

Photographic identification in reptiles: a matter of scales

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Abstract. Photographic identification is a promising marking technique alternative to the toe-clipping, since it is completely harmless, cheap, and it allows long time identification of individuals. Its application to ecological studies is mainly limited by the time consuming to compare pictures within large datasets and the huge variation of ornamentation patterns among different species, which prevent the possibility that a single algorithm can effectively work for more than few species. Scales of Reptiles offer an effective alternative to ornamentations for computer aided identification procedures, since both shape and size of scales are unique to each individual, thus acting as a fingerprint like ornamentation patterns do. We used the Interactive Individual Identification System (I³S) software to assess whether different individuals of two species of European lizards (*Podarcis muralis* and *Lacerta bilineata*) can be reliably photographically identified using the pattern of the intersections among pectoral scales as fingerprints. We found that I³S was able to identify different individuals among two samples of 21 individuals for each species independently from the error associated to the ability of the operators in collecting pictures and in digitizing the pattern of intersections among pectoral scales. In a database of 1043 images of *P. muralis* collected between 2007 and 2008, the software recognized 98% of recaptures within each year, and 99% of the recaptures between years. In addition, 99% and 96% of matches were ranked among the top five, and no more than 5 minutes were needed for digitizing and processing each image. The lepidosis of reptiles is a reliable alternative to ornamentation patterns in photographic identification of reptiles, which can be effectively analysed using the I³S software. This result represents a significant improvement in photographic identification of reptiles since (i) this procedure can be easily extended to most other species of reptiles, (ii) all kind of individuals within a species can be marked (i.e., young, subadults and adults) despite the differences in ornamentation patterns, and (iii) it is the only technique for species, like the western green lizard, which lack a clear ornamentation pattern.

Keywords: Capture-recapture, individual-identification, *Lacerta bilineata*, lizards, marking, photographic identification, *Podarcis muralis*.

Introduction

Many types of ecological studies and conservation strategies need accurate estimates of population size, which can be obtained only through the identification of single individuals, usually achieved by marking. Artificially marking of animals involves capturing and handling, which can stress individuals and/or lead to injury and infections. In case of amphibians and reptiles, artificial marking usually involves scale clipping, branding, tattooing, subcutaneous elastomer injections and pit tags of painting (see reviews in Ferner, 1979 and Don-

nelly et al., 1994). However, the toe-clipping, i.e., the removal of a unique combination of digits from each individual (Ferner, 1979; Hero, 1989; Waichman, 1992), is the most frequently used, as it is relatively easy and inexpensive (Donnelly et al., 1994).

Different studies concerning the effects of these marking techniques on behaviour and survival rates have reported conflicting results, indicating that it may be difficult to make broad generalizations about the effects of these techniques on amphibians and reptiles.

Particularly, the effectiveness of the toe-clipping has been questioned, since the base assumption that it does not influence animal's survival or behaviour has been shown to be frequently violated (Parris and McCarthy, 2001; McCarthy and Parris, 2004). Indeed, the toe-clipping may cause inflammation, infection of

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feet and limbs, mobility reduction and mortality increasing (Bustard, 1968, 1971; Clarke, 1972; Humphries, 1979; Golay and Durrer, 1994; Lemckert, 1996; Reaser and Dexter, 1996; Williamson and Bull, 1996; Davies and Ovaska, 2001; Bloch and Irschick, 2004).

For example, Clarke (1972) found an inverse correlation between the number of toes removed from Fowler's toads, *Bufo woodhousei fowleri*, and the recapture rate, and concluded that this was likely to be the result of increased mortality, whereas Golay and Durrer (1994) reported that 18% of recaptured natterjack toads, *B. calamita*, experienced infection or necrosis following toe-clipping. They reported that inflammation did not occur immediately post-amputation; in 50% of cases, inflammation was not detected until at least one month after toe-clipping.

With regards to reptiles, Bloch and Irschick (2004) found that climbing ability in *Anolis carolinensis* decreased dramatically after toe-clipping, and Bustard (1971) reported that 69% out of 150 individuals marked throughout the year were never seen again, while only 12.7% were recaptured during the month following marking.

On the other hand, several other studies have found no negative effects of toe-clipping (Huey et al., 1990; Dodd, 1993; Hudson, 1996; Van Gelder and Strijbosch, 1996; Williamson and Bull, 1996; Ott and Scott, 1999; Paulissen and Meyer, 2000; Borges-Landaez and Shine, 2003), suggesting that the effects of this technique may vary among species and must be assessed accordingly (Funk, Donnelly and Lips, 2005).

The negative effect of toe-clipping on survival as well as on common behaviours of marked individuals potentially affects the quality of the data collected during field researches (McCarthy and Parris, 2004; Bell and Pledger, 2005). Indeed, both altered behaviour and increased mortality resulting from marking violate the basic assumption underlying most capture-recapture methods, i.e., that recapture probability is not affected by marking (Caughley and Sinclair, 1994).

Moreover, natural toe loss is common enough in some species of skinks and lizards to potentially cause difficulties with possible misidentifications of individuals marked by toe-clipping (Rand, 1965; Schoener and Schoener, 1980; Middelburg and Strijbosch, 1988; Hudson, 1996). For example, Middelburg and Strijbosch (1988) found that 8% of common lizards (*Zootoca vivipara*) out of a sample of 900 individuals showed naturally loss toes, and amputations were three time more frequent in females than in males. Similarly, natural amputation in *Podarcis sicula* might be especially frequent in dense population, due to increased intraspecific competition (Vervust and Van Damme, 2009; Vervust et al. 2009). The frequency of toe loss may be considerable high in some cases (up to 34% in females of the Australian skink *Pseudemoia entrecasteauxii*, Hudson, 1996), and some individual may lost up to six toes (Hudson, 1996).

Irrespectively to the negative effects on individuals, there are also ethical and conservation implications that lead to consider toe-clipping with caution as a marking technique (McCarthy and Parris, 2004), and mark-induced mortality or sub-lethal effects on fitness (e.g., reduced reproduction, decreased growth rate) are particularly unacceptable when the concerned species is endangered.

In this scenario, the photographic identification is an emergent technique with a promising future for marking Amphibians and Reptiles, since it is completely harmless, cheap, and it allows long time identification of individuals. This approach is based on the identification of regular and individually specific patterns of ornamentation within well identified body regions of individuals, which do not change over time despite skin moults. Numerous efforts have been made to identify animals individually using photo-identification methods in many Amphibians and Reptiles (Fox, 1975; Gosá, 1987; Elbing and Rykena, 1996; Schmidt-Loske, 1996; Steinicke et al., 2000; Bradfield, 2004; Perera and Perez-Mellado,

2004; Voros et al., 2007; Buonantony, 2008; Gamble et al., 2008). However, the ornamentations used in some lizards for photographic identification have been shown to subtly change over the whole life of individuals, thus limiting the possibility of recognizing them over long time period (Henle et al., 1997; Sacchi et al., 2006).

