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Non-invasive UV-A photo-identification for individual recognition in olms (genus *Proteus*)

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Abstract

The cave salamander *Proteus* represents a flagship and keystone genus of the subterranean ecosystems of the Dinaric Karst. Currently classified as "Vulnerable" by the International Union for Conservation of Nature, its long-term monitoring is essential for understanding subterranean ecosystem dynamics and for guiding evidence-based conservation strategies.
Given its protected status and the strict permitting requirements for research and monitoring, the development of non-invasive protocols is of paramount importance.

The present study aims to present a non-invasive, underwater-capable photographic tracking method for this highly vulnerable subterranean species that minimizes handling-related stress and potential impacts on animal welfare. Specifically, this work explores the application of UV photography as a novel tool for the detection and characterization of individual skin patterns, enabling the recognition of over 300 individual specimens based on unique and naturally occurring morphological features. By providing a reliable means of distinguishing individuals without the need for invasive or potentially stressful traditional marking techniques, such as physical tagging, UV-based imaging may represent a valuable alternative for the long-term monitoring of olm populations.

This approach contributes to the development of scientifically grounded, welfare-centered monitoring protocols that effectively balance conservation requirements with the highest standards of animal care while improving the accuracy, repeatability, and ethical sustainability of individual-based ecological studies. Importantly, although recent evidence has revealed the presence of at least two distinct *Proteus* species within the study area, the present imaging-based approach remains effective for the individual identification of specimens across both species. This demonstrates that the method captures sufficient inter-individual variation to reliably distinguish individuals, irrespective of their species identity, highlighting its robustness and broad applicability in sympatric *Proteus* spp. populations.

Key Words

365 nm UV light, 395 nm UV light, biofluorescence, cryptic species, individual recognition, olm, UV-A

Introduction

Over recent decades, the well-documented global decline of amphibians (Gibbons et al. 2000; Stuart et al. 2004; Luedtke et al. 2023) has shown no clear signs of abating. Effective monitoring and conservation of threatened species increasingly rely on methods for individual identification, which underpin a wide range of ecological and demographic studies, including growth analyses, population estimates, behavioral studies, and capture-recapture models (Otis et al. 1978; Royle and Nichols 2003; Bailey et al. 2004; Wisz et al. 2013).

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While marking and tracking techniques have substantially advanced ecological research and biodiversity conservation, they remain controversial due to their intrusiveness and impacts that are often insufficiently evaluated prior to application. Reliable but relatively unintrusive marking methods are required to observe populations in their natural habitat over long periods of time (Paulissen and Meyer 2000). Ideally, individual marks should be permanent, without influencing the animal's chance of survival due to changes in performance, behavior, or conspicuousness (Ricker 1956); be easy to apply and read; cause minimum pain or stress to the animal; and be applicable to animals of various sizes (Lewke and Stroud 1974). Ethical and scientific responsibility demands that handling procedures minimize, whenever possible, stress, injury, and potential pain, particularly in long-lived and ecologically specialized species such as the olm.

Proteus spp. are neotenic cave salamanders endemic to the Dinaric Karst and are widely regarded as a priority genus of exceptional conservation and scientific value. The genus is currently classified as "Vulnerable" by the International Union for Conservation of Nature and is listed in Annex II (requiring special conservation areas) and Annex IV (requiring strict protection of habitats and species) of the EU Habitats Directive (92/43/EEC) as *P. anguinus* s.l., and research and monitoring are conducted only under special permits issued by national authorities. As obligate stygobiotic vertebrates inhabiting oligotrophic, well-oxygenated groundwater systems in limestone and dolomitic formations, these salamanders are highly sensitive to environmental perturbations, including groundwater contamination, hydrological alterations, and disruption of aquifer connectivity (Sket 1997; Trontelj et al. 2009; Tesařová et al. 2022).

Recently, nine distinct species have been recognized. Among them, the Ljubljanica and Kras/Carso lineages, previously identified by Recknagel et al. (2023), are now recognized as *Proteus xanthostictus* (Freyer, 1847) or *P. haidingeri* (Fitzinger, 1850) and as *P. maurii* (Manenti, Barzaghi, Dufresnes & Jablonski, 2026) or *P. scopolii* (Trontelj, Gorički, Kostanjšek, Zakšek, Delić, Bressi, Mauri, Lukić Bilela, Jalžić & Recknagel, 2026), both inhabiting the Classical Karst in Italy (Dufresnes et al. 2026 and Trontelj et al. 2026, respectively). Toe-clipping (Ferner 2007) and the application of PIT tags (Whiteman et al. 2016; Ribas et al. 2022) are often used methods for tracking individual amphibians. However, these techniques might be unsuitable for the olm for several reasons, including the potential impact of invasive procedures on small, long-lived populations and the risk of inflammation and tissue damage. Furthermore, the effectiveness of PIT tags is species-specific, meaning that a method that performs well in one species may be ineffective or associated with higher risks in another. For example, PIT tag retention was reported to be 100% in fire salamanders (*Salamandra salamandra*) but only 33.3% in Pyrenean brook newts (*Calotriton asper*) (Ribas et al. 2022). To current knowledge, PIT tag retention has not yet been evaluated in the olm. Toe-clipping is also particularly problematic in this species because, as a paedomorphic salamander, the olm has a high capacity for digit regeneration, leading to misidentification and rendering the procedure unsuitable for long-term demographic or capture-mark-recapture (CMR) studies (Carpaneto et al. 2004).

