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Habitat features and distribution of *Salamandra salamandra* in underground springs

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Abstract. Subterranean habitats are among the less known terrestrial habitats, but can reveal an unexpected biodiversity, and can play an underestimated role for amphibians. The fire salamander *Salamandra salamandra* is sometimes found in underground environments, but the factors affecting its distribution in subterranean spaces remain substantially unexplored. We repeatedly surveyed some hypogeous springs, such as draining galleries and "bottini" in NW Italy, in order to evaluate the relationship between environmental features and distribution of *S. salamandra* in these underground springs. We performed visual encounter surveys to assess the occurrence of larvae, juveniles or adults in springs. We also recorded four habitat variables: easy of access, isolation, macrobenthos richness and forest cover of the surrounding landscape. We used generalized linear models to evaluate the relationships between habitat features and occurrence of larvae. We observed larvae of *S. salamandra* in 13 out of 22 springs; their presence was associated to springs with high easy of access and with relatively rich macrobenthos communities. In underground springs, larval development apparently required longer time than in nearby epigeous streams. Nevertheless, *S. salamandra* can attain metamorphosis in this environment. The occurrence of *S. salamandra* in underground environments was not accidental, but repeated in the time and interesting from an ecological point of view, confirming the high plasticity of the species.

Keywords. Amphibians, cannibalism, cave, draining galleries, ecology, fire salamander, habitat, larval development.

INTRODUCTION

In the last years, biospeleology has had a remarkable development. Biospeleology has extended its attention not only to cave habitats, but also to interstitials ones, especially ground waters (Danielopol and Griebler, 2008). An increasing number of studies has shown that the subterranean domain, formerly considered as a species-poor environment, often reveals an unexpected biodiversity (Botosaneanu and Stock, 1997; Bottazzi et al., 2008). Subterranean communities are composed by both hypogean and epigean taxa (Danielopol and Griebler, 2008). The occurrence of amphibians in underground habitats is reported in both the herpetological and biospeleological literature (Gimenez-Lopez and Guarner Deu, 1982). The life cycle of some taxa is completely (e.g., *Proteus*) or partially (e.g., *Speleomantes*) connected with caves and interstitial habitats. Furthermore, several amphibians can use underground habitats even if they do not complete their life cycle there, i.e., they are occasional trogloxenes (Romero, 2001; Sket, 2008). A number of European amphibians have been more or less regularly found inside caves. Multiple mechanisms can explain the underground presence of amphibians: Underground spaces can be winter shelters, hiding places during the active season, and feeding habitats (Baumgart, 1981; Uhrin and Lesinsky, 1997). Furthermore, caves sometime act as natural traps where amphibians fall or are transported by the water flow (Uhrin and Lesinsky, 1997).

The fire salamander *Salamandra salamandra* has been repeatedly found in underground environments (Baumgart, 1981; Gimenez-Lopez and Guarner Deu, 1982; Uhrin and Lesinsky, 1997; Razzetti et al., 2001). Adults and metamorphs are often observed during latency periods, with fidelity of the individuals to shelter places (Feldman, 1967; Baungart, 1981). Furthermore, some studies reported the presence of larvae in subterranean damp biotopes. Gimenez-Lopez and Guarner Deu (1982) point out the finding of a larva in natural cave of Catalonia; Uhrin and Lesinsky (1997) suppose that salamanders reproduce in Slovakian underground spaces on the basis of records of larvae in caves and galleries; and Veith (1986) supposes that reproduction in underground environments is common in the Rhine valley. In Italy, several authors have observed larvae of *S. salamandra* in both natural and artificial underground environments (Bressi, 1995; Bressi and Dolce, 1999; Razzetti et al., 2001). Nevertheless, data on the occurrence and development of *S. salamandra* in hypogeous habitats remains anecdotic. Furthermore, the factors affecting the distribution of salamanders in subterranean environments are substantially unexplored.

Recent observations showed that *S. salamandra* sometimes lays larvae inside the brooks or in the pools of some artificial hypogeous biotopes, such as draining galleries and other subterranean springs in Northern Italy (Manenti, 2007, 2008). The aim of this study was evaluating the distribution of *S. salamandra* in subterranean springs, to understand the ecological determinants that affect their occurrence, and whether these environments can be a suitable reproductive habitat.

