SCIENTIFIC DATA

Check for updates

OPEN

DATA DESCRIPTOR

Photographic database of the European cave salamanders, genus *Hydromantes*

Enrico Lunghi [1,2], Simone Giachello[3 ✉], Yahui Zhao [1 ✉], Claudia Corti[2], Gentile Francesco Ficetola [3,4] & Raoul Manenti [3]

European *Hydromantes* are a group of eight salamander species often occurring in subterranean habitats, which are a difficult environment to explore. All *Hydromantes* are strictly protected species and thus, low-impact methodologies to study these salamanders are strongly needed. Here we used a photographic technique to produce a large dataset of European *Hydromantes*, providing standardised pictures of 1,052 individuals belonging to the eight species, including hybrids as well. With our methodology we were able to reduce the handling time of individuals, and produce high quality pictures useful to investigate multiple life traits of these endangered species. Furthermore, the standardised photos provided here can be used for future comparisons of individuals from the surveyed populations.

Background & Summary

The European cave salamanders (genus *Hydromantes*; see[1] for taxonomic discussion) are a group of eight amphibians species endemic to Italy and to a small part of south-eastern France[2]. Three species (*H. strinatii*, *H. ambrosii* and *H. italicus*) are distributed along the northern and central Apennine chain (*H. strinatii* being the only species present in France), whereas five (*H. flavus*, *H. supramontis*, *H. imperialis*, *H. genei* and *H. sarrabusensis*) are endemic to Sardinia island, where geomorphology represents the main drive of their allopatry[2,3]. Only two mainland species, *H. ambrosii* and *H. italicus*, come into contact naturally and hybrid populations occur in a small area[4]. *Hydromantes* salamanders often have epigean activity during cold and wet seasons, but exploit subterranean habitats (such as caves, mines, small cervices and springs) to avoid unfavourable climatic conditions (when too hot and/or dry)[5–7]. These salamanders are lungless and require a specific combination of relatively low temperature and high moisture to efficiently carry out their cutaneous respiration;[2,8] these conditions are often found in subterranean habitats[9], thus *Hydromantes* are able to maintain stable populations and even reproduce there[10,11], moving outdoors mostly to reach areas with high prey abundance[12,13].

During the last few decades several studies have shed light on some life history traits of *Hydromantes*, such as the reproductive behaviour, trophic niche and population dynamics[14–17]. However, to collect data on these species can be extremely complex. First, the subterranean habitats are not human-friendly; some can be explored only by skilled speleologists and, in any case, the constant low temperature and the air moisture close to saturation represent a challenge for researchers spending prolonged time there[18,19]. Second, all the *Hydromantes* are strictly protected by both national and international laws[20,21] and thus, studies cannot be performed without the proper authorisations. These salamanders are sensitive to multiple treats such as climate change, habitat degradation and poaching[2,22]. Furthermore, *Hydromantes* are sensitive to the deadly chytrid fungus, *Batrachochytrium salamandrivorans*, thus manipulation must be limited and must adhere to strict protocols to avoid spreading pathogens[23]. Indeed, researchers are continuously developing and testing different methodologies allowing them to limit their impact on these animals, without reducing the quality of their scientific researches[24–26]. Here we used a non-invasive method to build a large dataset related to *Hydromantes*, which can be used to investigate some of their unexplored life traits avoiding manipulation. Using photography, we here provide a dataset covering all *Hydromantes* species occurring in Europe, which also represents one of the few photographic datasets available

[1]Key Laboratory of the Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing, China. [2]Museo di Storia Naturale dell'Università degli Studi di Firenze, Museo "La Specola", Firenze, Italy. [3]Dipartimento di Scienze e Politiche Ambientali, Università degli Studi di Milano, Milano, Italy. [4]Univ. Grenoble Alpes, CNRS, Univ. Savoie Mont Blanc, LECA, Laboratoire d'Ecologie Alpine, F-38000, Grenoble, France. ✉e-mail: simgia. sg@gmail.com; zhaoyh@ioz.ac.cn

