 5.70, 5.86, 5.101, 5.117, 5.134, 5.135)

Diagnosis (female). This species is distinguished from other members of the *I. cardinalis* species-group by the following combination of characters: antennal scrobe carina arcuate (figs 5.40, 5.86), antennal rim tuberculate, genal process triangular and weakly curved with posterior genal carina sinuate (figs 5.34, 5.70), with carina anterior to process weakly crenulate, anterodorsolateral margin of pronotum outcurved (fig. 5.101), dorsolateral margin of mesosoma, from pronotal carina to propodeal spiracle, distinctly sinuate and undulating in outline in dorsal view (fig. 5.101), tubercle anterior of propodeal spiracle present (fig. 5.101), width of propodeum greater than distance between propodeal spiracles in dorsal view (fig. 5.101), mesopleuron with micropunctures throughout, the interpunctal space smooth, not coarsely sculptured (figs 5.38, 5.117), and integument of dorsum of head, mesosoma, and center of T2 orange-red (figs 5.14, 5.134).

Description (female). Body length 10.54–10.67 mm.

Head: Head 1.25–1.27 × as wide as mesosoma. Vertex and frons contiguously punctate. Antennal scrobe carina arcuate, with inner tip of carina separated from antennal rim. Frons not transversely recessed and concave below antennal scrobe carina, antennal rim consequently not recessed and is visible when head viewed laterally. Antennal rim apically glabrous, tuberculate. F1 1.95–2.09 × as long as F2. Clypeus concave, with transverse arcuate carina broadly interrupted medially, area merely punctate, resulting in bilateral carinate processes each with enlarged tubercle ventrad to process. Mandible acuminate. Distance from posterior margin of eye to posterolateral corner of head 1.48–1.5 × maximum diameter of eye. Genal process triangular and weakly curved, posterior genal carina sinuate, carina anterior to process weakly crenulate. Gena densely punctate laterally, punctures large and nearly contiguous, interpunctal space smooth. Postgena transversely rugose-striate medially.

Mesosoma: Mesosoma 1.14–1.19 × as wide as long. Dorsum and posterior face of mesosoma densely, contiguously punctate, surfaces coarse. Anterodorsolateral margin of pronotum outcurved. Pronotal carina prominent, glabrous, weakly crenulate, visible both dorsally and laterally, not strongly protruding posteriorly in dorsal view and not entirely obscuring apical portion of pronotal-mesopleural suture. Dorsolateral margin of mesosoma, from pronotal carina to propodeal spiracle, distinctly sinuate and undulating in outline in dorsal view; tubercle anterior of propodeal spiracle present, prominent. Width of propodeum greater than distance between propodeal spiracles in dorsal view. Lateral face of pronotum rugose-striate, with moderate punctures between rugae, lateral face posteriorly carinate along pronotal-mesopleural suture. Mesopleuron with micropunctures throughout, interpunctal space smooth. Mesopleuron with vertical column of punctures not tuberculate, not forming anterior and posterior carinae. Mesopleuron posteriorly carinate along ventral half of mesopleural-metapleural suture, dorsal portion of suture absent. Metapleuron weakly rugose-striate dorsad metacoxa, with few micro- and moderate punctures, remainder of sclerite weakly microgranulate, near glabrous to glabrous. Metapleural-propodeal suture with some overlapping striae at ventral one-third and dorsal one-third. Lateral face of propodeum posteriorly and with ventral one-third rugose-striate, with moderate punctures between rugae, dorsal two-thirds weakly microgranulate, near glabrous to glabrous, medially with cluster of moderate punctures. Coxae coarsely sculptured, with small to moderate deep punctures.

Metasoma: T1–5 densely, contiguously punctate. Visible portion of pygidial plate not obscured by setal hood rugose-granulate. S2 densely punctate, punctures near contiguous in some areas, basolateral concave area sparsely punctate, nearly glabrous between punctures. S3–6 densely punctate, coarsely microgranulate between punctures. Hypopygium with slightly arcuate transverse row of setae near apical margin, lateral setae longer than median setae.

Integument coloration: Orange, except the following ranging from dark red-brown to black: antenna, antennal rim, apex and base of mandible, tip of genal process, legs including coxae and trochanters, and metasoma (excluding two conjoined orange maculae on T2).

Pubescence: Frons ventrally with whitish setae, medially with few fuscous setae. Frons dorsally and vertex mostly covered with decumbent, and some raised, orange setae, with scattered raised fuscous setae. Posterolateral corner of head with sparse patch of fuscous setae. Mandible with dorsal and ventral longitudinal row of light orange setae. Remainder of head with whitish setae. Dorsum of mesosoma mostly covered with decumbent orange-red setae, dorsolateral edges with raised fuscous setae. Pronotum with dorsal transverse band of black setae, except area between epaulet and pronotal spiracle with whitish setae. Remainder of mesosoma including legs with whitish setae (except tarsi with orange bristles). Anterior face of T1 with whitish setae. T1 apically fringed with wide median band of black setae, laterally fringed with whitish setae. T2 with decumbent orange setae overlapping conjoined orange integumental maculae, with maculae anteriorly and posteriorly surrounded with black setae. Felt line of T2 and surrounding lateral area with whitish setae. T2 apically fringed with wide band of black setae medially, laterally fringed with whitish setae. T3 covered and fringed with whitish setae, few scattered raised fuscous setae present. T4 similar to T3 except with patch of black setae medially. T5 mostly covered and fringed with black setae medially, laterally fringed with whitish setae. T6 with orange setae surrounding pygidial plate, partially obscuring basal half. Remainder of metasoma with whitish setae.

MALE. Unknown.

Etymology: The specific epithet is derived from the type locality of Cuernavaca, Morelos, Mexico.

Distribution: Mexico (Morelos).

Biogeography: Neotropical region (Balsas Basin province).

Host(s): Unknown.

Remarks: This species is known only from the holotype and a paratype, which share the same collection data. It is apparently closely related to *I. cardinalis*, which also occurs in Cuernavaca. Both species share a posteriorly sinuate genal carina, an outcurved anterodorsolateral margin of pronotum, a sinuate and undulating dorsolateral margin of mesosoma in dorsal view, and a propodeal width greater than distance between propodeal spiracles in dorsal view.

Material examined (*I. cuernavaca*, 2♀).

Holotype: ♀ (0000032–CASC) [F9 and F10 of right antenna missing], [label 1 (tan):] *CUERNAVACA* / *12 JULY, 1963* [// label 2 (tan):] JB [// label 3 (white, folded):] J.B. is Joseph A. Beatty of Southern / Illinois, University. [// label 4 (red):] HOLOTYPE ♀ / Invreiella cuernavaca / Waldren, 2018 / GCW_HYM0000032. [// label 5 (white):] *Invreiella* / *curoei* / *Quintero & Cambra 2011* / Det K.A. Williams 2012. [(**MEXICO: Morelos:** Cuernavaca, [18.924°N 99.221°W], 12.Jul.1963, J.A. Beatty (1♀–0000032–CASC))]

Paratype: MEXICO: Morelos: Cuernavaca, [18.924°N 99.221°W], 12.Jul.1963, J.A. Beatty (1♀–0000033–CASC).

Invreiella cephalargia species-group

(Fig. 5.138)

Members of this female-based species-group are recognized by the following combination of character states:

1. Antennal scrobe carina position: inner tip well-separated from antennal rim.
2. Frons and antennal rim position: frons not transversely recessed and concave, antennal rim consequently not recessed and is visible when head viewed laterally.
3. Antennal rim: rounded to weakly tuberculate.
4. Clypeus: concave, with transverse arcuate carina complete, with small lateral tubercle ventrad to carina.
5. Genal process: triangular, posterior genal carina nearly straight.
6. Pronotal carina: weakly present, with cluster of dense, contiguous punctures in its place, edges of punctures tuberculate and crenulate, simulating carina.
7. Lateral face of pronotum sculpture: striate-rugose, with moderate punctures amid the striae.
8. Vertical column of punctures of mesopleuron: puncture edges tuberculate, forming crenulate anterior and posterior carinae.
9. Mesopleuron, metapleuron, and lateral face of propodeum sculpture, where present: conspicuously striate-rugose, with moderate punctures amid striae.

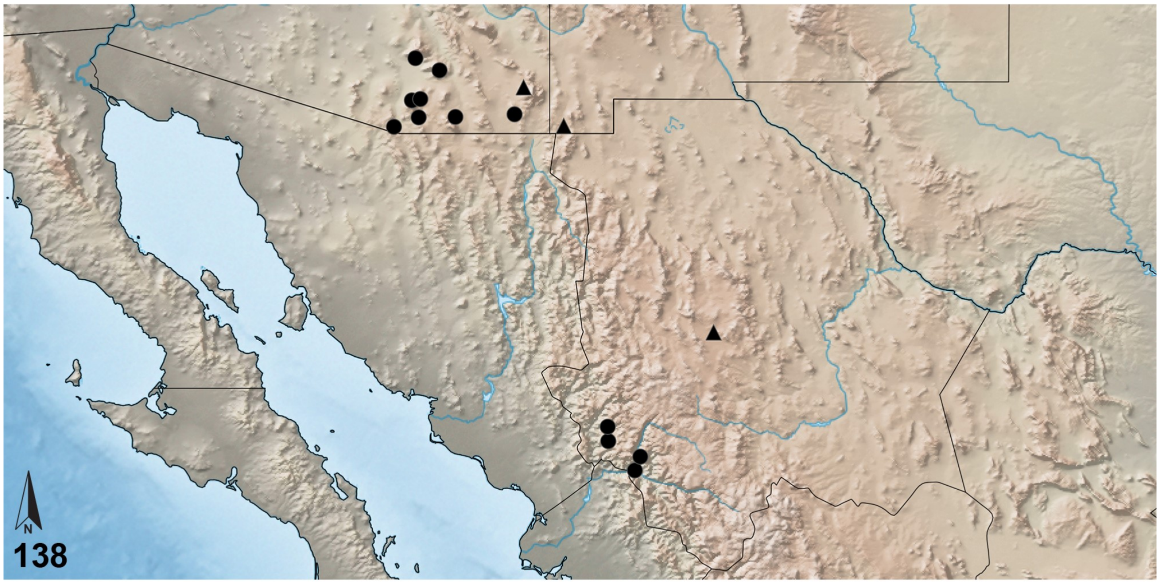
Species included: *I. cephalargia* (Mickel, 1924), **comb. nov.** and *I. manleyi* Waldren, **sp. nov.**

Distribution: Mexico (Chihuahua); USA (Arizona and New Mexico).

Biogeography: Nearctic region (Chihuahuan Desert province); Mexican transition zone (Sierra Madre Occidental province).

136
I. cephalargia
(0000024)
●

137
I. manleyi
(0000037)
▲



FIGURES 5.136–5.138. Female habitus, dorsal view: *I. cephalargia* species-group. Scale bars = 2 mm. **5.138**–Geographic distribution of the *I. cephalargia* species-group.

7. *Invreiella cephalargia* (Mickel, 1924), comb. nov.

(Figs 5.15, 5.55, 5.71, 5.87, 5.102, 5.118, 5.136, 5.138)

Pseudomethoca cephalargia Mickel, 1924: 17 [holotype ♀ (AMNH – lost)]. Mickel 1935: 384; Krombein 1951: 759, 1979: 1302.

Diagnosis (female). This species is distinguished from the other member of the *I. cephalargia* species-group, *I. manleyi*, **sp. nov.**, by the following combination of characters: the vertex is densely covered with decumbent golden-yellow setae (figs 5.15, 5.136), the dorsum of T2 with a central patch, or two median patches, of golden setae (sometimes sparse) (figs 5.15, 5.136), the integument of the antennae, pleura, and legs is orange-red (figs 5.15, 5.87, 5.118, 5.136), and the striations of the meso- and metapleura and lateral face of propodeum are less pronounced (fig. 5.118).

Redescription (female). Body length 7.49–11.56 mm.

Head: Head 0.93–1.15 × as wide as mesosoma. Vertex and frons contiguously punctate. Antennal scrobe carina arcuate, with inner tip of carina well-separated from antennal rim. Frons not transversely recessed and concave below antennal scrobe carina, antennal rim consequently not recessed and is visible when head viewed laterally. Antennal rim apically glabrous, rounded. F1 1.56–2.79 × as long as F2. Clypeus concave, with transverse arcuate carina complete, with small lateral tubercle ventrad to carina. Mandible acuminate. Distance from posterior margin of eye to posterolateral corner of head 0.93–1.56 × maximum diameter of eye. Genal process triangular, posterior genal carina nearly straight. Gena densely, contiguously punctate laterally. Postgena transversely rugose-striate.

Mesosoma: Mesosoma 1.19–1.3 × as wide as long. Dorsum and posterior face of mesosoma densely, contiguously punctate, surfaces coarse. Anterodorsolateral margin of pronotum outcurved. Pronotal carina weakly present, with cluster of dense, contiguous punctures in its place, edges of punctures tuberculate and crenulate, simulating carina. Tubercle anterior of propodeal spiracle obscure. Width of propodeum scarcely greater than distance between propodeal spiracles in dorsal view. Lateral face of pronotum rugose-striate, with moderate punctures amid striae, lateral face posteriorly carinate along pronotal-mesopleural suture. Mesopleuron finely striate-rugose anteriorly, microgranulate and with micropunctures, posteriorly striate-rugose. Mesopleuron with vertical column of punctures tuberculate at edges, forming crenulate anterior and posterior carinae. Mesopleuron posteriorly carinate along ventral half of mesopleural-metapleural suture, dorsal portion of suture absent. Metapleuron with ventral half striate-rugose, with few moderate punctures amid striae, dorsal half microgranulate and nearly glabrous. Metapleural-propodeal suture with short striae perpendicularly overlapping suture mostly throughout its length. Lateral face of propodeum with ventral two-thirds striate-rugose, with few moderate punctures amid striae, dorsal one-third microgranulate to striate-rugose, with few moderate punctures. Coxae coarsely sculptured, with small to large punctures.

Metasoma: Dorsum of T1–5 densely, contiguously punctate. Visible portion of pygidial plate not obscured by setal hood rugose-granulate. S2 densely punctate, punctures moderate to large, interpunctal space smooth, basolateral concave area sparsely punctate, nearly glabrous between punctures. S3–6 densely punctate, coarsely microgranulate between punctures. Hypopygium with slightly arcuate transverse row of setae near apical margin, lateral setae longer than median setae.

Integument coloration: Orange-red, except apical 1/2–1/3 of mandible and T6, dark brown-red.

Pubescence: Frons ventrally with whitish setae, medially with few fuscous setae. Frons dorsally and vertex with dense, decumbent golden-yellow setae. Posterolateral corner of head with sparse patch of black setae. Mandible with dorsal and ventral longitudinal row of pale orange setae. Remainder of head with whitish setae. Dorsum of mesosoma mostly covered with decumbent orange setae, dorsolateral edges with sparse raised fuscous and whitish setae. Pronotum with dorsal transverse band of black setae, except area between epaulet and pronotal spiracle with whitish setae. Additional patch of whitish setae surrounding or just posterior to propodeal spiracle and another patch sometimes present on scutum. Remainder of mesosoma including legs with whitish setae (except tarsi with dense orange bristles). Anterior face of T1 with whitish setae. T1 apically fringed with moderately wide band of black setae medially, laterally fringed with whitish setae. T2 with median transverse patch of whitish setae, or with two median round patches of whitish setae, remainder of dorsum of T2 covered with orange to black setae surrounding median whitish setae. Felt line of T2 and surrounding lateral area with whitish setae. T2 apically fringed with narrow patch of black setae medially, laterally fringed with whitish setae. T3 covered and fringed with whitish setae. T4 similar to T3 except with moderately wide patch of black setae medially. T5 mostly covered and fringed with black setae medially, laterally fringed with whitish setae. T6 with light orange setae surrounding pygidial plate, partially obscuring basal half. Remainder of metasoma with whitish setae.

MALE. Unknown.

Etymology: The specific epithet is in reference to the large head of this species.

Distribution: Mexico (Chihuahua); USA (Arizona).

Biogeography: Nearctic region (Chihuahuan Desert province); Mexican transition zone (Sierra Madre Occidental province).

