HERPETOZOA 29 (1/2): 27 - 35
Wien, 30. Juli 2016

Parasitism of the leech, *Batracobdella algira* (MOQUIN-TANDON, 1846), on Sardinian cave salamanders (Genus *Hydromantes*)
(Caudata: Plethodontidae)

Parasitismus des Egels *Batracobdella algira* (MOQUIN-TANDON, 1846)
auf Sardischen Höhlensalamandern der Gattung *Hydromantes*
(Caudata: Plethodontidae)

RAOUL MANENTI & ENRICO LUNGHI & CLAUDIA CANEDOLI & MORENA BONACCORSI & GENTILE FRANCESCO FICETOLA

KURZFASSUNG

Wenig ist über die Beziehungen zwischen Sardischen Höhlensalamandern (Gattung *Hydromantes*; Untergattungen *Speleomantes* und *Atylodes*) und anderen Höhlenbewohnern bekannt. Man weiß, daß Höhlensalamander vom Egel *Batracobdella algira* parasitiert werden, aber nichts über die Prävalenz des Befalls oder Faktoren, die ihn fördern. Die Autoren untersuchten 55 Höhlen im Verbreitungsgebiet von *H. flavus*, *H. genei*, *H. imperialis*, *H. sarrabusensis* und *H. supramontis*, analysierten die standortbedingten Unterschiede in der Parasitierung der Salamander durch *B. algira* und fanden den Parasiten bei *H. flavus*, *H. genei* und *H. imperialis*. Der Befall war bei allen Arten gering (Prävalenz < 3 %) außer bei *H. flavus* (9 %). Die Befallsprävalenzen waren standortbezogen stark autokorreliert, aber ohne Bezug zu abiotischen Höhlenparametern. Am häufigsten waren große Salamanderindividuen parasitiert. Weiters berichtet die Arbeit darüber, daß *H. sarrabusensis* wohl zur Beute der Spinne *Tegenaria* sp. zählt. Die Komplexität der Beziehungen zwischen Höhlensalamandern und *B. algira* ist durch starke populationsspezifische und individuelle Unterschiede gekennzeichnet.

ABSTRACT

Very little information is available on relationships between Sardinian cave salamanders (genus *Hydromantes*; subgenera *Speleomantes* and *Atylodes*) and other cave-dwelling organisms. Cave salamanders are known to be parasitized by the leech, *Batracobdella algira*, but no information exists on the prevalence of this parasite on cave salamanders or the factors that promote it. The authors surveyed 55 cavities covering the range of *H. flavus*, *H. genei*, *H. imperialis*, *H. sarrabusensis* and *H. supramontis*, analyzed the spatial variation of parasitism by *B. algira*, and detected the leech in *H. flavus*, *H. genei* and *H. imperialis*. Infestation was low for all species (prevalence < 3 %) except *H. flavus* (9 %). Prevalences showed strong spatial autocorrelation, and were unrelated to cave abiotic features. Large individuals were the most frequently parasitized. Moreover, predation on *H. sarrabusensis* by the spider *Tegenaria* sp. was observed. The relationships between cave salamanders and *B. algira* show a complex pattern, with strong heterogeneity among species, populations and individuals.

KEY WORDS

Amphibia: Caudata: Plethodontidae; *Hydromantes flavus*, *Hydromantes sarrabusensis*; host-parasite interactions, predation, parasites, parasitism, *Batracobdella algira*, spatial autocorrelation, caves; ecology, parasitology, veterinary medicine, Sardinia, Italy

INTRODUCTION

European cave salamanders (genus *Hydromantes*, subgenera *Speleomantes* and *Atylodes*; see VIEITES et al. 2011 for justification) are among the European amphibians showing the highest rate of endemism (SILLERO et al. 2014). Sardinia hosts the highest richness of *Hydromantes*, with five currently recognized species (SILLERO et al. 2014); among them, the species with the smallest range, *Hydromantes sarrabusensis* LANZA, LEO, FORTI, CIMMARUTA, CAPUTO & NASCETTI, 2001, was elevated to the species rank in 2008 (CARRANZA et al. 2008).

