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## Molecular and morphological evidence for a new subspecies of Fazila's Lycian Salamander *Lyciasalamandra fazilae* in South-west Anatolia

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A new subspecies of the Fazila's Lycian Salamander *Lyciasalamandra fazilae* is described based on material from ten localities in the Köyceğiz, Ortaca and Dalaman area in south-western Turkey. It is distinguished from the nominotypical subspecies by differences in the colouration pattern, morphometry and the mitochondrial molecular marker 16S rRNA. The distribution area of the new subspecies is located mainly in the western part of Dalaman River except for two seemingly intermediate populations (Şerefler and Sarsala-Kapikargin). New localities for the species are reported.

**Keywords:** Lycian Salamander; Caudata; new subspecies; Turkey

### Introduction

The first specimens of Fazila's Lycian Salamander were found by Başoğlu and Freytag (1958) in the area of Gökçeovacık village in Fethiye district of the Turkish province of Muğla, and diagnosed as *Mertensiella luschnani*. Sixteen years later, this population was assigned to a new subspecies, *Mertensiella luschnani fazilae* (Başoğlu & Atatürk, 1974). In 2004, Lycian salamanders were assigned to a new genus, *Lyciasalamandra*, by Veith and Steinfartz (2004) using genetic data, and most former subspecies, except for the today's *L. luschnani* subspecies *luschnani*, *basoglu* and *finikensis*, were elevated to species rank based on comprehensive molecular and non-molecular investigations (see also Weisrock, Macey, Uğurtaş, Larson, & Papenfuss, 2001).

During the last decade, several new populations of *Lyciasalamandra* have been found and some of them has been described as new subspecies (Göçmen, Arikhan, & Yalçınkaya, 2011; Göçmen & Akman, 2012; Göçmen et al., 2013; Akman & Godmann, 2014; Godmann, Karış, & Göçmen, 2016; Oğuz, Göçmen, & Yalçınkaya, 2016; Tok, Afsar, & Yakin, 2016; Göçmen & Karış, 2017). Up to now, only the Greek *L. helverseni* and the Turkish Fazila's Lycian Salamander, *L. fazilae*, remained monotypic, although for both substantial intraspecific genetic variation has been reported (e.g., Eleftherakos, Sotioropoulos, & Polymeni, 2007; Veith et al., 2008, 2016). *Lyciasalamandra fazilae* inhabits a comparatively large distribution area, with several populations living between south of Sultaniye, Köyceğiz district and northwest of İncirköy, Fethiye district as well as some nearby offshore islands. Population altitudes range from sea level up to ca. 1060 m asl (Veith et al., 2001 and references therein; Kordges, Thiesmeier, Meinig, & Eckstein, 2005; Polat & Başkale, 2018; present study).

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Between 2011 and 2017 we conducted comprehensive field studies in Muğla province to clarify the range limits of the species and to find new *L. fazilae* populations. As we recognised distinct morphological differences between some populations, we describe here a new subspecies based on differences in morphology and molecular markers.

## Material and Methods

**Collection of material.** For the morphological analysis, a total of 130 specimens of *Lyciasalamandra fazilae* was collected between February 2011 and March 2017. They comprise 49 (14♂, 25♀, 10 juv.) specimens of *L. f. fazilae* from seven localities and 81 (28♂, 35♀, 18 juv.) specimens of the new subspecies from ten localities. The salamanders were searched under rocks in areas with a high amount of karstic limestones. Specimens were kept in terrariums for colouration analysis and photography.

Tissue samples were taken from the distal end of the toes of collected specimens after anaesthesia with Tricaine (MS-222, Sigma). Specimens were afterwards fixed by a 96% ethanol injection into their body and deposited at the Zoology Museum of Adiyaman University (ZMADYU) in Adiyaman province, Turkey. The material and data on collection sites is given in Table 1.

**Statistics.** Statistics were calculated with SPSS 15.0 for Windows. Raw metric characters and indexed values of PERCRA (percent's of rostrum-anus length; [each metric character / RC] × 100) were tested with a Student's *t*-Test according to Werner (1971), which allowed to refined the analysis (Supplementary Tables 1-2). Kolmogorov-Smirnov and Shapiro-Wilk were used to test whether samples of measurements show a normal distribution, and differences between the mean values of normally distributed samples was tested Student's *t*-test. For the comparison of mensural (metric) characters we used all adults (males and females with a Total Body Length >90 mm) to avoid effects of allometry. The significance level of statistical evaluations was  $p \leq 0.05$ .

**Morphometry.** Metric characters were measured with a Mitutoyo digital caliper of 0.02 mm sensitivity, except total body length, rostrum-cloaca length and tail length, which were measured by a millimetric ruler. The following measurements of body parts and ratios were taken in line with the methods described e.g. by Akman et al. (2011) and Göçmen and Karış (2017). Mensural (metric) characters: Total Body Length (TBL): length of the whole body including the tail; Rostrum-Anus Length (RC): length from the snout to the anterior tip of the cloacal opening; Length of Trunk (LT): length from gular fold to the anterior edge of cloacal opening; Tail Length (TL); Nostril-Eye Distance (NED); Distance Between Nostrils (DBN); Eye Diameter (ED); Head Length (HL): distance from the snout to the gular fold; Head Width (HW); Parotoid Length (PL); Parotoid Width (PW); Fore Limb Length (FLL); Hind Limb Length (HLL); Distance between Fore- and Hind Limbs (DFHL); Height of dorsal protuberance on base of the tail [for males only] (HDPBT). Computed characters (ratios): Ratios of the characters HW/HL, TL/TBL, PW/PL, NED/HL and all characters / RC × 100 (PERCRA) were also computed and compared with Student's *t*-Test.

