INTRODUCTION

Earth is experiencing the sixth mass extinction (Barnosky et al., 2011), an event widely exacerbated by human actions (Ceballos et al., 2015). Amphibians are among the most affected species as they often show the combined effects of high sensitivity to environmental alteration and low dispersal ability (Beebee & Griffiths, 2005; Catenazzi, 2015). Pathogenic fungi of the genus *Batrachochytrium* (*B. dendrobatidis* and *B. salamandrivorans*) are a particularly serious threat to amphibians, as they can wipe out entire populations (Garner et al., 2006; Martel et al., 2014). Many countries are now adopting rigorous policies to protect biodiversity (European Community, 1992), and researchers are developing survey techniques to minimize their impact on study species (Ficetola, Barzaghi, et al., 2018; Ficetola, Manenti, & Taberlet, 2019; Sharifi, Naderi, & Hashemi, 2013).

Handling wild animals does not only facilitate transfer of pathogens (Garner et al., 2006; Martel et al., 2014) but induces stress, provoking a cascade of negative effects on their immune system...
The most common method used to measure salamanders is snout–vent length (SVL), a method that requires handling individuals. This measurement is taken ventrally from the snout tip to the posterior opening of the cloaca (Bingham, Papenfuss, Lindstrand, & Wake, 2018). SVL aids in distinguishing life stage and size in salamanders; thus, SVL can be used to differentiate adults from juveniles by using the smallest sexually mature adult as a reference (Lanza, Pastorelli, Laghi, & Cimmaruta, 2006). This method is particularly useful for differentiating between life stages of plethodontid salamanders with direct development because adults and juveniles exhibit few other morphological differences (Wells, 2007). Generally, accurately measuring individuals in the field is challenging and aiming for high measurement precision usually leads to a high error rate because individuals continuously squirm when handled (Luiseili, 2005; Setser, 2007; Zweig, Mazzotti, Rice, Brandt, & Abercrombie, 2004). Terrestrial plethodontids require high humidity and cool temperatures to respire efficiently (Ficetola, 2019; Mammola et al., 2019; Martel et al., 2014); therefore, specific authorizations are needed to conduct studies on these species. European Hydromantes, like most other plethodontids, are surface-dwelling species able to maintain stable populations in subterranean habitats where they can find a suitable and stable microclimate (Camp & Jensen, 2007; Lunghi, Manenti, & Ficetola, 2015) as well as a safe place to reproduce (Bradley & Eason, 2019; Lunghi et al., 2018). However, the intrinsic features of subterranean environments (e.g., narrow passages, air moisture near saturation) represent a natural challenge for researchers (MacNeil & Brcic, 2017), making it very difficult to collect data on subterranean populations. Using noninvasive methods, such as photographing individuals for measurements and identification, may not only limit negative effects incurred by wildlife, but may also alleviate the complexities of prolonged surveys performed in "nonhuman friendly" conditions that characterize subterranean and forest environments where plethodontids are found.

### 2 | MATERIALS AND METHODS

The European Hydromantes are comprised of eight threatened species distributed in Italy and France (Lanza et al., 2006). These salamanders are strictly protected by national and international laws (European Community, 1992; Rondinini, Battistoni, Peronace, & Teofili, 2013) because multiple threats, such as habitat degradation, climate change, spread of pathogens, and poaching, are negatively impacting their populations (Lunghi, Corti, Manenti, & Ficetola, 2019; Mammola et al., 2019; Martel et al., 2014); therefore, specific authorizations are needed to conduct studies on these species. European Hydromantes, like most other plethodontids, are surface-dwelling species able to maintain stable populations in subterranean habitats where they can find a suitable and stable microclimate (Camp & Jensen, 2007; Lunghi, Manenti, & Ficetola, 2015) as well as a safe place to reproduce (Bradley & Eason, 2019; Lunghi et al., 2018). However, the intrinsic features of subterranean environments (e.g., narrow passages, air moisture near saturation) represent a natural challenge for researchers (MacNeil & Brcic, 2017), making it very difficult to collect data on subterranean populations. Using noninvasive methods, such as photographing individuals for measurements and identification, may not only limit negative effects incurred by wildlife, but may also alleviate the complexities of prolonged surveys performed in "nonhuman friendly" conditions that characterize subterranean and forest environments where plethodontids are found.

