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VENTRAL AND LATERAL SPOT PATTERNS DIFFERENTIATION BETWEEN THREE SMOOTH NEWT SPECIES (AMPHIBIA: SALAMANDRIDAE: *LISSOTRITON*)

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The location, shape and area of dark spots on the belly, throat and sides of the body of three closely related species of smooth newts (*Lissotriton kosswigi*, *L. schmidtleri* and *L. vulgaris*) were analysed. Differences were found between species and sexes in the location and shape of dark spots on the belly, throat and lateral surfaces of the body. Discriminant analysis of these spot characteristics allows to identificate of males of all three species with a high degree of confidence (85–91%). In females, only *L. vulgaris* was correctly distinguished from the other two species (accuracy 81–94%). Anatolian and Thracian populations of *L. schmidtleri* have very similar patterns of dark spots, which confirm their conspecificity. The differences in the location and size of dark spots make possible successful identification of the species during field research and study of museum specimens. The developed method could be useful for comparative studies of other animal species which have spotted camouflage or aposematic colouration.

Key words: tailed amphibians, morphological variation, external characters, spotted colouration, newt, *Lissotriton*.

INTRODUCTION

The taxonomic challenge posed by cryptic species has been recognised for a long time, but the advent of molecular methods has given new tools for detecting morphologically similar species (Casu & Curini-Galletti 2004, Bickford *et al*. 2007, Neusser *et al*. 2011). Recently, numerous distinct lineages have been revealed in various groups of animals, including amphibians, for which morphological identification is still problematic (e.g., Borkin *et al*. 2004, Stuart *et al*. 2006, Dufresnes *et al*. 2018, Trevisan *et al*. 2020). The presence of a large number of such poorly distinguished species has led to increased interest in the study of their morphology (Arntzen *et al*. 2013, Wielstra & Arntzen 2016, Üzüm *et al*. 2019). Sometimes, a detailed re-examination of morphological characteristics unveils that suitable diagnostic traits are presented but have been overlooked before (Mann & Evans 2008, Litvinchuk *et al*. 2021, Papežík *et al*. 2021). Previous genetic studies of newts and salamanders of the family Salamandridae have revealed several cryptic species (Wielstra *et al*. 2013, van Riemsdijk *et al*. 2017, 2021, Sequeira *et al*. 2019). For example, morphologically similar species are known in such genera as *Salamandra*, *Salamandrina*, *Ommatotriton* and *Triturus* (Mattoccia *et al.* 2005, Wielstra & Arntzen 2016, Rodríguez *et al.* 2017, van Riemsdijk *et al.* 2017), as well as among newts of the genus *Lissotriton* (Martínez-Solano *et al.* 2006, Skorinov *et al*. 2008, Wielstra *et al.* 2018).

The genus *Lissotriton* consists of ten species widely distributed in Europe and penetrating into western Asia (Frost 2022). The genus includes *L. italicus*, *L. helveticus*, the *L. boscai* complex (*L. boscai* and *L. maltzani*), and most abundant, the *L. vulgaris* complex. The last is represented by the Carpathian newt (*L. montandoni*) and five closely related species of smooth newts, which was previously considered as subspecies of *L. vulgaris*, but were raised to the species level as a result of molecular studies (Skorinov *et al*. 2011, Pabijan *et al*. 2017, Wielstra *et al*. 2018, Speybroeck *et al*. 2020).

Secondary sexual characters, which are present only in males of smooth newts during the aquatic reproduction period, could be divided into two groups. The first one includes two species, *L. kosswigi* (Freytag, 1955) and *L. graecus* (Wolterstorff, 1906). During the breeding season, males of these species have a poorly developed dorsal crest, but a well-developed filament at the end of a tail and toe webbing. Males of the second group, consisting *L. vulgaris* (Linnaeus, 1758), *L. lantzi* (Wolterstorff, 1914), and *L. schmidtleri* (Raxworthy, 1988), have opposite states of these characteristics. They have the well-developed dorsal crest and the moderate-developed toe webbing, but the thin tail filament is absent. Only minor differences in the degree of development of these characteristics have been observed between species within these groups. Morphological identification of species among females, immature individuals and males outside the breeding season is extremely difficult.

It was previously shown that the lateral and ventral dark spot patterns do not change much over time in adults (Mettouris *et al*. 2016). Therefore, species of the genus *Lissotriton* can be successfully identified by their lateral and ventral colouration in both live individuals and specimens from museum collections. In the previous study (Skorinov & Litvinchuk 2013), the colouration of two cryptic species of smooth newts (*L. lantzi* and *L. vulgaris*) was analysed. It was found that their patterns significantly differ, allowing them to be reliably distinguished. Unfortunately, in this study, other smooth newt species were represented by small samples (*L. schmidtleri*) or were not included (*L. kosswigi*). Therefore, the main aim of our present paper was to update the previous study and analyse the dark spot pattern and test if we can

Table 1. Localities, coordinates and sample size of *Lissotriton kosswigi*, *L. schmidtleri* and *L. vulgaris*.

