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Cite this article: Muñoz-Ramírez CP, Bitton P-P, Doucet SM, Knowles LL. 2016 Mimics here and there, but not everywhere: Müllerian mimicry in *Ceroglossus* ground beetles? *Biol. Lett.* **12**: 20160429.
<http://dx.doi.org/10.1098/rsbl.2016.0429>

Received: 21 May 2016

Accepted: 3 September 2016

Subject Areas:

evolution, taxonomy and systematics

Keywords:

Müllerian mimicry, colour quantification, colour mismatch, Carabidae, Southern South America

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Electronic supplementary material is available online at <https://dx.doi.org/10.6084/m9.figshare.c.3470028>.

Evolutionary biology

Mimics here and there, but not everywhere: Müllerian mimicry in *Ceroglossus* ground beetles?

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The ground beetle genus *Ceroglossus* contains co-distributed species that show pronounced intraspecific diversity in the form of geographical colour morphs. While colour morphs among different species appear to match in some geographical regions, in others, there is little apparent colour matching. Mimicry is a potential explanation for covariation in colour patterns, but it is not clear whether the degree of sympatric colour matching is higher than expected by chance given the obvious mismatches among morphs in some regions. Here, we used reflectance spectrometry to quantify elytral coloration from the perspective of an avian predator to test whether colour similarity between species is, indeed, higher in sympatry. After finding no significant phylogenetic signal in the colour data, analyses showed strong statistical support for sympatric colour similarity between species despite the apparent lack of colour matching in some areas. We hypothesize Müllerian mimicry as the responsible mechanism for sympatric colour similarity in *Ceroglossus* and discuss potential explanations and future directions to elucidate why mimicry has not developed similar levels of interspecific colour resemblance across space.

1. Introduction

The existence of multiple geographical races is a common phenomenon in aposematic species [1–4] that can be maintained by localized frequency-dependent selection. When multiple closely related or phenotypically similar aposematic species co-occur, phenotypic convergence is locally favoured, because species benefit from sharing the costs of teaching predators about their unpalatability. This phenomenon, known as Müllerian mimicry, is documented in several systems exhibiting geographical mosaics of mimetic forms [1–3] and is best exemplified by *Heliconius* butterflies [2] where species mimic each other across extensive geographical areas with remarkable accuracy. Although geographical variation in aposematic coloration can also occur in the absence of mimicry [4], extreme variation seems to be relatively frequent in Müllerian systems [5].

In this study, we investigate whether species of ground beetles in the genus *Ceroglossus*, which are conspicuously coloured and chemically defended species endemic to the temperate forests of southern South America [6], are a possible new candidate system of mimicry. The taxa are widely co-distributed and characterized by high intraspecific variation ([5]; figure 1). In addition, some striking covariation in elytral coloration has been noted among co-occurring species [7]. For example, up to three different species with similar coloration can be collected at some localities, suggestive of colour convergence. However,