Column	Data description	Typology of data
1	ID	The salamander's database code
2	Site	"Forest", "Cave", "Mine" or "Spring"
3–4	Latitude and Longitude	Low resolution coordinates of the site
5	Population	The population code
6–8	Country, Region and Province	The relevant information for each site
9–10	Month and Year	The date in which the picture was taken
11	Species	The species to which the individual belongs
12	N_photo	The unique file number corresponding to each individual
13	Age_class	Juvenile (0) or adult (1)
14	Sex	Adult males (M), adult females (F), juveniles (J)
15	Total_lenght	The total length of the individual (mm)
16	Eggs	Indicates if the female was gravid (1) or not (0). For males and juveniles (NA = not applicable)
17	Tail_issue	Indicates whether the tail is shown for its entire length (0) or not (1)
18	Scale_bar	Indicates the size of the picture scale bar (mm)

Table 1. Qualitative data of the *Hydromantes* photographic dataset[50]. Information related to each photographed salamander and relative location. When the distance between two sites was <97 m, individuals were considered belonging to the same population[53]. Coordinates of the sites are not reported for species protection[37].

for the animal kingdom. Our dataset is not made up of simple pictures (e.g.[27,28]), but we have adopted an ad hoc methodology to obtain standardised photos that can be used for future comparisons and analysed with multiple software[29–31]. There is a growing demand for publication of standardised dataset[32–34], and those relating to endangered or not yet assessed species have a particular value[35,36]; however, special care should be taken when publishing data on sensitive species[37]. This dataset represents a snapshot of multiple *Hydromantes* populations, providing information on the minimum population size[38], the age of individuals and morphometry[39,40], and the variability of dorsal colouration[41–43]. Digitisation is a practice adopted to make manuscripts stored in various collections easily accessible to the public[44–46]. More recently, this trend also considers animal species[31,47], providing undeniable help for the entire scientific community, as well a window for the public to discover the wonders of nature. Therefore, with our dataset we will provide the first digital collection showing the morphological diversity of all European *Hydromantes* species.

Methods

Experimental design. We collected photographic information on all European *Hydromantes* species, also including hybrid populations. For each species and for the hybrid zone between *H. italicus* and *H. ambrosii*, we photographed ≥55 individuals, collecting data from at least two different sites (Table 1 and Fig. 1); this allowed us to gather information on a large number of individuals from different areas[2,4]. We also considered a population introduced into the French Pyrenees, which probably includes individuals of different *Hydromantes* species, if not hybrids;[48] there, we photographed 47 individuals. The surveyed sites included forest, natural caves, mines and artificial springs (see Table 1); in all of them, the presence of *Hydromantes* was previously assessed[4,48,49]. Each site was surveyed once between August and October 2018 in order to avoid repeated photos on the same individual. Prior to each survey, equipment and shoes were cleaned and disinfected with bleach to avoid the spread of potential pathogens.

Individuals sampling. In a dark area of the cave we built a photographic set to take standardised photos of salamanders. We used a soft white fabric box (hereafter, soft box) with a piece of white and flat plexiglass covering the inner bottom of the box. The soft box was placed suspended on the floor of the cave, with flash units on the left, on the right and under the soft box; such arrangement allows the light to be evenly distributed, limiting the presence of shadows on the subject (Fig. 2). The bottom flash produces a clear white background, a condition enabling a quick and easy way to isolate the salamander from the background in post-production. The two lateral flashes were arranged above the subject and inclined with 45° to fully and evenly illuminate it. The salamanders were collected and placed in fauna boxes until they were photographed. Before being photographed, each salamander was visually inspected and all debris adhering to the skin were removed. The salamanders were then placed in the soft box on the plexiglass and a photo was taken from above, keeping the camera perpendicular to the surface of the plexiglass. Salamanders were photographed with a Pantone colour card (see below) next to them to have a standard size reference and to correctly calibrate the colours and light during post-production. After the photo shoot, the salamanders were released where they were collected.

