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Efficiency of photo identification of inguinal color patterns of *Pithecopus gonzagai* (Anura: Phyllomedusidae) from northeastern Brazil

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Abstract

Efficiency of photo identification of inguinal color patterns of *Pithecopus gonzagai* (Anura: Phyllomedusidae) from northeastern Brazil. Animal monitoring research involving mark-recapture techniques increasingly requires non-invasive methods of individual identification. The photographic identification method (PIM) is an excellent tool for this purpose and has been applied successfully to many taxa. However, the utility of PIM is a function of species-specific features that are judged suitable for a given target-species. Herein, the suitability of inguinal color patterns for photo identification of individuals of *Pithecopus gonzagai* are evaluated by comparing two widely used computer-assisted photographic matching programs (I³S and Wild.ID). Both programs accurately identified more than 70% of individuals in the top 20 potential matching photographs. Wild.ID was slightly better than I³S in matching efficiency and has a faster processing time. Thus, PIM is useful to identify individual *P. gonzagai;* however, before implementing the technique in animal-monitoring studies of other taxa, one must evaluate the suitability PIM for the target species and calibrate the relative efficiency of the software programs in identifying individuals.

Keywords: Anurans, individual identification, mark-recapture method, I³S, Wild.ID.

Resumo

Eficiência da fotoidentificação dos padrões de cores inguinais de *Pithecopus gonzagai* (Anura: Phyllomedusidae) do nordeste do Brasil. A pesquisa de monitoramento de animais envolvendo técnicas de marcação-recaptura requer cada vez mais métodos não invasivos de identificação individual. O método de identificação fotográfica (PIM) é uma excelente ferramenta para esse propósito e tem sido aplicado com sucesso a diversos táxons. No entanto, a utilidade do

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PIM é em função de características específicas da espécie que são julgadas adequadas para uma determinada espécie-alvo. Aqui, a adequação dos padrões de cores inguinais para identificações fotográficas de indivíduos de *Pithecopus gonzagai* é avaliada comparando dois programas amplamente usados de correspondência fotográfica assistida por computador (I³S e Wild.ID). Ambos os programas identificaram com precisão mais de 70% dos indivíduos nas 20 melhores fotografias possíveis. Wild. ID foi ligeiramente melhor do que I³S em eficiência de correspondência e tem um tempo de processamento mais rápido. Assim, PIM é útil para identificação individual de *P. gonzagai*; entretanto, antes de implementar a técnica em estudos de monitoramento animal em outros táxons, deve-se avaliar a adequação do PIM para as espécies-alvo e calibrar a eficiência relativa dos programas de software na identificação de indivíduos.

Palavras-chave: anuros, identificação individual, método de recaptura de marca, I3S, Wild.ID.

Introduction

Individual recognition is essential to several kinds of biological studies, such as faunal monitoring, population demography, and behavioral ecology. However, to select the most appropriate way to identify individuals of a target species, one must carefully evaluate the benefits and disadvantages of various techniques for the specific system; biological constraints, financial costs, and any inherent ethical issues associated with a given taxon should be considered (Langkilde and Shine 2006, Caorsi *et al.* 2012). For example, despite the widespread usage of toe clipping and subcutaneous implants (elastomer or tags) in anurans (Brow 1997), these methods are invasive and controversial because their use may be harm the frog, especially in arboreal species in which adhesive discs are essential to perch (e.g., Clarke 1972, May 2004, Funk *et al.* 2005).

The Photographic Identification Method (PIM) is a useful, non-invasive marking alternative that can be used to distinguish individuals of species that possess characteristic features or natural markings (Würsig and Würsig 1977, Long and Azmi 2017). Many computer-assisted systems (pattern recognition or photographic matching software) have been developed that enable researchers to process large numbers of photographic images in relatively short timeframes. Thus, due to its biological, logistic, financial and ethical advantages, PIM has been increasingly used in research studies of an array of taxa, such as insects and sea stars (Chim and Tan 2012, Caci *et al.* 2013), sharks and rays (Tienhoven *et al.* 2007, Marshall and Pierce 2012), marine and freshwater teleost fishes (Correia *et al.* 2014, Dala-Corte *et al.* 2016), aquatic and terrestrial mammals (Kniest *et al.* 2010, Bolger *et al.* 2012), and turtles and lizards (Knox *et al.* 2013, Long and Azmi 2017), as well as toads and salamanders (Gamble *et al.* 2008, Caorsi *et al.* 2012).