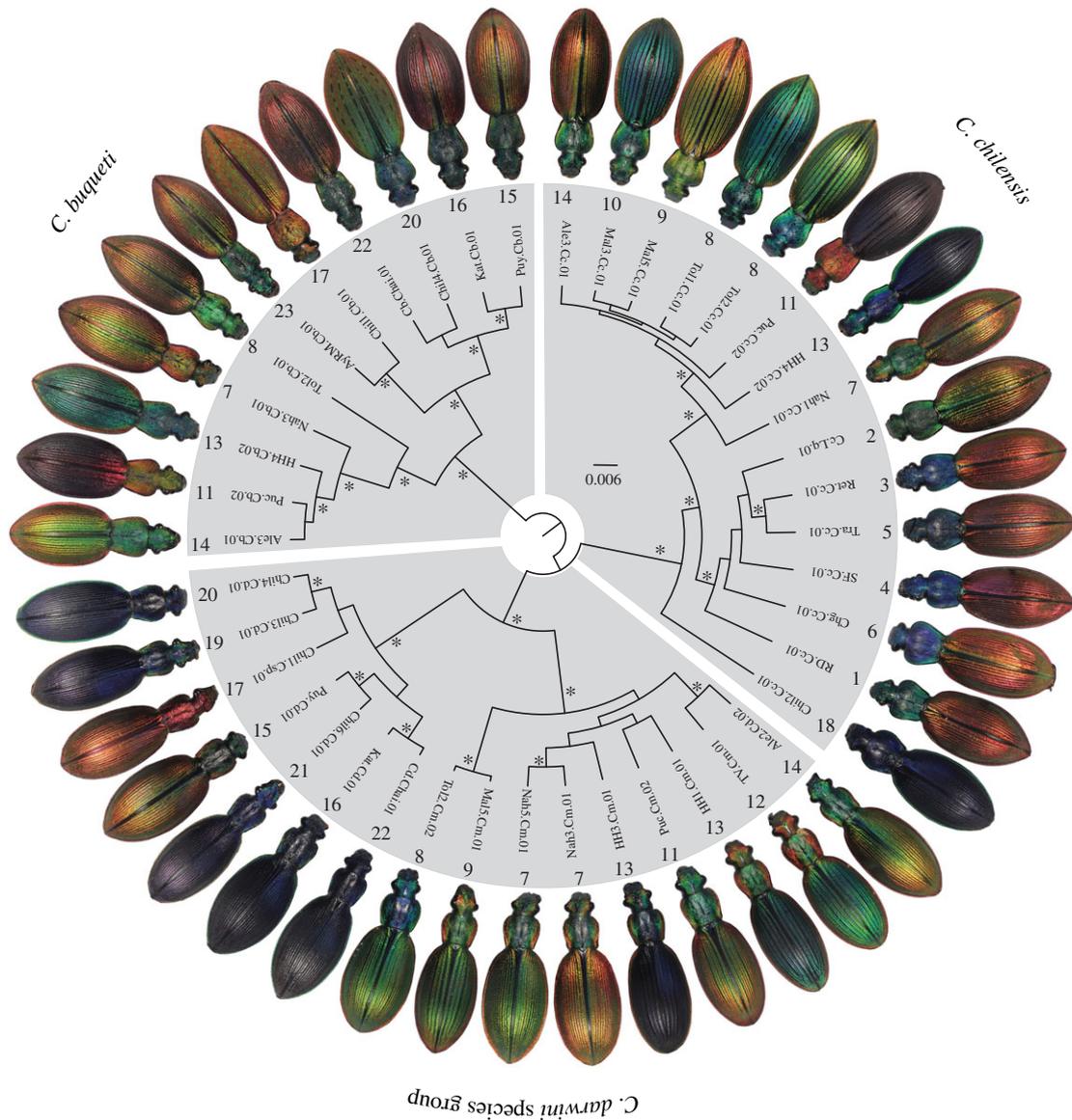


Figure 1. Colour diversity across the three *Ceroglossus* taxa analysed in this study. Relationships based on a Bayesian tree for COI recovered each of the three taxa as monophyletic (highlighted in grey). The asterisk symbol indicates Bayesian posterior probabilities above 0.9; numbers in front of the beetles indicate sample localities shown in the electronic supplementary material, table S3.

such interspecific colour matching is weak or lacking at other sites (figure 2a) calling into question whether mimicry actually explains the pronounced phenotypic variation in *Ceroglossus*.

To examine whether mimicry plays a predominant role in the *Ceroglossus* beetles, we collected reflectance data and tested whether the degree of colour matching among coexisting species is greater than expected by chance. Failure to reject the null hypothesis would preclude the need for invoking deterministic explanations posited by mimicry. By contrast, rejecting the null hypothesis of chance would lend preliminary support for a role of mimicry.

2. Methods

We collected colour data from 195 individuals belonging to three of the most widely distributed taxa that overlap to varying degrees: *Ceroglossus chilensis* ($n = 57$), *Ceroglossus buqueti* ($n = 71$) and the *Ceroglossus darwini* species group ($n = 67$; more sampling details can be found in the electronic supplementary material, tables S1 and S2). Species were identified following the diagnostic

characters described by Jiroux [6] (e.g. the number and position of antennal carinas).

