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An affordable method to measure animal-background contrast using digital images

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Abstract
Current color metrics commonly used to study the animal-substrate contrast, require researchers perform the analyses under controlled conditions of light. We argue that sometimes this approach can be suboptimal because it does not use the same light source as natural habitats, ignores the proportion of the different colors of the pattern, and ignores the behavioral repertoire displayed by animals when they are signaling. Here we present an affordable method to quantify animal-background contrast using photos taken under natural conditions. The method combines the use of free software to extract the color information and calculate the Color Overlapping Index (COI), which represent the degree of overlap between the color classes displayed by the animal and substrate. We showed that COI accurately reflects the degree of animal-substrate contrast seen in an image and that COI is a robust metric, yielding consistent results even for images with low resolution or with the un-calibrated color balance.

Keywords: animal coloration; aposematism; crypsis; COI; color metrics; background matching.

1. Introduction
Since Darwin's time, animal coloration has been of great interest among evolutionary biologists, natural historians, and animal behaviorists. Many studies have demonstrated the role of colors in survival, maintenance, and reproduction of individuals from different taxa [1–3].

Overall, there are two ways which animal's coloration appears: contrasting or matching with the background [4]. In the former case, the color pattern is called conspicuous, and may be used for aggressive displays toward sexual rivals, warning signaling (aposematic animals), or partner attraction [5, 6]. In the latter case, the pattern is said to be cryptic (or camouflaged), being related to attack and defense strategies [7, 8].

A variety of methods have been developed to study animal colors [9–12]. Fueled by advances in knowledge of and technological tools for colorimetry, purely categorical methods have been replaced by quantitative methods [13–16]. These methods aim to quantify animal-background contrasts in order to estimate the extent in which animals' color patterns are similar (or dissimilar) to their background. Nevertheless, current methods have many limitations regarding practical and theoretical issues.

An important practical limitation of most color metrics is the need to perform measurements under laboratory or under controlled light conditions [11, 17–19]. This causes a number of drawbacks, including those associated with collecting and transporting specimens and substrates, reduction of sample size by using only specimens successfully captured (in contrast with those "observed" in the field), acquiring collection permits (sometimes difficult because the conservation status of species), and difficulty in handling some animals and substrates due their size or morpho-physiological features. In some situations, there are financial constraints as well: many methods require a relatively expensive device called the spectrophotometer [5, 20–22], which makes its application impractical for researchers who lack funding. Hence, methods utilizing digital cameras have been increasingly adopted as a less expensive and versatile alternative to study animal and plant coloration [9–11, 16, 23, 24].

Despite these practical issues, our major criticism of current color metrics rests on theoretical grounds. By making measurements away from natural conditions, researchers may recreate scenarios that are fundamentally different from the conditions under which animals typically find themselves. We focus on three theoretical arguments that we judge as the most critical to the study of animal coloration.

First, whereas standardized conditions of light may ensure precision to describe and compare

colors, it can poorly simulate the animal-background contrast seen in natural conditions. Natural light assumes a particular spectrum that varies throughout the day, seasons, and is modified by natural filters (e.g., forest canopy), which affect how colors are perceived by signal receivers [25–27]. However, artificial light sources are frequently not identical to the spectrum of light found in the species habitats and its source comes generally from an electronic flash, creating misleading shadows not found in the natural environment. The problem is that by using a different illuminant one can promote metamerism, i.e., a phenomenon caused when two objects spectrally different match under a given light source [28]. In this sense, two scenarios are possible: either metamerism may artificially increase the resemblance between the subjects measured, or the metameric effect may in fact be used by organisms in nature (e.g., to enhance camouflage [29]). However, the method will be precluded from detecting such use. Finally, laboratory conditions cannot replicate the patterns of shadow and light of natural environments even though it is known that species can use such patterns to increase the efficiency of attack or defense strategies [27, 30]. In all these cases, bringing animals into captivity and measuring their coloration under artificial lights will not allow the full nature of camouflage to be studied.

