ÖGH Herpetozoa
Austrian Herpetological Society

Herpetozoa 32: 1–@ (2019)
DOI 10.3897/herpetozoa.32.e39030

On the stability of the dorsal pattern of European cave salamanders (genus *Hydromantes*)

Enrico Lunghi[1,2,3,4], Daniele Romeo[5], Manuela Mulargia[6], Roberto Cogoni[7], Raoul Manenti[8], Claudia Corti[3], Gentile Francesco Ficetola[8,9], Michael Veith[2]

1 *Key Laboratory of the Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beichen West Road 1, 100101, Beijing, China*
2 *Universität Trier Fachbereich VI Raum-und Umweltwissenschaften Biogeographie, Universitätsring 15, 54286 Trier, Germany*
3 *Museo di Storia Naturale dell'Università degli Studi di Firenze, sede "La Specola", Via Romana 11, 50125 Firenze, Italia*
4 *Natural Oasis, Via di Galceti 141, 59100 Prato, Italia*
5 *Università degli Studi di Firenze, Dip. di Biologia, Via La Pira 4, 50121 Firenze, Italia*
6 *CEAS Santa Lucia Siniscola. Via Isalle 4, 08029 Siniscola, Italia*
7 *Unione Speleologica Cagliaritana, via Scarlatti, 11, 09045 Quartu Sant'Elena, Italia*
8 *Dipartimento di Scienze e politiche ambientali, Università degli Studi di Milano, via Celoria 2, 20133 Milano, Italia*
9 *Laboratoire d'Ecologie Alpine (LECA), Université Grenoble-Alpes, F-38000 Grenoble, France*

http://zoobank.org/

Corresponding author: *Enrico Lunghi* (enrico.arti@gmail.com)

Academic editor: *Eva Ringler* ♦ **Received** 13 August 2019 ♦ **Accepted** 11 September 2019 ♦ **Published** @ ##### 2019

Abstract

Photographic identification is an emerging method for recognising wild animals. This harmless methodology allows researchers to identify "naturally marked" individuals and therefore study their specific ecology and behaviour. However, before incurring potential data loss, it is recommended to test the methodology on the target species and evaluate the pros and cons. We assessed the reliability of photographic identification in adult *Hydromantes* salamanders from three species. Specifically, we assessed whether the dorsal pattern of adult salamanders changed over time, thus evaluating its potential use as a reliable marking methodology. We used capture-mark-recapture and controlled conditions (i.e. individuals kept in fauna boxes) to evaluate potential changes in the dorsal pattern of *Hydromantes* through time. We did not observe any change in the dorsal pattern in the three species during the study period. Photographic identification might be a useful marking technique for these endangered species. However, these animals are usually found in environments generally lacking light and thus, researchers must be careful in setting up proper light conditions to produce suitable pictures for individual identification of *Hydromantes*.

Key Words

Capture-mark-recapture, Cave biology, Colouration, Individual recognition, Photo, Pattern, *Speleomantes*, Biospeleology, Amphibians

Introduction

Photographic identification of wild animals is gaining popularity amongst researchers conducting studies on single individuals (e.g. home range, migration) (Salvidio et al. 1994; Sharifi and Afroosheh 2014) and population dynamics (Martin-Smith 2011; Morrison et al. 2011). The popularity of this approach is mainly due to the lack of individual mutilations (and potential negative side effects) and for its potential low cost (Davis and Ovaska 2001;

McCarthy and Parris 2004; Sacchi et al. 2010). Therefore, photographic identification is an excellent approach that raises only minimal ethical issues and that can be applied to endangered species (Sharifi et al. 2013). Computer programmes, designed to improve and speed up individual photo recognition, make it easier to apply photographic identification to large populations (Bendik et al. 2013; Town et al. 2013; Sacchi et al. 2016; Treilibs et al. 2016). However, photographic recognition also has some limitations. First, this method only works well for species that have a defined pattern but its application is challenging in species having uniform colouration (Carafa and Biondi 2004; Martin-Smith 2011; Rocha et al. 2013; Carpentier et al. 2016). In addition, it does not allow monitoring the development of young individuals into adults in species where the pattern changes during ontogenesis (Lanza et al. 2006). Furthermore, photos taken in poor lighting conditions and with individuals improperly positioned (e.g. with covered body areas) cannot be used. In the worst case, this only becomes evident after returning from the field, risking significant data loss (Bailey 2004; Morrison et al. 2011). Finally, animals must be handled to get appropriate pictures, which may cause considerable stress depending on the handling time (Lunghi et al. 2016; Samimi et al. 2016; but see Bradley and Eason 2018).

