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Evaluation of a new AmphIdent module and sources of automated photo identification errors using data from *Salamandra infraimmaculata*

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Capture-mark-recapture (CMR) studies are an important tool in animal population biology (LEBRETON et al. 1992). Individual identification allows the quantification of various life history traits and fitness parameters, as well as population size estimates and displacements studies (SCHMIDT et al. 2007, BOLGER et al. 2012, UNGLAUB et al. 2015). Non-invasive individual identification methods are becoming increasingly popular, and the photographic identification of natural colour patterns is one of the most commonly used non-invasive methods. For manual (non-automated) individual identification and recapture analysis methods (i.e., comparing photos visually by a trained observer) the handling time per photo and the recognition errors increase strongly with the size of the dataset of photographs to be compared (KATONA & BEARD 1990). Research based on large photographic datasets therefore benefits greatly from computer-assisted automated analysis methods. Computer-assisted photo identification has been applied to studies based on individual colour patterns in various animal systems, including giraffes, cheetahs, marbled salamanders, and ventral fluke patterns in humpback whales (KATONA & BEARD 1990, KELLY 2001, SCHMIDT et al. 2005, GAMBLE et al. 2008, BOLGER et al. 2012, BENDIK et al. 2013).

AmphIdent[®] is a dedicated high-performance software for automated amphibian photo analysis (<http://www.amphident.de>, MATTHE et al. 2008, DRECHSLER et al. 2015). AmphIdent is based on an algorithm for modified cross-correlation comparisons of specific signal patterns

within a large set of images. Specific AmphIdent modules are already available for the ventral spot patterns of adult crested newts, *Triturus cristatus* (LAURENTI, 1768) (MATTHE et al. 2008), and Fire-Bellied Toads of the genus *Bombina* (OKEN, 1816). These modules have been proven to be rather tolerant of shifts, distortions and other minor differences in the images (DRECHSLER et al. 2015). Here we present the first results of a new module for individual salamander identification, which will in principle be applicable to all taxa with a cylindrical body and a yellow spotted dorsal pattern (including spotted members of the *Salamandra* LAURENTI, 1768 species complex as well as many *Ambystoma* TSCHUDI, 1838 species and potentially also certain lizard species such as *Heloderma* WIEGMANN, 1829 and certain snakes). The new salamander module can be requested from the developer and will soon be made available online. Because AmphIdent identifies all yellow pixels, it makes use of the full extent of the yellow pattern. AmphIdent is therefore more precise than, for instance, the grey-scale circle or ellipse-based approach of the Interactive Individual Identification System (I3S, VAN TIENHOVEN et al. 2007). In addition, it is conceptually simpler than Scale Invariant Feature Transform (SIFT) procedures, which require the identification of grey-scale feature points with calculated properties: scale, orientation, and descriptor (BOLGER et al. 2012). The new AmphIdent salamander module may be a valuable tool for conservation purposes, especially for detecting population declines in the face of emerging diseases such as the salamander-

specific chytrid fungus *Batrachochytrium salamandrivorans* (SPITZEN-VAN DER SLUIJS et al. 2013, MARTEL et al. 2014).

The six species of the *Salamandra* complex form a non-monophyletic group within the Salamandridae with some species being black and yellow (the so-called Fire Salamanders), and others completely melanistic (the so-called Alpine Salamanders) (STEINFARTZ et al. 2000, VENCES et al. 2014). The black-and-yellow spotted dorsal pattern of Fire Salamanders has been used previously for the visual identification of individuals in ecological studies of a number of salamander species (e.g., DOODY 1995, CARAFA & BIONDI 2004, WARBURG 2006). The Near East Fire Salamander (*Salamandra infraimmaculata* MARTENS, 1885) is distributed in Turkey, Iran, Iraq, Syria, and Lebanon; its range reaches the southernmost limit of any *Salamandra* species in Israel (EISELT 1958, STEINFARTZ et al. 2000). This species is considered endangered in Israel and near-endangered worldwide due to its small estimated population sizes, localized occurrence, and dependence on suitable aquatic habitats for reproduction (DOLEV & PEREVOLOTSKY 2004, IUCN 2014). Concern for the species' conservation, as well as reports of long-distance migration between breeding and terrestrial sites (BAR-DAVID et al. 2007) have prompted recapture studies to estimate population sizes and migration rates; such studies thus far have utilized visual methods of individual spotted dorsal pattern recognition (DEGANI 1996, WARBURG 2006, SEGEV et al. 2010). However, analyses of large photo databases and between-database comparisons are difficult using visual methods.