**Genetic analysis.** For molecular analysis we studied 87 specimens of *L. fazilae* from 19 localities. We integrated already published sequences from Veith et al. (2008) as well as 62 new samples from 13 additional localities. We used a fragment of the mitochondrial 16S rRNA gene as molecular marker. For hierarchical outgroup rooting we used one representative of the other *Lyciasalamandra* species, *Salamandra salamandra* and *Chioglossa lusitanica* (Supplementary Table S3).

For rRNA isolation we used the Qiagen Blood and Tissue Kit following the manufacturer's protocol. We sequenced a fraction of the 16S rRNA gene using the primers 16SAL and 16SBH of Palumbi, Martin, Romano, McMillan, Stice, and Grabowski (2002). PCR reactions were prepared using the 5Prime Master Mix, and PCR products were purified using the High Pure PCR Product Purification Kit of Roche: initial denaturation step for 2 min at 94°, 33 cycles of denaturation for 30 sec at 94° and elongation for 60 sec at 65°, final elongation for 10 min at 65°.

We automatically aligned the sequences with MAFFT (version 7, Katoh & Standley, 2013) using the iterative refinement method (Berger & Munson, 1991; Gotoh, 1993) and the

Table 1. Voucher numbers and localities of *L. f. ulsetae* ssp. n. and *L. f. fazilae* with descriptions of new localities for *L. fazilae*. TT = *terra typica*. A star (\*) indicates the populations which are morphologically affiliated to nominate subspecies but genetically affiliated to *L. f. ulsetae* ssp. n. Altitude (Alt.) given in m. The numbers give the registration numbers in ZMADYU.

	Numbers	Localities	Alt.	Coordinates	Date, no. of specimens
<i>L. f. fazilae</i>	2012/25:1	NW of İncirköy, Fethiye [a]	1030	36°47'N 29°11'E	27.02.2012, 1♀
	2011/87:1-5	S of Kavacık, Dalaman [b]	665	36°50'N 29°00'E	28.02.2011, 2♂, 3♀
	2017/204:1-6	Gökçevacık, Fethiye (TT) [c]	530	36°47'N 28°59'E	09.02.2017, 1♂, 2♀, 3 juv.
	2013/181:1-2	Gökçevacık, Fethiye (TT) [c]	530	36°47'N 28°59'E	16.02.2013, 1♂, 1♀
	2017/210:1-11	Domuz island, Darboğaz [d]	40	36°39'N 28°54'E	11.02.2017, 4♂, 5♀, 2 juv.
	2017/202:1-7	Cataldağ, Darboğaz (Kapıdağ peninsula) [e]	25	36°38'N 28°54'E	09.02.2017, 2♂, 2♀, 3 juv.
	2017/209:1-6	Göbüñ cove (Kapıdağ peninsula) [f]	10	36°38'N 28°53'E	11.02.2017, 2♂, 4♀
	2017/203:1-11	Kleopatra-Manastır cove (Kapıdağ peninsula) [g]	40	36°38'N 28°51'E	09.02.2017, 2♂, 7♀, 2 juv.
<i>L. f. ulsetae</i> ssp. n.	2017/207:1-9	S of Sultanije, Köyceğiz [1]	335	36°52'N 28°34'E	10.02.2017, 2♂, 3♀, 4 juv.
	2017/208:1-11	Ülemez-Horozlar, Köyceğiz (TT) [2]	120	36°50'N 28°37'E	10.02.2017, 2♂, 6♀, 3 juv.
	2017/206:1-6	Okçular, Ortaca [3]	30	36°50'N 28°40'E	10.02.2017, 2♂, 2♀, 2 juv.
	2015/141:1-5	Gökbel, Ortaca [4]	165	36°46'N 28°39'E	18.04.2015, 2♂, 2♀, 1 juv.
	2012/26:1-3	Gökbel, Ortaca [4]	165	36°46'N 28°39'E	26.02.2012, 1♂, 1♀, 1 juv.
	2017/213:1-7	Kargıcak cove, Ortaca [5]	200	36°45'N 28°38'E	11.02.2017, 3♂, 4♀
	2017/212:1-10	Aşı cove, Ortaca [6]	90	36°43'N 28°40'E	11.02.2017, 4♂, 4♀, 2 juv.
	2017/211:1-6	Mergenli, Ortaca [7]	55	36°44'N 28°41'E	11.02.2017, 3♂, 3♀
	2017/214:1-5	SW of Güzelyurt, Ortaca [8]	35	36°44'N 28°42'E	10.03.2017, 3♂, 2♀
	2017/205:1-9	(*)Şerefler, Dalaman [9]	165	36°44'N 28°50'E	09.02.2017, (2♂, 5♀, 2 juv.
	2012/24:1-10	(*)Sarsala-Kapıkargin, Dalaman [10]	200	36°40'N 28°50'E	27.02.2012, (4♂, 3♀, 3 juv.

