



Are diurnal iguanian lizards the evolutionary drivers of New World female velvet ant (Hymenoptera: Mutillidae) Müllerian mimicry rings?

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Although several recent studies have described a vast mimicry complex among New World mutillid wasps (velvet ants), little is known about the potential predators that could be driving the colour convergence in these wasps. Identifying potential predators can be a necessary part of understanding the evolution of large mimicry complexes because predation pressures likely are a key evolutionary driver of aposematism in these systems. However, pinpointing potential predators is difficult given the rarity of observing predation events in the wild. Furthermore, laboratory-based feeding trials are difficult to design without a priori information about which potential predators should be investigated. In the present study, we explore the potential predator communities that may have driven the evolution of the large North American velvet ant mimicry complex. We hypothesize that potential predators can be identified by examining: (1) distributional similarities between predators and prey; (2) similarities of predator assemblages in areas where convergently coloured prey occur; (3) known dietary preferences of potential predators; and (4) evolutionary concordance (both spatially and temporally) of predator and prey clades. We find that iguanians are likely predators of two of the described mutillid mimicry rings. We hypothesize that the warning coloration of the Black-headed Timulla and Tropical mimicry rings is particularly directed towards dactyloids (anoles). © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **00**, 000–000.

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INTRODUCTION

Recent studies have identified female diurnal New World velvet ants (Hymenoptera: Mutillidae), particularly in North America (Nearctic and Mesoamerica), as examples of one of the largest known Müllerian mimetic complexes, consisting of eight distinct mimicry rings: Black-headed Timulla, Desert, Eastern, Madrean, Red-headed Timulla, Texan, Tropical, and Western (Wilson *et al.*, 2012, 2015). Although the Eastern, Madrean, Western, and Red-headed Timulla mimicry rings have broad Nearctic distributions, the Black-headed Timulla, Desert, Texan, and Tropical mimicry rings are more geographically restricted on the continent (Wilson *et al.*, 2015).

Müllerian mimetic systems benefit both prey and predators by quickly training predators with respect to the threats posed by attacking well-defended prey, typically via venom or poison delivery systems, unpalatable taste or tissue injuring/damaging characteristic(s). This therefore limits, or prevents, future predation attempts and encounters, which could injure or kill the aposematic prey (Beatty, Beirincx & Sherratt, 2004; Williams, 2007; Wilson *et al.*, 2012).

Female mutillids, being apterous (i.e. wingless) wasps, have a fast-paced, erratic scurrying locomotion and are noncentral-place foragers, covering large areas during their daily active period(s) in search of host nests and broods (Schmidt & Blum, 1977; Vandersal, 2008). The rapid locomotion of velvet ants, along with the large areas covered by

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individuals, generates a greater likelihood of being noticed by potential predators (Schmidt & Blum, 1977; Vitt & Cooper, 1988; Pianka & Vitt, 2003). Female mutillids also resemble ants (Formicidae) in general *bauplan*, locomotion, and, in some cases, size. As a result of the similarities between mutillids and ants, predators, specifically those that exploit ants as a large portion of their diet and use visual cues and movement to detect prey, could initially misidentify mutillids as ants. The presence of aposematic coloration not only may be an adaptation to warn predators that the female is a well-defended organism, but also offers a contrast in appearance from ants, which are more often of uniform or subdued coloration than female mutillids (and are typically not densely pubescent: Hölldobler & Wilson, 1990; Pacheco & Mackay, 2013).

Within other studies of Müllerian mimicry rings (i.e. *Heliconius* Kluk), birds have often been considered the main driver for maintenance or development of such rings (Mallet & Gilbert, 1995; Langham, 2006). Mammalian carnivores, in addition to birds, are implicated in coral snake (*Micrurus* Wagler) Müllerian mimicry rings as predatory drivers (Beckers, Leenders & Strijbosch, 1996; Pfennig, Harcombe & Pfennig, 2001). In the mimicry complexes of New World mutillids, however, no effective predator has been identified among the various vertebrate and invertebrate taxa (Schmidt & Blum, 1977), although diurnal lizards that live in similar environments and whose activity periods coincide with velvet ants are considered the likely key agents for the development and maintenance of aposematic coloration in these wasps (Vitt & Cooper, 1988; Wilson *et al.*, 2012, 2015). Although at least one record exists of an attempted predation of a mutillid by a bird (Manley, 2000), there is no evidence that birds regularly attack female velvet ants. Pinpointing the specific predator group(s) likely driving the development and maintenance pressure of this mutillid Müllerian mimetic complex and component rings is challenging. Although rare, reports of female mutillids being ingested by lizards, particularly iguanians, are known, including in studies by Best & Pfaffenberger (1987), Manley (2000), Manley & Sherbrooke (2001), and Endriss (2003).

Relatively few feeding trials have been carried out aiming to determine which potential lizard predators feed on mutillids, with most studies finding that few predators are successful in any predation attempts (Schmidt & Blum, 1977; Vitt & Cooper, 1988; Manley, 2000; Manley & Sherbrooke, 2001; Edwards, 2008). Additional feeding trials are needed to test theoretical hypothesis regarding which lizard groups may be selective agents in the evolution of the

velvet ant mimicry complex. However, feeding trials alone as a way of determining which lizard groups feed on mutillids can be challenging because of the diversity of potential predatory groups, and the diversity of prey. Prior to feeding trials, investigations into which groups may be potential predators are needed.

