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A Simple Index To Quantify And Compare The Magnitude Of Intraspecific Geographic Plumage Colour Variation In Typical Antbirds (Aves: Passeriformes: Thamnophilidae)

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A simple index to quantify and compare the magnitude of intraspecific geographic plumage colour variation in typical antbirds (Aves: Passeriformes: Thamnophilidae)

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Intraspecific geographic phenotypic variation is a crucial theme in evolutionary biology. Comparing its magnitude across species can provide insights into its ecological and genetic correlates. Here, we developed an index, which we dub the *V* index, to quantify intraspecific plumage colour variation in typical antbirds (Thamnophilidae), a family which has long interested ornithologists due to a high prevalence of intraspecific variation. The *V* index is based on a bivariate colour space defined by brightness and redness. Its value for each species equals the mean area occupied by each of its subspecies in that colour space, divided by the area of the species. Lower values indicate greater intraspecific geographic variation. Based on this index, *Thamnophilus caerulescens* (Variable Antshrike) was exceptionally geographically variable compared to other thamnophilids, as previously suggested based on qualitative evidence. In general, we found that the most variable species had disjunct distributions and deep phylogeographic structure, suggesting an effect of historical population dynamics in producing geographic variation. The *V* index can be adapted for use with other taxa, traits, and taxonomic levels, and we expect it will instigate novel ways of thinking about phenotypic variation in birds and other animals.

ADDITIONAL KEYWORDS: coloration – plumage – spectrophotometry – subspecies – *Thamnistes anabatinus* – *Thamnophilus caerulescens*.

INTRODUCTION

Intraspecific geographic variation in phenotypic traits, particularly in vertebrates, has long played a central role in evolutionary biology (Mayr, 1963; Endler, 1977; Zamudio *et al*., 2016). Species vary widely in the amount of geographic variation they exhibit (Mayr, 1963; Johnson, 1966; Mayr & Diamond, 2001; Winger & Bates, 2015). Quantifying and comparing the magnitude of intraspecific variation represents a natural first step towards understanding why some species display more variation than others (Mayr, 1963; Haffer & Fitzpatrick, 1985) and, by investigating selected highly variable species, understanding the genetic and ecological correlates of that variation.

For example, Mayr & Vaurie (1948) and Mayr (1963) investigated the amount of geographic variation in species of drongos (Passeriformes: Dicruridae), finding that out of 20 species in the family, 13 showed pronounced geographic variation. Interestingly, of the seven that did not show geographic variation, five were endemic to small islands. Interspecific comparisons of the magnitude of geographic variation can provide insights into the evolutionary and ecological forces underlying that variation.

The typical antbirds (Thamnophilidae) comprise a family of approximately 220 exclusively Neotropical species of relatively small, unobtrusive, insectivorous, sedentary passerines (Zimmer & Isler, 2003). All but a few thamnophilid species are sexually dichromatic, and their plumages are typically coloured in subdued shades of grey or black in males and brown in females (Zimmer & Isler, 2003; Marcondes & Brumfield, 2019). Intraspecific geographic variation in plumage colour is prevalent in the Thamnophilidae (Zimmer & Isler, 2003). In this, as in many other bird families, the subspecies category is widely used to accommodate geographically partitioned variation below the species level (Remsen, 2005; Winker, 2010; Patten, 2015).

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Thamnophilids have an average of 2.3 subspecies per species (Cracraft, 2014); however, there are also extremely variable species, as illustrated by the 18 subspecies of *Dysithamnus mentalis* Temmimck, 1823, ten of *Microrhopias quixensis* (Sclater, 1862) and ten of *Pyriglena leuconota* (von Spix 1824) (Cracraft, 2014). Extreme geographic variation in Amazonian species has been attributed to antbirds' sedentary nature coupled with a long history of occupation of the Amazonian landscape (Haffer & Fitzpatrick, 1985; Zimmer & Isler, 2003; Smith *et al*., 2014; Naka & Brumfield, 2018).

High levels of intraspecific geographic plumage variation in the antbirds have interested ornithologists since the classical works of John Zimmer (Zimmer, 1933) and Carl Hellmayr (Cory & Hellmayr, 1924; Hellmayr, 1929). Recent decades have seen a renewed spike of taxonomic work in this family, prompted by an innovative system employing objective plumage and vocal variation yardsticks to delimit taxa (e.g. Isler *et al*., 1997, 1998; Remsen, 2005; Isler & Whitney, 2011); with the advent of molecular systematics that system has also been successfully used in conjunction with genetic data (Isler *et al*., 2012). Here, our major goal is to demonstrate a straightforward way to quantify and compare the magnitude of phenotypic variation across species of thamnophilids, and to illustrate evolutionary insights that may be gained from such comparisons.