Second, the proportion of specific colors in animals and background patterns are usually not taken into account in the current color metrics (but see [14]). Measurements are commonly made by quantifying a few point samples of animals and their background, and pairwise animal-background contrast analyses are calculated, by comparing the distance of the wavelengths [23, 31, 32]. The flaw of this approach is that colors typically do not occur in the same proportions throughout the patterns, which can affect their relative importance for signaling [14]. For instance, imagine a hypothetical animal that has two colors in the integument resting on a substrate that has exactly the same colors as the animal. However, whereas the animal exhibits these colors in a proportion of 9:1, the substrate exhibits them in a proportion of 1:9 (i.e., the reverse). By using the current methods, one would obtain a value suggesting that there is 100% background matching, when in fact this animal may seem very conspicuous on this background. Predators search for discontinuity in visual background to detect prey [33, 34]. Thus, even sharing the same colors, divergent color proportions among animals and background could provide important cues to the predator vision.

Finally, color metrics applied in a laboratory or on fixed specimens ignore the behaviors animals engage in while signaling. To be successful for signaling (e.g., camouflage, warning signal, and intersexual communication), a body coloration must be associated with an appropriate behavior [4, 35]. For example, many lizards display a cryptic pattern on the dorsum to avoid detection by predators, but a conspicuous coloration on the ventrolateral abdomen surfaces or on the throat are revealed by postural changes during intraspecific communications. These lizards should adopt different postures depending on the signal. Animals may also modify their position in a patch or with respect to the observer to improve camouflage or conspicuousness [4, 8, 35]. By ignoring these aspects, researchers might artificially increase or decrease the estimate of animal-background contrast.

Aiming to overcome the problems raised here, we developed an affordable method to measure color pattern contrast using digital photos taken under natural conditions of illumination and animal behavior. After extraction of the color information on animal and background by the free software Image J [36], the degree of similarity between animal and background is given by an index that calculates the overlap between their color classes (Color Overlapping Index, COI), which is calculated by a COI function coded in R [37]; a free and widely used programming language. We tested the performance of our method by quantifying the background matching of seven lizard species on varied substrates. We also tested the robustness of the method by applying it under several camera settings and image resolutions.

2. Materials and methods

2.1 Taken the photos in the field

We used a Canon EOS 350D digital camera (8.0 Mp) with 18-55 mm lens. To make the images comparable we set the camera to ISO 800 with aperture priority (Av) fixed at f 5.6 to guarantee that any change in brightness from the environmental conditions would be compensated by exposure time. Flash was not used. The photos were taken from October 2008 to October 2010 between 10:00 h and 16:00 h under varied weather conditions. We photographed seven lizard species with different color patterns on varied substrates (Fig. 1), which provided varied animal-background contrasts to evaluate the index's performance. All photos were taken on warm days with temperature ranging from 29 to 35 °C.

When a lizard was sighted, the experimenter turned to the lizard, stopped for 10 s, and then approached the subject in a straight line under a constant speed (approximately 0.5 m/s) until getting between 0.5 and 2 m from the lizard. Lizards were photographed in the angle at which they naturally stopped in relation to experimenter eyes (from 100 and 70 degrees). Only adults were photographed.

All photos were stored as a RAW file (3504 x 2336 pixels) and subsequently converted to full quality TIFF with *Canon* Digital Photo Professional software v. 3.7.1.1 (Canon Inc., Japan). The white balance of images was corrected using XRITE Color Checker Passport card with software v. 1.0.2 (X-Rite Inc. Grand Rapids, MI 49512, USA).