	Locality	Coordinates	Male (belly/throat)	Female (belly/throat)
		L. kosswigi		
1	Hacılar, Turkey	41.496°N, 32.088°E	15/15	17/17
2	Kocaeli, Turkey	40.750°N, 30.051°E	9/9	11/11
3	Mollafeneri, Turkey	40.893°N, 29.508°E	5/5	11/11
4	Alibahadır Köyü, Turkey	41.187°N, 29.205°E	6/6	8/8
		L. schmidtleri		
5	Halkalı, Turkey	41.060°N, 28.770°E	19/22	38/40
6	Dursunköy, Turkey	41.213°N, 28.645°E	4/4	2/2
7	Baklalı, Turkey	41.268°N, 28.658°E	5/5	3/3
8	Yassıören, Turkey	41.251°N, 28.590°E	5/5	11/11
9	Soğucak, Turkey	41.616°N, 27.663°E	1/1	2/2
10	Domurcalı, Turkey	41.817°N, 26.818°E	4/4	4/4
11	Sazlıdere, Turkey	41.605°N, 26.659°E	2/2	4/4
12	Çardak, Turkey	40.382°N, 26.727°E	6/6	7/7
13	Şahinli, Turkey	40.297°N, 26.772°E	2/2	5/5
14	Lapseki, Turkey	40.264°N, 26.772°E	5/5	9/9
15	Muratlar, Turkey	39.940°N, 26.794°E	0/0	2/2
16	Yeşilköy, Turkey	39.819°N, 26.805°E	0/0	2/2
17	Evciler Bucağı, Turkey	39.787°N, 26.760°E	1/1	4/4
18	Çalıoba Akçakıl, Turkey	39.779°N, 26.704°E	3/3	3/3
19	Çavuşlu, Turkey	39.752°N, 26.727°E	6/6	2/2
20	Çınarcik, Turkey	39.693°N, 27.209°E	3/3	7/7
21	Türkmen Köyü, Turkey	38.749°N, 27.183°E	10/10	10/10
22	Karacabey, Turkey	40.218°N, 28.426°E	2/3	15/17
		L. vulgaris		
23	Gatchina, Russia	59.559°N, 30.117°E	60/60	39/39
24	Chur, Russia	57.100°N, 52.983°E	22/22	27/27
25	Saransk, Russia	54.203°N, 45.201°E	31/31	15/15
26	Minai, Ukraine	48.583°N, 22.284°E	25/25	35/35
27	Batevo, Ukraine	48.366°N, 22.399°E	41/41	26/26
28	Solotvina, Ukraine	47.951°N, 23.899°E	14/14	13/13

successfully identify three closely related smooth newt species (*L. kosswigi*, *L. schmidtleri* and *L. vulgaris*), which have presumably parapatric distributional ranges with yet undefined boundaries in the eastern Balkans and western Anatolia (Fig. 1).

MATERIAL AND METHODS

Material

In total, 646 specimens from 28 localities were studied (Fig. 1, Table 1), including 78 males and 134 females of *L. schmidtleri* (18 localities), 193 males and 155 females of *L. v. vulgaris* (6 localities), and 35 males and 47 females of *L. kosswigi* (4 localities). We performed the species delimitation based on previously known distribution ranges (Wielstra *et al*. 2015, Wielstra *et al*. 2018) and external morphology of breeding males (for *L. kosswigi* only).

Since the dark spot pattern stabilises only after maturation (Hagström 1973, Mettouris *et al*. 2016), all studied specimens were adults. Vouchers are stored in herpetological collections of Institute of Cytology of Russian Academy of Sciences (St. Petersburg, Russia)

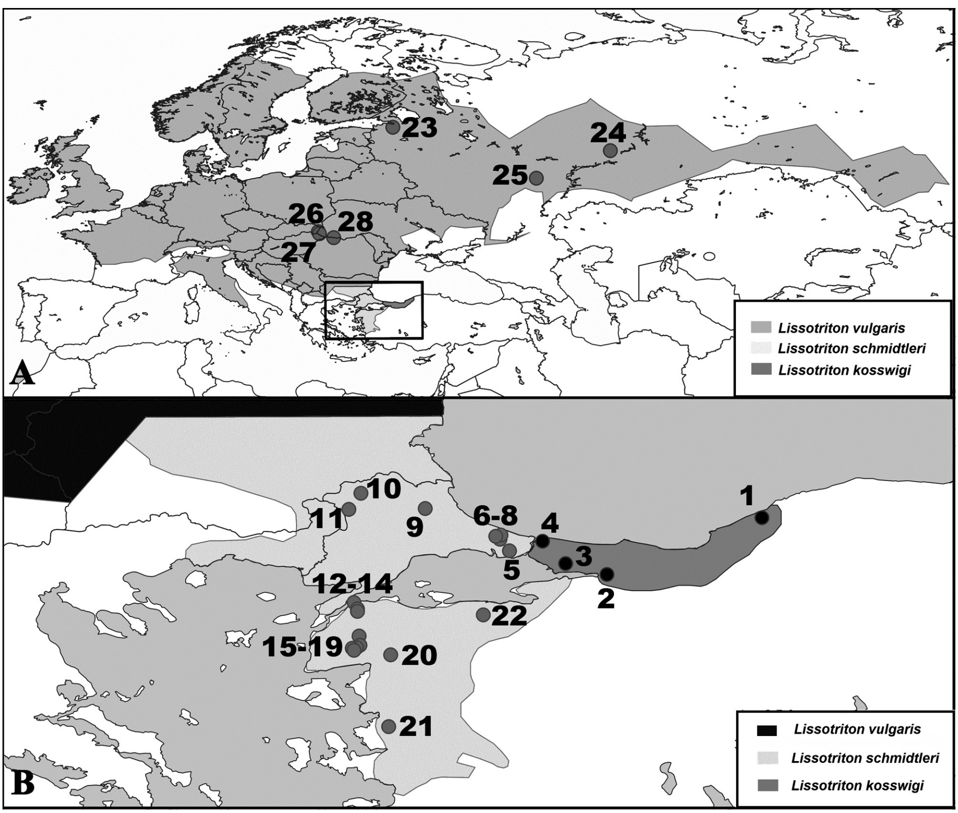

Fig. 1. Distribution of *Lissotriton vulgaris*, *L. schmidtleri* and *L. kosswigi* in the Western Palearctic (A) and western Turkey (B). Numbers for localities are given in Table 1

and Department of Biology, Faculty of Science and Arts, Aydın Adnan Menderes University (Aydın, Turkey). The colour of museum specimens (for example, stored in formalin) can change during the long-term storage in collections, but dark spots on the belly and sides of the body (Fig. 2) usually remain clearly distinguishable.