Examination and determination of the aposematic coloration of Caribbean female mutillids was not included in the studies by Wilson *et al.* (2012, 2015). Interestingly, species of *Dasymutilla* Ashmead from this geographical region of North America appear to be similar to the Black-headed Timulla Müllerian mimetic ring, which is unusual because this coloration is rare within the dasymutillines (Manley & Pitts, 2007; Williams, 2012; Williams & Pitts, 2013; Wilson *et al.*, 2015). The presence of this similar and rare coloration within Caribbean *Dasymutilla* (Fig. 1), as well as the restricted geographical area in which it occurs, provides an insight into which predatory lizard groups may be affecting aposematic coloration amongst these female mutillids because the potential predatory lizard fauna is less diverse (on a familial scale) than the continental fauna (Köhler, 2008; Hedges, 2015).

In the present study, we investigated potential predators of the mutillid mimicry complex by focusing our search on two geographically limited mutillid mimicry rings (Tropical and Black-headed Timulla) and the similarly coloured Caribbean fauna. We hypothesize that it is possible to recognize hypothetical predator candidates as evolutionary drivers by examining: (1) distributional similarities between potential predators and prey; (2) similarities of predator assemblages in areas where convergently coloured prey occur; (3) known dietary preferences of potential predators; and (4) evolutionary concordance (both spatially and temporally) of predator and prey clades.

MATERIAL AND METHODS

DISTRIBUTION ANALYSIS

Distribution maps of the Black-headed Timulla and Tropical Müllerian mimicry rings were modified from Wilson *et al.* (2012, 2015) (Fig. 1). In addition, the distribution of Caribbean *Dasymutilla* species was included (Fig. 1). Distribution maps were created for the two most speciose and diverse predatory iguanian families present in North America: the Dactyloidae (the anoles) (Fig. 2) and the Phrynosomatidae (horned-lizards, spiny-lizards, and allies) (Fig. 3). Distribution maps for dactyloids and phrynosomatids were created by overlapping, in Photoshop (Adobe Systems), known species distributions

