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Batracobdella leeches, environmental features and *Hydromantes* salamanders

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ABSTRACT

Leeches can parasitize many vertebrate taxa. In amphibians, leech parasitism often has potential detrimental effects including population decline. Most of studies on the host-parasite interactions involving leeches and amphibians focus on freshwater environments, while they are very scarce for terrestrial amphibians. In this work, we studied the relationship between the leech *Batracobdella algira* and the European terrestrial salamanders of the genus *Hydromantes,* identifying environmental features related to the presence of the leeches and their possible effects on the hosts. We performed observation throughout Sardinia (Italy), covering the distribution area of all *Hydromantes* species endemic to this island. From September 2015 to May 2017, we conducted > 150 surveys in 26 underground environments, collecting data on 2629 salamanders and 131 leeches. Water hardness was the only environmental feature correlated with the presence of *B. algira*, linking this leech to active karstic systems. Leeches were more frequently parasitizing salamanders with large body size. Body Condition Index was not significantly different between parasitized and non-parasitized salamanders. Our study shows the importance of abiotic environmental features for host-parasite interactions, and poses new questions on complex interspecific interactions between this ectoparasite and amphibians.

1. Introduction

Leeches, with around 700 different species, represent a specialized group of annelids widespread across the world (Sket and Trontelj, 2008). One particular group, the order Rhynchobdellida, includes leeches lacking jaws and teeth, which are commonly feeding on vertebrate blood (Sawyer, 1981; Moser et al., 2009). Because of their particular feeding strategy, research on hematophagous leeches is very active, as they may cause both negative and positive effects on hosts' health (Nehili et al., 1994; Wells, 2007; Al-Khleif et al., 2011; Elliot and Kutschera, 2011; Brisola Marcondes et al., 2017). Hematophagous leeches are able to parasitize a wide range of vertebrates and, in some circumstances, may be specialized on specific taxa (Sawyer, 1981). After attachment to their host, leeches can induce a chemical reaction to attenuate the host immune response (Salzet et al., 2000; DuRant et al., 2015). This particular feeding strategy can promote host vulnerability to further infections (Rigbi et al., 1987; Daszak et al., 1999). However, the biology of many leech species and the interspecific interactions between leeches and their hosts remain poorly studied.

Interactions between leeches and amphibians have been described several times (Trauth and Neal, 2004; Beukema and de Pous, 2010; Tiberti and Gentilli, 2010). Leeches feed on Amphibian larvae, adults and possibly eggs as well (Howard, 1978; Mock and Gill, 1984; Veith and Viertel, 1993; Romano and Di Cerbo, 2007; Wells, 2007), and may contribute to a species decline (Stead and Pope, 2010). Interactions between leeches and amphibians seem to be opportunistic (Elliot and Dobson, 2015). Generally, leeches use amphibians as food source without leading to host death (Getz, 2011; Rocha et al., 2012). Nevertheless, some studies documented that the leech *Hirudo medicinalis* represented a serious threat for local amphibian populations (Hoffmann, 1960; Wilkin and Scofield, 1990; Merilä and Sterner, 2002; Elliot and Dobson, 2015), even though the strong negative effects of leeches may be enhanced by environmental changes (Berven and Boltz, 2001; Ayres and Comesaña Iglesias, 2008).

Interactions between amphibians and leeches were mostly observed in freshwater environments, while data from terrestrial environments

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remain scarce (Rocha et al., 2012). During several herpetological activities performed in Madagascar, Rocha et al. (2012) documented the parasitism of *Malagabdella* leeches on at least four species of Mantellid tree frogs. In their report, authors highlighted the scarcity of observations carried out on leeches-amphibians interaction in terrestrial environments. Very few data exist on the interactions between the *Hydromantes* terrestrial salamanders (for furhter discussion on nomenclature see Wake, 2013) and the leech *Batracobdella algira*. Salamanders of the genus *Hydromantes* are amphibians of high conservation interest, including species endemic to very small areas (Lanza et al., 2006). While environmental and human factors affecting their distribution have been investigated, few studies have been devoted to the interaction with pathogens and parasites (Pasmans et al., 2013). Until now, reports of interactions between leeches and *Hydromantes* species is limited to a list of records on the occurrence of this ectoparasite, with little information on interspecific interactions, on ecological factors that can promote parasitism, and on their potential impacts (Lanza et al., 2006; Manenti et al., 2016).