European *Hydromantes* comprise eight species for which there is a growing interest in their ecology and life history, as they are all of conservation concern (Rondinini et al. 2013). These species show particular pigmented patterns (Ambrogio and Mezzadri 2017), yet less conspicuously than in other salamanders, so it is unclear if their patterns can be reliably used for individual identification. In only one study, the photographic identification was used to individually recognise *Hydromantes*, although no test assessing its reliability on this genus was ever performed (Salvidio et al. 1994). In this study, we assessed the stability of the dorsal pattern in the European cave salamanders (genus *Hydromantes*; see Wake 2013 for taxonomic discussion).

Methods

We combined photographic identification with two other methods to independently ascertain the identity of individual salamanders: *i*) Individuals were additionally marked with Visual Implant Alpha tags or Visual Implant Elastomers, methodologies providing unequivocal salamander identification (Lunghi and Veith 2017; Lunghi and Bruni 2018); *ii*) Salamanders were kept individually in fauna boxes and regularly photographed for several months.

We used three species of *Hydromantes*, two from Sardinia (*H. flavus*, *H. sarrabusensis*) and one from mainland Italy (*H. italicus*). We focused only on the dorsal pattern of adults as, in juveniles, it changes throughout development (Lanza et al. 2006; Ambrogio and Mezzadri 2017). Marked individuals of *H. flavus* ($N = 65$) and *H. italicus* ($N = 28$) (Lunghi and Veith 2017; Lunghi and Bruni 2018) were photographed and released in the wild; for *H. flavus* we performed 12 surveys over a period of ten months (September 2015 – June 2016), while for *H. italicus,* 14 surveys over a period of five months (April – August 2018). During each survey, recaptured marked individuals were photographed with digital cameras to check the stability of the dorsal pattern. Furthermore, two adult females of *H. sarrabusensis* were kept in a controlled environment (i.e. fauna boxes inside the cave) for twelve months (May 2016 – April 2017; Lunghi et al. 2018); invertebrates were monthly provided to feed salamanders and, on those occasions, potential changes in dorsal pattern were assessed. Considering that we did not use a standardised methodology to take pictures, photos were manually checked in order to identify areas recognisable for their combination of both dark and light pigments (hereafter: shapes). For each individual, at least four different shapes (up to twelve) were identified and used as a diagnostic character (Fig. 1). When pictures did not clearly show the salamanders' dorsal pattern (Figs 2B–C), they were discarded and not used in any further comparison.

Results

We captured three adult individuals of *H. flavus* at least twice (up to 3 times; average number of captures = 2.33 ± 0.58 SD), with a maximum time of eight months between the first and last observation (Fig. 1A–B). We captured eight individuals of *H. italicus* at least twice (up to 4 times; average number of captures = 2.5 ± 0.76 SD), with a maximum time of three months between the first and last encounter. On several occasions, individuals did not remain still during photo shooting, so we needed to position them several times, considerably increasing handling time (usually > 15 seconds per picture). The recaptured individuals did not exhibit changes in their dorsal pattern and were clearly recognisable in images of adequate quality (Fig. 2). Individuals of *H. sarrabusensis,* kept in fauna boxes, did not exhibit changes in their dorsal pattern in the course of the year of monitoring (Fig. 1C–D).

Discussion

Adult *Hydromantes* showed a stable dorsal pattern throughout the study period. The photographic recognition is therefore a potential method for individual identification of these endangered species. This method was used only once in *Hydromantes strinatii* (Salvidio et al. 1994); researchers photographed the back limbs of adult salamanders, but tests on the reliability of this approach in these salamanders are still lacking. Even considering the limited sample size ($N = 13$), our data suggests that adults' dorsal pattern does not change over time. A similar