Photo calibration. Correctly balancing the white is crucial to obtain standardised and comparable images. To guarantee an accurate and standardised white balance to our images, we shot a reference photo in RAW format (.CR2) of the Pantone colour card X-Rite Colorcheker Passport 2 at the beginning of each photographic session; shooting in RAW creates high quality files containing all the unprocessed data captured by the sensor. Images were then uploaded on a computer and organized in folders, each corresponding to the single photographic session. Using the function "White Balance Tool" of the software Adobe Camera Raw, for each site we created a
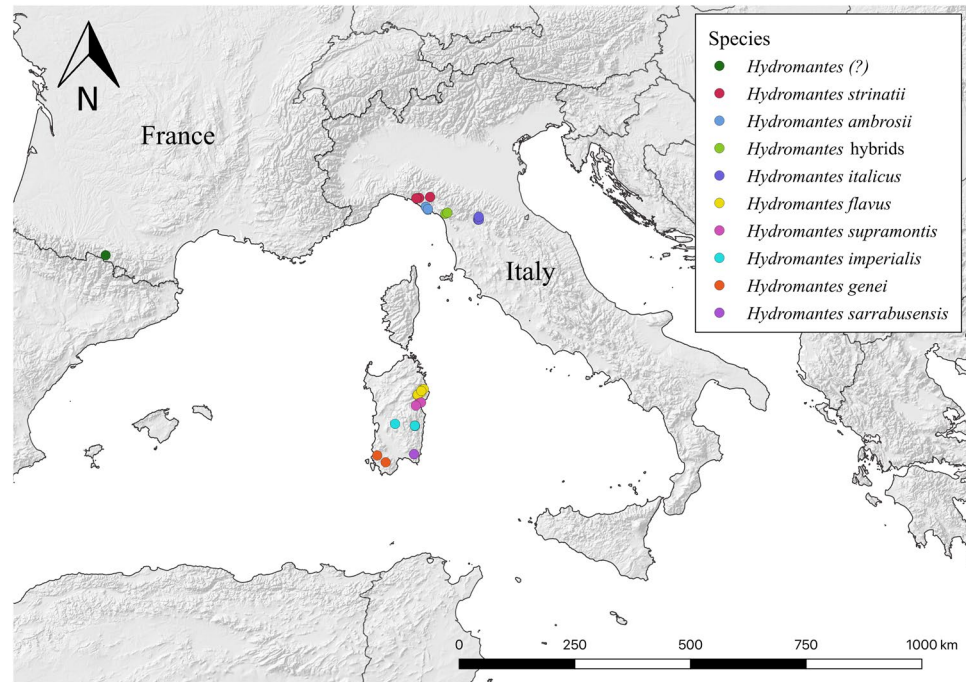
Fig. 1 Map showing the locations where the *Hydromantes* salamanders were photographed.

Fig. 2 Example of *Hydromantes* picture from the database. Here an individual of *H. flavus* photographed using the described methodology; scale bar 10 mm.

custom white balance profile using the respective picture of the Pantone colour card as reference. We then applied the profiles to the respective images and converted them into JPEG format, which enables reduction in the size of pictures without compromising their quality.

Data records

The dataset (The European *Hydromantes* salamanders[50]) includes 1,052 photographed individuals of all species and hybrids of European *Hydromantes*. Sites are within species natural range if not differently stated[2]. In particular, the dataset is composed as follows:

- *H. strinatii* (Strinati's cave salamander): 82 individuals (40 females, 33 males, 9 juveniles) from five sites, two outside its natural range;
- *H. ambrosii* (Ambrosi's cave salamander): 137 individuals (60 females, 47 males, 30 juveniles) from four sites;
- *H. italicus* (Italian cave salamander): 141 individuals (54 females, 53 males, 34 juveniles) from four sites;
- *H. flavus* (Monte Albo cave salamander): 171 individuals (64 females, 73 males, 34 juveniles) from six sites;
- *H. supramontis* (Supramonte cave salamander): 112 individuals (46 females, 25 males, 41 juveniles) from three sites;
- *H. imperialis* (Imperial cave salamander): 116 individuals (26 females, 63 males, 27 juveniles) from four sites;
- *H. genei* (Gene's cave salamander): 122 individuals (43 females, 51 males, 28 juveniles) from two sites;
- *H. sarrabusensis* (Sette Fratelli cave salamander): 69 individuals (30 females, 27 males, 12 juveniles) from two sites;
- *Hydromantes* hybrids: 55 individuals (32 females, 14 males, 9 juveniles) from two sites within the hybrid zone[4];
- *Hydromantes* (?) allochthonous population: 47 individuals (28 females, 11 males, 8 juveniles) from one site[48].