We were motivated by plumage variation in the Variable Antshrike, *Thamnophilus caerulescens* (Vieillot, 1816), a species whose common name emphasizes its inordinate geographic variation in colour (Meyer de Schauensee, 1966), and which has been described anecdotally as the single most polytypic species of antbird (Brumfield, 2005). Its eight subspecies vary in male coloration from almost all-black *Thamnophilus caerulescens melanchrous* (Sclater & Salvin, 1876) to *Thamnophilus caerulescens paraguayensis* (Hellmayr, 1904), which has a white belly and breast. There are also mostly grey subspecies in eastern South America (*Thamnophilus caerulescens caerulescens*, *gilvigaster*, *ochraceiventer* and *cearensis* (Cory, 1919)), and a subspecies (*Thamnophilus caerulescens dinellii* (von Berlepsch, 1906)) with extensive brown in the venter (Cory & Hellmayr, 1924; Zimmer, 1933; Meyer de Schauensee, 1966; Zimmer & Isler, 2003; Brumfield, 2005; Marcondes *et al*., in review). Finally, *Thamnophilus caerulescens aspersiventer* is similar to *melanchrous* but has white barring in the venter. Despite this impressive variation, plumage colour in this species has never been quantitatively compared to other thamnophilids to ascertain whether it is in fact extraordinary in the context of its family.

MATERIALS AND METHODS

Colour data

To quantify colour across thamnophilids, we used the dataset of Marcondes & Brumfield (2019), which includes reflectance data for seven plumage patches (crown, back, rump, tail, belly, breast and throat) for 227 species (90% complete at the species level). We did not include in this dataset the semiconcealed white interscapular plumage patch found in males of some thamnophilid species. In that previous study, we were not interested in intraspecific variation, so we generally included only one subspecies per species (Marcondes & Brumfield, 2019). For the present study, we supplemented that dataset with additional reflectance data for 105 subspecific thamnophilid taxa for females and 116 subspecific taxa for males, bringing the total number of taxa to 332 for females and 343 for males. This larger dataset does not include every subspecies in the family; however, we made an effort to include all subspecies with significant colour variation, such as those with obviously distinct hue or brightness in an entire plumage patch. We excluded subspecies with only minor plumage variation, for example, in number or colour of wing bars, or subtly different brightness in just one or a few plumage patches. We did not sample some taxa that were unavailable to us; however, all eight subspecies of *Thamnophilus caerulescens* were represented. Throughout this study, we followed the species- and subspecies-level taxonomy of Cracraft (2014).

Reflectance data consist of colour spectra giving the percentage of light reflected between wavelengths from 300 nm to 700 nm. We used an Ocean Optics USB2000 spectrophotometer coupled with a bifurcating optical fibre probe to an Ocean Optics PX-2 pulsed xenon light source to take three readings from each plumage patch in each specimen (Marcondes & Brumfield, 2019). We then used the R package *pavo* (Maia *et al*., 2013) to correct artefactual negative reflectance values, smooth spectra with a smoothing parameter of 0.4, and finally average spectra for each patch across all specimens of each subspecies. From the mean spectrum of each plumage patch in each specimen, we calculated brightness and redness. Brightness (variable B2 in *pavo* and in Montgomerie (2006)) is the total amount of light, in percent, reflected by an object and describes the position of a colour in a pure black (0%) to pure white (100%) continuum. Redness, or red chroma (variable S1R in *pavo*), in turn, is the percentage of the total light reflected that is between 605 nm and 700 nm. Greater percentages represent redder colours. We note that "redder", here, represents pheomelanin-produced colours that appear brown, or at most rufous, to human eyes (Figs 1-2). No members of the antbird family present highly chromatic, pure red plumage