Although regular patterns of ornamentation may supply a useful way to individually recognize animals, photographic identification is a time consuming technique, particularly when a large number of individuals is involved, since the number of paired comparisons for each picture increases exponentially according to the sample size (Whitehead, 1990; Kelly, 2001; Sacchi et al., 2006). For this reason, this method must be improved to reduce the time and/or the number of comparisons required for identification.

A feasible approach to solve this problem could be the analysis of pictures through pattern recognising algorithms, and several efforts have been done in recent years to develop software for reducing the time to match images within large databases (Hiby and Lovell, 2001; Kelly, 2001; Arzoumanian et al., 2005; Van Tienhoven et al., 2007; Voros et al., 2007; Buonantony, 2008; Gamble et al., 2008).

However, most of the algorithms used by these software rely on species-specific patterns of ornamentation, and consequently cannot be applied to species other than that for which they had been thought. This occurs because the patterns of ornamentation may be extraordinarily complex and largely different among species, so that it is unlikely that a single algorithm could effectively work for more than few species.

The scales covering the body surface of reptiles offer an alternative to the ornamentation for photographic identification, as the number and the shape of scales are highly variable among individuals, even though the number and distribution of scales in same body region can be fixed (e.g., the ventral scales in lizards and the head scales in most saurians and ophidians). In parti-

cular, the points of connection among scales are unique to each individual, and simultaneously highly divergent among different individuals for both number and position (it is largely unlikely that two individuals could share both the same number of scales and the same shape and position) thus potentially functioning as fingerprints. For example, the pattern of head scales of *Lacerta bilineata* has been shown to be exclusive for each individual, and does not vary over time (Fox, 1975; Elbing and Rykena, 1996), and the scale pattern of the ventral scales is suitable for recognizing individuals of six species of lacertids (*L. agilis*, *L. bilineata*, *L. viridis*, *L. perspicillata*, *Zootoca vivipara* and *Podarcis muralis*; Steinicke et al., 2000; Perera and Perez-Mellado, 2004). However, scale variation over time has been reported at least for vipers (Tomovic et al., 2008).

Therefore, a matching algorithm using the distribution of points connection among scales within a specific region of the body should be able to compare the images of different individuals as it happens for other procedures concerning the patterns of ornamentation.

In this paper we used the Interactive Individual Identification System (I³S Classic ver. 2.0, Van Tienhoven et al., 2007), a free software (download at: <http://www.reijns.com/i3s/index.html>) previously developed for computer-aided identification of whale sharks in order to determine whether it can be also applied to reptiles' identification through their scale patterns. We believe that this new approach offers new opportunities in capture-recapture studies and in conservation projects, since using lepidosis rather than ornamentations as identification tool (1) this procedure can be virtually applied to all species of reptiles (all reptiles have scales), (2) all kind of individuals (i.e., young, adult males and females) of a given species can be effectively marked despite they can lack ornamentation (as it can occur in subadults or in sexually dimorphic species), and (3) it is the only technique that can be applied to those species

that do not possess a fixed ornamentation as chameleons.

The specific purpose of I³S is to support the researcher to match the image of an unknown individual with a collection of images of known sharks, using the white's spot patterns on the shark's flanks (Van Tienhoven et al., 2007). The identification procedure assumes that the spot pattern of each individual is a unique distinguishing feature. By pointing out the most distinguishing spots in the image, a researcher builds a 'fingerprint file' which is matched with the spot patterns of all the known individuals in the database. To be able to correct for differences in viewing angle and distance, the user is also required to point out three reference points. Using a linear transformation, two shark images are compared in roughly the same coordinate system. A comparison of two individuals basically comes down to finding corresponding spot pairs in this coordinate system. From these pairs a distance metric is calculated to be able to rank each shark image in the database.

The software can be easily applied to the lepidosis of reptiles whereas the researcher points out the intersections among scales rather than the spots of the ornamentation. By this way, a fingerprint of a reptile can be obtained and compared with those stored within the database.

Therefore, our objectives were: (1) to determine, in two species of European lizards (the common wall lizard, *Podarcis muralis* and the Western green lizard, *Lacerta bilineata*), whether the I³S recognition algorithm applied to the pectoral scales can be used to individually distinguish lizards; (2) to demonstrate the utility of I³S in a long-term capture-recapture study on the common wall lizard, and (3) to describe the advantage of using lepidosis relative to ornaments for reptile identification.

Materials and methods

Model species

Our application of the software I³S to reptiles is based on two common species, occurring in most regions of west-central Europe, which can be assumed as representative of saurians of small to medium-size.

The common wall lizard is a small (50-70 mm adult snout-vent length, SVL; Arnold and Burton, 1978) diurnal lizard of central and south-eastern Europe. Ornamentation patterns are highly variable, being different both between sexes (e.g., black spots on ventral scales are present in adult males but only in few adult females, and never appear in subadults, Henle et al., 1997; Sacchi et al., 2006), and also among individuals of the same sex (e.g., back ornamentation may vary from striped to finely spotted, Sacchi and Scali, unpublished), thus limiting the application of ornamentation based photographic identification (Sacchi et al., 2006).

The western green lizard is one of the largest European lizards (adult SVL up to 13 cm, Arnold and Burton, 1978) occupying the western portion of Europe from Iberian Peninsula to France, Switzerland, Germany and Italy. It is a ubiquitous thermophilous species that lives in open habitats and edges, especially in ecotones with eastern and southern exposure. During breeding season males display a more or less evident light blue dominance colouring on the throat, which is a territorial or dominance signal to conspecifics. Except for this blue colouration, both males and females appear uniformly bright green, lacking any specific ornamentation pattern on the back as well as on the belly. In addition, juveniles are frequently uniformly brown or brown with light spot on the flanks and two-four thin white stripes on the back. Thus, western green lizard does not supply many opportunities for photoidentification through ornamentation patterns. In contrast, the pattern of head scales of lizard species is exclusive for each individual (Fox, 1975; Elbing and Rykena, 1996), as do the scale pattern of pectoral scales (Steinicke et al., 2000; Perera and Perez-Mellado, 2004).

Individual identification of lizards by I³S

In order to compare the markings on two individuals, their images have to be translated in a common reference system. I³S uses a two-dimensional affine transformation for the mapping into the common space, which can incorporate scaling, rotation, translation and correction of perspective. The transformation requires that each individual is strictly regarded as two-dimensional, and involves the x , y coordinates of three reference points that have to be the same for each individual (see Van Tienhoven et al., 2007 for details on formulae and calculations).

After the images are translated in a common space, point-pairs are accepted as a match if the nearest alternative point is at least twice the distance of the current match; then I³S computes an Euclidean distance of images as the sum of the distances between matched points divided by the square of the number of pairs (see Van Tienhoven et al., 2007 for details). A low score in the distance indicates a better match than a high score, so the images stored in the database can be ranked from the more similar (that showing the lowest distance) to the more different (that showing the highest distance) to that of the unknown individual.