Together with the photographic dataset we include information relating to each individual and their location (Qualitative data of the *Hydromantes* photographic dataset[50]; Table 1). Some information related to the salamanders (i.e., age, sex and total length) is critical to increase the quality of the dataset. Adult *Hydromantes* can be sexed with high confidence only by checking the presence of the mental gland on the chin of sexually mature males;[2] this is a part of the body not visible from our images. With this additional information, salamanders can be divided into juveniles, adult males and adult females.

Technical Validation

This dataset shows a unique collection of multiple individuals belonging to all *Hydromantes* species present in Europe. Collecting data on these salamanders can be challenging, as all species are strictly protected[20], and even simple manipulation requires ministerial authorisations. The single survey performed on each site provides data on 1,052 different individuals. The methodology applied here enables production of standardised high quality images with low impact on the species[29,30]. The overall time required to shoot each salamander was usually <15 seconds, thus limiting the stress caused by handling[51]. The white calibration before each session avoided potential divergence in light condition and thus, providing standardised pictures. Our methodology allowed to avoid the hurdles due to the use of flash on animals with moist skin, being thus widely applicable. Blind measurements of salamanders were performed to reduce possible bias[52]. Salamanders were measured entirely or at the furthest visible point. In few cases, the tail was severed or its tip covered; these cases are indicated in Table 1 [50]. Possible outliers were identified by plotting the data; the measurement of related individuals was taken twice to check whether the abnormal value was due to measurement errors. Considering that in these species individuals are aged according to their body size, and adults are sexed basing on the presence/absence of the male mental gland[2], we added such information for each individual in Table 1 [50]. To identify juveniles, we used the size of the smallest male observed as reference. Mainland species, hybrids and *H. genei* have all comparable size[35], therefore we used 68 mm as threshold for these salamanders. The other four Sardinian species are defined as "giant"; for these species the size of the smallest male was 77 mm. All salamanders smaller than the respective reference male were considered juveniles. *Hydromantes* can live more than 10 years[2,53] and thus, considering that our dataset provides a snapshot of individuals from 2018, it can be employed in comparative studies for multiple years ahead. The morphometrics obtained from our dataset can be compared with those of the same species published >20 years ago[54] to assess whether any potential change occurred and which may be the cause[55,56].

Usage Notes

The pictures can be used in R environment (http://www.R-project.org/) to perform analyses on colouration (e.g.[57]) and geometric morphometrics (e.g.[58]), and with the program ImageJ to record multiple salamanders' morphometrics (e.g.[59]). Furthermore, considering that, at least, in adult *Hydromantes* the dorsal pattern does not change throughout time[60], this dataset can be used over time as a reference for the recognition of individuals belonging to the same population[61,62], allowing to study growth rate, home range and other life traits. This also limits the manipulation of individuals, which represents a potential source of both stress and pathogens[23,51,63,64].

Code availability

No code was used in this study.

