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CLIP OR SNAP? AN EVALUATION OF TOE-CLIPPING AND PHOTO-IDENTIFICATION METHODS FOR IDENTIFYING INDIVIDUAL SOUTHERN RED-BELLIED TOADS, *MELANOPHRYNISCUS CAMBARAENSIS*

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Abstract. The most common method for identifying individual amphibians is toe-clipping (TC), whereby captured individuals are marked by a unique combination of amputated phalanges that corresponds to a unique alphanumeric code. However, ethical and methodological objections to this method have been raised and there is broad interest in developing alternative methods. One alternative is to use photo-identification methods (PIMs) to identify individuals based on their natural markings. We tested the efficacy of TC and two PIMs — visual matching (VM) and computer-assisted matching (CAM) using the software Wild-ID — in identifying individual adults of the endangered southern red-bellied toad, *Melanophryniscus cambaraensis.* We collected data over 5 mo at Floresta Nacional de São Francisco de Paula, Rio Grande do Sul, Brazil. All specimens were toe-clipped and photographed. The total dataset included 492 captures of 147 individuals. VM was most accurate (99.4%), followed by TC (95.3%) and CAM (90.9%); VM was significantly more accurate than TC and CAM and TC was significantly more accurate than CAM. CAM accuracy diminished as dataset size increased but was considerably faster than VM. All CAM and VM errors were false negatives but involved different images; all TC errors were cross-identifications. Given that misidentifications occurred using both PIMs and TC, our results suggest that studies that require high accuracy should employ at least two methods to allow cross-validation. The performance of each method and the impacts of different kinds and rates of error on inferences depend on the organisms, field conditions, dataset sizes, and study questions. As such, researchers must carefully evaluate the trade-offs of each method before investing significant time and resources in collecting field data.

Keywords. Individual identification; mark-recapture; visual matching; computer-assisted matching; misidentification; Amphibia; Anura; Bufonidae.

Introduction

Many wildlife studies require that individuals be identified in order to draw inferences from repeated observations or eliminate pseudoreplicates prior to analysis. For amphibians, the most widely used method of individual identification is toe-clipping, whereby captured individuals are marked by a unique combination of amputated phalanges that corresponds to a unique alphanumeric code (Donnelly *et al.,* 1994). Toe-clipping is quick, easy, inexpensive, and has become established through decades of use. Although ethical and methodological objections have been raised (Perry *et al.,* 2011), other methods of artificially marking amphibians (*e.g.,* external tags, passive internal transponder tags, brands, tattoos, subcutaneous elastomers) are afflicted by similar or worse problems. In the absence of a clearly superior method, the conservation importance of the ecological and demographic information obtained from toe-clipping studies clearly outweighs their potential negative impacts (Funk *et al.,* 2005). Nevertheless, there is broad interest in developing quick, easy, inexpensive, and reliable individual identification methods that avoid artificial marking.

Photo-identification methods (PIMs), which use photographs of unique natural markings to identify individuals (Bradfield, 2004), avoid most of the ethical objections to artificial marking methods, and it can be quick, easy, and inexpensive to generate images in the field. However, PIM utility also depends on the researcher's ability to quickly and accurately identify individuals. Assuming persistent natural markings occur, visual matching can be highly effective for small datasets but becomes increasingly onerous and inaccurate as image databases grow, which has led to the development and application of a variety of image matching algorithms (Kelly, 2001; Arzoumanian *et al.,* 2005; Speed *et al.* 2007; Gamble *et al.,* 2008; Hastings *et al.* 2008; Hiby *et al.* 2009; Sherley *et al.,* 2010). Recently, Bolger *et al.* (2011) released the software Wild-ID, which combines both approaches by scoring the pairwise similarity of all images and presenting the user with the 20 top-ranked matches for visual match confirmation.

