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The Yellow Spot Pattern of Salamander (*Salamandra infraimmaculata*) in Various Habitats at the Southern Border of Its Distribution in Israel

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Abstract

The present study describes the different color-pattern phenotypes of yellow spots on the black back of *Salamandra infraimmaculata* in various habitats at the southern border of its distribution in Israel. At Tel Dan, we photographed 454 salamanders in moist habitats where water flows year round; 100 of these were sampled to measure the percentage of yellow and black color on the back, and the number of spots on the head. At Kibbutz Sasa, 201 salamanders were photographed, of which 62 were sampled for the measurements. In Kibbutz Yehiam, 200 salamanders were photographed, and 60 were sampled for the measurements. At all sites, about a third of the salamanders were photographed more than once. For all three populations, yellow spots on the salamander back were found in one row, two rows or scattered. For two indices (proportion of yellow/black and number of spots on the head), the Dan population (under wet, running water all year round conditions) differed from the two other populations of salamanders (under semi-arid mountain conditions). The number of yellow spots on the head of the salamanders in the three populations varied from 1 to 7. In all populations, 4 spots pattern was the most common. In the Dan population, there were significantly more salamanders with 1 to 3 spots on their head than in the Sasa or Yehiam populations. No difference was found in the number of head spots for Sasa vs. Yehiam salamanders. The percentage of yellow on the black back was significantly larger for the Dan salamanders vs. the two other populations. The

main question examined was whether there is an effect of the habitat conditions in isolated populations on the spot pattern on the salamander back. The answer is positive and is supported by previous studies.

Keywords

Salamandra infraimmaculata, Color-Pattern, Habitats, Spots, Yellow

1. Introduction

The genus *Salamandra* Garsaut, 1764, belonging to the terrestrial Urodeles, is widely distributed in Europe and reaches, at its southern border, North Africa and the Middle East [1] (**Figure 1**). The systematics of this genus has undergone many changes, from its division into subspecies to its generally agreed upon division into six species [2] [3] [4]. Due to the large variation in salamander types, classification into species and subspecies is complicated and ambiguous. Some of the six species of the genus *Salamandra* are defined as subspecies in their wide distribution in Europe, North Africa and Asia, including Asia Minor and Israel [4] (**Figure 1**).

This division, and the large variation in colored spot patterns on the back among populations, sometimes make it difficult to determine the species, or subspecies, with any certainty [1]. Steinfartz *et al.* (2000) [2] suggested classifying the species in Europe, North Africa and The Levant into six groups, based on their mitochondrial DNA: *S. salamandra*, *S. infraimmaculata*, *S. corsica*, *S. atra*, *S. lanzai* and *S. algira.* Accordingly, the fire salamander (*S. infraimmaculata*) is located in Israel and *S. algira* in North Africa [9] [10]. Karahisar *et al.* [11] studied the differences between the fire salamander in Israel (*S. infraimmaculata*) and in Turkey. For comparison, salamanders in Israel were taken from Mount Carmel. Those researchers gave the first description of the karyotype of the fire salamander in Israel, finding 12 pairs of chromosomes [11].

In Israel, where the salamanders are located on the southern diaspora border, there are isolated populations which have survived in the north part of the country. Due to the different environmental conditions in their habitats, a comparison of these populations could be interesting [1] [6] [12] in terms of morphological [13] and physiological [14] [15] [16] variations, larval growth [17], and genetic variations [5] [18] [19] [20].

Salamandra infraimmaculata in Israel shows a fragmented distribution in various types of habitats, including mountain ranges that are 130 to more than 1000 m above sea level [1] [21]. Due to polymorphic coloration and diversity of reproductive modes, it is very difficult to separate the species according to these variables [5]. Genetic studies have focused mainly on tadpoles in various populations. Samples of salamanders after metamorphosis usually consist of relatively small numbers, and determining the relationship between the morphological

