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Treefrog lateral line as a mean of individual identification through visual and software assisted methodologies

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Abstract

Background: Ecological research often requires monitoring of a specific individual over an extended period of time. To enable non-invasive re-identification, consistent external marking is required. Treefrogs possess lateral lines for crypticity. While these patterns decrease predator detection, they also are individual specific patterns. In this study, we tested the use of lateral lines in captive and wild populations of *Dryophytes japonicus* as natural markers for individual identification. For the purpose of the study, the results of visual and software assisted identifications were compared.

Results: In normalized laboratory conditions, a visual individual identification method resulted in a 0.00 rate of false-negative identification (RFNI) and a 0.0068 rate of false-positive identification (RFPI), whereas Wild-ID resulted in RFNI = 0.25 and RFNI = 0.00. In the wild, female and male data sets were tested. For both data sets, visual identification resulted in RFNI and RFPI of 0.00, whereas the RFNI was 1.0 and RFPI was 0.00 with Wild-ID. Wild-ID did not perform as well as visual identification methods and had low scores for matching photographs. The matching scores were significantly correlated with the continuity of the type of camera used in the field.

Conclusions: We provide clear methodological guidelines for photographic identification of *D. japonicus* using their lateral lines. We also recommend the use of Wild-ID as a supplemental tool rather the principal identification method when analyzing large datasets.

Keywords: Lateral lines, Photographic individual identification, Treefrog, Wild-ID, Visual identification, Software assisted identification

Background

Individual identification of animal is critical in behavioral ecology. Numerous research subjects such as fitness, life history, territoriality, social behavior, and long-term monitoring require repeated identification of individuals. Traditionally, individual identification was conducted through uniquely applied markings (Amstrup et al. 2010). Markings can be natural or artificial. For amphibians, traditional marking techniques include toe-clipping, branding, tattooing, subcutaneous elastomer injections, and subcutaneous pit tags (Ferner 1979, Donnelly et al., 1994). One of the most frequently used method is toe-clipping because of its easy and inexpensive use (Donnelly et al., 1994, Waichman 1992). However, its use is currently debated as an invasive method, potentially causing infections and altering behaviors, especially for small species like hylids (Clarke 1972, Golay and Durrer 1994, Lemckert 1996, Waddle et al. 2008, Guimaraes et al. 2014). As a result, the environmental administration of the Federative Republic of Brazil has considered a ban on toe-clipping (Corrêa 2013). In contrast, the use of natural markers in photographic identification method (PIM) has gained popularity because of technological advances, being relatively inexpensive, and its non-invasive quality.

Amphibians are under aggravated threats (Stuart et al. 2004, Wake 2012), and the use of individual natural markings in PIM is a popular alternative method for non-invasive individual identification. To date, herpetological

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PIM has been used to identify individuals from a wide range of species with unique natural patterns, including frogs (Lama et al. 2011), toads (Elgue et al. 2014), salamanders (Church et al. 2007, Gamble et al. 2008), geckos (Knox et al. 2012), and leatherback turtles (*Dermochelys coriacea*; McDonald et al. 1996) among others.

The use of PIM with dorsal patterns of adult green-eyed treefrogs (*Litoria genimaculata*) as a natural marker was successfully demonstrated in the wild (Kenyon et al. 2009). The natural markers commonly used for hylid species are dorsal, ventral, and leg patterns. These patterns are stable over time, with the exception of juveniles, and are thus appropriate for PIM studies (Bolger et al. 2012). In contrast, colors are less stable and are not reliable markers.

More recently, computer-assisted PIM is used in an expanding number of mark and recapture studies throughout a broad range of species. When the image catalog for previous captures are large, the visual inspection becomes not only time consuming but unreliable. Therefore, advances on digital image analysis tools and pattern recognition algorithms play a significant role in the development and spreading the use of PIM (Bolger et al. 2012). However, the efficiency and accuracy of PIM and photographic analysis tool should always be tested before being implemented.

Here, we tested the use of lateral lines as natural markers for individual identification of wild and captive populations of *Dryophytes japonicus*. PIM was conducted by both visual identification method and a computerized assisted photograph-matching program: Wild-ID. Our goals were to assess the use of *D. japonicus* lateral line as a natural marker in individual identification, assess the validity of our methodology in a scientific research setting, provide protocols for the collection of photographic data, and demonstrate limitations of Wild-ID in identifying an individual *D. japonicus* using its lateral line as a natural marker.