Few studies have compared toe-clipping and PIMs, and none has compared the accuracy of toe-clipping, visual matching, and computer-assisted matching. As such, the primary objective of this study was to compare the accuracy of toe-clipping and two PIMs — visual matching and computer-assisted matching using Wild-ID — to identify adult individuals of the endangered southern red-bellied toad, *Melanophryniscus cambaraensis* Braun and Braun, 1979. To better assess the trade-offs associated with these two PIMs, we also compared the time required for visual and computer-assisted matching and evaluated the numerical performance of Wild-ID.

Materials and Methods

Data collection

Melanophryniscus cambaraensis is a small (*ca.* 35 mm snout-vent length), poisonous toad that migrates diurnally (Santos and Grant, 2011). Dorsal coloration is bright green and varies little among individuals. Ventral coloration is predominantly red with highly variable green, grey, or black blotches and white tubercles (Fig. 1; Braun and Braun, 1979). Coloration and tuberculation are not sexually dimorphic; however, recently metamorphosed individuals lack bright coloration (Fig. 2) and the ontogeny of pigmentation is unknown. As such, we focused exclusively on adults.

We studied *Melanophryniscus cambaraensis* at Floresta Nacional de São Francisco de Paula, southern Brazil (29°25'41.3"S, 50°23'44.5"W, 866 m above sea level), from October 2008-February 2009 (139 d from first to last sampling day). The study site and capture methods are described in Santos *et al.* (2010) and Santos and Grant (2011). All captured specimens were first weighed to 0.1 g and examined for overall health. Digits were removed according to Waichman's (1992) alphanumeric system using surgical scissors sterilized by flaming and cleaning in 100% ethanol, and 1% silver sulfadiazine antibiotic cream was immediately applied to the wound. Amputated digits were preserved in 100% ethanol. Previously toe-clipped specimens were examined for digit regeneration (Ursprung *et al.,* 2011) and their unique alphanumeric code was immediately recorded.

All specimens were subsequently photographed with a digital camera (Sony DSC-H1 5.1 MP, Sony DSC-W210 12.1 MP, or Sony DSC-W90 8.1 MP) using the built-in flash. We photographed entire venters by placing specimens on their backs on white paper next to a ruler (0.5 mm precision) for scale. Previous studies highlighted the importance of obtaining high quality images (Forcada and Aguilar, 2000; Gowans and Whitehead, 2001), so we ensured animals were clean (*i.e.,* free of debris that could obscure natural marks), dry (to avoid flash reflections), and in a position that did not conceal ventral markings, and we took 2-3 images per specimen to ensure proper focus and framing. Individuals were observed for at least 5 min prior to release. Images were later screened for quality and lighting and a single image was selected from each capture event. Selected images were cropped to eliminate as much of the background as possible and were saved in a new directory for individual identification.

Individual identification

Toe-clipping identification was based on the alphanumeric code recorded in the field. PIM identification was performed without knowledge of the specimens' alphanumeric codes. Visual image matching was accomplished by comparing each image to all others and examining the coloration of the belly, throat, arms, and legs. For computer-assisted image matching we used the Java program Wild-ID (Bolger *et al.,* 2011), which uses the scale invariant feature transform algorithm (SIFT; Lowe, 2004) for pattern extraction, compares the geometric arrangement of the SIFT features of each pair of images, and calculates a match score. The software then shows the 20 top-ranked matches for visual confirmation.

Given that no identification method is necessarily error-free, we used cross-validation to definitively establish specimen identity. To measure the accuracy of each method, we scored each identification as correct or incorrect relative to the cross-validated identification. We performed a χ^2 test to determine if the accuracy of the three methods differed significantly, assuming a significance level of 0.05 and Bonferroni correction for multiple comparisons. We also classified each error as (1) false negative (misidentification of a recaptured individual as a previously uncaptured individual), (2) false positive (misidentification of a previously uncaptured individual as a recaptured individual), or (3) cross-identification (misidentification of one previously captured individual as another previously captured individual).