We chose to examine the scales in the pectoral area due to the highly variability of shape and size of these scales among individuals (Steinicke et al., 2000; Perera and Perez-Mellado, 2004) and the ease with which photographers can identify this area. The pectoral area is also rigid enough

to be less likely to be distorted when taking photos. It is a trapezoidal shaped region delimited by the collar scales frontally, by the shoulders and flanks laterally, and by a line of at least five scales just in front of the ventral scales (hereafter, the first line of pectorals). As reference points we chose the base of the two shoulders (at the insertion point of the anterior portion of the forelegs) and the back of the central scales in the first line of pectorals (fig. 1). These points are easily recognizable in all images of both species, and all connection points among scales were afterward digitized (but two connection points closer each other not clearly defining a scale side, were considered as only one point).

In order to assess whether I³S can be used to distinguish lizard individuals we have to demonstrate that the variability of distance among individuals is significantly larger than that within individual. In addition we have to evaluate the effect of the two main sources of error a researcher can incur during the computation of the distance between two images, i.e.: (1) the error in selecting both reference points and intersection among scales, and (2) the error in positioning lizard under the camera. Finally, we have also to assess the effect of the number of points selected in each image on the distance between images, since it can vary from one image to another.

During spring-summer 2008 we captured by noosing 21 adult common wall lizards in a historical garden within the town of Cesano Maderno (Northwards Milan, 45°37'N-9°8'E) and 21 western green lizards in the Monticchie Natural Reserve (Southwards Lodi, 45°8'N-9°38'E). For each individual, we collected two images at 10 minutes interval; each image was processed by I³S two times at daily interval by an operator that first highlighted the three reference points and then the centre of the most obvious intersections among scales within the reference area delimited by the reference points (fig. 1). For each image the four following variables were computed: the number of points inserted in the fingerprint file, the distance from its replicate (Drep1), the distance from the second image of the same individual (Drep2), and the mean of the distances from the images of all other individuals within the sample (Dpopulation).

Field validation

As a part of a long-term population study on common wall lizards in Cesano Maderno, continuous capture-recapture by noosing have been done since 2004. Following a standard protocol, all captured lizards are photo-identified using ventral ornamentation (Sacchi et al., 2006) and marked on the back using a unique combination of non-toxic colour inks.

In order to validate the I³S recognition algorithm applied to the pectoral scales, we used the capture history of 2007, which included 652 single captures from 358 individuals (adult males: 201, adult females: 154, juveniles: 3) and that of 2008, which included 391 captures from 230 individuals (adult males: 154, adult females: 76); 61 individuals were captured in both years. All lizards were photographed for photoidentification through traditional methods (Steinicke et al., 2000; Perera and Perez-Mellado, 2004; Sacchi et al., 2006).

All the 1043 images were updated within the I³S database. Then, in order to test the performance of the software, we simulated a within year capture-recapture study by randomly selecting 50 images of lizards of both sexes that were captured almost twice during the 2008; these images were analysed by I³S, and for each one we noticed whether it was correctly identified by the software (i.e., it was ranked within the top 50 images) and both the rank and the distance to the image of the same individual matched within the database. This procedure was a blind reading, i.e., the human operator did not know the identity of the recaptured individuals. In a second test we used the same procedure to simulate a between-year capture-recapture study by analysing 50 images of lizards randomly selected among that captured in 2007 and recaptured during 2008. As for previous test, we noticed if each image was correctly identified and their ranks and distances from the other images of the same individuals matched within the I³S database.

Statistical analyses

All the distances as well the number of points were normally distributed. Therefore, we firstly computed the repeatability of all the three distances to assess the consistency of the measures. Then, we used a paired *t*-test to compare Dpopulation with Drep1 and Drep2 respectively. The same test was then used to compare Drep1 and Drep2.

Finally, we used a mixed-model analysis of variance to assess the effect of the number of points on the difference of Dpopulation from Drep1 and Drep2 respectively. The dependent variable was the distance, the number of points was a covariate, whereas the type of distance (i.e., Dpopulation vs Drep1 in a first model and Dpopulation vs Drep2 in a second model) was the fixed factor. The individual identity entered as random factor to account for repeated measures within the same individual. The number of points entered the model also as quadratic term, and all two-way interaction terms were considered. All models were subjected to a stepdown simplification procedure, where non-significant ($P > 0.05$) terms were removed at each step, starting from the least significant interaction terms, until a minimal adequate model, containing only significant terms ($P < 0.05$), was obtained (Crawley, 1993). All analyses were performed using R ver. 2.6.1 (R Development Core Team, 2007). Unless otherwise stated, means and parameter estimates are reported through the text together with their associated standard errors.

Results

Identification of lizards

In both species Drep1 was significantly greater than 0 (common wall lizard: 0.073 ± 0.004 , $n = 42$, one sample *t*-test, $t = 18.26$, $P < 0.001$;

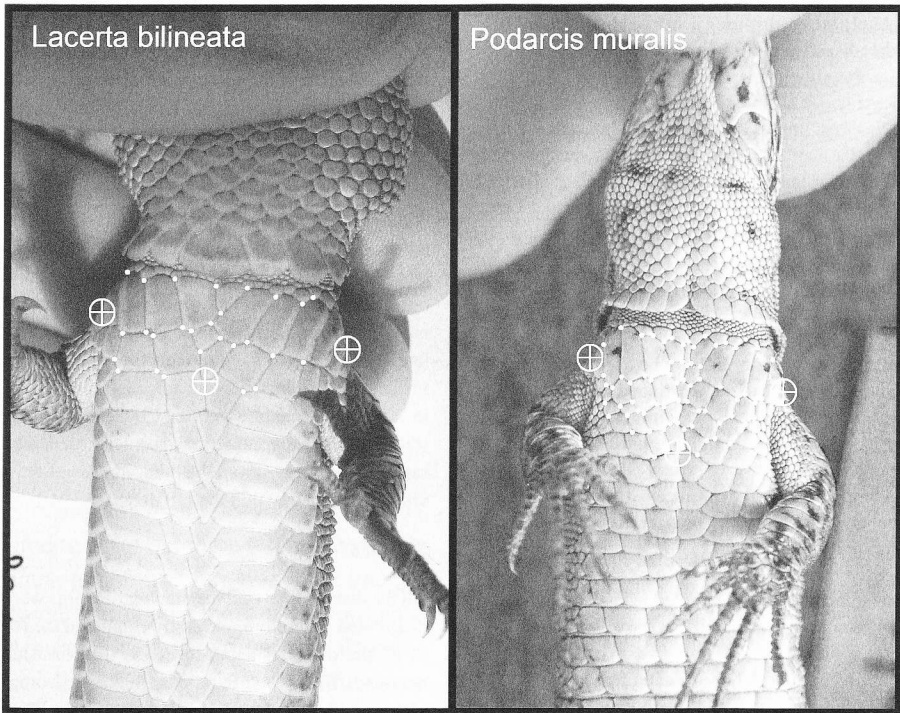


Figure 1. Example of lizard images after acquisition within the I³S database: the larger circular symbols are the three reference points, whereas the small white dots correspond to the intersection among scales.

western green lizard: 0.18 ± 0.01 , $n = 42$, one sample t -test, $t = 16.32$, $P < 0.001$, fig. 2), but highly consistent for a given individual (common wall lizard: repeatability = 0.94, $F_{20,62} = 3.79$, $P < 0.001$; western green lizard: repeatability = 0.88, $F_{20,62} = 3.13$, $P < 0.001$). This result suggested that the operator was able to recognize the three reference points for each individual, even though committed a small but non relevant error (i.e., the distance was not zero), mainly due to the different number of intersections selected in the two images (mean difference, common wall lizard: 2.6 ± 0.6 , western green lizard: 0.6 ± 0.2) rather than to the pointing on the three reference marks.

