

Phenotypic analysis of aposematic conoderine weevils (Coleoptera: Curculionidae: Conoderinae) supports the existence of three large mimicry complexes

SALVATORE S. ANZALDO^{1,*}, JOSEPH S. WILSON² and NICO M. FRANZ¹

¹*School of Life Sciences, Arizona State University, Tempe, AZ, USA*

²*Department of Biology, Utah State University, Tooele, UT, USA*

Received 30 October 2019; revised 16 December 2019; accepted for publication 18 December 2019

The Conoderinae (Coleoptera: Curculionidae) are one of the most distinctive Neotropical weevil groups in behaviour and appearance, attracting numerous hypotheses regarding the evolution and function of widespread apparent mimetic convergence. Conoderines have a poorly documented natural history, and a large fraction of the diversity of the group remains undescribed, presenting challenges to their study. In this analysis, 128 species of conoderine weevils previously or herein hypothesized to belong to three mimicry complexes are analysed in the first quantitative test of conoderine mimicry. Fifteen continuous and categorical characters describing the size, shape and coloration of these weevils were analysed using non-metric multidimensional scaling while statistically testing the resulting clusters in ordination space. Three similar, putatively mimetic complexes are recognized: (1) the ‘red-eyed fly’ complex of weevils, which are hypothesized to be evasively mimetic on various species of red-eyed flies; (2) the ‘striped/spotted’ complex, composed of weevils with a brightly coloured pronotum and red to white elytral stripes or spots; and (3) the ‘shiny blue’ complex of species with iridescent blue to blue–green pronotal scales. Each of these groups covers a wide geographical distribution and has evolved independently in multiple genera, although the red-eyed fly complex appears to be both the most species rich and widely distributed phylogenetically. Groupings were found to be statistically significant, although variation within each group suggests that the similarity in appearance of species in each group could be attributable to independent convergence on different, but phenotypically similar, models. Several avenues for future research on conoderine mimicry are discussed.

ADDITIONAL KEYWORDS: convergent evolution – evasive mimicry – Lechriopini – Zygopini.

INTRODUCTION

Weevils (Coleoptera: Curculionidae) are one of the most diverse animal radiations on the planet, with an estimated 220 000 species in existence (Oberprieler *et al.*, 2007). Unsurprisingly, this extreme species-level richness is also reflected in diverse evolved strategies to minimize predation. The Conoderinae Schoenherr, 1833 are among the largest subfamilies of weevils (Anzaldo, 2017) and are especially numerous in the New World tropics, where they have been recognized for their distinctive behaviours and convergently similar colour patterns (e.g. Champion, 1906: 1, 87). These colour patterns have prompted hypotheses on their adaptive significance (Hespenheide, 1973, 1980,

1984, 1986, 1987, 1995, 1996a, 2005, 2017, 2018); yet, despite the charismatic and often vibrantly coloured species of these weevils, no experimental or quantitative analyses of conoderine mimicry have been undertaken. This is probably attributable to the difficulty in studying conoderines: host plants and larval stages are, for the most part, entirely unknown, making it impossible at present to rear them in the laboratory without considerable advances in the knowledge of their natural history; they are infrequently collected in large numbers, and many species are known from only few localities; most species are not described (e.g. only 42% of the species used in the present study can be identified to a described species-level taxon); and the type of mimicry and the main predators driving selection for certain colour patterns are unknown, inhibiting the design and execution of experimental studies.

*Corresponding author. E-mail: sanzaldo@asu.edu

In the present study, we provide a first quantitative test of phenotypic similarity in conoderine mimicry complexes taking an approach following several recent studies testing mimetic convergence and mimetic fidelity to a model species (e.g. Wilson *et al.*, 2012; Rodriguez *et al.*, 2014; Smith *et al.*, 2015). We aim to establish formally the focal mimicry complexes and their currently known diversity and phenotypic variation. Confirmation of the phenotypic distinctiveness, or lack thereof, of previously hypothesized groupings of mimetic conoderines in the present analyses will allow these weevils to be tested further, with progressively stronger hypotheses regarding the evolution of their mimicry than was previously possible.

MIMICRY COMPLEXES IN THE NEW WORLD CONODERINAE

Throughout this paper, we follow the classificatory framework for Conoderinae proposed by Anzaldo (2017). Hespenheide (1995) provided the first overview of mimicry in this group of weevils and hypothesized several complexes based on the criteria of having distinctive, bright colour patterns that are similar to the patterns of unrelated species. Although the percentage of species thought to be mimetic increases in tropical areas, the majority of conoderine species are thought to be cryptic or non-mimetic (Hespenheide, 1995). Three prevalent hypotheses of mimetic similarity are focused on in the present analysis: (1) the 'red-eyed fly' complex; (2) the 'striped/spotted' complex; and (3) the 'shiny blue' complex. These complexes make up most of the conoderine species that have colour patterns considered aposematic, but (as our analysis reconfirms) each contains sufficient internal variation to prevent a very precise delimitation of subgroups.

Red-eyed fly complex (e.g. Figs 1, 2A–H, 3A–H)

First proposed by Hespenheide (1973), this mimicry complex is also found in several other families of beetles (e.g. Cleridae Latreille, 1802; Hespenheide 1973; Mawdsley, 1994) and other groups of weevils (e.g. Anthribidae Billberg, 1820; Hespenheide, 1973; Perger & Guerra, 2016) and has recently been proposed in jumping spiders (Salticidae Blackwall, 1841; Perger & Rubio, 2018). The greatest number of species and the greatest number of hypothesized independent origins of red-eyed fly mimicry occurs in the New World Conoderinae, where this pattern occurs in > 14 presently recognized genera and many undescribed species not assignable to described genera, and is distributed in the tribes Lechriopini Lacordaire, 1865 and Zygopini Lacordaire, 1865 (Anzaldo, 2017). Much phylogenetic and taxonomic progress is needed before the evolutionary history of conoderine

red-eyed fly mimicry (including putative convergence and reversals) can be assessed more thoroughly in a phylogenetic framework.

Given that neither the flies nor the beetles are thought to be distasteful to (likely or primarily vertebrate) predators, it has been hypothesized that this apparent mimetic convergence is based not on unpalatability, as in classical Batesian or Müllerian mimicry, but on evasiveness; the distinctive pattern is associated with unprofitability for predators that pursue unsuccessfully the fast-flying flies and the fast-flying weevils as prey (Hespenheide, 1973, 1995; Gibson, 1974; Ruxton *et al.*, 2004).