In this paper, we provide a brief overview of both *Hydromantes* species and *B. algira*, followed by new data on the ecology of *B. algira*, reporting for the first time information related to its effects on the Sardinian *Hydromantes*. In particular, we tested whether leeches may be detrimental to salamanders' body condition.

2. Materials and methods

2.1. The ectoparasite: Batracobdella algira

After the first description of *Batracobdella algira* by Moquin-Tandon (1846), few studies dealt with this species, mostly describing development and distribution. *B. algira* is a greenish dorsoventrally flattened leech belonging to the group of jawless leeches (Ben Ahmed et al., 2015). It is mostly distributed in the Mediterranean basin (Minelli, 1979; Nesemann, 1991; Ben Ahmed et al., 2015), where it parasitizes several species of amphibians (Ben Ahmed et al., 2015; Manenti et al., 2016); it is the only known ectoparasite of the Sardinian *Hydromantes* salamanders (Lanza et al., 2006). This leech has also been identified as a vector of some pathogenic micro-parasites, such as the protists *Lankesterella* and the protozoan *Trypanosoma* (Jiménez Sánchez, 1997). *Batracobdella algira* has been reported for different freshwater habitats (both lentic and lotic) and was even found in subterranean ones, generally attached to hosts like *Discoglossus* and *Pelophylax* frogs (Minelli, 1979). In both surface and subterranean terrestrial habitats it was found parasitizing *Hydromantes* species in different localities of Sardinia (Mertens, 1929; Manenti et al., 2016). However, ecological observations on *B. algira* remain scarce, and almost no investigations have been carried out on the factors affecting its distribution and phenology (Nesemann, 1991). Breeding occurs only once in life: adult individuals ($\geq$20 mm) mostly breed during spring, producing several eggs which hatch after ~3 weeks (Ben Ahmed et al., 2009; Romdhane et al., 2015). Parents provide pre- and post-hatch parental care and die 2–3 weeks after the hatch (Romdhane et al., 2015).

2.2. The hosts: Sardinian Hydromantes salamanders

Sardinian *Hydromantes* (*H. flavus*, *H. supramontis*, *H. imperialis*, *H. sarrabusensis*, *H. genei*) are lungless salamanders endemic to Sardinia (Sillero et al., 2014), showing allopatric distributions (Chiari et al., 2012). Salamanders of the genus *Hydromantes* are mostly epigean species which exploit a variety of different terrestrial habitats (Lanza et al., 2006; Manenti, 2014; Costa et al., 2016). However, these salamanders have a narrow thermal niche and require high humidity (Spotila, 1972). They are strongly influenced by environmental conditions (Lunghi et al., 2016a), and retreat underground when external conditions become too hot and dry (Ficetola et al., 2012; Lunghi et al., 2014a, 2015a).

These salamanders have direct development and mostly reproduce in underground habitats (Lanza et al., 2006; Lunghi et al., in press). Females lay about 6–14 eggs in caves characterized by stable microclimate and low predation pressure (Lunghi et al., 2014b, 2015b; Salvidio et al., 2017); the mother attends and protects eggs and hatchlings for more than nine months (Oneto et al., 2010, 2014). The Italian IUCN Red List classifies the majority of the Sardinian *Hydromantes* (*Speleomantes*) species as vulnerable to extinction risks (Rondinini et al., 2013); all these species therefore deserve protection.