Among anurans, hylid treefrogs comprise one of the largest families, with more than 750 described species (Frost 2018). Hylidae, Pelodryadidae (ca. 210 sp.), and Phyllomedusidae (ca. 70 sp.) compose the Arboranae lineage, which includes primarily arboreal species (Duellman *et al.* 2016). Many arboranan species have distinctive color patterns on the flanks, groins, or legs; these patterns do not change and thus, are suitable to track individuals in mark-recapture studies (e.g., Kenyon *et al.* 2009, Del Lama *et al.* 2011, Kim *et al.* 2017). We choose the arboreal phyllomedusid *Pithecopus gonzagai* Andrade, Haga, Ferreira, Recco-Pimentel, Toledo, and Bruschi, 2020 [previously included with *P. nordestinus* (Caramaschi, 2006) (Caramaschi 2006, Vilaça *et al.* 2011, Andrade *et al.* 2020)] as a target species because its morphological, ecological, and ethological features make it an excellent model for behavioral studies (Brasileiro *et al.* 2020, 2021). First, like most phyllomedusids, *P. gonzagai* has contrasting and colorful inguinal patterns (Figure 1) which make it highly suitable for individual identification (Oliveira *et al.* 2012). Second, individual frogs are easily habituated to humans, allowing for detailed recording of several stereotyped behaviors (e.g., fighting, Brasileiro *et al.* 2020). To validate the suitability and efficiency of PIM in *P. gonzagai,* we (1) assessed the use of inguinal color patterns in this species as a natural mark for individual identification and (2) evaluated the relative efficiency of two popular photographic image-matching programs for recognition of individual frogs.

Figure 1. Inguinal color pattern of *Pithecopus gonzagai* composed of black stripes on an orange background. The region used for individual identification is delimited with a rectangle.

Materials and Methods

Fieldwork was carried out in a temporary pond (03°52'4.02" S, 40°22'12.06" W; WGS84; 110 m a.s.l.; 1.058 m² of perimeter) belonging to the Rio Acaraú Basin, in the municipality of Groaíras, state of Ceará, northeastern Brazil. The study site is dominated by herbaceous vegetation (Figure 2) but is surrounded by open phytophysiognomies typical of the Caatinga Domain, including thorny and deciduous shrublands, as well as Carnaúba [*Copernicia prunifera* (Mill.) H.E.Moore] forest (Moro *et al.* 2015). Data were collected from February–June 2017. We captured individual *Pithecopus gonzagai* by active visual and acoustic searches around the margins of ponds where the tree frogs vocalized and spawned from 18:00–00:00 h, for a total of 37 h of sampling effort. The specimens were captured at random throughout the sampling period. For each frog captured/recaptured, one of us (FLA) took standardized photographs of the flanks with the inguinal color-pattern exposed (Figure 1). We released the individuals at their site of capture. Photographs were taken with a Sony DSC HX200V digital camera (f/4.5, 1/160s, ISO-400). All the sampling procedures were performed under authorization of Brazilian environmental agencies (ICMBio license nº 13587).

We selected the best photograph of each frog based on the focal quality and light exposure (but including slightly different angles) and cropped the images to encompass only the left inguinal region (Figure 1). The images were then compiled in a catalog to be submitted to matching process as follows. First, we performed a visual identification method (VIM) in which three researchers did a pairwise comparison of each photograph with the entire catalogue without any computer assistance. Second, we performed computer-assisted PIM, submitting the catalogue to two independent photographic image-matching programs, I³S (Tienhoven *et al.* 2007) and Wild.ID (Bolger *et al.* 2012).

The I³S software—Interactive Individual Identification System (available at http://www.reijns.com/i3s/)—delimits key-points of matching in each image, and presents a list of potential matching photographs, from which the user can identify the exact matching; see application in Town *et al.* (2013). The Wild.ID software (available at http://wildid.teamnetwork.org/index.jsp) automatically performs pattern recognition and matching, and also presents a rank of 20 most similar photographs classified according similarity; see application in Dala-Corte *et al.* (2016). The subset of the 20 most likely matches generated by each program was followed by inspection by human observers to evaluate a possible correct match in the lists.