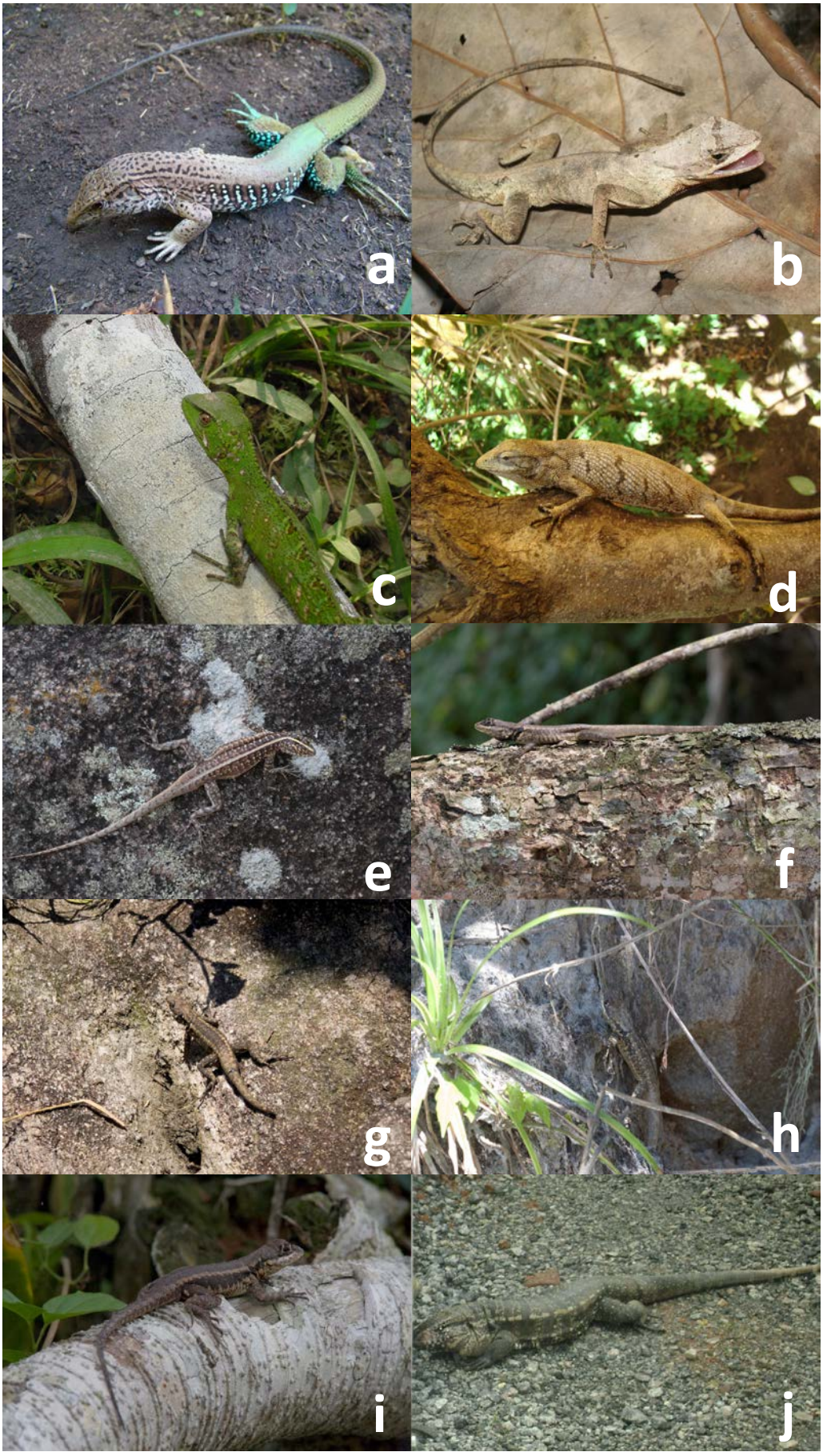

Fig 1: The ten lizard images used to quantify animal-background contrast using the Color Overlapping Index (COI). (a) *Ameiva ameiva* on dirt floor; (b) *Anolis chrysolepis* on brown litter; (c) *Enyalius perditus* on white bark; (d) *Polychrus acutirostris* on beige bark; (e) *Tropidurus semitaeniatus* on rock with lichens; (f) *Tropidurus torquatus* on brown bark; (g) *T. torquatus* on rock; (h) *T. torquatus* on sand dune; (i) *T. torquatus* on white bark; (j) *Tupinambis merianae* on stony ground.

2.2 Sampling the color information of the images

Digital photographic systems can now work with images with 12 or 14 bits by channel, although images seen in most LCD screens have 8 bits by channel or 256 shades of gray (2^8), which allow the representation of up to 16,777,216 hues. The next step to quantify animal-background contrasts was to extract this color information (both for the animal and the background) and group them into color classes.

To do so, we first opened the digital images in Image J and selected an area comprising the animal's body using the rectangular selections tool (note: any shaped selection tool can be used; Fig. 2a). Second, we extracted the color information from the selected image using the Color Inspector 3D v. 2.3 [38]; a plugin that must be previously installed in the Image J (download in http://rsb.info.nih.gov/ij/plugins/color-inspector.html). This plugin quantifies the RGB of every pixel of the selected area, assigns them to their associated color class, and outputs a list with these data (Fig. 2b-c). In *display mode* > *histogram* > *number of color cells* we set the scrollbar according with the intervals desired to partitioning the RGB space in the color classes. All measurements of the present study were performed with a color interval of 30 (the default of the program). However, we conducted a separate analysis using a color interval of 22 to test the behavior of the index when different color intervals are set. We then press the button *LUT* (lookup table) to display the list with the color information and pasted it an Excel spreadsheet. Importantly, the list pasted in the spreadsheet must contain only five columns: three columns for the RGB values, one column for the absolute frequency, and one column for the relative frequency - reader and row numbers should not be included in the spreadsheet (Fig. 2d). Fourth, we selected a representative area of the substrate and repeated the same steps used for animal. Importantly, the same color interval used in animal image must be assigned to the substrate. The LUT list of the substrate must be pasted in the same spreadsheet of the animal data (just right; Fig. 2d). It is not required that the animal and substrate list have the same length.