Drep2 was significantly greater than 0 for both species (common wall lizard: 0.13 ± 0.009 , $n = 42$, one sample t -test, $t = 14.47$, $P < 0.001$; western green lizard: 0.37 ± 0.03 , $n = 42$, one sample t -test, $t = 12.77$, $P < 0.001$) and also significantly higher than Drep1 (paired

t -test, common wall lizard: $t = 18.26$, $n = 42$, $P < 0.001$; western green lizard: $t = 8.17$, $n = 42$, $P < 0.001$, fig. 2). Therefore, the non corrected positioning of lizards under the camera represented an additional source of error. Despite this, Drep2 resulted highly repeatable within individual for both species (common wall lizard: repeatability = 0.89, $F_{20,62} = 4.86$, $P < 0.001$; western green lizard: repeatability = 0.75, $F_{20,62} = 2.24$, $P = 0.008$) suggesting that the two sources of error combined (i.e., digitizing and capturing images) did not significantly affect the process of updating the I³S database with the image of a new unknown individual.

Finally, Dpopulation in both species resulted significantly greater than both Drep1 (common wall lizard: 0.47 ± 0.01 , paired t -test, $t = 29.85$, $df = 41$, $P < 0.0001$; western green lizard: 1.23 ± 0.05 , paired t -test, $t = 20.08$, $df = 41$, $P < 0.0001$, fig. 2) and Drep2 (common wall lizard: 0.43 ± 0.01 , paired t -test, $t = 24.01$,

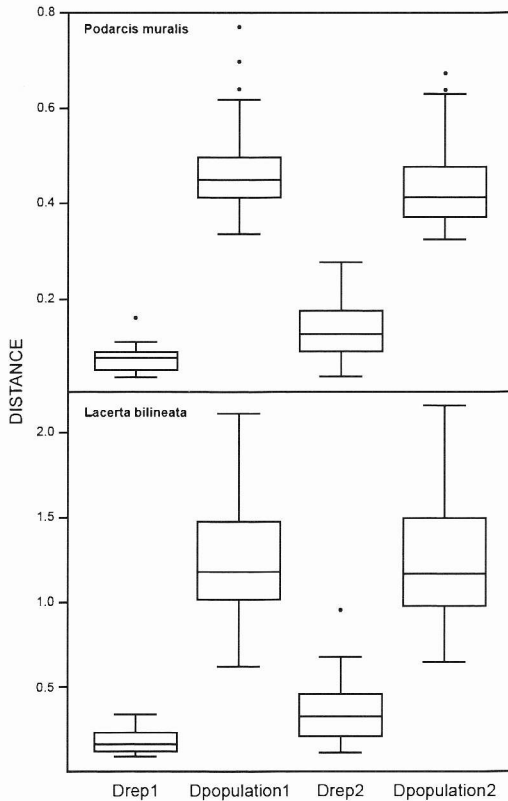


Figure 2. Mean values computed by I³S for the distance between two digitisations of the same image (Drep1), the distance between two different images of the same individual (Drep2), and the corresponding distances between an image from those of all other individuals in the sample (Dpopulation1 and Dpopulation2).

$df = 41$, $P < 0.0001$; western green lizard: 1.28 ± 0.08 , paired t -test, $t = 12.26$, $df = 41$, $P < 0.0001$, fig. 2) indicating that the software was able to discriminate a specific individual within the population.

The mean number of marked points for each image ranged from 26 to 64 for common wall lizards and from 14 to 36 in western green lizards (table 1) and significantly differed among individuals (ANOVA one-way: replicates of the same image, common wall lizard: $F_{20,21} = 22.02$, $P < 0.0001$, western green lizard, $F_{20,21} = 185.72$, $P < 0.0001$; two images of the same individual, common wall lizard: $F_{20,21} = 6.29$, $P < 0.0001$, western green lizard: $F_{20,21} = 53.7$, $P < 0.0001$).

The difference between Drep1 and Dpopulation in common wall lizards was not independent from the number of marked points since the minimum adequate mixed model on one hand confirmed that Drep1 was significantly lower than Dpopulation, on the other hand showed two significant interactions with the number of marked points and its quadratic terms (table 2), which stood for a different variation of Drep1 and Dpopulation according to the number of marked points (fig. 3a). Actually, the difference between Drep1 and Dpopulation was highest at 25-35 marked points and progressively decreased to reach a minimum for more than 50 points (fig. 3a). Therefore, increasing the number of marked points does not lead to an increased ability of I³S in discriminating the images of different individuals. However, the effect of the number of marked points is not enough relevant to significantly affect photographic identification by the software.

The same results were obtained from the mixed model analysis carried out for the comparison between Drep2 and Dpopulation (table 2, fig. 3b): the difference between Drep2 and Dpopulation was significantly affected by the number of marked points and was higher when fewer points were selected by the operator. As in the previous case, this effect was not large enough to compromise the ability of I³S to discriminate different common wall lizard individuals (fig. 3b).

By contrast, the effect of the number of marked points was marginal to non relevant in the case of the western green lizard. For that concerning the comparison between Drep1 and Dpopulation, the minimum adequate mixed model included the effect of the comparison and its interaction with the number of marked points (table 2), which accounted for a different effect of point number on the two kinds of distances. Actually, the difference between Drep1 and Dpopulation increased with increasing the number of marked points, but this effect was rather limited (fig. 4a).

Table 1. Mean number and intervals of between-scales intersections marked for each set of pictures during image acquisition within the I³S database.

	Marked intersections ($n \pm SE$)	Range
<i>Common wall lizard</i>		
1st image	43.0 \pm 2.0	27-56
replicate of 1st image	40.8 \pm 1.8	26-54
2nd image	44.6 \pm 2.3	25-64
<i>Western green lizard</i>		
1st image	27.2 \pm 1.4	14-35
replicate of 1st image	27.1 \pm 1.4	15-35
2nd image	27.2 \pm 1.3	15-36

Table 2. Minimal adequate mixed models assessing for both species the effect of the number of marked points on the difference between Dpopulation and respectively Drep1 and Drep2.