For analytical purposes, we assumed that VIM precisely recovered all the recaptures of

Figure 2. Location of the study site, a temporary pond at the municipality of Groaíras, state of Ceará in northeastern Brazil.

our dataset. To evaluate the efficiency of software tested in the PIM of *Pithecopus gonzagai,* we compared the performance of each program with respect to two criteria: (1) the rate of correct matching (number of correct identification/total number of recaptures by VIM); and (2) how long the image processing took (the average time in seconds to import the photographs from the catalog and to select the area of interest—i.e., the inguinal region). The rate of correct identification was calculated based in the entire catalogue (123 photographs), checking if each target photograph was included among the 20 first potential matching photographs provided by both programs. The duration of image processing was calculated for each program based in average time spent to import and select the inguinal region of 60 images.

Results

We recorded 26 recaptures by VIM; therefor, this total was used as the reference to compare the efficiency of the photographic image-matching programs. The rates of correct matching were quite similar. The Wild.ID (Figure 3) calculated 76.9% (*N* = 20 recaptures) correctly, and the I^{3}S (Figure 4) calculated 73.1% (*N* = 19 recaptures) correctly.

Image processing in Wild.ID was much faster than in I^{3}S. The Wild.ID took 105 seconds to import the 60 photographs (average of 1.75 seconds per image), whereas I^{3}S required more than 100 minutes (average of 100.72 seconds per image).

Discussion

Inguinal color patterns of *Pithecopus gonzagai* can be used to identify individual frogs, thereby allowing the implementation of photographic identification methods. Visual identification without computer assistance may be more accurate than some computer-assisted programs (Kim *et al.* 2017); however this technique requires so much time to analyze a large database, that it is practically unfeasible (Markowitz *et al.* 2003, Carter *et al.* 2014). Thus, many authors recommend its use as a supplement to computer-assisted systems of pattern recognition (Gamble *et al.* 2008, Del Lama *et al.* 2011).

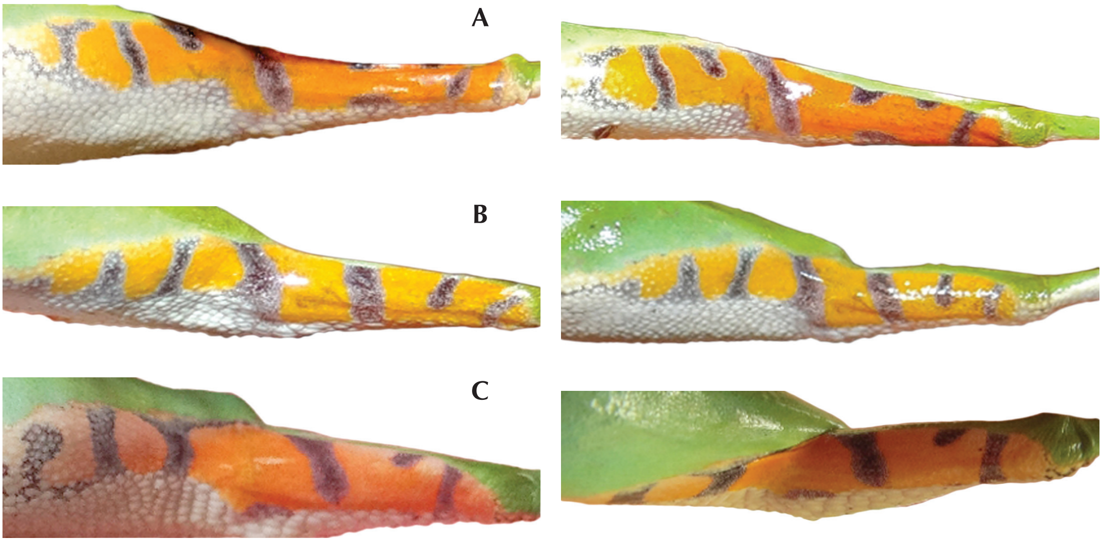

Figure 3. Results of three recapture matches with Wild.ID. Images in the left column correspond to focal images, and those in the right column correspond to the images with a high level of correspondence. Images (**A**) and (**B**) have the best level of correspondence (level 01 of 20), whereas image (**C**) has a relatively low level of correspondence (level 18 of 20), probably a result of the angle of the photograph.