Because all specimens were toe-clipped and photographed simultaneously, we were unable to compare

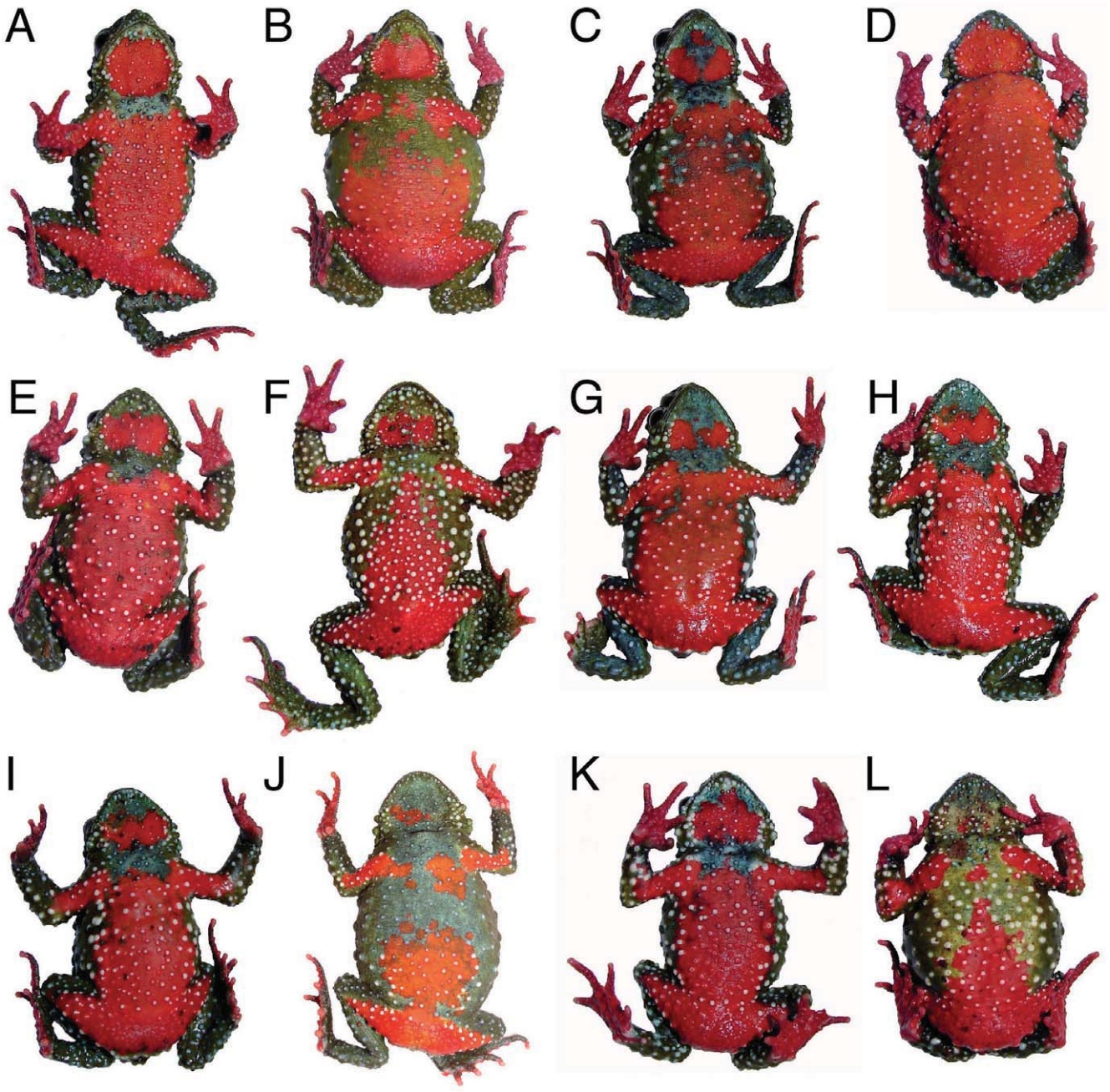

Figure 1. Ventral view of a sample of 11 adult individuals of Melanophryniscus cambaraensis from Floresta Nacional de São Francisco de Paula, RS, Brazil. (A) A1 C2 (male, SVL = 29.2 mm). (B) A2 D3 (female, SVL = 32.4 mm). (C) A3 D3 (female, SVL = 31.6 mm). (D) B3 C1 (female, SVL = 34.7 mm). (E) C1 D3 (male, SVL = 28 mm). (F) C1 D4 (male, SVL = 27.9 mm). (G) C3 D5 (male, SVL = 29.6 mm). (H, I) C4 (male, SVL =29.9 mm). (J) B3 D1 (female, SVL = 31.8 mm). (K) C4 D2 (male, SVL = 29.1 mm). (L) D1 (male, SVL = 31.3 mm).

field-processing times for the two methods. However, we compared the time required to perform visual and computer-assisted (Dell Inspiron N5110, Intel Core i5-2410M 2.3 GHz 2.30 GHz CPU, 6 GB RAM, Windows 7) matching of 100 randomly selected images. To better understand the performance of the Wild-ID software, we examined the rank of the correct matches among the 20 top-matches and (Bolger *et al.,* 2011) and assessed the accuracy of computer-assisted analyses of reduced datasets by analyzing the first 25%, 50%, and 75% of captures.

Results

The total dataset included 492 images of 147 individuals. We observed neither digit regeneration nor indication of infection, necrosis, or deterioration of

Figure 2. Ventral view of a metamorphosing individual (8.4 mm SVL) of *Melanophryniscus cambaraensis.*

health attributable to toe-clipping. However, we did observe several apparently unrelated injuries and malformations, including partial absence of unclipped digits, recently injured and infected unclipped digits, and inability to use the right leg of a previously uncaptured individual.

The greatest accuracy was achieved by visual matching (VM), which correctly identified all but three captures (99.4%). Toe-clipping (TC) was second, with 95.3% accuracy, followed by computer-assisted matching (CAM), with 90.9% accuracy for the entire dataset. The differences in accuracy for the analyses of the entire dataset were highly significant (VM-CA: $\chi^2 = 38.635$, $p = 0.0001$; VM-TC: $\chi^2 = 15.802$, $p = 0.0001$; CA-TC: $\chi^2 = 7.646$, $p = 0.0057$; corrected significance level = 0.017). All CAM and VM errors were false negatives but involved different images. All TC errors were cross-identifications.