Host(s): Unknown.

Remarks: The holotype is lost and is not present at AMNH; fortunately, this species is among the most easily recognized in the genus. *I. cephalargia* is the first known *Invreiella* to occur in the United States. In his 1924 revision of the *Pseudomethoca* (*sensu lato*) fauna of the United States and Canada, C. Mickel noted the unusual morphology of *I. cephalargia*: “This species is not closely related to any other of the forms treated herein. It will likely be found represented in the Mexican fauna. It may be easily recognized by the structure of the head at the sides beneath.” (Mickel 1924: 18)

Material examined (*I. cephalargia*, 15♀).

Holotype (lost): ♀ (AMNH). [(**USA: Arizona:** *Pima Co.*: Santa Catalina Mountains, Sabino Basin, [32.312°N 110.798°W], 08–20.Jul.1916 (1♀–AMNH))]

Additional specimens (non-types, examined or referenced*): MEXICO: Chihuahua: Divisadero, 5 km upstream along Rio Urique, 26.929°N 107.938°W, 301 m, 18.Mar.1997, B. Pickering, MSB 13565 (1♀–MSBA*); El Tejabán, 29 mi. SW along Urique River [in Copper Canyon], [27.108°N 107.867°W], 23–27.May.1991, R.E. Stecker (1♀–0000028–CASC); Témoris, 2 mi. N, [27.311°N 108.285°W], 22.Aug.1968, T.A. Sears, R.C. Gardner, & C.S. Glaser (1♀–0000022–DGMC); Témoris, 15 mi. N, [27.499°N 108.292°W], 22.Aug.1968, T.A. Sears, R.C. Gardner, & C.S. Glaser (1♀–0000021–DGMC). **USA: Arizona:** *Cochise Co.*: Leslie Canyon NWR, [31.579°N 109.506°W], 23.Apr.2001, W.R. Radke (1♀–0000025–DGMC), 10.Aug.2001, W.R. Radke (1♀–0000026–DGMC), 24.Sep.2008, W.R. Radke (1♀–0000027–DGMC); Sierra Vista, [31.545°N 110.277°W], 01.Aug.1961, R.F. Sternitzky (1♀–0000024–DGMC), 21.Aug.2001, R.F. Sternitzky (1♀–0000023–DGMC). *Pima Co.*: Santa Rita Experimental Station, 31.761°N 110.844°W, 1340 m, 01.Jul.1970, M.A. Kolner & S.L. Szerlip, ASUHIC 0059505 (1♀–ASUHIC*); Santa Rita Mountains (north end), Hwy 83, 2.8 mi. W on FS 62, 31.778611°N 110.734444°W, 08.Oct–03.Dec.2011, black pitfall, W.B. Warner (1♀–0000030–EMUS); Tucson, Rincon Mountains, [32.153°N 110.484°W], 16.Sep.1937, E.D. Ball (1♀–0000019–EMUS). *Santa Cruz Co.*: Patagonia, [31.539°N 110.756°W] (1♀–0000029–CASC); Peña Blanca Canyon, [31.416°N 111.078°W], 07–11.Sep.1978, W.P. Nye (1♀–0000020–EMUS).

8. *Invreiella manleyi* Waldren, sp. nov.

(Figs 5.16, 5.42, 5.47, 5.61, 5.72, 5.88, 5.103, 5.119, 5.137, 5.138)

Diagnosis (female). This species is distinguished from the other member of the *I. cephalargia* species-group, *I. cephalargia*, by the following combination of characters: the vertex is mostly covered with decumbent orange setae (figs 5.16, 5.137), the dorsum of T2 without a central patch/patches of golden setae, mostly covered with orange-red setae (figs 5.16, 5.137), the integument of the antennae, pleura, and legs is dark red-brown (figs 5.16, 5.88, 5.119, 5.137), and the striations of the meso- and metapleura and lateral face of propodeum more pronounced (figs 5.61, 5.119).

Description (female). Body length 7.49–8.76 mm.

Head: Head 1.12–1.22 × as wide as mesosoma. Vertex and frons contiguously punctate. Antennal scrobe carina moderately arcuate, with inner tip of carina well-separated from antennal rim. Frons not transversely recessed and concave below antennal scrobe carina, antennal rim consequently not recessed and is visible when head viewed laterally. Antennal rim apically glabrous, weakly tuberculate. F1 1.6–1.82 × as long as F2. Clypeus concave, with transverse arcuate carina complete, with small lateral tubercle ventrad to carina. Mandible acuminate. Distance from posterior margin of eye to posterolateral corner of head 1.18–1.64 × maximum diameter of eye. Genal process triangular, posterior genal carina nearly straight. Gena densely, contiguously punctate laterally. Postgena transversely rugose-striate.

Mesosoma: Mesosoma 1.09–1.18 × as wide as long. Dorsum and posterior face of mesosoma densely, contiguously punctate, surfaces coarse. Anterodorsolateral margin of pronotum outcurved. Pronotal carina obscurely present, with cluster of dense, contiguous punctures in its place, edges of punctures tuberculate and crenulate, simulating carina. Tubercle anterior of propodeal spiracle present. Width of propodeum greater than distance between propodeal spiracles in dorsal view. Lateral face of pronotum rugose-striate, with moderate punctures amid striae, lateral face posteriorly carinate along pronotal-mesopleural suture. Mesopleuron finely striate-rugose anteriorly, microgranulate and with micropunctures, posteriorly striate-rugose. Mesopleuron with vertical column of punctures tuberculate at edges, forming crenulate anterior and posterior carinae.

Mesopleuron posteriorly carinate along ventral half of mesopleural-metapleural suture, dorsal portion of suture obscure. Metapleuron with ventral half striate-rugose, dorsal half microgranulate and nearly glabrous. Metapleural-propodeal suture with short striae perpendicularly overlapping suture mostly throughout its length, dorsal third of suture overlapped by deep punctures or grooves between striae. Lateral face of propodeum with ventral two-thirds striate-rugose, dorsal one-third microgranulate to striate-rugose, with few moderate punctures. Coxae coarsely sculptured, with small to large punctures.

Metasoma: Dorsum of T1–5 densely, contiguously punctate. Visible portion of pygidial plate not obscured by setal hood rugose-granulate to transversely rugose-granulate. S2 densely punctate, punctures moderate, interpunctal space smooth, basolateral concave area sparsely punctate, nearly glabrous between punctures. S3–6 densely punctate, coarsely microgranulate between punctures. Hypopygium with slightly arcuate, transverse row of setae near apical margin, lateral setae longer than median setae.

Integument coloration: Frons, vertex, gena, venter of head, basal 2/3 of mandible, dorsum of mesosoma, lateral face of pronotum, most of T2, and middle of S2, orange, with remaining integument dark red-brown.

Pubescence: Frons ventrally with whitish setae, medially with few fuscous setae. Frons dorsally and vertex mostly covered with decumbent and raised orange-red setae, with scattered raised fuscous setae. Clypeus with fuscous and whitish setae. Mandible with dorsal and ventral longitudinal row of pale orange setae. Remainder of head with whitish setae. Dorsum of mesosoma mostly covered with decumbent orange setae, dorsal edges (except pronotum) with sparse raised fuscous setae. Pronotum without black setae, rather with orange setae. Posterior face of propodeum with black setae. Remainder of mesosoma including legs with whitish setae (except tarsi with dense orange bristles). Anterior face of T1 with black setae. T1 apically fringed with wide band of black setae medially, laterally fringed with whitish setae. T2 dorsally covered with decumbent orange setae, anteriorly with transverse band of black setae overlapping with T1 apical fringe, posteriorly with transverse band of black to whitish setae before apical fringe. Felt line of T2 and surrounding lateral area with whitish setae. T2 apically fringed with sparse median patch of black setae, laterally fringed with whitish setae. T3–5 covered with pale and some raised fuscous setae, mostly fringed with whitish setae, medially with small patch of black setae. T6 with light yellow-orange setae surrounding pygidial plate, partially obscuring basal half. Remainder of metasoma with whitish setae.

MALE. Unknown.

Etymology: This species is named in honor of Dr. Donald G. Manley in recognition of his contributions to mutillid systematics and biology since the 1970s. Dr. Manley also collected one of the paratypes in Hidalgo Co., New Mexico, the first record of *Invreiella* for the state.

Distribution: Mexico (Chihuahua); USA (Arizona and New Mexico).

Biogeography: Nearctic region (Chihuahuan Desert province); Mexican transition zone (Sierra Madre Occidental province).

Host(s): Unknown.

Remarks: This is the second known female-based *Invreiella* species from the United States (after *I. cephalargia*).

Material examined (*I. manleyi*, 3♀).

Holotype: ♀ (0000037–DGMC) [tarsus of right mesoleg missing], [label 1 (tan):] *1*5 mi.w.Portal / Chiricahua Mts / Ariz.VIII-*7*-58 [// label 2 (tan):] C.G. Moore / Collector [// label 3 (red):] HOLOTYPE ♀ / Invreiella manleyi / Waldren, 2018 / GCW_HYM0000037 [// label 4 (white):] Mutillidae: * / Pseudomethoca sp. ♀ / det. D.G. Manley 19*99*. [(**USA: Arizona:** *Cochise Co.*: Portal, 15 mi. W at Chiricahua Mountains, [31.931°N 109.388°W], 07.Aug.1958, C.G. Moore (1♀–0000037–DGMC))]

Paratypes: MEXICO: Chihuahua: Colonia Álvaro Obregón, 1.1 mi. S, [28.730°N 106.912°W], 7800 ft., 21.Jul.1973, R.R. & M.E. Murray (1♀–0000039–TAMU). **USA: New Mexico:** *Hidalgo Co.*: Animas, 40 mi. S at Gray Ranch, [31.426°N 108.865°W], 21.Aug.1992, D.G. Manley (1♀–0000038–DGMC).

***Invreiella satrapa* species-group**

(Fig. 5.142)

Members of this female-based species-group are recognized by the following combination of character states:

1. Antennal scrobe carina position: inner tip separated from antennal rim.
2. Frons and antennal rim position: frons weakly to moderately transversely recessed and concave below antennal scrobe carina, antennal rim consequently visible or not when head is viewed laterally.
3. Antennal rim: weakly tuberculate.
4. Clypeus: plate-like and medially flattened, rugose-granulate, with central cluster of long raised setae, with two medioapical, anterad-projecting tubercles.

5. Genal process: triangular to spinose, posterior genal carina weakly sinuate to nearly straight.
6. Pronotal carina: present, glabrous, visible both dorsally and laterally.
7. Lateral face of pronotum sculpture: rugose-striate, with moderate punctures amidst the striae.
8. Vertical column of punctures of mesopleuron: puncture edges weakly tuberculate, not forming anterior or posterior carinae.
9. Mesopleuron, metapleuron, and lateral face of propodeum sculpture, where present: conspicuously striate-rugose, granulate, and microgranulate, with few moderate punctures amid sculpture.

Species included: *I. breviclypeata* Waldren, **sp. nov.**, *I. chihuahuensis* Waldren, **sp. nov.**, and *I. satrapa* (Gerstaecker, 1874).

Distribution: Mexico (Chihuahua, Coahuila, Durango, Guanajuato, Hidalgo, Mexico, Michoacan, San Luis Potosi, Sinaloa, Veracruz, and Zacatecas).

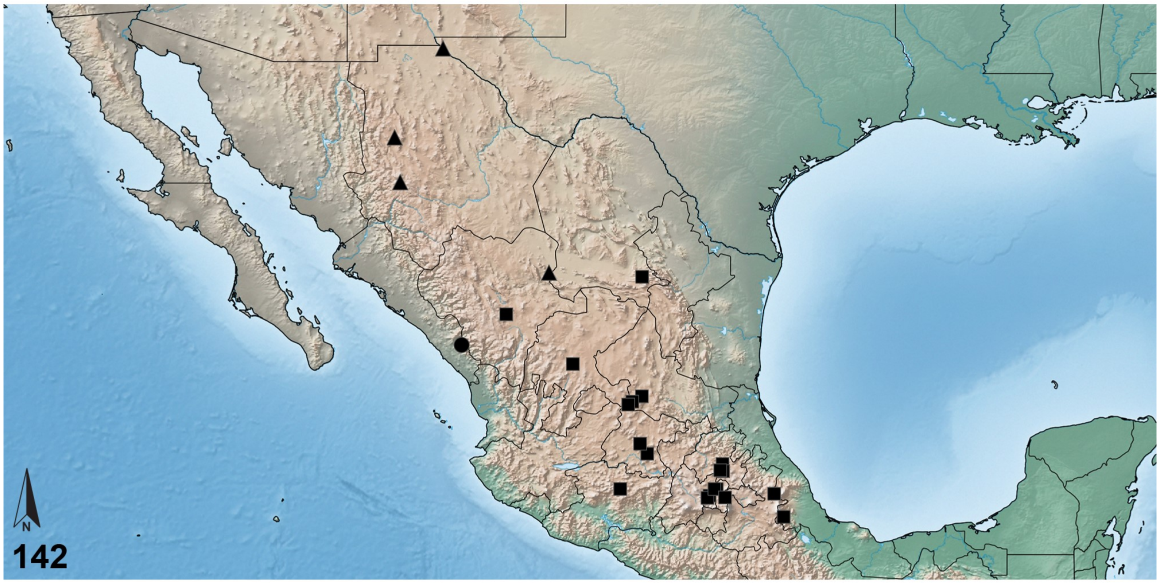
Biogeography: Nearctic region (Chihuahuan Desert province); Mexican transition zone (Sierra Madre del Sur province; Sierra Madre Occidental province; Sierra Madre Oriental province, Transmexican Volcanic Belt province).

Remarks: The form of the clypeus in this species-group is noteworthy in that it is plate-like and often elongate, medially ending in two tubercles (figs 5.24, 5.25, 5.89–5.91). This protruding clypeal form is convergent among several unrelated species of Pseudomethocini, as noted by Cambra *et al.* (2014): *Hoplocrates centromaculata* (Cresson, 1902), *Hoplognathoca jinotega* Cambra, Quintero, & Brothers, 2014 and *Invreiella satrapa* (Gerstaecker, 1874). We add to this list *I. breviclypeata*, **sp. nov.** and *I.*

139
I. breviclypeata
(0000003)
●

140
I. chihuahuensis
(0000031)
▲

141
I. satrapa
(0000049)
■



FIGURES 5.139–5.142. Female habitus, dorsal view: *I. satrapa* species-group. Scale bars = 2 mm. **5.142**–Geographic distribution of the *I. satrapa* species-group.

chihuahuensis, **sp. nov.**, as well as *Hoplocrates lingulatus* Mickel, 1941 and *Pseudomethoca nephele* (Fox, 1899), the latter being a small species that occurs in south Texas and adjacent northern Mexico. In *Hc. lingulatus*, *Hg. jinotega*, and *P. nephele*, the clypeal process is anterad-projecting, whereas in the *Invreiella satrapa* species-group it is ventrad-projecting, with the two apical tubercles anterad-projecting, forming an apparent "scoop" in *I. chihuahuensis*, **sp. nov.** and *I. satrapa*. The function of this clypeal form is presently unknown.

Also of note is the transverse recession of the frons below the scrobe carinae, which results in the recession of the antennal rims and clypeus. This condition is variably observed in this species-group as well as the *I. suarezi* species-group (see remarks under the latter).

9. *Invreiella breviclypeata* Waldren, sp. nov.

(Figs 5.17, 5.25, 5.73, 5.89, 5.104, 5.120, 5.139, 5.142)

Diagnosis (female). This species is distinguished from other members of the *I. satrapa* species-group by the following combination of characters: clypeus with the apical margin only slightly longer medially than laterally and coming to a shallow apex with the two median tubercles (figs 5.25, 5.89), the tubercles being semi-circular in shape in dorsal view and with a distinct gap present between them, genal process triangular (fig. 5.73), pronotal carina not strongly lobate posteriorly and not projecting away from mesosoma, not obscuring dorsal portion of pronotal-mesopleural suture (figs 5.104, 5.120), and apical margin of T2 with wide transverse band of near-black integument mostly covered with black setae (figs 5.17, 5.139).

Description (female). Body length 10.41 mm.