Among the methods available for individual identification in amphibians and other taxa, Visible Implant Elastomer (VIE) tagging is one of the most widely used in salamanders (Phillips and Fries 2009; Moon et al. 2022). The technique involves the subcutaneous injection of a silicone-based polymer that cures *in situ*, creating a long-lasting individual mark. Owing to its low cost and rapid application, VIE tagging has been extensively employed across a wide range of taxa, including amphibians, reptiles, crustaceans, cephalopods, gastropods, arachnids, and small mammals (Phillips and Fries 2009; Fouilloux et al. 2020; Knapp et al. 2023). However, tag retention and readability may vary among species and life stages, and potential tag migration has been reported in some amphibians (Brannelly et al. 2013; Cabot et al. 2021).

In olm research, VIE is currently the most commonly applied marking technique (Balázs et al. 2015, 2020; Barzaghi et al. 2026). Balázs et al. (2015) were the first to apply VIE tags to *Proteus* sp. *in situ*, using cave divers to mark individuals directly underwater without removing them from their habitat. Nevertheless, such specialized expertise is not always available, and VIE tagging generally requires capture, handling, and injection procedures, often involving the temporary removal of olms from the aquatic environment (Barzaghi et al. 2026).

Currently, UV photography or filming is applied to several taxa, even those largely unrelated to one another. Mammals, such as the eastern mole (*Scalopus aquaticus*) and various flying squirrels and bats, exhibit bright, species-specific or individual-specific pink, green, or yellow fluorescence on their fur when exposed to 365–395 nm UV light (Reinhold 2023; Roberson et al. 2025).

Studies have found that 37 out of 45 bird-of-paradise species exhibit biofluorescence in their feathers. Specific areas, such as the beaks of Atlantic puffins (Dunning et al. 2018) or the facial feathers of budgerigars (*Melopsittacus undulatus*) (Pearn et al. 2001), glow under UV, offering potential personalized identification markers. The bony casques (head ornaments) of cassowaries show unique biofluorescent patterns that differ between species (e.g., *Casuarius bennetti*, *C. casuarius*) (Green et al. 2026). Sea turtles, such as the hawksbill, have also been documented exhibiting red and green fluorescence. Fishes have also been studied for their individual-specific UV-reflecting facial patterns, as in the case of the Ambon damselfish display complex, which researchers believe are used for recognizing neighbors or species members (Partridge and Cuthill 2010).

For invertebrates, UV light is often used by taxonomists to visualize specific fluorescent patterns on the scales (elytra) of polychaete worms (*Harmothoe impar*), and UV light also aids in detecting otherwise invisible invertebrates, such as mites on a brittlestar (Koken and Grall 2011).

The patterns revealed under different wavelengths have been shown to be highly effective in distinguishing natural markings and morphological features, thereby enabling the identification of intraspecific variation within a population. The recognition method used in this study exploits the biofluorescence of proteids as a distinctive feature, enabling their identification based on the emitted optical signal. This approach is supported by previous observations of biofluorescence in amphibians, with Lamb and Davis (2020) documenting this phenomenon in several species of salamanders and newts, highlighting its broader occurrence within this group.

As part of an ongoing monitoring program of the *Proteus* spp. populations in the Classical Karst of Italy, approximately 300 individual olms have so far been photographed under visible light, infrared light, and ultraviolet light at two different wavelengths. The aim is to enable reliable individual identification without the need for more invasive marking techniques. The present study proposes this UV-A imaging-based approach as a reliable method for the individual identification of specimens belonging to at least two *Proteus* species. Its virtually non-invasive nature, cost-effectiveness, and ease of application make it particularly suitable for research groups, whereas its effectiveness in enabling the individual identification of animals from both sympatric species demonstrates its broad applicability within the study area. Moreover, the method does not require highly specialized equipment or substantial financial investment, making it an accessible and practical approach for long-term monitoring and conservation programs. Importantly, although recent evidence (Recknagel et al. 2023; Dufresnes et al. 2026) has revealed the presence of at least two distinct *Proteus* species within the study area, the present imaging-based approach remains effective for the individual identification of specimens across both species. This demonstrates that the method captures sufficient inter-individual variation to reliably distinguish individuals, irrespective of their species identity, highlighting its robustness and broad applicability in sympatric *Proteus* populations.

Methods

Area of study and olms sampling

Three hundred and three olms collected from nine different caves in the Italian Classical Karst (Trieste and Gorizia provinces, Italy) (Fig. 1, Table 1) were subjected to morphological measurements (total length and body weight) and photographed under visible light (Kikko lamp, 1300 lumen, 4000 K), UV-A light (365 and 395 nm), and infrared light (850 nm). All the light sources employed operate at commercially available wavelengths and are safe for operator use, requiring no special safety precautions under normal operating conditions.