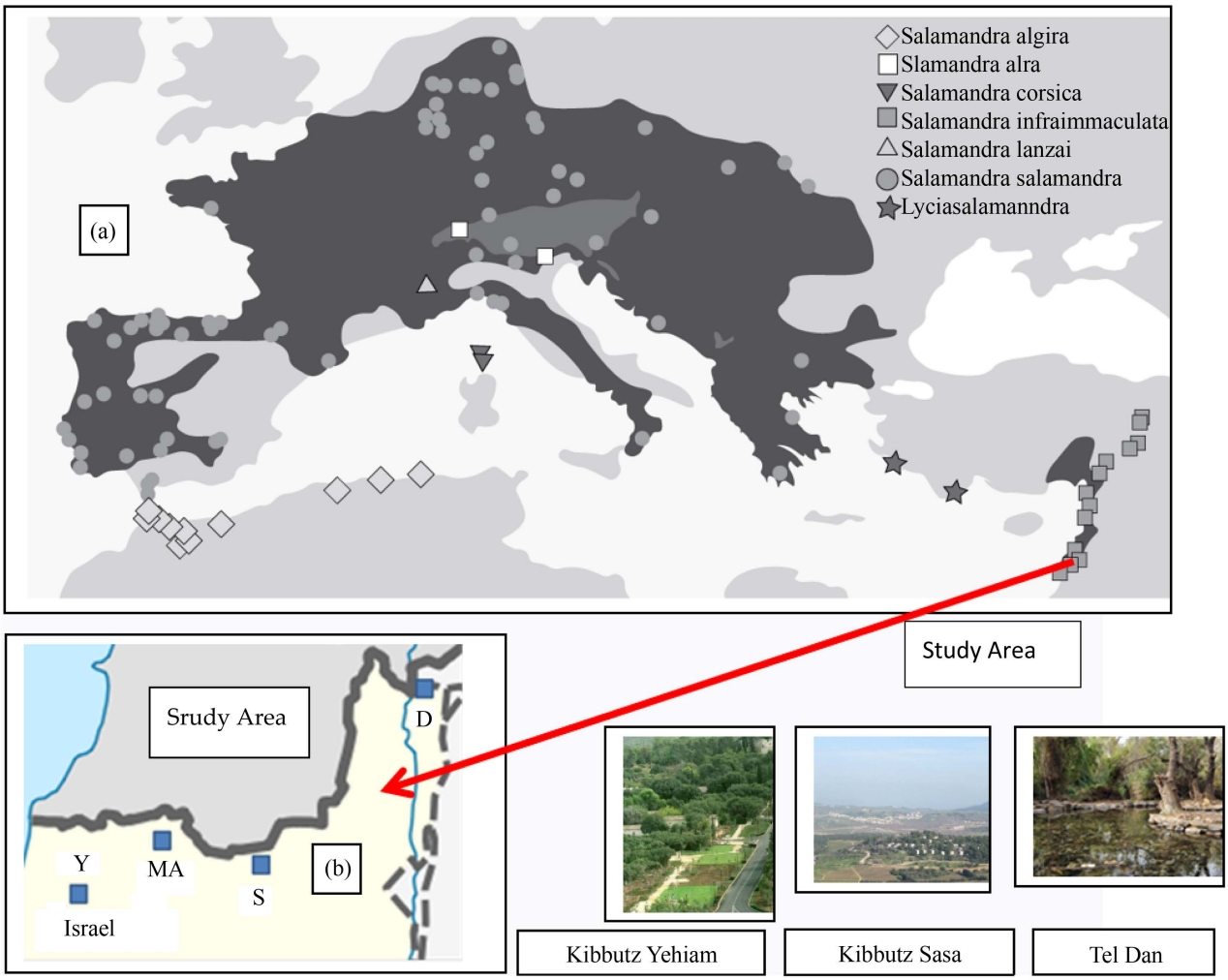

Figure 1. Distribution of the genus *Salamandra* in Europe [2] [5]. (a) *Salamandra infraimmaculata* distribution. (b) study area [6]. Locations of the studied populations (Study sites) appear on this map: Kibbutz Sasa (33.0277E, 35.3964N; 890 m above sea level [m.a.s.l.]) [7], Kibbutz Yehiam (32.9960E, 35.2227N, 350 m m.a.s.l.), Tel Dan (33.2485E, 35.6535N; 190 m.a.s.l.) [8].