Head: Head 1.18 × as wide as mesosoma. Vertex and frons contiguously punctate. Antennal scrobe carina straight, slightly more prominent internally, with inner tip of carina separated from antennal rim. Frons somewhat transversely recessed and concave below antennal scrobe carina, antennal rim consequently slightly recessed and slightly visible when head viewed laterally. Antennal rim apically glabrous, weakly tuberculate. F1 2.10 × as long as F2. Clypeus plate-like and medially flattened, coarsely rugose-granulate, with central cluster of long raised setae, with apical margin only slightly longer medially than laterally and coming to shallow apex, with two close medioapical tubercles that are anterad-projecting, dorsoventrally semi-circular in shape, and with distinct gap present between them. Mandible acuminate. Distance from posterior margin of eye to posterolateral corner of head 1.45 × maximum diameter of eye. Genal process triangular, posterior genal carina weakly sinuate. Gena densely, nearly contiguously punctate laterally, interpunctal space smooth. Postgena transversely rugose-striate.

Mesosoma: Mesosoma 1.28 × as wide as long. Dorsum and posterior face of mesosoma densely, contiguously punctate, surfaces coarse. Anterodorsolateral margin of pronotum outcurved. Pronotal carina prominent, glabrous, visible both dorsally and laterally, not strongly lobate posteriorly and not projecting away from mesosoma, not obscuring dorsal portion of pronotal-mesopleural suture. Tubercle anterior of propodeal spiracle present. Width of propodeum greater than distance between propodeal spiracles in dorsal view. Lateral face of pronotum rugose-striate, with moderate punctures between striae, lateral face posteriorly carinate along pronotal-mesopleural suture. Mesopleuron anteriorly microgranulate to granulate, with few punctures, posteriorly with ventral one-fourth of sclerite striate-rugose. Mesopleuron with vertical column of punctures weakly tuberculate, not forming anterior or posterior carinae. Mesopleuron posteriorly carinate along ventral half of mesopleural-metapleural suture, dorsal portion of suture obscure, faintly present. Metapleuron with ventral one-third striate-rugose, dorsal two-thirds mostly glabrous. Metapleural-propodeal suture with striae perpendicularly overlapping suture along ventral half. Lateral face of propodeum posteriorly with ventral half striate-rugose, anteriorly with dorsal half mostly glabrous, with few microstriae. Coxae coarsely sculptured, with small to large, deep punctures.

Metasoma: T1–5 densely, contiguously punctate. Visible portion of pygidial plate not obscured by setal hood rugose-granulate. S2 densely punctate, punctures large, interpunctal space smooth, basolateral concave area sparsely punctate, nearly glabrous between punctures. S3–6 densely punctate, coarsely microgranulate between punctures. Hypopygium with slightly arcuate, transverse row of setae near apical margin, lateral setae longer than median setae.

Integument coloration: Orange, except the following ranging from dark red-brown to black: antenna, antennal rim, apical one-third and basal one-fourth of mandible, legs including coxae and trochanters, T1 and S1, apical margin of T2 and S2 transversely, and T3–6 and S3–6 entirely.

Pubescence: Frons ventrally with few whitish setae. Frons dorsally, vertex, and posterolateral corner of head covered with mostly decumbent orange-red setae, posterolateral corner with few scattered raised fuscous setae. Mandible with dorsal and ventral longitudinal row of light orange setae. Remainder of head with whitish setae. Dorsum of mesosoma mostly covered with decumbent orange-red setae, dorsal edges (except pronotum) with sparse raised fuscous setae, particularly at top of posterior face of propodeum. Pronotum without black setae, rather with orange setae. Remainder of mesosoma including legs with whitish setae (except tarsi with dense orange bristles). Anterior face of T1 with whitish setae. T1 apically fringed with wide band of black setae medially, laterally fringed with whitish setae. Dorsum of T2 mostly covered with orange setae. Felt line of T2 and surrounding lateral area with whitish setae. T2 apically fringed with wide band of black setae medially, coming to median point, laterally fringed with whitish setae. T3–5 mostly covered and apically fringed with whitish setae, with few scattered dark raised setae and apical fringe with median patch of black setae, these patches becoming sequentially wider from T3–5. T6 with dark yellow-orange setae surrounding pygidial plate, partially obscuring basal half. Remainder of metasoma with whitish setae.

MALE. Unknown.

Etymology: The specific epithet is derived from the Latin *brevis* and *clypeus* in reference to the shortened clypeus which helps distinguish it from *I. chihuahuensis* and *I. satrapa*.

Distribution: Mexico (Sinaloa).

Biogeography: Mexican transition zone (Sierra Madre Occidental province).

Host(s): Unknown.

Remarks: This species is known only from the holotype.

Material examined (*I. breviclypeata*, 1♀).

Holotype: ♀ (0000003–DGMC) [F9 and F10 of right antenna missing; tarsomere #5 of right metaleg missing], [label 1 (white):] MEX:Sinaloa, / 5 mi W El / Palmito 6000'

/ x-13-1975 [// label 2 (white):] J Powell / J Chemsak / T Eichlin & / T. Friedlander [// label 3 (red):] HOLOTYPE ♀ / Invreiella breviclypeata / Waldren, 2018 / GCW_HYM0000003 [// label 4 (white):] Mutillidae: / Pseudomethoca sp. ♀ / det. D.G. Manley 20*03*. [(**MEXICO: Sinaloa:** El Palmito, 5 mi. W, [23.562°N 105.916°W], 6000 ft., 13.Oct.1975, J. Powell, J. Chemsak, T. Eichlin, & T. Friedlander (1♀–0000003–DGMC))]

10. *Invreiella chihuahuensis* Waldren, sp. nov.

(Figs 5.18, 5.24, 5.28, 5.30, 5.74, 5.90, 5.105, 5.121, 5.140, 5.142)

Diagnosis (female). This species is distinguished from other members of the *I. satrapa* species-group by the following combination of characters: clypeus elongate, extending well-beyond base of mandibular socket (figs 5.24, 5.90), with medioapical tubercles basally conjoined and dorsoventrally triangular in shape, genal process spinose and curved (figs 5.28, 5.74), pronotal carina not strongly lobate posteriorly and not projecting away from mesosoma, not obscuring dorsal portion of pronotal-mesopleural suture (figs 5.30, 5.105, 5.121), and extreme apical margin of T2 with dark red-brown integument fringed medially with black setae (figs 5.18, 5.140).

Description (female). Body length 8.0–12.45 mm.

Head: Head 1.08–1.18 × as wide as mesosoma. Vertex and frons contiguously punctate. Antennal scrobe carina weakly arcuate, slightly more prominent internally, with inner tip of carina well-separated from antennal rim. Frons weakly transversely recessed and concave below antennal scrobe carina, antennal rim consequently slightly recessed and slightly visible when head viewed laterally. Antennal rim apically glabrous, rounded and not tuberculate. F1 1.88–2.3 × as long as F2. Clypeus plate-like and medially flattened, coarsely rugose-granulate, with central cluster of long raised setae, apical margin medially elongate and coming to distinct apex, with two close medioapical tubercles that are anterad-projecting, dorsoventrally triangular in shape, and basally conjoined. Mandible acuminate. Distance from posterior margin of eye to posterolateral corner of head 1.48–2.13 × maximum diameter of eye. Genal process spinose and curved, posterior genal carina nearly straight. Gena with separate yet close moderate punctures laterally, interpunctal space smooth. Postgena transversely rugose-striate.

Mesosoma: Mesosoma 1.18–1.26 × as wide as long. Dorsum and posterior face of mesosoma densely, contiguously punctate, surfaces coarse. Anterodorsolateral margin of pronotum outcurved. Pronotal carina prominent, glabrous, visible both dorsally and laterally, not strongly lobate posteriorly and not projecting away from mesosoma, not overlapping and obscuring dorsal portion of pronotal-mesopleural suture. Tubercle anterior of propodeal spiracle present. Width of propodeum greater than distance between propodeal spiracles in dorsal view. Lateral face of pronotum weakly rugose-striate, with scattered moderate punctures, lateral face posteriorly carinate along pronotal-mesopleural suture. Mesopleuron coarsely microgranulate to granulate anteriorly, with few punctures, posteriorly striate-rugose. Mesopleuron with vertical column of punctures weakly tuberculate, not forming anterior or posterior carinae. Mesopleuron posteriorly carinate along ventral two-thirds of mesopleural-metapleural suture, dorsal portion of suture obscure, faintly present. Metapleuron with ventral one-third striate-rugose with scattered punctures, dorsal two-thirds glabrous. Metapleural-propodeal suture with striae perpendicularly overlapping suture along ventral one-third. Lateral face of propodeum posteriorly with ventral one-third to one-half striate-rugose with few large punctures, anteriorly with dorsal one-half to two-thirds glabrous. Coxae coarsely sculptured, with small to large, deep punctures.

Metasoma: T1–5 densely, contiguously punctate. Visible portion of pygidial plate not obscured by setal hood rugose-granulate. S2 densely punctate, punctures near contiguous in some areas, basolateral concave area less densely punctate, nearly glabrous between punctures. S3–6 densely punctate, coarsely microgranulate between punctures. Hypopygium with slightly arcuate transverse row of setae near apical margin, lateral setae longer than median setae.

Integument coloration: Orange, except the following ranging from dark red-brown to black: antenna, antennal rim, medioapical tubercles of clypeus, apical one-fourth to one-third of mandible, pleura, propodeum, legs, T1, extreme apical margins of T2 and S2, T3–6, and apical margins of S3–6.

Pubescence: Frons ventrally with orange setae. Frons dorsally and vertex (including posterolateral corner of head) covered with mostly decumbent orange-red setae, with few scattered fuscous setae. Clypeus with long fuscous and whitish setae medially. Mandible with dorsal and ventral longitudinal row of light orange setae. Remainder of head with whitish setae. Dorsum of mesosoma mostly covered with decumbent orange-red setae, dorsal edges (except pronotum) with few raised fuscous setae, particularly at top of posterior face of propodeum. Pronotum without black setae, rather with orange setae. Remainder of mesosoma including legs with whitish setae (except tarsi with dense orange bristles). Anterior face of T1 with pale and fuscous setae. T1 apically fringed with wide band of black setae medially, laterally fringed with whitish setae. Dorsum of T2 covered with orange-red setae. Felt line of T2 and surrounding lateral area with whitish setae. T2 apically fringed with moderately wide band of black setae medially, not coming to median point, laterally fringed with whitish setae. T3–5 mostly covered and apically fringed with whitish setae, with few scattered dark raised setae and apical fringe with median patch of black setae, these black setal patches mostly consistent in width between T3–5, with T5 black setal patch sometimes covering median section of sclerite. T6 with pale yellow-orange setae surrounding pygidial plate, partially obscuring basal half. Remainder of metasoma with whitish setae.

MALE. Unknown.

Etymology: The specific epithet is derived from the ecoregion this species is known to inhabit—the Chihuahuan Desert.

Distribution: Mexico (Chihuahua and Durango).

Biogeography: Nearctic region (Chihuahuan Desert province); Mexican transition zone (Sierra Madre Occidental province).

Host(s): Unknown.

Remarks: None.

Material examined (*I. chihuahuensis*, 4♀).

Holotype: ♀ (0000031–DGMC) [tarsomere #5 of left mesoleg missing], [label 1 (white):] *CD. JUAREZ CHIH / 25/Sep/85 / Roberto Huartos* [// label 2 (red):] HOLOTYPE ♀ / Invreiella chihuahuensis / Waldren, 2018 / GCW_HYM0000031 [// label 3 (yellow):] *Compared with / HOLOTYPE / M. macrocephala* [// label 4 (white):] *Invreiella / satrapa*. [(**MEXICO: Chihuahua:** Ciudad Juárez, [31.690°N 106.424°W], 25.Sep.1985, R. Huartos (1♀–0000031–DGMC))]

Paratype: MEXICO: Durango: Ciudad Lerdo, [25.544°N 103.526°W], H. Höge (1♀–0000057–NHMUK).

Additional specimens (non-types, examined): MEXICO: Chihuahua: San José Babícora, [29.252°N 107.750°W], 7100 ft., 05.Jul.1947 (1♀–MIUP); Km. 7 Creel Rd. [=Carretera a Creel], [28.024°N 107.597°W], V. den Berghe (1♀–MIUP).

11. *Invreiella satrapa* (Gerstaecker, 1874)

(Figs 5.5–5.8, 5.19, 5.29, 5.31, 5.63, 5.75, 5.91, 5.106, 5.122, 5.141, 5.142)

Mutilla satrapa Gerstaecker, 1874: 65 [lectotype ♀ (ZMHB)]. Dalla Torre 1897: 83.

-*Sphaerophthalma* [sic] *satrapa*: Cameron 1895: 364.

-*Ephuta* (*Ephuta*) *satrapa*: André 1902: 63.

-*Pseudomethoca satrapa*: Mickel 1937: 181; Mickel 1964: 168.

-*Invreiella satrapa*: Suárez 1966: 475; Nonveiller 1990: 41; Pagliano 2005: 272; Lelej & Brothers 2008: 29; Quintero & Cambra 2011: 219; Cambra *et al.* 2014: 296; Pagliano *et al.* 2020: 102.

Mutilla macrocephala Smith, 1879: 223 [holotype ♀ (NHMUK)]. Junior subjective synonym of *Pseudomethoca satrapa* (Gerstaecker, 1874) according to Mickel 1964: 168. **Synonym status confirmed.**

-*Sphaerophthalma* [sic] *macrocephala*: Blake 1886: 190, 256.

-*Ephuta* (*Ephuta*) *macrocephala*: André 1902: 61.

-?*Pseudomethoca macrocephala*: Mickel 1924: 46.

Mutilla ganahlii Dalla Torre, 1897: 42 [new name for *Mutilla macrocephala* Smith, 1879, *nec* Olivier, 1811]. Junior subjective synonym of *Pseudomethoca satrapa* (Gerstaecker, 1874) according to Mickel 1964: 168. **Synonym status confirmed.**

Diagnosis (female). This species is distinguished from other members of the *I. satrapa* species-group by the following combination of characters: clypeus elongate, extending well-beyond base of mandibular socket (*i.e.* fig. 5.24; fig. 5.91), with medioapical tubercles basally conjoined and dorsoventrally triangular in shape, genal process spinose and straight (figs 5.29, 5.75), pronotal carina strongly lobate posteriorly and projecting away from mesosoma, overlapping and obscuring dorsal portion of pronotal-mesopleural suture (figs 5.31, 5.106, 5.122), and extreme apical margin of T2 with dark orange integument (figs 5.19, 5.141).

Redescription (female). Body length 8.26–12.95 mm.

Head: Head 1.03–1.19 × as wide as mesosoma. Vertex and frons contiguously punctate. Antennal scrobe carina straight to weakly arcuate, slightly more prominent internally, with inner tip of carina well-separated from antennal rim. Frons ranging from weakly to moderately transversely recessed and concave below antennal scrobe carina, antennal rim consequently slightly recessed and slightly visible to recessed and not visible when head viewed laterally. Antennal rim apically glabrous, rounded and not tuberculate. F1 1.67–2.13 × as long as F2. Clypeus plate-like and medially flattened, coarsely rugose-granulate, with central cluster of long raised setae, apical margin medially elongate and coming to distinct apex, with two close medioapical tubercles that are anterad-projecting, dorsoventrally triangular in shape, and basally conjoined. Mandible acuminate. Distance from posterior margin of eye to posterolateral corner of head 1.37–1.9 × maximum diameter of eye. Genal process spinose and straight, posterior genal carina nearly straight. Gena with separate yet close moderate punctures laterally, interpunctal space smooth. Postgena transversely rugose-striate.