The existence and prevalence of evasive mimicry has been a contentious issue in the literature published over the past few decades (Srygley, 1994; Brower, 1995; Ruxton *et al.*, 2004; Pinheiro & Freitas, 2014; Pinheiro *et al.*, 2016). This type of mimicry has been considered most likely to occur when predator learning is aided by aposematic coloration (Ruxton *et al.*, 2004) or behavioural mimicry, such as locomotor mimicry (Srygley, 1999). Both features are suspected to occur in this system; e.g. red coloration, walking style and threat response (Hespenheide, 1973). Red-eyed fly-mimicking weevils generally have a three-part dorsal pattern (Hespenheide, 1973) consisting of: (1) an anterior red patch, which resembles the eyes of the flies; (2) a variably contrasting middle section, resembling the often longitudinally striped thorax of red-eyed flies; and (3) a mottled or unicoloured, sometimes iridescent, posterior area resembling the reflective wings of flies.

Striped/spotted complex (e.g. Figs 2I–P, 3I–P)

As defined here, this group is likely to be composed of several different and possibly not closely related mimicry rings that are currently difficult to distinguish. They share an apparent aposematic signal of a red to pink pronotum and lack the general pattern of the red-eyed fly mimics, often having white to red elytral spots or stripes on a largely black background. The 'red-spotted' group of Hespenheide (1995) is included here, in addition to variants mentioned with white spots or stripes (Hespenheide, 2017). Some species bear a resemblance to several widespread mutillid mimicry rings (e.g. the 'red-headed *Timulla*' and 'black-headed *Timulla*' mimicry rings; cf. Wilson *et al.*, 2015: fig. 1), and others resemble potentially chemically defended Chrysomelidae Latreille, 1802 (e.g. *Omophoita* Chevrolat, 1836; A. Deczynski, personal communication). Mimicry of both of these potential models is widespread in insect Batesian mimicry systems, with mimicry of mutillids also having been hypothesized to occur in other weevil groups (O'Brien, 1969; Lanteri & del Río, 2005; del Río & Lanteri, 2012) and in many other arthropods (e.g. Nentwig, 1985; Mawdsley, 1994; Schultz, 2001).

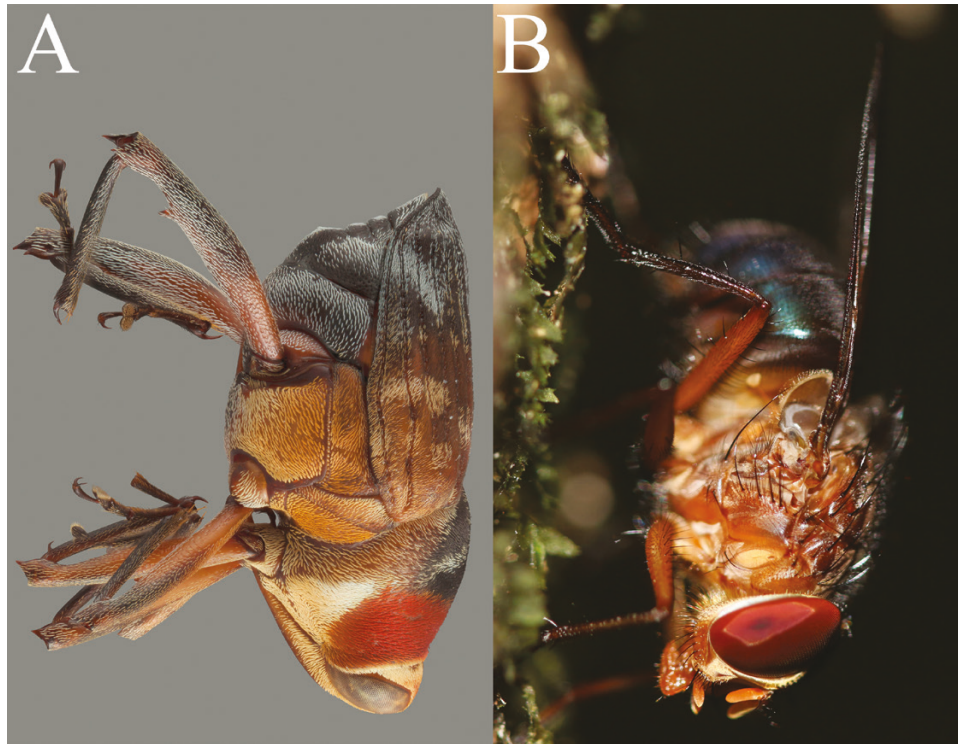


Figure 1. A, *Mnemynurus championi* Heller, 1933, a putatively fly-mimicking conoderine observed and collected while perching head-down on a tree trunk (Supporting Information, Figs S3, S4, species 65). B, potential model fly species of *M. championi*, photographed at Darién National Park, Panama by Salvatore Anzaldo.

Shiny blue complex (Figs 2Q–X, 3Q–X)

Referred to as the ‘blue-thorax’ group by Hespenheide (1995), this group was characterized by the iridescent blue to blue–green scales on the prothorax and smaller body size on average. Species have been proposed to have dolichopodid flies of the widespread genus *Medetera* Fischer von Waldheim, 1819 as their model (Hespenheide, 1995, 2005). Functionally, this system might represent another unexplored case of evasive mimicry, because dolichopodids have been recorded as having extraordinarily fast reflex responses (Sourakov, 2011).

The species included in this complex were initially treated as variants of the red-eyed fly complex (Hespenheide, 1973), because there are species that have both red and blue scales on the prothorax (e.g. Fig. 2Q–T). In a later study, these red and blue species were considered as a variant of an all-blue group (Hespenheide, 1995; Fig. 2V–X), but they were ultimately treated as a separate complex owing to their association with a distinct microhabitat from species with only blue scales (Hespenheide, 1996b, 2005). Despite these differences, the two subgroups are difficult to distinguish based on the presence/absence of red coloration, because several species contain small amounts of red on the head, and others have reddish

orange, orange or yellow. As such, the shiny blue conoderine weevils are treated here as one complex defined by the presence of iridescent blue scales.

MATERIAL AND METHODS

SPECIES SELECTION AND MORPHOLOGICAL ANALYSIS

Images of the dorsal and lateral habitus of 128 species of conoderine weevils (Supporting Information, Figs S1–S4; Table S1) were obtained using a Visionary Digital Passport II imaging system equipped with a Canon EOS 5D Mark II camera and aligned and stacked with Zerene Stacker v.1.04. All measurements were taken in Adobe Photoshop CS6 v.13.0. O’Brien & Wibmer (1982) and Wibmer & O’Brien (1986) served as the taxonomic reference for species-level entities, with the exception of more recently described species (Hespenheide, 2005, 2017, 2018).