Needleman-Wunsch algorithm (Needleman & Wunsch, 1970) with default parameter settings. Ambiguous areas in the alignment were excluded using Gblocks (version 0.91b, Castresana, 2000). Gaps are half allowed, the remaining settings are default settings. The best-fitting model of nucleotide substitutions was determined from a set of 88 nested models using jModeltest (version 2.1.3, Darriba, Taboada, Doallo, & Posada, 2012; Guindon & Gascuel, 2003) under the Akaike Information Criterion (AIC).

We generated a haplotype network for the mitochondrial 16S rRNA dataset. We sorted the dataset into haplotypes using FaBox (Villesen, 2007) and used Arlequin (Excoffier, Laval, & Schneider, 2005) for calculating the distance matrix and HapStar (version 07, Teacher & Griffiths, 2011) for gene tree visualization. We performed phylogenetic analysis on the haplotype dataset using Maximum Likelihood (ML) and Bayesian Inference (BI). In RAxML we used the GTRGAMMA model running 2,000 bootstrap replicates using rapid bootstrapping and the greedy algorithm (Stamatakis, 2014). BI was performed with MrBayes (version 3.2.6; Ronquist & Huelsenbeck, 2003; Ronquist et al., 2012) and with the best-fitting substitution model. Two runs were started separately in order to avoid local maxima, each analysis running four independent Markov Chain Monte Carlo (MCMC) analyses with one cold and three heated chains for 10 million generations. We sampled a tree every 1,000<sup>th</sup> generation with a burn-in of 20%.

The present study was carried out according to the Animal Ethical Committee of Ege University (#2011-091) and approved by the Republic of Turkey Ministry of Forestry and Water Affairs (#2014-62406).

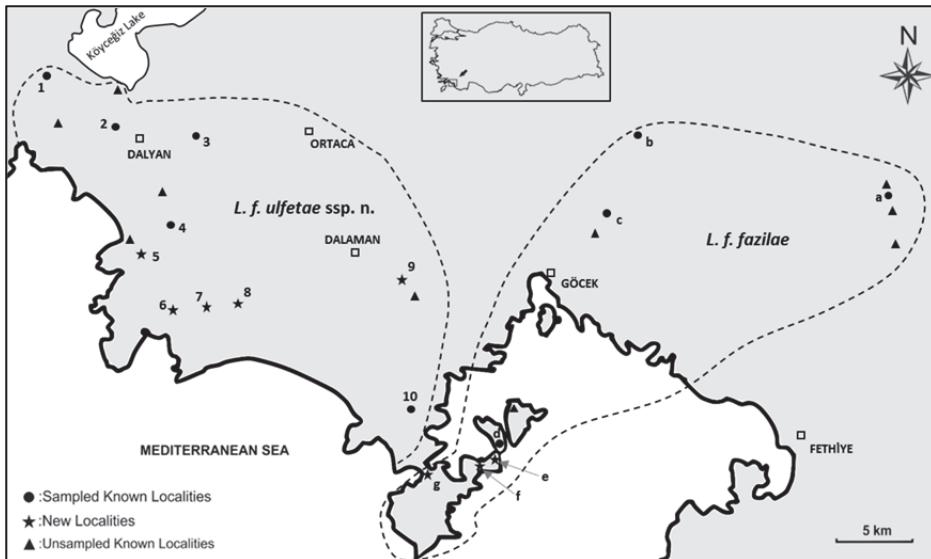


Figure 1. Distribution of *Lyciasalamandra fazilae*. Letters indicate ssp. *fazilae*, numbers indicate *L. f. ulfetae* ssp. n. For locality details with explanation of letters and numbers see Table 1.

## Results and Discussion

### Intraspecific variation in *Lyciasalamandra fazilae*

The current distribution of the *Lyciasalamandra fazilae* with the new localities is given in Figure 1. In terms of colour and pattern, specimens from western Dalaman stream populations differ from eastern Dalaman stream populations. The specimens from western populations are dominated by larger area of dark blackish colouration on dorsum than eastern populations. Inside of this pattern, specimens from western populations have numerous tiny white spots while the specimens from eastern populations have fewer and relatively bigger white spots (Figure 3).

Significant differences were found in some morphometric characters between males and females (see Supplementary Tables S1-2). A comparison of the two subspecies (Table 2) showed that males differ significantly ( $p \leq 0.05$ ) in PW (raw data) and TBL, TL, and PW (PERCRA values). Females differ significantly in the ratios HW/HL and NED/HL. Juveniles significantly differ in almost all characters ( $p \leq 0.05$ ), but morphometry of juveniles can be variable as expected. As a result of comparison of the combined adults (males + females), we only found PERCRA index value of PW statistically different ( $p \leq 0.05$ ). According to these results, especially parotoid width (PW) is considerably different between the two subspecies.

In total, we analysed a 16S rRNA gene fragment of 483 basepairs. GBlocks removes five basepairs of the original dataset (238-242 bp), and according to jModeltest we used TVM+I+G as the best-fitting substitution model. The morphometric differences between the western and eastern Dalaman stream populations were clearly supported by the molecular analysis.

According to the genus tree analysis, the dataset was collapsed into 16 haplotypes and we identified 13 differences between the two populations separated by the Dalaman river (Supplementary Table S4, Figure 4). The phylogenetic analysis using ML and BI



Figure 2. Habitus of the holotype of *Lyciasalamandra fazilae ulfetae* ssp. n. (2017/208-2♂).

also supports the differences with Bayesian Posterior Probability of 1 and bootstrap value of 81 (Figure 5).

Based on the aforementioned results, we describe the western population of *L. fazilae* as new subspecies.