Methods

The species used for this study was *Dryophytes japonicus*, previously assigned to *Hyla japonica* (Duellman et al., 2016), and recognized as synonymous to *D. ussuriensis* and *D. stepheni* (Dufresnes et al. 2016). The species is present in North East Asia and is the most common amphibian species breeding in rice paddies in the Republic of Korea (Roh et al. 2014). The photographic data for this study were collected over two independent periods, once in the laboratory and once in the field. In photographs, a lateral line was defined as starting from the posterior tip of the tympanum and finishing at the skin folds leading to the rear legs. For each individual, photographs were taken in series while the individual was held presenting its dextral lateral line parallel to the camera. Care was taken to including the totality of the lateral line on the picture by carefully holding the individual's legs between thumb and index finger while supporting the body with the thumb (Fig. 1a), and thus preventing the frogs from sitting naturally and bending its legs.

Before using the photographs for PIM, the best quality photograph with the lateral line clearly visible was selected for each individual frog. The selected photographs were then cropped such that the totality of the lateral line was included with a minimum amount of background (Fig. 1b). The cropping process excluded the majority of the background to remove external cues such as frog legs or dorsal patterns and in some cases the fingertips of the researcher.

The processed photographs were analyzed twice, once with visual identification and the other with PIM. For the visual identification method, a group of participants identified matching lateral lines without a computerized matching program. For PIM, a group of separate participants used Wild-ID to identify identical lateral lines. The computerized matching program Wild-ID 1.0 (Version 1.0; Dartmouth College; Hanover, New Hampshire, USA) used in this study is a pattern extraction matching program for photographs (Bolger et al. 2012). The software employs the Scale Invariant Feature Transform (SIFT) operator, and for each photograph, it returns the 20 most similar photograph in rank according to the matching score of similarity (1.0 = 100% similarity, 0.0 = 0% similarity). All photographs were

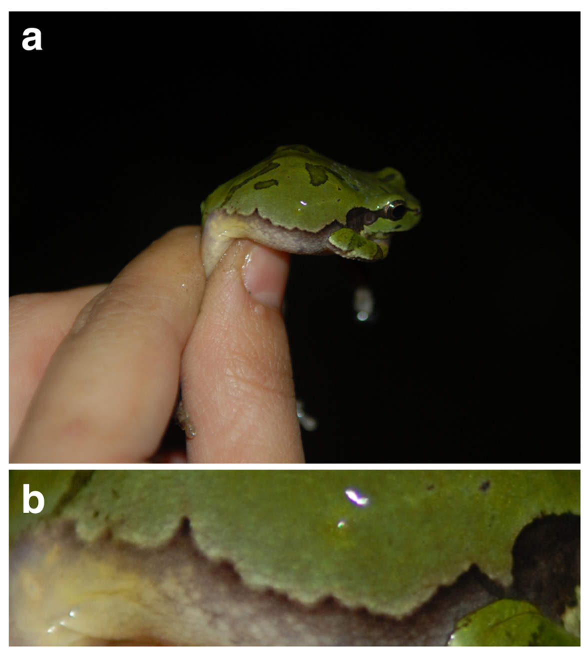

Fig. 1 Photographic illustration on how to take a photograph of the lateral line of an individual frog to be used for the PIM. **a** How frogs are held to show the totality of lateral line in the photograph. **b** Cropped photograph with the lateral line and with minimum amount of background to be used for the analysis

processed by Wild-ID according to the suggested procedure (Boldger et al. 2012).

Before looking at the dataset of the study, all participants were given a set of ten randomly selected photographs with two matching individuals and were asked to identify them either with or without a computerized matching program. All participants in this study were field trained, familiar with frog patterns, and without prior interaction with the photographs used in this study.

Photographs of lab-raised individuals

In order to conduct the photographic identification in laboratory conditions, 27 *D. japonicus* were caught in the city of Paju, Republic of Korea (37.7519° N, 126.7253° E), in September 2013. Individuals were subsequently housed in cylindrical PVC boxes (15 cm diameter × 15 cm high), with vertical aerations until November 2013. Individuals were photographed every 14 days, with a tripod mounted camera (DSLR, NIKON D200 and AF-S NIKOR 17–55 mm 1:2.8). The photographs were taken under a normalized light condition averaged at 52.3 lx.

