Yellow patterns polymorphism of the fire salamander *Salamandra salamandra* in Poland

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**Abstract.** We analysed variation of dorsal yellow patterns in 2077 individuals that represented 23 populations from the northern parts of the Sudetes and the Carpathian Mountains. We distinguished four types of yellow patterns: spotted (50.1%), spotted-and-striped (42.8%), zig-zag (5.8%), and striped (1.3%). Spotted form dominated in the east (69.2%, the Carpathians) and its frequency decreased to the west (46.4%, the Sudetes), where spotted-and-striped forms became more common. The rarest in both mountain ranges (0.9% in the Carpathians, 1.3% in the Sudetes) was striped type. The exception was the westernmost population represented by 15.8% of the striped individuals. We did not find evidence of differences between yellow colouration variants and genotypes of 557 individuals defined by 10 microsatellite loci. No differences were found between females and males. We concluded that patterning does not constitute a unique feature at population and mountain ranges levels.

**Keywords.** Amphibians, colouration, dorsal yellow patterns, Poland, *Salamandra salamandra*. 

**INTRODUCTION**

The fire salamander *Salamandra salamandra* (Linnaeus, 1758) is highly polytypic and widely distributed urodelan in Europe (Kuzmin et al., 2009). The species consists of over a dozen divergent geographical subspecies traditionally distinguished by various patterns of yellow dots and/or stripes on the black background of the dorsal side, with a great variety observed at regional, local, and individual levels (e.g., Freytag, 1955; Eiselt, 1958; Veith, 1992; Thiesmeier, 2004; Velo-Antón and Buckley, 2015). For many years, yellow-black colouration had been explained as a cryptic colouration closely linked to climate and quality of the substrate or background of habitats (e.g., Frish, 1920). Nowadays, it is known that phenotype depends on heritable genetic variation, and functions as an aposematic colouration (Thiesmeier, 2004; Thiesmeier and Grossenbacher, 2004). The differences among populations are explained by evolutionary processes, such as natural selection. Among presumed factors, e.g., van Alphen and Arntzen (2016) mentioned predation intensity or thermal conditions.

Current classification of *Salamandra* species and subspecies was supported by molecular studies, and some morphologically recognizable subspecies were also genetically differentiated (Steinfartz et al., 2000; Steinfartz and Tautz, 2003). On the other hand, phenotypical similarities exist between closely related species e.g., *S. salamandra* and *S. infraimmaculata*, or subspecies e.g., *S. s. salamandra* and *S. s. terrestris* (Degani, 1986; Thiesmeier, 2004). Colour polymorphism is mostly evident among the fire salamanders inhabiting the Iberian Peninsula (Alcobendas et al., 1996; Thiesmeier and Grossenbacher, 2004; Reis et al., 2011; Velo-Antón and Buckley, 2015), but no genetic or geographical
divergence was recently revealed between phenotypically differentiated subspecies \textit{S. s. bernardezi} or \textit{S. s. alfredschmidtii} (Beukema et al., 2016). Therefore the authors suggested to revoke subspecies status of the latter taxon.

Poland is inhabited by the nominative subspecies \textit{S. s. salamandra}, characterized by yellow, or rarely orange, irregular spots and stripes on the dorsal part of the body (Juszczyk, 1987; Zakrzewski, 2007). The classification of dorsal yellow patterns described by Eiselt (1958) was further applied by Juszczyk (1987) and Zakrzewski (2007) for individuals collected in the Carpathians. Originally it contained four forms: spotted, striped, striped-spotted, and spotted-striped. Furthermore Paluch and Profus (2004) proposed to distinguish the fifth, i.e., zig-zag form. According to available data, spotted and striped-spotted forms dominate, and striped one is the rarest among the fire salamanders in Poland (Juszczyk, 1987; Paluch and Profus 2004; Zakrzewski, 2007). In addition, zig-zag form was described as extremely rare (Zakrzewski, 2007).

Here, we present diversity and distribution of dorsal yellow patterns of the fire salamanders from the species entire range in Poland. The aim of this study was to characterize differences at population and mountain range levels, and between females and males. We assume that a gradient of dorsal patterns exists with domination of spotted form in the east (the Carpathians), and striped form in the west (the Sudetes). We also tested the possible relationship between pattern types and genotypes based on 10 microsatellite loci.