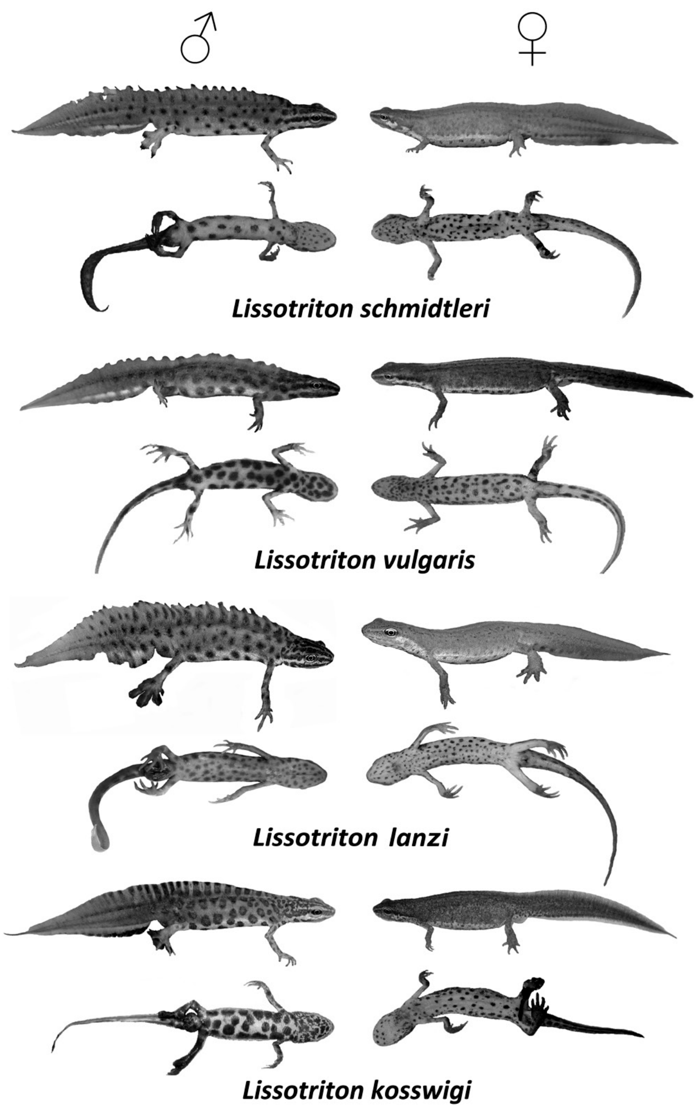

Fig. 2. Color patterns of smooth newt species. *Lissotriton schmidtleri*: males from Domurcalı (lateral view) and Dursunköy (ventral view), females from Yassıören (lateral view) and Karacabey (ventral view), Turkey; *L. vulgaris*: Gatchina, Russia; *L. kosswigi*: males from Mollafeneri (lateral view) and Hacılar (ventral view), females from Alibahadır Köyü, Turkey; *L. lanzi*: males from Ldzaa (lateral view) and Sukhum (ventral view), females from Ldzaa (lateral view) and Machara (ventral view), Abkhazia

Photographs of ventral and lateral surfaces of each specimen were taken with a Canon IXUS 130 digital camera. Pictures were made from a tripod (camera at the height of 12.5 cm) at a resolution of 14.1 megapixels with a dimension of 2592 × 3456 pixels. The resulting JPEG files were processed using program ImageJ 1.45s (http://imagej.nih.gov/ij/). Determination of the lower threshold of shading spots was taken "by default" and was automatically performed by the program. Spots were highlighted in red (8-bit colour) for convenience. In cases where the program itself could not clearly distinguish a border of spots (i.e., too pale spots or a deformed ventral surface), the threshold was selected manually. Spots with a diameter of less than 20 pixels were excluded from the analysis because they were almost invisible.

Analysis of ventral and lateral spot patterns

To analyse dark spot patterns on the ventral surface of newts, two polygons (belly and throat) were selected (Fig. 3A). Three main ventral spot parameters were analysed. The first parameter was the number of dark spots (N). The second parameter was the shape

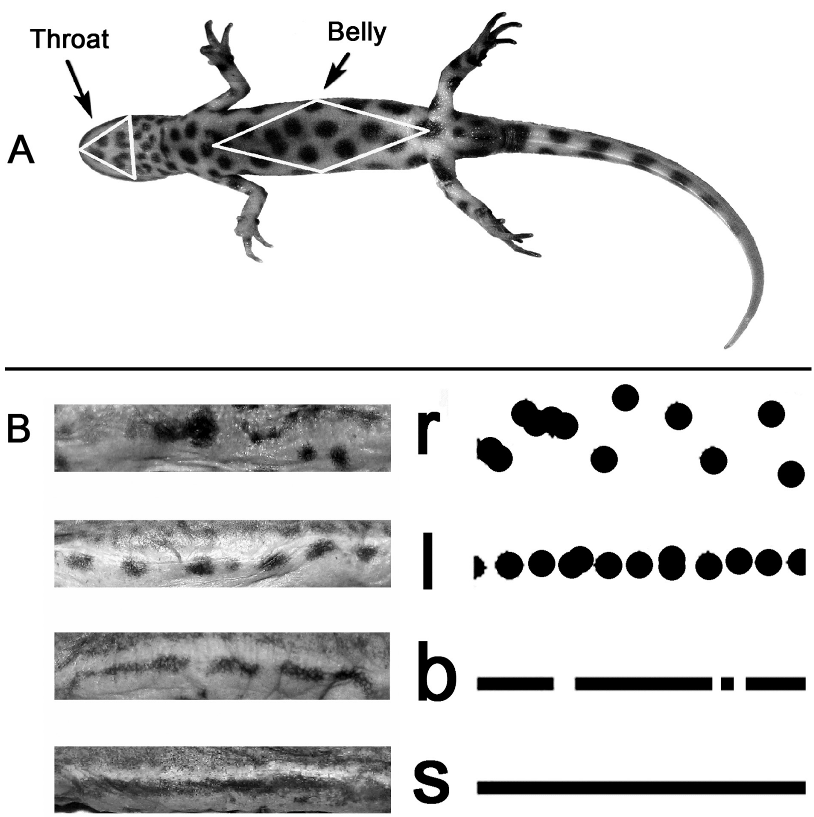

Fig. 3. Polygons selected on the ventral surface of newts (A) and types of dark spot arrangements located on the border between lateral and ventral surfaces (B), where r is random spots, l is line of spots, b is broken stripe, and s is continuous stripe

of dark spots, which was estimated using two characteristics: mean circularity (ØC; 1 is a perfect circle) and mean roundness (ØR; degree of evenness of the spot's edge). ØC and ØR were calculated for each spot using the formulas $4\pi \times \text{area}/\text{perimeter}^2$ and $4 \times \text{area} / (\pi \times \text{length of greatest diameter}^2)$, respectively. The third parameter was the area of dark spots, which was estimated using two characteristics: proportion of dark spots in relation to the entire area of the polygon (AS; in percent) and the index of relative spot area (IRSA), which was calculated using the formula S/L^2 and was multiplied by 10^4 for convenience. In this formula, S is the average dark spot area, and L is the body length which was measured from the anterior tip of the lower jaw to the anterior edge of the cloaca (Skorinov & Litvinchuk 2013). All the measurements were taken in pixels. For practical purposes, we calculated the average diameter of dark spots (D; in mm) using the formula: the mean length of greatest diameter of spots (in pixels) / the body length (in pixels) × the body length (in mm). This parameter was estimated for each specimen, and then we calculated the average D for the species.