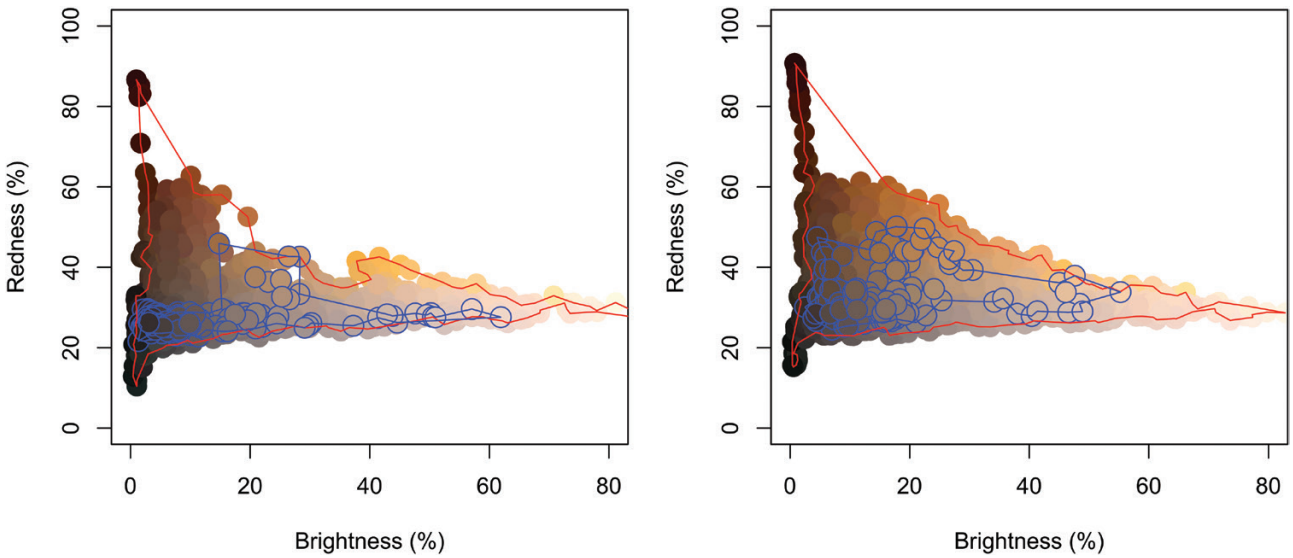

Figure 1. Bivariate colour space displaying brightness and redness values from each plumage patch in each male (left) and female (right) specimen. The outer red polygons are concave polygons delimited by all specimens belonging to all species of Thamnophilidae; the blue inner polygons and the points with blue edges are *Thamnophilus caerulescens*. The colour of each point is an approximation of the actual specimen colour calculated with the *pavo* function *spec2rgb* (Maia *et al*., 2013).

colours typically produced by carotenoid pigments. Because the vast majority of antbirds are coloured exclusively in shades of grey, black, white and brown, our two variables of brightness and redness capture essentially all colour variation present in the family.

Quantifying intraspecific geographic plumage colour variation

To quantify and compare the magnitude of intraspecific colour variation in each species of thamnophilid, we started by constructing a simple bidimensional colour space with redness on the *y*-axis and brightness on the *x*-axis. We plotted into that colour space the values for each plumage patch for each specimen we measured (total: 1082 male and 982 female specimens; 7076 individual plumage patches for males and 6444 individual plumage patches for females). Then, for each sex, we used the R package *concaveman* (Gombin *et al*., 2017) to calculate the area of a concave polygon delimited by all points for all taxa, thus representing the colour space extent occupied by the entire family. Next, we calculated the areas of polygons delimited by each species and subspecies separately. We used concave instead of convex hulls because the latter would cause large portions of colour space not actually occupied by any points to be included in the polygons. For example, because the colour space occupied by thamnophilid males is approximately L-shaped (Fig. 1), a convex polygon would necessarily take an approximately triangular shape, including the unoccupied regions of colour space between the two legs of the L. For all polygons, we set the concavity argument in *concaveman* to 2 and the length threshold argument to 4. We chose these values because in preliminary trials they produced the best balance between overly complex polygons with extremely large numbers of very small edges, versus simpler polygons that covered large areas of colour space not actually including any points.

Finally, to obtain a simple metric of the magnitude of colour variation among subspecies in each species (i.e. geographic variation), we took the mean of the colour areas of each subspecies within a species and divided it by the colour area of the entire species. We denote this ratio the *V* index. Values near 1 mean that the various subspecies within a species, on average, occupy a similar colour area as the entire species. Values closer to 0 mean that each subspecies, on average, occupies a small proportion of the colour area of the entire species. Thus, lower *V* values denote greater geographic variation in plumage colour within a species.