Choice-rank in CAM was high, with > 90% of the correct matches ranked in the top 3 (74% ranked first, 10% second, 5% third). The time required to match 100 randomly selected images was 180 min for VM and 20 min for CAM, including the time required to visually confirm each match. CAM accuracy was 95% for the 25% (n = 123 captures), 50% (n = 246 captures) datasets and 93% for the 75% (n = 369 captures) dataset.

Discussion

None of the tested methods was error-free, but visual image matching (VM) was significantly more accurate than toe-clipping (TC) and computer-assisted image matching (CAM), and TC, in turn, was significantly more accurate than CAM. Our finding that VM was significantly more accurate than TC differs from that of Kenyon *et al.* (2009), who reported that TC was considerably, albeit not significantly, more accurate than VM. This difference probably owes to differences in the conspicuousness of the natural markings in the two species. Indeed, Kenyon *et al.* (2009) underscored the difficulty in visually identifying individuals that lacked distinctive dorsal hourglass patterns, which constituted the majority of their sample, whereas the bright ventral patterns of *Melanophryniscus cambaraensis* were well defined in all sampled individuals.

Although both photographic identification methods (PIMs) exhibited the same class of error (viz., false negatives), the specific images that were misidentified differed. As such, by combining the two PIMs all errors were eliminated. TC errors were exclusively cross-identifications caused by human errors when recording alphanumeric codes in the field. Given that misidentifications occurred using both PIMs and TC, we recommend that studies that require high accuracy employ at least two methods to allow cross-validation.