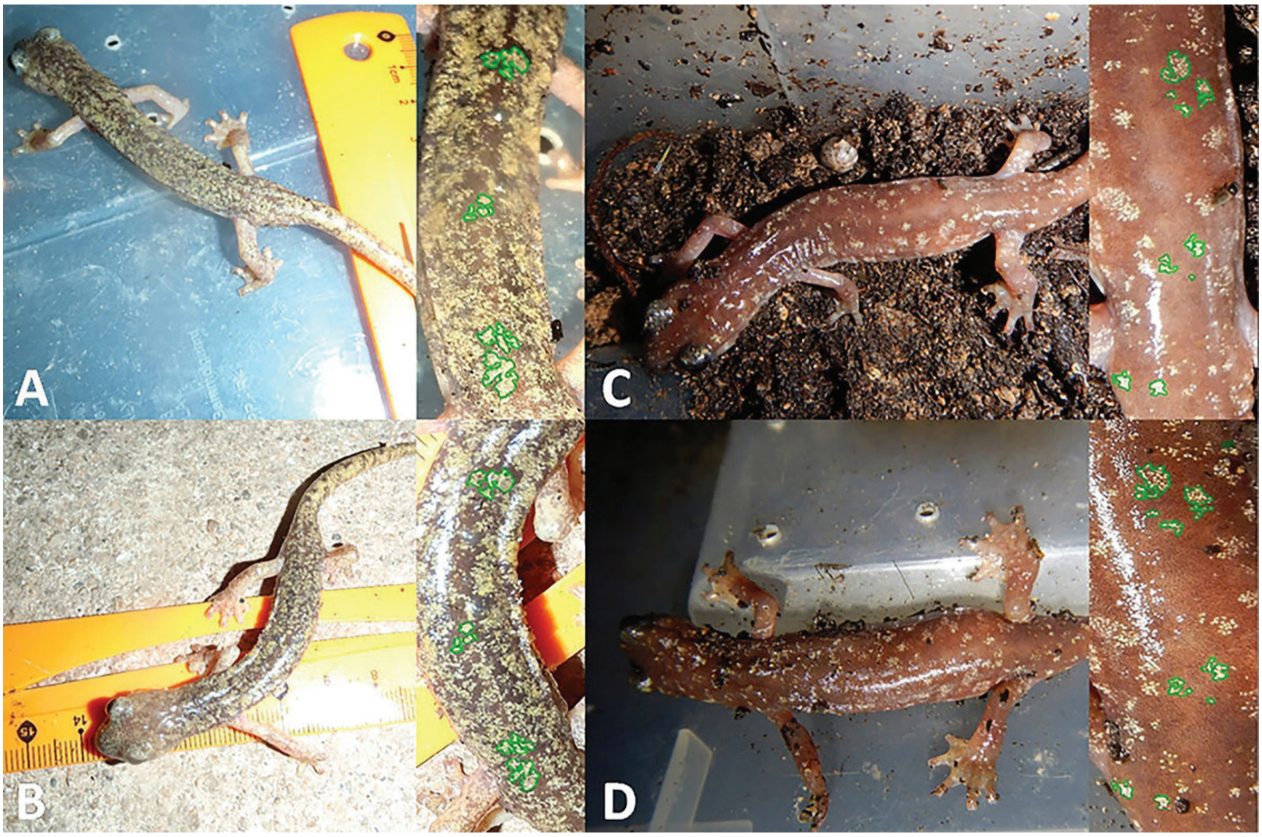

Figure 1. Two examples of individual photographic identification. A–B) An individual of *H. flavus* recaptured; C–D) An individual of *H. sarrabusensis* kept in a fauna box and photographed during prolonged monitoring. Dorsal pattern (i.e. combination of dark and light pigments) that can be used for individual recognition are encircled in green.

sample size provided useful information on the stability of the dorsal pattern in the Tiger salamander *Ambystoma tigrinum* (Waye 2013). We therefore believe that the dorsal pattern of *Hydromantes* is more suitable for recognition than that of their limbs (Fig. 1), as the latter shows less surface (and shapes as well) and occurs in a highly mobile body part, complicating individual recognition. Although photographic identification of cave salamanders is potentially useful for individual recognition (Bradley and Eason 2018), some challenges deserve attention.

