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The lateral stripe – a reliable way for software assisted individual identification for *Hyla arborea*

Simeon Lukanov[1,*], Andrey Kolev[2], Angel Dyugmedzhiev[1], Miroslav Slavchev[1]

[1] *Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Tzar Osvoboditel 1, Sofia, Bulgaria*
[2] *Faculty of Biology, Sofia University "St. Kliment Ohridski", Dragan Tzankov 8, Sofia, Bulgaria*
[*] *Corresponding author.* Email: simeon_lukanov@abv.bg

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Abstract. Traditional mark-recapture methods for amphibians and reptiles involve the application of invasive physical markers, such as toe clipping. Photographic identification methods are non-invasive alternative that use natural colouration of individuals for inexpensive, reliable identification. The relatively small species of the Hylidae family are susceptible to toe clipping – so dorsal, ventral, or leg patterns have been used in studies on different hylid species. The present study aims to test the usefulness of the lateral stripe in the Common tree frog *Hyla arborea* in software-assisted image recognition, which would allow for a reliable and time-efficient individual identification. A total of 258 adult tree frogs from a pond near the village of Oshtava, SW Bulgaria, were captured by hand in twelve sessions throughout the spring (April-May) of 2022 and 2023. The right and left side of each frog was photographed and the animals were released at the site of capture. Images were loaded into Hotspotter – free software for image recognition that has been demonstrated to work very well for several amphibian species. Results revealed 108 recaptures of 46 individual tree frogs (including 11 tree frogs captured more than twice and three recaptures between years). Only 12% of all frogs had similar right and left lateral stripes, with the others displaying significant variations. This study highlights both the applicability of the lateral stripe for individual recognition in this species and the need for consistency in image processing.

Keywords. Colouration, Common tree frog, recognition, variation.

For several decades, individual identification of animals has been a crucial aspect of conservation biology and behavioural ecology. Studies focused on life-history traits and long-term monitoring require to repeatedly and reliably identify captured individuals. Traditional mark-recapture methods for amphibians and reptiles involve the application of physical markers with varying degrees of invasiveness – from paint marking to toe clipping (review in Ferner, 2007). For amphibians in particular, popular markings include toe clipping and subcutaneous injections/pit tags, with toe clipping being the most widespread for many decades because of its affordability. However, toe clipping does inflict temporary (i.e., newts regenerate toes) or permanent physical harm to the animal, and it is well-established that it could cause diminished survival and altered behaviour in some smaller species (e.g., Clarke, 1972; Guimaraesh et al., 2014). Alternatives to toe clipping have been implemented since the turn of the century, incl. visible implant elastomers (VIE) (Pittman et al., 2008; Antwis et al., 2014) and visible implant tags (Pittman et al., 2008). VIE provide reliable and rapid identification, but are expensive so their usefulness is often limited by their cost (Le Chevalier et al., 2017). During the past decade, there has been a growing number of studies investigating the potential of using the natural colouration pattern of various amphibian spe-

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cies in photographic identification methods (PIM), as the rapid advance in technology allows for inexpensive, reliable and non-invasive identification (Kenyon et al., 2009; Bendik et al., 2013; Elgue et al., 2014; Schoen et al., 2015; Sannolo et al., 2016; Kim et al., 2017; Renet et al., 2019; Lukanov, 2022). The relatively small species of the Hylidae family are susceptible to toe clipping, as it has a negative effect on their survival and capture probability, especially when multiple toes are removed (Waddle et al., 2008) – so dorsal, ventral or leg patterns have been used in PIM studies on some hylid species (Kim et al., 2017). While manual visual identification is very useful for smaller samples, it becomes unreliable and time-consuming for larger catalogues, and in recent years, there have been developed some specialized software packages aimed at image pattern recognition (Speed et al., 2007; Bolder at al., 2012; Crall et al., 2013; Matthé, 2018). As the usefulness of software-assisted image recognition for *Hyla* sp. has not been investigated, the present study aims to test whether photographs of the lateral stripe in the Common tree frog *Hyla arborea* (L., 1758) would allow for a reliable and time-efficient individual identification.