Despite a growing interest on these salamanders, comprehensive studies on their ecology and on their interactions with other components of the underground communities are limited. For instance, there is no information on the predators of most of Sardinian *Hydromantes* (Lanza et al. 2006, pag. 42). Large cave spiders (genus *Meta*) are known to prey on the juveniles of the continental species *H. italicus* (Dunn, 1923) (Pastorelli & Laghi 2007), whereas information on the interactions between spiders and the Sardinian species are lacking. Leeches frequently parasitize amphibians (reviewed by Romano & Di Cerbo 2007). The leech, *Batracobdella algira* (Moquin-Tandon, 1846), is the only ectoparasite known for the genus, and has been "frequently found" on the north-eastern Sardinian species *H. flavus* (Stefani, 1969), *H. imperialis* (Stefani, 1969) and *H. supramontis* (Lanza, Nascetti & Bullini, 1986) (Lanza 1999a; Lanza et al. 2006). However, there is no information on the actual frequency of this parasite, nor on the ecological factors related to its occurrence.

The present study reports the results of a survey on the five species of Sardinian *Hydromantes* with regard to these questions: 1) What is the prevalence of the ectoparasite *B. algira* on cave salamander populations? 2) Is the prevalence homogeneously distributed across populations and species? 3) Is the prevalence related to environmental features of caves, or to morphological features of individuals? As additional data on interspecific relationships, the first information on the predators of *H. sarrabusensis* is reported.

MATERIALS AND METHODS

In June 2013 and June 2014 the authors surveyed 120 Sardinian caves and other underground cavities, to assess the presence of cave salamanders. *Hydromantes* salamanders are not necessarily restricted to the cave environments, and during fresh and humid seasons (from autumn to early spring) may present relevant outdoor activity (Lanza et al. 2006). However, they move to the humid underground environments when external conditions would be too harsh (e.g., dry, hot) for lungless terrestrial salamanders (Cimmaruta et al. 1999; Lunghi et al. 2015). Therefore, during summer periods they are easily detectable in caves, where they sometime reach high densities (e.g., Lanza 1991; Lanza et al. 2006; Salvidio 2007; Lindstrom et al. 2010; Ficetola et al. 2012b).

Visual encounter surveys (Crump & Scott 1994) were used to assess the distribution of salamanders. In each survey, three to six trained people actively searched for salamanders perched on the floors and walls of the caves. These were entered and explored as far as feasible, given the available climbing equipment. For each salamander observed, the presence of parasitizing *Batrachobdella algira* and, if possible, total length were recorded. Each cave was subdivided in 3-m longitudinal intervals (hereafter: sectors) for the measurement of environmental features (Ficetola et al. 2013). The size of sectors approximately corresponded to the home ranges known for *Hydromantes* species (Salvidio et al. 1994). The micro-habitat in each 3-m sector was characterized by three measured parameters representing the abiotic conditions: air temperature (°C), relative humidity (%) and illuminance (i.e., intensity of incident light, measured in lux) (see Ficetola et al. 2013 for additional methodological details).

Moran's *I* was used to assess whether the prevalence of *B. algira* showed significant spatial autocorrelation. Spatial autocorrelation occurs when neighboring localities have similar values for the measured parameters, such as species distribution data or abiotic features. Spatial autocorrelation of species distribution may arise because of endogenous biological processes (e.g., aggregation, dispersal, transmission of parasites between nearby hosts) or because the distribution of individuals is determined by environmental features which, in turn, are autocorrelated (e.g., altitude, climate) (Wagner & Fortin 2005; Legendre & Legendre 2012; Ficetola et al. 2012a). In this study case, tests of spatial autocorrela-