2.3. Surveys

From September 2015 to May 2017, we performed surveys throughout the known distribution range of the Sardinian *Hydromantes* species, exploring underground environments where individuals are easily detectable (Ficetola et al., 2012; Lunghi et al., 2016b). To prevent the spread of pathogens (Stegen et al., 2017), salamanders were handled using disposable gloves, and instruments were disinfected with bleach before every survey. We searched for cave salamanders using Visual Encounter Surveys (Crump and Scott, 1994). Salamanders were hand-inspected in order to detect *Batracobdella algira*. When leeches were observed, we first recorded the salamander body part at which the ectoparasites were attached and then leeches were removed using forceps. For each leech we estimated the body size, after relaxation, ascribing individuals to one of the four identified categories: Small: up to 5 mm; Medium: 5–10 mm; Large: 10–20 mm; X-Large > 20 mm). Leech relaxation was induced by warming up its body, as a sudden strong heat induces a temporary thermal shock in ectotherm species (Lunghi et al., 2016a). After having hold the leech in hands for 30 s, it was placed on a plasticized millimeter paper sheet and estimated body size during its inactivity. When feasible, we recorded the linear distance from the cave entrance (measured by KKmoon RZE-70 laser meter, accuracy ~2 mm; hereafter depth) to salamander capture point, snout-vent length (SVL) and total length (TL) (using a transparent plastic rule) and weight to 0.01 g (salamanders were placed in a plastic container and weighted with a portable digital scale G&G TS-B + G 100 g/0,01 g) after removing leeches. Salamander age class was estimated on the basis of SVL, considering as juveniles all individuals with SVL < 45 mm (< 40 for *H. genei*) (Lanza et al., 2006). Adults were sexed on the basis of the presence/absence of male secondary sexual characters (mental gland and premaxillary teeth) (Lanza et al., 2006). Body Condition Index (BCI) was estimated using the Residual Index, calculated through a regression analysis using log-transformed weight and the total length of salamanders (i. e., logBM and logBL) (Băncilă et al., 2010; Labocha et al., 2014). We extracted the residual of each individual to obtain the difference between observed and predicted body mass of salamanders for a given size. We considered the total length because tail is usually used as energy storage (Scott et al., 2007). When dripping water was present at *H. flavus* sites, we measured pH, permanent hardness, and NO_2 using multi-parameter colorimetric strips (Sera 5in1 Quick test). Because dripping water was not constant across caves and time, we calculated the average value for each parameter. After measurements, some leeches were let attach again to their previous host, while 17 specimens were put in pure ethanol and preserved in the collection of the Natural History Museum of the University of Florence, section of Zoology "La Specola" for future genetic analyses.

2.4. Statistical analyses

For each site where leeches were observed, we calculated the average prevalence of parasitized salamanders. For the three species for which we recorded "depth", we used a binomial generalized linear mixed model (GLMM) to test if the presence of leeches on salamanders was related to depth, age class and sex of the host. In a first GLMM we used as dependent variable the presence/absence of the ectoparasite, with both salamander age class and depth as independent variables; we

used the year, month of survey, cave identity and host-species as random factors. We did not consider the size of salamanders (total length) as it was strongly correlated with age class ($r = 0.85$, $P < .001$). We repeated the analysis replacing age class with sex to test if adult males and females are parasitized with the same prevalence. Before using the salamander BCI in statistical analyses, we tested whether the Residual Index shows any correlation with both salamanders' total length and species. We ran a GLMM using BCI as dependent variable, salamander total length and species as independent variables, and cave identity and both month and year of survey as random factors. Moreover, we added as a further independent variable the interaction between species and salamander total length, to avoid biases caused by eventual interspecific differences in allometric growth (Adams and Nistri, 2010). We then tested whether leech presence is related to salamander BCI. To avoid pseudoreplications, we only considered measures from marked individuals and from just one survey per cave (Lunghi and Veith, 2017). We ran a binomial GLMM to evaluate the BCI differences between parasitized and non-parasitized salamanders; the presence/absence of leeches was used as dependent variable, salamander BCI as independent variable and cave identity, year, month of survey and host-species as random variables. Given that the number of leeches parasitizing a single salamander was variable (see results), we ran a GLMM considering only parasitized salamanders to assess if BCI varied in relation to the number of leeches attached to the host. We used leech abundance (log-transformed) as dependent variable, while salamander BCI and leech size were independent variables; year, month of survey, cave identity and host-species were used as random factors. For the caves where *Hydromantes flavus* was present, we merged data from all surveys and used a binomial generalized linear model to relate the presence of leeches to water pH, hardness and NO_2. We used the presence of leeches inside caves as dependent variable (if the leeches were observed at least once, they were considered present), while the average water parameters were used as independent variable.