RESULTS

Into a bivariate colour space, we plotted redness and brightness data for seven plumage patches from 1082 male specimens representing 343 species or subspecies of thamnophilid (Fig. 1). The area of the concave polygon delimited by all points was 1455.9. The *Thamnophilus caerulescens* polygon, including all its subspecies, had an area equal to 40% of the family polygon (Table 1),

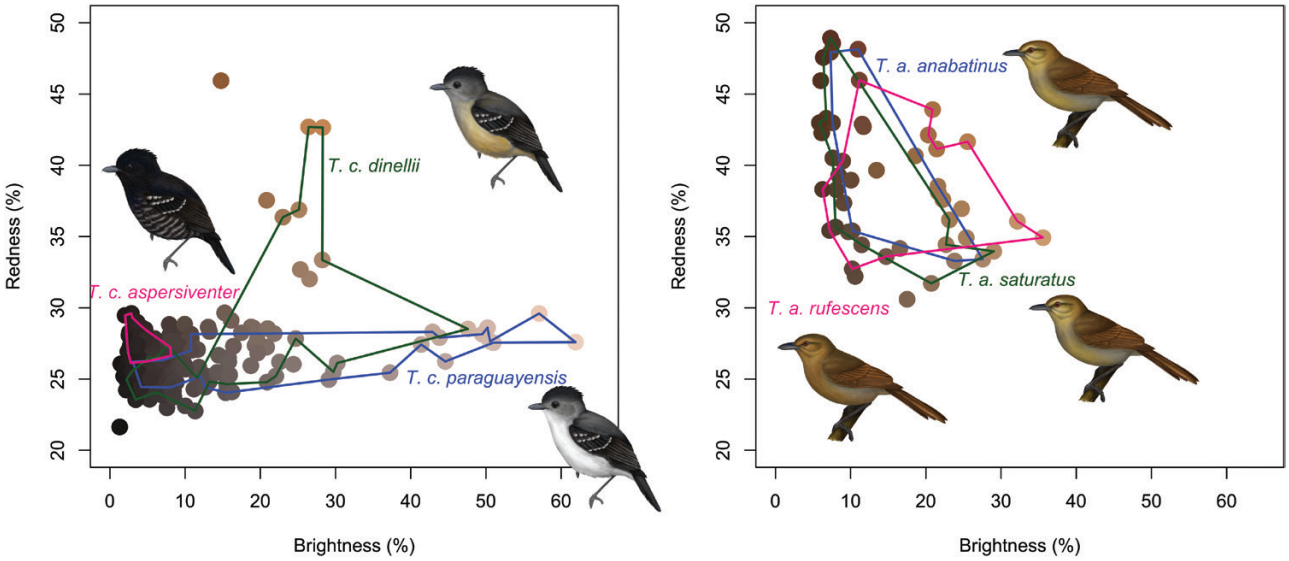

Figure 2. Comparison of colour polygons of subspecies of male *Thamnophilus caerulescens* (left) and *Thamnistes anabatinus* (right). *Thamnophilus caerulescens* ($V = 0.2004$) displays greater geographic variation than *Thamnistes anabatinus* ($V = 0.7541$), because there is less overlap between the colour spaces of each of its subspecies. Both species contain more subspecies than depicted; we selected only some to plot here for illustrative and clarity reasons. The colour of each point is an approximation of the actual specimen colour calculated with the *pavo* function *spec2rgb* (Maia *et al*., 2013).

ranking it 2nd within the Thamnophilidae. For females (Fig. 1), the family polygon was slightly larger, with an area of 1667.78, and *Thamnophilus caerulescens* occupied 40.3% of that area (Table 1), also making it the species with the 2nd largest colour space.

For the *V* index, representing the magnitude of geographic variation within each species, *Thamnophilus caerulescens* had the lowest value among all Thamnophilids for males, and the 3rd lowest for females (Table 1), indicating it is in fact extraordinarily geographically variable in relation to other species in the family. Some species had a *V* index greater than 1 (Supporting Information, Tables S1-S2); this is an artefact related to how concave polygons are calculated. Because a larger number of points tends to produce more intricate polygons than smaller numbers of points, an area of colour space encompassed by some subspecies' polygons (but not including any actual points) might not be included when the entire species' polygon is generated, with a greater number of points. In any case, this will only happen to species whose subspecies encompass a large proportion of their colour areas, therefore those species with $V > 1$ should be interpreted as having minimal amounts of geographic variation.