(a) Quantification of colour

Colour data were quantified with a USB4000 spectrophotometer (Ocean Optics) under a deuterium–tungsten light source (Ocean Optics) and in relation to a white reference standard (WS-1 Ocean Optics), from 300 to 700 nm. Data were processed with the R-package ‘pavo’ [8] to represent the visual system of potential predators (avian with ultraviolet sensitive (UV) and violet sensitive (VS) short-wavelength photoreceptors and a reptile visual system) following [9,10]. Additional methodological details and data can be found in the electronic supplementary material.

(b) Testing phylogenetic signal

A 680 bp fragment of the COI gene was obtained for 42 individuals following the protocol described in [11]. Sequences were used to estimate a Bayesian phylogeny using MRBAYES v. 3.2.6 [12]. Because our colour data are multidimensional, phylogenetic signal of trait values was tested by both the Mantel test [13] and a multivariate implementation of Pagel’s lambda test available in RPHYLIPARS [14]. See the electronic supplementary material for

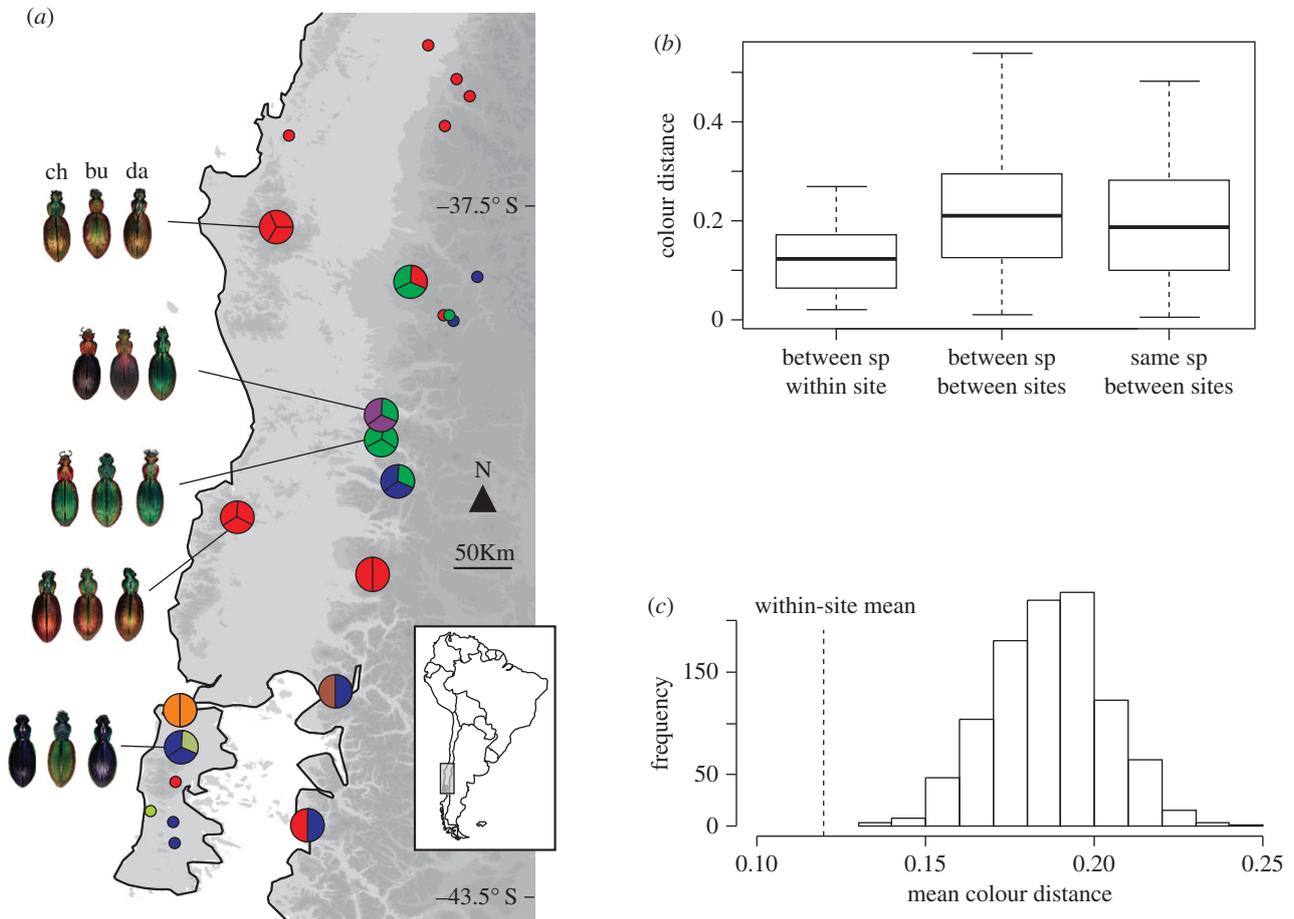


Figure 2. (a) Distribution map of *Ceroglossus* beetles highlighting by reference to sampled localities for three species (ch, *Ceroglossus chilensis*; da, *Ceroglossus darwini* group and bu, *Ceroglossus buqueti*) geographical areas where the taxa co-occur (shown by pie diagrams). Colour in the pie diagrams represents approximate species coloration in that area. The small coloured dots refer to localities where only a single species was collected. (b) Boxplots show how within-site colour distances compared with between-site distances. (c) Randomization test showing the distribution of random colour-distance means versus the empirical within-site distance mean (dashed line).

details about sequenced individuals and GenBank accession numbers (electronic supplementary material, table S3) and data (electronic supplementary material, table S4).

(c) Randomization test of colour matching

Because of the lack of phylogenetic signal in colour indicated by both the Mantel test and the Pagel's lambda test (electronic supplementary material, table S5), we tested whether colour similarity between species was higher within than between sites using randomization tests. Specifically, we calculated all pairwise interspecific colour distances—the Euclidean distance between the colour–space coordinates [15] of each population's centroid—to test whether 'within-site' distances were lower than 'between-site' distances. Posteriorly, to test whether the mean of the within-site colour distances ($n = 29$) was lower than expected by chance, we compared the observed mean against a random distribution generated from 1000 mean colour values calculated by randomly picking 29 distance values (i.e. the same number of within-site distances calculated from the empirical data) from the total pool of distances without replacement.