MATERIALS AND METHODS

Underground springs considered

We considered two typologies of subterranean artificial springs: draining galleries and the so called ‘bottini’. Draining galleries (Fig. 1A) are characterized by an almost horizontal tunnel that penetrates the side of a slope, to catch the subterranean water of a spring and bring it outward. Draining galleries are associated to traditional agriculture, and are found in Europe, Asia and Northern Africa (Balland, 1992). Even if they are less known than other spring typologies, draining

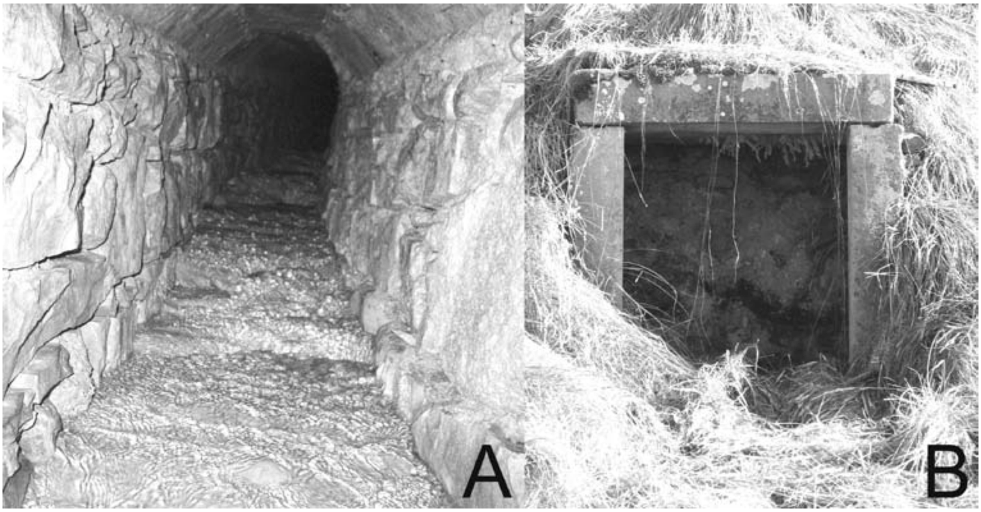

Fig. 1. Example of the access tunnel of (A) a draining gallery and (B) a "bottino".

galleries are widespread in Italy. Some of them are very ancient, such as the Etruscan spring flow tunnels (Caponetti, 2005) or the galleries in the towns of Matera and Siena (Kucher, 2005). They house small brooks and/or water reservoirs. 'Bottini' (Fig. 1B) are small buildings with limited subterranean development and with basins for water collecting. Both typologies are frequently obsolete and unused. In most cases, they are hidden and difficult to reach (Manenti, 2008). In the study area, draining galleries have a limited width (max 2 m); and a length varying from 5 up of 100 meters. The brooks and the pools within galleries usually have limited depth (average 20 cm). The length of Bottini is usually 3-4 m; the average water depth is 41.7 cm.

Study area and surveys

We surveyed 22 subterranean springs in Lombardy (Northern Italy) between the districts of Lecco, Como, and Milan (Fig. 2). The study area is comprised in the catchment basins of the Lambro, Adda and Seveso rivers. It is characterized by hilly and mountainous reliefs with a good cover of broadleaved woodlands. In order to identify and reach the springs, we used information available in local studies on troglophyle molluscs and springs features (Nardo and Guglielmin, 1996; Pezzoli, 2007), we collected information from local people and local environmental organisation, and we directly explored the countryside. All springs were permanent or nearly permanent.

From September 2007 till January 2009, we surveyed each springs 2-8 (median: 3) times. Most of springs were surveyed 2-5 times; two springs with larvae were surveyed 7-8 times, at intervals of one-two months, to better monitor the larval development. The occurrence of larvae in springs was not related to the number of surveys (logistic regression, $\chi^2_1 = 1.05$, $P = 0.31$), indicating that variation in the number of surveys did not bias the results of our analyses. We performed visual encounter surveys to assess the presence and the abundance of *S. salamandra* larvae, juveniles and adults. The surveyed waterbodies are usually small, always have very clear water, therefore visual census allow a reliable estimation of presence/absence of larvae in the water. When larvae of *S. salamandra* are present, their per-visit detection probability is > 90% (Manenti et al., 2009), therefore with two-three surveys the cumulative probability of undetecting present larvae is < 1%. Larvae