Figure 1. Map of the study area. The provinces of Trieste and Gorizia are highlighted in yellow, while the Classical Karst, which extends beyond the Italian border, is shown in shades of red. Blue lines represent rivers. The principal underground flow path of the Reka–Timavo River is indicated from A to B, and dotted blue arrows schematically illustrate diffuse groundwater inflows from the surrounding karst aquifer.

Table 1. Sampled caves where olms were recorded, alongside their unique National and Regional Cadastral Number (N/RCN) and the total number of olms recorded.

Cave	National/Regional Cadastral Number	Number of specimens recorded
Trebiciano	3/17VG	198
Luftloch	7477/6442VG	30
Abisso di Repen	906/4035VG	6
Pozzo della Ferrovia	214/226VG	17
Grotta del Proteo	1191/4112VG	9
Pozzo di Vermegliano	4306/5386VG	28
Cavernetta presso Comarie	1287/4221VG	2
Pozzo presso Jamiano	273/360VG	11
Grotta Andrea	2391/4804VG	2

Sampling was conducted as part of an ongoing long-term monitoring program covering the target area, which started on 1 August 2025 and is still continuing. In addition, a small portion of the tail fin was collected from each individual to obtain high-quality DNA for subsequent genetic analyses (Manfrin et al. in revision).

Equipment

The required equipment included a mobile phone or camera, a headlamp, already part of the standard equipment necessary for cave exploration, UV-A torches (365 and 395 nm), and an IR-light torch. The simplest camera apparatus used to acquire olm pictures was a 12 MP camera on a mobile phone (Samsung Galaxy S21), and the most professional camera used was an OM-5 Mark II with an M.Zuiko 12-45/4 Pro 20.4 MP lens (OM System). Pictures were collected in RAW or JPG formats, based on the apparatus available during each field activity.

Procedure

Repeated visits to the same caves throughout the monitoring campaign enabled the identification of individual olms and the documentation of recapture events, with a total field effort of 77 days. Following morphometric measurements, exposure to UV-A light was kept to a minimum, with less than 30 s required per individual to acquire multiple photographs of the dorsal side. Upon first capture, each olm underwent tail clipping for genetic analyses (Manfrin et al. in revision). Individual identification was based on the distinctive skin pigmentation patterns visible under UV illumination rather than on tail clipping. Particular attention was paid to the head and dorsal body regions, where patterns were assessed based on the presence, position, and configuration of distinctive markings. Each olm was photographed under each light condition, with a minimum of three and up to 10 images per condition. Photographs were subsequently organized into folders corresponding to identified individuals. For each specimen, photographs were systematically compared with the complete reference collection of previously identified individuals, displayed simultaneously on separate screens. Thus, each newly captured individual was assessed against all previously identified specimens rather than against a subset of individuals. Identification involved the assessment of both concordant and discordant patterns to determine whether the newly captured individual matched a previously identified specimen. Individuals captured only once also exhibited highly distinctive and readily discernible patterns that allowed them to be distinguished from other individuals sampled at the same cave. To ensure the reliability and independence of the identification process, each photographic comparison was carried out independently and blindly by at least two observers, each working without knowledge of the other's assignment. At least one observer had participated in the field sampling, whereas the other had not and was therefore unaware of tail-clipping information. The latter assessments were based exclusively on photographic records, together with information on the capture site and morphometric measurements (e.g., body length and weight) of previously recorded and newly captured specimens. During fieldwork, samples were labeled only with progressive numbers and were not assigned individual IDs. Individual IDs were subsequently assigned through photographic analysis. Consequently, information on tail clipping available to field observers was not used to assign individual identities and could only be compared retrospectively with photo-based identifications, providing an independent posterior validation of the UV-based identification method. An individual was classified as a recapture only when its photographic characteristics were considered consistent with those of a previously identified individual by at least two independent observers. Tail clipping was therefore not used as a primary criterion for individual identification but rather as a secondary mark assessed retrospectively to avoid false rejection, i.e., failure to recognize a previously captured individual. Although the tail can regenerate, the regenerated portion remains distinguishable from the previously clipped portion because of its different pigmentation (see Suppl. material 1: supplementary information 3), providing an additional indication of previous capture and further reducing the risk of false rejection.

Results

Individual recognition

To date, 303 olms have been captured during 77 days of fieldwork across the provinces of Trieste and Gorizia, within the Italian Classical Karst, accounting for a total of 350 capture events. All individuals were photographed and photo-identified before being released. To assess whether different wavelengths could enhance the visibility of individual morphological features, images

of each specimen were acquired under different wavelengths (Fig. 2). No differences were observed between images obtained using a smartphone and those acquired with a professional camera or between images saved in RAW and JPG formats, indicating that professional photographic equipment is not required for reliable individual identification. Both in the field and during subsequent laboratory examination, UV-A photography proved to be particularly effective for recognizing olm individuals irrespective of the species identity of the two species occurring in the study area.