variance and molecular variability of several markers is problematic [5] [21] [22] [23]. The color pattern of *S. salamandra* has been relatively well-described in many articles (see for review [24]). The arrangement of the colored segments on the black back varies from two yellow lines on *S. terrestris* to variously shaped yellow spots on *S. salamandra*, and there is great variability among individual details, including the mixing of these two models [5] [24]. The differences between the color-pattern phenotypes in *S. salamandra* and *S. terrestris* are very large—not only between species, but also among individuals. In Israel, where the species *S. infraimmaculata* lives under the most extreme conditions, and where the differences between habitats may affect the variability of the populations, morphological differences were studied in several areas (Tel Dan, Upper Galilee [Mount Meron], Western Galilee, and Mount Carmel), based on a relatively small number of adult salamanders [1] [3] [13]. The results of these studies of *S. infraimmaculata* in Israel support the hypothesis that the environmental condi-

tions of the different habitats have an effect on the morphology and physiology of the populations. The body size of the salamanders from the Tel Dan population was found to be significantly smaller than that of salamanders from the other areas. Nevertheless, no difference were found in mean body measurements. The standard deviation, calculated as described in [3] [13], covered the means of the other three areas (Upper Galilee, Western Galilee and Mount Carmel), where the habitats are similar. These differences are also supported by studies of the genetic differences between the Tel Dan population and salamanders in other parts of Israel, living under more extreme ecological conditions [1] [20]. In the studies on the pattern of yellow spots on the back of salamanders between different regions of Israel (Tel Dan, Upper Galilee, Western Galilee and Mount Carmel), no clear differences have been found between the regions [3]. The present study describes the different color-pattern phenotypes on the black back of *S. infraimmaculata* in various habitats: Tel Dan (under stable wet conditions throughout the year), Kibbutz Sasa in Mount Meron region and Kibbutz Yehiam in the Western Galilee (both under semi-arid changing conditions). It further compares the color-pattern phenotypes of *S. infraimmaculata* to those of other salamander species [5] (**Figure 1**).

2. Material and Methods

The area of study includes one of the highest mountains in Israel, Mount Meron, kibbutz Sasa and Kibbutz Yehiam both of them are semi- arid habitats (**Figure 1**). The winter is longer and rainier than in most of the country, with annual precipitation reaching 1000 mm. The summer is comparatively dry, no rain. The Tel Dan Nature Reserve is in the northeast of the country; it lies among hills and is bounded by mountains to the north, but it is only 180 m above sea level. The area as a whole is characterized by numerous spring-fed streams running water.

Field observations, samples and photographs of salamanders for coloration pattern determination were from populations that have been intensively studied and for which all other parameters (body weight and length) have been well-described [1]. Of all the salamanders that were photographed, only the salamanders whose photography had a clear difference between black and yellow were sampled for this study. The salamander individuals were characterized one by one by their unique pattern of yellow spots on their head and back. Observations were conducted on rainy nights when the salamanders are active on the surface in the three habitats and are easy to follow [7] [25]. Salamanders were studied in Tel Dan [8], Kibbutz Sasa [7], and Kibbutz Yehiam [1] [26] (**Figure 1**). In the Tel Dan population (Dan), 454 salamanders were photographed, of which 100 were sampled to measure the percentage of yellow and black coloring on their back, and for counting their head spots. In Kibbutz Sasa (Sasa), 201 salamanders were photographed, and 62 were sampled for the measurements. In Kibbutz Yehiam (Yehiam), 200 salamanders were photographed, of which 60

were sampled for the measurements. In all locations, about a third of the salamanders were photographed more than once. The ratio of black to yellow color was calculated using the formula [Yellow/(Yellow + Black)] × 100. Each salamander was photographed and the image was copied into Word Object Design software. The background of the image has become white. The image was copied to the software Cool PHP Tools Image Color Extract Tow Colors. With its help the percentage of yellow on the back of the salamander was determined.

Statistical Analysis

All statistical processing was done using JAMOVI 2.0.0 software. To test the significance of the results, we used a one-way analysis of variance (ANOVA) and a post-hoc Student t-test for each pair of averages without correction, based on multiple tests. The number of salamanders with different color patterns in the various populations was estimated by Z-proportion analysis. The significance threshold was $p < 0.05$. Statistical processing was done with AMOVI software, using χ^2, t-test, Z-test and ANOVA.