Model	df	F	P
Comparison between Drep1 and Dpopulation			
<i>Common wall lizard</i>			
Intercept	1,58	1910.01	<0.0001
Comparison	1,58	2554.06	<0.0001
Num. of points	1,58	45.40	<0.0001
(Num. of points) ²	1,58	1.67	0.20
Comparison \times Num. of points	1,58	57.90	<0.0001
Comparison \times (Num. of points) ²	1,58	6.92	0.010
Random effect (individual): $LR\chi^2 = 6.87$, df = 1, $P = 0.009$			
<i>Western green lizard</i>			
Intercept	1,60	310.93	<0.0001
Comparison	1,60	599.74	<0.0001
Num. of points	1,60	0.09	0.76
Comparison \times Num. of points	1,60	5.07	0.028
Random effect (individual): $LR\chi^2 = 13.44$, df = 1, $P < 0.0001$			
Comparison between Drep2 and Dpopulation			
<i>Common wall lizard</i>			
Intercept	1,58	8.14	0.006
Comparison	1,58	28.06	<0.0001
Num. of points	1,58	1.81	0.18
(Num. of points) ²	1,58	0.79	0.37
Comparison \times Num. of points	1,58	7.40	0.009
Comparison \times (Num. of points) ²	1,58	4.35	0.041
Random effect (individual): $LR\chi^2 = 3.77$, df = 1, $P = 0.052$			
<i>Western green lizard</i>			
Intercept	1,62	215.97	<0.0001
Comparison	1,62	149.50	<0.0001
Random effect (individual): $LR\chi^2 = 5.56$, df = 1, $P = 0.018$			

In the comparison between Drep2 and Dpopulation, the number of points had no significant effect as the minimum adequate mixed model included the only effect of the kind of comparison (table 2, fig. 4b). Therefore, the ability of the software I³S in discriminating the images of different western green lizard individuals was not notably affected by the number of

intersections among scales selected by the operator.

Field validation

In the within-year analysis the software I³S recognized 98% of the images (all but one), whereas 99% of matches were among the top five images ranked by the software (41 being

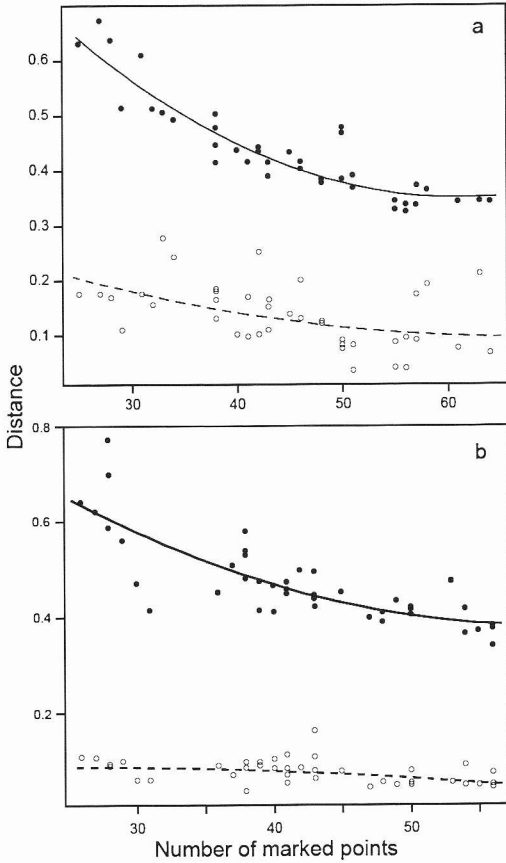


Figure 3. Variation of (a) Drep1 (open circle) and Dpopulation1 (filled circles) and (b) Drep2 (open circle) and Dpopulation2 (filled circles) in common wall lizards according to the number of points marked by the operator during the acquisition of the images within the I³S database.

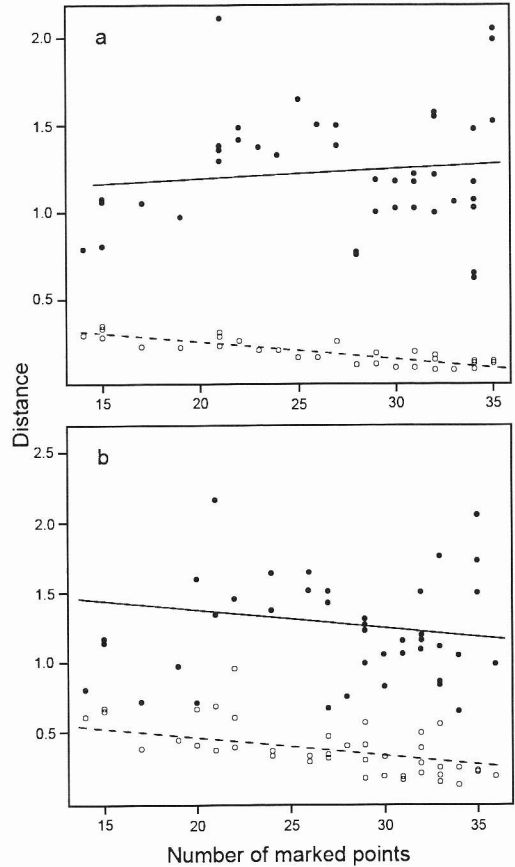


Figure 4. Variation of (a) Drep1 (open circle) and Dpopulation1 (filled circles) and (b) Drep2 (open circle) and Dpopulation2 (filled circles) in western green lizards according to the number of points marked by the operator during the acquisition of the images within the I³S database.

the top ones); the mean distance (\pm SE) of matched images was 0.107 ± 0.06 , and the only case of unsuccessful recognition probably occurred because the lizards were not enough flattened under the camera (the images were not purposely collected to be analysed by I³S).

A good performance of the software was obtained also in the between-years analysis: 49 out of 50 images (98%) were correctly identified and 96% of matches were among the top five ranked images (38 being the top ones). The mean distance (\pm SE) in this test was 0.126 ± 0.17 . As for previous test, bad quality of the image was probably the main reason for the unique case of missed classification.

Discussion

This study confirms that photographic identification is a useful marking technique for lizards (Steinicke et al., 2000; Perera and Perez-Mellado, 2004; Sacchi et al., 2006), and can be considered an effective alternative to the toe-clipping. Indeed, our data confirm that the mismatching of photographic identification is low and much less than the error intrinsic to the toe-clipping technique arising from natural toe loss (see, for example, Hudson, 1996). In both simulation tests of capture-recapture studies we failed the recognition of only 1 out of 50 recaptures (2%) whereas Hudson (1996) in his study on 12 Australian skink species showed that 19%

of females (83 out of 445) naturally lost toes, and in some populations this feature increased to more than 30%.