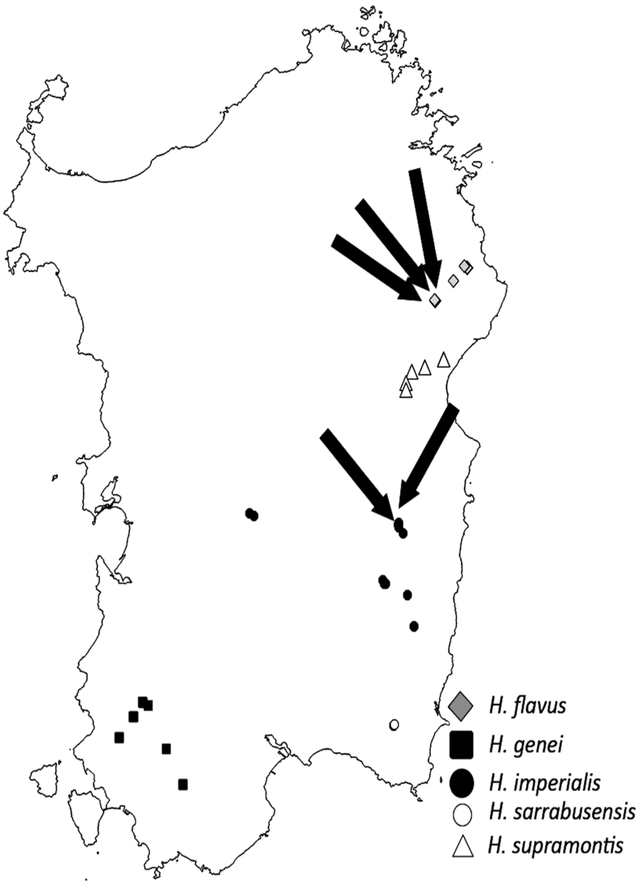

Fig. 1: Location of the studied *Hydromantes* populations in Sardinia, Italy. The black arrows indicate the populations in which parasitism by the leech, *Batracobdella algira* (MOQUIN-TANDON, 1846) was detected.

Abb. 1: Die Lage der untersuchten *Hydromantes*-Populationen auf Sardinien, Italien. Schwarze Pfeile weisen auf Populationen, in denen der Egel *Batracobdella algira* (MOQUIN-TANDON, 1846) als Parasit auftrat.

tion allow to assess whether more parasitized populations are geographically closer to each other than expected by chance (LEGENDRE & LEGENDRE 2012). Subsequently, the method of Bayesian equal-tailed Jeffreys prior intervals was used to estimate 95 % confidence intervals (CI) of the parasite prevalence in the five study species and in the infected populations (BROWN et al. 2001). Comparative analyses showed that Jeffreys intervals have a good performance in estimating binomial CI, even if sample size is limited (BROWN et al. 2001).

Generalized linear mixed models (GLMM) that assume binomial error distribution, were used to assess whether the prevalence of parasites within cave sectors was related to environmental variables (temperature, humidity and illuminance). This analysis was limited to the caves occupied by *H. flavus* and *H. imperialis*, as *B.*

Fig. 2: A - *Hydromantes flavus* (STEFANI, 1969), B - *Hydromantes imperialis* (STEFANI, 1969) and C - *Hydromantes genei* (TEMMINCK & SCHLEGEL, 1838) parasitized by the leech, *Batracobdella algira* (MOQUIN-TANDON, 1846).

Abb. 2: A - *Hydromantes flavus* (STEFANI, 1969), B - *Hydromantes imperialis* (STEFANI, 1969) and C - *Hydromantes genei* (TEMMINCK & SCHLEGEL, 1838), die alle vom Egel *Batracobdella algira* (MOQUIN-TANDON, 1846) parasitiert sind.

Table 1: Frequency of individuals parasitized by the leech *Batracobdella algira* (MOQUIN-TANDON, 1846) (prevalence) across species and populations of Sardinian *Hydromantes*. CI - confidence interval; * - None of the individuals surveyed in caves during summer cave monitoring was parasitized. However, *B. algira* was found parasitizing one adult *H. genei* (TEMMINCK & SCHLEGEL, 1838) in outdoor activity during the wet season.

Tab. 1: Häufigkeit der vom Egel *Batracobdella algira* (MOQUIN-TANDON, 1846) parasitierten Individuen (Prävalenz) bei Arten und Populationen Sardischer *Hydromantes*-Arten. CI – Konfidenzgrenzen; * - Keines der während der sommerlichen Höhlenbegehungen untersuchten Individuen war befallen. Doch fand sich *B. algira* an einem adulten *H. genei* (TEMMINCK & SCHLEGEL, 1838), der in der feuchten Jahreszeit außerhalb der Höhle angetroffen wurde. Cave – Höhle; sinkhole near the cave – Karsttrichter nahe der Höhle.