The 128 included species belong to 20 different genera in three tribes of Conoderinae (and several species not placeable into a currently recognized genus). As many different genera were analysed as possible to maximize the inclusion of patterns potentially representing independent origins of mimicry, although species representing the occurrence of these mimicry

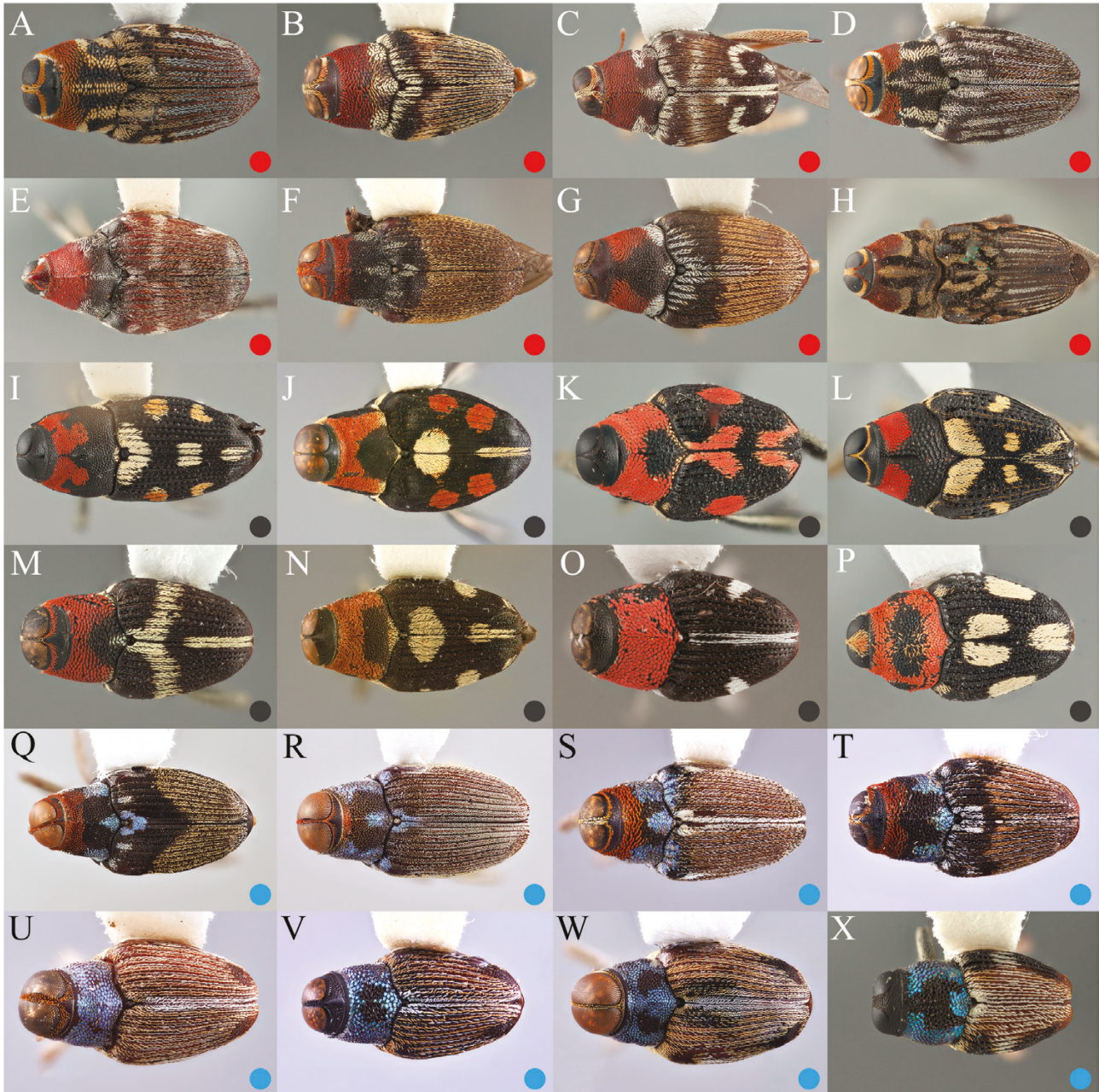


Figure 2. Dorsal habitus of putatively mimetic weevils assigned to mimicry complex. Colour code: red, red-eyed fly; black, striped/spotted; blue, shiny blue. The number in square brackets corresponds to the species number in the [Supporting Information](#). A, *Copturomimus* Heller, 1895 sp. 1 [1]. B, *Hoplocopturus* Heller, 1895 sp. 11 [6]. C, *Hoplocopturus* sp. 14 [9]. D, *Macrocopturus* Heller, 1895 sp. 12 [14]. E, *Mnemyrnus* Heller, 1895 sp. 11 [25]. F, *Macrocopturus* sp. 16 [16]. G, *Macrocopturus* sp. 17 [19]. H, *Zygops rufomaculatus* Champion, 1906 [70]. I, *Macrocopturus* sp. 24 [74]. J, *Macrocopturus richardpackeri* (Hespenheide, 2017) [82]. K, *Copturus coccinatus* (Champion, 1906) [105]. L, *Copturus* Schoenherr, 1825 sp. 1 [75]. M, *Macrocopturus* sp. 25 [96]. N, *Macrocopturus* sp. 29 [79]. O, *Lechriopini* gen. sp. 2 [98]. P, *Copturus* sp. 3 [85]. Q, *Macrocopturus torquatus* (Heller, 1895) [111]. R, *Macrocopturus* sp. 6 [112]. S, *Macrocopturus* sp. 7 [114]. T, *Macrocopturus* sp. 3 [116]. U, *Macrocopturus* nr. *lamprothorax* sp. 4 [118]. V, *Macrocopturus* nr. *lamprothorax* sp. 2 [119]. W, *Macrocopturus* nr. *lamprothorax* sp. 1 [121]. X, *Macrocopturus* sp. 5 [125].

complexes in other genera not analysed here are known. Species were identified a priori into mimicry complexes based on perceived visual similarity or

dissimilarity to those previously designated to a mimicry complex (Hespenheide, 1973, 1995, 2005, 2017, 2018). Accordingly, the examined species pertain