Mesosoma: Mesosoma 1.2–1.42 × as wide as long. Dorsum and posterior face of mesosoma densely, contiguously punctate, surfaces coarse. Anterodorsolateral margin of pronotum outcurved. Pronotal carina prominent, glabrous, visible both dorsally and laterally, strongly lobate posteriorly and projecting away from mesosoma, overlapping and obscuring dorsal portion of pronotal-mesopleural suture. Tubercle anterior of propodeal spiracle present. Width of propodeum greater than distance between propodeal spiracles in dorsal view. Lateral face of pronotum rugose-striate, with scattered moderate punctures, lateral face posteriorly carinate along pronotal-mesopleural suture in addition to aforementioned lobate structure of pronotal carina. Mesopleuron microgranulate to granulate anteriorly, with few punctures, posteriorly striate-rugose and weakly glabrous. Mesopleuron with vertical column of punctures weakly tuberculate, not forming anterior or posterior carinae. Mesopleuron posteriorly carinate along ventral one-half to two-thirds of mesopleural-metapleural suture, dorsal portion of suture obscure, faintly present. Metapleuron with ventral one-third to one-half striate-rugose with scattered punctures, dorsal one-third to one-half microgranulate and shining. Metapleural-propodeal suture with striae perpendicularly overlapping suture along ventral one-third to one-half. Lateral face of propodeum posteriorly with ventral one-third to one-half striate-rugose with scattered punctures, anteriorly with dorsal one-third to one-half microgranulate and shining. Coxae coarsely sculptured, with small to large, deep punctures.

Metasoma: T1–5 densely, contiguously punctate. Visible portion of pygidial plate not obscured by setal hood transversely rugose to rugose-granulate. S2 densely punctate, punctures near contiguous in some areas, basolateral concave area less densely punctate, nearly glabrous between punctures. S3–6 densely punctate, coarsely microgranulate between punctures. Hypopygium with slightly arcuate transverse row of setae near apical margin, lateral setae longer than median setae.

Integument coloration: Orange, except the following ranging from dark orange to black: antenna, antennal rim, medioapical tubercles of clypeus, apical one-third to near entirety of mandible, legs, T1, extreme apical margins of T2 and S2, T3–6, and apical margins of S3–6; pleura, coxae, and S1 vary between orange and dark red-brown; posterior and lateral faces of propodeum vary between orange and nearly black.

Pubescence: Frons ventrally with pale to light orange setae. Frons dorsally and vertex (including posterolateral corner of head) covered with mostly decumbent orange-red setae, with few scattered fuscous setae. Clypeus with long pale or fuscous setae medially. Mandible with dorsal and ventral longitudinal row of light orange setae. Remainder of head with whitish setae. Dorsum of mesosoma mostly covered with decumbent orange-red setae, dorsal edges (except pronotum) with sparse raised fuscous setae, particularly at top of posterior face of propodeum. Pronotum without black setae, rather with orange setae. Remainder of mesosoma including legs with whitish setae (except tarsi with dense orange bristles). Anterior face of T1 with whitish setae. T1 apically fringed with wide band of black setae medially, laterally fringed with whitish setae. Dorsum of T2 covered with orange-red setae. Felt line of T2 and surrounding lateral area with whitish setae. T2 apically fringed with moderately wide band of black setae medially, not coming to median point, laterally fringed with whitish setae. T3–5 mostly covered and apically fringed with whitish setae, with few scattered dark raised setae and apical fringe with median patch of black setae, these black setal patches mostly consistent in width between T3–5, with T5 black setal patch usually covering median section of sclerite. T6 with pale yellow-orange setae surrounding pygidial plate, partially obscuring basal half. Remainder of metasoma with whitish setae.

MALE. Unknown.

Etymology: The specific epithet is likely derived from the Greek *satrápēs*, meaning governor of a province.

Distribution: Mexico (Coahuila, Durango, Guanajuato, Hidalgo, Mexico, Michoacan, San Luis Potosi, Veracruz, and Zacatecas).

Biogeography: Nearctic region (Chihuahuan Desert province); Mexican transition zone (Sierra Madre del Sur province; Sierra Madre Occidental province; Sierra Madre Oriental province, Transmexican Volcanic Belt province).

Host(s): Unknown.

Remarks: Gerstaecker (1874) based his description of *M. satrapa* on at least two specimens, as he provided a length range of “11–14 mill.” Upon examining the type of *M. satrapa*, it was observed that C. Mickel designated it as a lectotype, which he never explicitly published. Two additional syntypes were discovered at ZMHB (L. Kirschey, pers. comm.). In Mickel (1964), implicit reference is made to the location of the type of *M. satrapa*, and the word ‘type’ is missing from the treatment of this species. According to the ICZN (1999), article 74.5: Lectotype designations before 2000: “In a lectotype designation made before 2000, either the term "lectotype," or an exact translation or equivalent expression (*e.g.* "the type"), must have been used or the author must have unambiguously selected a particular syntype to act as the unique name-bearing type of the taxon." Consequently, Mickel's unpublished lectotype designation for *M. satrapa* is here validated. There is some natural wear to the lectotype of *I. satrapa*: the apices of the mandibles and medioapical tubercles of the clypeus are worn down. This gives the impression that the clypeal tubercles are dorsoventrally semi-circular in shape, rather than triangular, which is not the case for this species. Additionally, the apices of both genal processes are broken off. Among the specimens of *I. satrapa* that have been examined, minor asymmetry in the shape of the genal carinae and processes has been observed to varying degrees.

The holotype specimen of *Mutilla macrocephala* Smith, 1879 and its collection data are noteworthy and are here expanded upon. Smith (1879) noted the distribution of this species as "Orizaba," a city in Veracruz, Mexico; interestingly, the only locality included on the holotype's label data is "Mex." Orizaba is a common locality among Hymenoptera that F. Smith had described and we maintain this locality here despite the type not explicity being labeled as such. Additionally, the holotype of *M. macrocephala* is unusual in that its frons is more transversely recessed and concave below the scrobe carinae than most other specimens of *I. satrapa*.

Lastly, there are two records of this species being collected on plants. One female was collected on *Bidens pilosa* Linnaeus (beggar-ticks/Spanish needle) and another on a pepper tree (both SEMC).

Material examined (*I. satrapa*, 28♀).

Lectotype (*Mutilla satrapa* Gerstaecker) (designated by C.E. Mickel (unpublished), here designated) (examined, figs 5.5–5.8): ♀ (ZMHB) [apices of both genal processes broken off; entire right proleg missing], [label 1 (green):] Mexico / Ehrenberg S. [// label 2 (tan):] 6585 [// label 3 (red):] Type [// label 4 (red):] Lectotypus / *C.E. Mickel* [// label 5 (tan):] Zool. Mus. / Berlin [// label 6 (white):] Mutilla satrapa. [(**MEXICO: Unknown:** Ehrenberg S. (1♀–ZMHB))]

Syntypes (*Mutilla satrapa* Gerstaecker) (examined via photographs, consequently not designated as paralectotypes): MEXICO: Unknown: Ehrenberg S. (2♀–ZMHB).

Holotype (*Mutilla macrocephala* Smith) (examined): ♀ (NHMUK) [F2–10 of left antenna missing; entire right antenna missing; most of femur, entire tibia, and tarsus of left proleg missing; tarsus missing of left mesoleg missing; tarsomere #5 of left metaleg missing; tarsus of right proleg missing; tarsomeres #4–5 of right mesoleg missing; tarsomere #5 of right metaleg missing], [label 1 (circular, light blue, obverse):] *Mex* [label 1 (reverse):] *65 / 31* [// label 2 (tan):] *Mutilla / macrocephala / (Type) Sm.* [// label 3 (circular, tan center with red outline):] Type [// label 4 (tan, folded, inner side):] *Smith's description of / macrocephala does / not correspond with / the type specimen / The latter is con- / -specific with jocularis, Cameron / C.L.B.* [label 4 (outer side):] *This is not / correct. / C.E. Mickel / 1930* [// label 5 (tan):] B.M. TYPE / HYM. / *15.1043.* [(**MEXICO: Veracruz: [**Orizaba], [18.850°N 97.103°W] (1♀–NHMUK))]

Additional specimens (non-types, examined or referenced*): MEXICO: Coahuila: Saltillo, [25.430°N 100.977°W], Gribodo, MNCN_Ent 171986 (1♀–MNCN*). **Durango:** Victoria de Durango, 31.7 mi. N, [24.403°N 104.696°W], 22.Jul.1982, A.J. Gilbert (1♀–0006578–CSCA). **Guanajuato:** Roque, [20.581°N 100.838°W], 31.Oct.1964, H. Velasco, “HOSP. plantas silvestres” (1♀–0000045–EMUS); San José de Allende, [20.856°N 101.033°W], oak forest, 9000 ft., 10.Aug.1988, G.B. Edwards (1♀–0000047–FSCA). **Hidalgo:** Atotonilco El Grande, Sanctórum, [20.301°N 98.777°W], 12.Sep.1992, L. Godinez, “#735, ex. *Bidens pilosa* L.” (1♀–SEMC); Pachuca, [20.104°N 98.763°W], 7900 ft., 28.Jul.1954, University of Kansas Mexico Expedition (1♀–DJBC*); Pachuca, 4 mi. W, [20.128°N 98.838°W], 7900 ft., 16.Jun.1961, University of Kansas Mexico Expedition (1♀–DJBC*). **México:** Mexico City, [19.432°N 99.133°W], H. Höge (1♀–0000040–UMSP; 1♀–0000058–NHMUK); Mexico City, Mixcoac, [19.372°N 99.190°W], H.F. Wickham (1♀–0000041–UMSP); Tepexpan, [19.614°N 98.936°W], 26.Jul.1963, F.D. Parker & L.A. Stange (1♀–0000046–EMUS; 1♀–0006577–UCDC); Venta de Carpio, 0.6 mi. SE, [19.615°N 98.999°W], 7800 ft., 06.Aug.1972, R.R. & M.E. Murray, “ME-68” (1♀–0000043–TAMU; 1♀–0000044–GCWC); Zoquiapan [National Park], [19.383°N 98.708°W], 7000 ft., 14.Jul.1953, University of Kansas Mexico Expedition, “taken on pepper tree” (1♀–SEMC). **Michoacán:** Tzintzunzán, 1 km S, [19.616°N 101.576°W], 03.Nov.1992, F.A. Noguera & A. Rodríguez (1♀–0000042–EBCC). **San Luis Potosí:** [San Luis Potosí], [22.156°N 100.985°W], E. Palmer (2♀–0000059, 0007210–NHMUK); San Luis Potosí, 20 mi. SW, [22.009°N 101.247°W], 6800 ft., 25.Jul.1962, University of Kansas Mexico Expedition (1♀–DJBC*; 1♀–SEMC); San Luis Potosí, 29 mi. SW, [21.929°N 101.358°W], 6800 ft., 25.Jul.1962, University of Kansas Mexico Expedition (1♀–DJBC*). **Unknown:** (1♀–0000050–NHMD). **Veracruz:** Perote, 10 mi. SW, [19.484°N 97.364°W], 27.Jul.1974, Clark, Murray, Ashe, & Schaffner (1♀–0000049–DGMC).

Zacatecas: Fresnillo, 9 mi. S, [23.040°N 102.874°W], 20.Aug.1956, D.D. Linsdale, EMEC 1157454 (1♀–0000048–EMEC).

***Invreiella suarezi* species-group**

(Fig. 5.146)

Members of this female-based species-group are recognized by the following combination of character states:

1. Antennal scrobe carina position: inner tip well-separated from antennal rim.
2. Frons and antennal rim position: frons transversely recessed and concave below antennal scrobe carina, antennal rim consequently recessed and not visible when head viewed laterally.
3. Antennal rim: rounded.
4. Clypeus: concave, recessed, with transverse arcuate carina complete, with small lateral tubercle ventrad to carina.
5. Genal process: triangular, posterior genal carina weakly sinuate.
6. Pronotal carina: absent, with cluster of dense contiguous punctures in its place, edges of punctures may be tuberculate and crenulate, simulating weak carina.
7. Lateral face of pronotum sculpture: rugose-striate, with moderate punctures amid the striae.
8. Vertical column of punctures of mesopleuron: puncture edges tuberculate, forming crenulate anterior and posterior carinae.

9. Mesopleuron, metapleuron, and lateral face of propodeum sculpture, where present: microgranulate to striate-rugose, with micropunctures and few moderate punctures.

Species included: *I. erythrocephala* Waldren, **sp. nov.**, *I. suarezi* Waldren, **sp. nov.**, and *I. tequila* Waldren, **sp. nov.**

Distribution: Mexico (Guerrero, Jalisco, and Morelos).

Biogeography: Mexican transition zone (Sierra Madre del Sur province, Sierra Madre Occidental province); Neotropical region (Balsas Basin province).

Remarks: The transverse recession of the frons below the antennal scrobe carinae is noteworthy for this species-group, which results in the antennal rims being recessed and essentially not visible when the head is viewed laterally; the clypeus is consequently also recessed. Members of the *I. satrapa* species-group also have this same transverse recession below the antennal scrobe carinae to varying degrees. Further, it is also observed in some unrelated Pseudomethocini, such as several undescribed *Pseudomethoca* (*sensu lato*) species, wherein the frons at or dorsad the antennal scrobe carinae is swollen, resulting in recessed antennal rims. The recessed antennal rim condition is manifested in its most extreme form in members of *Horcomutilla* Casal, 1962 where the frons at the antennal scrobe carinae is swollen and anterad-projecting to varying degrees, with the antennal rims consequently being highly recessed.

12. *Invreiella erythrocephala* Waldren, sp. nov.

(Figs 5.20, 5.62, 5.76, 5.92, 5.107, 5.123, 5.143, 5.146)

Diagnosis (female). This species is distinguished from other members of the *I. suarezi*

143
I. erythrocephala
(0000034)
●

144
I. suarezi
(0000051)
▲

145
I. tequila
(0000053)
■

FIGURES 5.143–5.146. Female habitus, dorsal view: *I. suarezi* species-group. Scale bars = 2 mm. **5.146**–Geographic distribution of the *I. suarezi* species-group.

species-group by the following combination of characters: vertex, mesosoma, and T2 covered mostly with decumbent orange-red setae (figs 5.20, 5.143), T2 without a distinct dorsal setal pattern (figs 5.20, 5.143), and the vertical column of punctures of the mesopleuron are weakly tuberculate anteriorly and prominently tuberculate posteriorly, forming a single, crenulate carina (*i.e.* fig. 5.57).

Description (female). Body length 11.18 mm.

Head: Head 1.13 × as wide as mesosoma. Vertex and frons contiguously punctate. Antennal scrobe carina arcuate, with inner tip well-separated from antennal rim. Frons strongly transversely recessed and concave below antennal scrobe carina, antennal rim consequently recessed and not visible when head viewed laterally. Antennal rim apically glabrous, rounded and not tuberculate. F1 2.2 × as long as F2. Clypeus concave, recessed, with transverse arcuate carina complete, with small lateral tubercle ventrad to carina. Mandible acuminate. Distance from posterior margin of eye to posterolateral corner of head 1.6 × maximum diameter of eye. Genal process triangular, posterior genal carina weakly sinuate. Gena densely punctate laterally, punctures close, interpunctal space smooth. Postgena transversely rugose-striate.

Mesosoma: Mesosoma 1.3 × as wide as long. Dorsum and posterior face of mesosoma densely, contiguously punctate, surfaces coarse. Anterodorsolateral margin of pronotum outcurved. Pronotal carina absent, with cluster of dense, contiguous, crenulate punctures in its place, simulating weak carina. Tubercle anterior of propodeal spiracle present. Width of propodeum greater than distance between propodeal spiracles in dorsal view. Lateral face of pronotum rugose-striate, interspersed with moderate punctures, lateral face posteriorly carinate along pronotal-mesopleural suture. Mesopleuron mostly covered with micropunctures, interpunctal space smooth, weakly rugose-striate posteriorly dorsad middle coxa. Mesopleuron with vertical column of punctures tuberculate at edges, forming weak anterior and strong posterior crenulate carinae. Mesopleuron posteriorly carinate along ventral half of mesopleural-metapleural suture, carina diverging from suture roughly at midpoint and merging with dorsal carina formed by posterior tuberculate edges of vertical row of punctures, carina terminating shortly before dorsolateral margin, dorsal portion of suture obscure, faintly present at groove halfway to dorsolateral tubercle and absent remaining distance to tubercle. Metapleuron with dorsal half mostly glabrous, ventral half with some micropunctures and moderate punctures. Metapleural-propodeal suture with rugae perpendicularly overlapping suture along ventral one-fourth. Lateral face of propodeum mostly micropunctate throughout, punctures well-separated, with moderate punctures posteriorly. Coxae coarsely sculptured, with small to moderate punctures.