Hydromantes Species	Location / Fundort	Prevalence of *B. algira* / Prävalenz von *B. algira*	95 % CI
H. flavus	Whole range / Gesamtes Verbreitungsgebiet	0.092	0.039-0.180
	Cave «Grotta degli Antenati», Lula, NU	0.50	0.123-0.877
	Sinkhole near the cave «Grotta degli Antenati»	0.50	0.061-0.939
	Cave «Su Enapru», Lula, NU	0.10	0.021-0.284
H. genei	Whole range / Gesamtes Verbreitungsgebiet	0*	0-0.009
H. imperialis	Whole range / Gesamtes Verbreitungsgebiet	0.004	0.001-0.009
	Cave «Genna 'E Ua», Gairo Taquisara, OG	0.222	0.049-0.544
	Cave «Sa Rutta Noa», Gairo Taquisara, OG	0.013	0.001-0.061
H. sarrabusensis	Whole range / Gesamtes Verbreitungsgebiet	0	0-0.030
H. supramontis	Whole range / Gesamtes Verbreitungsgebiet	0	0-0.018

algira was detected on populations of these two species only. Furthermore, the authors tested whether there were differences in body size between the parasitized and non-parasitized individuals. This analysis was limited to the individuals from the caves where *B. algira* was detected. In mixed models, cave and species identity were included as random factors. Significance of terms was assessed using a likelihood-ratio test. Before analyses, if needed, variables were transformed using logarithms (body size, illuminance), or square-root-arcsine (humidity) to reduce skewness and improve homoscedasticity. The analysis of spatial autocorrelation was performed using SAM 4.0 (RANGEL et al. 2010); Jeffreys intervals and GLMM were computed in R using the packages lme4 and MKmisc (R DEVELOPMENT CORE TEAM 2014).

RESULTS

Parasitism by *Batracobdella algira*.- Overall, 1380 *Hydromantes* individuals from 55 cavities were inspected (Fig. 1). *Batrachobdella algira* was detected on six individuals of *H. flavus* and three *H. imperialis* from five cavities (Table 1, Fig. 1). The frequency of parasitized individuals showed significant spatial autocorrelation (Moran's $I = 0.202$, $P < 0.001$), as caves with parasites were geographically clustered (Fig. 2). Furthermore, the frequency of parasitized individuals was significantly different among species (likelihood ratio test: $\chi^2_1 = 35.2$, $P < 0.001$), as *H. flavus* showed much higher frequencies than the other species (Table 1).

No relationship was found between environmental features and the frequency of parasitism, as the frequency of parasitism was unrelated to temperature (GLMM: $\chi^2_1 = 0.04$, $P = 0.84$), humidity ($\chi^2_1 = 0.97$, $P = 0.33$) or illuminance ($\chi^2_1 = 0.07$, $P = 0.79$). Within the five cavities with *B. algira*, body length of 119 individuals was measured. Parasitized individuals were significantly larger than the non-parasitized salamanders (GLMM: $\chi^2_1 = 14.9$, $P < 0.001$, Fig. 3).

Predation.- One dead juvenile *H. sarrabusensis* was detected within the sheet web of a funnel-web spider of the genus *Tegenaria* (Fig. 4). The individual was relatively large (Total length 6.5 cm).

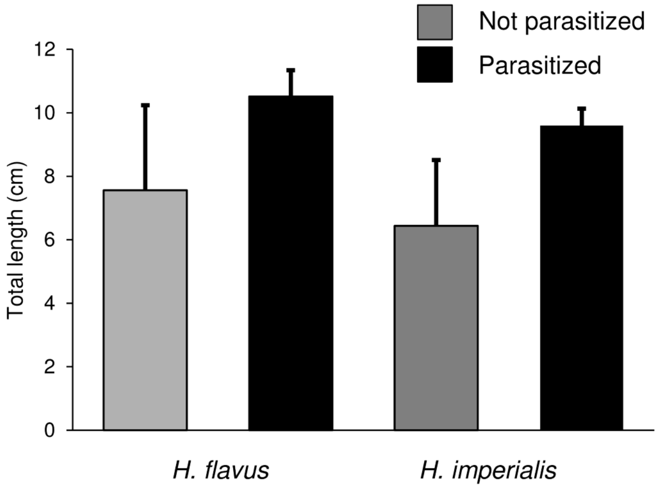

Fig. 3: Differences in body size (cm) between *Hydromantes* cave salamanders parasitized and non-parasitized by *Batracobdella algira* (Moquin-Tandon, 1846). Error bars are standard deviations.