Specifically, visible light allowed the recognition of amputated appendages, hematomas on the body, and other morphological malformations. Among the tested wavelengths, infrared (IR) illumination was the least effective and did not enhance the detection of individual body patterns or improve identification success (Fig. 2B). By contrast, photographs taken under 365 nm UV illumination revealed unique epidermal fluorescence patterns that were consistently present in each specimen (Fig. 2C). Illumination at 395 nm further highlighted additional dermal morphological features (Fig. 2D), including greenish spots, which are described in more detail in Fig. 4. The short exposure time required for UV-A imaging (less than 30 s) minimizes stress and UV-related impacts on the animals while ensuring the acquisition of high-quality images suitable for individual recognition.

Comparison of UV pictures allowed the recognition of individual-specific body signatures, enabling reliable individual photo-identification of all captured olms. Consequently, long-term monitoring is being conducted exclusively using visible-light and 365/395 nm UV-light photography.

Body signatures recorded under 365 nm and 395 nm UV light

At 365 nm, the highest fluorescence was observed, highlighting body signatures that were very useful for individual recognition (Figs 3, 5).

Moreover, using 395 nm UV light, it was possible to define additional spots that were less abundant but characteristic for individual recognition (Fig. 4).

Regardless of species, the non-uniform distribution of these fluorescent compounds across the skin surface generates stable spot patterns that appear to be unique to each specimen (Fig. 5).

No detectable shifts in the position, shape, or arrangement of the fluorescent spots were observed, allowing the unequivocal recognition, as exemplified in Fig. 6, of an individual first captured on 17 August 2025 and subsequently recaptured on 25 February 2026 (A and B, respectively). Additional pictures are available in Suppl. material 1: supplementary information 1.

During the ongoing monitoring, the longest time elapsed between capture and recapture was approximately 12 months. No modification in skin pattern was observed under 365 nm UV illumination, even in juveniles (Fig. 6C, D). The latter specimen weighed 3.20 g and had a total length of 12.00 cm and reached a weight of 3.60 g and a total length of 12.12 cm one year later. The smallest olm identified so far weighed 2.60 g and had a total length of 11 cm.

To date, a total of 303 olms have been documented, totaling 350 captures, across nine caves (Table 1). The highest recapture rates were observed in Pozzo presso Jamiano

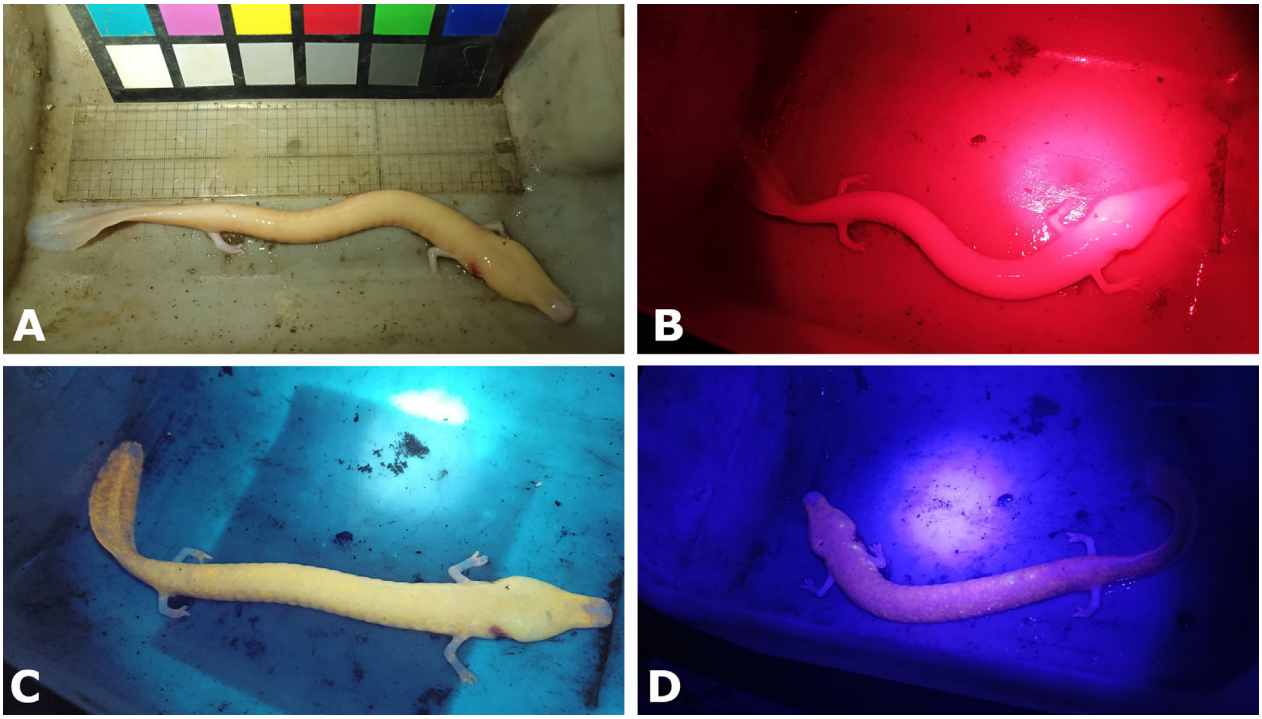

Figure 2. Same olm pictured under different wavelengths: **A.** Visible white light; **B.** Infrared light; **C.** 365 nm UV light; and **D.** 395 nm UV light. Photo credit: Marco Restaino.