\textbf{MATERIAL AND METHODS}

\textit{Sampling and study area}

2077 individuals were collected during active seasons (April-October) in the years 2004-2016 from 23 populations in Poland. The study area represents the Western, Central and Eastern Sudetes Mountains (16 populations), and the Outer Western and Outer Eastern Carpathian Mountains (seven populations). They constitute two main mountain ranges in the country, isolated by the Moravian Gate (depression between the Eastern Sudetes and the Western Carpathians). Polish populations are located at the northern margin of the species range and inhabit mountainous areas including their foothills.

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Population number & Mountain range & Locality & Altitude (m a.s.l.) & Years of samples collection (number of collected individuals) \\
\hline
1 & the Sudetes & Eastern Upper Lusatia & 283 & 2015 (22), 2016 (16) \\
2 & the Sudetes & Izera Mts. & 470 & 2008 (51), 2009 (59) \\
3 & the Sudetes & Katzbach Mts. & 494 & 2008 (24), 2009 (71) \\
4 & the Sudetes & Kaczawskie Piedmont & 392 & 2008 (46), 2009 (57) \\
5 & the Sudetes & Kaczawskie Piedmont & 394 & 2005 (116), 2006 (20), 2008 (7), 2009 (53) \\
6 & the Sudetes & Wałbrzyskie Mts. & 509 & 2008 (26), 2009 (58) \\
7 & the Sudetes & Stołowe Mts. & 589 & 2008 (40), 2009 (64) \\
8 & the Sudetes & Ślęża Massif & 263 & 2004 (139), 2005 (28) \\
9 & the Sudetes & Bardzie Mts. & 407 & 2008 (42), 2009 (60) \\
10 & the Sudetes & Bardzie Mts. & 356 & 2006 (59), 2007 (38) \\
11 & the Sudetes & Bardzie Mts. & 279 & 2008 (41), 2009 (52) \\
12 & the Sudetes & Bardzie Mts. & 418 & 2008 (45), 2009 (67) \\
13 & the Sudetes & Bardzie Mts. & 294 & 2008 (40), 2009 (53) \\
14 & the Sudetes & Śnieżnik Massif & 372 & 2007 (5), 2008 (42), 2009 (54) \\
15 & the Sudetes & Golden Mts. & 496 & 2007 (122), 2009 (57), 2014 (44), 2015 (18) \\
16 & the Sudetes & Opawskie Mts. & 401 & 2007 (122), 2009 (57), 2014 (44), 2015 (18) \\
17 & the Carpathians & Bielsko-Biała city, Silesian Piedmont & 394 & 2015 (35), 2016 (43) \\
18 & the Carpathians & Roźnów Piedmont & 351 & 2014 (4), 2016 (38) \\
19 & the Carpathians & the Ciężkowice Piedmont & 378 & 2014 (8), 2015 (9), 2016 (41) \\
20 & the Carpathians & Strzyżów Piedmont & 326 & 2014 (8), 2015 (9), 2016 (41) \\
21 & the Carpathians & Lower Beskids & 417 & 2014 (2), 2016 (42) \\
22 & the Carpathians & Lower Beskids & 440 & 2014 (12), 2015 (40) \\
23 & the Carpathians & Bieszczady Mts. & 615 & 2016 (60) \\
\hline
\end{tabular}
\caption{Characteristic of sampling sites, years and the number of the collected individuals of the fire salamander \textit{Salamandra salamandra} from Poland.}
\end{table}
Polymorphism of S. salamandra from Poland

(Głowaciński and Zakrzewski, 2003). Dominant vegetation component in the Sudetes sampling sites are mixed or deciduous forests (including Dentario enneaphylli-Fagetum) with predominance of the European beech Fagus sylvatica and admixture of e.g. spruce Picea sp., fir Abies sp. or sycamore Acer sp. (see Ogrodowczyk et al., 2010). In the Carpathians, salamanders inhabit large beech Dentario glandulosae-Fagetum or mixed forests with F. sylvatica as dominant species and highly shaded bottom with thick layer of litter. The exception is population no. 17 that inhabits small, highly isolated and contaminated heterogeneous forest, located within urbanized area of Bielsko-Biała city (for details see Najbar et al., 2017).

Although the Carpathians reach higher altitudes than the Sudetes, populations from both ranges are located at an average altitude of 400 m above the sea level. Altitudes of each study site were determined in the center of a sampled site using Google Earth software (Google Inc.). Detailed description of the study area is presented in Table 1 and Fig. 1. Straight line minimum distance was approx. 1.1 km from population no. 11 to population no. 13 in the Sudetes, and maximum distance reached approx. 580.6 km between population no. 1 and 23 (Fig. 1). Distances between populations were designated in Quantum GIS 2.18.2 software (QGIS Development Team 2013). In each population, transects were established by a breeding stream and salamanders were collected in the buffer of approx. 50 m up to 100 m on its both sides.