3. Results

Analyses using the avian UV, the avian VS and the reptile model data produced nearly identical results. Therefore, here we present the results only for the UV model. Results for the other models can be found in the electronic supplementary

material, figures S1 and S2. Spectral reflectance plots for sites containing all three species can be found in the electronic supplementary material, figure S3. Interspecific colour distances were lower within sites ($n = 29$; mean = 0.126; s.d. = 0.072) than between sites ($n = 706$; mean = 0.214; s.d. = 0.108), and this difference was statistically significant (one-way ANOVA, $F_{2,1078} = 11.9$; $p < 0.001$; figure 2b). Furthermore, within-site colour distances between species were on average also lower than between-site colour distances among populations of the same species ($n = 346$; mean = 0.194; s.d. = 0.11). This pattern was statistically supported by the randomization test, showing that the mean colour distance within sites was much lower than random expectations (figure 2c).

4. Discussion

Even though *Ceroglossus* species exhibit little evidence of colour matching in some areas (figure 2a), the degree of covariation of elytral coloration among species nonetheless deviates from random expectations (figure 2c), providing preliminary support for the potential role of mimicry in these beetles. Because all *Ceroglossus* have glands that produce noxious chemical compounds [6], we hypothesize that this pattern may represent a case of Müllerian mimicry. Although alternative, non-mimetic explanations for colour variation can be invoked including sexual or natural selection for

certain colours in similar light environments, high colour variation across areas in close geographical proximity with similar forest (and therefore light environment) types suggests these mechanisms are unlikely to explain colour matching patterns in *Ceroglossus*. Similarly, the evolution of certain colours to match the background substrate (crypsis) seems also unlikely, because many *Ceroglossus* beetles appear conspicuously coloured relative to a forest background (i.e. bright metallic red and blue) and they are very active predators in nearly constant motion [6], which should make them easily visible to predators.

Although further studies are necessary to confirm mimicry, including toxicity quantification and field/laboratory experiments, this study represents the first step in establishing the Müllerian mimicry hypothesis as a primary determinant of colour variation across these species (figure 1). In the following, we highlight aspects of the *Ceroglossus* beetle system—in particular, and perhaps somewhat ironically, the variation in the degree of matching across geography (figure 2a)—that speak to its potential promise for providing insights about the evolutionary dynamics associated with mimicry.