Figure 3. Details of skin pigments observed under 365 nm UV light in an olm. Other fluorescent materials in the tank are fibers present in the water. Photo credit: Chiara Manfrin.

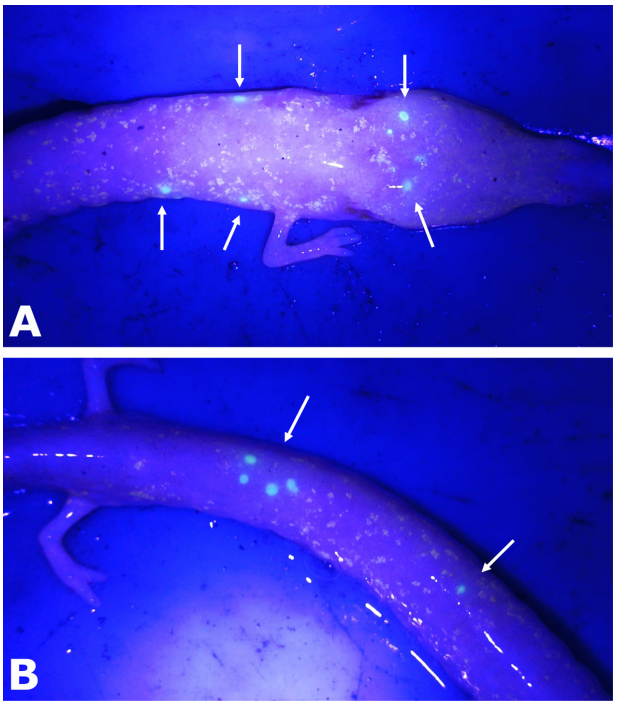

Figure 4. Olm skin details observable under 395 nm UV light (white arrows). **A.** Dorsal view of the head and anterior trunk; **B.** Spots present in the posterior trunk. Photo credit: Marco Restaino.

(273/360VG), where 64% of documented individuals were recaptured, and in Pozzo della Ferrovia (214/226VG), where 53% of sampled olms were recaptured. Some individuals at both sites were recaptured on three or four separate occasions.

Over the course of a year of monitoring, none of the recaptured specimens showed any changes or reductions in their skin markings, whether juveniles or adults (Fig. 6). This result suggests that the proposed method can be reliably applied over longer time periods than those tested to date. Studies aimed at verifying this hypothesis are still ongoing. Moreover, the technique does not necessarily require direct handling of the amphibian, since the olm is collected by net, placed in a box for documentation, and then released. To further reduce handling stress, photographs were also obtained while animals remained submerged in the water. Successful identification using skin markers was also possible under underwater conditions, demonstrating that the fluorescent epidermal signatures can be reliably detected without removing specimens from the water (Fig. 7).

Comparison of UV-A photography and VIE tagging

The UV-picture-based method is sustainable in comparison with the costs of VIE tagging (Table 2). Based on an estimate for a project marking 1,000 olms:

VIE tagging: (i) initial kit: €245–1,138 (US$280–1,300); (ii) additional elastomer may be needed depending on the number of marks/colors; and (iii) significant labor for injection.

UV photography: (i) UV flashlight: €15–150 (US$17–169); (ii) digital camera or smartphone (often already available); (iii) essentially no consumable costs; and (iv) faster processing in the field.

Overall, both methods enable reliable long-term individual recognition. However, UV-photo identification offers several practical advantages: it is non-invasive, requires minimal handling, is suitable for small individuals, involves no consumables, has negligible per-animal costs, and can be applied to all captured specimens.

Discussion

In this study, the reliability of UV-A-assisted photographic identification as a non-invasive method for recognizing a large set of *Proteus* spp. was evaluated, with 303 olms individually recognized. Because the olm is a blind cave salamander adapted to absolute darkness, limited and very brief (<30 s) UV-A light exposure was maintained within a strict time limit, and no behavioral flight responses were observed.