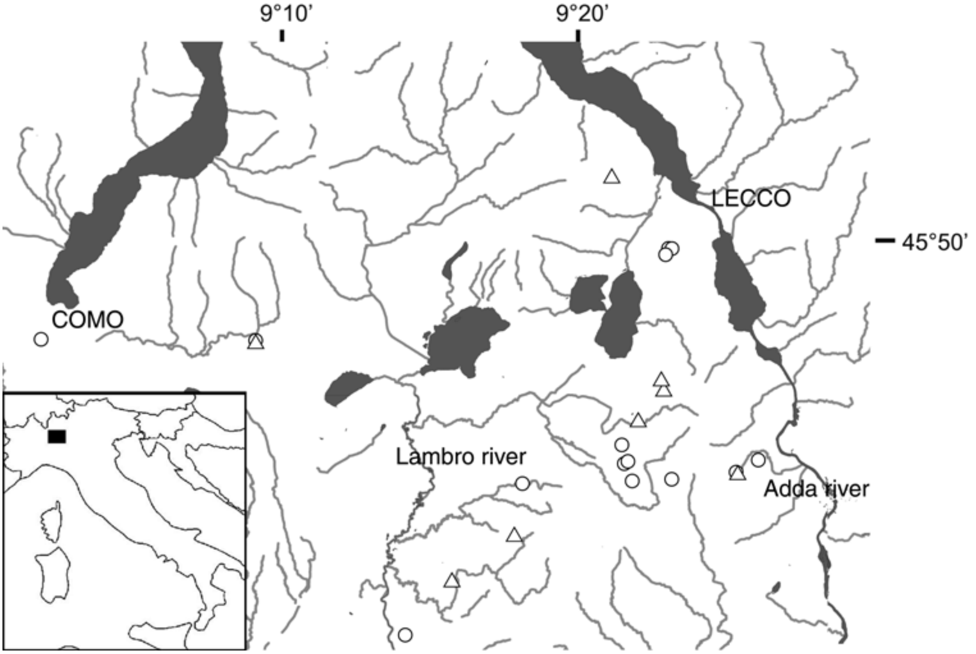

Fig. 2. Study area. White circles show the location of the draining galleries, while triangles of the "bottini"; grey: hidrographic network. Some circles and triangles are partially superimposed, due to geographic proximity.

were assigned to three development stages on the basis of total length and morphology (Jusczcyk and Zakrzewski, 1981): 27-45 mm; 46-65 mm; metamorphs with body size > 60 mm.

We recorded four environmental variables to describe these subterranean habitats and to evaluate the relationship between habitat features and *S. salamandra* (Manenti et al., 2009): a) easy of access for salamanders, measured using a rank scale (1 = completely closed by doors or other obstacles and apparently inaccessible; 2 = difficult access because of doors or other obstacles; 3 = open and accessible); b) isolation, measured as the distance between each spring and the nearest known laying site of *S. salamandra*, obtained from previous studies (Manenti et al., 2008; Ficetola et al., 2009; Manenti et al., 2009) or by direct observations during surveys; c) richness of the community of benthonic macro-invertebrates, measured as the number of taxonomic units (see Ghetti, 1997; in every spring, we sampled macrobenthos by moving the substrate for 5-10 minutes, and we used a thin-mesh dip net to collect the invertebrates); d) forest cover measured as forest cover percentage within 400 m from each sampling point on the basis of the 1:10000 Vector Map of Lombardy, using the ESRI ArcView 3.2 GIS. Forests are the major habitat of adults, and forest cover at this scale is strongly associated with populations of *S. salamandra* (Ficetola et al., 2009).

Statistical analyses

We used generalized linear models (GLMs), assuming binomial error, to evaluate the relationships between habitat features and occurrence of larvae. First, we analyzed all univariate relationships. We also tried to build GLMs describing the relationships between multiple habitat features and occurrence of *S. salamandra*, subjected to the constraint that all variables in a given GLM must have variance inflation factor ≤ 5 (Bowerman and O'Connell, 1990). We built GLMs with all combinations of two or three variables, testing also for interactions. However, none of these GLMs with multiple variables showed Akaike Information Criterion lower than the best univariate model, probably because of the limited sample size (see Burnham and Anderson, 2002). Therefore, in the results we report the univariate models only. We also calculated Nagelkerke's R^2 (R^2_N) as a measure of the variance explained by GLMs. If necessary, we transformed variables to improve normality (see Table 1).