The most important limit to the application of the photographic identification is the large amount of time needed to compare the pictures of a given sample, which rises exponentially as the number of individuals involved increases (Sacchi et al., 2006). This constraint has been the main reason used to prefer the toe-clipping as marking technique, particularly in long term studies (i.e., Ott and Scott, 1999), and manual photoidentification approaches have been feasible only in small-scale studies involving low sample size (Stenhouse, 1985). Our results demonstrate that the application of I³S to the lizard images is largely time-efficient, even though the visual comparison is necessary to confirm the match between two images. Digitizing image process by the operator required no more than 1-2 minutes whereas the comparison of one image to the database (i.e., 652 images in the within-year simulation, and 391 images in the between-year simulation) by I³S took on average (\pm SE) 44 ± 4 s, ranging from 7 s up to 1 min 38 s. In addition, the training for a new user required no more than few minutes for all steps of the identifying process. In the case of the within-year simulation the analysis through unaided manual matching of images would have required more than 32 000 comparisons (i.e., 50×652 images) corresponding roughly 90 h of work at an estimate of 10 s per comparison.

The performance of I³S software applied to lizard images compared favourably to other published algorithms developed for improving photo-identification of individuals in wildlife studies. For example, the method proposed by Arzoumanian et al. (2005) is based on a modification of the algorithm designed for star identification for the photoidentification of whale sharks reported 92% of correct identification of 27 images in a set of nearly 450 images; the 3D-approach developed by Hiby and Lovell (1990)

to identify cheetahs (*Acinonyx jubatus*) reported a 93.5% of matches had similarity coefficient above the threshold of 0.45, but low-quality images were discarded from the analysis. More recently, Gamble et al. (2008) developed an algorithm for the identification of marble salamanders which misclassified less than 5% of images in a set of 1008 images.

The approach used by I³S to match the images is essentially a geometric method, which uses the connection points among scales as descriptors. This is a quite simple approach, which uses only a part of the information included in the "texture" or "patterning" of the images. In fact, in literature there is a large amount of alternative methods for obtaining information (see, for example, Kirby and Sirovich, 1990; Hiby and Lovell, 2001; Gamble et al., 2008). All these procedures are probably able to obtain more information from the images by respect to the geometric approach used by I³S. Despite this limitation, using the only information held in the geometric net defined by the connection points among scales, the I³S software allows the correct identification of the images within a large database. The other relevant merits that lead to choose I³S, despite its limitation in obtaining information from the images, are that it is easy to use, fast in digitizing the images, as well in searching images within the database.

Our study has two important improvements by respect to all the other methods of software-aided photoidentification proposed up to now, i.e., (i) we used the lepidosis rather than the ornamentation pattern for identifying individuals, and (ii) we used a set of scales (the pectoral scales) that is shared by a large number of lizard species other than the common wall lizard and the western green lizard.

The most important limitation to software-aided algorithms developed up to now is that they are based on ornamentation patterns, which are highly species-specific and may change during ontogeny or differ between sexes or among different age classes (Gosà, 1987; Henle et al., 1997; Sacchi et al., 2006). Furthermore, these

algorithms cannot work at all with species, as the western green lizards, which lack defined ornamentation or are uniformly coloured. Consequently, each algorithm can be used for a very limited number of species (i.e., all that sharing the same pattern of ornamentation), despite their high reliability. A second limitation is that the spots in the ornamentation are not always clearly bounded (e.g., the pineal spots of leatherback *Dermochelys coriacea*, Buonantony, 2008), so algorithms are forced to implement complex approximations which may increase the probability to incur in errors. All these problems in reptiles are completely got through using the lepidosis: all species of reptiles are covered by scales and the variability of their size and shape is so wide that the probability of finding two individuals with the same number and shape of scales is virtually null. Thus, scale shape and size have a potential to singly mark an individual at least equal to that of ornamentations, but, contrary to the ornamentations, the scale patterns are shared by all individuals within a species despite sex or age, do not change during ontogeny, and frequently are similar among individual of different species. A second useful advantage is that the mathematical formulations implemented in the algorithms are largely simpler than those used for ornamentations, since the only information required for describing a scale pattern are the x , y coordinates of the intersection points among the scales. By contrast, the mathematical description of the ornamentation patterns necessarily need of complex procedures for defining boundaries, shapes, colours or positions of the spots (Hiby and Lovell, 1990; Buonantony, 2008; Gamble et al., 2008), with the only exception of the ornamentations with spots like a dot (e.g., Arzoumanian et al., 2005; Van Tienhoven et al., 2007).

Despite the software I³S was originally developed for analysing the ornamentation pattern of sharks (Van Tienhoven et al., 2007), it can be applied without any modification to the analysis of scale patterns of lizards as the ornamentation

of sharks is composed by small white dots on a dark background and the data acquired by the software are only the x , y coordinates of spots and three reference points common to all picture. Our analysis showed that the three points we selected as references (i.e., the shoulders and the central scale) are easy to identify and highly repeatable, as well as the intersections among the pectoral scales. A second result confirming the high discrimination power of this software is that the rank of the matched image in the two simulation tests was among the top five in 99% and 96% of cases respectively, and the top one in more than 75% of cases.

Moreover, we showed that the matching of two photos of the same individual is only marginally affected by the number of intersections marked by the operator, suggesting that the software is able to recognize individuals despite what and how many points are stored in the fingerprint files. This result sensibly increases the reliability of I³S for lizard photoidentification.

Finally, the two cases of misclassification were mainly due to a moderate quality of the pictures (slightly out of focus or not perfectly aligned under the camera), suggesting that a strict standardization of the image collection process might further reduce the possibility to incur in misclassifications.

The second important improvement of this study is that we obtained similar results from two quite different species of lizards, which suggests that our procedure of photoidentification may be efficiently applied to all other species of lizards that possess pectoral scales. This pattern of scales is widely shared among laceratids, teiids, and cordilids (fig. 5). More generally, the matching of images within large databases basing on software-aided procedures applied to the lepidosis has the potential to be expanded to other non lizard reptiles, provided that they possess a body region showing an enough variable pattern of scales, e.g., the throat in colubrids, the head scale in iguanids or vipers etc. However, specific studies have to be preliminary carried out to assess whether the lepidosis