Metasoma: T1–5 densely, contiguously punctate. Visible portion of pygidial plate not obscured by setal hood rugose-granulate. S2 densely punctate, punctures near contiguous in some areas, basolateral concave area less densely punctate, nearly glabrous between punctures. S3–6 densely punctate, coarsely microgranulate between punctures. Hypopygium with slightly arcuate transverse row of setae near apical margin, lateral setae longer than median setae.

Integument coloration: Orange, except the following ranging from dark brown-red to black: antenna, antennal rim, base of clypeus, apex of mandible, lateral and posterior face of propodeum, legs excluding most of femora and coxae, and metasoma (except T2 mostly covered with two large coalescing orange maculae).

Pubescence: Frons ventrally with whitish setae, medially with few fuscous setae. Frons dorsally and vertex with mostly decumbent and some raised orange-red setae. Posterolateral corner of head with scattered raised fuscous setae. Mandible with dorsal and ventral longitudinal row of light orange setae. Remainder of head with whitish setae. Dorsum of mesosoma mostly covered with decumbent orange-red setae, dorsolateral edges with sparse raised fuscous setae. Pronotum with dorsal transverse band of black setae, except area between epaulet and pronotal spiracle with few whitish setae. Dorsum of propodeum with inconspicuous longitudinal row of black setae. Remainder of mesosoma including legs with whitish setae (except tarsi with orange bristles). Anterior face of T1 with whitish setae. T1 apically fringed with wide median band of black setae, laterally fringed with whitish setae. T2 with decumbent orange setae overlapping large coalescing orange integumental maculae, remainder of dorsum of T2 with red-brown to black setae surrounding maculae, with asetose punctate patch of integument present just posterior to middle of maculae, surrounded by red-brown setae. Felt line of T2 and surrounding lateral area with whitish setae. T2 apically fringed with narrow median patch of dark red-brown setae, laterally fringed with whitish setae. T3 covered and fringed with whitish setae, medially with few red-brown setae. T4 covered and fringed with red-brown setae medially, with whitish setae laterally. T5 mostly covered and fringed with black setae medially, with whitish setae laterally. T6 with dark orange setae surrounding pygidial plate, partially obscuring basal half. Remainder of metasoma with whitish setae.

MALE. Unknown.

Etymology: The specific epithet is derived from the Greek *erythraios* and *kephale* in reference to the reddish head that helps distinguish this species from the other two members of the *I. suarezi* species-group.

Distribution: Mexico (Morelos).

Biogeography: Neotropical region (Balsas Basin province).

Host(s): Unknown.

Remarks: This species is known only from the holotype. The collection locality for this specimen of "Puebla: 11km NW. of Tepexco" actually places it just within the state boundary of Morelos.

Material examined (*I. erythrocephala*, 1♀).

Holotype: ♀ (0000034–CASC) [right proleg, except procoxa, missing], [label 1 (white):] MEXICO: Puebla: 11km / NW. Tepexco, 1280m / 1-XI-1976 / Edward S. Ross / Cal.Acad.Sci.Coll. [// label 2 (yellow):] *PF071* [// label 3 (yellow):] CAS [// label 4 (red):] HOLOTYPE ♀ / Invreiella erythrocephala / Waldren, 2018 / GCW_HYM0000034 [// label 5 (white):] *Invreiella* / *cardinalis* / *(Gerstaecker) 1874* / Det K.A. Williams 2012. [(**MEXICO: Morelos:** Tepexco (Puebla), 11 km NW, [18.704°N 98.771°W], 1280 m, 01.Nov.1976, E.S. Ross (1♀–0000034–CASC))]

13. *Invreiella suarezi* Waldren, sp. nov.

(Figs 5.21, 5.57, 5.60, 5.77, 5.93, 5.108, 5.124, 5.144, 5.146)

Diagnosis (female). This species is distinguished from other members of the *I. suarezi* species-group by the following combination of characters: vertex covered with decumbent light yellow setae (figs 5.21, 5.144), T2 with patch of light yellow setae roughly in the shape of an exaggerated “W” overlapping slightly lighter integument of the same “W” shape (figs 5.21, 5.144), and the vertical column of punctures of the mesopleuron are weakly tuberculate anteriorly and strongly tuberculate posteriorly, forming a single, crenulate carina (fig. 5.57).

Description (female). Body length 8.89–9.91 mm.

Head: Head 1.11–1.13 × as wide as mesosoma. Vertex and frons contiguously punctate. Antennal scrobe carina arcuate, with inner tip of carina well-separated from antennal rim.Frons transversely recessed and concave below antennal scrobe carina, antennal rim consequently recessed and barely visible when head viewed laterally. Antennal rim mostly apically glabrous, rounded and not tuberculate. F1 2.06–2.44 × as long as F2. Clypeus concave, recessed, with transverse arcuate carina complete, with small lateral tubercle ventrad to carina. Mandible acuminate. Distance from posterior margin of eye to posterolateral corner of head 1.11–1.27 × maximum diameter of eye. Genal process triangular, posterior genal carina weakly sinuate. Gena densely punctate laterally, punctures close, interpunctal space weakly microgranulate to smooth. Postgena transversely rugose-striate, with punctures between striae.

Mesosoma: Mesosoma 1.26–1.28 × as wide as long. Dorsum and posterior face of mesosoma densely, contiguously punctate, surfaces coarse. Anterodorsolateral margin of pronotum outcurved. Pronotal carina absent, with cluster of dense, contiguous, crenulate punctures in its place, simulating weak carina. Tubercle anterior of propodeal spiracle present. Width of propodeum greater than distance between propodeal spiracles in dorsal view. Lateral face of pronotum rugose-striate, interspersed with moderate punctures, lateral face posteriorly carinate along pronotal-mesopleural suture. Mesopleuron microgranulate with micropunctures mostly throughout, posteriorly rugose-striate dorsad mesocoxa. Mesopleuron with vertical column of punctures tuberculate at edges, forming weak anterior and strong posterior crenulate carinae. Mesopleuron posteriorly carinate along ventral half of mesopleural-metapleural suture, carina diverging from suture roughly at midpoint and merging with dorsal carina formed by posterior tuberculate edges of vertical row of punctures, carina terminating shortly before dorsolateral margin, dorsal portion of suture faintly present as groove. Metapleuron microgranulate to smooth in areas, weakly rugose-striate dorsad metacoxa with few moderate punctures. Metapleural-propodeal suture with weak rugae perpendicularly overlapping suture along ventral one-fifth. Lateral face of propodeum mostly lightly micropunctate throughout, punctures well-separated, with moderate punctures posteriorly. Coxae coarsely sculptured, with small to moderate punctures.

Metasoma: T1–5 densely, contiguously punctate. Visible portion of pygidial plate not obscured by setal hood rugose-granulate. S2 densely punctate, punctures near contiguous in some areas, basolateral concave area less densely punctate, nearly glabrous between punctures. S3–6 densely punctate, coarsely microgranulate between punctures. Hypopygium with transverse row of setae near apical margin, lateral setae longer than median setae.

Integument coloration: Dull red-brown, except the following ranging from very dark brown-red to black: antennal rim, antenna and most or all flagellomeres, apex of mandible, pygidial plate, and apical portion of hypopygium; T2 with slightly lighter integumental pattern, corresponding with setal pattern that is roughly in shape of exaggerated “W.”

Pubescence: Frons ventrally with whitish setae, medially with few fuscous setae. Frons dorsally and vertex covered with mostly decumbent light yellow setae. Posterolateral corner of head with triangular patch of mostly decumbent black setae. Mandible with dorsal and ventral longitudinal row of light orange setae. Remainder of head with whitish setae. Dorsum of mesosoma covered with light yellow setae, with prominent ring of black setae that begins at dorsum of pronotum. Dorsal edges of mesosoma excluding most of pronotum with light yellow setae. Remainder of mesosoma including legs with whitish setae (except tarsi with orange bristles). Anterior face of T1 with whitish setae. T1 apically fringed with wide median band of black setae, laterally fringed with whitish setae. T2 medially with patch of light yellow to orange setae roughly in shape of exaggerated “W” overlapping lighter integument, tip of outer arm nearly reaching apical margin of T1, remainder of dorsum of segment with black setae. Felt line of T2 and surrounding area with whitish setae. T2 apically fringed with narrow patch of black setae medially, mostly fringed with whitish setae. T3 covered and fringed with whitish setae, medially with very few black setae. T4 similar to T3 except with more black setae medially. T5 mostly covered and fringed with black setae medially, laterally covered and fringed with whitish setae. T6 with pale orange setae surrounding pygidial plate, partially obscuring basal half. Remainder of metasoma with whitish setae.

MALE. Unknown.

Etymology: This species in named in honor of Dr. Francisco J. Suárez (1926–1985), author of the genus *Invreiella*.

Distribution: Mexico (Guerrero).

Biogeography: Mexican transition zone (Sierra Madre del Sur province); Neotropical region (Balsas Basin province).

Host(s): Unknown.

Remarks: This species is only known from two specimens collected in the Mexican state of Guerrero.

Material examined (*I. suarezi*, 2♀).

Holotype: ♀ (0000051–UNAM), [label 1 (white):] México: Guerrero / Atlixtac Km. 39 Chilapa- / Tlapa 1425msnm / 17°35’47” N 99°01’06” W / 11-XII-2006 / L. Cervantes C. Mayorga [// label 2 (white):] Colección del Instituto / de Biología UNAM. / México, D. F. [// label 3 (red):] HOLOTYPE ♀ / Invreiella suarezi / Waldren, 2018 / GCW_HYM0000051 [// label 4 (white):] *Invreiella* / *sp. nov.* / Det K.A. Williams 2012. [(**MEXICO: Guerrero:** Atlixtac Km 39 Chilapa-Tlapa, 17.59639°N 99.018333°W, 1425 m, 11.Dec.2006, L.C.C. Mayorga (1♀–0000051–UNAM))]

Paratype: MEXICO: Guerrero: San Agustín Oapan, 3 km N, 17.998056°N 99.459167°W, 2500 ft., 11.Dec.2007, J. Amith & P. Pantaleón, JDA #00382 (1♀–0000052–EMUS).

14. *Invreiella tequila* Waldren, sp. nov.

(Figs 5.22, 5.43, 5.48, 5.50, 5.56, 5.58, 5.59, 5.78, 5.94, 5.109, 5.125, 5.145, 5.146)

Diagnosis (female). This species is distinguished from other members of the *I. suarezi* species-group by the following combination of characters: frons and part of vertex with a large central patch of black setae surrounded by decumbent coppery-orange setae on the vertex (figs 5.22, 5.145), T2 with patch of light yellow setae roughly in the shape of an exaggerated “W” overlapping slightly lighter integument of the same “W” shape (figs 5.22, 5.145), and the vertical column of punctures of the mesopleuron are strongly tuberculate both anteriorly and posteriorly, forming parallel, crenulate carinae bounding the punctures (fig. 5.58). Lastly, it is distinguished from all other *Invreiella* by its medioapically expanded mandible, with the dorsal carina preceding the middle tooth and the ventral carina both expanded (fig. 5.59).

Description (female). Body length 11.94–12.57 mm.

Head: Head 1.04–1.07 × as wide as mesosoma. Vertex and frons contiguously punctate. Antennal scrobe carina arcuate, with inner tip of carina well-separated from antennal rim. Frons transversely recessed and concave below antennal scrobe carina, antennal rim consequently recessed and barely visible when head viewed laterally. Antennal rim apically glabrous, rounded and not tuberculate. F1 2.14–2.39 × as long as F2. Clypeus concave, recessed, with transverse arcuate carina complete, with small lateral tubercle ventrad to carina. Mandible medioapically expanded in width, with both dorsal carina preceding middle tooth and ventral carina expanded. Distance from posterior margin of eye to posterolateral corner of head 1.44–1.65 × maximum diameter of eye. Genal process triangular, posterior genal carina weakly sinuate. Gena densely punctate laterally, punctures close, nearly contiguous, interpunctal space smooth. Postgena transversely rugose-striate, with punctures between striae.

Mesosoma: Mesosoma 1.22–1.29 × as wide as long. Dorsum and posterior face of mesosoma densely, contiguously punctate, surfaces coarse. Anterodorsolateral margin of pronotum outcurved. Pronotal carina absent, with cluster of dense, contiguous, crenulate punctures in its place, simulating weak carina. Tubercle anterior of propodeal spiracle obscure. Width of propodeum greater than distance between propodeal spiracles in dorsal view. Lateral face of pronotum rugose-striate, interspersed with moderate punctures, microgranulate, lateral face posteriorly carinate along pronotal-mesopleural suture. Mesopleuron coarsely microgranulate, with scattered small punctures, posteriorly rugose-striate dorsad mesocoxa. Mesopleuron with vertical column of punctures strongly tuberculate anteriorly and posteriorly, forming parallel, crenulate carinae bounding the punctures. Mesopleuron posteriorly carinate along ventral half of mesopleural-metapleural suture, carina diverging from suture roughly at midpoint and near seamlessly merging with dorsal carina formed by posterior tuberculate edges of vertical row of punctures, carina continuing to and terminating at dorsolateral margin of mesosoma, dorsal portion of suture present, although weakly, and terminating at tubercle anterior to propodeal spiracle. Metapleuron with dorsal half obscurely microgranulate with few micropunctures, ventral half micropunctate, rugose-striate dorsad metacoxa with few moderate punctures. Metapleural-propodeal suture with rugae perpendicularly overlapping suture along ventral one-third. Lateral face of propodeum posteriorly with ventral one-third striate-rugose with few moderate punctures, anteriorly with dorsal two-thirds microgranulate with micropunctures and few moderate punctures. Coxae coarsely sculptured, with small to moderate punctures.

Metasoma: T1–5 densely, contiguously punctate. Visible portion of pygidial plate not obscured by setal hood transversely rugose to rugose-granulate. S2 densely punctate, punctures near contiguous in some areas, basolateral concave area less densely punctate, nearly glabrous between punctures. S3–6 densely punctate, coarsely micropunctate between punctures. Hypopygium with slightly arcuate transverse row of setae near apical margin, lateral setae longer than median setae.

Integument coloration: Dull red-brown, except the following ranging from very dark brown-red to black: antennal rim, antenna, apex of mandible, pygidial plate, and apical portion of hypopygium; T2 with slightly lighter integumental pattern, corresponding with setal pattern that is roughly in shape of exaggerated “W.”

Pubescence: Frons ventrally with whitish setae, medially with few fuscous setae. Frons dorsally and part of vertex with large central patch of black setae, surrounded by coppery-orange setae on the vertex. Posterolateral corner of head with triangular patch of mostly decumbent black setae. Mandible with dorsal and ventral longitudinal row of light orange setae. Remainder of head with whitish setae. Dorsum of mesosoma mostly covered with black setae, with median patch of light yellow setae. Area between epaulet and pronotal spiracle with light yellow setae. Dorsal face of propodeum laterally with light yellow setae. Remainder of mesosoma including legs with whitish setae (except tarsi with orange bristles). Anterior face of T1 with whitish setae. T1 apically fringed with wide median band of black setae, laterally fringed with whitish setae. T2 medially with patch of light yellow setae roughly in shape of exaggerated “W” overlapping lighter integument, tip of outer arm nearly reaching apical margin of T1, remainder of dorsum of segment covered with black setae. Felt line of T2 and surrounding lateral area with whitish setae. T2–4 apically fringed with moderately wide median patch of black setae, laterally fringed with whitish setae. T5 mostly covered and fringed with black setae medially, laterally covered and fringed with whitish setae. T6 with pale orange to yellow setae surrounding pygidial plate, partially obscuring basal half. Remainder of metasoma with whitish setae.

MALE. Unknown.

Etymology: The specific epithet is derived from the type locality of Tequila, Jalisco, Mexico.

Distribution: Mexico (Jalisco and Morelos).

Biogeography: Mexican transition zone (Sierra Madre Occidental province); Neotropical region (Balsas Basin province).

Host(s): Unknown.