Table 1. Habitat features of springs with and without larvae, univariate relationships between habitat features and presence / absence of larvae of *Salamandra salamandra*, and comparison with epigeous streams with larvae from the same study area (sites in Ficetola et al., 2009). For easy of access, we report the median and the range of variation; for isolation, macrobenthos richness and forest cover, we report mean ± SE. R^2_N: Nagelkerke's R^2.

	Presence of larvae		χ^2_1	P	R^2_N	Epigeous streams
	Yes (n = 13)	No (n = 9)				(n = 45)
Easy of access	3 (2-3)	2 (1-3)	10.57	0.001	0.51	-
Isolation (m)[1]	475 ± 250	471 ± 292	0.177	0.674	0.01	-
Macrobenthos taxa[2]	2.38 ± 0.39	1.33 ± 0.62	5.48	0.019	0.30	14.9 ± 1.5
Forest cover[3]	0.66 ± 0.07	0.64 ± 0.09	0.06	0.810	0.00	0.65 ± 0.03

[1]: log transformed prior to analyze; [2]: square-root transformed prior to analyze; [3]: square-root arcsine transformed prior to analyze

RESULTS

We observed larvae of *S. salamandra* in 13 out of 22 springs. Furthermore, we found a recently metamorphosed individual (65 mm) in one spring where we did not record larvae. We found adults in five tunnels. We observed recently laid larvae in November 2007, May-April 2008 and December 2008. In nearly all the cases, these larvae overlapped with older larvae previously laid. All the springs with larvae in autumn 2007 received at least a new deposition in spring or autumn 2008. In all springs, we observed multiple development stages during the same visit, indicating the presence of larvae from different clutches. The richness of macrobenthos was usually low. The average number of taxonomic units observed was 1.95 per spring (SE = 0.35), and was much lower than observed in epigeous laying streams (Manenti et al., 2009; Table 1).

Univariate tests showed that the presence of larvae was associated to the springs with high easy of access and with rich macrobenthos. The relationships with isolation and forest cover were non significant (Table 1). Easy of access was the variable with the largest explanatory power, and explained 51% of variation of the distribution of *S. salamandra* larvae (Table 1).

We observed metamorphs in four springs. Furthermore, in all springs with larvae we recorded the presence of late development stages. However, in four sites, the exit of metamorphs from springs seemed to be very difficult. We directly observed episodes of cannibalism in two galleries, in which larvae at late development stages fed on small larvae.

DISCUSSION

The occurrence of *S. salamandra* in the hypogeous springs was not accidental, but repeated in time and interesting from an ecological point of view. All the study springs are underground headwaters. Therefore, the presence of larvae can not be explained by drifting or trapping from epigeous environments. Instead, the presence of larvae suggests that these headwaters are used by females for laying. Furthermore, in all springs with larvae we observed repeated laying during multiple seasons, indicating that laying in these sites was not occasional.

Easy of access was the variable most strongly related to the presence of larvae. In practice, we observed larvae in most of springs accessible to adults. This suggests that underground springs are selected by females for laying. Underground springs have permanent hydroperiod, and can be particularly important in the areas where epigeous streams are temporary. Furthermore, we observed a significant association with macrobenthos richness. Benthonic invertebrates constitute the major prey items of larvae, and therefore this relationship is not surprising (see Manenti et al., 2009). The presence of benthos can be particularly important in these habitats, where invertebrates never reach high densities. It should also be noted that easy of access and macrobenthos richness were positively related (Spearman's correlation, $r_S = 0.55$, $P = 0.008$), probably because easy of access increases also colonization by insects. Therefore, it is possible that these two variables have a synergic effect.

Nevertheless, the richness of invertebrates remained low in all springs (Table 1). This can be a critical factor for the survival and development of larvae in this environment. Indeed, the scarcity of shelter and of invertebrates might favours cannibalism. During our surveys, we observed some episodes of cannibalism; furthermore, in several cases we observed larvae with wounds and bite marks in areas without predator insects (see below), suggesting that cannibalism can be frequent. *S. salamandra* often performs cannibalism; in suboptimal habitats, cannibalism can be the only strategy allowing some larvae (usually the oldest ones) to reach metamorphosis (Eitam et al., 2005).