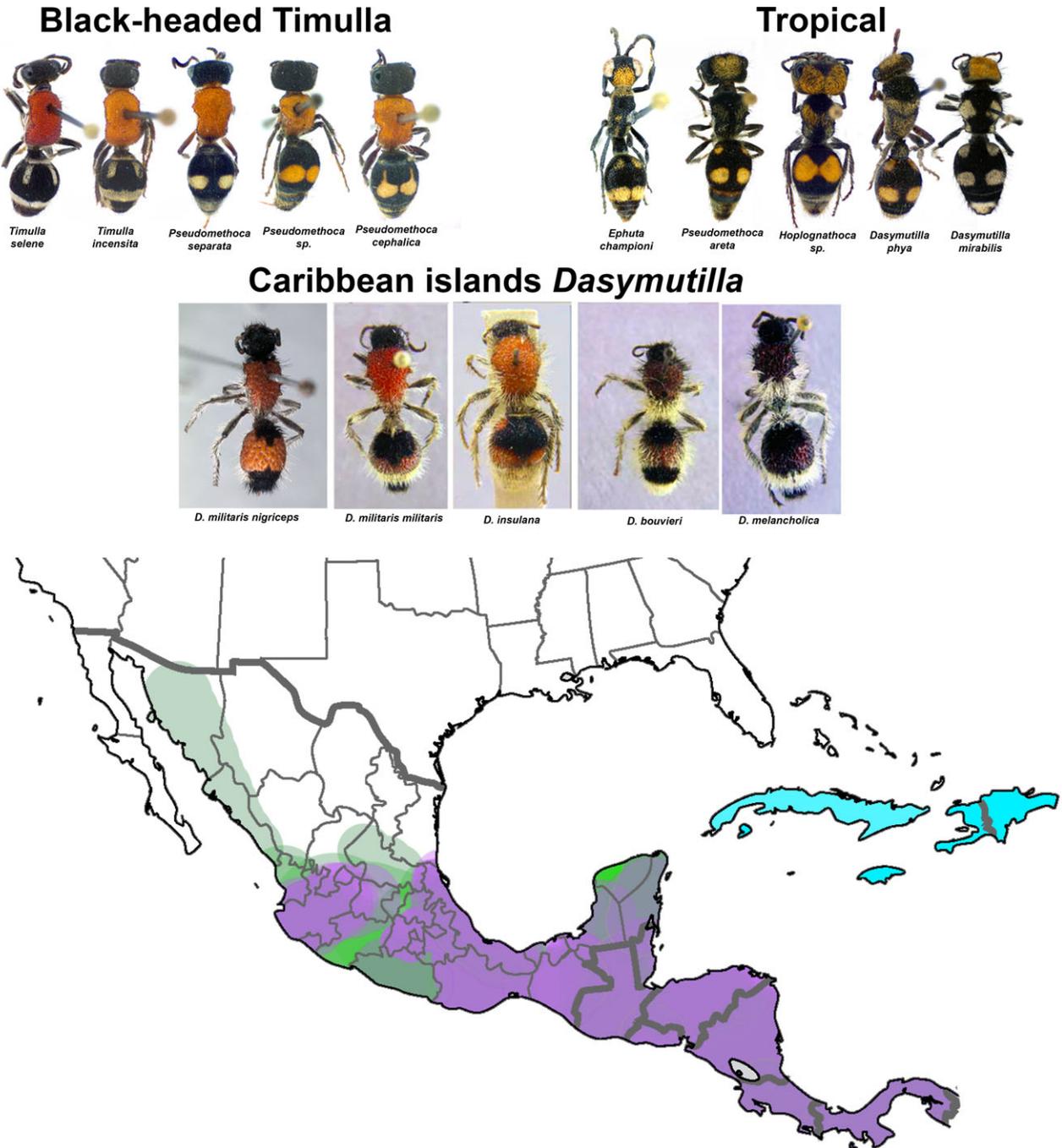


Figure 1. Representative specimens showing the coloration of the Black-headed *Timulla* and Tropical mimicry rings, as well as *Dasymutilla* specimens from the Caribbean showing the morphological similarity to the aforementioned mimicry rings. Additionally, distribution maps are provided showing the Black-headed *Timulla* ring (purple) and the Tropical mimicry ring (green) and the overlap between these rings (grey). The distribution of the Caribbean mutillids is also shown (blue).

based on published distribution maps and species descriptions (Köhler, 2008; Köhler *et al.*, 2014; IUCN, 2015) (Figs 2, 3). Mutillid mimicry species distributions were based on label data from over 20 000 specimens from the Utah State University

Insect Collections and published distributions (Manley & Pitts, 2007; Wilson *et al.*, 2012, 2015; Williams & Pitts, 2013). Similarities in distributions of predators and prey were visually compared for concordance (Figs 1, 2, 3).



Figure 2. Map showing the distribution of dactyloids (anoles). The darker the shade, the greater the number of species found in that area.

EXAMINATION OF PREDATOR ASSEMBLAGES OF CONVERGENT MIMICRY RINGS

First, morphological analyses were performed to determine whether Caribbean taxa were convergent with other published mutillid mimicry rings. Morphological characters of female mutillids were analyzed to investigate phenotypic similarities among species. Characters and methodology followed that described by Wilson *et al.* (2012). Specifically, we quantified 13 colour characters and one setal character using digital images of each species. Characters included head primary (background) colour,

head secondary colour, mesosoma background colour, mesosoma secondary colour, petiole colour, presence or absence of a contrasting setal spot on the petiole, metasoma background colour, metasoma secondary colour, presence of a third colour on the second tergite of the metasoma (T2), contrasting dark and light pattern on T2, light setal fringes on the apical tergites (T3–T5), integument colour, leg primary colour, and setal length. All colour characters were coded as black, white, yellow, orange or red. Although the differences between red, orange, and yellow can be difficult to determine, and they