Abb. 3: Unterschiede in der Körpergröße (cm) zwischen vom Egel *Batracobdella algira* (Moquin-Tandon, 1846) parasitierten und nicht parasitierten *Hydromantes*-Individuen. Fehlerbalken zeigen die Standardabweichung an.

DISCUSSION

A strong heterogeneity was observed in the frequency of ectoparasites on *Hydromantes* populations: the leech *Batracobdella algira* was detected on multiple populations, but its prevalence was uneven across populations, species and individuals. First of all, the prevalence of parasites showed strong spatial autocorrelation. The five populations parasitized by *B. algira* were clustered in two very small areas, whereas the majority of populations were apparently unaffected (Fig. 1). Conversely, no relationship was found between the recorded abiotic features of caves and prevalence. Spatial autocorrelation of species distribution data is often high, and can be caused by endogenous population processes determining clustering, such as dispersal and interactions between individuals, or by exogenous, autocorrelated factors determining species distribution (Wagner & Fortin 2005; Ficetola et al. 2012a). Spatial autocorrelation for the distribution of parasites or of infections is frequent (Vaughn & Taylor 2000; Fink & Kofoet 2005; Slater & Michael 2013). Indeed, other studies showed that spatial autocorrelation may be more important than environmental heterogeneity to explain the prevalence of parasites, suggesting a major role of the population-level processes of transmission of parasites occurring among hosts (Vaughn & Taylor 2000; Fink & Kofoet 2005; Slater & Michael 2013). Furthermore, spatial autocorrelation may also occur because the frequency of parasites is correlated to environmental features not explicitly considered, such as climatic variation (exogenous factors). Unfortunately, the limited number of localities with *B. algira* precluded analyses of relationships between prevalence and climate.

Prevalence was also strongly variable among species. *Hyromantes flavus* showed the highest prevalence, with overall values distinctly higher than any of the other species (Table 1), while prevalence values were relatively low and homogeneous among the

Fig. 4: Juvenile *Hydromantes sarrabusensis* Lanza, Leo, Forti, Cimmaruta, Caputo & Nascetti, 2001, within a *Tegenaria* spider web.

Abb 4: Juveniler *Hydromantes sarrabusensis* Lanza, Leo, Forti, Cimmaruta, Caputo & Nascetti, 2001, im Gespinst einer *Tegenaria* Spinne.

remaining species. For instance, *B. algira* was not detected on populations of *H. supramontis*, even if previous studies showed that this salamander can be parasitized (Lanza 1999b). Nevertheless, the observed prevalence in *H. imperialis* (0.004) fell within the 95 % CI estimated for *H. supramontis* (0-0.018, Table 1). The authors are not aware of previous studies observing parasitism of *B. algira* on *H. genei* (Temminck & Schlegel, 1838) (Lanza et al. 2006). However, during occasional surveys performed in the wet season (October 2013; Mt. Tamara, Nuxis municipality), *B. algira* was seen parasitizing one adult *H. genei* in outdoor activity (Fig. 2C), so this species might have a low prevalence, comparable to the one of *H. imperialis* (95 % CI estimated for *H. genei*: 0-0.009 Table 1).

Despite the authors' attempt to comprehensively cover the range of all the species by sampling during the dry season when the salamanders' density in hypogeous habitats is maximum, the strong spatial heterogeneity makes it difficult to generalize; thus, it is quite possible that unsampled populations show different prevalence values. Furthermore, the relationships between *Batracobdella* and *Hydromantes* life cycles should also be investigated during the autumn and winter months, when the salamanders are in the outside environment, even if their concentration and detectability might be lower.

Leeches prevalence was also heterogeneous among *Hydromantes* individuals. Within the populations with prevalence > 0, the parasitized individuals were significantly largest (Fig. 3). Unfortunately, the sample size was limited, and the interactions between *B. algira* and cave salamanders are largely unknown (Minelli 1979), therefore it is difficult to unravel the causes of such differences. Body size differences might occur because the large individuals are able to withstand the parasite load longer, or might be related to other unknown processes of the host-parasite relationship.