(a) Potential insights on mimicry from *Ceroglossus* beetles

Instances of imperfect mimicry may shed new light on the processes that mediate mimicry evolution [16,17]. Potential processes preventing mimicry might include ecological differences across space, high levels of maladaptive gene flow and the stability of co-distributions among taxa.

Because predators are the selective force maintaining mimicry [18], shifts in predator communities across regions might change the selective landscape for mimicry causing strong selection for colour convergence in some areas versus weak selection for colour convergence in others [5]. Alternatively, positive frequency-dependent selection on similarly abundant aposematic morphs [19], or microhabitat partitioning of mimicry patterns to exploit different predator assemblages [20] could also preclude selection for convergence. In *Ceroglossus*, differential predation pressures are possible given that the beetles are distributed across a relatively large geographical area with potentially different predator communities [21]. However, information on potential predators is scarce. Although mammals and reptiles may prey on beetles, birds are among the most abundant visual predators in Chilean forests, with beetles being commonly found in their diet [22,23], suggesting they may play a dominant role in the evolution of colour patterns in *Ceroglossus*. However, without additional data, especially on the abundance of *Ceroglossus* and their predators, it remains unclear whether differences in predator communities or positive frequency-dependent selection on multiple aposematic local morphs might explain the observed variation.

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Alternatively, increased connectivity between populations might counteract the evolution of mimicry if gene flow introduces colour genes that do not match local phenotypes [24]. In *Ceroglossus*, this possibility seems unlikely owing to the flightlessness of the beetles. In addition, the geographical proximity of some different colour morphs suggests selection is strong enough to override the effect of gene flow (figure 2a). Nevertheless, this possibility could be investigated in the future with detailed genetic analyses in areas with different degrees of mimicry.

Lastly, Pleistocene glaciations may have also played a role in preventing the evolution of mimicry. Postglacial expansions may have resulted in mismatching of phenotypes, with ‘incomplete’ mimicry possibly reflecting recent co-occurrence, or reduced rates of evolution resulting from limited standing genetic variation owing to bottlenecks (i.e. a possible example of genetic constraint [25]). The distribution of *Ceroglossus* species encompasses regions known to have been impacted by glaciations [26,27], suggesting investigations into the role of stability should be considered in future studies.

A potential new mimicry complex among *Ceroglossus* ground beetles would complement well-studied systems such as *Heliconius*. In particular, the variation in degree of phenotypic matching among the species provides a potential window into the mechanisms that drive, or conversely impede, the evolution of mimicry, in contrast with taxa that show uniform patterns of morphological variation clearly consistent with mimicry predictions.

Ethics. The research conducted in this study did not require approval from an ethics committee.

Data accessibility. Full colour data and colour data associated with the tree: electronic supplementary material, tables S2 and S4. The phylogenetic tree and sequence alignment: TreeBASE accession number S19767 [28].

Authors' contributions. C.P.M.-R. carried out fieldwork sampling, molecular data collection, statistical analyses and drafted the manuscript. L.L.K. provided substantial input on study design, analyses and writing the manuscript. P.-P.B. and S.M.D. contributed with colour sampling design, colour data processing and critical revisions of the manuscript. P.-P.B. also contributed writing a portion of the Methods. All authors gave final approval for publication and agree to be held accountable for the content herein.

Competing interests. We have no competing interests.

Funding. This research was supported by NSF (DEB-1601260 to C.P.M.-R. and L.L.K.), the Ammerman Endowment (Insect Division, Museum of Zoology, University of Michigan), NSERC Canada (scholarship to P.-P.B., grants to S.M.D.) and the Ontario Ministry of Research and Innovation (grant to S.M.D.).

Acknowledgements. We thank Viviane Jerez, Alvaro Zuñiga, Christian Muñoz, Alfonso Jara and many others for field assistance and donation of specimens, Rabee Kaheel for helping with colour data collection and Stephen Smith, Evan Twomey, Erik Svensson, the Knowles laboratory, and three anonymous reviewers for insightful comments on the manuscript. Fieldwork permits were granted by *Corporación Nacional Forestal* (CONAF, Chile).

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