To test the performance of the method using a different sampling strategy, we varied the number and size of areas selected in the background. In each image, we selected one large area to be analyzed in the *pairs* mode of the COI function, and five minor areas to be analyzed in the *fuzzy* mode (see details in "*The COI function*"). All spreadsheets were then converted to .txt file (but .csv files are also accepted by the COI function).

2.3 The COI index

The COI index is an adaptation of the Renkonen similarity index [39], widely used in studies of community ecology [40]. COI is calculated as the sum of the lowest relative frequencies among the color classes shared by animal and substrate. Its formula is written as:

$$COI = \sum \min(p_{1i}, p_{2i})*100$$

where, p_{1i} and p_{2i} are the relative frequencies of the i*th* color class of the animal (p1) and the substrate (p2). The only differences in relation to Renkonen index is that COI is given as a per cent overlap and is based on classes of values (i.e., the color classes). COI values close to 0% mean that animal pattern is very conspicuous, whereas COI values close to 100% mean that animal is very cryptic.

2.4 The COI function

We calculated the COIs using an algorithm written in R, the COI function (available in the supplementary material). To avoid the need of creates separate data files to each image, the function permits many image data be compared from a unique file. Still, there are two ways for COI function comparing the data of the images, each mode requiring a specific way to organize the data in the spreadsheet. The *pairs* mode calculates one COI for each animal-background pair. This mode requires the substrate data should be placed just right of the animal data which it should be contrasted. The *fuzzy* mode, in turn, contrasts a single animal's data with two or more sets of background data. In the fuzzy mode, the first five columns should report animal data and the following columns (to the immediate right) should contain the background data. In both modes, the file should not contain empty columns between the data.

The COI function requires two arguments to be run: the data object and the comparison mode ("pairs" or "fuzzy"). To avoid one always import the data file to R before running the function, the COI function permits the user to write directly the full name of file (in quotes) in place of the data object argument. The requirement is that file is in the same directory of the COI code. For example, to calculate the COI index in pairs mode to the "ameiva.csv" file, the user can simply write: coi ("ameiva.csv","pairs").

The COI function outputs the COI values, the lowest and largest COI, the sample size (i.e., the number of indices), and, for the fuzzy mode, the mean and standard deviation of the indices (Fig. 2e). The results are outputted both in the R console and as a .csv file.

2.5 Robustness test: color balance

In the images used here we applied the white balance correction, which is a procedure that ensures fidelity in the color reproductions in digital images [23]. However, the device required to perform the white balance correction represents an additional cost to the researcher. To test the robustness of our method to uncorrected images, we compared the COIs of images before (the original photos) and after the white balance correction (corrected). Additionally, we calculated the COIs of images in which the color temperature was altered (using the *color balance tool*) to colder (cyan 20 and cyan+blue 20) and warmer color temperatures (red 20 and red+yellow 20). This modification on color temperatures aimed to simulate varied degrees of nonconformity in relation to the standard white balance that one could find in their cameras.

2.6 Robustness test: image resolution

To test whether our method is equally suitable to analyze photos with high and low resolutions, we measured the COI of the images with reduced size. To do so, we resized the ten lizard images using the *size* tool of Image J. The image reduction was made in four steps. At each step we reduced the image in 20% in relation to the previously reduced image. Specifically, we reduced the original photo in 20% (=1x), then we reduced this latter image in 20% (=2x), and so on.

2.7 Statistical Analyzes

All statistical analyses were performed in R. Assumptions of the models were assessed with diagnostic plots [41, 42]. Differences between COIs calculated from different color intervals (30 and 22) were tested with a paired t test [43]. We used one-way ANOVA with repeated measures to test for

differences between COIs of the color balance and image resolution treatments [43]. We calculated 95% confidence intervals to infer whether COIs calculated using a single sample of the substrate differed from COIs using multiple samples of the substrate [43,44]. Significance tests were two-tailed with $\alpha = 0.05$.