Information on prey-predator relationships for the recently described *H. sarrabusensis* are essentially lacking. *Tegenaria* spiders are widespread across Europe and make typical sheet webs which extend from a tubular retreat in different kind of habitats including rocky surfaces, walls and anthropic buildings (Roberts 2001). Different species have been observed at cave entrances or in hypogeous artificial habitats, where it is supposed that they can interact with other organisms dwelling in the cave twilight zone (Isaia et al. 2011). While the predation of cave spiders of the genus *Meta* on *Hydromantes* juveniles was reported for the continental salamander species, there is no information on the interaction between spiders and the Sardinian *Hydromantes* species. The present study provides the first evidence that funnel-web spiders of the genus *Tegenaria* may prey on *Hydromantes* salamanders and give insights on predators that may affect *H. sarrabusensis* populations.

Recent studies on Sardinian cave salamanders contributed to the knowledge of their evolution, taxonomy, distribution, life history, disease risk and ecological relationships (e.g., Carranza et al. 2008; van der Meijden et al. 2009; De Pous et al. 2012; Chiari et al. 2013a, 2013b; Pasmans et al. 2013; Lunghi et al. 2014), but knowledge of these highly endemic species remains far from being complete. The relationships between cave salamanders and *B. algira* show a complex pattern, with strong heterogeneity among species, populations and individuals. More studies are required to understand the host-parasite dynamics and the processes promoting differences in prevalence among species and individuals.

AKNOWLEDGMETNS

This study was allowed by the Italian Ministry of the Environment, notwithstanding of the DPR 357/97, Prot. 0040002. The authors are grateful to the many speleologists and friends that helped in cave location and during the field research: A. Acca, G. Altea, T. Buschettu, C. Carta, D. Carta, S. Coda, R. Curreli, A. R. Deidda, G. Di Falco, A. Lai, S. Manca, P Meloni, G. Merella, V. Mirimin, M. Mulargia, C. Mulas, R. Murgia, M. Murru, F. Orru, S. Papinuto, A. Pasella, P. Pilisi, G. Pirisi, Mr. Saba, S. Solinas, G. Zanda, and the Cooperativa Grotte Taquisara.

REFERENCES

Brown, L. D. & Cai, T. T. & Dasgupta, A. (2001): Interval estimation for a binomial proportion.- Statistical Science, Hayward; 16: 101-117.

Carranza, S. & Romano, A. & Arnold, E. N. & Sotgiu, G. (2008): Biogeography and evolution of European cave salamanders, *Hydromantes* (Urodela : Plethodontidae), inferred from mtDNA sequences.- Journal of Biogeography, Oxford; 35: 724-738.

Chiari, Y. & van der Meijden, A. & Mucedda, M. & Lourenço, J. M. & Hochkirch, A. & Veith, M. (2013a): Phylogeography of Sardinian cave salamanders (genus *Hydromantes*) is mainly determined by geomorphology.- PloS one, Lawrence; 7: e32332.

Chiari, Y. & van der Meijden, A. & Mucedda, M. & Wagner, N. & Veith, M. (2013b): No detection of the pathogen *Batrachochytrium dendrobatidis* in Sardinian cave salamanders, genus *Hydromantes*.- Amphibia-Reptilia, Leiden; 34: 136-141.

Crump, M. L. & Scott, N. J. (1994): Visual encounter surveys; pp. 84-92. In: Heyer, W. R. & Donnelly, M. A. & McDiarmid, R. W. & Hayek, L.-A. C. & Foster, M. S. (Eds.): Measuring and monitoring biological diversity: standard methods for Amphibians. Washington (Smithsonian Institution Press).

De Pous, P. & Speybroeck, J. & Bogaerts, S. & Pasmans, F. & Beukema, W. (2012): A contribution to the atlas of the terrestrial herpetofauna of Sardinia.- Herpetology Notes, Braunschweig; 5: 391-405.

Ficetola, G. F. & Manenti, R. & De Bernardi, F. & Padoa Schioppa, E. (2012a): Can patterns of spatial autocorrelation reveal population processes? An analysis with the fire salamander.- Ecography, Oxford; 35: 693-703.

Ficetola, G. F. & Pennati, R. & Manenti, R. (2012b): Do cave salamanders occur randomly in cavities? An analysis with *Hydromantes strinatii*.- Amphibia-Reptilia, Leiden; 33: 251-259.