The aim of this study was to evaluate the performance of the new AmphIdent Fire Salamander module i) compared to manual analysis using data from a previous study, and ii) by comparing results from opportunistic and standardized procedures of photography. The results presented here indicate that visual analysis by a trained observer performed better at analysing datasets consisting of variable photo quality (not standardized in terms of the angle of photography), while automated analysis provided highly reliable results for datasets consisting of photos with a standardized angle of photography.

We tested the performance of the new AmphIdent Fire Salamander module using two independent photographic databases of *S. infraimmaculata* in Israel. The first dataset (dataset A) consisted of 307 photo records of individual salamander encounters in the field during the reproductive periods in 2002–2004 in the vicinity of three reproduction sites in Israel: Ein El Balad, Manof, and Secher. Dataset A was collected with the intent of a manual recapture analysis (SEGEV et al. 2010). The visual analysis of dataset A was performed previous to this study without any intent of comparison to automated analysis. This dataset excludes six photos that were of insufficient quality (in terms of focus and lighting) to be successfully loaded into AmphIdent. Of the 307 photo encounters included, 277 encounters were recorded by multiple photos. The dorsal side of animals was

photographed inside a standard-size plastic box (length \times width \times depth: 23 \times 15 \times 7 cm) using a Sony DSR PD100AP camera (0.45 Megapixel). Body postures were rather variable and the angle of photography ranged approximately between 60 and 90° to the dorsal face in various directions.

The second dataset (dataset B) consisted of 144 individual salamander encounters in Tel Dan that were recorded using a standardized photographic procedure during the reproductive period 2013–2014 with the intent of employing AmphIdent automated identification analysis. These data were recorded using a Panasonic Lumix DMC-TZ camera (12 Megapixel). Care was taken to maintain a consistent perpendicular angle of photography between the camera and the plane of the salamander dorsal face. Animals were photographed straight from above during paused natural movement on level ground. We found this to be a reliable approach, allowing for lateral variation in body posture but avoiding torso rotations. Multiple photos per encounter were available for all 144 encounters.

Salamander spotted dorsal patterns are standardized in AmphIdent by adjusting the automatically generated body contour points during the process of uploading a new photo in the software. Examples of adjusted body contour points are shown in the original photos of Figure 1. Adjusting the contour points should be done so that the midpoint-line follows the spine of the animal (see Fig. 1) to prevent lateral shifts in the resulting standardized patterns. This procedure also corrects for lateral variation in body posture and distance from the camera. Subsequently, pixels with a significant amount of yellow are identified and used for pattern identification. Automatic comparisons are made with all existing standardized patterns in the database, and a ranking of the best matches – based on the number of matching yellow pixels – is presented on the screen. The user finally classifies a new photo as either a recapture or a new entry, based on the best matches presented. The AmphIdent workflow is identical to previous modules (DRECHSLER et al. 2015). Computation times did not exceed a few seconds for the datasets in this study (up to ~300 pictures).

The number of recaptures (a new encounter of a previously observed individual) in dataset A was identified using i) visual comparison of hardcopy printed photos (SEGEV et al. 2010), as well as ii) the newly developed Fire Salamander module of the photo recapture software AmphIdent for automated analysis of digital photos. Comparing the results of these two approaches allowed us to identify and investigate the possible reasons for any mismatches between these two methods. If the visual approach did not identify a recapture that was correctly identified by AmphIdent (confirmed by detailed inspection), we recorded this as a manual false rejection (MFR). If, on the other hand, AmphIdent did not identify a recapture (within the best eight matches provided by AmphIdent) that was correctly identified visually (confirmed by careful double checking), we recorded this as an automated false rejection (AFR).

The misidentification error rate of AmphIdent was additionally estimated by calculating the independent self-

test false rejection rate (FRR) using the method suggested by BOLGER et al. (2012). The FRR is an objective self-test measure of error for automated analyses and defined as the number of false rejections divided by the number of known true matches (DRECHSLER et al. 2015). Essentially, the FRR represents the probability that a photo of an individual will not match other photos known to depict that same individual. In the current study, we made use of the fact that most salamander encounters were documented by more than a single photo. Even though these photos were taken during the same encounter, there were differences between photos, e.g., in terms of body posture or from photo flash reflections on the animal's skin. If two photos from the same encounter (i.e., the same individual) were not classified as a recapture by the software, it was recorded as a false rejection and used for FRR calculations.

The identification of a recapture is finally determined by the human user of AmphIdent (who ultimately classifies the photo as either a match or a new entry) and false acceptances can therefore not be self-tested objectively.

However, previous studies reported false acceptance rates by AmphIdent to be zero for crested newts (DRECHSLER et al. 2015), and between-observer comparisons within this study also suggested zero false acceptances.