3. Results

We performed 162 surveys in 26 different caves (average 6.38 ± 5.1 surveys per cave), overall exploring 1366 m of underground environments, and examining 2629 salamanders (Table 1); we measured and weighed 513 salamanders (*Hydromantes flavus*: $N = 287$; *H. supramontis*: $N = 66$; *H. imperialis*: $N = 76$; *H. sarrabusensis*: $N = 84$) and, for them, we calculated BCI. The BCI of salamanders was not related to species, salamander length nor to the interaction between species and length (all $P > .05$).

We observed 131 individuals of *Batracobdella algira* (Small = 35, Medium = 16, Large = 31, X-Large = 43) in nine caves inhabited by four *Hydromantes* species (*H. flavus* = 5; *H. supramontis* = 1; *H. imperialis* = 2; *H. sarrabusensis* = 1) (Table 1); 92 salamanders were found with parasites (5 juveniles, 43 females, 31 males and 13 unsexed adults). About one-third of the observed leeches (32.82%) were adults, as only our largest category (XL) corresponds with good confidence to the size of sexually mature leeches (Romdhane et al., 2015). Almost all the observed leeches (130) were parasitizing *Hydromantes* (Fig. 1a), while one was found attached to a stone located 6.1 m from the cave entrance (Fig. 1b). Leeches were found attached to the hosts' tail ($N = 12$), limbs ($N = 24$), back ($N = 14$), belly ($N = 7$), flanks ($N = 36$) and head ($N = 11$). Infection prevalence varied among populations from < 1% to > 30% (chi-squared test; $\chi^2_3 = 19.475$, $P < .001$) (Table 1).

The presence of leeches was significantly higher in adult salamanders ($\chi^2 = 17.44$, df = 1, $P < .001$) and in individuals close to the cave entrance ($\chi^2 = 715.37$, df = 1, $P < .001$). Analysis carried out on adults only showed significant relationships with both sex ($\chi^2 = 98619.26$, df = 1, $P < .001$) and depth ($\chi^2 = 310.28$, df = 1, $P < .001$); leeches were more frequently found on females and in adults close to the cave entrance.

Overall, BCI was not significantly different between parasitized and non-parasitized salamanders ($\chi^2 = 0.33$, df = 1, $P = .568$) (Fig. 2). Parasitized salamanders carried in average 1.57 ± 0.23 leeches (max: 11). Among parasitized salamanders, those with higher parasite load showed lower BCI ($F_{1,80.41} = 7.21$, $P = .009$), while the smallest leeches were found to be more aggregated than the largest ones ($F_{3,82.95} = 46.94$, $P < .001$).

Among caves inhabited by *H. flavus*, the presence of leeches was significantly higher in sites with high water hardness ($\chi^2 = 7.59$, df = 1, $P < .004$). We found no significant correlation with other water parameters (for both pH and NO_2, $P > 0,5$).

4. Discussion

Our study adds new information on the ecology of *Batracobdella algira* and on its relationships with Sardinian *Hydromantes* species, including the first description of *B. algira* on *Hydromantes sarrabusensis*. Because Sardinian *Hydromantes* species show an allopatric distribution (Chiari et al., 2012), there are likely distinct genetic differences among the leeches associated with each species, unless other hosts, such as the frog *Discoglossus sardus*, contribute to the widespread distribution of *B. algira*. However, no information exists on the gene flow between leech populations. It remains to be determined if leeches parasitizing the different *Hydromantes* species are genetically isolated and have greater genetic diversity than previously assumed.