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

Fink, M. & Kofoet, A. (2005): A two-dimensional stochastic model of downy mildew of radish.- Ecological Modelling, Amsterdam; 181: 139-148.

Isaia, M. & Paschetta, M. & Lana, E. & Pantini, P. & Schonhofer, A. L. & Christian, E. & Badino, G. (2011): Subterranean arachnids of the Western Italian Alps. Torino (Museo Regionale di Scienze Naturali di Torino), pp. 325.

Lanza, B. (1991): Note faunistiche sulle grotte di Samugheo e di Asuni, in particolare sul geotritone *Speleomantes imperialis*; pp. 67-72. In: Bartolo, G. & Muzzetto, G. (Eds.): Il Castello di Medusa: ambiente, leggende, grotte. Cagliari (Guido Bartolo Editore).

Lanza, B. (1999a): *Speleomantes flavus* (Stefani, 1969) - Monte-Albo-Höhlensalamander; pp. 137-144. In: Grossenbacher, K. & Thiesmeier, B. (Eds.): Handbuch der Reptilien und Amphibien Europas. Bd. 4/I. Schwanzlurche (Urodela) I (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I: *Pleurodeles*, *Salamandrina*, *Euproctus*, *Chioglossa*, *Mertensiella*) Wiesbaden (AULA-Verlag).

Lanza, B. (1999b): *Speleomantes supramontis* (Lanza, Nascetti und Bullini, 1986) - Supramontes Höhlensalamander; pp. 175-204. In: Grossenbacher, K. & Thiesmeier, B. (Eds.): Handbuch der Reptilien und Amphibien Europas. Bd. 4/I. Schwanzlurche (Urodela) I (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I: *Pleurodeles*, *Salamandrina*, *Euproctus*, *Chioglossa*, *Mertensiella*) Wiesbaden (AULA-Verlag).

Lanza, B. & Pastorelli, C. & Laghi, P. & Cimmaruta, R. (2006): 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, Trieste; 52 (Suppl.): 5-135.

LEGENDRE, P. & LEGENDRE, L. (2012): Numerical Ecology, 3rd Edition Amsterdam (Elsevier), pp. XVI, 990.

LINDSTROM, J. & REEVE, R. & SALVIDIO, S. (2010): Bayesian salamanders: analysing the demography of an underground population of the European plethodontid *Speleomantes strinatii* with state-space modelling.- BMC ecology, London; 10: 4 [Online resource available at http://www.biomedcentral.com/bmcecol/content].

LUNGHI, E. & MANENTI, R. & MANCA, S. & MULARGIA, M. & PENNATI, R. & FICETOLA, G. F. (2014): Nesting of cave salamanders (*Hydromantes flavus* and *H. italicus*) in natural environments.- Salamandra, Rheinbach; 50: 105-109.

LUNGHI, E. & MANENTI, R. & FICETOLA, G. F. (2015): Seasonal variation in microhabitat of salamanders: environmental variation or shift of habitat selection?- PeerJ, London, Corte Madera etc.; 3: e1122. DOI:10.7717/peerj.1122.

MINELLI, A. (1979): Hirudinea. Fauna d'ltalia, vol. XV. Bologna (Calderini), pp. 152.

PASMANS, F. & VAN ROOIJ, P. & BLOOI, M. & TESSA, G. & BOGAERTS, S. & SOTGIU, G. & GARNER, T. W. J. & FISHER, M. C. & SCHMIDT, B. R. & WOELTJES, T. & BEUKEMA, W. & BOVERO, S. & ADRIAENSEN, C. & ONETO, F. & OTTONELLO, D. & MARTEL, A. & SALVIDIO, S. (2013): Resistance to chytridiomycosis in European plethodontid Salamanders of the Genus *Speleomantes*.- PloS one, Lawrence; 8: e63639.

PASTORELLI, C. & LAGHI, P. (2007): Predation of *Speleomantes italicus* (Amphibia: Caudata: Plethodontidae) by *Meta menardi* (Arachnida: Araneae: Metidae); pp. 45-48. In: BOLOGNA, M. A. & CAPULA, M. & CARPANETO, G. M. & LUISELLI, L. & MARANGONI, C. & VENCHI, A. (Eds.) Atti del 6° Congresso Nazionale - Societas Herpetologica Italica. Latina (Belvedere) [Series "La Scienze" No. 6].