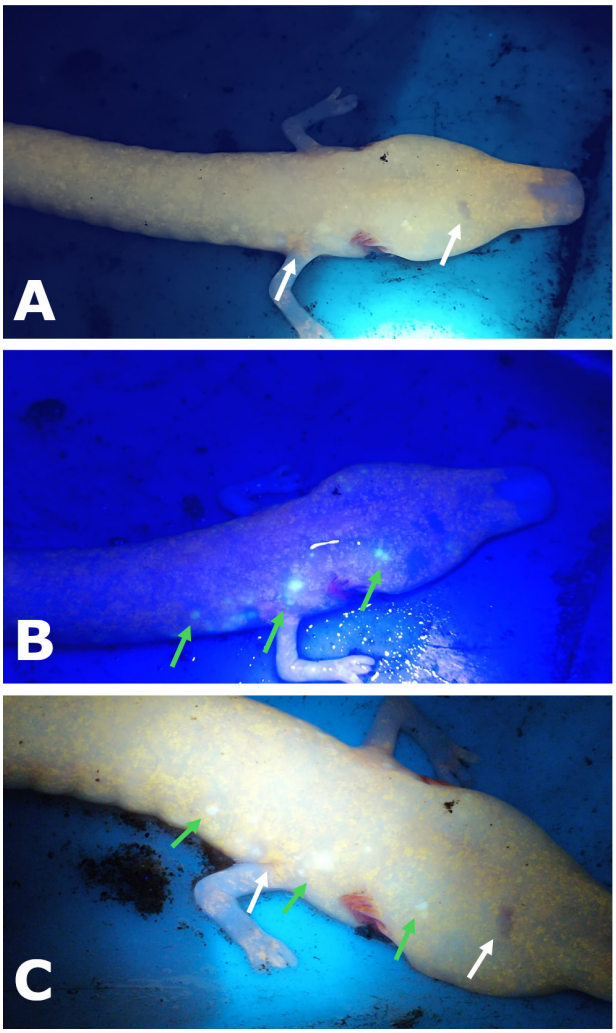

Figure 5. Pictures of the same olm. **A.** 365 nm UV light. White arrows indicate hypopigmentation (head) and hyperpigmentation (limb insertion); **B.** 395 nm UV light. Green arrows indicate specific greenish spots; **C.** Mixed UV-A light with both individual markers shown with corresponding-colored arrows. Photo credit: Marco Restaino.

The approach requires no animal marking or handling beyond routine morpho-photographic documentation and relies on naturally occurring fluorescent skin patterns visible under ultraviolet illumination. Similar fluorescence- or pattern-based approaches have been successfully applied in other amphibians and wildlife species, where naturally occurring skin or integument patterns provide stable individual identifiers (Sacchi et al. 2010; Bendik et al. 2013).

All recaptured olms were successfully identified through comparison of their UV fluorescence patterns, demonstrating that these markings provide sufficient variation for reliable individual recognition. Across 303 examined individuals distributed over nine hypogean sites, no two specimens exhibited the same arrangement of fluorescent spots, suggesting that these patterns function as natural "fingerprints." The method was also effective in juveniles and subadults, with distinctive patterns clearly visible in individuals approximately 10 cm in total length. This represents an important advantage over conventional marking techniques such as Visible Implant Elastomer (VIE) tags or Passive Integrated Transponder (PIT) tags, which become increasingly invasive or technically challenging in small-bodied animals.

The fluorescent markings are likely associated with riboflavin-like compounds naturally present in the skin of *Proteus* (Istenič and Ziegler 1974). Riboflavin and related flavins are characterized by strong yellow-green fluorescence when excited by ultraviolet light. However, the precise biochemical basis of the observed patterns remains unknown. Because skin sampling was beyond the scope of this study and would require specific authorization, the chemical composition of the fluorescent spots was not investigated. Future histological and biochemical studies will be necessary to identify the molecules responsible for the observed fluorescence and to determine the mechanisms underlying their spatial distribution.

From a practical perspective, UV-A-assisted photographic identification offers several advantages over traditional marking techniques. The method requires only portable UV light sources and a digital camera or smartphone, eliminating the need for consumable marking materials, specialized equipment, or artificial identification codes. Following the initial equipment purchase, the per-animal cost is essentially negligible, making the approach particularly attractive for long-term monitoring programs involving repeated surveys.

Importantly, the method removes the need for invasive marking procedures when the objective is solely individual identification. Although tissue sampling remains essential for studies requiring high-quality DNA or RNA, including population genomics, transcriptomics, and parentage analyses, it is unnecessary for routine identification because naturally occurring fluorescence patterns provide sufficient discriminatory power. Consequently, UV-assisted photographic identification complements rather than replaces molecular sampling by restricting invasive procedures to studies in which they are scientifically justified.

The method also proved practical for relatively large datasets. To date, this monitoring campaign represents the largest sampling ever conducted on olm populations in their natural distributional range, to current knowledge (Holtze et al. 2017 sampled 13 olms; Balázs et al. 2020 monitored 19 adults; and Barzaghi et al. 2026 collected 76 olms). Three hundred and three olms were photographed under UV-A light, of which approximately 200 individuals from a single cave were successfully distinguished using manual photographic comparisons. Given that many documented *Proteus* populations consist of only a few dozen observed individuals per site, this level of performance suggests that manual identification is feasible for routine monitoring.