Previous studies showed that, in epigeous streams, larvae are associated with high forest cover, probably because forests are the major habitat of adults (Ficetola et al., 2009; Manenti et al., 2009). The lack of a significant relationship (Table 1) does not mean that forest presence is unimportant. Most of the studied streams are in highly forested landscapes (Table 1); the average forest cover across all sites (65%) was similar to the cover in epigeous streams used for laying by salamanders (Table 1: Ficetola et al., 2009; Manenti et al., 2009). Therefore, forest presence was not a limiting factor in the landscapes studied. We did not observe a relationship with isolation, and we observed reproductions also in springs very far from the nearest occupied epigeous stream (Table 1). Amphibians often live in network of metapopulations, and isolation has strong negative effects on the occupancy of wetlands (Ficetola and De Bernardi, 2004; Zanini et al., 2009). However, our knowledge of breeding streams is probably incomplete, and we might have missed nearby sites.

Amphibians living in cave environments usually have delayed development (Clergue-Gazeau, 1975). We found a similar pattern, with the coexistence of multiple larval stages. Our observations suggest that larval development in these caves can require more than eight months. This is more than twice the age at metamorphosis in epigeous streams (usually 3-4 months: Nöllert and Nöllert, 1992). The slow development rate can be caused by cold temperature, lack of light, and scarcity of food items (e.g., macrobenthos). On the other hand, underground habitats can have advantages, for example because of the constant thermal environment, which allows development also during winter, or the scarcity of predators. For instance, during surveys and macrobenthos samplings, we never observed fish or predatory insects, even those that typically inhabits other *S. salamandra* breeding sites (e.g., *Nepa cinerea*; larvae of *Cordulegaster boltoni* or other Odonata). The observation of metamorphs in several springs confirm that these areas can be suitable habitat for larval development. Nevertheless, some of the springs can act as traps. In three sites, the vertical banks did not allow the adults or metamorphs to leave the springs, and we found drown animals.

S. salamandra shows high plasticity, has surprising local adaptations, and can perform larval development in habitats ranging from fast running streams to ponds (Weitere et al., 2004). The observation of larvae in underground springs is a further confirmation of this plasticity. Indeed, the occurrence of *S. salamandra* in underground, artificial springs is also known for other countries (e.g., Switzerland: K. Grossenbacher pers. comm.) and similar breeding sites are probably present also in other areas of its distribution range (Baumgart, 1981). Underground environments are among the less known terrestrial habitats, and can play an underestimated role for amphibians. Draining galleries and other underground springs could be very useful to study plasticity and adaptations in *S. salamandra*.

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REFERENCES

Balland, D. (1992): Les eaux cachées. Études géographiques sur les galleries drainantes souterraines. Dèpartement de Géographie, Université Sorbonne, Paris.

Baumgart, G. (1981): Observations sur l'hibernation de quelques amphibiens dans les anciennes mines vosgiennes: Salamandre tachetée, Grenouille rousse et Crapaud commun. Aquarama **58**: 42-72.

Botosaneanu, L., Stock, J.H. (1997): Stygofauna of Oman .1. A new freshwater stygobiont Cyathura (Isopoda, Anthuridae), from interstitia of coarse wadi sediments in Oman. Ann. Limnol.-Int. J. Limnol. **33**: 79-84.

Bottazzi, E., Bruno, M.C., Mazzini, M., Pieri, V., Rosetti, G. (2008): First report on Copepoda and Ostracoda (Crustacea) from northern Apenninic springs (N. Italy): a faunal and biogeographical account. J. Limnol. **67**: 56-63.

Bowerman, B.L., O'Connell, R.T. (1990): Linear statistical models. PWS-Kent, Boston.

Bressi, N. (1995): Catalogo della Collezione Erpetologica del Museo civico di Storia Naturale di Trieste. I-Amphibia. Museo civico di Storia Naturale di Trieste, Trieste.

Bressi, N., Dolce, S. (1999): Osservazioni di Anfibi e Rettili in grotta. Riv. Idrobiol. **38**: 475-481.

Burnham, K.P., Anderson, D.R. (2002): Model selection and multimodel inference: a practical information-theoretic approach. Springer Verlag, New York.

Caponetti, L. (2005): Gallerie drenanti e sistemi idraulici Etruschi: il caso di Tuscania. Ecomusei Regione Piemonte, Trino (VC).

Clergue-Gazeau, M. (1975): Effets de la vie cavernicole sur la reproduction des Amphibiens. Bull. Soc. Zool. Fr. **100**: 665-666.

Danielopol, D.L., Griebler, C. (2008): Changing Paradigms in Groundwater Ecology - from the 'Living Fossils' Tradition to the 'New Groundwater Ecology'. Int. Rev. Hydrobiol. **93**: 565-577.