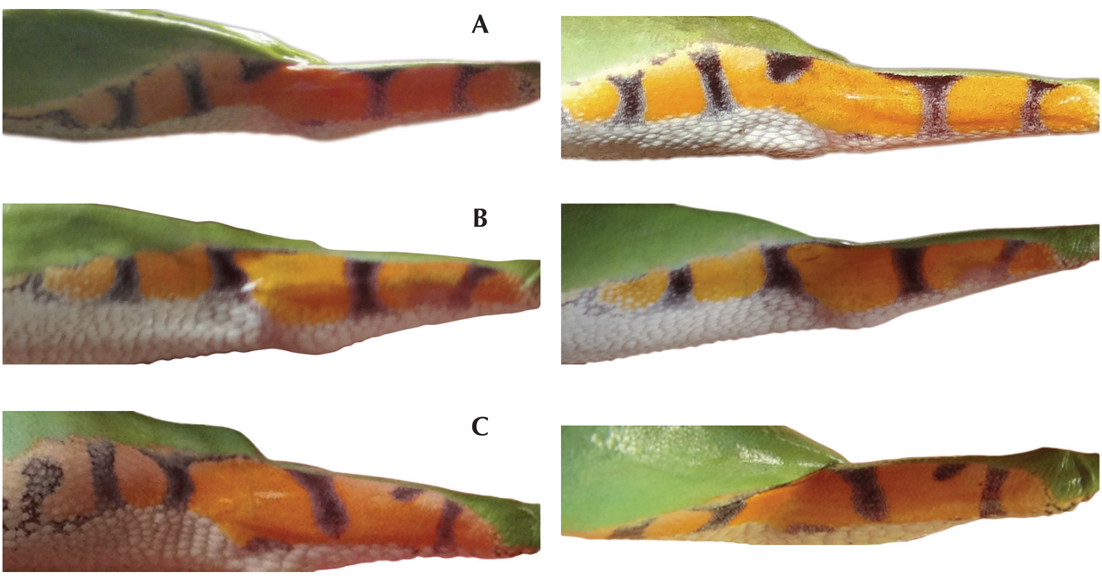

Figure 4. Results of three correspondence recaptures in the I³S software. Images are presented as in Figure 3. Images (**A**) and (**B**) matching the best, whereas image (**C**) had a relatively low level of correspondence, probably due to the difference of angulation.

The two photographic image-matching programs that we tested identified correct recaptures in the 70% range, but with a performance difference of 3.8%. The 70% value is reasonably efficient and and about the same as those reported in other studies of the performance of computer-assisted photographic identification (e.g., Kenyon *et al.* 2009, Beirão *et al.* 2014). Although both programs only performed at the 70% level, the enhanced processing speed is beneficial. Thus, the use of PIM software seems to be a suitable alternative for many scientific purposes (e.g., avoidance of pseudo-replication).

The Wild.ID software is much more efficient than I³S because it imports photographs so much more rapidly than does I³S (6946% slower than in Wild.ID), thereby decreasing the amount of time significantly to analyze the dataset. Several other studies also have commended the processing speed of Wild.ID relative to that of other software (Bendik *et al.* 2013, Halloran *et al.* 2015).

We conclude that both Wild.ID and I³S are suitable to identify individual *Pithecopus gonzagai* by the inguinal color pattern of the thighs. The results reinforce the suitability of photographic identification methods for studies of phyllomedusid frogs (e.g., Oliveira *et al.* 2012, Oliveira 2017). However, it is worth noting that some photographic variables, such as photo angle and exposure, can adversely affect the accuracy of the software performance. We recommend standardizing the photography procedure to eliminate variants that will affect the performance of the software. Additionally, researchers should consider the sizes of their datasets. If the sample is large, then the speed of Wild.ID is advantageous, whereas I³S is adequate for smaller samples.

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