#### *Lyciasalamandra fazilae ulfetae* ssp. n. (Figure 2)

*Holotype:* Adult male, ZMADYU 2017/208-2 from Ülemez-Horozlar (ca. 2 km NE of Çandır village), Köyceğiz district, Muğla province, Turkey, 120 m asl (36°50'N, 28°37'E), 10.ii.2017, B. Göçmen, M. Karış & D. Şener leg. (for habitat photograph see Supplementary Figure S1). – *Paratypes:* 80 specimens from ten localities collected from districts of Köyceğiz, Ortaca and Dalaman, Muğla province; they were deposited as paratypes. For locality details and collection numbers see Table 1.

*Diagnosis.* *Lyciasalamandra fazilae ulfetae* ssp. n. is clearly distinct from all other *Lyciasalamandra* species by its colouration and pattern except from the nominotypic subspecies (*L. f. fazilae*). Due to its size (close to *L. f. fazilae*) and colour pattern traits (orange red ground colouration), the new taxon belongs to *L. fazilae*. It differs from the nominate subspecies by having a somewhat darker orange-red coloured ground of dorsum and head; absence of the continuous white lateral bands on both flanks (except the two seemingly intermediate populations in Şerefler and Sarsala). Besides, it has a larger area of the dark blackish pattern with numerous tinsel-like tiny white dots on dorsum and head except parotoids, while the nominate subspecies has a basically lighter orange-red coloured dorsum (however showing more or less variations), head, and tail. Also, *L. f. fazilae* specimens mostly have lighter coloured upper eyelids except



Figure 3. Colour-pattern variations on some populations of the two subspecies of *L. fazilae*. Sultaniye, Ülemez, Okçular, Kargıcak, Mergenli, Şerefiler and Sarsala belong to *L. f. ulfetae* ssp. n. while Manastır, Göbüñ, Çataldağ, Domuz and Gökçeovacık belong to *L. f. fazilae*.

Gökçeovacık specimens, dark brown pattern covers less space, with fewer but bigger white spots on the ground colour of dorsum, also almost all specimens of the populations have continuous lateral white band on both flanks. All adenoid gland openings (black dots) are clearly visible, forming a longitudinal row along the median part of the dorsum, while irregularly scattered on the parotoids (Figure 2).

*Description of the holotype.* Head flat, longer than broad (HW/HL 0.69). Snout rounded. Parotoids long and narrow (PW/PL 0.34), the posterior part broader than the anterior part. Gular fold distinct (folded). The cloacal region shows a very slight swelling. The finger-like projection above the base of the tail about 1.45 mm (2.20 PERCRA), pointed and curved forward at its apex. Tail shorter than rostrum-cloaca (TL 53 mm, 80.30 PERCRA (= length of parameter (L) in percent of rostrum-cloaca length; RC 66 mm). TBL 119 mm [180.30 PERCRA], LT 50.88 mm (77.09 PERCRA), eyes big, eye diameter 4.37 mm [6.62 PERCRA]. HL 15.12 mm, HW 10.48 mm [22.91 and 15.88 PERCRA]. Parotoids distinct, PL 7.56 mm [11.45 PERCRA], PW 2.58 mm [3.91 PERCRA]. Hind limb longer than fore limb (HLL 23.37 mm, 35.41 PERCRA; FLL 20.10 mm, 30.45 PERCRA). Nostril-eye distance 2.26 mm [3.42 PERCRA],

Table 2. Comparison of mean values of the mensural characters and indexes between *L. f. fazilae* and *L. f. ulfetae* ssp. n. populations with P-values (significance of Student's *t*-Test). Characters with significant differences ( $p \leq 0.05$ ) in bold. 1: according to the values in raw data; 2: according to the values in PERCRA index. Abbreviations of the characters are given in Materials and Methods.