A diamond shape of the belly's sector (Fig. 3A) was selected to avoid the occasional capture of lateral dark spots (or stripes) and/or to study curved specimens (if the curvature is lateral). Corners of this diamond were selected manually. Front and back corners were at the centre of the line connecting the posterior parts of the foreleg and anterior parts of the hind digit bases. Lateral corners were at the middle of the belly surface edges, located between fore- and hind limbs. In the throat triangle sector, two lateral corners corresponded to the articulations of the jaws, and the front corner was the anterior edge of the lower jaw.

Additionally, we analysed dark spot arrangements (Ar) located on the border between the lateral and ventral sides of the body (Fig. 3B). The following types were distinguished: r (random spots), l (line of spots), b (broken stripe), s (continuous stripe), and 0

Table 2. Variability (X±SD) of characters in *Lissotriton kosswigi*, *L. schmidtleri* and *L. vulgaris*. N is the number of dark spots, AS is the proportion of dark spots in relation to the entire area of the polygon, IRSA is the index of relative spot area, ØC is mean circularity, ØR is mean roundness, and D is the average diameter of spots (in millimetres).

Surface	Species	Character					
		N	AS	IRSA	ØC	ØR	D
Belly ♂	*kosswigi*	12.4±2.9	25.6±11.3	10.3±5.6	0.37±0.08	0.56±0.07	0.97±0.24
	schmidtleri	11.9±3.5	19.1±6.0	5.6±2.5	0.41±0.10	0.54±0.09	0.79±0.20
	vulgaris	10.8±3.1	32.8±9.3	17.3±8.9	0.39±0.08	0.55±0.08	1.25±0.29
Throat ♂	*kosswigi*	8.8±3.4	33.3±17.1	6.3±4.8	0.45±0.09	0.57±0.08	0.75±0.25
	schmidtleri	8.1±2.8	18.7±7. 9	3.2±1.9	0.57±0.12	0.62±0.10	0.58±0.17
	vulgaris	6.4±2.0	39.2±11.4	9.1±5.7	0.49±0.10	0.56±0.08	0.91±0.25
Belly ♀	*kosswigi*	17.3±7.8	10.4±5.3	3.3±1.8	0.54±0.09	0.61±0.06	0.60±0.14
	schmidtleri	23.0±9.3	13.2±7.2	2.7±2.1	0.58±0.10	0.62±0.06	0.51±0.16
	vulgaris	18.4±8.7	15.9±9.7	5.3±4.6	0.44±0.11	0.57±0.08	0.64±0.25
Throat ♀	*kosswigi*	8.3±5.6	7.1±6.8	1.0±0.6	0.48±0.22	0.51±0.21	0.30±0.15
	schmidtleri	8.7±5.0	10.4±10.0	1.4±1.5	0.55±0.20	0.57±0.19	0.35±0.17
	vulgaris	7.4±5.0	11.5±10.1	1.8±1.8	0.46±0.18	0.51±0.18	0.35±0.18

Table 3. Significance of differences (the Kolmogorov-Smirnov test) between smooth newt species, where k is *Lissotriton kosswigi*, s is *L. schmidtleri*, and v is *L. vulgaris*. Differences are significant if P < 0.001 (+++), P < 0.01 (++), P < 0.05 (+); – is insignificant differences. N is the number of dark spots, AS is the proportion of dark spots in relation to the entire area of the polygon, IRSA is the index of relative spot area, ØC is mean circularity, and ØR is mean roundness.

Surface	Species	Character					
		N	AS	IRSA	ØC	ØR	D
Belly ♂	*k/s*	–	++	+++	–	+	+
	k/v	+	+++	++	–	–	+++
	s/v	–	+++	+++	–	–	+++
Throat ♂	*k/s*	–	+++	+++	+++	++	++
	k/v	+++	++	+++	–	–	+++
	s/v	+++	+++	+++	+++	+++	+++
Belly ♀	*k/s*	+++	+	++	–	–	++
	k/v	+++	+++	+++	–	–	–
	s/v	+	+++	+++	+++	–	+++
Throat ♀	*k/s*	–	–	+	+	++	–
	k/v	–	–	+++	–	–	–
	s/v	–	–	++	+++	+++	–

(spots are absent). Types of the arrangements on the right and left sides were analysed separately (in percents), and then the average frequencies of their occurrence were calculated for each specimen.

The significance of mean differences (N, IRSA, ØC, ØR and AS) between species and sexes was calculated by the Kolmogorov-Smirnov test and lateral dark spot arrangements (Ar) on both sides of the body by the Mann–Whitney U-test using Statistica 8.0. Canonical discriminant analysis (centroids for samples) was performed to understand whether species form distant clusters. Samples containing less than three specimens were not included in the analysis. In order to estimate the percentage of correct identifications, we used the discriminant analysis with species as a grouping variable (separately for females and males).

RESULTS

Analysis of spot patterns on the throat and belly

Noticeable interspecies differences were found in the location and shape of dark spots on both the belly and throat (Tables 2–3). All three species of both sexes are significantly different from each other by the index IRSA only (Table 3). The most significant differences by the index (P < 0.001) were observed for bellies between *L. schmidtleri* and *L. vulgaris* of both sexes. The proportion of dark spots (AS) was also different in all species (except the throat in females). In pairwise comparisons, the greatest number of significantly different characteristics (4–5) was revealed for male throats and female bellies in *L. schmidtleri* and *L. vulgaris*, as well as for male throats in *L. kosswigi* and *L. schmidtleri* (Table 3). The greatest differences between species were observed for throats in males, for which *L. kosswigi* was characterised by numerous dark spots of irregular shape, *L. schmidtleri* by numerous small round spots, and *L. vulgaris* by a small number of large round spots.