In a previous study, Lunghi et al. (2020) produced a photographic database of European Hydromantes, providing high-quality pictures of the dorsal pattern for more than 1,000 individuals of all eight species. The authors photographed salamanders in situ placing them in a white soft box to obtain high-quality photograph (Lunghi et al., 2020). During this study, 22 individuals (~3 per species) were randomly selected and photographed ventrally (Figure 1a). Before photographing the salamanders, Lunghi et al. (2020) held them for 30 s in hand to increase their body temperature, which caused a short thermal shock that made them calm for a few seconds (Lunghi et al., 2020).
These digital images were used to measure the 22 salamanders using the program ImageJ (Figure 1a). Dorsal and ventral images of each individual were paired. We measured SVL of each individual using images from the ventral view and used it as reference of a “true” measure (Margenau et al., 2018). Then, we asked 31 volunteers with different backgrounds (students or experienced herpetologists) to use ImageJ and estimate the SVLe from the dorsal view (Figure 1b). Measures of SVL and SVLe were taken to the nearest mm. All participants were given a manual that explained the measuring process, particularly how to recognize the diagnostic characters indicating the position of *Hydromantes*’ cloaca from the dorsal view, herein described. Located ventrally in the area between the hind legs and the tail is the cloaca (Figure 1a), which is the landmark used to measure SVL. Dorsally, this area has a conical shape that narrows from the hind legs to the tail base (Figure 2a). In some individuals, this area shows lateral “folds,” of which the third from the hind limb roughly corresponds to the tail base (Figure 2b), and thus the posterior end of the cloaca used in SVL. Photographs were provided without any additional information to ensure unbiased measurements (MacCoun & Perlmutter, 2015).

We used linear mixed models (LMMs) (function *lme* of the R package nlme; Pinheiro, Bates, DebRoy, Sarkar, & Team, 2016; R Development Core Team, 2019) to evaluate the relationship between SVL and SVLe in European *Hydromantes*. The salamanders’ SVLe was used as the dependent variable and the SVL as the independent variable. The experience of operators (yes/no), together with the identity of operators and salamanders, and species, were assigned as random factors. The correlation coefficient between SVLe and SVL was high (Figure 2a) and the SVL e was on average 2.69 mm (±0.08 SE) smaller than SVL. We found a weak, although significant correlation between MSE and salamanders’ SVL ($F_{1,464} = 115.78$, $p < .001$; $R^2_m = 0.14$) with a regression slope of 0.008 (95% CI 0.007–0.001), while no effect of operator experience was detected ($F_{1,29} = 1.83$, $p = .186$); the MSE was slightly higher in large salamanders (Figure 2b). For each salamander, there was an overall deviation from the average SVL e of only 1.6 mm (±0.03), corresponding to 2.7% of the average salamanders’ SVLe.

**FIGURE 2** Diagnostic characters indicating where the trunk of *Hydromantes* ends. (a) The conical end of the salamander’s body indicates the base of the tail; a natural “line” helps in identifying this point (*H. flavus*, individual 1074390). (b) In some cases, a few folds are visible behind the hind limbs; the posterior end of the cloaca roughly corresponds to the third fold (*H. genei*, individual 1033580)

**RESULTS**

The correlation between salamander SVLe and SVL was high ($F_{1,464} = 3,154.56$, $p < .001$; $R^2_m = .81$) with a regression slope of 0.87 (95% CI 0.83–0.90) (Figure 3a). The MSE was significantly different from zero (one-way t test, $t = 19.719$, $df = 681$, $p < .001$; 95% CI 0.102–0.124) and the SVL e was on average 2.69 mm (±0.08 SE) smaller than SVL. We found a weak, although significant correlation between MSE and salamanders’ SVL ($F_{1,464} = 115.78$, $p < .001$; $R^2_m = 0.14$) with a regression slope of 0.008 (95% CI 0.007–0.001), while no effect of operator experience was detected ($F_{1,29} = 1.83$, $p = .186$); the MSE was slightly higher in large salamanders (Figure 3b). For each salamander, there was an overall deviation from the average SVL e of only 1.6 mm (±0.03), corresponding to 2.7% of the average salamanders’ SVLe.