The Common tree frog is distributed from the Southern Balkans to North-Western Europe. In Bulgaria, it is present in the Struma river basin, whereas the Eastern tree frog *Hyla orientalis* (Bedriaga, 1890) occurs across the rest of the country (Dufresnes et al., 2015). The current study is part of an ongoing project on tree frog morphology and distribution in Bulgaria, which aims to compare populations across the country. The present study pond is located near the village of Oshtava in South-Western Bulgaria, with a surface area around 0.50 hectares and fluctuating water level (spring maximums and summer minimums, incl. desiccation). It is surrounded by grassy meadows and mixed forests, and water vegetation is dominated by reeds (*Phragmites* sp.). While the maximum depth is over 1m, there are shallow waters along the edges of the pond, and this combination provides excellent breeding conditions for many amphibian and reptile species: Agile frog *Rana dalmatina* Fitzinger, 1838, Balkan spadefoot toad *Pelobates balcanicus* Karaman, 1928, Common tree frog *Hyla arborea* (L., 1758), Green toad *Bufotes viridis* (Laurenti, 1768), Common toad *Bufo bufo* (L., 1758), Marsh frog *Pelophylax ridibundus* (Pallas, 1771), Buresch's crested newt *Triturus ivanbureschi* Arntzen & Wielstra, 2013, Grass snake *Natrix natrix* (L., 1758).

A total of 258 tree frogs (51 females, 207 males) were captured in 12 sessions in the period April-May of 2022 (141 frogs from six sessions) and 2023 (117 frogs from six sessions). In addition to this number, in each year, 10 of the captured tree frogs were randomly chosen and kept in individual containers until the end of the session; these frogs were photographed each night for the duration of the sessions and the images were used as control for the individual recognition tests. Tree frogs were collected at night (between 21:30h and 23:30h), by hand or by using a fishing hand-net, in both shallow (both male and female tree frogs swimming or sitting in the shallows) and in deeper water (male tree frogs calling from the reeds at up to 1m depth). Each frog was held in hand and photographed laterally from both sides to document the shape of its lateral stripe, after which it was released at the site of capture. We chose the lateral view, rather than the dorsal or the ventral, as the lateral stripe has been proven to be individually specific for other tree frog species (Kim et al., 2017), while colouration, in contrast, is known to be varying and unreliable (Bolger et al., 2012). For individual identification, we used the software Hotspotter v. 1.0 (Crall et al., 2013), which has been demonstrated to work very well for some amphibian species (e.g., Naumov and Lukanov, 2018; Patel and Das, 2020; Burgstaller at al., 2021; Lukanov, 2022). The region of interest (ROI) which is used for comparison between the images, was set as close around the frog body as possible to minimize external factors, but at the same time to allow all elements of the lateral stripe to be clearly visible (for a detailed description of image processing and analysis, see Crall et al., 2013; Naumov and Lukanov, 2018). Suggested matches by the program were always manually verified by a human observer.

Results revealed 108 recaptures of 46 individual tree frogs (26 in 2022 and 20 in 2023, or a recapture rate of approximately 42% and 41%, respectively), incl. 11 tree frogs captured more than twice (all males) and three recaptures between years (one female and two males). In order to assess the software match, the images from the control groups were compared to the ones taken in the field and were very consistent, with the verified matches always appearing as the first suggested matches. Unlike coloration, the shape of the lateral stripe remained constant (at least in the short-term, as evidenced by frogs from the control group) and allowed for easy individual identification (Fig. 1). As demonstrated for other species (Burgstaller et al., 2021), the type of camera did not matter and photos from different cameras performed equally (incl. smartphones, as we used Xiaomi Redmi Note 8 and Mi A2, as well as Huawei P Smart, with default settings and flashes turned on).