Additionally, in males, *L. vulgaris* significantly differed from both *L. schmidtleri* and *L. kosswigi* by the average diameter of dark spots (D) on both the belly and the throat, where the first species was characterised by the largest diameter (1.25 mm *vs* 0.79–0.97 mm for belly and 0.91 mm *vs* 0.58–0.75 mm for throat; Tables 2–3). In females, significant differences were found in the average diameter of dark spots on the throat in *L. schmidtleri* and *L. kosswigi*, where the last species had the smallest diameter (0.30 *vs* 0.35 mm).

Analysis of lateral spot patterns

The analysis of the lateral dark spot arrangements (Ar) showed that random spots were predominately observed in males of *L. kosswigi* (80%) and *L. schmidtleri* (55%), whereas males of *L. vulgaris* were usually characterised by linear series of spots (57%; Table 4). Females of *L. kosswigi* and *L. schmidtleri* were characterised by a predominance of spotted patterns (r + l = 75–91%), but *L. vulgaris* by the presence of stripes (b + s = 84%). An obvious asymmetry ($P < 0.05$) between spot patterns on the left and right sides of the body was only

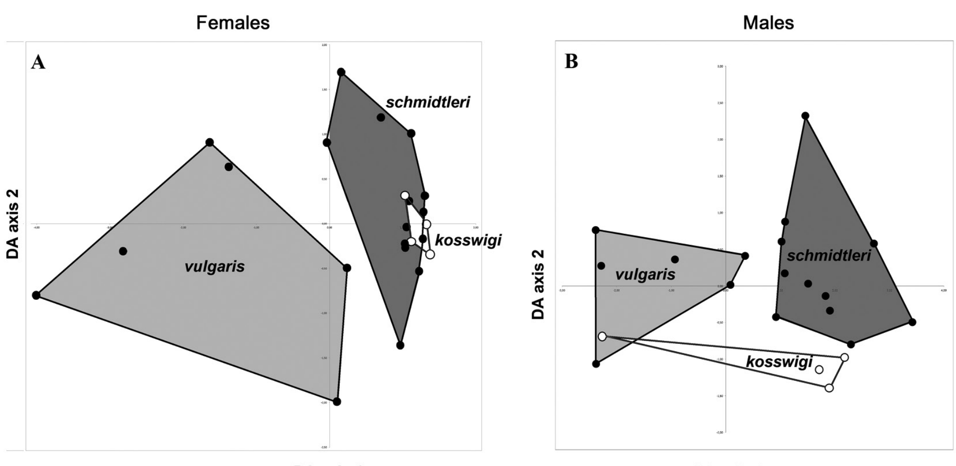

Fig. 4. Plot of centroids for females (A) and males (B) of *Lissotriton kosswigi*, *L. schmidtleri* and *L. vulgaris* in the space of the first and second canonical discriminant axes

Table 4. Average percentages of dark spot arrangement type occurrences on lateral surfaces of three species of smooth newts of the genus *Lissotriton*, where r is random spots, l is line of spots, b is broken stripe, s is continuous stripe, and 0 is absence of spots.

Sex	Species	Character state				
		r	l	b	s	0
Male	*kosswigi*	80	19	1	0	0
	schmidtleri	55	42	3	0	0
	vulgaris	39	57	5	0	0
Female	*kosswigi*	47	44	9	0	0
	schmidtleri	38	37	25	0	0
	vulgaris	8	7	49	35	1

observed in males of *L. kosswigi* (on the left side percentage of r state was 92%, l=8%, b=0%, but on the right side r = 77%, l = 20%, b = 3%).

Multivariate analyses of spot patterns

Using the discriminant analysis (species as a grouping variable), we estimated the percentage of correct identifications of species using the belly, throat and lateral surface characteristics. In males, all species can be successfully determined with an accuracy of 85% (*L. kosswigi* and *L. vulgaris*) and 91% (the other two pairs). In females, the best values of successful identification were observed for the pair *L. kosswigi* and *L. vulgaris* (94%). Lower accuracy (81%) was registered for females of *L. schmidtleri* and *L. vulgaris*. Only females of *L. kosswigi* and *L. schmidtleri* did not differ from each other.

Table 5. Significance of sex differences (the Kolmogorov-Smirnov test) in *Lissotriton kosswigi*, *L. schmidtleri* and *L. vulgaris*. Difference are significant if P < 0.001 (+++), P < 0.01 (++), P < 0.05 (+); – is insignificant differences. N is the number of dark spots, AS is the proportion of dark spots in relation to the entire area of the polygon, IRSA is the index of relative spot area, ØC is mean circularity, and ØR is mean roundness.

Sex	Species	Character				
		N	AS	IRSA	ØC	ØR
Belly	*kosswigi*	+++	+++	+++	+++	+++
	schmidtleri	+++	+++	+++	+++	+++
	vulgaris	+++	+++	+++	+++	+++
Throat	*kosswigi*	–	+++	+++	+	–
	schmidtleri	–	+++	+++	–	–
	vulgaris	+++	+++	+++	–	–

The canonical discriminant analysis (populations as a grouping variable) of both sexes showed that samples of *L. vulgaris* always formed a distinct cluster (Fig. 4). The only exception was observed in males, where the centroid of the *L. kosswigi* sample from Hacılar (Table 1) was located within *L. vulgaris* samples. Males of *L. kosswigi* and *L. schmidtleri* also formed distinct clusters, but females have strongly overlapping clusters (Fig. 4). Within these species, we did not find a relationship between the geographical location of samples and their spot pattern.

Significant differences between sexes were found for all five belly characteristics in all three smooth newt species (Table 5). The greatest differences were observed for two characteristics: the proportion of dark spots (AS) and the index of relative spot area (IRSA). As a rule, males had larger spots than females. The throat pattern turned out to be less informative.