Figure 3. Lateral habitus of putatively mimetic weevils assigned to mimicry complex. Colour code: red, red-eyed fly; black, striped/spotted; blue, shiny blue. The number in square brackets corresponds to the species number in the [Supporting Information](#). A, *Copturomimus* sp. 1 [1]. B, *Hoplocopturus* sp. 11 [6]. C, *Hoplocopturus* sp. 14 [9]. D, *Macrocopturus* sp. 12 [14]. E, *Mnemynurus* sp. 11 [25]. F, *Macrocopturus* sp. 16 [16]. G, *Macrocopturus* sp. 17 [19]. H, *Zygops rufomaculatus* [70]. I, *Macrocopturus* sp. 24 [74]. J, *Macrocopturus richardpackeri* [82]. K, *Copturus coccinatus* [105]. L, *Copturus* sp. 1 [75]. M, *Macrocopturus* sp. 25 [96]. N, *Macrocopturus* sp. 29 [79]. O, *Lechriopini* gen. sp. 2 [98]. P, *Copturus* sp. 3 [85]. Q, *Macrocopturus torquatus* [111]. R, *Macrocopturus* sp. 6 [112]. S, *Macrocopturus* sp. 7 [114]. T, *Macrocopturus* sp. 3 [116]. U, *Macrocopturus* nr. *lamprothorax* sp. 4 [118]. V, *Macrocopturus* nr. *lamprothorax* sp. 2 [119]. W, *Macrocopturus* nr. *lamprothorax* sp. 1 [121]. X, *Macrocopturus* sp. 5 [125].

to the three focal complexes: the red-eyed fly complex (73 species in 14 different genera and 3 tribes); the spotted/striped complex (34 species in 8 genera and

3 tribes, including several lechriopine species not placeable into a current genus); and the shiny blue complex (21 species in 5 genera and 2 tribes). The

shiny blue complex was split further into two groups in a separate analysis (Supporting Information, Fig. S6) to test the hypothesis that species with red scales pertain to a distinct group.

Each species was scored for four continuous and 11 discrete (binary or multistate) phenotypic characters (Table S2). The preferred method of coding characters that applied to only a subset of taxa (e.g. character 4) was to treat them as inapplicable for the taxa without the character (Strong & Lipscomb, 1999; Franz, 2014). Alternative coding methods, such as composite coding, would have resulted in increased similarity among taxa that lack these features. As an example, taxa that were scored as not having red/orange/pink on the pronotum/head (character 3) were not scored as a separate state of 'absent' for character 4 or as '0%' red/orange/pink for character 5; instead, they were classified as inapplicable ('NA') for these structurally contingent characters and states.

NON-METRIC MULTIDIMENSIONAL SCALING AND PERMUTATIONAL MULTIVARIATE ANALYSIS OF VARIANCE

The obtained character matrix was transformed into a distance matrix using Gower distances with the isoMDS function of the MASS package (Venables & Ripley, 2002) in R (R Core Team, 2014; e.g. Wilson *et al.*, 2018). The emergent signal was visualized in three-dimensional graphical space with non-metric multidimensional scaling (NMDS), a robust ordination method that allows for the incorporation of categorical and continuous data. The similarity between groups or individuals was represented by their proximity in graphical space.

The grouping of species to their a priori assignments was tested using permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001), a non-parametric statistical method used to test the null hypothesis that the groupings are not different, as implemented by the adonis function of the vegan package in R (Oksanen *et al.*, 2018). This method tests for the overall effect of the groupings in addition to pairwise relationships between all complexes. If the groupings are shown to be significant as a categorical variable, a distinct clustering of the points pertaining to each complex is expected.

PHENOTYPIC CHARACTERS

Reference to specific character states of discrete characters in the text adopts the following syntactic convention: '3:0' refers to character 3, state 0.

1. Body length. Coded as a continuous character and measured as the dorsal length from the anterior

margin of the head (excluding the rostrum) and the posterior margin of the elytra or pygidium.

2. Body length/width ratio. Coded as a continuous character. Body length measured as in character 1, and width measured as the distance across the elytral humeri.
3. Red/orange/pink colour on pronotum and/or head: (0) absent; (1) present.
4. Shape of red/orange/pink patch on pronotum, dorsal view: (0) a single transverse patch mostly in the anterior two-thirds of the pronotum (e.g. Fig. 2B); (1) a single patch covering all or nearly all of the pronotum (e.g. Fig. 2O); (2) two separate anterolateral patches (e.g. Fig. 2G); (3) more than two separate patches; (4) a patch encircling a transversely ovoid median black area (e.g. Fig. 2P); (5) a single patch extending completely or almost completely to the posterior margin at the posterolateral corners, enclosing a semicircular black space along the posterior margin at the middle (e.g. Fig. 2J); (6) coloration present at anterior margin unconnected with coloration at posterior margin. This character was scored as inapplicable (NA) for taxa without red on the pronotum and/or head (3:0).
5. Percentage of red/orange/pink on dorsal surface. Coded as a continuous character. This character was scored as inapplicable (NA) for taxa without red on the pronotum and/or head (3:0).
6. Posterior red/orange/pink (on abdominal sternites, pygidium or elytral apex): (0) absent; (1) present, in one or two spots; (2) present, dominant colour on abdominal sternites.
7. Iridescent blue to blue–green scales on pronotum/head/elytral base: (0) absent; (1) present.
8. Percentage of blue/blue–green scales on dorsal surface. Coded as a continuous character. This character was scored as inapplicable (NA) for taxa without blue scales (7:0).
9. Dominant elytral colour (by rough percentage), dorsal view: (0) black; (1) white/grey; (2) brown/tan; (3) red/orange/pink; (4) yellow.
10. Secondary elytral colour (by rough percentage, dorsal view): (0) black; (1) white/grey; (2) brown/tan; (3) red/orange/pink; (4) yellow.
11. Elytral sutural stripe: (0) absent; (1) present, incomplete (e.g. Fig. 2J); (2) present, complete (e.g. Fig. 2O).
12. Short, transverse bar across elytral suture at middle to one-third from apex: (0) absent; (1) present (e.g. Fig. 2C).
13. Lateral/ventral pattern: (0): mottled/indistinctly contrasting (e.g. Fig. 3F); (1) strongly contrasting, mostly white and black (e.g. Fig. 3J).
14. Prothoracic area bordering red or blue patch (lateral view): (0) indistinctly contrasting, completely or mostly mottled with white (e.g.

Fig. 3F); (1) contrasting, with dense white patch at least partly along ventrolateral margin (between the procoxa and the ventral part of head), bordered posteriorly by black (e.g. Fig. 3J); (2) white absent or not prominent.