We compared image scores (values assigned by the program based on image similarity, see Crall et al., 2013) between First match (score of the first suggested match for verified matches), Last match (aggregate score for all verified matches after the first suggested match, when more

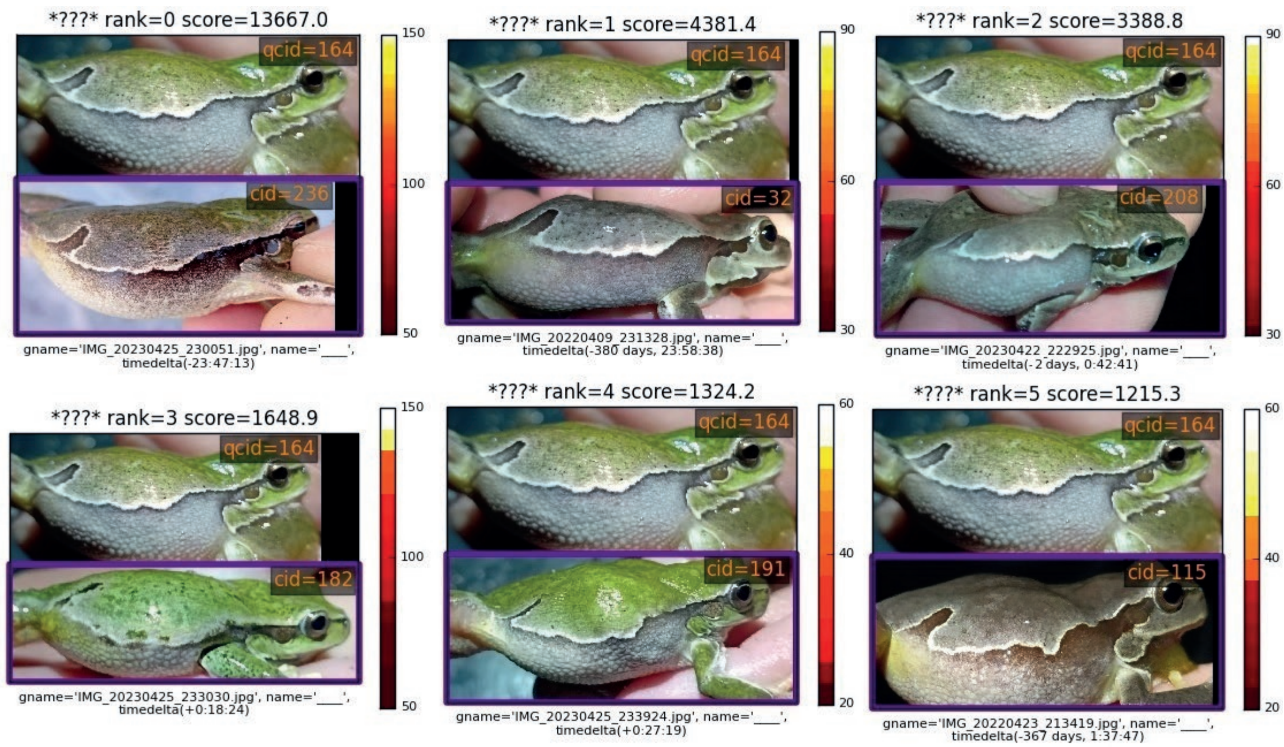

Fig. 1. Results from Hotspotter showing a male first captured on April 9th 2022 and then recaptured on 22nd, 24th and 25th April 2023. Scores for recaptures (first row) are 2-10 times higher than the other three suggestions (second row).

than one verified match was registered), and No match (score of the first suggested match for individuals without verified recapture). As data was not normally distributed, we used a Kruskal-Wallis and Multiple comparisons test in Statistica v. 7 (StatSoft, Inc. 2004), with level of statistical significance set at $P < 0.05$. The First match had an average similarity score of 13 735, with min-max range of 3590-52 541; these values were 3814 (1619-6482) for the Last match and 1314 (573-1805) for the No match, respectively. The Kruskal-Wallis test revealed significant differences between the three groups ($H_2 = 34.60$, $P < 0.001$), and Multiple Comparisons test confirmed differences between First match and No match ($P < 0.001$), Last match and No match ($P < 0.001$), but not between First match and Last match ($P = 0.075$). Although the average value for Last match was higher than the minimum of First match, there were no false matches between the first and the last suggested match. This is probably due to the fact that only 11 frogs were captured more than two times (i.e., in most cases there was no other match than the first), and of these, only five were captured four times (i.e., with three consecutive matches).