3. Results

The color of the spots differed among individual salamanders in the same population, with various different shades of yellow. The different patterns of the yellow spots on the backs of the salamanders (one row, two rows or scattered) in the different populations (Dan, Sasa and Yehiam) are shown in **Figure 2**. The size and shape of the spots differed; some were round and others had different shapes. It was difficult to discern the differences in spot patterns between the three populations (**Figure 2**). The patterns of yellow spots on the backs of the salamanders (one row, two rows and scattered) between the habitats (Dan, Sasa and Yehiam) are represented in **Figure 3**. Differences between the habitats were not significant (χ^2, t-test, Z-test and ANOVA portion, $p > 0.05$).

For two indices (proportion of yellow/black and the number of spots on the head), the Dan population differed from the two other populations of salamanders (χ^2, t-test, Z-test and ANOVA, $p < 0.05$). The ratio between yellow and black differed significantly between the Dan population and the other two populations, with the percentage of yellow on the back being significantly larger for the former salamanders (**Figure 4**) (χ^2, t-test, Z-test and ANOVA, $p < 0.05$).

Various numbers of yellow spots, from 1 to 7, were found on the heads of the salamanders in the three populations (**Figure 5**). In all populations, there were more salamanders with 4 spots than with any other number of spots. However, in the Dan population, there were significantly more salamanders with 1 to 3 spots than in Sasa or Yehiam (ANOVA, $p < 0.05$) (**Figure 5**). No difference was found in the number of yellow spots on the head between Sasa and Yehiam salamanders (ANOVA, $p > 0.05$).

The color of the spots varied from light yellow (yellow with white hue) to yellow with a red hue in all populations studied (**Figure 6**).

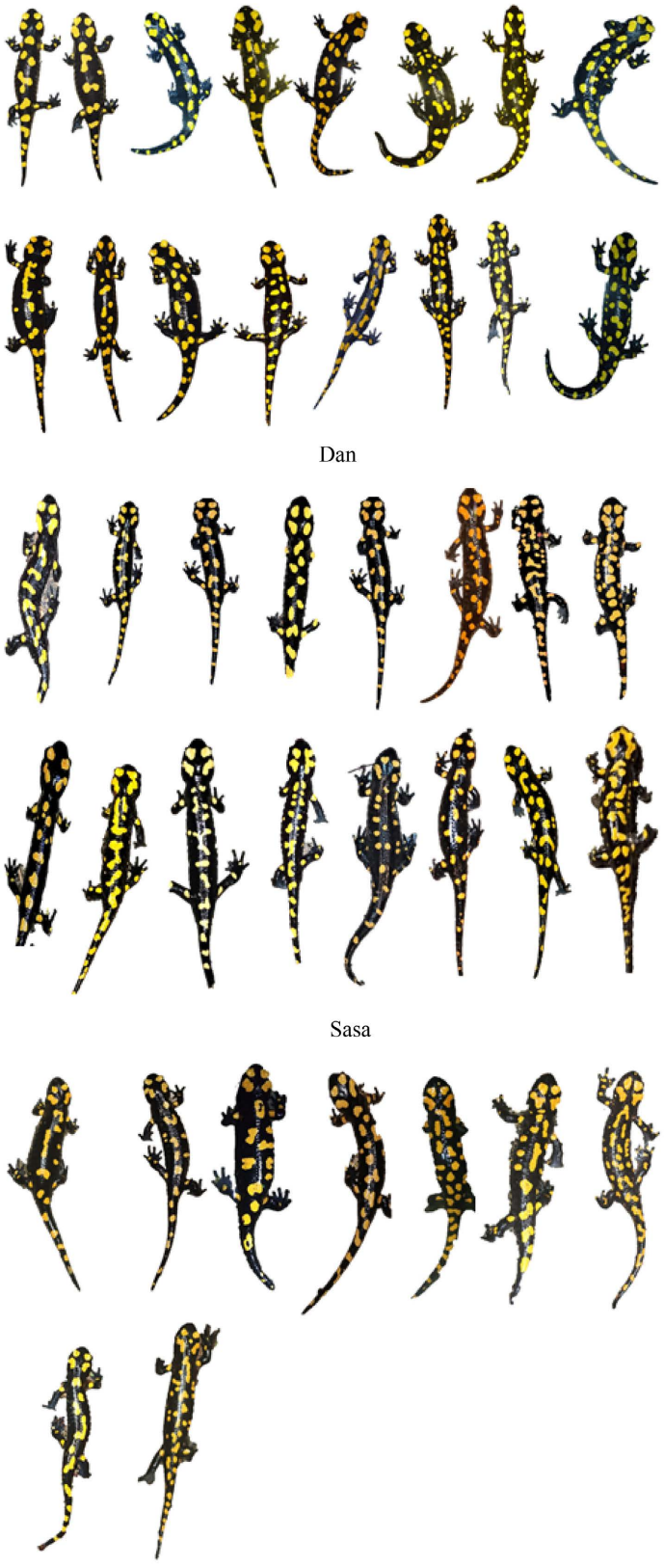

Figure 2. Different dispersion of spots on the back of the salamanders in the Dan, Sasa and Yehiam populations.