IDENTIFICATION KEY

Males

1 Numerous dark spots of irregular shape on the throat, poorly developed dorsal crest, well developed filament at the end of a tail (Fig. 2) *L. kosswigi*

– Round spots on the throat, well developed dorsal crest, no thin filament at the end of a tail (Fig. 2) 2

2 Spots on the throat and belly has nearly the same size *L. lantzi*

– Spots on the belly are obviously larger than on the throat 3

3 Spots on the belly are big (as a rule, larger than 1 mm) *L. vulgaris*

– Spots on the belly are small (less than 1 mm) *L. schmidtleri*

Females

1 Lateral dark spot arrangement is striped (Fig. 3: b, s) *L. vulgaris*

– Lateral dark spot arrangement is spotted (Fig. 3: r, l) 2

2 Mean diameter of spots on the belly is approximately two times larger than on the throat: *L. kosswigi*

– Diameter of spots on the belly is usually the same or little bigger than on the throat 3

3 Spots on the belly are small (usually less than 0.4 mm) *L. lantzi*

– Spots on the belly are big (more than 0.4 mm) *L. schmidtleri*

DISCUSSION

The ventral and lateral dark spot patterns are very useful for distinguishing smooth newt species during field research and study of museum specimens. It was previously shown that both sexes of *L. vulgaris* and *L. lantzi* have significant interspecific differences by the index S/L^2, proportion of dark spots (AS) on the belly and degree of evenness of the spot's edge (ØR) on the throat (Skorinov & Litvinchuk 2013). Additionally, obvious differences in the type of lateral dark spot arrangement in females were observed. Females of *L. lantzi* are characterised by a predominance of spotted patterns, but *L. vulgaris* by the presence of stripes.

In the present study, we found that both sexes of *L. kosswigi*, *L. schmidtleri* and *L. vulgaris* are significantly different from each other by the index IRSA which is the modification of previously used the index S/L^2. Thus, our results showed that the index IRSA is the best characteristic for identifying smooth newt species. Such characteristics as AS and ØR, as well as the type of lateral dark spot arrangement, could be essential as well.

It should be noted that among males *L. schmidtleri* and *L. vulgaris* had the most distinct both belly and throat patterns (small and large spots, respectively). This is very important because males of these species are similar in terms of the development of their dorsal crest, tail filament and toe webbing during the breeding season (Raxworthy 1990, Skorinov *et al*. 2008). Males of *L. kosswigi* differ from the other two smooth newt species by numerous spots of irregular shape on the throat. A single exclusion concerned males of *L. kosswigi* from Hacılar (the eastern side of the species' range). Among females, *L. schmidtleri* and *L. kosswigi* were different from *L. vulgaris* in the size of dark spots on the belly and throat (small in the first two and large in the last species), as well as by dark spot arrangements on lateral surfaces (spots and stripes, respectively). Thus, the use of dark spot pattern characteristics makes it possible to distinguish specimens of these three smooth newt species with a high degree of accuracy (degree of confidence is 81–94%). A single exception is females of *L. schmidtleri* and *L. kosswigi*, which are obviously differed from each other by relative differences in the diameter of spots on the belly and throat (differences in size of the spots by 2 and 1.5 times, respectively; Table 2). Based on the studied spot pattern characters (and some secondary sexual characters in males), we proposed an identification key to identify both males and females of four smooth newt species (including *L. lantzi*).

It was previously considered that *L. schmidtleri* occurs only in the Anatolian part of Turkey (with *L. vulgaris* distributed in Thrace) (Raxworthy 1988, Raxworthy 1990, Babik *et al*. 2005). However, later molecular studies (Wielstra *et al*. 2018) showed that *L. schmidtleri* inhabits both the Anatolian and Thracian parts of Turkey and even occurs in neighbouring regions of southeastern Bulgaria and eastern Greece (Fig. 1). Our results support this point of view. Both Anatolian and Thracian populations of *L. schmidtleri* have similar spot patterns, morphological (Eiselt 1966, Yilmaz 1983, Olgun *et al*. 1999) and genetic (Skorinov *et al.* 2008, Nadachowska & Babik 2009, Pabijan *et al*. 2015, 2017, Wielstra *et al*. 2018) characteristics and, therefore, should be assigned to the same species. It should be noted that in western Anatolia, species of crested newts (*Triturus anatolicus* and *T. ivanbureshi*) has nearly similar boundaries of ranges (Wielstra *et al*. 2012, Wielstra & Arntzen 2016) to that of *L. kosswigi* and *L. schmidtleri*. Perhaps, this reflects the speciation peculiarities of these tailed amphibians in the region.

Finally, it should be noted that the method we used could also be applied to comparative studies of other animal species with bright spotted camouflage or aposematic colouration.

*

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REFERENCES

Arntzen, J. W., McAtear, J., Recuero, E., Ziermann, J. M., Ohler, A., van Alphen, J. & Martínez-Solano, I. (2013): Morphological and genetic differentiation of Bufo toads: two cryptic species in Western Europe (Anura, Bufonidae). – *Contributions to Zoology* **82**: 147–169. https://doi.org/10.1163/18759866-08204001

Babik, W., Branicki, W., Crnobrnja-Isailović, J., Cogălniceanu, D., Sas, I., Olgun, K., Poyarkov, N. A., Garcia-París, M. & Arntzen, J. W. (2005): Phylogeography of two European newt species—discordance between mtDNA and morphology. – *Molecular Ecology* **14**: 2475–2491. https://doi.org/10.1111/j.1365-294X.2005.02605.x

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., Ingram K. K. & Das, I. (2007): Cryptic species as a window on diversity and conservation. – *Trends in Ecology & Evolution* **22**: 148–55. https://doi.org/10.1016/j.tree.2006.11.004

Borkin, L. J., Litvinchuk, S. N., Rosanov, Yu. M. & Skorinov, D. V. (2004): On cryptic species (an example of amphibians). – *Entomological Review* **84**(Suppl. 1): 75–98.

Casu, M. & Curini-Galletti, M. (2004): Sibling species in interstitial flatworms: a case study using Monocelis lineata (Proseriata: Monocelididae). – *Marine Biology* **145**: 669–679. https://doi.org/10.1007/s00227-004-1367-x

Eiselt, J. (1966): Ergebnisse zoologischer Sammelreisen in der Türkei: Amphibia caudata. – *Annalen des Naturhistorischen Museums in Wien* **69**: 427–445.