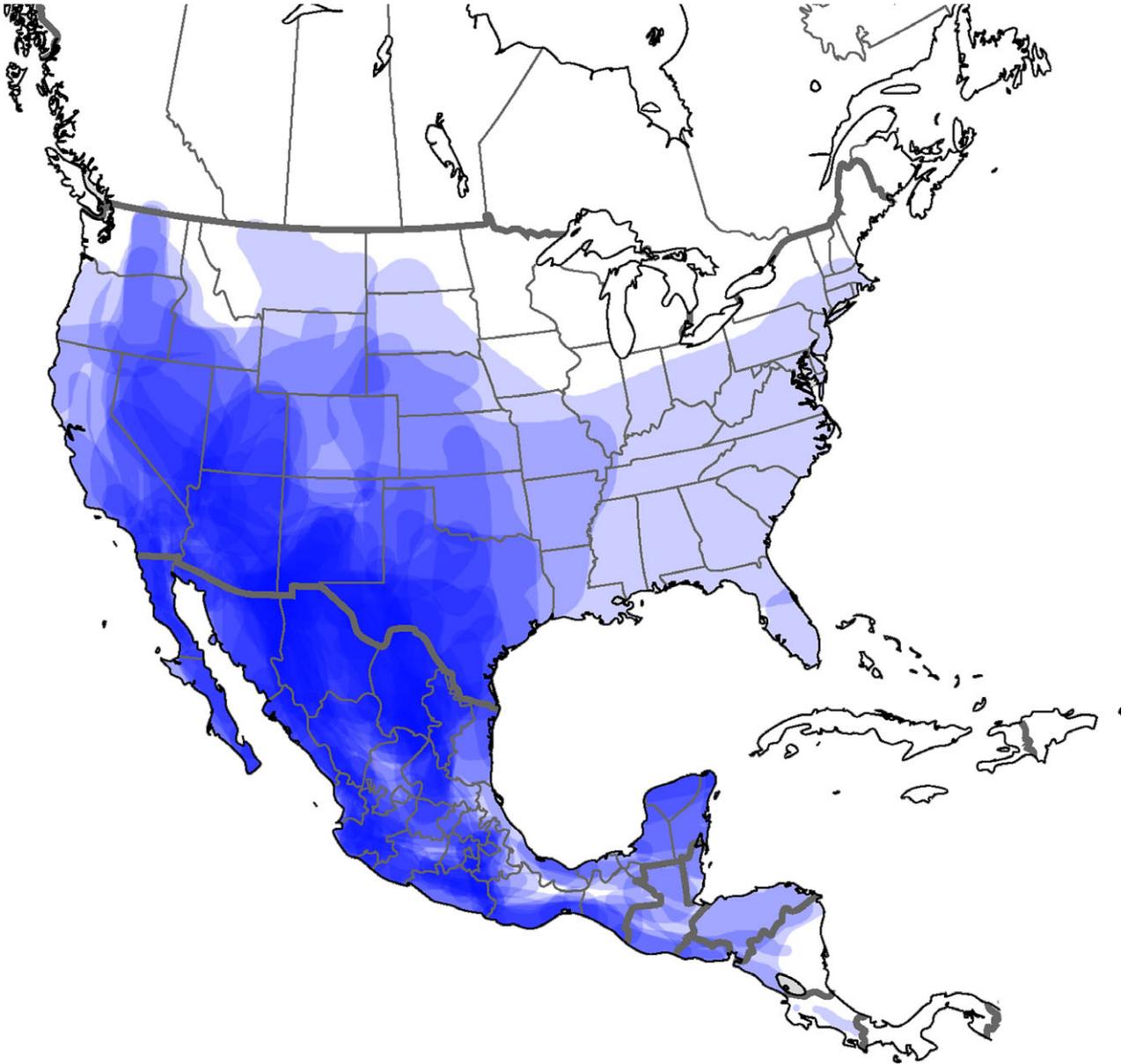


Figure 3. Map showing the distribution of phrynosomatids (horned lizards, spiny lizards, and kin). The darker the shade, greater the number of species found in that area.

likely represent a continuum, the results of the analysis indicate that the differences between these colours did not play a major role in the assignments of species to mimicry rings (i.e. yellow, orange, and red species all were placed into the Western Mimicry Ring). Other characters, where applicable, were coded as present or absent. Setal length was coded as a continuous character from 1 to 4, with 1 being hairless and 4 being the longest setae.

Morphological characters were compared with non-metric multidimensional scaling (NMDS) calculated

using the `isoMDS` function in the `MASS` package in R (R Foundation for Statistical Computing). For the NMDS, a Gower distance matrix was utilized, which is appropriate for categorical data, using the `daisy` function in the `cluster` package in R.

Predator assemblages were compared for areas where the Black-headed Timulla and Tropical Mullerian mimicry rings exist, as well as for the Caribbean taxa by examining distributions of insectivorous vertebrate taxa (including squamates and birds). Predatory groups (often at the family level) were considered potential predators if they

were well represented in areas where the target mimicry rings occur.

DIETARY PREFERENCES OF POTENTIAL PREDATORS

The dietary preferences of potential predators were examined by searching the literature describing the diet and hunting strategies of various insectivorous vertebrates that are found in areas where the target mutillids live.

SPATIAL AND TEMPORAL EVOLUTIONARY CONCORDANCE BETWEEN PREDATORS AND PREY

To examine the concordance of evolutionary histories of predator and prey, we searched the literature for proposed evolutionary ages of both predator and prey groups. Similarities in evolutionary ages could allow for some coevolution of predator and prey groups or at least some degree of historical interaction that could have influenced the evolution of mimicry. Spatial concordance was determined by investigating the literature for historical biogeographical descriptions of target taxa. This was important because current distributional similarities, if only recently developed, may not provide ample evidence for historic interactions that could be responsible for the evolution of mimicry.

RESULTS

DISTRIBUTION ANALYSIS

Diversity-distribution maps of the Dactyloidea and Phrynosomatidae differ from each other. Dactyloids are mainly restricted to south of the Trans-Mexican Volcanic Belt, with few species reaching the Mexican Plateau, and versants of the Sierra Madre Occidental [*Anolis nebulosus* (Wiegmann)] and Sierra Madre Oriental [*Anolis naufragus* (Campbell, Hillis, & Lamar)], and one native species occurring in the southern USA (*Anolis carolinensis* Voigt) (Fig. 2). The family is particularly species-rich on the Yucatan Peninsula, along the Sierra Madre del Sur, in Central America, and especially in the Caribbean, with several species occurring on each island of the Greater Antilles (Fig. 2) (Losos, 2011; Hedges, 2015). The family also extends into South America occurring as far south as southern Brazil (Nicholson *et al.*, 2012). The distribution map of the Phrynosomatidae indicates that the family is particularly species rich in western North America and northern and central Mexico, with few species represented in Central America (all represented by *Sceloporus* Wiegmann, except for *Phrynosoma asio* Cope in Guatemala), particularly south of northern Nicaragua. The family

does not occur in the Caribbean or extend into South America (Wiens, Kozak & Silva, 2013) (Fig. 3).