In order to test if the difference in resolution (Mega-pixel) between the cameras used in the two datasets had an influence on AmphIdent recapture analysis, we digitally resized (downsized) all 144 suitable photos from dataset B to 700×600 pixels (0.42 MP), which is similar to the resolutions used in dataset A. The FRR was subsequently recalculated and compared.

Based on the combination of both visual and automated analysis, a total of 94 recapture events were identified among the 307 photo encounters in dataset A. Manual false rejections (unidentified recaptures that were correctly identified and matched by AmphIdent) numbered 3 out of 94 (MFR = 3.2%). Conversely, AmphIdent false rejections (unidentified recaptures that were correctly identified and matched using the visual method) numbered 44 (AFR = 46.8%).



Figure 1. Examples of two photographic encounters of the same *Salamandra infraimmaculata* individual showing: i) original photos with contour points for body posture correction on the left, and ii) the resulting AmphIdent standardized dorsal patterns on the right. On the left pane, marked with A, photos are shown of a recapture from dataset A. In this example the recapture was not recognized as such by AmphIdent, possibly due to differences in torso rotation leading to a lateral shift and foreshortened compression of the standardized dorsal pattern. On the right pane, marked with B, an example is shown from dataset B. In this case, the individual was recognized by AmphIdent as a recapture (first match) despite differences in lateral body posture and photo flash reflection on the skin.

The AmphIdent self-test false rejections in dataset A numbered 80 (FRR = 35.2%). This high FRR indicates that the AmphIdent algorithm struggled to identify photos from the same individual in this photographic dataset. This may also explain the relatively high number of AFR in comparison to the visual analysis. Among the 144 photo encounters in dataset B, 82 recaptures (56.9%) were identified. In contrast to dataset A, the AmphIdent self-test FRR of dataset B was faultless (FRR = 0%). Reducing the resolution of the dataset B photos to 0.42 MP did not affect the FRR (again 0%).

Comparison of manual and automated photo analysis showed that the more flexible eye of a trained observer performed better in analysing datasets consisting of photos of varying quality (not optimally standardized in terms of the angle of photography), as was indicated by lower manual false rejection rates (MFR = 3.2% vs AMR = 46.8%). However, for the dataset consisting of standardized photos, automated analysis provided highly reliable results. The marked difference in self-test error rates between the variable photo dataset A (FRR = 35.2%) and the standardized dataset B (FRR = 0%) indicated that a standardized procedure of photography in the field is important for subsequent analysis with automated methods, such as the AmphIdent approach.

A possible source of variability between photos in dataset A could be a difference in the angle between the camera and the salamander dorsal face, which may lead to lateral shifts or foreshortened compressions of the colour pattern (see Fig. 1). It may even obscure lateral parts of the dorsal pattern, as a salamander body is largely cylindrical. Differences in the photographic angle may in some cases simply be caused by a degree of torso rotation. The use of a small plastic box for taking photos of salamanders in dataset A may have contributed to a higher variability in torso rotation, especially as individuals of *S. infraimmaculata* can be quite large. Torso rotation has been much less of a problem for previous modules of AmphIdent, because the target animals (Crested Newts and Fire-Bellied Toads) are usually photographed through a glass surface against which the ventral side of the animal is gently pressed using a wet sponge (see DRECHSLER et al. 2015). This ensures that the ventral face is flattened, causing differences in photo angle or torso rotation to be much less influential. However, this approach is not suitable for salamanders, as their dorsal face is inherently rounded by their rib cage. The dorsal face cannot be flattened without causing undesirable amounts of stress or even damage to the animal. Other studies have focused on spotted patterns on the head of salamander larvae (BENDIK et al. 2013) where rotations or distortions are less likely, but the perspective of the photo (= angle) was still considered a possible source of identification error.

Digital resizing of 14-MP photos to 0.42 MP did not affect the automated recapture recognition performance. This is likely due to the fact that the AmphIdent software standardizes the pixel grid when it generates standardized

dorsal patterns. Differences in resolution therefore do not pose problems for AmphIdent automated identification analysis. Most other studies on automated photo identification did not distinguish between photo resolution and perspective as the source of errors (e.g., BOLGER et al. 2012, BENDIK et al. 2013), although perspective was suggested to be a key factor in a study on cheetahs (KELLY 2001). We suspect that – unless the colour pattern is flattened – perspective is more important than resolution also in other photo identification studies using other software.

We conclude that AmphIdent is a robust pattern recognition algorithm for the analysis of salamander spotted dorsal patterns. The new Fire Salamander module of AmphIdent is sensitive to torso rotations, but resilient to lateral differences in body posture (within the photo focal plane) or flash reflection. For reliable results in automated photo identification analysis we recommend that a perpendicular photographic angle to the colour-patterned face is maintained with as much accuracy as possible and torso rotation or surface distortion are minimized.

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