	Males						Females					
	<i>L.f. fazilae</i>		<i>L.f. ulfetae</i> ssp.n.		P	<i>L.f. fazilae</i>		<i>L.f. ulfetae</i> ssp.n.		P		
	Mean	SD	Mean	SD		Mean	SD	Mean	SD			
TBL	<b>1</b> 117.29	7.09	115.39	7.68	0.43	114.84	12.15	116.26	13.70	0.67		
	<b>2</b> 181.01	3.55	178.28	4.69	<b>0.04</b>	176.95	9.54	179.00	4.61	0.33		
RC	<b>1</b> 64.79	3.49	64.71	3.78	0.95	65.04	7.21	64.91	7.21	0.95		
LT	<b>1</b> 49.90	2.83	49.87	3.23	0.98	49.94	5.76	49.67	5.81	0.86		
	<b>2</b> 77.02	0.71	77.05	1.41	0.92	76.75	1.06	76.46	1.00	0.29		
TL	<b>1</b> 52.50	3.96	50.86	4.25	0.23	50.96	6.28	51.34	6.85	0.83		
	<b>2</b> 81.01	3.55	78.55	3.97	<b>0.05</b>	79.46	4.06	79.00	4.61	0.69		
NED	<b>1</b> 2.52	0.26	2.61	0.29	0.31	2.74	0.34	2.65	0.34	0.33		
	<b>2</b> 3.89	0.28	4.04	0.43	0.17	4.21	0.29	4.09	0.32	0.13		
DBN	<b>1</b> 4.37	0.37	4.46	0.31	0.46	4.54	0.52	4.53	0.44	0.92		
	<b>2</b> 6.74	0.30	6.89	0.35	0.15	7.00	0.49	7.01	0.48	0.95		
ED	<b>1</b> 4.29	0.31	4.35	0.26	0.54	4.34	0.43	4.32	0.39	0.84		
	<b>2</b> 6.62	0.25	6.73	0.42	0.28	6.70	0.43	6.68	0.44	0.90		
HL	<b>1</b> 14.89	0.83	14.66	0.75	0.40	15.09	1.59	15.25	1.54	0.69		
	<b>2</b> 22.98	0.71	22.69	1.04	0.29	23.24	1.06	23.55	1.00	0.26		
HW	<b>1</b> 10.78	0.62	10.64	0.57	0.48	10.97	1.15	10.88	1.15	0.76		
	<b>2</b> 16.64	0.35	16.46	0.57	0.20	16.90	0.79	16.79	0.71	0.58		
PL	<b>1</b> 7.26	0.71	7.52	0.67	0.27	7.72	0.96	7.82	1.05	0.72		
	<b>2</b> 11.21	0.84	11.62	0.72	0.13	11.88	0.76	12.03	0.75	0.45		
PW	<b>1</b> 2.12	0.43	2.40	0.32	<b>0.04</b>	2.39	0.44	2.47	0.44	0.49		
	<b>2</b> 3.26	0.57	3.71	0.49	<b>0.02</b>	3.67	0.50	3.81	0.56	0.32		
FLL	<b>1</b> 19.71	1.45	19.44	1.23	0.56	19.28	1.72	19.30	1.82	0.97		
	<b>2</b> 30.42	1.42	30.06	1.20	0.42	29.74	1.36	29.81	1.25	0.85		
HLL	<b>1</b> 22.10	1.25	22.30	1.07	0.60	22.26	2.22	22.60	2.12	0.55		
	<b>2</b> 34.11	0.72	34.51	1.37	0.22	34.29	1.37	34.91	1.49	0.10		
DFHL	<b>1</b> 31.71	2.60	31.86	2.88	0.87	31.69	4.22	32.16	4.45	0.68		
	<b>2</b> 48.92	2.19	49.17	2.48	0.74	48.63	2.17	49.43	2.41	0.19		
HDPBT	<b>1</b> 1.57	0.28	1.66	0.35	0.40							
	<b>2</b> 2.42	0.36	2.56	0.53	0.30							
HW/HL	<b>1</b> 0.72	0.02	0.73	0.03	0.83	0.73	0.02	0.71	0.03	<b>0.04</b>		
TL/TBL	<b>1</b> 0.45	0.01	0.44	0.01	0.06	0.43	0.03	0.44	0.01	0.29		
PW/PL	<b>1</b> 0.29	0.05	0.32	0.04	0.11	0.31	0.05	0.32	0.05	0.58		
NED/HL	<b>1</b> 0.17	0.01	0.18	0.02	0.07	0.18	0.01	0.17	0.01	<b>0.02</b>		

distance between nostrils 4.80 mm [7.27 PERCRA], distance between fore- and hind limbs 32.72 mm [49.58 PERCRA]. TL/TBL = 0.45, NED/HL 0.15.

Dorsal ground is orange-red coloured (in life). The extremities and the tail the same, but lighter coloured. Dark blackish pattern with numerous tinsel-like tiny white dots on lateral part of dorsum and head except parotoids (only openings have blackish colouration). Blackish arrow-like pattern on the head towards to neck (Figure 2). Anterior and median parts of the upper eyelids darker than posterior part. White discontinuous lateral band on the flanks absent, only some irregularly scattered white speckles on ventrolateral sides of the trunk. Ventral side of the body flesh-coloured.

**Variation.** Variation observed in some morphometric characters and ratios within the populations; sexual dimorphism ( $p \leq 0.05$ ) was found regarding HL, HW, PL in raw data or PERCRA values (Supplementary Table S2). All specimens of populations from the western part of Dalaman plain and stream are agreeing with the diagnosis. Two