The scores assigned by the Hotspotter algorithm tend to vary depending on the species and the size of the image set, with small image sets producing higher scores (Crall et al., 2013). For this reason, match score values cannot be used as a sole indicator of a positive match, and manual verification is required. Nevertheless, there were no false positives (i.e., similarly high scores for different individuals) and a careful manual examination of all photos confirmed there were also no false negatives (i.e., low scores for images of the same individual, or identical individual image not suggested as a potential match by the program).

Results from comparisons between the right and left lateral stripes were virtually identical, i.e., all frogs that were identified as recaptures from images of the right side of their body, were also identified from images of the left side, and vice versa. This was reflected in the similarity scores, which were very similar (Mann Whitney U test: First match $U = 51.0$, $P = 0.732$; Last match $U = 19.0$, $P = 0.335$; No match $U = 32.0$, $P = 0.270$). However, there were often contrasting differences between the left and right lateral stripes in the same individual (Fig. 2A), and in only 12% of all frogs the right and left lateral stripes were similar (but still not identical) (Fig. 2B).

Our results lead to two conclusions: 1) the lateral stripe in the Common tree frog could be reliably used in PIM for both sexes, at least for short-term studies (the number of recaptures between years was very low and no definite conclusions could be made) and 2) a soft-

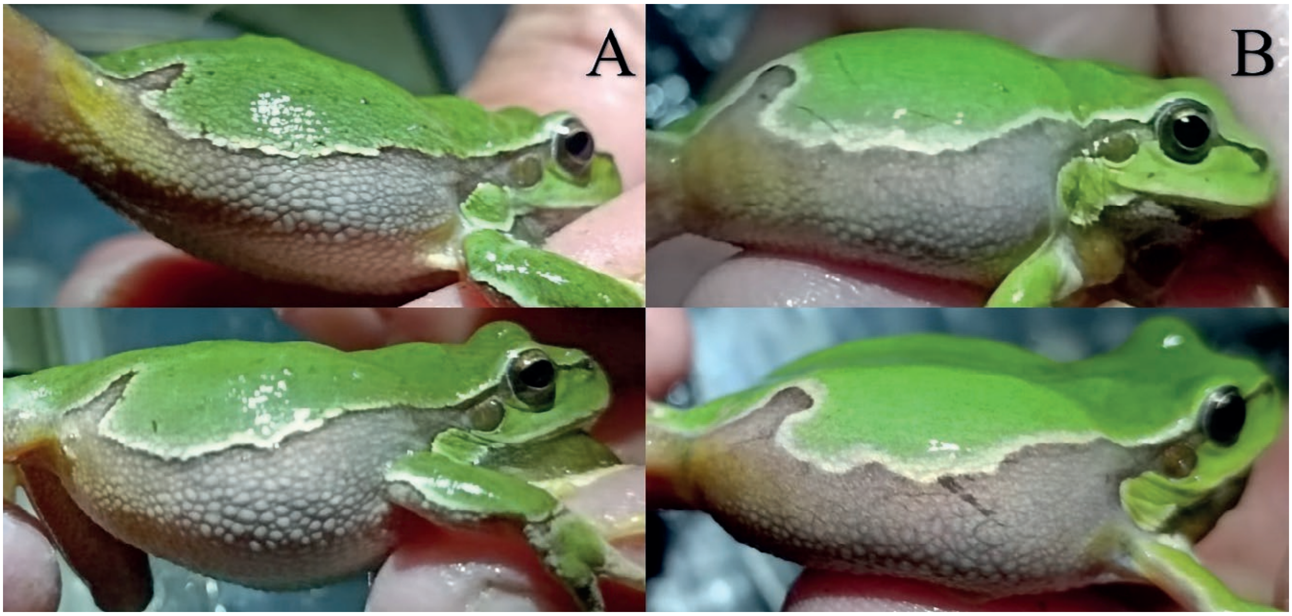

Fig. 2. A) Individual with very different right (top) and left (bottom) lateral stripes; B) Individual with similar right (top) and left (bottom) lateral stripes. Images of the left side are reversed to the right for easier comparison.