Mutillid species possessing aposematic coloration that falls within the Black-headed Timulla and Tropical mimicry rings have a subtropical/tropical distribution (Fig. 1). These Müllerian mimicry rings overlap greatly, being found in the Trans-Mexican Volcanic Belt region and throughout southern Mexico and Central America (Fig. 1). The Tropical ring extends farther north than the Black-headed Timulla ring, up the versant of the Sierra Madre Occidental, based on a single species, *Dasymutilla pulchra* (Smith), near the US border, although the Tropical ring is much more diverse below the Trans-Mexican Volcanic Belt. In addition, in eastern Mexico, the Tropical ring extends into northern San Luis Potosi in the southern Sierra Madre Oriental based on the distribution of another species, *Dasymutilla arachnoides* (Smith) (Manley & Pitts, 2007) (Fig. 1).

EXAMINATION OF PREDATOR ASSEMBLAGES OF CONVERGENT MIMICRY RINGS

Based on our NMDS analysis of morphological data, the Caribbean *Dasymutilla* are phenotypically similar to both the Black-headed Timulla and Tropical Müllerian mimicry rings compared to the other mimetic rings (Fig. 4).

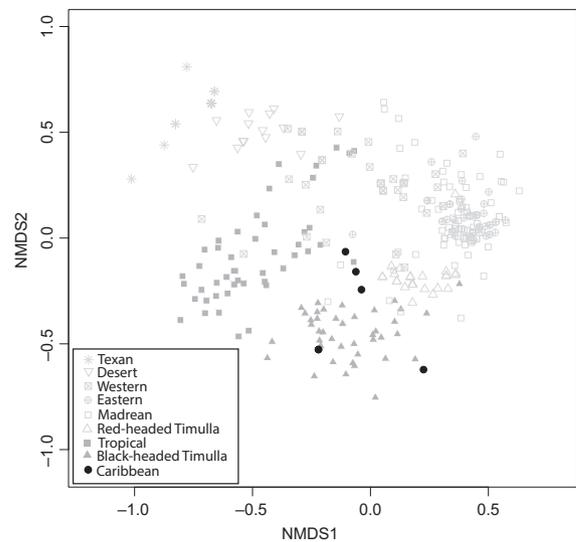


Figure 4. Non-metric multidimensional scaling (NMDS) plot showing the members of the mutillid mimicry complex. Mimicry rings restricted to the Nearctic are coloured light grey, mimicry rings found in the Neotropics are coloured dark grey, and the Caribbean taxa are coloured black. Each symbol represents an individual species. The plot shows the similarity between the Caribbean species and the Black-headed Timulla mimicry ring.

As indicated by our distributional analyses, predator assemblages show some concordance in areas where the Black-headed Timulla, Tropical, and Caribbean Müllerian mimicry rings occur, with Dactyloidae being one of the most diverse lizard taxa in these regions. Although it has been suggested that phrynosomatid lizards may feed on mutillids (Manley & Sherbrooke, 2001), the distribution of these lizards indicates that they are likely not a major part of the predator assemblage of these tropical mutillid groups.

DIETARY PREFERENCES OF POTENTIAL PREDATORS

Iguanians were considered potential predators primarily because insectivorous iguanians are sit-and-wait foraging visual predators and dietary analyses conducted by Vitt *et al.* (2003) and Vitt & Pianka (2005) indicate that hymenopterans, especially ants, and coleopterans make up a much greater percentage of prey in both desert and Neotropical iguanians than in other squamate groups, which generally exploit orthopterans, isopterans, lepidopterans, immature insects (larvae, pupae, and eggs), and spiders in their diet. These iguanian diet preferences also exist on a global scale (Pianka & Vitt, 2003; Vitt *et al.*, 2003; Vitt & Pianka, 2005; Pyron, Burbrink & Wiens, 2013; Vitt & Caldwell, 2014; Reeder *et al.*, 2015). Scincoid, lacertoid, and anguimorph lizards are typically active foragers with well-developed chemosensory systems that allow them to detect cryptic or stationary prey (i.e. termites, spiders, and insect larvae: Pianka & Vitt, 2003; Vitt *et al.*, 2003).

Although a number of insectivorous birds are present in the geographical regions encompassed by female mutillids with Black-headed Timulla and Tropical Müllerian mimicry coloration, most of these groups (families) are not likely major predators or evolutionary drivers of mutillid Müllerian mimicry rings based on the very few reports of mutillids being attacked or ingested by birds, avian hunting strategies (among these families) that generally preclude female mutillids as prey, and/or overall geographical distribution (Judd, 1899; Manley, 2000; Gabriel & Pizo, 2005). The Tyrannidae, a species rich family of insectivorous suboscine passerine birds in the Nearctic (and New World), are mainly aerial hawk or sally-glean predators, often concentrating on flying insects or those found on leaves (Fitzpatrick, 1980; Garrido & Kirkconnell, 2000; Gabriel & Pizo, 2005). In addition, the extensive New World distribution of the family overlaps with all eight mutillid Müllerian mimicry rings, and a mechanism that would explain the development, division, and the maintenance of all these rings based on predation by this family is not likely. Also, many typical Nearctic and

Neotropical insectivorous or ground-dwelling omnivorous birds (i.e. tyrannids and odontophorids) are poorly represented, or do not occur (i.e. Conopophagidae, Formicariidae, Grallariidae), in the Caribbean and likely do not play a major role in the aposematic coloration of mutillids in the West Indies (Garrido & Kirkconnell, 2000; Madge & McGowan, 2002). As a result of the factors listed above, the role of birds as drivers of aposematic coloration in female mutillids is not considered further in the present study.