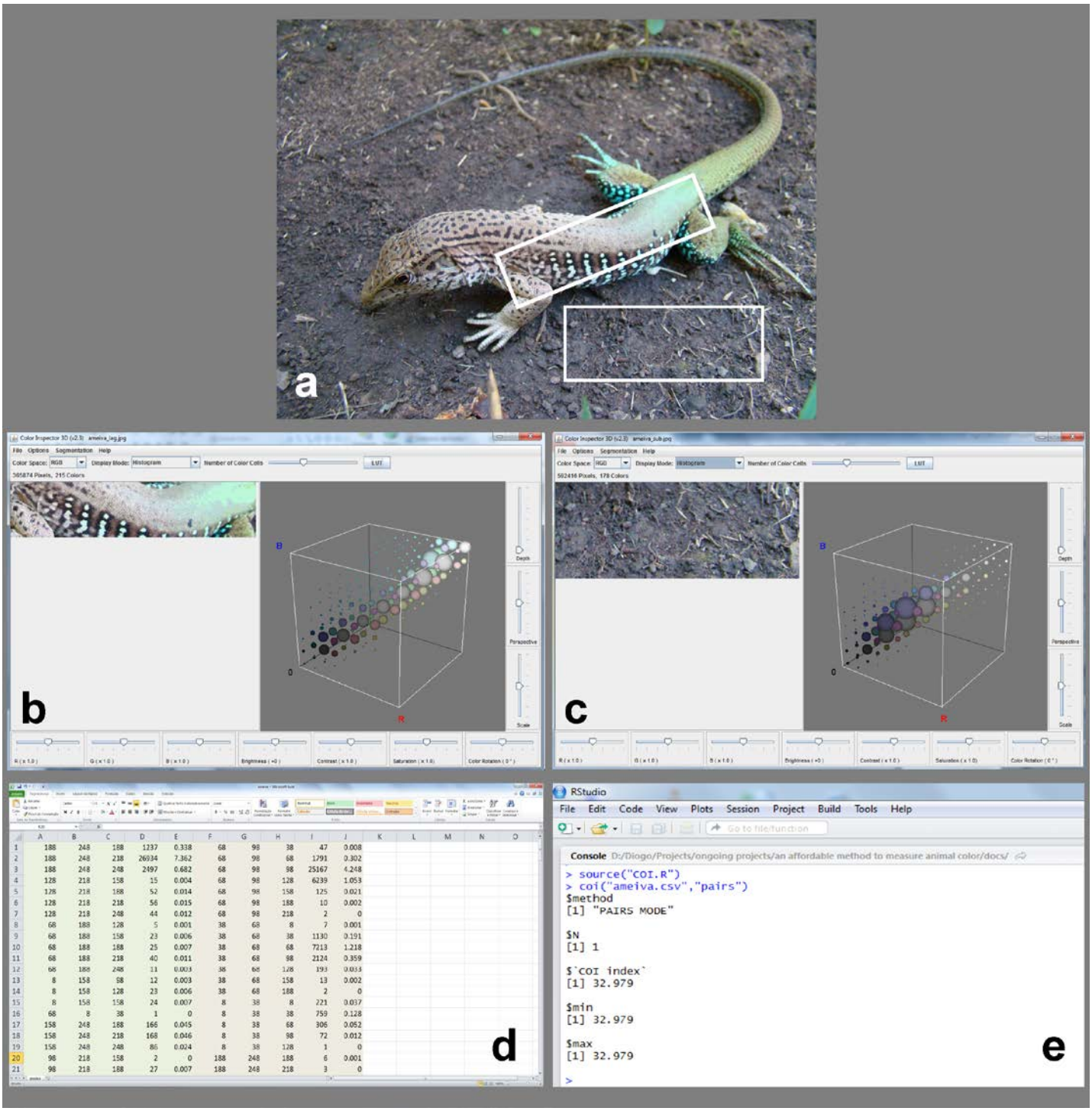

Fig 2: Illustration of the steps to quantify the animal-background contrast using the Color Overlapping method. (a) Sampled area of the lizard (*Ameiva ameiva*) and the substrate (white rectangles). (b-c) Windows of the Color Inspector 3D; a plugin of Image J used to extract the color data of both (b) lizard and (c) substrate areas. The spheres inside the cubes represent the relative frequency of each color interval of the sampled areas. (d) Spreadsheet containing the color data of the lizard (green columns) and substrate (brown columns) imported from the LUT tables of the Color Inspector. (e) Command lines and output of the COI function. See text to additional details.