Analysis of lab photographs

A set of photographs for 27 different *D. japonicus* and one matching photograph of one individual taken at two different dates were formatted for the analysis ($n = 28$). This allows for true individual match known to the researcher. For the analysis, eight participants were given the set of photographs and were asked to visually identify matching lateral lines. Eight different participants were then asked to identify identical individuals through Wild-ID.

Photographs of field-caught individuals

The second data set used was acquired during weekly surveys throughout the breeding season of *D. japonicus*, from 18 April to 3 July 2014 (for the full protocol, see Kim 2015). During each survey, every individual seen was caught and photographed for its lateral pattern using three different types of methods: (1) a photo-box that provided normalized setting with Samsung compact cameras (ES95; Samsung, People's Republic of China) and illuminated by mini motion light (average = 146 lx; Koninklijke Philips Electronics N.V., People's Republic of China) covered with neutral gray paper sheets; 2) Nikon D200 and mounted lens AF-S NIKKOR 17-55 mm 1:2.8 with its own momentary flash (average = 395 lx), and (3) Sony digital camera (Alpha NEX-C3; Sony, Thailand) with its own flash (average = 148 lx).

Analysis of field photographs

For the purpose of this study, photographs of all females caught throughout all the weekly surveys ($n = 75$) were used as the first dataset, while only males caught on 11 June 2014 and 18 June 2014 ($n = 318$) were used as the second dataset. For the analysis, two participants were given both datasets and asked to visually identify matching lateral lines. Then, two different participants were given the same two datasets and were asked to identify identical lateral lines using Wild-ID.

Performance of identification method

To evaluate the identification rate of both visual identification and PIM, we calculated the rate of false-negative identification (RFNI: failure to match two images of the same individual; RFNI = number of false-negative identification/number of identification attempts) and the rate of false-positive identification (RFPI: match of two images of different individuals; RFPI = number of false-positive identification/number of identification attempts).

Finally, the photographs from the recaptured males were run alone into Wild-ID for an ad-hoc test to compare matching scores and camera types between recaptures. The matching score provided by Wild-ID for matched photographs represents the relative closeness of the match, with 1.0 being the highest match value. The resulting scores were correlated with the continuity of the type of camera used (Table 1). The statistical analysis were run with SPSS v21.0 (SPSS, Inc., Chicago, IL, USA).

Table 1 Matching scores ($1.0 \geq n \geq 0.0$) given by Wild-ID for recaptured males for the two consecutive weeks with the type of camera used to take the lateral line photograph for each field date

Matching score	Camera used in 11 June 2014	Camera used in 18 June 2014
0.000000	Samsung ES95	Samsung ES95
0.000000	Samsung ES95	Samsung ES95
0.000000	Samsung ES95	Samsung ES95
0.000000	Samsung ES95	Samsung ES95
0.000000	Samsung ES95	NIKON D200
0.000000	Sony Alpha NEX-C3	Samsung ES95
0.000000	Sony Alpha NEX-C3	Sony Alpha NEX-C3
0.000003	Sony Alpha NEX-C3	NIKON D200
0.000003	Sony Alpha NEX-C3	NIKON D200
0.000003	Sony Alpha NEX-C3	NIKON D200
0.000000	NIKON D200	Samsung ES95
0.000000	NIKON D200	Samsung ES95
0.000000	NIKON D200	Samsung ES95
0.000000	NIKON D200	Samsung ES95
0.000000	NIKON D200	Samsung ES95
0.000000	NIKON D200	Samsung ES95
0.000004	NIKON D200	Samsung ES95
0.131066	NIKON D200	NIKON D200

Results

Photographs of lab-raised individuals

The visual identification method resulted in eight positive matches, with a RFNI of 0.00 and 21 false-positive matches with a RFPI of 0.0068. The photographic analysis using Wild-ID resulted in six positive matches with RFNI = 0.25 and no false-positive matches with RFNI = 0.00. The matching pair found through Wild-ID received a matching score of 0.072732, with the picture ranked first from the list of 20 potential matches.

Photographs of field caught individuals

The visual identification for the female data set had a RFNI of 0.00 and RFPI of 0.00, whereas the RFNI was 1.00 and RFPI was 0.00 through Wild-ID. An ad-hoc run on Wild-ID to assess the score given by the software for a match of the two photographs returned a value of 0.000002, a score too low to be considered as a potential match in Wild-ID. It was observed that when matching scores were low for all photographs, the 20 potential matches given by Wild-ID followed the order of input in the database folder. The visual identification of photographs of males from the two consecutive weeks identified 18 recaptures with RFNI of 0.00 and RFPI of 0.00. Using Wild-ID, no recaptured individuals were identified, resulting in RFNI = 1.0 and RFPI = 0.0.