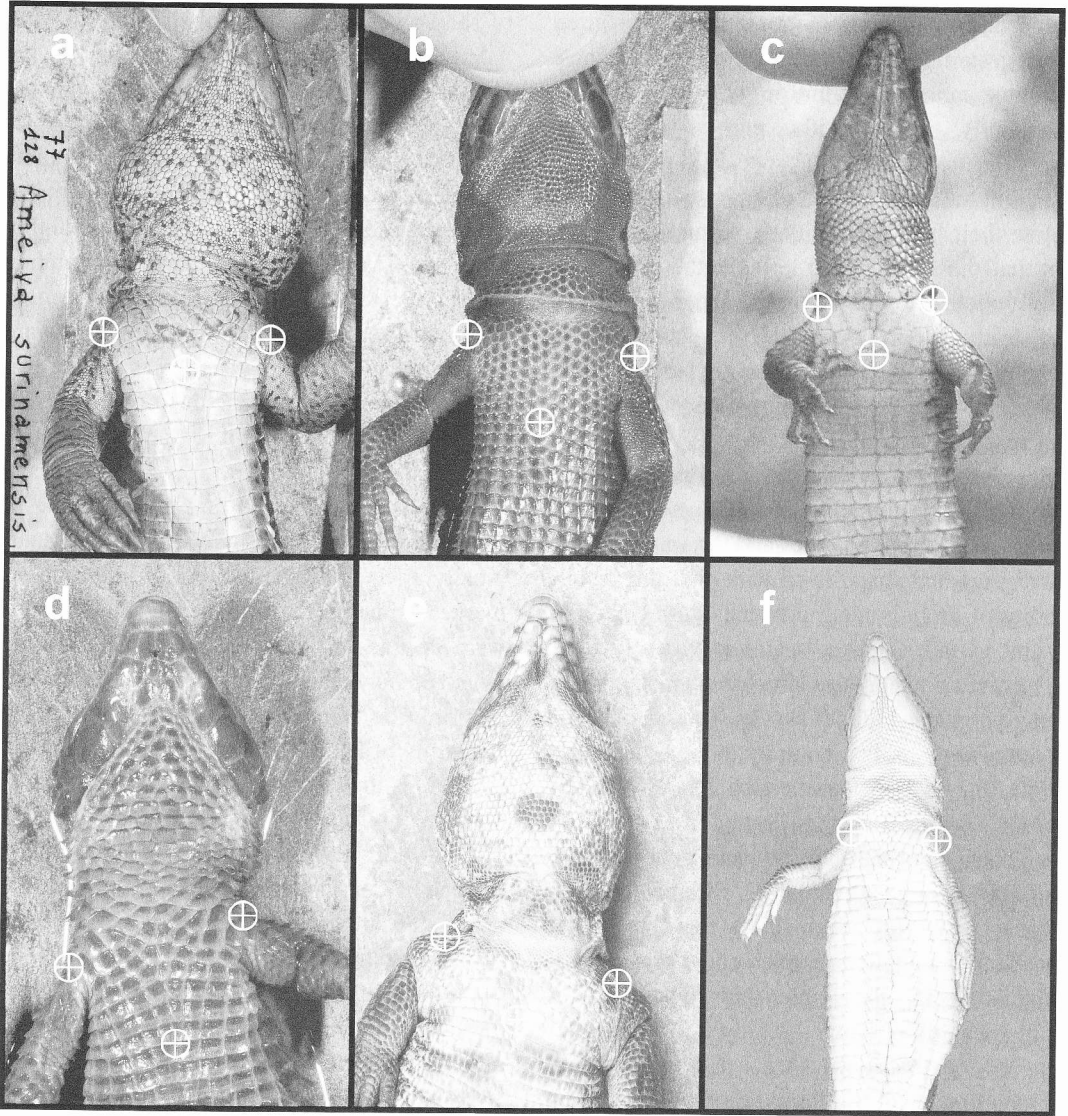


Figure 5. Example of species that might be photographically identified using the lepidosis and the I³S software: the teiids (a) *Ameiva surinamensis*, (b) *Kentropyx calcaratus* and (c) *Tupinambis rufescens* from Southern America; the lacertids (c) *Algyroides nigropunctatus* and (f) *Pseudoeremias smithi* from Europe and Africa respectively and (d) the cordilid *Zonurus cordylus* from Southern America. Symbols as in fig. 1.

sis is unique to individuals and it does not vary within individual over time, as it occurs for example in the meadow vipers (Tomovic et al., 2008).

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References

- Arnold, E.N., Burton, J.A. (1978): A Field Guide to the Reptiles and Amphibians of Britain and Europe. London, Collins.
- Arzoumanian, Z., Holmberg, J., Norman, B. (2005): An astronomical pattern-matching algorithm for computer-aided identification of whale sharks *Rhincodon typus*. *J. Applied Ecol.* **42**: 999-1011.
- Bell, B.B., Pledger, S. (2005): Does toe clipping affect the return rates of the terrestrial frog *Leiopelma pakeka* on Maud Island, New Zealand? *New Zeal. J. Zool.* **32**: 219-220.