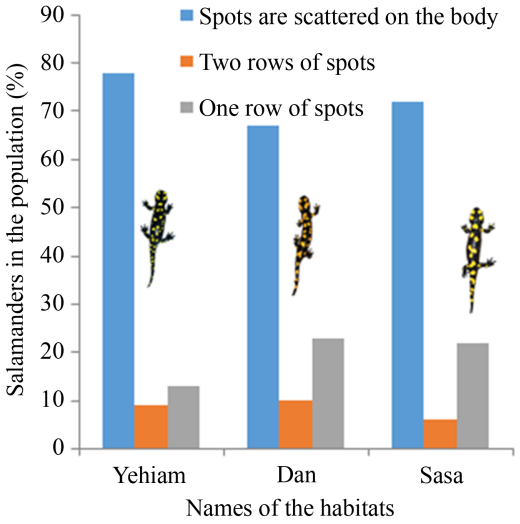

Figure 3. Comparison of the patterns of yellow spots on the backs of the salamanders (one row, two rows and scattered) between habitats (Dan, Sasa and Yehiam). Differences were not significant (Z-test portion, $p > 0.05$).

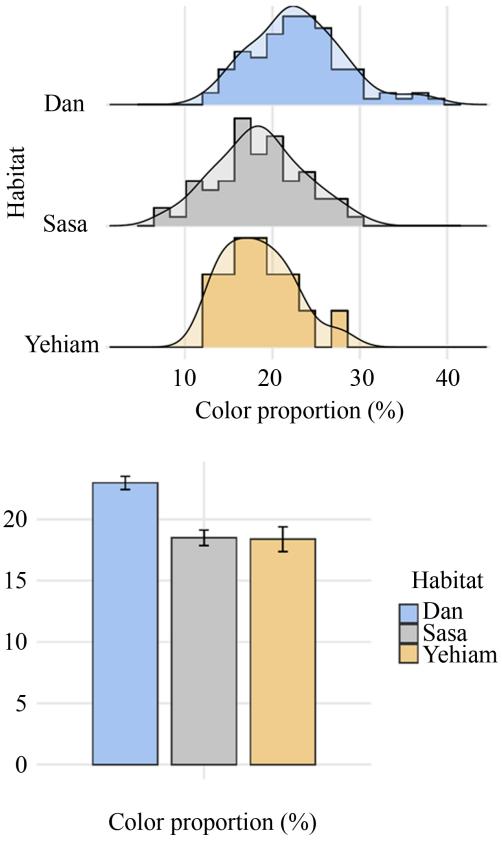

Figure 4. Comparison of percentage of yellow spots on the black back of salamanders in the different habitats (populations) using the JAMOVI statistical program (ANOVA and t-test). No significant differences were found between the Yehiam and Sasa populations. Both populations differed from Dan salamanders. The formula for calculated the % of the yellow spots' area (yellow proration) is: % of yellow = $[A/(A + B)] \times 100$. A is the area of the yellow spots and B is the area of the black back skin.