First, individuals need to be handled in order to accurately photograph their dorsal pattern (Bradley and Eason 2018). This produces high stress, especially during prolonged handling (Lunghi et al. 2016; Samimi et al. 2016). Handling time is strongly reduced in other marking methods (i.e. Visual Implant Elastomers; Davis and Ovaska 2001; Lunghi and Bruni 2018) and thus, apart from the first tag injection, a quick and reliable individual recognition method may reduce stress to the animals, thereby increasing data quality. Furthermore, cave salamanders are generally found in underground environments or outside at night when the lack of light makes it difficult to obtain photos of high quality useful for individuals' recognition (see Fig. 2). The images we shot in a controlled environment using appropriate tools (i.e. soft box, multiple flashes) always resulted in high quality (Fig. 2A). On the contrary, many pictures did not show enough quality in the absence of the above-mentioned conditions (Fig. 2B–C), requiring a significant amount of time to confirm individual recognition. Photo previews on camera screens do not always allow a proper quality check, so incorrect photo settings may only be detected post hoc in the lab. This can irreversibly reduce data reliability. Plethodontid salamanders must keep their skin moist because of cutaneous respiration (Spotila 1972; Lanza et al. 2006). As a consequence, light reflection on the skin adds further constraints since part of the salamander's dorsal pattern is often covered by the light's reflection (Figs 2B–C). Finally, when a large number of individuals are photographed, identification may become complex because of the large number of pictures that have to be compared. In this circumstance, the use of appropriate software can greatly speed up picture comparisons (Sacchi et al. 2016; Treilibs et al. 2016). However, the use of such method requires researchers to follow a specific standardised data collection along with individuals' manipulation (Bradley and Eason 2018).

We conclude that the dorsal pattern could be employed in individual identification of sexually mature *Hydromantes* (Lanza et al. 2006; Lunghi et al. 2018), while

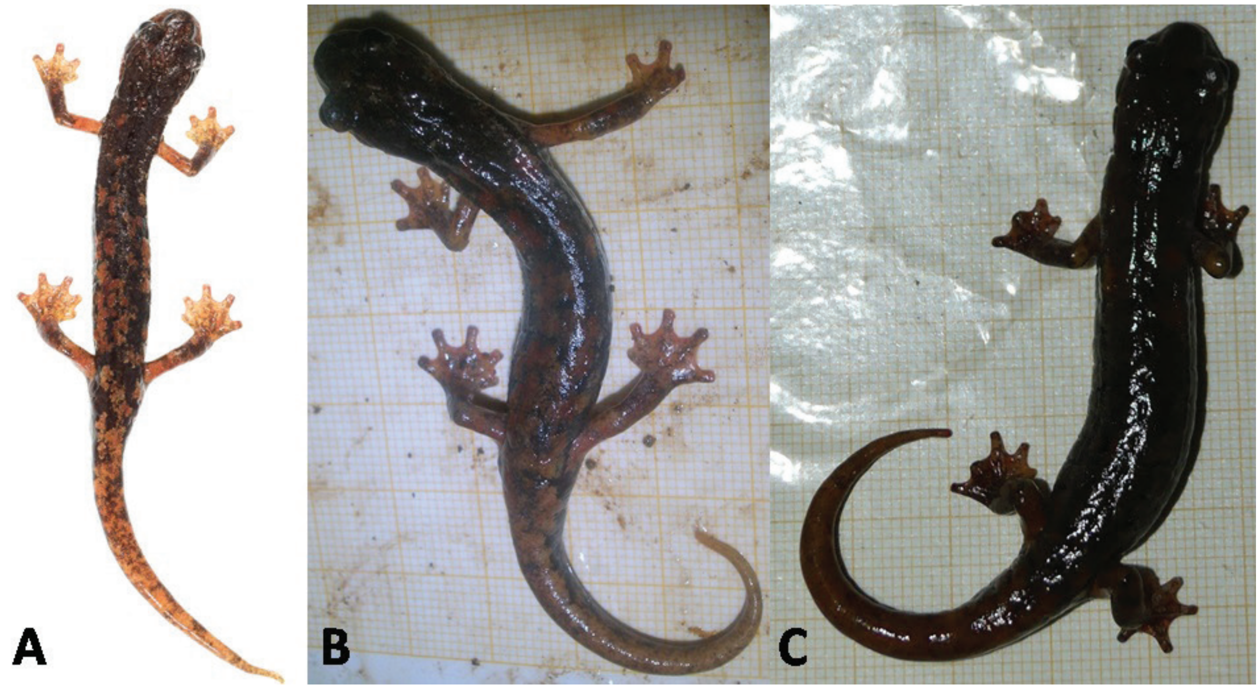

Figure 2. Example pictures of *H. italicus*. A) Picture of high quality, suitable for photographic recognition (photo by S. Giachello); B) picture of poor quality but still useful; C) picture of bad quality useless for recognition.