A comparison of the results for males of *Thamnophilus caerulescens* ($V = 0.2004$; eight subspecies) and a less variable species, the russet antshrike, *Thamnistes anabatinus* ($V = 0.7541$; four subspecies), illustrates how this metric conveys information about geographic variation. The total colour area of *Thamnophilus caerulescens* is 423.6, and its *V* index indicates that each of its subspecies occupies, on average, only 20.0% of that area. In contrast, the colour area for *Thamnistes anabatinus* is 225.0 and each subspecies occupies on average 75.4% of it. Plotting polygons for selected subspecies of each species reveals great overlap between the subspecies of *Thamnistes anabatinus*, but much less so of *Thamnophilus caerulescens* (Fig. 2). For example, *T. c. dinellii* occupies a large region of colour space that is not occupied by either *T. c. aspersiventer* or *T. c. paraguayensis*. Moreover, the colour space occupied by *T. c. aspersiventer* has almost no overlap with either *T. c. dinellii* or *T. c. paraguayensis*. This demonstrates that the overall colour space of *Thamnophilus caerulescens* is generated by the sum of largely nonoverlapping colour areas of subspecies with disparate colours (i.e. geographic variation), whereas the overall colour space of *Thamnistes anabatinus* is similar to that of each of its subspecies individually.

DISCUSSION

Characterizing the patterns and explaining the causes of intraspecific phenotypic variation is a major theme in evolutionary biology (Mayr, 1963; Mayr & Diamond, 2001). We developed a simple, easily interpretable metric, which we dub the *V* index, to compare the magnitude of intraspecific plumage colour variation in species of the Thamnophilidae.

Table 1. Metrics of intraspecific plumage colour variation in thamnophilids. Species colour area is the area occupied by a species, including all its subspecies, in a colour space delimited by brightness and redness. Mean subspecies colour area is the mean colour area occupied by each subspecies within a species. The *V* index, our metric of geographic variation, is the ratio of the mean subspecies colour area by the respective species' colour area. Smaller numbers represent greater plumage colour differentiation among subspecies within a species. Shown are the ten species with the smallest *V* indices (greatest geographic variation) for each sex; an exhaustive list is in the Supporting Information (Tables S1-S2).

	Rank	Species	Species colour area	% of family colour area	Number of subspecies	Mean subspecies colour area	*V* index
Males							
	1	*Thamnophilus caerulescens*	423.63	30.97	8	84.89	0.2004
	2	*Thamnophilus aethiops*	103.20	7.55	6	32.58	0.3157
	3	*Dysithamnus mentalis*	343.53	25.12	9	109.68	0.3193
	4	*Cercomacra nigrescens*	36.06	2.64	5	12.12	0.3362
	5	*Myrmotherula axillaris*	42.27	3.09	6	14.42	0.3411
	6	*Pyriglena leuconota*	37.16	2.72	6	13.04	0.3510
	7	*Myrmotherula menetriesi*	62.40	4.56	4	22.46	0.3600
	8	*Myrmelastes leucostigma*	55.87	4.08	3	20.19	0.3614
	9	*Rhegmatorhina melanosticta*	86.64	6.33	3	34.09	0.3935
	10	*Willisornis poecilinotus*	90.99	6.65	4	35.92	0.3948
Females							
	1	*Percnostola rufifrons*	285.88	17.89	2	68.54	0.2398
	2	*Willisornis poecilinotus*	421.96	26.40	5	118.21	0.2802
	3	*Thamnophilus caerulescens*	644.06	40.30	7	183.96	0.2856
	4	*Microrhopias quixensis*	176.82	11.06	5	61.21	0.3462
	5	*Thamnophilus aethiops*	316.57	19.81	6	123.38	0.3898
	6	*Pyriglena leuconota*	351.11	21.97	6	137.19	0.3907
	7	*Thamnistes anabatinus*	371.70	23.26	6	158.01	0.4251
	8	*Myrmoborus myotherinus*	408.75	25.58	3	226.05	0.5530
	9	*Poliocrania exsul*	146.46	9.16	4	81.60	0.5571
	10	*Myrmeciza hemimelaena*	480.20	30.61	2	276.53	0.5652

Patterns and origins of intraspecific plumage colour variation in the Thamnophilidae