SPATIAL AND TEMPORAL EVOLUTIONARY CONCORDANCE BETWEEN PREDATORS AND PREY

The Mutillidae have a poor fossil record, with all definitive fossils of the family representing crown taxa (*Dasymutilla* and *Ephuta*) from Oligo-Miocene aged Dominican amber (Manley & Poinar, 1991, 1999; Brothers, 2003). Based on relaxed molecular clock estimates, Wilson *et al.* (2013) estimated the Mutillidae may have originated in the late Cretaceous (with estimate ranges from 65 to 105 Mya). However, based on the re-assignment and evaluation of the fossil record of pompilids (a family that is closely related, and possibly sister to the velvet ants) and estimated divergence times within the family, it is likely that crown mutillids did not begin radiating until the Eocene (Williams, 2012; Wilson *et al.*, 2013; Rodriguez *et al.*, 2016).

Iguanian lizards have a fossil record that extends back to the Early Jurassic (Liassic Age; Evans, Prasad & Manhas, 2002). The earliest record of the suborder in the New World (Brazil) is from the Late Cretaceous (Turonian-Campanian; Simões *et al.*, 2015). Two Maastrichtian taxa, *Lamiasaura* Longrich *et al.* and *Pariguana* Longrich *et al.*, represent the earliest record of Iguania in North America from dentaries located in Wyoming (Longrich, Bhullar & Gauthier, 2012, 2013). During the Eocene, a number of iguanian clades that are now associated with the Neotropical region are present in western North America (Wyoming, North Dakota), including stem or possibly crown, corytophanids, dactyloids, and polychrotids (Smith, 2006, 2009; Conrad, Rieppel & Grande, 2007; Smith & Gauthier, 2013; Wiens *et al.*, 2013). The earliest definitive records of crown anoles are of late Oligocene or early Miocene age from Florida, Hispaniola, and Mexico (Lazell, 1965; Castañeda, Sherratt & Losos, 2014; Chovanec, 2014). Based on the limited fossil record and molecular clock estimates, it is likely that the stem dactyloids are no older than Late Cretaceous (Campanian) in age and perhaps as young as the early Eocene (Mulhally *et al.*, 2012; Castañeda *et al.*, 2014). In either case, crown dactyloids appear to have radiated at about the same time as mutillids in North America.

DISCUSSION

The results of the present study support our initial hypothesis that it is possible to recognize hypothetical predator candidates as evolutionary drivers by examining: (1) distributional similarities between potential predators and prey; (2) similarities of predator assemblages in areas where convergently coloured prey occur; (3) known dietary preferences of potential predators; and (4) evolutionary concordance (both spatially and temporally) of predator and prey clades. Based on our results, we further hypothesize that the Black-headed Timulla and Tropical mimicry coloration of the Nearctic and Mesoamerican regions of North America is an aposematic adaptation that is particularly effective at defending against tropical/subtropical dactyloid iguanian lizards.

DISTRIBUTIONAL SIMILARITIES

The results of the present study show that mutillids with aposematic coloration categorized within the Tropical Müllerian ring in North America have a similar geographical distribution to the Black-headed Timulla ring, with most taxa restricted to, or found south of, the Trans-Mexican Volcanic Belt. Our analysis also indicates that a number of mutillid species endemic to the Caribbean have aposematic coloration very similar to these two mimicry rings (Figs 1, 4).

Interestingly, there appears to be a phylogenetic component to the Black-headed Timulla mimicry ring. Most species within the Black-headed Timulla mimicry ring belong within the Mutillinae (*Ephuta* Say, *Timulla* Ashmead) or pseudomethocine sphaerophthalmines (*Calomutilla* Mickel, *Horcomutilla* Casal, *Pertyella* Mickel, *Lophomutilla* Mickel, *Pseudomethoca* Ashmead; Pitts, Wilson & von Dohlen, 2010) (Fig. 1). The only dasymutilline mutillid included by Wilson *et al.* (2015) in this mimicry ring is an unnamed *Traumatomutilla* André from the Canal Zone of Panama. However, despite the overall rarity of this coloration in this clade, three species of Caribbean *Dasymutilla*, the widespread *D. militaris* from the Bahamas (Great Exuma), Cuba, Hispaniola (Dominican Republic), and Jamaica, *D. bouvieri* André from Hispaniola, and *D. insulana* from the Cayman Islands and Cuba (including the Isle of Pines), have females with aposematic coloration that, based on our NMDS results, closely resembles the Black-headed Timulla mimicry ring (Mickel, 1928; Manley & Pitts, 2007; Wilson *et al.*, 2012, 2015; Williams & Pitts, 2013) (Figs 1, 4). These species have black heads, red (to violet) mesosomata, and black metasomata with yellow–orange markings (Manley & Pitts, 2007; Williams & Pitts, 2013) (Fig. 1).