3. Results

3.1 Performance of index and method

The COI values matched with visual perception of how much lizards' contrasted against their background (Table 1 and Fig. 1). Lizards that seem more camouflaged in their substrates (*Anolis chrysolepis* on brown litter, *T. torquatus* on brown bark, and *T. torquatus* on rock) had higher COIs than lizards whom seemed more conspicuous (*Enyalius perditus* and *T. torquatus* on white bark, and *Ameiva ameiva* on dirt floor; Table 1).

COIs calculated using smaller color intervals (22) were lower than COIs using larger color intervals (30) (t = 3.21, df = 9, P = 0.01; Table 1). With the exception of *T. torquatus* on white bark, all lizards had the background matching reduced when COIs were calculated from a color interval of 22 (Table 1). However, although statistically significant, the overall difference between COIs from different color intervals was small (mean = 3.49, SD = 3.44).

Evaluation of the confidence intervals showed that all COIs calculated using a single sample of the substrate differed significantly from those COIs calculated using multiple samples of the substrate (Table 2).

Table 1: Color Overlapping Indices (COI) of the ten lizard images analyzed. For each image, the COIs were calculated after partitioning the RGB color space in intervals of 30 and 22.

species (substrate)	30	22
Ameiva ameiva (dirt floor)	32.98	30.86
Anolis chrysolepis (brown litter)	74.58	72.21
Enyalius perditus (white bark)	4.23	2.53
Polychrus acutirostris (beige bark)	64.61	60.89
Tropidurus semitaeniatus (rock with lichens)	51.79	41.51
Tropidurus torquatus (brown bark)	80.80	76.19
Tropidurus torquatus (rock)	74.68	66.19
Tropidurus torquatus (sand dune)	60.47	59.19
Tropidurus torquatus (white bark)	15.51	15.85
Tupinambis merianae (stony ground)	65.22	64.56

Table 2: Color Overlapping Indices (COI) calculated using single and multiple (five) samples of the substrate. CI = 95% confidence interval.

	single sample	**multiple samples**	
species (substrate)		**mean**	**CI**
***Ameiva ameiva* (dirt floor)**	32.98	27.17	23.88 - 30.45
***Anolis chrysolepis* (brown litter)**	74.58	71.53	69.64 - 73.41
***Enyalius perditus* (white bark)**	4.23	5.37	4.66 - 6.08
***Polychrus acutirostris* (beige bark)**	64.61	59.38	56.13 - 62.61
***Tropidurus semitaeniatus* (rock with lichens)**	51.79	49.67	48.35 - 50.98
***Tropidurus torquatus* (brown bark)**	80.80	77.88	76.06 - 79.69
***Tropidurus torquatus* (rock)**	74.68	73.51	72.78 - 74.23
***Tropidurus torquatus* (sand dune)**	60.47	61.39	60.81 - 61.95
***Tropidurus torquatus* (white bark)**	15.51	20.09	17.25 - 22.92
***Tupinambis merianae* (stony ground)**	65.22	70.28	67.14 - 73.41

Table 3: Color Overlapping Indices (COI) calculated from images before (original) and after (corrected) the white balance correction, and of images in which the color temperature were modified to colder (cyan 20 and cyan+blue 20) and warmer color temperatures (red 20 and red+yellow 20).

species (substrate)	**corrected**	**original**	**cyan 20**	**cyan+blue 20**	**red 20**	**red+yellow 20**
***Ameiva ameiva* (dirt floor)**	32.98	33.27	33.93	31.65	33.56	33.12
***Anolis chrysolepism* (brown litter)**	74.58	74.65	73.92	72.66	71.94	71.63
***Enyalius perditus* (white bark)**	4.23	3.50	3.27	3.27	3.92	3.56
***Polychrus acutirostris* (beige bark)**	64.61	63.71	63.96	64.95	63.54	62.51
***Tropidurus semitaeniatus* (rock with lichens)**	51.79	57.80	53.93	47.77	45.32	55.33
***Tropidurus torquatus* (brown bark)**	80.80	80.75	80.27	83.27	80.06	82.65
***Tropidurus torquatus* (rock)**	74.68	75.55	73.24	72.21	70.04	74.55
***Tropidurus torquatus* (sand dune)**	60.47	59.60	59.65	61.86	58.94	59.34
***Tropidurus torquatus* (white bark)**	15.51	14.07	14.79	15.78	16.08	14.28
***Tupinambis merianae* (stony ground)**	65.22	65.05	65.29	69.56	66.46	70.18

3.2 Robustness tests

COIs of images before (original) and after (corrected) the white balance correction did not differ (t = -0.46, df = 9, P = 0.657; Table 3). Moreover, the COIs of corrected images did not differ from COIs of images with colder and warmer color temperatures (F = 1.37, df = 4, P = 0.264; Table 3). Also, there was no difference between COIs of original and reduced images (F = 0.443, df = 4, P = 0.777; Table 4).