Eitam, A., Blaustein, L., Mangel, M. (2005): Density and intercohort priority effects on larval *Salamandra salamandra* in temporary pools. Oecologia **146**: 36-42.

Feldman, R. (1967): Nachweis der Ortstreue des Feuer-salamanders, *Salamandra salamandra terrestris* Lacépède, 1788, gegenuber seinem Winterquartier. Zool. Anz. Leipzig **178**: 42-48.

Ficetola, G.F., De Bernardi, F. (2004): Amphibians in an human-dominated landscape: the community structure is related to habitat features and isolation. Biol. Conserv. **119**: 219-230.

Ficetola, G.F., Padoa-Schioppa, E., De Bernardi, F. (2009): Influence of landscape elements in riparian buffers on the conservation of semiaquatic amphibians. Conserv. Biol. **23**: 114-123.

Ghetti, P.F. (1997): Indice Biotico Esteso (I.B.E.): Manuale di applicazione. Provincia Autonoma di Trento, Trento.

Gimenez-Lopez, S., Guarner Deu, N. (1982): Distribucion Hipogea de *Salamandra salamandra.* Laurenti (Amphibia Salamandridae) en San Lorenç del Munt i Serra de l'Obac (Terrasa, provincia de Barcelona, Espana). P. Cent. pir. Biol. Exp. **13**: 43-45.

Jusczcyk, W., Zakrzewski, M. (1981): External morphology of larval stages of the spotted salamander *Salamandra salamandra* (L.). Acta Biol. Cracoviensa **23**: 127-135.

Kucher, M. (2005): The water supply system of Siena, Italy. Routledge Ed., Florence.

Manenti, R. (2007): Considerazioni sulla cenosi erpetologica delle gallerie drenanti. Riv. Idrobiol. **43**: 114-118.

Manenti, R. (2008): Amphibiens des sources et galeries drainantes en territoire préalpin, l'exemple du Mont Barro et du Mont de Brianza (Lombardie, Italie). Bull. Soc. Herp. Fr. **128**: 25-40.

Manenti, R., Ficetola, G.F., De Bernardi, F. (2009): Water, stream morphology and landscape: complex habitat determinants for the fire salamander *Salamandra salamandra*. Amphibia-Reptilia **30**: 7-15.

Manenti, R., Ficetola, G.F., Padoa-Schioppa, E., De Bernardi, F. (2008): Spatial autocorrelation and distribution of *Salamandra salamandra* – a preliminary analysis. In: Herpetologia Sardiniae, pp. 341-344. C. Corti, Ed, Edizioni Belvedere, Latina.

Nardo, A., Guglielmin, M. (1996): Le Sorgenti del Barro. Quaderni del Parco Monte Barro, **3**: 1-48.

Nöllert, A., Nöllert, C. (1992): Die Amphibien Europas. Kosmos, Stuttgart.

Pezzoli, E. (2007): I molluschi e i crostacei delle sorgenti e delle acque sotterranee della Lombardia: censimento delle stazioni. Parco Regionale del Monte Barro, Galbiate (LC).

Razzetti, E., Bonini, L., Barbieri, F. (2001): Riproduzione in grotta di *Salamandra salamandra* e *Salamandrina terdigitata* negli Appennini settentrionali. Atti 3° Congresso nazionale SHI, (Pavia, 2000). Pianura **13**: 181-184.

Romero, A. (2001): The biology of hypogean fishes. Kluwer, Dordrecht.

Sket, B. (2008): Can we agree on an ecological classification of subterranean animals? J. Nat. Hist. **42**: 1549-1563.

Uhrin, M., Lesinsky, G. (1997): Mechanism of occurrence of amphibians in an underground spaces in Slovakia: preliminary data evaluation. Proceedings of the 12th International Congress of Speleology, La Chaux de fonds Switzerland: **3**: 325-327.

Veith, M. (1986): Feuersalamander - *Salamandra salamandra*. In: Die Amphibien und Reptilien in Rheinland-Pfalz A. Bitz, Ed, Ges. fur Naturschutz und Ornithologie Rheinland-Pfalz.

Weitere, M., Tautz, D., Neumann, D., Steinfartz, S. (2004): Adaptive divergence vs. environmental plasticity: tracing local genetic adaptation of metamorphosis traits in salamanders. Mol. Ecol. **13**: 1665-1677.

Zanini, F., Pellet, J., Schmidt, B. (2009): The transferability of distribution models across regions: an amphibian case study. Divers. Distrib. **15**: 469-480.