Furthermore, the phylogenetic relationship of *Dasymutilla militaris* within *Dasymutilla* is unresolved, although most topologies recover *D. militaris* as an early branching lineage (Williams, 2012). By contrast, *Dasymutilla bouvieri* and *D. insulana* are situated within the variable and more nested *Dasymutilla bioculata* species complex (Williams *et al.*, 2011; Williams & Pitts, 2013). The presence of distantly related Caribbean *Dasymutilla* species with very similar aposematic coloration to Black-headed Timulla mimicry ring (i.e. convergent evolution), notably a very rare coloration in North American dasymutilline taxa (< 2% of New World dasymutilline taxa, out of almost 400 species, have this coloration), provides an opportunity to examine potential evolutionary drivers of the Black-headed Timulla mimicry ring (Fig. 1) by examining the predator faunas of the Greater Antilles compared to the remaining New World tropics (Williams, 2012).

PREDATOR FAUNAL COMPARISONS

The lizard fauna of the Greater Antilles and Bahamas are dominated (in terms of species richness) by four families: the Anguidae (galliwasp), Dactyloidae (anoles), Leiocephalidae (curly-tailed lizards), and Sphaerodactylidae (dwarf geckos; Hedges, 2015). The anoles, however, are by far the most species-rich and ecologically dominant lizards in the Caribbean, with over 120 species in the Greater Antilles (Losos, 2011; Hedges, 2015). Anoles occur in all terrestrial environments and have radiated to fill numerous microhabitats, from ground to canopy dwelling species, on most islands (Losos, 2011; Hedges, 2015). In addition, the fossil record indicates that ecological niche partitioning amongst island anoles extends back to the early Miocene, between 20 and 15 Mya, indicative of the family's ecological dominance compared to other Caribbean squamate groups throughout the Neogene (Sherratt *et al.*, 2015). The family is also species rich and ecologically prevalent in Mesoamerica and Central America (Fig. 2), with anoles being the most common lizards in many areas and niche partitioning being observed amongst co-occurring mainland species as well (Köhler *et al.*, 2014; Charreau, Cedeño-Vázquez & Köhler, 2015).

PREDATOR DIET AND NATURAL HISTORY

Although, as a whole, Caribbean anoles show great variety in diet and prey items taken, a sizeable number of species (> 15%) are reported to include a large proportion of hymenopterans, particularly ants, in their diet (Losos *et al.*, 1990; Schettino, 1999; Huang, Norval & Tso, 2008; Henderson & Powell, 2009; Losos, 2011; McDowell *et al.*, 2012). Similarly, ants

and other hymenopterans also make up a large proportion of the diet in several species of anoles in Mesoamerica and Central America [i.e. *Anolis biporcatus* (Wiegmann), *Anolis cupreus* Hallowell, *Anolis nelsoni* Barbour, *Anolis oxylophus* Cope: Losos, 2011; Vitt *et al.*, 2001; McCranie & Köhler, 2015].

Other lizards in the Caribbean islands are typically more generalist predators. Galliwaspas are active foragers that commonly search for prey in the leaf litter, and their reported prey includes millipedes, beetles, cockroaches, earwigs, isopods, gastropods, and earthworms (Barbour, 1910; Thomas & Kessler, 1996; McDowell *et al.*, 2012; Vitt & Caldwell, 2014). The Caribbean endemic and terrestrial leiocephalids have a varied diet that includes ants, beetles, cockroaches, butterflies, frogs, lizards, and plant matter (Pianka & Vitt, 2003; Losos, 2011; Kircher, Robinson & Johnson, 2014). The sphaerodactylid geckos (i.e. *Aristelliger* Cope, *Gonatodes* Fitzinger, *Sphaerodactylus* Wagler) are small lizards and typically prey on psocopterans (barklice), orthopterans (crickets), cockroaches, beetles, collembolans (springtails), insect larvae, and small spiders (Gifford, Powell & Steiner, 2000; Charruau *et al.*, 2015). Almost all of these lizard families, except perhaps the leiocephalids, which are also iguanians, likely do not take formicids as a large portion of their diet.

Dactyloids have good colour vision, possessing two retinal foveae, high photoreceptor densities, and multiple spectral classes of cones, which play a major role in both prey acquisition and intraspecific recognition via dewlap patterns and displays (Losos, 2011). In terms of prey recognition and acquisition, a study by Sexton (1963) noted that anoles appeared to differentiate between uniform and polychoured prey insects and attempted to capture unicoloured insects more often. Interestingly, preference for unicolour vs. polychoured prey occurred regardless of whether the polychoured insects were noxious or not (Sexton, 1963).