more specific studies are needed to shed light on the variability occurring in juveniles. However, we urge that all necessary precautions must be taken to increase picture quality and reduce misidentification of individuals. The use of automatic recognition using standardised scoring softwares may speed up identification (Speed et al. 2007; Rocha et al. 2013; Bradley and Eason 2018), but its applicability on *Hydromantes* still needs to be tested.

Acknowledgements

We thank J. G. Bradley and another anonymous reviewer for their suggestions. Fieldwork was authorised by the Italian Ministry of Environment (9384/PNM of 12/05/2015 and further integrations) and by the Regione Autonoma della Sardegna (n° 6312 of 27/03/2017). At present Enrico Lunghi is supported by the Chinese Academy of Sciences President's International Fellowship Initiative for postdoctoral researchers.

References

Ambrogio A, Mezzadri S (2017) Geotritoni d'Italia – Cave Salamanders of Italy. Piacenza, Gavia Edizioni, 64 pp.

Bailey LL (2004) Evaluating elastomer marking and photo identification methods for terrestrial salamanders: marking effects and observer bias Herpetological Review 35(1): 38–41.

Bendik NF, Morrison TA, Gluesenkamp AG, Sanders MS, O'Donnell LJ (2013) Computer-assisted photo identification outperforms visible implant elastomers in an endangered salamander, *Eurycea tonkawae*. PLoS ONE 8(3): e59424. https://doi.org/10.1371/journal.pone.0059424

Bradley JG, Eason PK (2018) Use of non-invasive technique to identify individual cave salmanders, *Eurycea lucifuga*. Herpetological Review 49(4): 660–665.

Carafa M, Biondi M (2004). Application of a method for individual photographic identification during a study on *Salamandra salamandra gigliolii* in central Italy. Italian Journal of Zoology 71(S2): 181–184. https://doi.org/10.1080/11250000409356631

Carpentier AS, Jean C, Barret M, Chassagneux A, Ciccione S (2016) Stability of facial scale patterns on green sea turtles *Chelonia mydas* over time: a validation for the use of a photo-identification method. Journal of Experimental Marine Biology and Ecology 476: 15–21. https://doi.org/10.1016/j.jembe.2015.12.003

Davis TM, Ovaska K (2001). Individual recognition of amphibians: effects of toe clipping and fluorescent tagging on the salamander *Plethodon vehiculum*. Journal of Herpetology 35(2): 217–225. https://doi.org/10.2307/1566111

Lanza B, Pastorelli C, Laghi P, Cimmaruta R (2006) A review of systematics, taxonomy, genetics, biogeography and natural history of the genus *Speleomantes* Dubois, 1984 (Amphibia Caudata Plethodontidae). Atti del Museo Civico di Storia Naturale di Trieste 52: 5–135.

Lunghi E, Bruni G (2018) Long-term reliability of Visual Implant Elastomers in the Italian cave salamander (*Hydromantes italicus*). Salamandra 54(4): 283–286.

Lunghi E, Corti C, Manenti R, Barzaghi B, Buschettu S, Canedoli C, Cogoni R, De Falco G, Fais F, Manca A, Mirimin V, Mulargia M, Mulas C, Muraro M, Murgia R, Veith M, Ficetola GF (2018) Comparative reproductive biology of European cave salamanders (genus *Hydromantes*): nesting selection and multiple annual breeding. Salamandra 54(2): 101–108.

Lunghi E, Manenti R, Canciani G, Scarì G, Pennati R, Ficetola GF (2016) Thermal equilibrium and temperature differences among body regions in European plethodontid salamanders. Journal of Thermal Biology 60: 79–85. https://doi.org/10.1016/j.jtherbio.2016.06.010

Lunghi E, Veith M (2017). Are Visual Implant Alpha tags adequate for individually marking European cave salamanders (genus *Hydromantes*)? Salamandra 53(4): 541–544.