Frost, D. R. (2022): *Amphibian species of the world. Version 6.1.* https://amphibiansoftheworld.amnh.org/index.php. American Museum of Natural History, New York, USA. https://doi.org/10.5531/db.vz.0001

Dufresnes, C., Mazepa, G., Rodrigues, N., Brelsford, A., Litvinchuk, S. N., Sermier, R., Lavanchy, G., Betto-Colliard, C., Blaser, O., Borzée, A., Cavoto, E., Fabre, G., Ghali, K., Grossen, C., Horn, A., Leuenberger, J., Phillips, B. C., Saunders, P. A., Savary, R., Maddalena, T., Stöck, M., Dubey, S., Canestrelli, D. & Jeffries, D. L. (2018): Genomic evidence for cryptic speciation in tree frogs from the Apennine Peninsula, with description of Hyla perrini sp. nov. – *Frontiers in Ecology and Evolution* **6**: 1–18. https://doi.org/10.3389/fevo.2018.00144

Hagström, T. (1973): Identification of newt specimens (Urodela, Triturus) by recording the belly pattern and a description of photographic equipment for such registrations. – *British Journal of Herpetology* **4**: 321–326.

Litvinchuk, S. N., Svinin, A. O. & Dujsebayeva, T. N. (2021): Morphological differentiation between diploid and polyploid species of green toads (Anura: Bufonidae: Bufotes Rafinesque, 1815) in Central Asia. – *Bonn zoological Bulletin* **70**: 361–371. https://doi.org/10.20363/BZB-2021.70.2.361

Mann, D. G. & Evans, K. M. (2008): The species concept and cryptic diversity. Pp. 262–268. *In:* Moestrup, Ø., Doucette, G., Enevoldsen, H., Godhe, A., Hallegraeff, G., Luckas, B., Lundholm, N., Lewis, J., Rengefors, K., Sellner, K., Steidinger, K., Tester, P. & Zingone, A. (eds): *Proceedings of the 12th International Conference on Harmful Algae*. – International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO, Copenhagen.

Martínez-Solano, Í., Teixeira, J., Buckley, D., García-París, M. (2006): Mitochondrial DNA phylogeography of Lissotriton boscai (Caudata, Salamandridae): evidence for old, multiple refugia in an Iberian endemic. – *Molecular Ecology* **15**: 3375–3388. https://doi.org/10.1111/j.1365-294X.2006.03013.x

Mattoccia, M., Romano, A. & Sbordoni, V. (2005): Mitochondrial DNA sequence analysis of the spectacled salamander, Salamandrina terdigitata (Urodela: Salamandridae), supports the existence of two distinct species. – *Zootaxa* **995**: 1–19. https://doi.org/10.11646/zootaxa.995.1.1

Mettouris, O., Megremis, G. & Giokas, S. (2016): A newt does not change its spots: using pattern mapping for the identification of individuals in large populations of newt species. – *Ecological Research* **31**: 483–489. https://doi.org/10.1007/s11284-016-1346-y

Nadachowska, K. & Babik, W. (2009): Divergence in the face of gene flow: the case of two newts (Amphibia: Salamandridae). – *Molecular Biology and Evolution* **26**: 829–841. https:// doi.org/10.1093/molbev/msp004

Neusser, T. P., Jörger, K. M. & Schrödl, M. (2011): Cryptic species in tropic sands – Interactive 3D anatomy, molecular phylogeny and evolution of meiofaunal Pseudunelidae (Gastropoda, Acochlidia). – *PLoS ONE* **6**: e23313. https://doi.org/10.1371/journal.pone.0023313

Olgun, K., Baran, I. & Tok, C. V. (1999): The taxonomic status of Triturus vulgaris (Linnaeus, 1758) populations in western Anatolia, Turkey. – *Turkish Journal of Zoology* **23**: 133–140.

Pabijan, M., Zieliński, P., Dudek, K., Chloupek, M., Sotiropoulos, K., Liana, M. & Babik, W. (2015): The dissection of a Pleistocene refugium: phylogeography of the smooth newt, Lissotriton vulgaris, in the Balkans. – *Journal of Biogeography* **42**: 671–683. https://doi.org/10.1111/jbi.12449

Pabijan, M., Zieliński, P., Dudek, K., Stuglik, M. & Babik, W. (2017): Isolation and gene flow in a speciation continuum in newts. – *Molecular Phylogenetics and Evolution* **116**: 1–12. https://doi.org/10.1016/j.ympev.2017.08.003

Papežík, P., Kubala, M., Jablonski, D., Doležálková-Kaštánková, M., Choleva, L., Benovics, M. & Mikulíček, P. (2021): Morphological differentiation of endemic water frogs (Ranidae: Pelophylax) from the southwestern Balkans. – *Salamandra* **57**: 105–123.

Raxworthy, C. J. (1988): A description and study of a new dwarf sub-species of smooth newt, Triturus vulgaris, from western Anatolia, Turkey. – *Journal of Zoology* **215**: 753–763. https://doi.org/10.1111/j.1469-7998.1988.tb02409.x

Raxworthy, C. J. (1990): A review of the smooth newt (Triturus vulgaris) subspecies, including an identification key. – *Herpetological Journal* **1**: 481–492.

Rodríguez, A., Burgon, J. D., Lyra, M., Irisarri, I., Baurain, D., Blaustein, L., Göçmen, B., Künzel, S., Mable, B. K., Nolte, A. W., Veith, M., Steinfartz, S., Elmer, K. R., Philippe, H. & Vences, M. (2017): Inferring the shallow phylogeny of true salamanders (Salamandra) by multiple phylogenomic approaches. – *Molecular Phylogenetics and Evolution* **115**: 16–26. https://doi.org/10.1016/j.ympev.2017.07.009

Sequeira, F., Bessa-Silva, A., Tarroso, P., Sousa-Neves, T., Vallinoto, M., Gonçalves, H. & Martínez-Solano, I. (2019): Discordant patterns of introgression across a narrow hybrid zone between two cryptic lineages of an Iberian endemic newt. – *Journal of Evolutionary Biology* **33**: 202–216. https://doi.org/10.1111/jeb.13562

Skorinov, D. V. & Litvinchuk, S. N. (2013): Variation of ventral colour pattern in two cryptic newt species, Lissotriton vulgaris and L. lanzi (Amphibia: Salamandridae). – *Proceedings of the Zoological Institute RAS* **317**: 459–473.