Table 4: Color Overlapping Indices (COI) calculated from the lizard images with its original and reduced sizes. The original images were reduced four times, each time representing a reduction of 20% over the previously reduced image. Values within cells are COI [number of pixels of animal area, number of pixels of substrate area].

species (substrate)	original	1x	2x	3x	4x
***Ameiva ameiva* (dirt floor)**	32.98 [319, 521]	32.98 [246, 385]	33.06 [184, 279]	32.83 [135, 203]	32.64 [97, 150]
***Anolis chrysolepis* (brown litter)**	74.58 [319, 85]	74.77 [242, 65]	75.22 [173, 51]	75.79 [123, 40]	76.62 [88, 33]
***Enyalius perditus* (white bark)**	4.23 [158, 122]	3.95 [113, 93]	3.58 [83, 71]	3.12 [62, 56]	2.29 [47, 40]
***Polychrus acutirostris* (beige bark)**	64.61 [203, 131]	64.57 [192, 99]	64.42 [146, 75]	64.52 [108, 59]	64.69 [83, 47]
***Tropidurus semitaeniatus* (rock with lichens)**	51.79 [103, 147]	51.69 [79, 155]	51.89 [61, 117]	51.78 [48, 88]	51.39 [38, 69]
***Tropidurus torquatus* (brown bark)**	80.80 [83, 227]	80.85 [64, 182]	80.64 [50, 133]	81.09 [38, 98]	80.17 [31, 73]
***Tropidurus torquatus* (rock)**	74.68 [39, 88]	75.03 [34, 72]	75.00 [29, 58]	74.26 [25, 47]	73.99 [22, 40]
***Tropidurus torquatus* (sand dune)**	60.47 [104, 166]	60.55 [82, 128]	60.54 [62, 96]	60.78 [48, 71]	60.69 [38, 54]
***Tropidurus torquatus* (white bark)**	15.51 [150, 287]	15.53 [115, 205]	15.59 [83, 145]	15.47 [63, 104]	15.24 [48, 76]
***Tupinambis merianae* (stony ground)**	65.22 [92, 90]	65.20 [74, 71]	65.41 [58, 57]	65.60 [46, 46]	65.45 [37, 39]

4. Discussion

We developed an affordable method to quantify animal-background contrast using digital photos taken under natural conditions of light and animal behavior. The method combines the use of the free software Image J (used to extract the color information) and an algorithm written in R (used to calculate the Color Overlapping Index, COI). COI calculates the degree of overlap between the sets of color classes shared by animal and substrate. Our evaluation using photos with varied animal-background contrasts suggests that COI is accurate, reflecting the degree of background matching observed in an image. COI also proved to be a robust metric that yields consistent results even for images with incorrect white balance and low resolutions.

To ensure comparability among samples, the method requires the standardization of a series of procedures, such as: camera settings, the approach to the animal, and the assignment of the range used for partitioning the color space. By maintaining constant the lens aperture, we ensured that any variation in the photo brightness is an effect on environment conditions of habitat chosen by the animal. For example, during the approach, the *T. torquatus* photographed on sand dune moved from a sunny to a shaded patch of the substrate. It may potentially reflect a behavioral strategy to improve crypsis. Moreover, *Polychrus acutirostris* was observed on a tree trunk completely shaded by the canopy, and *Tupinambis merianae* was observed on an overcast cloudy day. Depending upon the research questions of interest, observations could be limited to similar environmental conditions (e.g., only sunny habitats) or habitat type could be used as a factor in the analysis (e.g., sunny x cloudy habitats).

Most color metrics relies on quantification of the distances between the dominant hues displayed by the animal and substrate surface [11, 18, 20, 45]. However, this does not reflect the way in which animals see the world around them because animals see patterns of colors, and not isolated colors [14]. The COI takes this fact into account by comparing *patterns* of colors present in animals and the substrate, i.e., their color classes and the relative frequency of these color classes.