Individual recognition was performed by comparing photographs from each capture event with all previously collected images, taking into account the overall skin

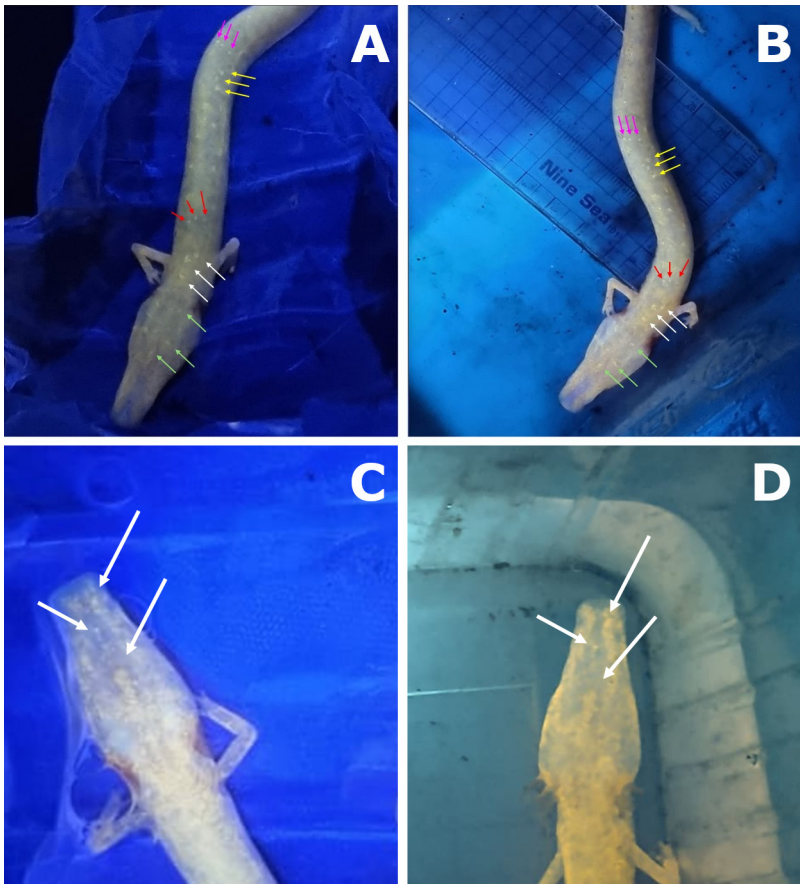

Figure 6. 365 nm UV-light images of the same olm taken on 17 August 2025 (**A**) and 25 February 2026 (**B**). Green arrows indicate individual spots on the head, white and red arrows indicate specific spot clusters on the anterior trunk, and yellow and purple arrows indicate spot clusters on the middle trunk. Juxtaposed images of the juvenile head of another specimen, showing the same individual at its first capture (**C**) and more than one year later (**D**), with arrows indicating persistent individual markers. Photo credit: Chiara Manfrin.

Figure 7. Olm pictured underwater using 395 nm UV light. Open gills are visible, indicating that the olm is in the water. Photo credit: Marco Restaino.

pattern rather than relying on a single mark. The tail clip was used as a secondary identification mark to minimize the risk of false rejections and to validate the method proposed in the present study; however, it is not required for the application of the proposed method. The creation of standardized photographic catalogs ("photo passports") facilitates rapid field identification and long-term monitoring. Although manual comparison was effective in the present study, future developments could further improve efficiency. Machine-learning and artificial intelligence approaches are currently being developed by the research group to automate the recognition of olm UV fluorescence patterns, and this approach has already been used to enable rapid matching among thousands of images while reducing observer bias (Takaya et al. 2023; Schulte et al. 2024; Dehaut et al. 2026; Rahmdel et al. 2026). Such tools will be particularly valuable for large-scale monitoring programs, and while AI will optimize future large-scale projects, the current manual "photo passport" matrix is highly reliable and readily accessible for standard population sizes without specialized computational knowledge.

With growing attention to animal welfare, recent studies increasingly highlight the value of photo-identification as a reliable and less invasive alternative to artificial tagging. In particular, photo-identification can be effectively applied to juvenile frogs (Nemesházi et al. 2026) and, when supported by automated software, can facilitate the management of large datasets, making it preferable to more invasive methods such as PIT tagging and VIE tagging whenever feasible (Dehaut et al. 2026). Although ultraviolet illumination has been successfully used to document biofluorescence in amphibians, including salamanders, previous studies have primarily focused on fluorescence patterns rather than on the potential physiological or welfare-related effects of UV exposure (Lamb and Davis 2020). To the best of the authors'

Table 2. Cost and operational comparison between VIE elastomer tagging and UV torch-based photographic identification for individual recognition. An explanation of the calculation of identification efficiency is provided in Suppl. material 1: supplementary information 2.