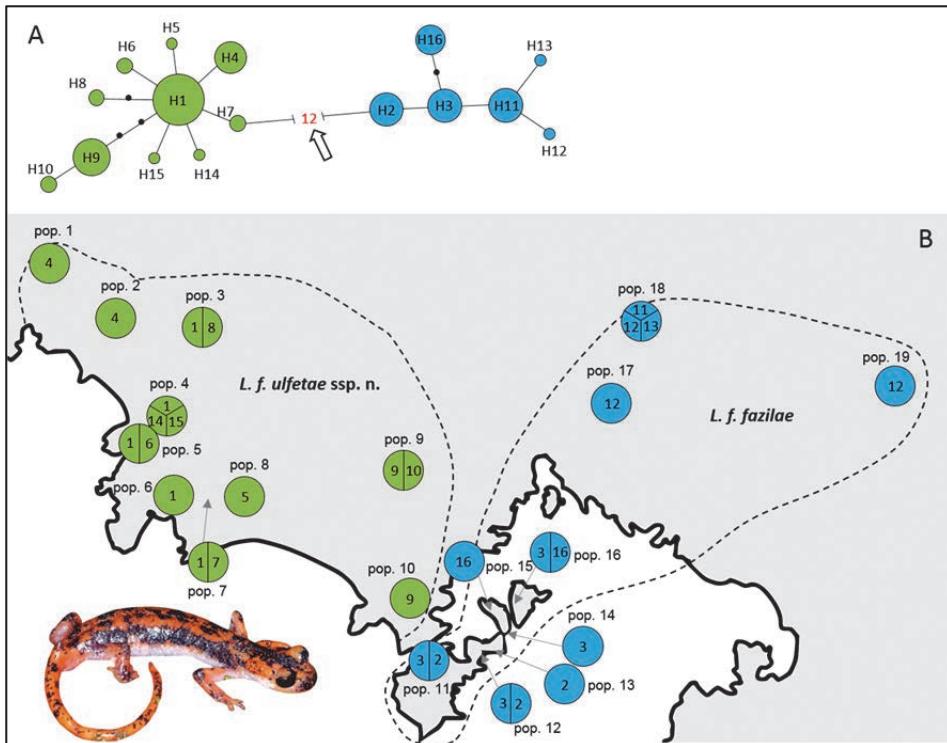


Figure 4. A) Haplotype network (the black arrow indicates the root of the haplotype network) and B) geographic haplotype distribution of *L. f. ulfetae* ssp. n. (green) and *L. f. fazilae* (blue); population (pop.) numbers refer to Table 3.

populations from the eastern part of Dalaman plain and stream (Serefler and Sarsala-Kapikargin) were also attributed to the new subspecies because of genetically affiliation to the new taxon, which morphologically more resemble to *L. f. fazilae*. Specimens from the Sarsala-Kapikargin population have the most extreme reddish dorsal ground colouration than any other populations within *L. fazilae*. Female specimens of the new subspecies have a larger area of dark blackish pattern on dorsum and head than males (Figure 3).

**Habitat and ecology.** *Lyciasalamandra fazilae* ssp. n. lives, like the other taxa of *Lyciasalamandra*, mostly under rocks in areas dominated by karstic limestone (Rödder et al., 2011). This landscape is a typical Mediterranean association of maquis groves and pine forests (*Pinus brutia*). Salamanders are also found in olive tree (*Olea europaea*) covered landscape surrounded by pine forests (Supplementary Figure S1). Activity of the specimens is highly dependent on temperature and humidity. During summer period, the specimens hide deep inside the karstic crevices and keep themselves safe from dehydration. In the Güzelyurt population, we observed a crab, most likely *Potamon rhodium*, feeding upon a male specimen of *L. f. ulfetae* ssp. n. (Supplementary Figure S2)

The habitat of the new subspecies extends from Sarsala-Kapikargin, Dalaman district, to the south of Sultaniye village, Köyceğiz district. The distribution range of the new subspecies stretches over approximately 32 km (air distance) in NW-SE direction

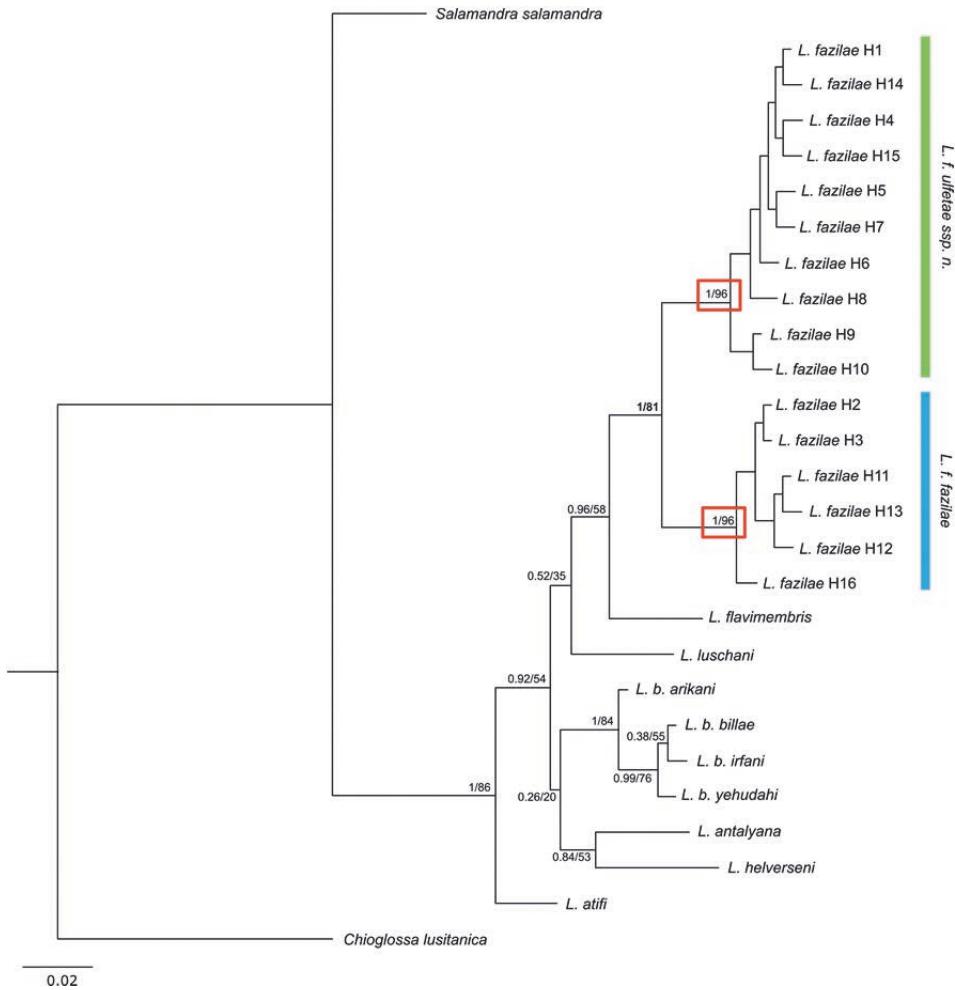


Figure 5. BI tree representing all sampled specimens of *L. fazilae*. The red boxes indicate the support values for the differentiation of *L. f. ulfetae* and *L. f. fazilae*. Node values represent Bayesian Posterior Probabilities/Likelihood values.