**DISCUSSION**

Besides the reliable estimation of the “true” SVL, the use of SVLe shows some noteworthy advantages. For example, the time available to measure SVLe from pictures is virtually endless, whereas data collection in the field is more time restrictive. As mentioned above, measuring SVL in the field is difficult and may be prone to increased error rate (Guo, Chen, Zhang, Pan, & Wu, 2016; Luiselli, 2005; Setser, 2007). Once a salamander has been released, SVL cannot be measured again, so unnoticed errors that occurred while measuring will become part of the dataset (Brown et al., 2018). Furthermore, prolonged handling stress produces negative effects on animal health (Allen-Blevins et al., 2017; Billey & Woodley, 2012; Caipang et al., 2014; Lunghi et al., 2016). Therefore, photographing salamanders and the post hoc measurement of SVLe facilitates easier measurements, requires shorter handling time with images of sufficient quality (Bradley, 2018; Lunghi et al., 2020), and is a method available to a wide number of operators (Margenau et al., 2018; Miyazaki et al., 2014). Nonetheless, such approach is particularly suitable for aquatic species, as operator can easily take measurements of individuals without removing them from water (Gutierrez, Guess, & Pierce, 2018).

The repeated SVLe measurements performed on each salamander provided information on the robustness of this method. An error of 1.6 mm occurring when multiple operators measure SVLe of each salamander (corresponding to 2.7% of the salamander “true” SVL) is likely lower than what can be obtained from a direct measurement of SVL for plethodontids in the field. Field measures of SVL (with no anesthetics) for snakes resulted in an average error of 2.5%
Error among operators in measuring SVL was about 1.5% for alligators (Zweig et al., 2004). In both cases, the size of measured animals was 8–30 times larger than our salamanders (on average ~62 mm); this highlights the ability of this approach to provide highly precise measurements for smaller animals. We observed that the MSE slightly increases with salamander size (Figure 3b). It may be possible that measurement inequality exists between SVL and SVLe, and such error proportionally increases with size (Hayek & Heyer, 2005).

Besides the multiple advantages of the proposed approach, a few general challenges should be noted. The main challenge is to obtain a picture of suitable quality to allow for the post hoc analysis. Artificial lighting is fundamental to highlight body details, and poor illumination can produce useless images (Lunghi, Romeo, et al., 2019). Photographs shot perpendicular to the longitudinal axis of the salamander increases the precision of measurements. *Hydromantes* are usually found under logs and stones or over complex surfaces (i.e., cave walls), and thus, their placement in a standardized area (i.e., flat surface allowing a perpendicular photograph) was crucial to completely flatten the animal and photograph from the proper angle (Lunghi et al., 2020). Nevertheless, some errors may also occur when measuring individuals from pictures. Indeed, particular attention should be paid during the setting of picture scale and when choosing start/end points, as multiple potential errors can accrue during this process.

**5 | CONCLUSIONS**

We provided evidence of the reliability of the estimation of SVL from the dorsal view in *Hydromantes* salamanders in a noninvasive manner. We also reported all positive aspects that justify the best trade-off between quality of data and disturbance caused to wild animals. Besides the reliability of measurements obtained with this approach, problems inherent to prolonged field activities may be alleviated and researchers can verify all measurements to spot any potential error.

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**CONFLICT OF INTERESTS**

None declared.
AUTHOR CONTRIBUTION
Enrico Lunghi: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Project administration (lead); Visualization (lead); Writing—original draft (lead); Writing—review & editing (lead). Simone Giachello: Investigation (supporting); Writing—review & editing (supporting). Raoul Manenti: Investigation (supporting); Writing—review & editing (supporting). Yahui Zhao: Investigation (supporting); Writing—review & editing (supporting). Claudia Corti: Investigation (supporting); Writing—review & editing (supporting). Gentile Francesco Ficetola: Investigation (supporting); Writing—review & editing (supporting). Joseph Gavin Bradley: Conceptualization (supporting); Investigation (supporting); Writing—review & editing (supporting).

DATA AVAILABILITY STATEMENT
Data used in this paper are provided as Appendix S1. To retrieve the original code of individuals: https://figshare.com/s/066225d0977ed93c1f6b.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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