The results of the present study clearly indicate that aposematic patterns of female mutillids in the West Indies are likely not an adaptation to warning the Phrynosomatidae, which are absent from this region of North America (Fig. 3). In addition, the family is poorly represented in Neotropical portions of Mesoamerica [with only 20 species (including 18 species of *Sceloporus*) occurring south of the Trans-Mexican Volcanic Belt] and absent from South America (Köhler, 2008; Jones & Lovich, 2009; Wiens *et al.*, 2013). The family, however, may play an important and prominent role in the coloration of Western North American North American female mutillid mimicry rings. Furthermore, the differences in predator communities among the eight distinct mutillid mimicry rings could help explain how each distinct coloration

pattern evolved, although future analyses are required to identify likely predator candidates for each ring.

FOSSIL RECORD, MOLECULAR CLOCKS, AND RADIATION TIMES

Although the contemporaneous radiation of crown dactyloids and mutillids in North America is notable, it is particularly interesting when taking into account the fossil record of ants (Formicidae). The earliest formicid fossils extend back to the early Late Cretaceous (Cenomanian), although fossils of the family are rare and do not become abundant until the early Eocene (Ypresian; Grimaldi & Engel, 2005; Moreau *et al.*, 2006; Wilson & Hölldobler, 2005; Sites, Reeder & Wiens, 2011; LaPolla, Dlussky & Perrichot, 2013). During the Eocene, the family became more prevalent, often representing 20% of the fossil insects in faunas and the radiation of the species rich and diverse myrmicine, formicine, and dolichoderine ants (Wilson & Hölldobler, 2005; Moreau *et al.*, 2006; LaPolla *et al.*, 2013). Today, ants are among the most prolific and ecologically dominant organisms in subtropical and tropical environments, often making up enormous proportions of the animal biomass in tropical forests (Hölldobler & Wilson, 1994; Wilson & Hölldobler, 2005). Fossils of ants from the Oligo-Miocene amber deposits of Hispaniola make up to 24–36% of the insect fossil fauna (LaPolla *et al.*, 2013).

The proliferation of ants from the Eocene onward created an abundant, readily available prey source for insectivorous predators, including squamates, to exploit. Based on modern observations, predatory strategies, diet preferences, ecological importance, and geographical distributions are not similar amongst squamate groups (Pianka & Vitt, 2003; Vitt *et al.*, 2003; Vitt & Pianka, 2005). In the New World, iguanian lizards are more species rich and diverse, and also play a more prominent ecological role in communities than other lizard groups; their preference for hymenopterans (including ants) and coleopterans, and a visual focused sit-and-wait hunting strategy suggests that focusing on this group of lizards is warranted to determine the possible evolutionary drivers of aposematic coloration in mutillid Müllerian mimicry rings (Pianka & Vitt, 2003; Vitt *et al.*, 2003; Vitt & Pianka, 2005; Pyron *et al.*, 2013; Vitt & Caldwell, 2014; Reeder *et al.*, 2015).

CONCLUSIONS

We have presented evidence supporting the hypothesis that that Black-headed *Timulla* and Tropical mimicry coloration found in 10 and nine mutillid genera, respectively, of the Nearctic and Mesoamerican

regions of North America is an aposematic adaptation mainly directed towards tropical/subtropical dactyloid iguanian lizards. This hypothesis is supported by the similarity in distributions of the two mimetic rings and the distribution of the anoles; the similar coloration of Caribbean *Dasymutilla* species that also possess aposematic coloration closely resembling that of the mainland Black-headed Timulla and Tropical mimicry rings; the importance of ants in the diets many dactyloid species; and the similar geographical ranges and evolutionary radiation ages of iguanians, mutillids, and ants.

ADDITIONAL NOTES

The main distributions of both of these mutillid mimicry rings and the dactyloids greatly overlap and the few areas where the mimicry ring and anoles do not overlap can be easily resolved based on a recent late Neogene dispersal of *A. carolinensis* to the south-eastern USA, or the presence of a widely distributed species, *A. nebulosus*, whose reported diet does not typically consist of ants (Williams, 1969; Holman, 1995; Glor, Losos & Larson, 2005; Boyd *et al.*, 2007; Köhler *et al.*, 2014).

Another aspect that should be noted is that an iguanian predator may recognize several mimicry rings in the complex as aposematic coloration. The study hypothesis proposing iguanians as possible evolutionary drivers of Black-headed Timulla and Tropical Müllerian mimicry rings does not preclude the recognition of other mimetic rings recognized by dactyloids or other iguanians. Also, although iguanian lizards are likely the major predatory group affecting female mutillid mimicry ring coloration in North America, other predatory groups could have driven the development and maintenance of other defensive characteristics in female mutillids (Schmidt & Blum, 1977; Manley, 2000). For example, the production of pungent exudate may provide additional defences against more chemosensory adapted groups such as scincoids and anguimorphs (Manley, 2000; Vitt *et al.*, 2003; Wilson *et al.*, 2012, 2015).

The results of the present study provide a basis for additional exploration into predatory evolutionary drivers of one of the largest recognized Müllerian mimetic complexes.

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