- Bloch, N., Irschick, D.J. (2004): Toe-clipping dramatically reduces clinging performance in a pad-bearing lizard (*Anolis carolinensis*). *J. Herpetol.* **37**: 293-298.
- Borges-Landaez, P.A., Shine, R. (2003): Influence of toe-clipping on running speed in *Eulamprus quoyii*, an Australian scincid lizard. *J. Herpetol.* **37**: 592-595.
- Bradfield, K.S. (2004): Photographic identification of individual Archey's frogs, *Leiopelma archeyi*, from natural markings. *DOC Sci. Int. Series* **191**: 1-36.
- Buonantony, D. (2008): An analysis of utilizing the leatherback's pineal spot for photo-identification. Duke University, Durham.
- Bustard, R.H. (1968): The ecology of the Australian gecko, *Gehyra variegata*, in northern New South Wales. *J. Zool.* **154**: 113-138.
- Bustard, R.H. (1971): A population study of the Eyed gecko, *Oedura ocellata* Boulenger, in Northern New South Wales. *Australia. Copeia* **1971**: 658-669.
- Caughley, G., Sinclair, A. (1994): *Wildlife Ecology and Management*. Cambridge, Massachusetts, Blackwell Science.
- Clarke, R.D. (1972): The effect of toe-clipping on survival in Fowler's toad (*Bufo woodhousei fowleri*). *Copeia* **1972**: 182-185.
- Crawley, M.J. (1993): *GLIM for Ecologists*. Oxford, Blackwell Science.
- Davis, T.M., Ovaska, K. (2001): Individual recognition of amphibians: Effects of toe clipping and fluorescent tagging on the salamander *Plethodon vehiculum*. *J. Herpetol.* **35**: 217-225.
- Dodd, C.K. (1993): The effect of toe-clipping on sprint performance of the lizard *Cnemidophorus sexlineatus*. *J. Herpetol.* **27**: 209-213.
- Donnelly, M.A., Guyer, C., Juterbock, J.E., Alford, R.A. (1994): Techniques for marking amphibians. In: *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Heyer, W.R., Donnelly, M.A., McDiarmid, R.W., Hayek, L.C., Foster, M.S., Eds, Washington, Smithsonian Institution Press.
- Elbing, K., Rykena, S. (1996): Analyse der Schuppenmerkmale bei *Lacerta viridis*. *Die Eidechse* **7**: 13-18.
- Ferner, J.W. (1979): A review of marking techniques for Amphibians and Reptiles. *SSAR Herp. Circ.* N°9.
- Fox, S.K. (1975): Natural selection on morphological phenotypes of the lizard *Uta stansburiana*. *Evolution* **29**: 95-107.
- Funk, W.C., Donnelly, M.A., Lips, K.R. (2005): Alternative views of amphibian toe-clipping. *Nature* **433**: 193.
- Gamble, L., Ravela, S., McGarigal, K. (2008): Multi-scale features for identifying individuals in large biological databases: an application of pattern recognition technology to the marbled salamander *Ambystoma opacum*. *J. Applied Ecol.* **45**: 170-180.
- Golay, N., Durrer, H. (1994): Inflammation due to toe-clipping in natterjack toads (*Bufo calamita*). *Amphibia-Reptilia* **15**: 81-96.
- Gosá, A. (1987): Observaciones sobre el colorido y diseño en poblaciones ibéricas de lagartija roquera, *Podarcis muralis* (Laurenti, 1768). *Rev. Esp. Herpetol.* **2**: 7-27.
- Henle, K., Kuhn, J., Podloucky, R., Schmidt-Loske, K., Bender, C. (1997): Individualerkennung und Markierung mitteleuropäischer Amphibien und Reptilien: Übersicht und Bewertung der Methoden; Empfehlungen aus Natur- und Tierschutzsicht. *Mertensiella* **7**: 133-184.
- Hero, J.M. (1989): A simple code for toe clipping anurans. *Herpetol. Rev.* **20**: 66-67.
- Hiby, L., Lovell, P. (2001): A note on an automated system for matching the callosity patterns on aerial photographs of southern right whales. *J. Cet. Res. Manag.* **2**: 291-295.
- Hudson, S. (1996): Natural toe loss in southeastern Australian skinks: Implications for marking lizards by toe-clipping. *J. Herpetol.* **30**: 106-110.
- Huey, R.B., Dunham, A.E., Overall, K.L., Newman, R.A. (1990): Variation in locomotor performance in demographically known population of the lizard *Sceloporus merriami*. *Phy. Biochem. Zool.* **63**: 845-872.
- Humphries, R.B. (1979): *Dynamics of a Breeding Frog Community*. Princeton, Princeton University Press.
- Kelly, M.J. (2001): Computer-aided photograph matching in studies using individual identification: an example from Serengeti cheetahs. *J. Mammol.* **82**: 440-449.
- Kirby, M., Sirovich, L. (1990): Application of the Karhunen-Loeve procedure for the characterization of human faces. *IEEE Trans. Pattern Anal. Mac. Intel.* **12**: 103-108.
- Lemckert, F. (1996): Effects of toe clipping on the survival and behaviour of the Australian frog *Crinia signifera*. *Amphibia-Reptilia* **17**: 287-290.
- McCarthy, M.A., Parris, K.M. (2004): Clarifying the effect of toe clipping on frogs with Bayesian statistics. *J. Applied Ecol.* **41**: 780-786.
- Middelburg, J.J., Strijbosch, H. (1988): The reliability of the toe-clipping method with the common lizard (*Lacerta vivipara*). *Herpetol. J.* **1**: 291-293.
- Ott, J.A., Scott, D.E. (1999): Effects of toe-clipping and PIT-tagging on growth and survival in metamorphic *Ambystoma opacum*. *J. Herpetol.* **33**: 344-348.
- Parris, K.M., McCarthy, M.A. (2001): Identifying effects of toe clipping on anuran return rates: the importance of statistical power. *Amphibia-Reptilia* **22**: 275-289.
- Paulissen, M.A., Meyer, H.A. (2000): The effect of toe-clipping on the gecko *Hemidactylus turcicus*. *J. Herpetol.* **34**: 282-285.
- Perera, A., Perez-Mellado, V. (2004): Photographic identification as a non invasive marking technique for Lacertid lizard. *Herpetol. Rev.* **35**: 349-350.
- R Development Core Team (2007): *R: A Language and Environment for Statistical Computing*. Vienna, Austria, R Foundation for Statistical Computing. Available at <http://www.R-project.org>.
- Rand, A.S. (1965): On the frequency and extent of naturally occurring foot injuries in *Tropidurus torquatus* (Sauria, Iguanidae). *Papeis Avulsos de Depto. Zool.* **35**: 87-96.
- Reaser, J.K., Dexter, R.E. (1996): *Rana pretiosa* (spotted frog). Toe clipping effects. *Herpetol. Rev.* **27**: 275-289.
- Sacchi, R., Scali, S., Fasola, M., Galeotti, P. (2006): The numerical encoding of scale morphology highly improves photographic identification in lizards. *Acta Herpetol.* **2**: 27-35.

- Schmidt-Loske, K. (1996): Fotografische identification von *Podarcis muralis* Laur., 1768. Möglichkeiten und Grenzen. *Die Eidechse* **7**: 7-12.
- Schoener, T.W., Schoener, A. (1980): Ecological and demographic correlates of injury rate in some Bahamian *Anolis* lizards. *Copeia* **1980**: 839-850.
- Steinicke, H., Ulbrich, K., Henle, K., Grosse, W.R. (2000): Eine neue Methode zur fotografischen Individualidentifikation mitteleuropäischer Halsbandeidechsen (Lacertidae). *Salamandra* **36**: 81-88.
- Stenhouse, S.L. (1985): Migratory orientation and homing in *Ambystoma maculatum* and *Ambystoma opacum*. *Copeia* **1985**: 631-637.
- Tomovic, L., Carretero, M.A., Ajtic, R., Crnobrnja-Isailovic, J. (2008): Evidence for post-born instability of head scutellation in the meadow viper (*Vipera ursinii macrops*) – pattern and taxonomical implications. *Amphibia-Reptilia* **29**: 61-70.
- Van Gelder, J.J., Stribosch, H. (1996): Marking amphibians: effects of toe clipping on *Bufo bufo* Anura: Bufonidae. *Amphibia-Reptilia* **17**: 169-174.
- Van Tienhoven, A.M., Den Hartog, J.E., Reijns, R.A. (2007): A computer-aided program for pattern-matching of natural marks on the spotted raggedtooth shark *Carcharias taurus*. *J. Applied Ecol.* **44**: 273-280.
- Vervust, B., Van Damme, R. (2009): Marking lizards by heat branding. *Herpetol. Rev.* **40**: 73-174.
- Vervust, B., Van Dongen, S., Grabac, I., Van Damme, R. (2009): The mystery of the missing toes: extreme levels of natural mutilation in island lizard populations. *Funct. Ecol.* **23**: 996-1003.
- Voros, J., Szalay, F., Barabas, L. (2007): A new method for quantitative pattern analysis applied to two European *Bombina* species. *Herpetol. J.* **17**: 97-103.
- Waichman, A.V. (1992): An alphanumeric code for toe clipping amphibians and reptiles. *Herpetol. Rev.* **23**: 19-21.
- Whitehead, H. (1990): Mark-recapture estimates with emigration and reimmigration. *Biometrics* **46**: 473-479.
- Williamson, I., Bull, C.M. (1996): Population ecology of the common frog *Crinia signifera*: adults and juveniles. *Wildlife Res.* **23**: 249-266.

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