Manenti et al. (2016) reported a strong spatial autocorrelation of caves in which *B. algira* was present, and also identified *H. flavus* as the species showing the highest frequency of parasitized salamanders. Considering only the distribution of *H. flavus* (limited to a single massif; see Lanza et al., 2006), we observed a particular distribution of leeches. Caves in which we observed *B. algira* were located along the major longitudinal length of the massif, mostly avoiding lower altitudes (min and max recorded altitude, 267 and 1029 m a.s.l.) (Fig. 3). The presence of *B. algira* decreased with altitude, where evergreen forests are replaced by sporadic xeric shrubs (AA. VV., 2006). Leech presence may be higher in forests because this environment can buffer seasonal environmental conditions and increase humidity, or because of other unidentified features affecting leeches distribution.

In this study we identified one cave parameter related to the presence of leeches: *B. algira* was associated with caves where the hardness of the dripping water was high. To our knowledge this is the first report concerning the relationship between *B. algira* and physical/chemical environmental features. Hard water can be an indicator of water flowing through in karst systems where water is actively altering rock rich in $CaCO_3$ (Culver and Pipan, 2009). We speculate that water flowing through active karst systems might play a role in the underground diffusion of *B. algira*.

Where *Hydromantes* salamanders first contact leeches ectoparasites is still unknown. Salamander flanks and limbs seem to be the body parts where leeches more frequently attached (chi-squared test; $\chi^2_5 = 38.03$, $P < .001$), while they are rarely observed on the belly. We suspect that leeches seldom remain on the ventral body parts of the salamanders, which generally remain in contact with the substrate (Bicca-Marques and Calegaro-Marques, 2016). We observed a higher proportion of parasitized salamanders in cave areas near the surface where prey availability for salamanders is higher (Ficetola et al., 2013; Manenti et al., 2015; Lunghi et al., 2017). However, it is not clear if salamanders encounter leeches more frequently in areas close to the cave entrance, or if the added burden of supporting the ectoparasite results in infected hosts investing more time in foraging. (Ficetola et al., 2013). We found a higher infection prevalence among female salamanders. Nevertheless, this correlation may be a function of body size, as female *Hydromantes* tend to be larger, there is a positive correlation between body size and leech prevalence (Lanza et al., 2006) and, indeed, previously was assessed that bigger salamanders can be the most parasitized by leeches (Manenti et al., 2016).

Table 1
Data of each monitored cave inhabited by *Hydromantes* species. In this table are shown: Coordinates; Elevation (meters a.s.l.); Length of the explored cave area (meters); average values (± ES) of the water parameters (pH, permanent Hardness and NO_2); number of the examined salamanders; total number of leeches observed; average frequencies (± ES) of the parasitized salamanders. * indicate one single data collection.