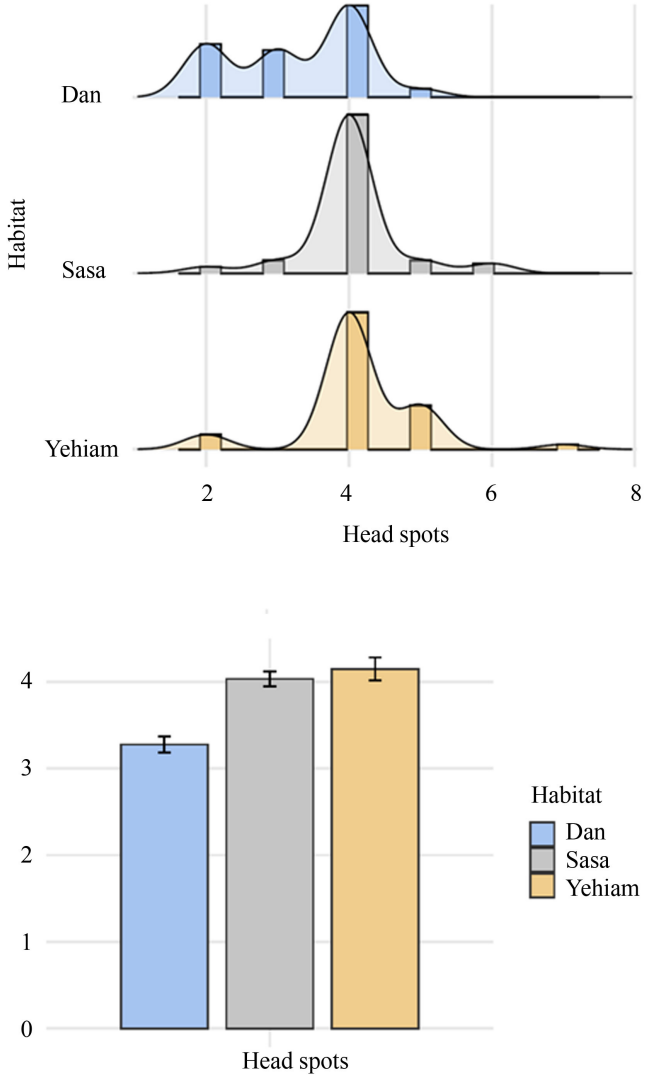

Figure 5. Number of spots on the heads of salamanders from the different populations (Habitats: Dan, Sasa, Yehiam).

Figure 6. Different shades of spots in Sasa salamanders.

4. Discussion

Results of this study of spot patterns on *S. infraimmaculata* at the southern border of its distribution in three habitats revealed differences compared to the salamanders in Europe (**Figure 7**), in agreement with a relatively large number of studies (e.g., [4] [5] [6]. The color pattern described for the various subspecies of *S. salamandra* (**Figure 7**) appeared in the findings of this work, as well as in the water salamander *S. algira* [9] [10] and *S. corsica* [5]. The distribution of *S. salamandra* is the widest among the species of the genus *Salamandra*. Most of the patterns of the dorsal spots appeared in both species *S. salamandra* and *S. terrestris*. In the past, *S. infraimmaculata* was classified as *S. salamandra* mainly according to the spots on the back, hence the importance of the holocaust for these two species [1].

Figure 7. Color patterns in the various subspecies of *S. salamandra*. Some of these patterns also appeared in populations of *S. infraimmaculata*. (a) Spot patterns of *S. salamandra* that did not appear in *S. infraimmaculata* in the present study. ((b) and (c)) Spot patterns of *S. salamandra* that also appeared in this study in *S. infraimmaculata* [5] [6] [28].

- The contribution of this work is in the relatively large number of *S. infraimmaculata* individuals sampled at the southern border of their distribution, in different habitats that are relatively close together geographally, but have different environmental conditions: Dan, where there is water available year round at constant temperatures [8], vs. Sasa and Yehiam, which are semi-arid habitats where it rains only a few months in the winter [1] [7] [25]. The hue of the spot colors found in this work appears in the genus *Salamandra*, but probably varies within the species [4] [5] [6] [27]. The differences found in the arrangement of the spots among the three habitats in this study are supported by other biological aspects among populations of salamanders on the southern border of their distribution: morphological (body size) [1] [13], physiological [14] [15] and genetic [1] [3] [18]. Similar phenomena have been found in salamanders of the same genus but of different species. In *S. algira*, at the southern border of its distribution in North Africa (Algeria) [28], also found differences among populations in both genetic variation and spots on the back. The pattern of spots found in Israel in *S. infraimmaculata* in the present study has also been seen in *S. algira* in North Africa at the southern border of its distribution; however, there are also different models and colors [5] [6] [28]. Effects of the ecological conditions on morphological variations are also found in other species of amphibians, for example in the Green Toad [29].

The differences in back spots between *S. infraimmaculata* populations in Israel, which are described in this work, may be affected by their different habitats' environments. These differences are supported by works from other fields of biology, and are typical to the genus *Salamandra*.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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