Statistical and molecular analysis

Statistical analyses were performed in STATISTICA 12 software (Statsoft, USA). Pearson’s χ² and post-hoc χ² were used to find differences in compositions of dorsal yellow patterns of individuals: a) from the Sudetes and the Carpathians, b) from westernmost population no. 1, the Sudetes and the Carpathians, and c) between males and females. Pearson’s χ² was not used to test differences between populations due to small number or absence of striped and zig-zag forms, but frequencies of dorsal yellow patterns are presented in Table 2 and Supplementary Table S1.

To determine possible relationship between genetic variation and type of dorsal colour pattern we used polymorphism of 10 microsatellite loci: Sal3, Sal23, Sal29, SalE5, SalE6, SalE7, SalE8, SalE11, SalE12, SalE14 (Steinfartz et al., 2004), that were obtained in our previous study concerning population genetics of S. salamandra in Poland (for protocol see Najbar et al., 2015; Konowalik et al., 2016). Genotypes of 557 individuals representing 19 populations from the entire Polish range were grouped according to five forms (see below preliminary results) and four forms in applied classification (Table 3). Values of F ST between pairs of colour variants were calculated in ARLEQUIN 3.5 (Excoffier and Lisher, 2010). Their significance was tested with 10 000 permutations.

Dorsal yellow patterns classification

According to the criteria of Eiselt (1958), then applied by Juszczyk (1987), Paluch and Profus (2004), and Zakrzewski (2007), the classification of S. salamandra dorsal yellow patterning includes five distinct forms: spotted, striped, zig-zag, striped-spotted and spotted-striped (Fig. 2, Supplementary Table S1). However, the last two (striped-spotted and spotted-
striped) reveal difficulties in their unquestionable recognition because intermediate forms may occur. To avoid significant mistakes while comparing various studies, we considered whether we were allowed to pool striped-spotted and spotted-striped forms into one spotted-and-striped form. Preliminary results supported simplification of the classification because 1) their frequencies were comparable in the entire studied area (Supplementary Table 2), and 2) no relationship between genotypes and patterning existed (Table 3A). Although we found significant differences in frequencies of striped-spotted and spotted-striped forms between the Sudetes and the Carpathians (post hoc $\chi^2 = 13.13$, $P = 0.001$), the above arguments supported this procedure.

Therefore, in this study we applied the simplified classification of yellow patterns including four forms: A) spotted, B) striped, C) zig-zag, and D) spotted-and-striped (Fig. 2).

All the collected salamanders were documented in the pictures and checked in order to avoid analysis of the same individual twice or more times. Moreover, selected salamanders from the entire range were marked by toe clipping for further research what helped to recognize previously sampled individuals.

## RESULTS

Spotted form dominated in the entire *S. salamandra* range and constituted 50.1% of all the collected individuals. However, its frequency was higher in the Carpathians (69.2%) than in the Sudetes (46.4%). The frequency of the remaining forms in the range was: 42.8% of spotted-and-striped form, 5.8% of zig-zag, and only 1.3% of striped form. The exception was population no. 1 where 15.8% of individuals represented striped form and the

### Table 2. Frequency of dorsal yellow patterns of the fire salamander *Salamandra salamandra* within populations, mountain ranges and sexes. The Carpathian populations are presented in bold.

<table>
<thead>
<tr>
<th>Location</th>
<th>n</th>
<th>Frequency [%]</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>the entire species range in Poland</td>
<td>2077</td>
<td>50.1</td>
<td>1.3</td>
<td>5.8</td>
<td>42.8</td>
</tr>
<tr>
<td>the Sudetes</td>
<td>1736</td>
<td>46.4</td>
<td>1.3</td>
<td>6.5</td>
<td>45.8</td>
</tr>
<tr>
<td>the Carpathians</td>
<td>341</td>
<td>69.2</td>
<td>0.9</td>
<td>2.3</td>
<td>27.6</td>
</tr>
</tbody>
</table>

in each population:

<table>
<thead>
<tr>
<th>Number</th>
<th>n</th>
<th>Frequency [%]</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38</td>
<td>13.2</td>
<td>15.8</td>
<td>13.2</td>
<td>57.9</td>
</tr>
<tr>
<td>2</td>
<td>110</td>
<td>60.0</td>
<td>0.0</td>
<td>4.5</td>
<td>35.5</td>
</tr>
<tr>
<td>3</td>
<td>95</td>
<td>46.3</td>
<td>1.1</td>
<td>4.2</td>
<td>48.4</td>
</tr>
<tr>
<td>4</td>
<td>103</td>
<td>65.0</td>
<td>0.0</td>
<td>2.9</td>
<td>32.0</td>
</tr>
<tr>
<td>5</td>
<td>196</td>
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<td>0.0</td>
<td>1.0</td>
<td>71.9</td>
</tr>
<tr>
<td>6</td>
<td>84</td>
<td>57.1</td>
<td>1.2</td>
<td>10.7</td>
<td>31.0</td>
</tr>
<tr>
<td>7</td>
<td>104</td>
<td>47.1</td>
<td>1.9</td>
<td>1.0</td>
<td>50.0</td>
</tr>
<tr>
<td>8</td>
<td>167</td>
<td>47.3</td>
<td>0.6</td>
<td>4.8</td>
<td>47.3</td>
</tr>
<tr>
<td>9</td>
<td>102</td>
<td>43.1</td>
<td>2.0</td>
<td>3.9</td>
<td>51.0</td>
</tr>
<tr>
<td>10</td>
<td>97</td>
<td>50.5</td>
<td>0.0</td>
<td>14.4</td>
<td>35.1</td>
</tr>
<tr>
<td>11</td>
<td>93</td>
<td>52.7</td>
<td>1.1</td>
<td>8.6</td>
<td>37.6</td>
</tr>
<tr>
<td>12</td>
<td>112</td>
<td>51.8</td>
<td>1.8</td>
<td>13.4</td>
<td>33.0</td>
</tr>
<tr>
<td>13</td>
<td>93</td>
<td>50.5</td>
<td>1.1</td>
<td>9.7</td>
<td>38.7</td>
</tr>
<tr>
<td>14</td>
<td>101</td>
<td>43.6</td>
<td>1.0</td>
<td>12.9</td>
<td>42.6</td>
</tr>
<tr>
<td>15</td>
<td>179</td>
<td>46.4</td>
<td>0.0</td>
<td>6.7</td>
<td>46.9</td>
</tr>
<tr>
<td>16</td>
<td>62</td>
<td>32.3</td>
<td>8.1</td>
<td>1.6</td>
<td>58.1</td>
</tr>
<tr>
<td>17</td>
<td>78</td>
<td>55.1</td>
<td>2.6</td>
<td>1.3</td>
<td>41.0</td>
</tr>
<tr>
<td>18</td>
<td>42</td>
<td>73.8</td>
<td>0.0</td>
<td>0.0</td>
<td>26.2</td>
</tr>
<tr>
<td>19</td>
<td>58</td>
<td>77.6</td>
<td>0.0</td>
<td>8.6</td>
<td>13.8</td>
</tr>
<tr>
<td>20</td>
<td>7</td>
<td>42.9</td>
<td>0.0</td>
<td>0.0</td>
<td>57.1</td>
</tr>
<tr>
<td>21</td>
<td>44</td>
<td>63.6</td>
<td>0.0</td>
<td>0.0</td>
<td>36.4</td>
</tr>
<tr>
<td>22</td>
<td>52</td>
<td>76.9</td>
<td>1.9</td>
<td>1.9</td>
<td>19.2</td>
</tr>
<tr>
<td>23</td>
<td>60</td>
<td>76.7</td>
<td>0.0</td>
<td>1.7</td>
<td>21.7</td>
</tr>
<tr>
<td>females</td>
<td>936</td>
<td>47.9</td>
<td>1.0</td>
<td>5.9</td>
<td>45.3</td>
</tr>
<tr>
<td>males</td>
<td>884</td>
<td>46.5</td>
<td>1.9</td>
<td>5.4</td>
<td>46.2</td>
</tr>
<tr>
<td>juveniles</td>
<td>252</td>
<td>70.2</td>
<td>0.0</td>
<td>7.1</td>
<td>22.6</td>
</tr>
</tbody>
</table>
lowest frequency (13.2%) of spotted salamanders was found. In addition, population no. 16 also revealed higher number (8.2%) of striped forms. Detailed predominance and frequency of dorsal colour patterns within populations are presented in Table 2 and Fig. 3A, and Supplementary Table S1.