15. Arrangement of blue or blue and red scales on the pronotum (dorsal view): (0) uniformly covered with blue scales; (1) with one black space (e.g. Fig. 2Q); (2) with two black spaces (e.g. Fig. 2W).

RESULTS

The NMDS analysis (Fig. 4, S5) shows the clustering of species by their assignment in a mimicry complex in three-dimensional space. The overall effect of these species groupings as a categorical variable was found to be significant ($F = 47.69$; $R^2 = 0.433$; $P < 0.001$). Ordination plots show the relative separation and clustering of species in each of the three complexes, supported by significant pairwise PERMANOVA results between all pairs (Table 1).

RED-EYED FLY COMPLEX

Nearly all of the species assigned to this group had a red region on the pronotum and/or head that was present in one transverse (4:0) or two lateral (4:2) anterior patches. A few species (11%) shared a red posterior patch (6:1), which was also found in some members of the shiny blue complex but not in any striped/spotted members. The remainder of the patterns observed were highly variable.

SPOTTED/STRIPED COMPLEX

Considerable variation was observed regarding the shape and extent of coverage of the bright red to pink pronotal patch. All encoded states for the shape of the pronotal patch were present in this group, although two states (4:4 and 4:5) were each present in nine included species and seem to be correlated with other features. There are numerous intermediate forms that prevent a clear subdivision of species in this group. Many species have a strongly contrasting, tri-coloured lateral region of the prothorax, with the anteroventral margin (between the procoxa and head) white, dorsally and anterodorsally red to pink, and black, usually bare cuticle, centrally and posteriorly (14:1).

SHINY BLUE COMPLEX

The analysis recovered one distinct group including species with both blue and red scales, despite evidence from natural history and some phenotypic difference suggesting that mimicry in this group is likely to

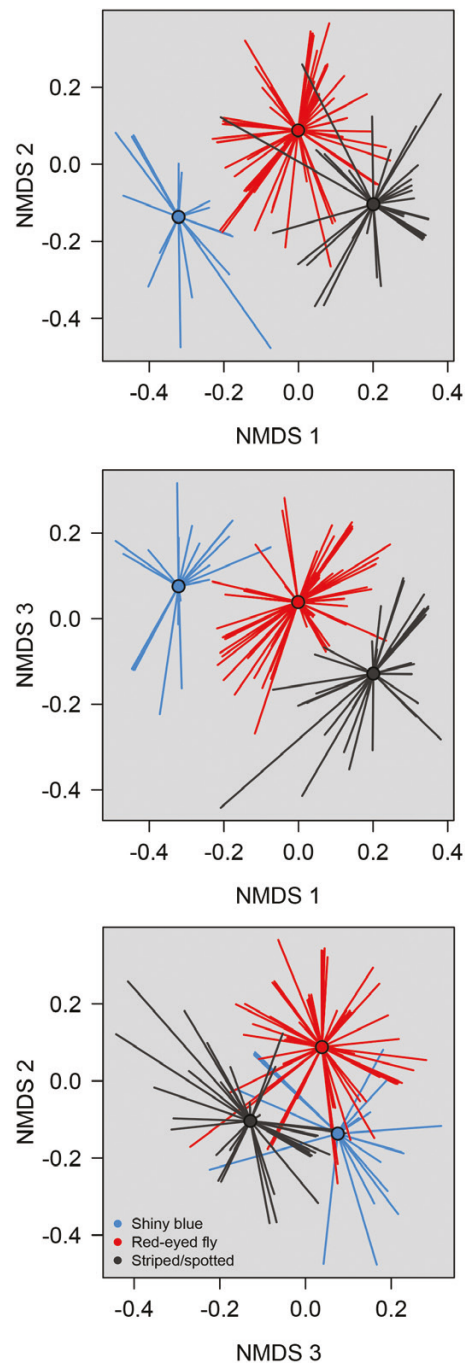


Figure 4. Non-metric multidimensional scaling analysis showing the clustering of points in ordination space. The mean value for each complex is represented by a circle, and lines are drawn from the mean to the values for individual species. Greater proximity of individual points (species) represents greater overall similarity. Colour code: red, red-eyed fly; black, striped/spotted; blue, shiny blue. See Material and Methods for further explanation.

be based on different models (Hespenheide, 2005). When split into two groups based on the presence or absence of red scales, the clusters of points were

Table 1. Pairwise PERMANOVA results between each mimicry complex

Complex 1	Complex 2	R^2	F	Residual d.f.	P -value
Shiny blue	Red-eyed fly	0.324	44.1	92	0.001
Shiny blue	Striped/spotted	0.538	61.8	53	0.001
Red-eyed fly	Striped/spotted	0.288	42.4	105	0.001

incompletely separated, and PERMANOVA results were non-significant (Supporting Information, Fig. S6). Two species (9.5%) shared the red posterior patch found in some red-eyed fly mimics. Although the group is defined by the presence of iridescent blue scales, there is considerable variation in the arrangement (character 15) and location on the body. All species lack the strongly contrasting lateral patterns found in many species of the other two groups (13:0).

DISCUSSION

Our analysis shows three diverse mimicry complexes of conoderine weevils to be distinct from each other based on many continuous and categorical phenotypic characters, representing the first rigorous, quantitative study of mimicry complexes in this diverse weevil taxon using transparent character coding and powerful statistical methods. This allows for a greater understanding of the diversity of and similarity in colour patterns using a dataset that covers a broad phylogenetic spectrum, which in turn can lead to sound evolutionary inferences. However, the evolution of conoderine mimicry complexes has probably been shaped by many complex and interrelated factors. Further exploration is hindered by a lack of knowledge about the natural history and phylogeny of these species. As these limitations are overcome, other analytical approaches not constrained by an initially subjective a priori sorting of species based on human perception of colour and pattern will be possible.

PREDATION PRESSURE

A bright red to pink coloration is a significant component of the mimetic pattern of most analysed species. It is therefore unlikely that arthropod predators, many of which lack a receptor for detecting long wavelengths, such as red (Briscoe & Chittka, 2001), are the main selective force driving the evolution of these mimicry complexes. To an insect predator, conspicuous long-wavelength aposematic signals could instead be cryptic (Fabricant & Herberstein, 2015). Considering the range in body size, wide geographical distribution and apparent specialization to different microhabitats (Hespenheide, 1996b) found within these mimicry complexes, it appears that many

predator species, probably birds and lizards, could act as a selective force driving the evolution of these colour patterns. Hespenheide (1973) posits that anoles are the most common predator. Iguanian lizards, such as anoles, have been shown to be the likely driver of several mutillid mimicry rings in Central America and the Caribbean (Pan *et al.*, 2017). As an additional supporting component of this interpretation, the overlap in range of the mutillids (including the 'black-headed *Timulla*' ring hypothesized to be a model for some species in the striped/spotted complex) and anoles shown by Pan *et al.* (2017) largely coincides with that of the weevils included in the present study.