R DEVELOPMENT CORE TEAM (2014): R: A language and environment for statistical computing Vienna, (R Foundation for Statistical Computing) [WWW document available at https://cran.r-project.org/manuals.html].

RANGEL, T. F. L. V. B. & DINIZ-FILHO, J. A. F. & BINI, L. M. (2010): SAM: a comprehensive application for Spatial Analysis in Macroecology.- Ecography, Oxford; 33: 46-50.

ROBERTS, M. J. (2001): Field guide to the spiders of Britain and Northern Europe. London (Collins), pp. 383.

ROMANO, A. & DI CERBO, A. (2007): Leech predation on amphibian eggs.- Acta Zoologica Sinica, Bei Jing; 53: 750-754.

SALVIDIO, S. (2007): Population dynamics and regulation in the cave salamander *Speleomantes strinatii*.- Naturwissenschaften, Berlin; 94: 396-400.

SALVIDIO, S. & LATTES, A. & TAVANO, M. & MELODIA, F. (1994): Ecology of a *Speleomantes ambrosii* population inhabiting an artificial tunnel.- Amphibia-Reptilia, Leiden; 15: 35-45.

SILLERO, N. & CAMPOS, J. & BONARDI, A. & CORTI, C. & CREEMERS, R. & CROCHET, P. A. & ISAILOVIĆ, J. C. & DENOËL, M. & FICETOLA, G. F. & GONÇALVES, J. & KUZMIN, S. & LYMBERAKIS, P. & DE POUS, P. & RODRÍGUEZ, A. & SINDACO, R. & SPEYBROECK, J. & TOXOPEUS, B. & VIEITES, D. R. & VENCES, M. (2014): Updated distribution and biogeography of amphibians and reptiles of Europe based on a compilation of countrywide mapping studies.- Amphibia-Reptilia, Leiden; 35: 1-31.

SLATER, H. & MICHAEL, E. (2013): Mapping, Bayesian geostatistical analysis and spatial prediction of lymphatic filariasis prevalence in Africa.- PloS one, Lawrence; 8: 14.

VAN DER MEIJDEN, A. & CHIARI, Y. & MUCEDDA, M. & CARRANZA, S. & CORTI, C. & VEITH, M. (2009): Phylogenetic relationships of Sardinian cave salamanders, genus *Hydromantes*, based on mitochondrial and nuclear DNA sequence data.- Molecular Phylogenetics and Evolution, San Diego; 51: 399-404.

VAUGHN, C. C. & TAYLOR, C. M. (2000): Macroecology of a host-parasite relationship.- Ecography, Oxford; 23: 11-20.

VIEITES, D. R. & ROMAN, S. N. & WAKE, M. H. & WAKE, D. B. (2011): A multigenic perspective on phylogenetic relationships in the largest family of salamanders, the Plethodontidae.- Molecular Phylogenetics and Evolution, San Diego; 59: 623-635.

WAGNER, H. H. & FORTIN, M.-J. (2005): Spatial analysis of landscape: concepts and statistics.- Ecology, Washington; 86: 1975-1987.

DATE OF SUBMISSION: October 20, 2014

Corresponding editor: Heinz Grillitsch

AUTHORS: Raoul MANENTI [1]; Enrico LUNGHI [2]; Claudia CANEDOLI [3]; Morena BONACCORSI [4] & Gentile Francesco FICETOLA (Corresponding author < francesco.ficetola@gmail.com >) [3, 5, 6]

[1] Dipartimento di Bioscienze, Università degli Studi di Milano. Via Celoria 26, 20133 Milano Italy.

[2] Natural Oasis, via di Galceti, 141 59100 Prato (PO), Italy.

[3] Dipartimento di Scienze dell'Ambiente e del Territorio, e di Scienze della Terra, Università degli Studi di Milano-Bicocca. Piazza della Scienza 1, 20126 Milano, Italy.

[4] Speleo-Club Nuxis, 09010 Nuxis (CI), Italy.

[5] Laboratoire d'Ecologie Alpine (LECA), Université Grenoble-Alpes. F-38000 Grenoble, France.

[6] CNRS (Centre national de la recherche scientifique), LECA (Laboratoire d'Ecologie Alpine), F-3800 Grenoble, France.