Statistical analyses showed significant differences in the frequencies of dorsal yellow patterns between salamanders from the Sudetes and the Carpathians ($\chi^2 = 60.72, P < 0.001$), and while comparing population no. 1 with western (the Sudetes) and eastern (the Carpathians) groups of populations ($\chi^2 = 138.48, P < 0.001$). Post-hoc

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**Table 3.** Dorsal yellow patterns pairwise $F_{ST}$ (below diagonal) for: A) classification of Eiselt (1958), applied by Juszczyk (1987), Paluch and Profus (2004) and Zakrzewski (2007) for Polish populations, and B) simplified classification used in this study. Above diagonal $F_{ST}$ $P$ values are presented.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>n</td>
<td>spotted</td>
<td>striped</td>
<td>zig-zag</td>
<td>striped-spotted</td>
</tr>
<tr>
<td>297</td>
<td>n</td>
<td>spotted</td>
<td>-</td>
<td>0.758</td>
<td>0.887</td>
</tr>
<tr>
<td>8</td>
<td>n</td>
<td>striped</td>
<td>-0.004</td>
<td>-</td>
<td>0.849</td>
</tr>
<tr>
<td>22</td>
<td>n</td>
<td>zig-zag</td>
<td>-0.003</td>
<td>-0.008</td>
<td>-</td>
</tr>
<tr>
<td>84</td>
<td>n</td>
<td>striped-spotted</td>
<td>0.001</td>
<td>-0.005</td>
<td>-0.002</td>
</tr>
<tr>
<td>146</td>
<td>n</td>
<td>spotted-striped</td>
<td>0.001</td>
<td>-0.009</td>
<td>-0.004</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B</th>
<th>n</th>
<th>spotted</th>
<th>striped</th>
<th>zig-zag</th>
<th>spotted-and-striped</th>
</tr>
</thead>
<tbody>
<tr>
<td>297</td>
<td>n</td>
<td>spotted</td>
<td>-</td>
<td>0.763</td>
<td>0.886</td>
</tr>
<tr>
<td>8</td>
<td>n</td>
<td>striped</td>
<td>-0.004</td>
<td>-</td>
<td>0.841</td>
</tr>
<tr>
<td>22</td>
<td>n</td>
<td>zig-zag</td>
<td>-0.003</td>
<td>-0.008</td>
<td>-</td>
</tr>
<tr>
<td>230</td>
<td>n</td>
<td>spotted-and-striped</td>
<td>0.001</td>
<td>-0.007</td>
<td>-0.003</td>
</tr>
</tbody>
</table>

**Fig. 2.** Classification of colour patterns of the fire salamander *Salamandra s. salamandra*. A – spotted form: irregular spots also on vertebral line; B – striped form: two rows of full or discontinuous stripes, vertebral line remains black without any spots; C – zig-zag form: continuous, rarely interrupted in one or two places, linear pattern that covers vertebral line; D – spotted-and-striped form: spots and striped, vertebral line remains black or may be disturbed by yellow spots; this intermediate form consists of striped-spotted and spotted-striped forms described by Eiselt (1958), and applied for Polish populations of the species by Juszczyk (1987), Paluch and Profus (2004) and Zakrzewski (2007).
\( \chi^2 \) results revealed differences between mountain ranges in the proportion of spotted (\( \chi^2 = 59.46, P < 0.001 \)), zig-zag (\( \chi^2 = 9.00, P = 0.003 \)), and spotted-and-striped individuals (\( \chi^2 = 38.69, P < 0.001 \)). Population no. 1 differed in the proportion of spotted (\( \chi^2 = 17.23, P < 0.001 \)) and striped (\( \chi^2 = 62.18, P < 0.001 \)) forms with salamanders from the Sudetes, and in the proportion of all forms with individuals collected in the Carpathians (for all \( P < 0.001 \)). We did not find differences between males and females (\( \chi^2 = 3.36, P = 0.340 \)).

Values of pairwise F\textsubscript{ST} between forms of dorsal patterning varied from -0.008 to 0.001 (for all \( P > 0.05 \); Table 3B). The results suggested no evidence of genetic differences between individuals that represent defined patterns at least according to variation of selected molecular markers used in this study.