ware-assisted identification would provide time-efficient and reliable results. We still need to stress the necessity of manual verification, as relying solely on matching scores could lead to false positives, especially in smaller samples. Under certain conditions, some amphibians may change their colour pattern over long periods (see Naumov and Lukanov, 2018), and the applicability of this method for long-term population studies on *Hyla* species remains to be confirmed – but it is undoubtedly a very useful tool for non-invasive individual recognition. One way to prove the long-term usefulness of colour patterns in this frog genus is to match it with results from VIE, as this method has been demonstrated to work well for up to six years in the salamander *Hydromantes italicus* (Lunghi and Bruni, 2018). It also needs to be noted that Hotspotter seems to outperform other image recognition programs such as Wild-ID, which did not produce satisfactory results for the Japanese tree frog *Dryophytes japonicus* (Günther, 1859), which was photographed in a manner that was similar to ours - being held in hand and released immediately (Kim et al., 2017). This would confirm the observations of Naumov and Lukanov (2018) and Burgstaller et al. (2021), who conclude that Hotspotter performs consistently better than the other image recognition software they tested (for *Triturus dobrogicus* (Kiritzescu, 1903) and *Bufotes viridis*, respectively). Importantly, Burgstaller et al. (2021) point out that camera type has a negligible effect on the performance of all tested image recognition programs. It has to be noted that Hotspotter may prove unreliable for larger samples (over 900–1000 images), as in such cases it is known to crash and disrupt the workflow (S. Lukanov, pers. obs.; S. Burgstaller, pers. comm. 2022); however, no problems of this kind were reported by Dunbar et al. (2021), who used 2136 images of Hawksbill turtles *Eretmochelys imbricata* (L., 1766).

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REFERENCES

Antwis, R.E., Purcell, R., Walker, S.L., Fidgett, A.L., Preziosi, R.F. (2014): Effects of visible implanted elastomer marking on physiological traits of frogs. Conserv. Physiol. **2**: cou042.

Bendik, N.F., Morrison, T.A., Gluesenkamp, A.G., Sanders, M.S., O'Donnell, L.J. (2013): Computer-assisted photo identification outperforms visible implant elastomers in an endangered salamander, *Eurycea tonkawae*. PloS ONE **8**: e59424.

Burgstaller, S., Gollmann, G., Landler, L. (2021): The Green toad example: a comparison of pattern recognition software. North-west. J. Zool. **17**: 96-99.

Bolger, D.T., Morrison, T.A., Vance, B., Lee, D., Farid H. (2012): A computer assisted system for photographic mark–recapture analysis. Methods Ecol. Evol. **3**: 813-822.

Clarke, R.D. (1972): The effect of toe clipping on survival in Fowler's toad (*Bufo woodhousei fowleri*). Copeia **1972**: 182-185.

Crall, J., Stewart, C., Berger-Wolf, T., Rubenstein, D., Sundaresan S. (2013): Hotspotter - patterned species instance recognition; pp. 230-237. In: Proceedings of the 2013 IEEE Workshop on applications of Computer Vision (WaCV 2013), January 15-17, 2013, Clearwater Beach. WWW internet resource and software available at http://cs.rpi.edu/hotspotter/

Dufresnes, C., Brelsford, A., Crnobrnja-Isailovic, J., Tzankov, N., Lymberakis, P., Perrin, N. (2015): Timeframe of speciation inferred from secondary contact zones in the European tree frog radiation (*Hyla arborea* group). BMC Evol. Biol. **15**: 155.