When comparing the continuity in the type of camera used and the best scores, the highest score was obtained for the recapture photographed both times with the DSLR camera (0.131066), whereas every other combination of camera type had 0.000004 for highest match score (Table 1). The correlation between repetition in camera type and scores was significant ($R = -0.67$, $n = 18$, $p = 0.014$), but it was not for camera type used for one of the two captures (first capture: $R = 0.24$, $n = 18$, $p = 0.332$; second capture: $R = -0.27$, $n = 18$, $p = 0.914$).

Discussion

Despite their popularity, the computerized photographic identification methods are difficult to use for identification of individual treefrogs based on their lateral lines. However, and especially for small datasets, using lateral lines as natural markers to identify individuals is possible through visual inspection.

One of the limitations of computer-assisted photographic identification is the production of misidentification errors that can severely bias studies (Lukacs and Burnham 2005, Yoshizaki et al. 2009). It is thus important to set an estimate of misidentification error as a step in evaluating the efficacy of computer-assisted photographic identification (Hastings et al. 2008). In this study, both false-negative identification and false-positive identification occurred, but more importantly, the identical individual photographs were not given as a potential match by the program.

Wild-ID is a pattern-matching program designed to find and extract distinctive image features (Bolger et al. 2012). Ever since the release of the program, it has been used for many species of animals, the majority of them with spots (Bolger et al. 2012, Bendik et al. 2013, Elgue et al. 2014, Dala-Corte et al. 2016). This study is uncommon in that it is testing the program on a linear pattern. Therefore, the result of this experiment could be an indication of the limitation on using Wild-ID with different pattern types.

The scores for Wild-ID were low, resulting in the absence of matching individuals in the ranked potential matches, which directly affected the rate of individual identification. The results of this study show that the matching scores were affected by the type of camera that was used to take photographs. The only high matching score in this study originated from photographs taken with the DSLR camera, providing high-quality photographs (Table 1). The quality of the photograph is an important factor in the successful use of Wild-ID, which means that high-end cameras should be used to obtain optimum quality photographs.

Despite the inability of Wild-ID to get matches, the software can be used to assist with very large datasets. Wild-ID gives 20 photographs as a potential matches, and therefore, if only 20 photographs are fed to Wild-ID at a time, the software can be used as a facilitating comparison tool, rather than a software picking matches from a database.

The visual identification method was successful in individual identification despite the dorsal coloration change in accordance with surrounding conditions (Choi and Jang 2014, Kang et al. 2016). The longest period between taking two photographs that were identified as matched by participants was 35 days. This suggests a possibility of using PIM in long-term ecological research of *D. japonicus.*

Our results also highlight the importance of normalized conditions. Taking pictures in laboratory conditions improved matches. Despite our efforts, it is difficult to maintain the same standard in the field. Finally, this experiment relied on photographing the lateral line of individuals, but focusing on other permanent morphological cues such as leg stripes may provide different results.

Conclusions

This study tested the use of lateral lines of *D. japonicus* in photographic individual identification and compared the performance of visual identification method and software assisted identification method (Wild-ID). The results demonstrate that lateral lines of *D. japonicus* could be used as a natural marker for individual identification; however, only visual identification method was proven to be reliable for identifying recaptured individuals.

The suggested key guidelines for using lateral lines of treefrog for photographic identification are as follows: 1. The entire lateral line of an individual should be captured through holding its legs back, 2. Constant high quality of photograph is crucial in getting the best results in identification of individuals and 3. In analyzing the data, we suggest use of visual identification method with using Wild-ID as a supplementary tool.

Abbreviation
PIM: Photographic identification method

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Availability of data and materials
The datasets are available from the corresponding author on a request.

Authors' contributions
MK carried out the study, performed the analysis, and wrote the manuscript. JK conducted the field data collection as well as part of PIM. AB participated in the design of the study, analyzed data, and wrote/reviewed the manuscript. YK contributed to draft the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The experiments in this study comply with the current law of the Republic of Korea (Ministry of Environment Permit Number: 2013–16).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interest.

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