The performance of Wild-ID (Bolger *et al.,* 2011) in identifying individual *Melanophryniscus cambaraensis* was similar to that of previously studied programs and organisms (*e.g.,* Kelly, 2001; Arzoumanian *et al.,* 2005; Speed *et al.,* 2007; Gamble *et al.,* 2008; Hastings *et al.,* 2008; Hiby *et al.,* 2009; Sherley *et al.,* 2010). Importantly, correct matches were ranked in the top 3 in the vast majority of

comparisons, which greatly facilitated visual confirmation. Although CAM accuracy diminished as dataset size increased and was significantly lower than for either VM or TC, CAM image matching was considerably faster and remained > 90% accurate.

We did not observe any variation in ventral coloration during the course of our study, which is consistent with most previous studies of adult anurans (Stephenson and Stephenson, 1957; Denton and Beebee, 1993; Kenyon *et al.,* 2010; but see Kenyon *et al.,* 2009). Nevertheless, we caution that the bright ventral pigmentation of *Melanophryniscus cambaraensis* is lacking at metamorphosis and is acquired over time. Insofar as we exclusively targeted migrating adults, we did not assess ontogenetic variation in ventral coloration or its effect on PIM accuracy. Similarly, although we did not observe digit regeneration in adults, we did not assess the potentially extensive regeneration in juveniles (*e.g.,* Richards *et al.,* 1975), which could also confound individual identification.

No method of individual identification can be guaranteed to be completely error-free, and the overall performance of each method and the impacts of different kinds and rates of error on inferences will depend on the organisms, field conditions, dataset sizes, and study questions. Toe-clipping has the ancillary advantages of generating tissue samples for DNA analysis and skeletochronology of phalanges, and, although accuracy was high in the present study, PIMs appear to be less effective for species with less conspicuous natural markings. As such, selection of the optimal method of individual identification is a scientific problem — not a legal or political one — that requires researchers to carefully evaluate the trade-offs of each method before investing significant time and resources in collecting field data.

Resumo

O método mais utilizado para a identificação individual de anfíbios é a marcação por amputação de falanges (AF), no qual cada indivíduo capturado é marcado através de uma combinação única de falanges amputadas de um ou mais dígitos de acordo com um código alfanumérico. Entretanto, alguns questionamentos éticos e metodológicos tem sido levantados a respeito deste método de marcação e existe um grande interesse em desenvolver métodos alternativos. Os métodos de fotoidentificação (MFI) são uma alternativa que permite identificar indivíduos a partir de padrões de coloração e marcas naturais dos animais estudados. Neste estudo, nós testamos a eficácia da AF e dois tipos de MFI — através da identificação visual (IV) e outro com o auxílio de computador (AC) usando o software Wild-ID — na identificação individual de adultos do sapinho-de-barriga-vermelha, *Melanophryniscus cambaraensis.* Os dados foram coletados durante um estudo de cinco meses realizado na Floresta Nacional de São Francisco de Paula, Rio Grande do Sul, Brasil. Todos os espécimes coletados foram marcados pelo método de AF e posteriormente fotografados. O banco de dados teve um total de 492 capturas correspondentes a 147 indivíduos. O método de IV foi o método mais acurado (99,4%), seguido de AF (95,3%) e AC (90,9%); IV foi significativamente mais acurado que os outros dois métodos, enquanto AC foi significativamente menos acurado. A acurácia de AC diminuiu conforme aumentou o banco de dados a ser analisado, entretanto, seu processamento foi consideravelmente mais rápido que IV. Todos os erros cometidos com AC e IV foram falsos negativos, porém envolveram diferentes imagens; já os erros da AF foram identificações cruzadas. Uma vez que os erros de identificação ocorreram tanto nos métodos MFI como AF, os resultados sugerem que estudos que requerem uma alta acurácia devem utilizar pelo menos dois métodos diferentes para permitir a validação cruzada. O desempenho de cada método, seus impactos e taxa de erros nas inferências dependem do organismo estudado, condições do trabalho em campo, tamanho do banco de dados e os objetivos do estudo. Sendo assim, os pesquisadores devem avaliar cuidadosamente as vantagens e desvantagens de cada método de identificação individual antes de investir recursos e tempo na coleta de dados em campo.

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