Cave	*Hydromantes* species	Coor_X	Coor_Y	Elevation	Explored area	pH	Hardness (°dH)	NO_2 (mg/L)	Examined salamanders	Total leeches	Parasitized salamanders (%)
Cave_fla1	*H. flavus*	9.59	40.49	267	147	7.8 ± 0.6	20	30 ± 2	112	67	31.06 ± 8.02
Cave_fla2	*H. flavus*	9.52	40.46	1029	105	7.87 ± 0.53	18.33 ± 1.67	20.00 ± 1.53	675	33	5.94 ± 2.01
Cave_fla3	*H. flavus*	9.61	40.51	116	99	7.73 ± 0.35	16.67 ± 3.33	101.67 ± 7.64	17	0	
Cave_fla4	*H. flavus*	9.61	40.51	116	15	8.00 ± 0.4	20.00	10.00 ± 1.33	12	0	
Cave_fla5	*H. flavus*	9.64	40.56	777	12				23	12	28.33 ± 13.84
Cave_fla6	*H. flavus*	9.61	40.51	107	18	7.60 ± 0.4	17.50 ± 2.5	10.00	2	0	
Cave_fla7	*H. flavus*	9.65	40.54	265	54				5	0	
Cave_fla8	*H. flavus*	9.68	40.58	94	48	8.00 ± 0.4	18.33 ± 1.67	33.33 ± 1.67	22	0	
Cave_fla9	*H. flavus*	9.62	40.55	902	9				32	3	9.37*
Cave_fla10	*H. flavus*	9.64	40.57	954	18				39	0	
Cave_fla11	*H. flavus*	9.53	40.47	679	45	8.4*	20*	0*	58	2	1.14 ± 1.14
Cave_fla12	*H. flavus*	9.68	40.56	116	24				7	0	
Cave_sup1	*H. supramontis*	9.34	40.08	937	90				40	5	10*
Cave_sup2	*H. supramontis*	9.33	40.17	142	30				170	0	
Cave_sup3	*H. supramontis*	9.29	40.15	206	36				191	0	
Cave_sup4	*H. supramontis*	9.36	40.19	200	150				81	0	
Cave_imp1	*H. imperialis*	8.57	39.53	180	33				304	0	
Cave_imp2	*H. imperialis*	9.27	39.51	833	39				170	3	2.14 ± 1.50
Cave_imp3	*H. imperialis*	9.27	39.51	820	72				25	1	3.33 ± 3.33
Cave_sar1	*H. sarrabusensis*	9.26	39.17	765	6				261	5	0.38 ± 0.26
Cave_sar2	*H. sarrabusensis*	9.26	39.17	780	6				26	0	
Cave_sar3	*H. sarrabusensis*	9.26	39.17	718	6				3	0	
Cave_gen1	*H. genei*	8.25	39.21	200	36				23	0	
Cave_gen2	*H. genei*	8.31	39.15	412	139				266	0	
Cave_gen3	*H. genei*	8.44	39.07	234	117				47	0	
Cave_gen4	*H. genei*	8.31	39.15	440	12				18	0	

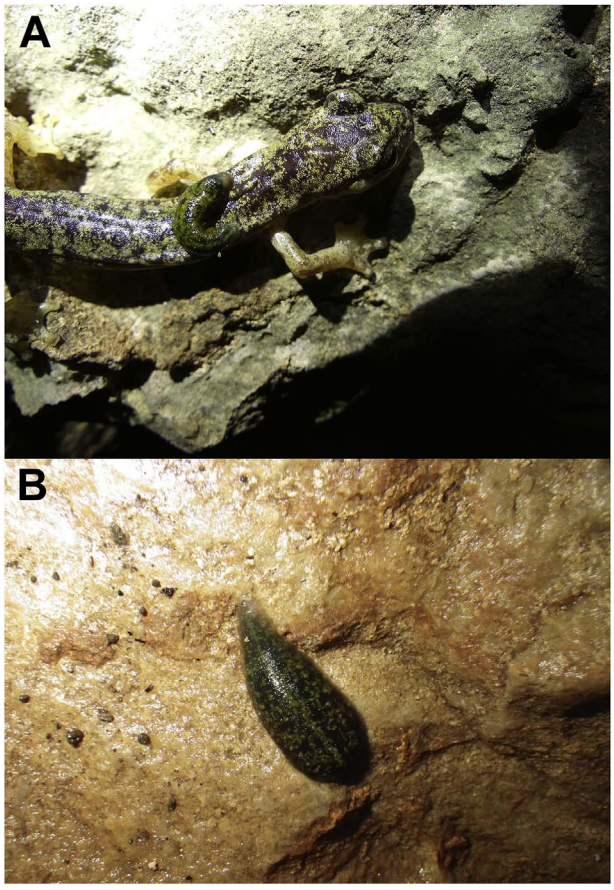

Fig. 1. Two adults of *Batracobdella algira*. (A) parasitizing *Hydromantes flavus*, (B) attached to a stone.