References

1. Wake, D. B. The enigmatic history of the European, Asian and American plethodontid salamanders. *Amphibia-Reptilia* **34**, 323–336 (2013).
2. Lanza, B., Pastorelli, C., Laghi, P. & Cimmaruta, R. 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 (2006).
3. Chiari, Y. *et al.* Phylogeography of Sardinian cave salamanders (genus *Hydromantes*) is mainly determined by geomorphology. *PLoS One* **7**, e32332, https://doi.org/10.1371/journal.pone.0032332 (2012).
4. Ficetola, G. F., Lunghi, E., Cimmaruta, R. & Manenti, R. Transgressive niche across a salamander hybrid zone revealed by microhabitat analyses. *Journal of Biogeography* **46**, 1342–1354, https://doi.org/10.1111/jbi.13621 (2019).
5. Ficetola, G. F., Pennati, R. & Manenti, R. Do cave salamanders occur randomly in cavities? An analysis with *Hydromantes strinatii*. *Amphibia-Reptilia* **33**, 251–259 (2012).
6. Lunghi, E., Manenti, R. & Ficetola, G. F. Do cave features affect underground habitat exploitation by non-troglobite species? *Acta Oecologica* **55**, 29–35, https://doi.org/10.1016/j.actao.2013.11.003 (2014).
7. Costa, A., Crovetto, F. & Salvidio, S. European plethodontid salamanders on the forest floor: local abundance is related to fine-scale environmental factors. *Herpetological Conservation and Biology* **11**, 344–349 (2016).
8. Ficetola, G. F. *et al.* Differences between microhabitat and broad-scale patterns of niche evolution in terrestrial salamanders. *Scientific Reports* **8**, 10575, https://doi.org/10.1038/s41598-018-28796-x (2018).
9. Culver, D. C. & Pipan, T. *The biology of caves and other subterranean habitats. Second edition* 336 (Oxford University Press, 2019).
10. Lunghi, E. *et al.* Comparative reproductive biology of European cave salamanders (genus *Hydromantes*): nesting selection and multiple annual breeding. *Salamandra* **54**, 101–108 (2018).
11. Lunghi, E., Manenti, R. & Ficetola, G. F. Seasonal variation in microhabitat of salamanders: environmental variation or shift of habitat selection? *PeerJ* **3**, e1122, https://doi.org/10.7717/peerj.1122 (2015).
12. Lunghi, E. *et al.* Environmental suitability models predict population density, performance and body condition for microendemic salamanders. *Scientific Reports* **8**, 7527, https://doi.org/10.1038/s41598-018-25704-1 (2018).
13. Manenti, R., Lunghi, E. & Ficetola, G. F. Distribution of spiders in cave twilight zone depends on microclimatic features and trophic supply. *Invertebrate Biology* **134**, 242–251, https://doi.org/10.1111/ivb.12092 (2015).
14. Lunghi, E. *et al.* What shapes the trophic niche of European plethodontid salamanders? *PLoS ONE* **13**, e0205672, https://doi.org/10.1371/journal.pone.0205672 (2018).

15. Ficetola, G. F., Pennati, R. & Manenti, R. Spatial segregation among age classes in cave salamanders: habitat selection or social interactions? *Population Ecology* **55**, 217–226 (2013).

16. Salvidio, S. Homing behaviour in *Speleomantes strinatii* (Amphibia Plethodontidae): a preliminary displacement experiment. *North-Western Journal of Zoology* **9**, 429–433 (2013).

17. Oneto, F., Ottonello, D., Pastorino, M. V. & Salvidio, S. In *Scripta Herpetologica. Studies on Amphibians and Reptiles in honour of Benedetto Lanza* (eds M. Capula & C. Corti) 129–138 (Edizioni Belvedere, 2014).

18. Zagmajster, M., Culver, D. C., Christman, M. C. & Sket, B. Evaluating the sampling bias in pattern of subterranean species richness: combining approaches. *Biodiversity and Conservation* **19**, 3035–3048, https://doi.org/10.1007/s10531-010-9873-2 (2010).

19. MacNeil, R. R. & Brcic, J. Coping with the subterranean environment: a thematic content analysis of the narratives of cave explorers. *Journal of Human Performance in Extreme Environments* **13**, Article 6, https://doi.org/10.7771/2327-2937.1089 (2017).

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

21. European Community. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. *Official Journal of the European Union* **L 206/7**, 1–44 (1992).

22. Mammola, S. *et al*. Scientists' warning on the conservation of subterranean ecosystems. *BioScience* **69**, 641–650, https://doi.org/10.1093/biosci/biz064 (2019).

23. Martel, A. *et al*. Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. *Science* **346**, 630, https://doi.org/10.1126/science.1258268 (2014).

24. Crovetto, F., Romano, A. & Salvidio, S. Comparison of two non-lethal methods for dietary studies in terrestrial salamanders. *Wildlife Research* **39**, 266–270, https://doi.org/10.1071/WR11103 (2012).

25. Ficetola, G. F. *et al*. N-mixture models reliably estimate the abundance of small vertebrates. *Scientific Reports* **8**, 10357, https://doi.org/10.1038/s41598-018-28432-8 (2018).

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

27. Miyazaki, Y. *et al*. Biological monitoring by citizens using Web-based photographic databases of fishes. *Biodiversity and Conservation* **23**, 2383–2391, https://doi.org/10.1007/s10531-014-0724-4 (2014).

28. Karanth, K. U., Kumar, N. S. & Vasudev, D. Photographic database informs management of conflict tigers. *Oryx* **48**, 481–485 (2014).