**DISCUSSION**

It has been recognized that spotted forms of *S. salamandra* are characteristic of Eastern Europe, while striped individuals with two yellow bands are dominant in the West (e.g., Eisely, 1958; Thiesmeier, 2004). In Poland, *S. salamandra* is mainly represented by individuals with irregular spots and/or stripes on the dorsal side of the body. In our study, spotted form was the most common, whereas striped form was the rarest (Table 2). Predominance of spotted and striped-spotted forms (a part of spotted-and-striped form according to classification applied for this study, Fig. 2D) was previously reported by Juszczyk (1987), Paluch and Profus (2004), and Zakrzewski (2007). This result complies with distribution of *S. salamandra* subspecies recognized on the basis of variety in regionally observed colouration.

In relation to other populations, substantial predominance of striped form, characteristic of *S. s. terrestris* (Lacépede, 1788), was observed in site no. 1. This population is located at the Polish-German border along the Lusatian Neisse (Najbar and Najbar, 2015), where ranges of these two subspecies contact (Thiesmeier, 2004). Frequency of striped form in this site was higher than in the remaining sites (Table 2), while spotted and zig-zag represented the minority of observed individuals. Significant dominance of striped patterns in the extremely western population no. 1 may suggest natural admixture with *S. s. terrestris*, as was also suggested by Thiesmeier (2004). Such results indicated an increase in the frequency of striped form towards the West. On the other hand, relatively large number of striped salamanders was observed also in population no. 16 located in the Opawskie Mountains (Fig. 1). Likewise, Paluch and Profus (2004) reported 4.8% of salamanders with the striped dorsal pattern in population from Morzyszów, which is distanced of about 130 km to the east of population no. 1. Although we observed striped and zig-zag salamanders in both the Sudetes and the Carpathians (Table 2), these patterns were confirmed to be rare similarly to other studies from Poland (Juszczyk, 1987; Paluch and Profus, 2004; Zakrzewski, 2007).

Phenotypes of several *Salamandra* species and their subspecies tend to be misleading in their recognition (e.g., Bickford et al., 2007), and the fire salamanders collected in this study resemble at least two subspecies, e.g. *S. s. terrestris* and *S. s. beschkovi* (Obst, 1981). Kowalski and Młynarski (1965) reported similarities in yellow patterning between some of the collected individuals from Poland and those representing western European subspecies *S. s. terrestris*, whereas Juszczyk (1987) found similarities to *S. s. algira, S. s. infraimmaculata* or *S. s. bejarae*.
Polymorphism of *S. salamandra* from Poland

According to subspecies classification of Eiselt (1958). The first two do not exist in the current systematics anymore (Joger and Steinfartz, 1995; Steinfartz et al., 2000) and were recognized as distinct species: *S. algira* (Bedriaga, 1883) and *S. infraimmaculata* (Mertens, 1948). On the other hand, the existence of several distinct phenotypes may be observed in Spanish populations of *S. s. bernardesi* (Beukema et al., 2016).

Sexual polymorphism has been frequently reported for amphibian species, including body size, colour dichromatism, pigmentation, quality or quantity of patterns (e.g., Kupfer, 2007; Davis and Grayson, 2008; Hoffman and Blouin, 2008). In *S. salamandra*, sex-related morphological features, such as size and shape of the body, were usually observed (e.g., Zakrzewski, 2007; Labus et al., 2013; Balogová and Uhrin, 2015). Our study did not reveal relationship between the occurrence of exact dorsal patterns and sexes, and correspond to previous conclusions of Zakrzewski and Wójcik (1982) that females and males did not differ in the topography of spots. However, a recent study of Balogová and Uhrin (2015) from the Slovakian Carpathians revealed the existence of sexual dichromatism in *S. s. salamandra*, and concluded that males were covered with larger yellow spots on dorsal side than females. Genetic diversity in 10 microsatellite loci did not show correlation with a variety of dorsal yellow patterns. However, the westernmost population no. 1 was not included to genetic analyses because the site was discovered later than when we conducted population genetics study (Najbar et al., 2015; Konowalik et al., 2016). Veith (1992) excluded the possibility to recognize of *S. s. salamandra* and *S. s. terrestris* by color patterns in wide zone of hybrid populations from Germany. Therefore, further genetic analyses should be performed to confirm our hypothesis about possible occurrence of the second subspecies, *S. s. terrestris*, in Poland.

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**SUPPLEMENTARY MATERIAL**

Supplementary material associated with this article can be found at [<http://www.unipv.it/webshi/appendix> manuscript number 22886].

**REFERENCES**