The degree of background matching of the lizards differed statistically when COI was calculated with different color intervals. Almost all lizards had a smaller COIs when a color interval of 22 was used instead of a color interval of 30. However, the difference between the indices was low. When we ranked the COIs, most species maintained themselves in the same position in both treatments. The only exception was *T. torquatus* on rock that changed its position with *A. chrysolepis* when analyzed with the color interval of 22, changing from the second to the third most cryptic.

The reduction of background matching as color interval decreased was theoretically expected. By reducing the ranges in which the color space is partitioned, one is moving towards higher resolution comparisons that even hues very close in the RGB space would be put into different classes. To illustrate it with an extreme example, imagine a hypothetical animal displaying 100% of its integument in the hue R=100, G=100, B=200. Now, imagine an equally homogeneous background displaying a unique hue of R=100, G=100, B=202. Even animal and background being practically impossible to discriminate with human eyes (and probably with any other species' eyes), the background matching would be zero if one use a color interval of 2. The reverse is also true when color intervals are very large (e.g., 128). For this reason, the assignment of the color intervals evokes parsimony. Thus, and despite our finding that COI values may vary a bit based on the color interval used, we suggest that it should not be a problem when applying the method in real cases because, to make the samples comparable, one should always perform the analyses using the same color interval for all images included in the study. It ensures that, if present, the decrease or increase in the COI values will be constant among all samples.

One advantage of using photos taken in natural conditions - in contrast with those made into laboratory - is that researchers can focus the measurements on the parts of animal's bodies that are exposed to a potential receiver during an encounter. However, the choice of the background area to be analyzed may be not so obvious. Should one select only the area close of the animal? Should one select the entire image, excluding the animal? The answer may depend on the specific biological question. Our results show that COIs calculated from a single sample differed from COIs calculated using five substrate samples. The multiple samples approach should be particularly useful when analyzing complex substrates. Cryptic preys should resemble a random sample of the background [46]. Thus, an alternative approach would be to partition the image using a grid and then randomize the background area (i.e., the cells of the grid) to be sampled.

Our findings suggest no difference between COIs when the white balance was corrected or not. The COIs also did not differ among the images with varied resolutions. This is because the animal-background comparison is made on a same image. Thus, any effects of changes in color temperature and resolution will affect equally both the pixels present in the animal and in the substrate.

The visual capacity of humans and other animal species may

differ substantially. For instance, an object seen as green to the human eyes may not be seen as green to bees, birds, and fishes [45]. This is the reason why many studies develop formal visual models to quantify animal-background contrasts [20, 32, 45]. Our method uses the RGB color system, which is based on the trichromatic vision of humans. The RGB system organizes millions of hues in a tridimensional color space so that similar hues are closer each other in the RGB space. Because of this organization of the hues, our method extends its applicability to non-human species. Although a pixel displaying a R=0, G=255, B=0 may not be seen as green to a non-human animal, a R=0, G=250, B=0 is certainly seen as having a very similar hue with the previous one to this same non-human animal. The crux of our method is that it is based on relative and not absolute terms. Moreover, a recent study testing many of current methods (including those parameterized to non-human vision) showed that the metrics based on human vision was the best predictor for the variation of anti-predator behavior in birds [47].

Some insects [45, 48], birds [49], and some lizards [19, p50] have tetrachromatic vision with UV-sensitive cones. Such vision capacity may provide predators enhance prey detection [51, 52]. To researchers interested in study visual ecology out of visible spectra, the method described here can be extended by using UV reflection photography [53]. It can be accomplished using UV-sensitive digital SLRs (several Nikon models are UV sensitive, or other brands with modified sensors) with an objective that does not absorb UV with a visible barrier filter and choosing the monochromatic option. Although the resulting images are presented in shades of gray, images can be compared using the same protocol. The limitation of this approach is that visible and UV spectra should be compared separately.

Along the text we stress the use of the COI index to quantify animal-background contrast, using lizards species as the model organism. However, we argue the index is obviously applicable to other animal taxa and other situations, as comparison between coloration of conspecific [18] and plant parts [11, 54].

5. Conclusion

We concluded that our method is an affordable way to quantify animal-background contrast using photos taken under natural conditions. The results showed that our method is accurate and robust to variations in color balance and image resolutions, representing an inexpensive and powerful tool for researchers engaged in study animal coloration.

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