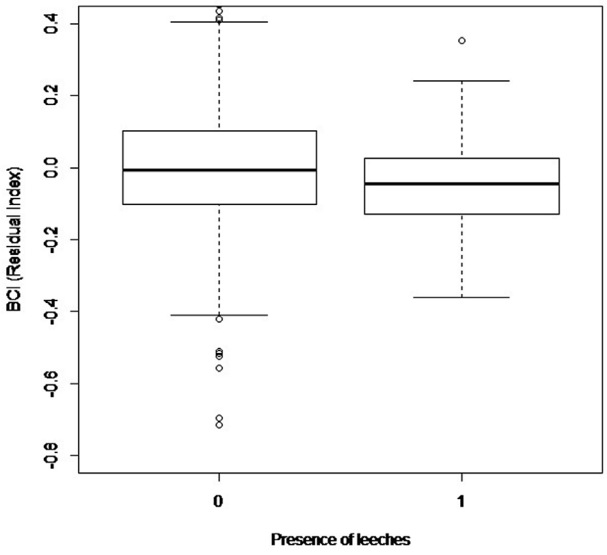

Fig. 2. Boxplots showing BCI of *Hydromantes*. On left side (0) BCI of salamanders free from leeches, while on the right side (1) BCI of the parasitized salamanders.

The BCI of parasitized salamanders was not significantly different from non-parasitized ones (Fig. 2) although we observed lower BCI in the individuals with more parasites. Several processes might explain why individuals with many small parasites simultaneously attached had the lowest BCI. For example, the aggregation of many small, rapidly growing leeches might be feeding more frequently and consuming more resources. It is also possible that individuals carrying several small leeches might actually have been fed on by the delivering mother leech, who not only fed but also delivered offspring to the host prior to dropping off and dying (Ben Ahmed et al., 2009; Romdhane et al., 2015). The host-parasite relationship is generally negative for the host, which is going to suffer the cost of the parasite nourishment, activity

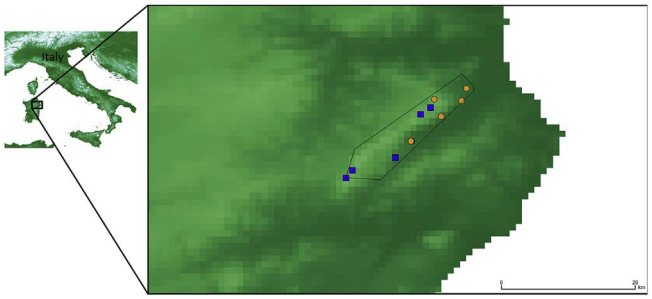

Fig. 3. Distribution of studied sites for *Hydromantes flavus*. The map shows the altitude levels of the studied area; the polygon represents the whole distribution area of *H. flavus*. Yellow circles indicate sites where *B. algira* was not observed, while green squares indicate sites in which the leech was present. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

mostly determined by both host tolerance and parasite invasiveness (DuRant et al., 2015; Budischak et al., 2017; Fleskes et al., 2017; Miller and Cotter, 2017). However, our observations were limited to the salamanders body condition, and future investigations are needed to explore other aspects of this host-parasite relationship such as the possible transfer of pathogenic micro-parasites, changes in the host immunologic response and in behavior.

5. Conclusion

The present study provides new information on the ecology of *Batracobdella algira* (Jiménez Sánchez, 1997; Ben Ahmed et al., 2015; Romdhane et al., 2015; Manenti et al., 2016). Given the wide distribution of this species (Ben Ahmed et al., 2015), further research considering the whole range of the species will be needed to better understand its ecology, in order to assess whether local adaptations are occurring. Our first assessment on *B. algira-Hydromantes* relationship did not find strong negative effects on salamanders body condition. However, further studies will serve to better understand the dynamics occurring between leeches and cave salamanders, especially the impact of *B. algira* on the long-term performance of *Hydromantes sp.*

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijppaw.2018.01.003.

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