Martin-Smith KM (2011) Photo-identification of individual weedy seadragons *Phyllopteryx taeniolatus* and its application in estimating population dynamics. Journal of Fish Biology 78: 1757–1768. https://doi.org/10.1111/j.1095-8649.2011.02966.x

McCarthy MA, Parris KM (2004) Clarifying the effect of toe clipping on frogs with Bayesian statistics. Journal of Applied Ecology 41: 780–786. https://doi.org/10.1111/j.0021-8901.2004.00919.x

Morrison TA, Yoshizaki J, Nichols JD, Bolger DT (2011) Estimating survival in photographic capture-recapture studies: overcoming misidentification error. Methods in Ecology and Evolution 2: 454–463. https://doi.org/10.1111/j.2041-210X.2011.00106.x

Rocha R, Carrilho T, Rebelo R (2013) Iris photo-identification: a new methodology for the individual recognition of *Tarentola* geckos. Amphibia-Reptilia 34: 590–596. https://doi.org/10.1163/15685381-00002918

Rondinini C, Battistoni A, Peronace V, Teo li C (Eds) (2013) Lista Rossa IUCN dei Vertebrati Italiani. Roma, Comitato Italiano IUCN e Ministero dell'Ambiente e della Tutela del Territorio e del Mare.

Sacchi R, Scali S, Mangiacotti M, Sannolo M, Zuffi MAL (2016) Digital identification and analysis. Reptile Ecology and Conservation. Kenneth Dodd CJ. Oxford University Press, Oxford, 59–72. https://doi.org/10.1093/acprof:oso/9780198726135.003.0005

Sacchi R, Scali S, Pellitteri-Rosa D, Pupin F, Gentilli A, Tettamanti S, Cavigioli L, Racina L, Maiocchi V, Galeotti P, Fasola M (2010) Photographic identification in reptiles: a matter of scales. Amphibia-Reptilia 31: 489–502. https://doi.org/10.1163/017353710X521546

Salvidio S, Latters A, Tavano M, Melodia F, Pastorino MV (1994) Ecology of a *Speleomantes ambrosii* population inhabiting an artificial tunnel. Amphibia-Reptilia 15: 35–45. https://doi.org/10.1163/156853894X00533

Samimi AS, Tajik J, Jarakani S, Shojaeepour S (2016) Evaluation of a five-minute resting period following handling stress on electrocardiogram parameters and cardiac rhythm in sheep. Veterinary Science Development 6: 6481.

Sharifi M, Afroosheh M (2014) Studying migratory activity and home range of adult *Neurergus microspilotus* (Nesterov, 1916) in the Kavat Stream, western Iran, using photographic identification (caudata: Salamandridae). Herpetozoa 27(1/2): 77–82.

Sharifi M, Naderi B, Hashemi R (2013) Suitability of the photographic identification method as a tool to identify the endangered Yellow spotted newt, *Neurergus microspilotus* (Caudata: Salamandridae). Russian Journal of Herpetology 20(4): 264–270.

Speed CW, Meekan MG, Bradshaw CJA (2007) Spot the match – wildlife photo-identification using information theory. Frontiers in Zoology 4: 2. https://doi.org/10.1186/1742-9994-4-2

Spotila JR (1972) Role of temperature and water in the ecology of lungless salamanders. Ecological Monographs 42: 95–125. https://doi.org/10.2307/1942232

Town C, Marshall A, Sethasathien N (2013) Manta Matcher: automated photographic identification of manta rays using keypoint features. Ecology and Evolution 3(7): 1902–1914. https://doi.org/10.1002/ece3.587

Treilibs CE, Pavey CR, Hutchinson MN, Bull CM (2016) Photographic identification of individuals of a free-ranging, small terrestrial vertebrate. Ecology and Evolution 6(3): 800–809. https://doi.org/10.1002/ece3.1883

Wake DB (2013) The enigmatic history of the European, Asian and American plethodontid salamanders. Amphibia-Reptilia 34: 323–336. https://doi.org/10.1163/15685381-00002893

Waye HL (2013) Can a tiger change its spots? A test of the stability of spot patterns for identification of individual Tiger salamanders (*Ambystoma tigrinum*). Herpetological Conservation and Biology 8: 419–425.