(Figure 1). Most of the populations were found at altitudes between 30 and 335 m asl (see Table 1), however, a population on the Ülemez mountain was found at ca. 500–600 asl. The populations of the new subspecies seem to be isolated from the nominotypical subspecies. At present, Dalaman plain and stream clearly divide the populations.

*Derivatio nominis.* The name of the newly described subspecies derives from the name of the senior author's late mother Ülfet Göçmen (1934–2017), who passed away on 11 February 2017, while the senior author was in the field to work on *L. fazilae* populations.

### Supplementary Material

Supplementary tables S1–S4 and Supplementary figures S1–S2 are given as a Supplementary Annex, which is available via the “Supplementary” tab on the article’s online page (<http://dx.doi.org/10.1080/09397140.2018.1511291>).

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### Disclosure statement

No potential conflict of interest was reported by the authors.

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**Supplementary Material to:**

**Molecular and morphological evidence for a new subspecies of Fazila's Lycian  
Salamander *Lyciasalamandra fazilae* in South-west Anatolia**

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Supplementary Table S1. Some mensural characters (in mm) and ratios of the *Lyciasalamandra fazilae fazilae*. 1: values in raw data; 2: values in PERCRA index; N: number of specimens; SD: standard deviation; SE: standard error of mean; P:  $p \leq 0.05$  value for sexual dimorphism. The other abbreviations of the characters are given in Materials and Methods.