Remarks: This species is only known from the Mexican states of Jalisco and Morelos, with two of the four known specimens collected in and around the city of Tequila, Jalisco.

Material examined (*I. tequila*, 4♀).

Holotype: ♀ (0000053–FSCA), [label 1 (white):] MEXICO,Jalisco / Tequila / Sept 27, 1991 / E. Giesbert, coll. [// label 2 (red):] HOLOTYPE ♀ / Invreiella tequila / Waldren, 2018 / GCW_HYM0000053 [// label 3 (white):] *Invreaella* / *sp. nov.* / Det K.A. Williams 2012. [(**MEXICO: Jalisco:** Tequila, [20.881°N 103.832°W], 27.Sep.1991, E. Giesbert.)]

Paratypes: MEXICO: Jalisco: San Luis Soyatlán, [20.196°N 103.306°W], 03.Sep.1941, J. Marquis (1♀–0000055–CASC); Tequila, 7 mi. NW, [20.950°N 103.908°W], 17.Aug.1960 (1♀–0000054–AMNH).

Additional specimen (non-type, referenced*): MEXICO: Morelos: Yuatepec, 7.3 mi. S, [18.786°N 99.041°W], 5400 ft., 01.Aug.1969, G. Gordh (1♀–DJBC*).

Nomen dubium

15. *Invreiella megacantha* (Cockerell & Casad, 1894), stat. resurr.

Sphaerophthalma [sic] *megacantha* Cockerell & Casad, 1894: 294 [holotype ♀ (MADUG – lost)]. Cameron 1895: 365; Cockerell 1895: 60. Junior subjective synonym of *Pseudomethoca cardinalis* (Gerstaecker, 1874) according to Mickel 1964: 166.

-*Mutilla megacantha*: Dalla Torre 1897: 60.

-*Ephuta* (*Ephuta*) *megacantha*: André 1902: 61.

Cockerell & Casad (1894) described two new species of Mutillidae based on material collected in Guanajuato, Mexico by Alfredo Dugès: *Sphaerophthalma* [sic] *dugesii* Cockerell & Casad and *S. megacantha* Cockerell & Casad. Shortly after, two additional species, *S. prunotincta* Cockerell and *S. rufosuffusa* Cockerell & Casad, were also described from material collected by Dugès in Guanajuato (Cockerell 1895; Cockerell & Casad 1895). The holotypes of *S. dugesii*, *S. megacantha*, *S. prunotincta*, and *S. rufosuffusa* have been considered lost for some time (Mickel 1928, 1964; Suárez 1966; Manley & Pitts 2007). Despite not having located and examined the holotype of *S. megacantha*, Mickel (1964) synonymized this species under *Pseudomethoca cardinalis* (Gerstaecker).

We sought to locate these four lost holotypes with the hunch that Cockerell and Casad may have returned the material to Dugès in Guanajuato, Mexico. Mr. Néstor Quezadas Tapia at the Museo de Historia Natural Alfredo Dugès (MADUG) kindly searched for these four type specimens and provided photographs to confirm their identity. The type of *S. rufosuffusa* was discovered (label: "*Sphaeropthalma* / *rufosuffusa,* / *Type.*"), as well as a specimen of *S. dugesii* that is not labeled as a type but could potentially be it (label: "*Sphaerophthalma* / *Dugesi, Ckll* ♀."). A specimen of *S. prunotincta* was also found but it lacks labels; this could be the type based on its match with Cockerell's original description, the apparent age of the specimen, and the appearance of the pin. It is worth noting that André (1898) mentioned that he examined the type of *S. prunotincta* through A. Dugès. No specimens labeled as *S. megacantha* were located at MADUG.

Based on the original description of *S. megacantha* by Cockerell & Casad (1894), it is likely a member of *Invreiella*; however, there is not enough information to determine with certainty its species-level identity. Due to the unexpected diversity of *Invreiella* discovered during the course of this study, we here treat *I. megacantha*, **stat. resurr.**, as a *nomen dubium* with the hope that type material will eventually surface. It is possible that *I. megacantha* is indeed a synonym of *I. cardinalis*, as A. Dugès' brother, Eugenio Dugès, collected a female of *I. cardinalis* in Guanajuato; this specimen is housed at RBINS. As for the other three types, Manley & Pitts (2007) designated a neotype for *S. dugesii*, a species that is now considered a synonym of *Dasymutilla foxi* (Cockerell, 1894) (Pilgrim *et al*. 2008). The species *S. prunotincta* was synonymized under *Mutilla* (*Ephuta*) *sicheliana* Saussure, 1867 (=*Dasymutilla sicheliana*) by André (1898). Lastly, the species *S. rufosuffusa* is likely a synonym of *Dasymutilla formosa* (Blake, 1871).

Discussion

The species diversity in this genus was expanded by 467% in this study (three species in Quintero & Cambra 2011; fourteen species in this treatment). This is initially surprising when compared with recent taxonomic treatments of other diurnal New World Mutillidae; these found numerous synonyms that resulted in reductions in species count for the treated taxa. A few examples are listed in Table 5.1. It is notable that each of these taxa belongs to the tribe Dasymutillini (*sensu* Brothers & Lelej 2017). Comparatively fewer revisions have been recently conducted with members of Pseudomethocini, which includes *Invreiella*. A recent revision of a Mesoamerican pseudomethocine genus, *Silvorientilla* Williams & Cambra in Williams *et al.* (2019), also resulted in a large increase in species count, raising the known diversity from one species to seven (an increase of 700%). In both *Invreiella* and *Silvorientilla*, the species are differentiated by finite structural features, rather than color patterns alone, which were used extensively to differentiate dasymutilline species by earlier workers. It should also be noted that each of the referenced dasymutilline revisions was based on hundreds or thousands of specimens, but the revisions of *Invreiella* and *Silvorientilla* were based on 100 and 41 specimens, respectively. It is not yet clear whether the disparity in taxonomic outcomes for these groups is based on phylogenetic differences of the tribes, comparative rarity of taxa, or some other factor, such as distribution or behavioral differences that inhibited collecting efforts by earlier authors.

Invreiella, along with several other New World mutillid genera such as *Dasymutilla* Ashmead, 1899, *Ephuta* Say, 1836, *Pseudomethoca* Ashmead, 1896, and *Timulla* Ashmead, 1899, were recently discovered to participate in one of the largest Müllerian mimicry complexes in the world (Wilson *et al*. 2012, 2013, 2015). Eight

Table 5.1. Comparison of taxonomic changes in recent revisions of dasymutilline Mutillidae.

Taxon	**References**	**Previous species**	**Synonyms / new species**	**Current species**	**Percent change**
Dasymutilla bioculata species-group	Williams *et al*. 2010; Williams & Pitts 2013	22	16 / 0	6	reduced to 27%
D. monticola species-group	Pilgrim *et al*. 2008; Williams *et al*. 2012	19	7 / 0	12	reduced to 63%
D. quadriguttata species-group	Pilgrim *et al*. 2009	22	10 / 0	12	reduced to 55%
Traumatomutilla americana species-group	Bartholomay *et al*. 2019	17	14 / 0	3	reduced to 18%
T. juvenilis species-group	Bartholomay *et al*. 2020	14	10 / 1	5	reduced to 36%

mimicry rings in North and Central America were described based on morphology and distribution. Additionally, several pompilid species in the genus *Psorthaspis* Banks were found to be members of several of these mimicry rings (Rodriguez *et al*. 2014). In maps for these studies there is a conspicuous gap in southern Central Mexico (Wilson *et al.* 2012: Fig. 1), where a limitation of examined specimens restrained the authors from recognizing the dominant mimicry ring in that region. The predominant distribution of *Invreiella* species allows us to revisit this map. Of the fourteen *Invreiella* species, six (including the two most commonly encountered species: *I. cardinalis* and *I. satrapa*) exhibit a predominantly reddish-orange pattern consistent with the Western Mimicry Ring and two (*I. mesomexicana* and *I. cuernavaca*) possess a pattern that is seemingly intermediate between the Western and Red-headed Timulla rings. These species bear a strong resemblance to *Dasymutilla erythrina* (Say, 1836), which has recently been revealed as the most commonly encountered mutillid in Central Mexico by citizen scientists on the website inaturalist.org (https://www.inaturalist.org/taxa/266465-Dasymutilla-erythrina). The Western mimicry ring, therefore, appears to be the dominant mimicry ring in Central Mexico, although the Madrean and Red-headed Timulla rings are also prevalent in this area. These mimicry rings are also represented by species treated here, including *I. cephalargia*, *I. suarezi*, and *I. tequila* in the Madrean ring, *I. bimaculata* in the Red-headed Timulla ring, and *I. australis* as an intermediate between the Red-Headed Timulla and Black-Headed Timulla rings.

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References

André, E. (1898) Étude sur les Mutillides du Muséum de Paris. *Annales de la Société Entomologique de France*, 67, 1–79.

André, E. (1902 [“1903”]) Fam. Mutillidae. *In*: Wytsman, P. (Ed.), *Genera Insectorum*, Fascicle 11. Bruxelles, 1–77, plates 1–3. https://www.biodiversitylibrary.org/item/107651#page/281/mode/1up

Ashmead, W.H. (1896) Descriptions of new parasitic Hymenoptera. *Transactions of the American Entomological Society*, 23 (2), 179–234. https://www.biodiversitylibrary.org/item/32356#page/199/mode/1up

Ashmead, W.H. (1899) Superfamilies in the Hymenoptera and generic synopses of the families Thynnidae, Myrmosidae, and Mutillidae. *Journal of the New York Entomological Society*, 7, 45–60. https://www.biodiversitylibrary.org/item/36372#page/57/mode/1up

Bartholomay, P.R., Williams, K.A., Luz, D.R. & Oliveira, M.L. (2018) New species of *Traumatomutilla* André in the *T. tabapua* and *T. integella* species-groups (Hymenoptera, Mutillidae). *Zootaxa*, 4433 (2), 361–385.

https://doi.org/10.11646/zootaxa.4433.2.7

Bartholomay, P.R., Williams, K.A., Lopez, V. & Oliveira, M.L. (2019) Taxonomic revision of the *Traumatomutilla americana* species group (Hymenoptera: Mutillidae). *Zootaxa*, 4608 (1), 1−34. https://doi.org/10.11646/zootaxa.4608.1.1

Bartholomay, P.R., Williams, K.A., Cambra, R.A. & Oliveira, M.L. (2020) Review of the *Traumatomutilla juvenilis* species group (Hymenoptera, Mutillidae). *Journal of Natural History*, 53 (43–44), 2639–2683. https://doi.org/10.1080/00222933.2020.1715501

Blake, C.A. (1871) Synopsis of the Mutillidae of North America. *Transactions of the American Entomological Society*, 3, 217–265, 358. https://www.biodiversitylibrary.org/item/32422#page/227/mode/1up

Blake, C.A. (1886) Monograph of the Mutillidae of North America. *Transactions of the American Entomological Society*, 13, 179–286. https://www.biodiversitylibrary.org/item/32291#page/189/mode/1up

Brothers, D.J. (1975) Phylogeny and classification of the aculeate Hymenoptera, with special reference to Mutillidae. *The University of Kansas Science Bulletin*, 50 (11), 483–648. https://www.biodiversitylibrary.org/item/25768#page/111/mode/1up

Brothers, D.J. (1995) Mutillidae. *In*: Gauld, I. & Hanson, P. (Eds.), *The Hymenoptera of Costa Rica*. Oxford University Press, Oxford, pp. 541–548.

Brothers, D.J. (1999) Phylogeny and evolution of wasps, ants and bees (Hymenoptera, Chrysidoidea, Vespoidea and Apoidea). *Zoologica Scripta*, 28 (1–2), 233–249.

https://doi.org/10.1046/j.1463-6409.1999.00003.x

Brothers, D.J. (2006a) Familia Mutillidae. *In*: Fernández, F. & Sharkey, M.J. (Eds.), *Introducción a los Hymenoptera de la Región Neotropical*. Sociedad Colombiana de Entomología y Universidad Nacional de Colombia, Bogotá D.C., pp. 577–594.

Brothers, D.J. (2006b) Capítulo 14.4. Familia Mutillidae. *In*: Hanson, P.E. & Gauld, I.D. (Eds.), *Hymenoptera de la Región Neotropical.* The American Entomological Institute, Gainesville, FL, pp. 586–593.

Brothers, D.J. & Lelej, A.S. (2017) Phylogeny and higher classification of Mutillidae (Hymenoptera) based on morphological reanalyses. *Journal of Hymenoptera Research*, 60, 1–97. https://doi.org/10.3897/jhr.60.20091

Cambra T., R.A., Quintero A., D. & Brothers, D.J. (2014) A new species of *Hoplognathoca* Suárez, 1962 from Central America and description of male of *Hoplognathoca costarricensis* Suárez, 1962 (Hymenoptera: Mutillidae). *Zootaxa*, 3884 (3), 295–300. https://doi.org/10.11646/zootaxa.3884.3.9

Cameron, P. (1894–1900) Fam. Mutillidae, *In*: Godman, F.D. & Salvin, D. (Eds.), *Biologia Centrali-Americana. Insecta. Hymenoptera.* (*Fossores*.), *Vol. II*. R.H. Porter, London, (1894) pp. 259–328; (1895) pp. 329–376; (1896) pp. 377–395; (1899) p. 404; (1900) pp. iii, v, x–xi, 405–413, plates 13–14. https://doi.org/10.5962/bhl.title.730

Casal, O.H. (1962) Mutillidae Neotropicales, IX (Hymenoptera). Un nuevo género de Sphaeropthalminae. *Notas del Museo de La Plata* (*Zoología*), 20 (194), 63–75.

Cockerell, T.D.A. (1894) Descriptions of new Hymenoptera. *Entomological News*, 5 (7), 199–201. https://www.biodiversitylibrary.org/item/20320#page/227/mode/1up

Cockerell, T.D.A. (1895) Descriptions of new Hymenoptera. *Entomological News*, 6, 60–64. https://www.biodiversitylibrary.org/item/79332#page/78/mode/1up

Cockerell, T.D.A. & Casad, J.E. (1894) New species of Mutillidae. *Entomological News*, 5, 293–296. https://www.biodiversitylibrary.org/item/20320#page/329/mode/1up

Cockerell, T.D.A. & Casad, J.E. (1895) Descriptions of new Hymenoptera. *Transactions of the American Entomological Society*, 22, 297–300. https://www.biodiversitylibrary.org/item/32378#page/319/mode/1up

Cresson, E.T. (1902) Descriptions of some *Mutilla* from Brazil. *Transactions of the American Entomological Society*, 28 (1), 1–82. https://www.biodiversitylibrary.org/item/32387#page/19/mode/1up

Dalla Torre, K.W. von [as C.G. de Dalla Torre] (1897) *Catalogus Hymenopterorum hucusque descriptorum systematicus et synonimicus*. *Volumen VIII*: *Fossores* (*Sphegidae*). Guilelmi Engelmann, Lipsiae [=Leipzig], viii, 749 pp. https://www.biodiversitylibrary.org/item/41193#page/7/mode/1up

Diller, E. (1990) Die von Spix und Martius 1817–1820 in Brasilien gesammelten und von J.A.M. Perty 1833 bearbeiteten Hymenopteren in der Zoologischen Staatssammlung München. *Spixiana*, 13 (1), 61–81. https://www.biodiversitylibrary.org/item/89701#page/71/mode/1up

Evenhuis, N.L. (2020) The insect and spider collections of the world website. Available from: http://hbs.bishopmuseum.org/codens/ (accessed 10 August 2020)

Ferguson, W.E. (1967) Male sphaeropthalmine mutillid wasps of the Nevada Test Site. *Brigham Young University Science Bulletin*, *Biological Series*, 8 (4), 1–26. https://scholarsarchive.byu.edu/byuscib/vol8/iss4/1/

Fernández C., F. (2001) Checklist of genera and subgenera of aculeate Hymenoptera of the Neotropical Region (Hymenoptera: Vespomorpha). *Biota Colombiana*, 2 (2), 87–130. https://www.redalyc.org/pdf/491/49120201.pdf

Fox, W.J. (1899) The North American Mutillidae. *Transactions of the American Entomological Society*, 25, 219–292. https://www.biodiversitylibrary.org/item/32379#page/239/mode/1up

Gerstaecker, A. (1874) Mutillarum Americae meridionalis indigenarum synopsis systematica et synonymica. *Archiv für Naturgeschichte*, 40, 41–77, 299–328. https://www.biodiversitylibrary.org/item/29638#page/49/mode/1up, https://www.biodiversitylibrary.org/item/29638#page/307/mode/1up

Halffter, G. (1976) Distribución de los insectos en la zona de transición Mexicana: Relaciones con la entomofauna de Norteamérica. *Folia Entomológica Mexicana*, 35, 1–64.