Skorinov, D. V., Litvinchuk, S. N., Borkin, L. J. & Rosanov, J. M. (2008): Genetic differentiation, genome size and morphological variation in newts of the Lissotriton vulgaris group. Pp. 375–383. *In:* Ananjeva, N. B., Danilov, I. G., Dunayev, E. A., Ishchenko, V. G., Lada, G. A., Litvinchuk, S. N., Orlova, V. F., Smirina, E. M., Tuniyev, B. S. & Khalikov, R. G. (eds): *The problems of herpetology*. – The A. M. Nikolsky Herpetological Society, St. Petersburg.

Skorinov, D. V., Litvinchuk, S. N., Borkin, L. J. & Rosanov, J. M. (2011): Systematics of newts of the Lissotriton vulgaris complex (Salamandridae). Pp. 235–240. *In:* Ananjeva, N. B., Danilov, I. G., Dunayev, E. A., Ishchenko, V. G., Lada, G. A., Litvinchuk, S. N., Orlova, V. F., Smirina, E. M., Tuniyev, B. S. & Khalikov, R. G. (eds): *The problems of herpetology*. – The A. M. Nikolsky Herpetological Society, St. Petersburg.

Speybroeck, J., Beukema, W., Dufresnes, C., Fritz, U., Jablonski, D., Lymberakis, P., Martínez-Solano, I., Razzetti, E., Vamberger, M., Vences, M., Vörös, J. & Crochet, P. -A. (2020): Species list of the European herpetofauna – 2020 update by the Taxonomic Committee of the Societas Europaea Herpetologica. – *Amphibia-Reptilia* **41**: 139–189. https://doi.org/10.1163/15685381-bja10010

Stuart, B., Inger, R. & Voris, H. K. (2006): High level of cryptic species diversity revealed by sympatric lineages of Southeast Asian forest frogs. – *Biology Letters* **2**: 470–474. https://doi.org/10.1098/rsbl.2006.0505

Trevisan, C. C., Batalha-Filho, H., Garda, A. A., Menezes, L., Dias, I. R., Solé, M., Canedo, C., Juncá, F. A. & Napoli, M. F. (2020): Cryptic diversity and ancient diversification in the northern Atlantic Forest Pristimantis (Amphibia, Anura, Craugastoridae). – *Molecular Phylogenetics and Evolution* **148**: 106811. https://doi.org/10.1016/j.ympev.2020.106811

Üzüm, N., Avci, A., Olgun, K., Bülbül, U., Fahrbach, M., Litvinchuk, S. N. & Wielstra, B. (2019): Cracking cryptic species: external characters to distinguish two recently recognised banded newt species (Ommatotriton ophryticus and O. nesterovi). – *Salamandra* **55**: 131–134.

van Riemsdijk, I., Arntzen, J. W., Bogaerts, S., Franzen, M., Litvinchuk, S. N., Olgun, K. & Wielstra, B. (2017): The Near East as a cradle of biodiversity: a phylogeography of banded newts (genus Ommatotriton) reveals extensive inter-and intraspecific genetic differentiation. – *Molecular Phylogenetics and Evolution* **114**: 73–81. https://doi.org/10.1016/j.ympev.2017.05.028

van Riemsdijk, I., Arntzen, J. W., Babik, W., Bogaerts, S., Franzen, M., Kalaentzis, K., Litvinchuk, S. N., Olgun, K., Wijnands, J. W. P. M. & Wielstra, B. (2021): Next-generation phylogeography of the banded newts (Ommatotriton): a phylogenetic hypothesis for three ancient species with geographically restricted interspecific gene flow and deep intraspecific genetic structure. – *Molecular Phylogenetics and Evolution* **167**: 107361. https://doi.org/10.1016/j.ympev.2021.107361

Wielstra, B. & Arntzen, J. W. (2016): Description of a new species of crested newt, previously subsumed in Triturus ivanbureschi (Amphibia: Caudata: Salamandridae). – *Zootaxa* **4109**: 73–80. https://doi.org/10.11646/zootaxa.4109.1.6

Wielstra, B., Beukema, W., Arntzen, J. W., Skidmore, A. K., Toxopeus, A. G. & Raes, N. (2012): Corresponding mitochondrial DNA and niche divergence for crested newt candidate species. – *PLoS ONE* **7**: e47771. https://doi.org/10.1371/journal.pone.0046671

Wielstra, B., Litvinchuk, S. N., Naumov, B., Tzankov, N. & Arntzen, J. W. (2013): A revised taxonomy of crested newts in the Triturus karelinii group (Amphibia: Caudata: Salamandridae), with the description of a new species. – *Zootaxa* **3682**: 441–453. https://doi.org/10.11646/zootaxa.3682.3.5

Wielstra, B., Bozkurt, E. & Olgun, K. (2015): The distribution and taxonomy of Lissotriton newts (Amphibia, Salamandridae) in Turkey. – *ZooKeys* **484**: 11–23. https://doi.org/10.3897/zookeys.484.8869

Wielstra, B., Canestrelli, D., Cvijanović, M., Denoël, M., Fijarczyk, A., Jablonski, D., Liana, M., Naumov, B., Olgun, K., Pabijan, M., Pezzarossa, A., Popgeorgiev, G., Salvi, D., Si, Y., Sillero, N., Sotiropoulos, K., Zieliński, P. & Babik, W. (2018): The distributions of the six species constituting the smooth newt species complex (Lissotriton vulgaris sensu lato and L. montandoni) – an addition to the New Atlas of Amphibians and Reptiles of Europe. – *Amphibia-Reptilia* **39**: 252–259. https://doi.org/10.1163/15685381-17000128

Yilmaz, I. (1983): A morphological and taxonomical investigations of Thracian urodeles (Urodela: Salamandridae). – *Doğa Bilim Dergisi, Temel Bilm* **7**: 119–130.

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