The *V* index can be used to illuminate commonalities among the most variable species of thamnophilids. Many of the thamnophilids with the lowest *V* indices (Table 1) are widely-distributed species encompassing multiple geographically isolated populations. For example, *Willisornis poecilinotus* (Cabanis, 1847), *Thamnophilus aethiops* (Sclater, 1858), *Epinecrophylla leucophthalma* (von Pelzeln, 1868), *Cercomacra nigrescens* (Cabanis & Heine, 1860), *Myrmotherula menetriesii* (d'Orbigny, 1837) and *Microrhopias quixensis* (Cornalia, 1849) are all Amazonian species with phenotypically distinct populations isolated from each other by rivers. These river-associated genetic splits are often deep, such as 1.3 Myr between subspecies of *Myrmotherula menetriesii* and 3.1 Myr between subspecies of *W. poecilinotus* (Naka & Brumfield, 2018). Other species among the most variable are either montane species with subspecies separated by lowland areas (*Thamnistes anabatinus* Sclater & Salvin, 1860), or species with a highly disjunct circum-Amazonian distribution (*Dysithamnus mentalis*, *Pyriglena leuconota*), a pattern that has, in other passerine families, been shown to be associated with deep phylogeographic structure (Lovette, 2004; Batalha-Filho *et al*., 2013; Savit & Bates, 2015). Altogether, the highly subdivided geographic and genetic structure of the most variable thamnophilids suggests a role for historical isolation in producing high levels of phenotypic variation.

Thamnophilus caerulescens might be an exception to the above interpretation. We confirmed, using the *V* index, that this species is exceptionally variable in the context of its family, as had been previously speculated (Brumfield, 2005). But, in contrast to most other species with low *V* indices, *Thamnophilus caerulescens* displays remarkable plumage colour variation across a largely continuous range. The geographically isolated subspecies *T. c. cearensis* is the only form that does not have a contact zone with at least one other subspecies. Discerning the relative roles of historical population dynamics versus local adaptation to environmental gradients in producing intraspecific phenotypic variation is challenging; however, this pattern of colour variation observed in *Thamnophilus caerulescens* might be more indicative of the latter.

Using and expanding the *V* index

Quantifying the magnitude of variation between species can help illuminate the correlates and origins of geographic variation (Mayr & Vaurie, 1948; Mayr, 1963; Haffer & Fitzpatrick, 1985). Here, we showed how a simple index can be used to compare and quantify the magnitude of intraspecific plumage colour variation in species of the Thamnophilidae; however, the *V* index can be easily adapted for use with other taxa, traits and taxonomic levels. All that is needed are two or more axes of variation on which to calculate a bi- or multidimensional phenotypic space. One can then simply divide the mean size of the phenotypic space for entities in a less inclusive category by the that of the encompassing more inclusive entity. Our two phenotypic axes were brightness and redness; other potentially interesting phenotypes to apply the *V* index include ecomorphological variables of birds (e.g. Bravo *et al*., 2014; Pigot *et al*., 2020), mammals (e.g. Nations *et al*., 2019) or reptiles (e.g. Losos, 2011).

The *V* index can also be applied at other taxonomic levels. For example, to ask how species within a genus partition phenotypic space, one can calculate phenotypic volumes for a genus and for all species it comprises, and then divide the mean species volume by the volume of the genus. Next, comparing that metric across genera in a family may reveal differences in how phenotypic variation is structured within each genus. This approach can also be adapted for use in a phylogenetic context, without reference to taxonomic levels, by comparing phenotypic variation across subclades in a set of more inclusive clades. These extensions of the *V* index approach, however, would not convey information about geographic variation, because higher taxonomic or phylogenetic levels lack the intrinsic geographic aspect of subspecies.

We recognize that our index is relatively simplistic. Its value is mainly heuristic. Akin to a researcher obtaining inspiration from browsing drawers of specimens in a natural history collection, we expect that qualitatively examining how the *V* index varies across the Thamnophilidae (Supporting Information, Tables S1-S2) will foster novel research questions and ways of thinking about intraspecific variation in this family, and potentially inspire similar work on other taxa and traits.

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SUPPORTING INFORMATION

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

Table S1. Metrics of plumage colour variation for thamnophilid species (females). Species colour area is the area occupied by a species, including all its subspecies, in a colour space delimited by brightness and redness. Mean subspecies colour area is the mean colour area occupied by each subspecies within a species. The *V* index, our metric of geographic variation, is the ratio of the mean subspecies colour area by the respective species' colour area. Smaller numbers represent greater plumage colour differentiation among subspecies within a species. Species are ranked by increasing *V* index (decreasing geographic variation).

Table S2. Metrics of plumage colour variation for thamnophilid species (males). Species colour area is the area occupied by a species, including all its subspecies, in a colour space delimited by brightness and redness. Mean subspecies colour area is the mean colour area occupied by each subspecies within a species. The *V* index, our metric of geographic variation, is the ratio of the mean subspecies colour area by the respective species' colour area. Smaller numbers represent greater plumage colour differentiation among subspecies within a species. Species are ranked by increasing *V* index (decreasing geographic variation).