29. Charbonnier, L., van Meer, F., van der Laan, L. N., Viergever, M. A. & Smeets, P. A. M. Standardized food images: A photographing protocol and image database. *Appetite* **96**, 166–173, https://doi.org/10.1016/j.appet.2015.08.041 (2016).

30. Stevens, M., Párraga, C. A., Cuthill, I. C., Partridge, J. C. & Troscianko, T. S. Using digital photography to study animal coloration. *Biological Journal of the Linnean Society* **90**, 211–237 (2007).

31. Moussi, A. & Tlili, H. Photographic database of North African Acridomorpha (Orthoptera, Caelifera) type specimens deposited at NHM London. *Metaleptea* **40**, 14–15 (2020).

32. Lunghi, E. *et al*. Cave morphology, microclimate and abundance of five cave predators from the Monte Albo (Sardinia, Italy). *Biodiversity Data Journal* **8**, e48623, https://doi.org/10.3897/BDJ.8.e48623 (2020).

33. Wynne, J. J., Howarth, F. G., Sommer, S. & Dickson, B. G. Fifty years of cave arthropod sampling: techniques and best practices. *International Journal of Speleology* **48**, 33–48, https://doi.org/10.5038/1827-806X.48.1.2231 (2019).

34. Tulloch, A. I. T. *et al*. A decision tree for assessing the risks and benefits of publishing biodiversity data. *Nature Ecology & Evolution* **2**, 1209–1217, https://doi.org/10.1038/s41559-018-0608-1 (2018).

35. Lunghi, E. *et al*. Field-recorded data on the diet of six species of European *Hydromantes* cave salamanders. *Scientific Data* **5**, 180083, https://doi.org/10.1038/sdata.2018.83 (2018).

36. Lunghi, E., Zhao, Y., Sun, X. & Zhao, Y. Morphometrics of eight Chinese cavefish species. *Scientific Data* **6**, 233, https://doi.org/10.1038/s41597-019-0257-5 (2019).

37. Lunghi, E., Corti, C., Manenti, R. & Ficetola, G. F. Consider species specialism when publishing datasets. *Nature Ecology & Evolution* **3**, 319, https://doi.org/10.1038/s41559-019-0803-8 (2019).

38. Barke, R. J., Schofield, M. R., Link, W. A. & Sauer, J. R. On the reliability of N-mixture models for count data. *Biometrics*, 1–9, https://doi.org/10.1111/biom.12734 (2017).

39. Salvidio, S. Estimating abundance and biomass of *Speleomantes strinatii* (Caudata, Plethodontidae) population by temporal removal sampling. *Amphibia-Reptilia* **19**, 113–124 (1998).

40. Black, C. E., Mumby, H. S. & Henley, M. D. Mining morphometrics and age from past survey photographs. *Frontiers in Zoology* **16**, 14, https://doi.org/10.1186/s12983-019-0309-x (2019).

41. Ambrogio, A. & Mezzadri, S. *Geotritoni d'Italia - Cave Salamanders of Italy*. 64 (Gavia Edizioni, 2017).

42. Lunghi, E. *et al*. Melanism in European plethodontid salamanders. *Spixiana* **40**, 157–160 (2017).

43. Lunghi, E. *et al*. Cases of albinism and leucism in amphibians in Italy: new reports. *Natural History. Sciences (Atti della Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale di Milano)* **4**, 73–80, https://doi.org/10.4081/nhs.2017.311 (2017).

44. Jareonruen, Y. & Tuamsuk, K. Lifecycle and requirements for digital collection management of Thai theses and dissertations. *Journal of Information Science Theory and Practice* **7**, 52–64, https://doi.org/10.1633/JISTaP.2019.7.3.5 (2019).

45. Marchionni, P. & Findlay, P. New models for open digital collections. *Insights* **30**, 44–50, https://doi.org/10.1629/uksg.375 (2017).

46. Pääkkönen, T. Crowdsourcing metrics of digital collections. *Liber Quarterly* **25**, 41–55, https://doi.org/10.18352/lq.10090 (2015).