Halffter, G. (1987) Biogeography of the montane entomofauna of Mexico and Central America. *Annual Review of Entomology*, 32, 95–114. https://doi.org/10.1146/annurev.en.32.010187.000523

Halffter, G. & Morrone, J.J. (2017) An analytical review of Halffter's Mexican transition zone, and its relevance for evolutionary biogeography, ecology and biogeographical regionalization. *Zootaxa*, 4226 (1), 1–46. https://doi.org/10.11646/zootaxa.4226.1.1

ICZN (1999) *International Code of Zoological Nomenclature*. Fourth Edition. The International Trust for Zoological Nomenclature, London, UK, 306 pp. http://www.iczn.org/iczn/index.jsp

Krombein, K.V. (1951) Family Mutillidae. *In*: Muesebeck, C.F.W., Krombein, K.V., & Townes, H.K. (Eds.), *Hymenoptera of America North of Mexico Synoptic Catalog* (*Agriculture Monograph No. 2*). United States Government Printing Office, Washington, D.C., pp. 749–773. https://doi.org/10.5962/bhl.title.65057

Krombein, K.V. (1972) Monograph of the Madagascan Mutillidae (Hymenoptera). Part I: Myrmillini, Mutillini and Smicromyrmini. *Annales, Musée Royal de l'Afrique Centrale* (*Série in 8°*), *Sciences Zoologiques*, 199, 1–61.

Krombein, K.V. (1979) Family Mutillidae. *In*: Krombein, K.V., Hurd, Jr., P.D., Smith, D.R., & Burks, B.D. (Eds.), *Catalog of Hymenoptera in America North of Mexico.* Smithsonian Institution Press, Washington D.C., pp. 1276–1314. https://doi.org/10.5962/bhl.title.5074

Krombein, K.V. (1992) Host relationships, ethology and systematics of *Pseudomethoca* Ashmead (Hymenoptera: Mutillidae, Andrenidae, Halictidae and Anthophoridae). *Proceedings of the Entomological Society of Washington*, 94 (1), 91–106. https://www.biodiversitylibrary.org/item/54937#page/97/mode/1up

Lelej, A.S. & Brothers, D.J. (2008) The genus-group names of Mutillidae (Hymenoptera) and their type species, with a new genus, new name, new synonymies, new combinations and lectotypifications. *Zootaxa*, 1889, 1–79. https://doi.org/10.11646/zootaxa.1889.1.1

Lelej, A.S. & Nemkov, P.G. (1997) Phylogeny, evolution and classification of Mutillidae (Hymenoptera). *Far Eastern Entomologist*, 46, 1–24.

Linnaeus, C. (1758) *Systema Naturae per Regna Tria Naturae, secundum Classes, Ordines, Genera, Species, cum Characteribus, Differentiis, Synonymis, Locis*. Tomus I. Editio Decima, Reformata. Laurentii Salvii, Holmiae [= Stockholm], pp. 1–823. https://www.biodiversitylibrary.org/item/10277#page/3/mode/1up

Luz, D.R., Waldren, G.C. & Melo, G.A.R. (2016) Bees as hosts of mutillid wasps in the Neotropical region (Hymenoptera, Apidae, Mutillidae). *Revista Brasileira de Entomologia*, 60 (4), 302–307. https://doi.org/10.1016/j.rbe.2016.06.001

Manley, D.G. & Pitts, J.P. (2002) A key to genera and subgenera of Mutillidae (Hymenoptera) in America north of Mexico with description of a new genus. *Journal of Hymenoptera Research*, 11 (1), 72–100. https://www.biodiversitylibrary.org/item/21557#page/370/mode/1up

Manley, D.G. & Pitts, J.P. (2007) Tropical and subtropical velvet ants of the genus *Dasymutilla* Ashmead (Hymenoptera: Mutillidae) with descriptions of 45 new species. *Zootaxa*, 1487, 1–128. https://doi.org/10.11646/zootaxa.1487.1.1

Michener, C.D. (1944) Comparative external morphology, phylogeny, and a classification of the bees (Hymenoptera). *Bulletin of the American Museum of Natural History*, 82 (6), 151–326. http://hdl.handle.net/2246/1272

Mickel, C.E. (1924) A revision of the mutillid wasps of the genera *Myrmilloides* and *Pseudomethoca* occurring in America north of Mexico. *Proceedings of the United States National Museum*, 64 (15), 1–51, plates 1–4. https://www.biodiversitylibrary.org/page/7539245#page/281/mode/1up

Mickel, C.E. (1928) Biological and taxonomic investigations on the mutillid wasps. *United States National Museum*, Bulletin 143, 1–351, plates 1–5. https://www.biodiversitylibrary.org/page/7835957#page/5/mode/1up

Mickel, C.E. (1935) Descriptions and records of Nearctic mutillid wasps of the genera *Myrmilloides* and *Pseudomethoca* (Hymenoptera: Mutillidae). *Transactions of the American Entomological Society*, 61, 383–398. https://www.jstor.org/stable/25077360

Mickel, C.E. (1937) New World Mutillidae in the Spinola collection at Torino, Italy (Hymenoptera). *Revista de Entomologia*, *Rio de Janeiro*, 7 (2–3), 165–207.

Mickel, C.E. (1941) Monograph of the South American mutillid genus *Hoplocrates* Mickel (Hymenoptera: Mutillidae). *Revista de Entomologia*, *Rio de Janeiro*, 11 (1–2), 341–414.

Mickel, C.E. (1964) Synonymical notes on Neotropical Mutillidae (Hymenoptera). *Proceedings of the Royal Entomological Society of London. Series B*, *Taxonomy*, 33, 163–171.

https://doi.org/10.1111/j.1365-3113.1964.tb01635.x

Morrone, J.J. (2014) Biogeographical regionalisation of the Neotropical region. *Zootaxa*, 3782 (1), 1–110. https://doi.org/10.11646/zootaxa.3782.1.1

Morrone, J.J. (2015) Halffter's Mexican transition zone (1962–2014), cenocrons and evolutionary biogeography. *Journal of Zoological Systematics and Evolutionary Research*, 53 (3), 249–257. https://doi.org/10.1111/jzs.12098

Morrone, J.J., Escalante, T. & Rodríguez-Tapia, G. (2017) Mexican biogeographic provinces: Map and shapefiles. *Zootaxa*, 4277 (2), 277–279. https://doi.org/10.11646/zootaxa.4277.2.8

Naumann, I.D. (1991) Hymenoptera (wasps, bees, ants, sawflies). *In*: Naumann, I.D. *et al.* (Eds.), *The Insects of Australia*: *A Textbook for Students and Research Workers* (2nd edition) (volume 2). Cornell University Press, Ithaca, pp. 916–1000.

Nonveiller, G. (1990) *Catalogue of the Mutillidae, Myrmosidae and Bradynobaenidae of the Neotropical region including Mexico* (*Insecta, Hymenoptera*). *Hymenopterorum Catalogus* (*Nova Editio*), *18*. SPB Academic Publishing, Den Haag, 150 pp.

Pagliano, G. (2005) I Mutillidae della collezione Spinola (Hymenoptera). *Bollettino del Museo regionale di Scienze naturali*, *Torino*, 23 (1), 267–322.

Pagliano, G., Brothers, D.J., Cambra T., R.A., Lelej, A.S., Lo Cascio, P., Matteini Palmerini, M., Scaramozzino, P.L., Williams, K.A. & Romano, M. (2020 ["2018"]) Checklist of names in Mutillidae (Hymenoptera), with illustrations of selected species. *Bollettino del Museo regionale di Scienze naturali di Torino*, 36 (1–2), 5–425.

Perty, J.A.M. (1833) Insecta Brasiliensia [*Mutilla*]. *In*: de Spix, J.B. & de Martius, C.F.Ph. (Eds.), *Delectus animalium articulatorum, quae in itinere per Brasiliam annis MDCCCXVII-MDCCCXX jussu et auspiciis Maximiliani Josephi I. Bavariae regis augustissimi peracto*, Fascicle 3. Frid Fleischer, Monachii [=München], pp. 137–138, pl. XXVII. https://www.biodiversitylibrary.org/item/265704#page/11/mode/1up

Pilgrim, E.M., Williams, K.A. & Pitts, J.P. (2008) Sex association and synonymy in southwestern U.S. species of *Dasymutilla* (Hymenoptera: Mutillidae). *The Pan-Pacific Entomologist*, 84 (1), 57–68. https://doi.org/10.3956/2007-13.1

Pilgrim, E.M., Williams, K.A., Manley, D.G. & Pitts, J.P. (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. https://doi.org/10.2317/JKES08.11.1

Pitts, J.P., Wilson, J.S. & von Dohlen, C.D. (2010) Evolution of the nocturnal Nearctic Sphaeropthalminae velvet ants (Hymenoptera: Mutillidae) driven by Neogene orogeny and Pleistocene glaciation. *Molecular Phylogenetics and Evolution*, 56 (1), 134–145. https://doi.org/10.1016/j.ympev.2010.03.033

Quintero A., D. & Cambra T., R.A. (1996) *Horcomutilla* Casal: Description of previously unknown males, new distribution records, and comments on the genus (Hymenoptera: Mutillidae). *Journal of Hymenoptera Research*, 5, 53–63. https://www.biodiversitylibrary.org/item/26143#page/61/mode/1up

Quintero A., D. & Cambra T., R.A. (2011) Revision of the Mexican endemic genus *Invreiella* Suárez (Hymenoptera: Mutillidae), with the description of a new species. *Transactions of the American Entomological Society*, 137 (1/2), 217–224. https://doi.org/10.3157/061.137.0114

Rodriguez, J., Pitts, J.P., von Dohlen, C.D. & Wilson, J.S. (2014) Müllerian mimicry as a result of codivergence between velvet ants and spider wasps. *PloS ONE*, 9 (11), e112942. https://doi.org/10.1371/journal.pone.0112942

Saussure, H. de (1867) Mutillarum novarum species aliquot. *Annales de la Société Entomologique de France*, 7, 351–364, pl. VIII. https://www.biodiversitylibrary.org/item/101298#page/355/mode/1up

Say, T. (1836) Descriptions of new species of North American Hymenoptera, and observation on some already described. *Boston Journal of Natural History*, 1 (3), 209–305. https://www.biodiversitylibrary.org/item/100882#page/212/mode/1up

Selander, R.B. & Vaurie, P. (1962) A gazetteer to accompany the "Insecta" volumes of the "Biologia Centrali-Americana." *American Museum Novitates*, 2099, 1–70. http://hdl.handle.net/2246/3415

Shorthouse, D.P. (2010) SimpleMappr, an online tool to produce publication-quality point maps. Available from: https://www.simplemappr.net/ (accessed 10 August 10 2020)

Smith, F. (1855) *Catalogue of Hymenopterous Insects in the Collection of the British Museum. Part III. Mutillidae and Pompilidae*. Taylor and Francis, London, pp. 1–206, pl. A, plates 1–5. https://www.biodiversitylibrary.org/item/36602#page/5/mode/1up

Smith, F. (1879) *Descriptions of new species of Hymenoptera in the collection of the British Museum*. Taylor and Francis, London, pp. xxi, 1–240. https://www.biodiversitylibrary.org/item/56822#page/7/mode/1up

Suárez, F.J. (1962) Datos sobre Mutílidos neotropicales. III. Un nuevo género de Centroamérica y paises noroccidentales de América del Sur (Hymenoptera). *Eos*, *Revista Española de Entomología*, *Madrid*, 38 (1), 115–146. http://hdl.handle.net/10261/157756

Suárez, F.J. (1966 [“1965”]) Datos sobre Mutílidos neotropicales. VI. Dos nuevos géneros de Pseudomethocini de Méjico y Brasil (Hymenoptera). *Eos*, *Revista Española de Entomología*, *Madrid*, 41 (2–3), 471–482. http://hdl.handle.net/10261/162095

Wheeler, Q.D. & Platnick, N.I. (2000) 5. The Phylogenetic Species Concept (*sensu* Wheeler and Platnick). *In*: Wheeler, Q.D. & Meier, R. (Eds.), *Species Concepts and Phylogenetic Theory*: *A Debate*. Columbia University Press, New York, pp. 55–69.

Williams, K.A., Manley, D.G., Pilgrim, E.M., von Dohlen, C.D. & Pitts, J.P. (2010) Multifaceted assessment of species validity in the *Dasymutilla bioculata* species-group (Hymenoptera: Mutillidae). *Systematic Entomology*, 36 (1), 180–191. https://doi.org/10.1111/j.1365-3113.2010.00555.x

Williams, K.A., Brothers, D.J. & Pitts, J.P. (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. https://doi.org/10.11646/zootaxa.3064.1

Williams, K.A., Manley, D.G., Deyrup, M., von Dohlen, C. & Pitts, J.P. (2012) Systematic review of the *Dasymutilla monticola* species-group (Hymenoptera: Mutillidae): Using phylogenetics to address species-group placement and sex associations. *Zootaxa*, 3554, 1–29. https://doi.org/10.11646/zootaxa.3554.1.1

Williams, K.A. & Pitts, J.P. (2013) Caribbean and Mexican additions to the *Dasymutilla bioculata* species-group. *Annals of the Entomological Society of America*, 106 (4), 429–436. https://doi.org/10.1603/AN12123

Williams, K.A., Cambra, R.A., Waldren, G.C., Quintero A., D. & Pitts, J.P. (2019 [“2018”]) *Silvorientilla*, gen. nov. a new Neotropical velvet ant genus (Hymenoptera: Mutillidae). *Folia Entomológica Mexicana* (*nueva serie*), 4 (3), 91−109.

Wilson, J.S., Jahner, J.P., Forister, M.L., Sheehan, E.S., Williams, K.A. & Pitts, J.P. (2015) North American velvet ants form one of the world's largest known Müllerian mimicry complexes. *Current Biology*, 25 (16), R704–R706. https://doi.org/10.1016/j.cub.2015.06.053

Wilson, J.S., Jahner, J.P., Williams, K.A. & Forister, M.L. (2013) Ecological and evolutionary processes drive the origin and maintenance of imperfect mimicry. *PloS ONE*, 8 (4), e61610. https://doi.org/10.1371/journal.pone.0061610

Wilson, J.S., Williams, K.A., Forister, M.L., von Dohlen, C.D. & Pitts, J.P. (2012) Repeated evolution in overlapping mimicry rings among North American velvet ants. *Nature Communications*, 3, 1272. https://doi.org/10.1038/ncomms2275

CHAPTER VI

CONCLUSION

Mutillidae are, indeed, a little-known group, as revealed by the results in each of my chapters. In this dissertation, I used molecular phylogenetics and revisionary taxonomy to elucidate the relationships and composition of taxa at various ranks. Further, the ancestral ages of various mutillid groups were inferred and biogeographic hypotheses were proposed. Notably, the family-group level diversity for Mutillidae and species-level diversity for *Invreiella* were both significantly underestimated and required expansion.