IMPERFECT MIMICRY

Another aspect to the variation seen in this group can potentially be explained by imperfect mimicry (e.g. Sherratt, 2002; Wilson *et al.* 2013). It is currently unknown whether species in, for example, the red-eyed fly complex are high-fidelity mimics of a specific fly model or share general characteristics that are similar enough to the diversity of model flies present in a given locality that they gain a selective advantage from it (i.e. they represent an 'averaging' of characteristics; Hespenheide, 1973). Some weevil species strongly resemble specific flies (Fig. 1). The appearance of this weevil–fly pairing, for example, is considerably different from another frequent pattern within the red-eyed fly complex, i.e. an appearance resembling many sarcophagid flies, with predominantly grey and black stripes and sometimes a red posterior abdominal spot (e.g. Fig. 2D; Vanin & Guerra, 2012; Guerra, 2019). Hespenheide (1973) reported several fly species at a specific locality that potentially serve as models for some of the beetle species considered to be red-eyed fly mimics.

With little known about the behaviour of individual weevil species, it is currently impossible to assess whether evasive Batesian or evasive Müllerian mimicry is more prevalent among these mimetic conoderines, or even whether the energetic cost of the pursuit of difficult-to-catch weevils and flies is a major factor driving the evolution of this phenomenon. Hespenheide (1973) discussed other weevils (Curculionidae: Baridinae Schoenherr, 1836) with a similar pattern that are 'sluggish' in their movements,

considering this as a type of Batesian mimicry, because they do not also share the fast-flying qualities of the flies they resemble but are potentially benefitting from the same protection against predation. Although many species of tropical Conoderinae are fast-flying, making the system presumably Müllerian for these species, at least one conoderine species ascribed to this mimicry complex is reported to be slow and first to resort to thanatosis ('playing dead') rather than flying away (Vanin & Guerra, 2012; Guerra, 2019).

PHYLOGENETIC CONSIDERATIONS

The question of how much similarity in the mimetic facies of these mimicry complexes is a product of common ancestry, convergent evolution or reversal cannot be addressed adequately until phylogenetic analyses target these lineages of Conoderinae. The knowledge of the relationships between and within conoderine tribes is very incomplete, and genera that contain a large number of mimetic species (e.g. *Macrocopturus* Heller, 1895) are likely not to be monophyletic as they are currently defined. Some closely related genera (e.g. *Mnemyrnurus* Heller, 1895, *Hoplocopturus* Heller, 1895 and *Balaninurus* Heller, 1895) contain many red-eyed fly-mimetic species, potentially representing one independent acquisition of this mimicry in a common ancestor (Hespenheide, 1995).

The red-eyed fly and striped/spotted complexes as defined here occur in three tribes of Conoderinae. Even taking into account the many classificatory changes necessary to redefine the tribes into natural groups (S. S. Anzaldo, unpublished data), these complexes are still present in three different tribes, although the majority of species and of potentially independent origins will probably be shown to occur in the Lechriopini. The species at present known to pertain to the shiny blue complex are currently distributed in the Lechriopini and Zygopini but will probably be restricted to the Lechriopini. Within the Lechriopini, many independent origins are probable for each complex, although more exact estimates are not possible with the current state of phylogenetic knowledge.

BIOGEOGRAPHICAL DISTRIBUTION OF CONODERINE MIMICRY

The focal mimicry complexes for this study are each widely distributed in the New World tropics (Hespenheide, 1995). Distributions of individual species of conoderine weevils are very poorly known. Many included species are known from only one or a few localities, restricting our ability to associate individual species with potentially co-occurring

models. Understanding both historical and extant species distribution patterns is an important factor when considering whether species are involved in the same mimicry complex. Advances in this knowledge will be likely to play a role in redefining these mimetic groups based on specific available model species.

Although apparently much less diverse, species that seem to pertain to the red-eyed fly and the striped/spotted complexes have been observed among specimens from the Old World tropics (Supporting Information, Figs S3, S4, species 71–73 and 103). Flies with red eyes are found in many dipteran groups worldwide and, considering how readily mimicry of them appears to have evolved in different New World taxa, it is not surprising that the Old World conoderine tribe Mecopini Lacordaire, 1865, a group with similar behaviours (Lyal, 1986) to their New World relatives in the Lechriopini and Zygopini, have also evolved a convergent phenotypic appearance. Both New World and Old World species of Buprestidae Leach, 1815 are also known from this mimicry complex (Hespenheide HA, personal communication). Mutillids are also a plausible group to serve as models for Old World conoderines, with very similar Old World mimicry rings to those found in the Neotropics (e.g. Wilson *et al.*, 2018).

Another important factor to be considered is the microhabitat within an individual locality where these weevils spend time and are thus exposed to predators. Hespenheide (1996b) discussed conoderine mimicry as being structured by plant communities, with proposed mimicry complexes occurring in separate microhabitats (e.g. on tree trunks, on the underside of leaves). If mimetic species are spending most of their time in a certain microhabitat, they are potentially more or less exposed to different types of predators, different types and densities of alternative prey for the predators to pursue instead, in addition to different mimic and model species that can influence the selective pressures faced by the weevils (Wilson *et al.*, 2013).

FUTURE DIRECTIONS AND CONCLUSIONS

The Conoderinae present an underexplored yet highly promising system to study the evolution of different types of mimicry, including evasive mimicry. Although the inferences that can be drawn from this study about the purpose and the evolution of mimetic patterns are limited, this analysis can be used as a starting point to drive further discoveries about conoderine mimicry and develop more informed hypotheses, including alternative explanations for putative similarity (e.g. Ruxton & Schaeffer, 2011). There are many types of analyses that can provide finer resolution for the delimitation of the mimicry complexes presented here

into smaller, more precisely defined groups that reflect their convergence as a product of shared selection pressures.