47. Erolin, C., Jarron, M. & Csetenyi, L. J. Zoology 3D: Creating a digital collection of specimens from the D'Arcy Thompson Zoology Museum. *Digital Applications in Archaeology and Cultural Heritage* **7**, 51–55, https://doi.org/10.1016/j.daach.2017.11.002 (2017).

48. Lunghi, E., Guillaume, O., Blaimont, P. & Manenti, R. The first ecological study on the oldest allochthonous population of European cave salamanders (*Hydromantes* sp.). *Amphibia-Reptilia* **39**, 113–119, https://doi.org/10.1163/15685381-00003137 (2018).

49. Ficetola, G. F., Lunghi, E. & Manenti, R. Microhabitat analyses support relationships between niche breadth and range size when spatial autocorrelation is strong. *Ecography* **43**, 1–11, https://doi.org/10.1111/ecog.04798 (2020).

50. Lunghi, E. *et al*. Photographic database of the European cave salamanders, genus *Hydromantes* (Amphibia: Caudata). *figshare* https://doi.org/10.6084/m9.figshare.c.4876788 (2020).

51. Lunghi, E. *et al*. 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 (2016).

52. MacCoun, R. & Perlmutter, S. Hide results to seek the truth. *Nature* **526**, 187–189, https://doi.org/10.1038/526187a (2015).

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

54. Lanza, B., Caputo, V., Nascetti, G. & Bullini, L. Morphologic and genetic studies of the European plethodontid salamanders: taxonomic inferences (genus *Hydromantes*). *Monografie del Museo Regionale di Scienze Naturali, Torino* **16**, 1–366 (1995).

55. Baken, E. K., Mellenthin, L. E. & Adams, D. C. Macroevolution of desiccation-related morphology in plethodontid salamanders as inferred from a novel surface area to volume ratio estimation approach. *Evolution* **74**, 476–486, https://doi.org/10.1111/evo.13898 (2020).

56. Ficetola, G. F. *et al*. Morphological variation in salamanders and their potential response to climate change. *Global Change Biology* **22**, 2013–2024, https://doi.org/10.1111/gcb.13255 (2016).

57. Weller, H. I. & Westneat, M. W. Quantitative color profiling of digital images with earth mover's distance using the R package colordistance. *PeerJ* **7**, e6398, https://doi.org/10.7717/peerj.6398 (2019).

58. Adams, D., Collyer, M. & Kaliontzopoulou, A. geomorph. Geometric Morphometric Analyses of 2D/3D Landmark Data. *R package version 3.2.1*, https://github.com/geomorphR/geomorph (2020).
59. Bingham, R. E., Papenfuss, T. J., Lindstrand, L. I. & Wake, D. B. Phylogeography and species boundaries in the *Hydromantes shastae* complex, with description of two new species (Amphibia; Caudata; Plethodontidae). *Bulletin of the Museum of Comparative Zoology* **161**, 403–427 (2018).
60. Lunghi, E. *et al.* On the stability of the dorsal pattern of European cave salamanders (genus *Hydromantes*). *Herpetozoa* **32**, 249–253, https://doi.org/10.3897/herpetozoa.32.e39030 (2019).
61. Treilibs, C. E., Pavey, C. R., Hutchinson, M. N. & Bull, C. M. Photographic identification of individuals of a free-ranging, small terrestrial vertebrate. *Ecology and Evolution* **6**, 800–809, https://doi.org/10.1002/ece3.1883 (2016).
62. Sharifi, M., Naderi, B. & Hashemi, R. 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**, 264–270 (2013).
63. Allen-Blevins, C. R., You, X., Hinde, K. & Sela, D. A. Handling stress may confound murine gut microbiota studies. *PeerJ* **5**, e2876, https://doi.org/10.7717/peerj.2876 (2017).
64. Samimi, A. S., Tajik, J., Jarakani, S. & Shojaeepour, S. Evaluation of a five-minute resting period following handling stress on electrocardiogram parameters and cardiac rhythm in sheep. *Veterinary Science Development* **6**, 6481, https://doi.org/10.4081/vsd.2016.6481 (2016).

Acknowledgements

Author contributions

E.L. and S.G. collected the data, processed the pictures and prepared the figures; E.L. drafted the manuscript and prepared dataset and table; all authors revised the ms and accepted its final version.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to S.G. or Y.Z.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.