Component	VIE-tagging	UV-photo identification	Conclusion
Identification efficiency	61 VIE-tagged olms = 84.7% (n = 72); four too small to tag, seven not tagged (Barzaghi et al. 2026)	All 303 olms were photographed and individually identified = 100% (n = 303; present study)	Both methods enable individual recognition, but UV-photo identification can be applied to all captured individuals
Estimated lifetime of the mark	≥ 4 years (Balázs et al. 2015)	≥ 1 year demonstrated; potentially persistent over the animal's lifetime	Both methods have potential for long-term monitoring
Minimum individual weight	5 g (Barzaghi et al. 2026)	2.6 g (present study)	UV-photo identification is suitable for smaller individuals
Initial equipment	Injection kit, syringes and VIE elastomer	UV torch and camera/smartphone	Both methods require simple equipment
Consumables	Elastomer must be regularly replenished; mixed aliquots have a working time of approximately 45 min–2 h, depending on temperature	No consumables; only electrical equipment requires recharging	UV-photo identification requires no consumables
Per-animal cost	Moderate: elastomer is consumed for each individual	Negligible after initial equipment purchase	UV-photo identification has lower per-animal costs
Processing time: field	Injection required (< 3 min per specimen)	Illumination and photography (< 30 s per specimen)	UV-photo identification requires less handling and processing time in the field
Processing time: identification	Time required to check tag codes; not reported	Visual comparison with the photographic database (1–15 min, depending on the number of specimens collected per cave)	Processing times for individual recognition are not directly comparable
Animal handling	Relatively long and invasive	Short and non-invasive when photographing directly; minimally invasive if temporary containment is required	UV-photo identification requires substantially less handling
Potential animal stress	Direct handling without anesthesia for < 3 min, plus light exposure during tag identification (Barzaghi et al. 2026)	Direct UV-A exposure for < 30 s at a distance of ≥ 35–40 cm	Both methods involve animal handling/light exposure, but VIE tagging involves longer handling
Individual identification	Excellent	Excellent: distinctive natural fluorescent patterns enabled the identification of > 300 olms	Both methods provide highly effective individual recognition; UV-photo identification relies on natural markers
Technical difficulty	Requires specific training in VIE tagging	Relatively simple photographic procedure	UV-photo identification requires less specialized training

knowledge, no published studies have specifically investigated the effects of short-term UV-A exposure on *Proteus* spp. Therefore, as a precautionary measure, UV illumination should be limited to the minimum duration necessary for photographic documentation, avoiding any unnecessary prolongation of exposure. The use of naturally occurring body patterns for individual recognition is well established in amphibian research, where dorsal or ventral pigmentation has been successfully employed for photographic identification (Sannolo et al. 2016; Lamb and Davis 2020; Patel and Das 2020; Santos et al. 2024). The present results extend this concept to *Proteus* spp., demonstrating that naturally occurring UV-fluorescent skin patterns provide an equally reliable means of individual recognition in a subterranean amphibian.

Conclusion

The ability to identify individual animals is fundamental to ecological research (Otis et al. 1978; Pollock et al. 1990). Reliable individual recognition enables capture-recapture studies to estimate key demographic parameters such as survival, population size, recruitment, and dispersal, while also supporting behavioral, ecological, and genetic investigations in both wild and captive populations (Otis et al. 1978; Pollock et al. 1990; Lemckert 1996; Brown 1997). Increasingly, these studies seek to minimize disturbance and handling, particularly when working with threatened species, making non-invasive identification methods highly desirable.

Overall, UV-assisted photographic identification combines high discriminatory power with minimal invasiveness, low cost, and ease of implementation. By eliminating the need for artificial, stressful, or physically invasive marking while maintaining reliable, long-term individual recognition, this method represents a highly valuable tool for capture-recapture studies and long-term conservation monitoring of vulnerable cave salamanders belonging to the genus *Proteus*.

The identification of stable, natural "fingerprints" using 365 nm and 395 nm UV wavelengths provides a solution to the welfare-related challenges historically associ-

ated with monitoring delicate, subterranean organisms. By facilitating rapid, low-stress documentation, even underwater, this approach sets a new standard for ethically conscious, non-invasive amphibian research.

Furthermore, the implications of this study extend beyond the Classical Karst area. The successful development of this photographic workflow suggests that UV-assisted identification may be readily applicable to other olm genetic lineages and rare, threatened, or cave-dwelling species that exhibit individually distinctive fluorescent structures or subtle pigmentation patterns. As wildlife monitoring increasingly prioritizes both high-resolution data collection and the highest standards of animal welfare, accessible methods like the one presented here will be crucial in balancing conservation priorities with non-invasive scientific inquiry.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

Field sampling and surveys of *Proteus a. anguinus* were conducted under Permits No. 144200 (1 August 2025) and No. 14324 (22 January 2026) issued by the Italian Ministry of Environment and Energy Security.

Artificial Intelligence (AI) use

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI.
During the preparation of this work, the authors used Claude.ai in order to improve the readability and language. Following the use of this tool, the authors reviewed and edited the content as necessary and take full responsibility for the content of the publication.

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Author contributions

MR and CM conceived the ideas, designed the methodology, and collected and analyzed the data; CM led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data availability

The complete photographic database compiled during this study is available from the corresponding author upon reasonable request. The authors welcome and encourage collaboration with fellow researchers working on Proteus spp. The authors are open to cross-referencing photographic catalogs, comparing regional spot-pattern variations, and providing technical support or guidance to groups interested in implementing this non-invasive monitoring method at their own study sites.

Supplementary material 1

Supplementary information

Authors: Marco Restaino, Chiara Manfrin
Data type: pdf
Explanation note: **1.** Representative examples of different olms recorded under the three selected lighting conditions. **2.** Recognition efficiency between VIE tagging and UV-photo identification: calculations. **3.** Detail of the regenerated tail.
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