In my second chapter, I tested the higher classification of Mutillidae proposed by Brothers & Lelej (2017) using ultraconserved elements. Overall, at the subfamily level, the classification of Brothers & Lelej (2017) was mostly congruent with the results of my phylogenetic analyses with two major exceptions: Myrmosinae was not recovered as a member of Mutillidae, and the *Odontomutilla* genus-group of Mutillinae: Mutillini: Ephutina is deserved of subfamily status. Myrmosidae is now recognized as a family, and Odontomutillinae is now recognized as a subfamily of Mutillidae. Another notable discovery was that the other component group of Ephutina, the *Ephuta* genus-group, was recovered as a lineage of Sphaeropthalminae; Ephutina was consequently transferred to that subfamily and raised to a tribe, Ephutini. Myrmillinae was recovered as polyphyletic, with two genera, *Ceratotilla* Bischoff and *Viereckia* Ashmead, recovered in the basal lineage of Mutillinae. The situation for the tribal classification, however, was far less stable than the subfamily classification, with all of the currently recognized tribes of

Mutillinae and Sphaeropthalminae recovered as non-monophyletic. In addition to the tribes proposed by Brothers & Lelej for these two subfamilies, three additional tribal lineages of Mutillinae and six tribal lineages of Sphaeropthalminae were recognized in my classification. The ages of Pompiloidea and its constituent families were inferred to be far older than previously thought, with Pompiloidea emerging at 154.11/144.27 Ma and Mutillidae emerging at 123.06/105.28 Ma. With the new classification proposed herein, new insights can be had and hopefully new, previously overlooked synapomorphies will be discovered for the subfamilies and tribes of Mutillidae.

In my third chapter, I reported on the novel observation of phoretic copulation in the sphaeropthalmine *Sphaeropthalma pensylvanica* (Lepeletier). The observation of this behavior was the first reported for Sphaeropthalminae, and served as an opportunity to critically review the mating strategies for Mutillidae. I developed new terminology to more accurately describe the mating strategies that are known to occur in mutillids: mandibular phoretic copulation, terminalic phoretic copulation, and *in situ* copulation. A comprehensive table of all known observations of mating strategies and relevant details was composed to allow for future study. Additionally, I ultimately regarded as erroneous a type of mating strategy described by O'Toole (1975). Lastly, I hypothesized that the present disjunct distributions for several sphaeropthalmine genera may be due to dispersal via phoretic copulation.

In my fourth chapter, I investigated the phylogeny of the mutilline tribe Trogaspidiini using ultraconserved elements. The goals of this study were to test if the Old World and New World trogaspidiine faunas were monophyletic, as multiple dispersal events may have occurred between the hemispheres in this cosmopolitan tribe.

Trogaspidiini was recovered as monophyletic. The New World fauna, represented by *Timulla* Ashmead, was recovered as monophyletic and was sister-goup to a clade of mixed Afrotropical and Oriental trogaspidiines. The Old World trogaspidiines fauna is paraphyletic with respect to *Timulla*, and there were multiple dispersal events between the Afrotropical and Oriental regions. Further, I inferred the ancestral areas for Trogaspidiini, which revealed a hypothesized Afrotropical ancestral emergence area. Multiple dispersal events occurred between the Afrotropical and Oriental regions, which may have been influenced by Indian monsoons (Goswami & Rajagopal 2003; Wang et al. 2003) and eustatic sea-level changes that exposed islands between India and East Africa (Warren et al. 2010), allowing an over-water, stepping-stone dispersal approach enhanced by the mandibular phoretic copulation conducted by these wasps. The ancestral area of *Timulla* is less clear, as depending on the analysis, the ancestor of clade 8 and clade 9 was inferred to be Afrotropical in the ML analysis, while Oriental in the MP analysis. Morphology of the basal lineage of *Timulla* suggests a close relationship to the Oriental trogaspidiines. When *Timulla* emerged at 7.65/6.01 Ma, the summer temperatures in Beringia had already been in the process of dropping and reached their current temperatures 5–6 Ma (Wolfe 1994). Additionally, the dominant biome in Beringia was boreal forest (Wolfe & Tanai 1980). As trogaspidiines are primarily tropical in distribution, the arrival of an Oriental trogaspidiine lineage into North America via Beringia is unlikely. With no land bridge option left, jump dispersal was likely how ancestral *Timulla* reached the New World. The ancestral area for *Timulla* was inferred to be Neotropical North America, with several subsequent dispersals into Nearctic North America and Neotropical South America. The phylogenetic hypotheses that were inferred will serve as a foundation for future revisionary and biogeographic work on Trogaspidiini.

Lastly, in my fifth chapter, I revised the pseudomethocine genus *Invreiella* Suárez. Fourteen species were recognized, an increase of 467% from the three species previously recognized in Quintero & Cambra (2011). *Invreiella curoei* Quintero & Cambra, was discovered to be a synonym of *Invreiella cardinalis* Gerstaecker. The species *Pseudomethoca cephalargia* Mickel was recognized as a member of *Invreiella* and was transferred to the genus. I recognized five species-groups and each were supported by a unique combination of character states. Eleven new species were described, with most known from fewer than five specimens. Lastly, I investigated the biogeography of the genus, which primarily occurs in the Mexican transition zone. This revision will give researchers a much-needed foundation for future work on this group, and will hopefully lead to the recognition of the unknown males.

A multi-faceted approach using molecules, morphology, biogeography, and biology is necessary to understand this complex and fascinating family. Foundational work has been laid in my dissertation for the higher classification of Mutillidae, characterization of the known mating strategies of mutillids, the systematic relationships and biogeographical history of trogaspidiines, and a genus-level revision of a rare genus. It is hoped that these findings will serve as a resource for future research.

References

Brothers, D.J. & Lelej, A.S. (2017) Phylogeny and higher classification of Mutillidae (Hymenoptera) based on morphological reanalyses. *Journal of Hymenoptera Research*, 60: 1–97.

Goswami, B.N. & Rajagopal, E.N. (2003) Indian Ocean surface winds from NCMRWF analysis as compared to QuikSCAT and moored buoy winds. *Journal of Earth System Science*, 112(1): 61–77.

Quintero A., D. & Cambra, R.A. (2011) Revision of the Mexican endemic genus *Invreiella* Suárez, with the description of a new species. *Transactions of the American Entomological Society*, 137(1+2): 217–224.

Wang, B., Clemens, S.C., & Liu, P. (2003) Contrasting the Indian and East Asian monsoons: Impacts on geologic timescales. *Marine Geology*, 201: 5–21.

Warren, B.H., Strasberg, D., Henrich Bruggemann, J., Prys-Jones, R.P., & Thébaud, C. (2010) Why does the biota of the Madagascar region have such a strong Asiatic flavor? *Cladistics*, 26: 526–538.

Wolfe, J.A. (1994) An analysis of Neogene climates in Beringia. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 108: 207–216.

Wolfe, J.A. & Tanai, T. (1980) The Miocene Seldovia Point flora from the Kenai Group, Alaska. *Geological Survey Professional Paper*, 1105: 1–52.

Appendices

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Systematic revision of the North American velvet ant genus *Invreiella* Suárez (Hymenoptera: Mutillidae) with description of eleven new species

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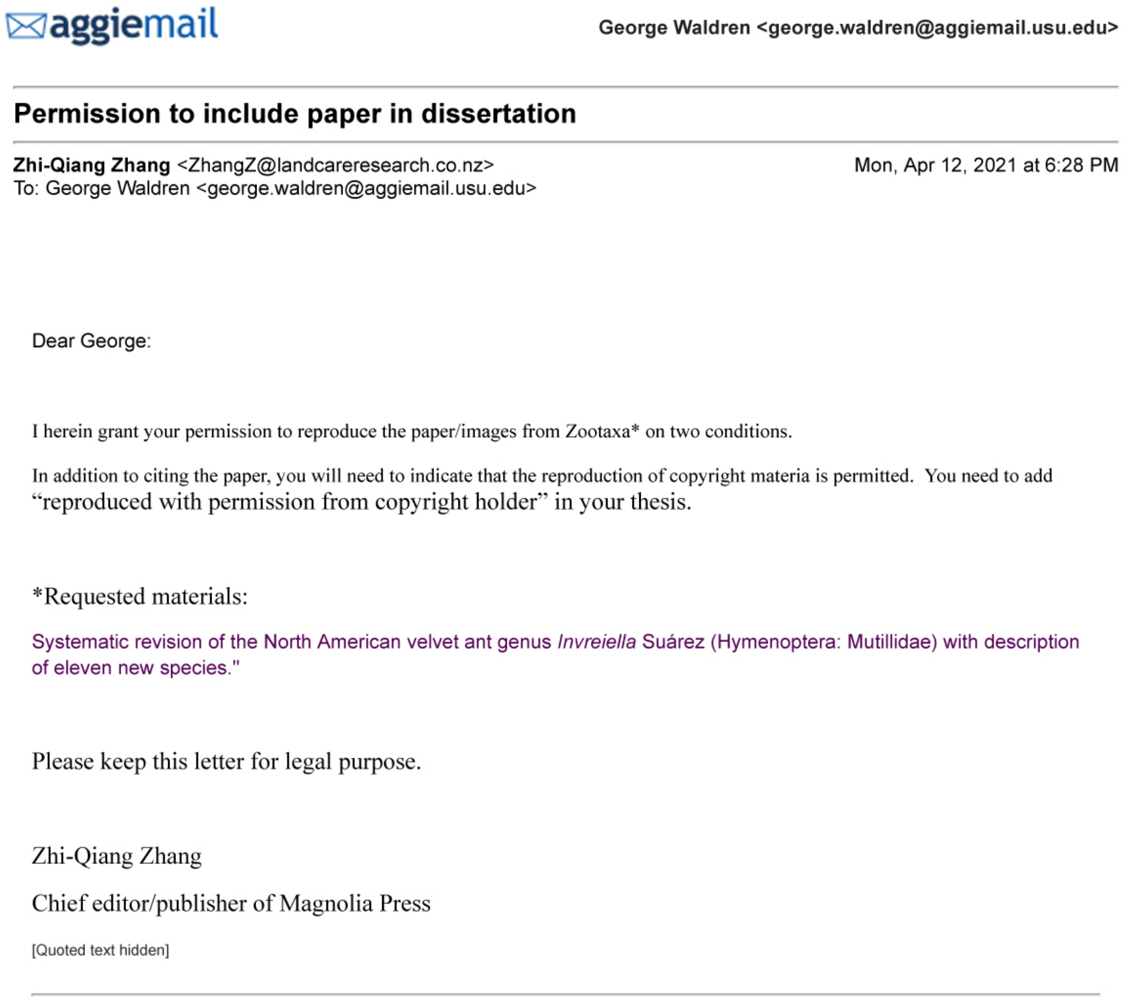

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Appendix C. Curriculum Vitae

Curriculum Vitae

GEORGE C. WALDREN

EDUCATION

Ph.D. Biology (Systematic Entomology). Utah State University, Logan, Utah (May 2021). Dissertation title: *The Velvet Ants (Hymenoptera: Mutillidae): Systematics, Biology, and Biogeography of a Little-Known Family*.

Advisor: Dr. James P. Pitts.

Master of Architecture Texas Tech University, Lubbock, Texas (2010).

B.Sc. Architecture Texas Tech University, Lubbock, Texas (2008).

PROFESSIONAL EXPERIENCE

2014–2020	**Laboratory Researcher/Assistant.** Utah State University, Department of Biology, Logan, Utah.
2015–2018	**Fire Ant Surveyor.** State of Utah, St. George, Utah.
2014–2020	**Laboratory Instructor.** Utah State University, Department of Biology, Logan, Utah.
2011–2013	**Architectural intern.** Randall Scott Architects, Dallas, Texas.
2010	**Student intern.** Overland Partners Architects, San Antonio, Texas.
2008–2010	**Laboratory Researcher/Assistant.** Invertebrate Collection, Natural Science Research Laboratory, Museum of Texas Tech University, Lubbock, Texas.

PEER-REVIEWED PUBLICATIONS

10. **Waldren, G.C.**, Williams, K.A., Cambra, R.A., & Pitts, J.P. (2020) Systematic revision of the North American velvet ant genus *Invreiella* Suárez (Hymenoptera: Mutillidae) with description of eleven new species. *Zootaxa*, 4894(2): 151–205. https://doi.org/10.11646/zootaxa.4894.2.1

9. **Waldren, G.C.**, Roberts, J.D., & Pitts, J.P. (2020) Phoretic copulation in the velvet ant *Sphaeropthalma pensylvanica* (Lepeletier) (Hymenoptera, Mutillidae): A novel behavior for Sphaeropthalminae with a synthesis of mating strategies in Mutillidae. *Journal of Hymenoptera Research*, 78: 69–89. https://doi.org/10.3897/jhr.78.55762

8. Williams, K.A., Cambra, R.A., **Waldren, G.C.**, Quintero A., D., & Pitts, J.P. (2019) *Silvorientilla*, gen. nov. a new Neotropical velvet ant genus (Hymenoptera: Mutillidae). *Folia Entomológica Mexicana* (*nueva serie*), 4(3): 91−109.

7. Cambra, R.A., Quintero A., D., **Waldren, G.C.**, Bartholomay, P.R., & Williams, K.A. (2018) Taxonomic revision of the genus *Timulla* Ashmead, 1899 of Panama (Hymenoptera: Mutillidae). *Tecnociencia*, 20(2): 23–57.

6. Bartholomay, P.R., **Waldren, G.C.**, & Oliveira, M.L. (2017) Observation of a mixed-sex, mixed-species aggregation of velvet ants, genus *Timulla* Ashmead, 1899 (Hymenoptera: Mutillidae) in the Brazilian Amazon, Roraima, with a new synonymy. *Zootaxa*, 4362(1): 135–140. https://doi.org/10.11646/zootaxa.4362.1.8

5. Luz, D.R., **Waldren, G.C.**, & Melo, G.A.R. (2016) Bees as hosts of mutillid wasps in the Neotropical region (Hymenoptera, Apidae, Mutillidae). *Revista Brasileira de Entomologia*, 60: 302–307. https://doi.org/10.1016/j.rbe.2016.06.001

4. Lohrmann, V., **Waldren, G.C.**, Reiß, M., & Engel, M.S. (2016) An anocellar polistine wasp (Hymenoptera, Vespidae, Polistinae) from Texas. *Zoosystematics and Evolution*, 92(2): 251–255. https://doi.org/10.3897/zse.92.10548

3. Auko, T.H., Bartholomay, P.R., Luz, D.R., **Waldren, G.C.**, & Williams, K.A. (2016) First records of the genus *Allotilla* Schuster, 1949 (Hymenoptera, Mutillidae) in Brazil. *Check List*, 12(3): 1–3. https://doi.org/10.15560/12.3.1898

2. Bartholomay, P.R., Williams, K.A., **Waldren, G.C.**, & de Oliveira, M.L. (2015) Corrections on the biology of *Traumatomutilla* André, 1901 (Hymenoptera: Mutillidae). *Zootaxa*, 3920(1): 198–200. https://doi.org/10.11646/zootaxa.3920.1.10

1. Pitts, J.P., Tanner, D., **Waldren, G.C.**, & Parker, F.D. (2010) Facultative size-dependent sex allocation in *Sphaeropthalma pensylvanica* Lepeletier (Hymenoptera: Mutillidae) with further host records. *Journal of the Kansas Entomological Society*, 83(1): 68–75. https://doi.org/10.2317/JKES0812.15.1

CONFERENCE PRESENTATIONS

Waldren, G.C. & Pitts, J.P. Molecular phylogenetics reveals paraphyly in the New World velvet ant genus *Pseudomethoca* Ashmead (Hymenoptera: Mutillidae). Graduate Poster Competition: SysEB – Phylogeny. 63rd Annual Meeting of the Entomological Society of America, November 2015, Minneapolis, Minnesota.

AWARDS

Joseph E. Greaves Memorial Scholarship. Utah State University (2018)

Lubbock AIA Scholarship. Texas Tech University (2009)

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International Society of Hymenopterists

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TEACHING EXPERIENCE

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Introductory Biology II, Laboratory Instructor, Utah State University (2014–2020)

Insect Systematics and Evolution, Laboratory Instructor, Utah State University (2018)

OUTREACH

2019−present	Contributing Specialist, www.iNaturalist.org
2012	Hymenopterist, Texas A&M Entoblitz, OUBS, Willis, OK
2011	Hymenopterist, Texas A&M Entoblitz, Elephant Mtn. WMA, Alpine, TX
2010	Habitat for Humanity, Texas Tech University, Lubbock, TX
2006−present	Contributing Editor, www.BugGuide.net

ENTOMOLOGICAL COLLECTION RESEARCH VISITS

Texas A&M University (2009, 2010, 2014, 2016)

University of Oklahoma Biological Station (2012)

University of Texas at Austin (2010, 2011, 2016)