Similarity in behaviours, occurrence in specific microhabitats, predation pressure, alternative prey, mimic and model densities and phylogenetic relationships (information that is currently unavailable for the vast majority of species) are all likely to play a role in shaping the evolution of convergent colour patterns in the Conoderinae. Experimental field studies will undoubtedly be instrumental in uncovering this crucial information about mimetic species that will allow new hypotheses to be formulated and tested. It is hoped that future analyses can build on the present study as more knowledge is accumulated on these fascinating weevils.

ACKNOWLEDGEMENTS

This research was primarily funded by the United States Department of Agriculture - Agricultural Research Service (agreement 58-1275-1-335) and the National Science Foundation Collections in Support of Biological Research award 1756327. Many specimens used in the analysis were generously provided by the late Dr Charles O'Brien. We would also like to thank Lourdes Chamorro (NMNH), Bob Anderson (CMNC), Hector Barrios (PCMENT), Max Barclay (NHMUK) and Olaf Jäger (MTD) for loans of additional specimens. Type specimens included in the analysis were imaged at the NHMUK and MTD with funds from the Harvard Museum of Comparative Zoology Ernst Mayr Travel Award, the Society of Systematic Biologists Mini-ARTS Award, and an Arizona State University Graduate Student Support award. Henry Hespenheide, Carlos Muñoz-Ramírez and an anonymous reviewer provided constructive feedback that improved the manuscript.

REFERENCES

- Anderson MJ. 2001.** A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26**: 32–46.
- Anzaldo SS. 2017.** Review of the genera of Conoderinae (Coleoptera, Curculionidae) from North America, Central America, and the Caribbean. *Zookeys* **683**: 51–138.
- Briscoe AD, Chittka L. 2001.** The evolution of color vision in insects. *Annual Review of Entomology* **46**: 471–510.
- Brower AVZ. 1995.** Locomotor mimicry in butterflies? A critical review of the evidence. *Philosophical Transactions of the Royal Society B: Biological Sciences* **347**: 423–425.
- Champion GC. 1906.** *Zygopina*. London: *Biologia Centrali-Americana*, Vol. 4, part 5, 1–130.
- Fabricant SA, Herberstein ME. 2015.** Hidden in plain orange: aposematic coloration is cryptic to a colorblind insect predator. *Behavioral Ecology* **26**: 38–44.
- Franz NM. 2014.** Anatomy of a cladistic analysis. *Cladistics* **30**: 294–321.
- Gibson DO. 1974.** Batesian mimicry without distastefulness? *Nature* **250**: 77–79.
- Guerra TJ. 2019.** Evasive mimicry: too beetle, or not too beetle? *Ecology* **100**: e02773.
- Hespenheide HA. 1973.** A novel mimicry complex: beetles and flies. *Journal of Entomology* **48**: 49–56.
- Hespenheide HA. 1980.** New species and a review of the genus *Helleriella* (Coleoptera: Curculionidae: Zygopinae). *The Coleopterists Bulletin* **34**: 323–332.
- Hespenheide HA. 1984.** New Neotropical species of putative ant-mimicking weevils (Coleoptera: Curculionidae: Zygopinae). *The Coleopterists Bulletin* **38**: 313–321.
- Hespenheide HA. 1986.** Mimicry of ants of the genus *Zacryptocerus* (Hymenoptera: Formicidae). *Journal of the New York Entomological Society* **94**: 394–408.
- Hespenheide HA. 1987.** A revision of *Lissoderes* Champion (Coleoptera: Curculionidae: Zygopinae). *The Coleopterists Bulletin* **41**: 41–55.
- Hespenheide HA. 1995.** Mimicry in the Zygopinae (Coleoptera: Curculionidae). *Memoirs of the Entomological Society of Washington* **14**: 145–154.
- Hespenheide HA. 1996a.** Clytrine chrysomelids as models of mimicry complexes. In: Jolivet PHL, Cox ML eds. *Chrysomelid biology, volume 2: ecological studies*. The Hague: SPB Academic, 1–2.
- Hespenheide HA. 1996b.** The role of plants in structuring communities of mimetic insects. In: Gibson AC, ed. *Neotropical biodiversity and conservation*. Los Angeles: University of California, 109–126.
- Hespenheide HA. 2005.** Weevils of the genera *Archocopturus* Heller and *Zygopsella* Champion: sibling species and mimetic homoplasy (Coleoptera: Curculionidae: Conoderinae). *Proceedings of the Entomological Society of Washington* **107**: 671–685.
- Hespenheide HA. 2017.** A striking new species of *Macrocopturus* Heller, 1895 (Coleoptera: Curculionidae: Conoderinae) from Panama: raising the question of coloration. *The Coleopterists Bulletin* **71**: 74–76.
- Hespenheide HA. 2018.** A review of *Philenis* Champion, 1906 (Coleoptera: Curculionidae: Conoderinae), with descriptions of new species from Central and South America. *Diversity* **10**: 84.
- Lanteri AA, del Río MG. 2005.** Taxonomy of the monotypic genus *Trichaptus* Pascoe (Coleoptera: Curculionidae: Entiminae), a potential weevil mimic of Mutillidae. *The Coleopterists Bulletin* **59**: 47–54.
- Lyal CHC. 1986.** Observations on zygopine weevil behavior (Coleoptera: Curculionidae: Zygopinae). *Journal of Natural History* **20**: 789–798.
- Mawdsley JR. 1994.** Mimicry in the Cleridae (Coleoptera). *The Coleopterists Bulletin* **48**: 115–125.
- Nentwig W. 1985.** A mimicry complex between mutillid wasps (Hymenoptera: Mutillidae) and spiders (Araneae). *Studies on Neotropical Fauna and Environment* **20**: 113–116.
- O'Brien CW. 1969.** A new species of *Gerstaeckeria* in Peru, first record for South America (Coleoptera, Curculionidae). *The Coleopterists Bulletin* **23**: 73–76.