Dunbar, S.G., Anger, E.C., Parham, J.R., Kingen, C., Wright, M.K., Hayes, C.T., Safi, S., Holmberg, J., Salinas, L., Baumbach D.S. (2021): HotSpotter: using a computer-driven photo-id application to identify sea turtles. J. Exp. Mar. Biol. Ecol. **535**: 151490.

Elgue, E., Pereira, G., Achaval-Coppes, F., Maneyro R. (2014): Validity of photo-identification technique to analyze natural markings in *Melanophryniscus montevidensis* (Anura: Bufonidae). Phyllomedusa **13**: 59-66.

Ferner, J.W. (2007): A review of marking and individual recognition techniques for amphibians and reptiles. Herpetological circular No35, Society for the study of amphibians and reptiles, Salt Lake City.

Guimaraes, M., Correa, D.T., Filho, S.S., Oliveira, T.A., Doherty, P.F., Sawaya R.J. (2014): One step forward: contrasting the effects of toe clipping and PIT tagging on frog survival and recapture probability. Ecol. Evol. **4**: 1480-1490.

Kenyon, N., Phillott, A.D., Alford, R.A. (2009): Evaluation of the photographic identification method (PIM) as a tool to identify adult *Litoria genimaculata* (Anura: Hylidae). Herpetol. Conserv. Biol. **4**: 403-410.

Kim, M.Y., Borzée, A., Kim, J.Y., Jang, Y. (2017): Treefrog lateral line as a mean of individual identification through visual and software assisted methodologies. J. Ecol. Environ. **41**: 42.

Le Chevalier, H., Calvez, O., Martínez-Silvestre, A., Picard, D., Guérin, S., Isselin-Nondedeu, F., Ribéron, A., Trochet, A. (2017): Marking techniques in the Marbled Newt (*Triturus marmoratus*): PIT-Tag and tracking device implant protocols. Acta Herpetol. **12**: 79-88.

Lukanov, S. (2022): Inter-pond migration during the aquatic phase by male *Triturus ivanbureschi*. Russ. J. Herpetol. **29**: 373-376.

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

Matthé, M. (2018): AmphIdent. Available from: http://www.amphident.de

Naumov, B., Lukanov, S. (2018): Notes on age-related changes in body size and colorpattern in captive *Triturus dobrogicus* (Kiritzescu, 1903). Herpetozoa **30**: 159-168.

Patel, N.G., Das, A. (2020): Shot the spots: A reliable field method for individual identification of *Amolops formosus* (Anura, Ranidae). Herpetozoa **33**: 7-15.

Pittman, S.E., Jendrek, A.L., Price, S.J., Dorcas, M.E. (2008): Habitat selection and site fidelity of Cope's gray treefrog (*Hyla chrysoscelis*) at the aquatic-terrestrial ecotone. J. Herpetol. **42**: 378-385.

Renet, J., Leprêtre, L., Champagnon, J., Lambret, P. (2019): Monitoring amphibian species with complex chromatophore patterns: a non-invasive approach with an evaluation of software effectiveness and reliability. Herpetol. J. **29**: 13-22.

Sannolo, M., Gatti, F., Mangiacotti, M., Scali, S., Sacchi, R. (2016): Photo-identification in amphibian studies: a test of I3S Pattern. Acta Herpetol. **11**: 63-68.

Schoen, A., Boenke, M., & Green, D. M. (2015): Tracking toads using photo identification and image-recognition software. Herpetol. Rev. **46**: 188-192.

Speed, C.W., Meekan, M.G., Bradshaw, C.J. (2007): Spot the match – wildlife photo-identification using information theory. Front. Zool. **4**: 2.

Waddle, J.H., Rice, K.G., Mazzotti, F.J., Percival, H.F. (2008): Modeling the effect of toe clipping on treefrog survival: beyond the return rate. J. Herpetol. **42**: 467-473.