- O'Brien CW, Wibmer GJ. 1982. Annotated checklist of the weevils (Curculionidae *sensu lato*) of North America, Central America, and the West Indies (Coleoptera: Curculionidae). *Memoirs of the American Entomological Institute* **34**: 1–382.
- Oberprieler RG, Marvaldi AE, Anderson RS. 2007. Weevils, weevils, weevils everywhere. *Zootaxa* **1668**: 491–520.
- Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner ESH. 2018. *vegan: community ecology package*. R package version 2.5-2. Available at: <https://CRAN.R-project.org/package=vegan>
- Pan A, Williams KA, Wilson JS. 2017. Are diurnal iguanian lizards the evolutionary drivers of New World female velvet ant (Hymenoptera: Mutillidae) Müllerian mimicry rings? *Biological Journal of the Linnean Society* **120**: 436–447.
- Perger R, Guerra F. 2016. The description of a new calyptrate fly mimicking species of the fungus weevil genus *Gymnognathus* Schönherr 1826 from the Southern Bolivian Andes (Coleoptera: Anthribidae: Anthribinae). *Zootaxa* **4084**: 277–284.
- Perger R, Rubio GD. 2018. A wolf in sheep's clothing: the description of a fly resembling jumping spider of the genus *Scoturius* Simon, 1901 (Araneae: Salticidae: Huriini). *PLoS ONE* **13**: e0190582.
- Pinheiro CEG, Freitas AVL. 2014. Some possible cases of escape mimicry in Neotropical butterflies. *Neotropical Entomology* **43**: 393–398.
- Pinheiro CEG, Freitas AVL, Campos VC, DeVries PJ, Penz CM. 2016. Both palatable and unpalatable butterflies use bright colors to signal difficulty of capture to predators. *Neotropical Entomology* **45**: 107–113.
- R Core Team. 2014. *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: <http://www.R-project.org/>
- del Rio MG, Lanteri AA. 2012. Redescription of the genus *Curiades* Pascoe 1880 (Coleoptera: Curculionidae: Entiminae: Naupactini), a potential mimic of Mutillidae from Brazil. *Zootaxa* **3570**: 82–88.
- Rodriguez J, Pitts JP, von Dohlen CD, Wilson JS. 2014. Müllerian mimicry as a result of codivergence between velvet ants and spider wasps. *PLoS ONE* **9**: e112942.
- Ruxton GD, Schaefer HM. 2011. Alternative explanations for apparent mimicry. *Journal of Ecology* **99**: 899–904.
- Ruxton GD, Speed M, Sherratt TN. 2004. Evasive mimicry: when (if ever) could mimicry based on difficulty to capture evolve? *Proceedings of the Royal Society B: Biological Sciences* **271**: 2135–2142.
- Schultz TD. 2001. Tiger beetle defenses revisited: alternative defense strategies and colorations of two Neotropical tiger beetles, *Odontocheila nicaraguensis* Bates and *Pseudoxyscheila tarsalis* Bates (Carabidae: Cicindelinae). *The Coleopterists Bulletin* **55**: 153–163.
- Sherratt TN. 2002. The evolution of imperfect mimicry. *Behavioral Ecology* **13**: 821–826.
- Smith AD, Wilson JS, Cognato AI. 2015. The evolution of Batesian mimicry within the North American Asidini (Coleoptera: Tenebrionidae). *Cladistics* **31**: 441–454.
- Sourakov A. 2011. Faster than a flash: the fastest visual startle reflex response is found in a long-legged fly *Condylostylus* sp. (Dolichopodidae). *The Florida Entomologist* **94**: 367–369.
- Srygley RB. 1994. Locomotor mimicry in butterflies? The associations of positions of centres of mass among groups of mimetic, unprofitable prey. *Philosophical Transactions of the Royal Society B: Biological Sciences* **343**: 145–155.
- Srygley RB. 1999. Incorporating motion into investigations of mimicry. *Evolutionary Ecology* **13**: 691–708.
- Strong EE, Lipscomb D. 1999. Character coding and inapplicable data. *Cladistics* **15**: 363–371.
- Vanin SA, Guerra TJ. 2012. A remarkable new species of flesh-fly mimicking weevil (Coleoptera: Curculionidae: Conoderinae) from Southeastern Brazil. *Zootaxa* **3413**: 55–63.
- Venables WN, Ripley BD. 2002. *Modern applied statistics with S*, 4th edn. New York: Springer. ISBN: 0-387-95457-0.
- Wibmer GJ, O'Brien CW. 1986. Annotated checklist of the weevils (Curculionidae *sensu lato*) of South America (Coleoptera: Curculionidae). *Memoirs of the American Entomological Institute* **39**: 1–563.
- Wilson JS, Jahner JP, Forister ML, Sheehan ES, Williams KA, Pitts JP. 2015. North American velvet ants form one of the world's largest known Müllerian mimicry complexes. *Current Biology* **25**: R704–R706.
- Wilson JS, Jahner JP, Williams KA, Forister ML. 2013. Ecological and evolutionary processes drive the origin and maintenance of imperfect mimicry. *PLoS ONE* **8**: e61610.
- Wilson JS, Pan AD, Limb ES, Williams KA. 2018. Comparison of African and North American velvet ant mimicry complexes: another example of Africa as the 'odd man out'. *PLoS ONE* **13**: e0189482.
- Wilson JS, Williams KA, Forister ML, von Dohlen CD, Pitts JP. 2012. Repeated evolution in overlapping mimicry rings among North American velvet ants. *Nature Communications* **3**: 1272.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1. Dorsal habitus images of species 1–64.

Figure S2. Lateral habitus images of species 1–64.

Figure S3. Dorsal habitus images of species 65–128.

Figure S4. Lateral habitus images of species 65–128.

Figure S5. Non-metric multidimensional scaling (NMDS) ordination plot, with individual species coloured by mimicry complex.

Figure S6. Non-metric multidimensional scaling (NMDS) ordination plot of analysis, showing a circle at the mean value of each complex and lines drawn from the mean to individual species. The 'shiny blue' complex is split into two groups based on the presence or absence of red scales on the head and/or pronotum.

Table S1. List of 128 species included in the analysis. Specimen identifiers from the NHMUK and the CASC are digitized in their own databases. Specimens from MTD were not databased. All other specimen occurrence records are in the Symbiota Collections of Arthropods Network (SCAN). Collection codes: ARTSYS, Arthropod Systematics Research Collection; ASUCOB, Arizona State University Charles W. O'Brien Collection, Tempe, AZ, USA; ASUHIC, Arizona State University Hasbrouck Insect Collection, Tempe, AZ, USA; CASC, California Academy of Sciences, San Francisco, CA, USA; CMNC, Canadian Museum of Nature Collection, Ottawa, ON, Canada; MTD, Museum für Tierkunde, Dresden, Germany; NHMUK, Natural History Museum, London, UK; NMNH, National Museum of Natural History, Washington, DC, USA; PCMENT, Programa Centroamericana de Maestria en Entomología, Universidad de Panamá, Panamá; SSAC, Salvatore S. Anzaldo Collection, Tempe, AZ, USA.

Table S2. Matrix of 128 species scored for 15 characters used in the non-metric multidimensional scaling (NMDS) analysis.