Characters	♂						t-test	♀						Juveniles						Sum Adults (♂+♀)						
	N	Min.	Max.	Mean	SD	SE		N	Min.	Max.	Mean	SD	SE	N	Min.	Max.	Mean	SD	SE	N	Min.	Max.	Mean	SD	SE	
TBL	1	14	104.00	131.00	117.29	7.09	1.894	0.43	25	95.00	141.00	114.84	12.15	2.429	10	74.00	89.00	83.40	5.36	1.694	39	95.00	141.00	115.72	10.57	1.693
	2	14	176.19	187.14	181.01	3.55	0.950	0.07	25	146.15	185.53	176.95	9.54	1.907	10	172.09	181.63	177.03	3.49	1.105	39	146.15	187.14	178.40	8.10	1.297
RC	1	14	58.00	71.00	64.79	3.49	0.933	0.88	25	53.00	78.00	65.04	7.21	1.443	10	43.00	51.00	47.10	2.69	0.849	39	53.00	78.00	64.95	6.09	0.975
	2	14	44.29	55.17	49.90	2.83	0.757	0.98	25	40.52	59.87	49.94	5.76	1.152	10	26.55	38.23	34.19	3.54	1.119	39	40.52	59.87	49.93	4.87	0.780
LT	1	14	75.98	78.05	77.02	0.71	0.189	0.36	25	74.28	78.87	76.75	1.06	0.213	10	54.18	76.04	72.63	6.57	2.077	39	74.28	78.87	76.85	0.95	0.152
	2	14	46.00	61.00	52.50	3.96	1.057	0.37	23	41.00	65.00	50.96	6.28	1.309	10	31.00	40.00	36.30	2.91	0.920	37	41.00	65.00	51.54	5.51	0.905
TL	1	14	76.19	87.14	81.01	3.55	0.950	0.23	23	68.85	85.53	79.46	4.06	0.847	10	72.09	81.63	77.03	3.49	1.105	37	68.85	87.14	80.04	3.90	0.641
	2	14	2.03	3.03	2.52	0.26	0.070	0.04	25	2.13	3.31	2.74	0.34	0.067	10	2.02	2.43	2.20	0.17	0.054	39	2.03	3.31	2.66	0.33	0.052
NED	1	14	3.27	4.33	3.89	0.28	0.076	0.00	25	3.63	4.81	4.21	0.29	0.059	10	4.40	5.15	4.68	0.28	0.089	39	3.27	4.81	4.09	0.33	0.052
	2	14	3.74	5.08	4.37	0.37	0.098	0.24	25	3.61	5.45	4.54	0.52	0.103	10	2.96	3.61	3.41	0.18	0.058	39	3.61	5.45	4.48	0.47	0.075
DBN	1	14	6.35	7.26	6.74	0.30	0.079	0.05	25	6.12	8.04	7.00	0.49	0.099	10	6.30	8.07	7.26	0.49	0.155	39	6.12	8.04	6.91	0.45	0.071
	2	14	3.73	4.80	4.29	0.31	0.082	0.65	25	3.60	5.21	4.34	0.43	0.086	10	3.28	3.76	3.46	0.14	0.043	39	3.60	5.21	4.32	0.39	0.062
ED	1	14	6.19	7.06	6.62	0.25	0.066	0.46	25	6.00	7.41	6.70	0.43	0.087	10	6.98	8.02	7.36	0.37	0.118	39	6.00	7.41	6.67	0.38	0.060
	2	14	13.71	16.29	14.89	0.83	0.221	0.60	25	11.82	18.13	15.09	1.59	0.318	10	11.25	12.77	11.91	0.51	0.163	39	11.82	18.13	15.02	1.35	0.217
HL	1	14	21.95	24.02	22.98	0.71	0.189	0.38	25	21.13	25.72	23.24	1.06	0.213	10	23.96	27.09	25.33	1.07	0.339	39	21.13	25.72	23.15	0.95	0.152
	2	14	9.65	11.74	10.78	0.62	0.165	0.50	25	8.77	12.96	10.97	1.15	0.231	10	7.93	9.39	8.49	0.51	0.160	39	8.77	12.96	10.91	0.99	0.159
HW	1	14	15.92	17.16	16.64	0.35	0.094	0.17	25	15.37	18.44	16.90	0.79	0.157	10	16.65	18.79	18.04	0.72	0.228	39	15.37	18.44	16.81	0.67	0.107
	2	14	6.16	8.60	7.26	0.71	0.190	0.10	25	6.03	10.03	7.72	0.96	0.192	10	4.21	6.18	5.33	0.56	0.178	39	6.03	10.03	7.56	0.90	0.144
PL	1	14	9.57	13.03	11.21	0.84	0.224	0.02	25	9.84	13.55	11.88	0.76	0.152	10	8.96	12.79	11.33	1.19	0.377	39	9.57	13.55	11.64	0.84	0.135
	2	14	1.43	2.98	2.12	0.43	0.114	0.07	25	1.47	3.53	2.39	0.44	0.087	10	1.35	1.87	1.54	0.21	0.065	39	1.43	3.53	2.29	0.45	0.071
PW	1	14	2.20	4.38	3.26	0.57	0.153	0.03	25	2.72	4.77	3.67	0.50	0.100	10	2.76	4.26	3.28	0.54	0.170	39	2.20	4.77	3.53	0.56	0.089
	2	14	17.35	22.49	19.71	1.45	0.387	0.42	25	16.03	22.69	19.28	1.72	0.343	10	13.30	15.58	14.52	0.60	0.189	39	16.03	22.69	19.44	1.62	0.259
FLL	1	14	27.11	31.93	30.42	1.42	0.380	0.16	25	27.50	32.17	29.74	1.36	0.272	10	28.73	32.74	30.86	1.15	0.365	39	27.11	32.17	29.98	1.40	0.224
	2	14	20.29	24.56	22.10	1.25	0.334	0.77	25	18.68	27.13	22.26	2.22	0.444	10	14.91	17.65	16.76	0.87	0.274	39	18.68	27.13	22.20	1.91	0.306
HLL	1	14	32.59	35.20	34.11	0.72	0.194	0.60	25	31.32	36.35	34.29	1.37	0.273	10	33.72	37.63	35.60	1.26	0.398	39	31.32	36.35	34.23	1.17	0.187
	2	14	28.51	38.13	31.71	2.60	0.695	0.98	25	24.28	39.89	31.69	4.22	0.845	10	19.16	25.90	22.95	1.88	0.594	39	24.28	39.89	31.70	3.69	0.590
DFHL	1	14	45.15	53.70	48.92	2.19	0.586	0.70	25	43.91	51.64	48.63	2.17	0.433	10	44.56	52.86	48.70	2.57	0.812	39	43.91	53.70	48.74	2.15	0.345
	2	14	1.14	2.12	1.57	0.28	0.076																			
HDPBT	1	14	1.97	2.99	2.42	0.36	0.095																			
	2	14	0.69	0.76	0.72	0.02	0.006	0.69	25	0.67	0.78	0.73	0.02	0.005	10	0.68	0.74	0.71	0.02	0.006	39	0.67	0.78	0.73	0.02	0.004
HW/HL	1	14	0.43	0.47	0.45	0.01	0.003	0.07	25	0.32	0.46	0.43	0.03	0.007	10	0.42	0.45	0.43	0.01	0.004	39	0.32	0.47	0.44	0.03	0.005
	2	14	0.19	0.38	0.29	0.05	0.015	0.31	25	0.23	0.41	0.31	0.05	0.009	10	0.22	0.36	0.29	0.04	0.014	39	0.19	0.41	0.30	0.05	0.008
NED/HL	1	14	0.14	0.19	0.17	0.01	0.003	0.01	25	0.16	0.20	0.18	0.01	0.002	10	0.17	0.21	0.19	0.02	0.005	39	0.14	0.20	0.18	0.01	0.002

Supplementary Table S2. Some mensural characters (in mm) and ratios of the *Lyciasalamandra fazilae ulfetae* ssp. n. 1: values in raw data; 2: values in PERCRA index; N: number of specimens; SD: standard deviation; SE: standard error of mean; P:  $p \leq 0.05$  value for sexual dimorphism. The other abbreviations of the characters are given in Materials and Methods.

Characters	$\delta$						$t$ -test						$\varphi$						Juveniles						Sum Adults ( $\delta + \varphi$ )					
	N	Min.	Max.	Mean	SD	SE	P	N	Min.	Max.	Mean	SD	SE	N	Min.	Max.	Mean	SD	SE	N	Min.	Max.	Mean	SD	SE					
TBL	1	28	98.00	134.00	115.39	7.68	1.451	0.75	35	90.00	140.00	116.26	13.70	2.316	18	49.00	89.00	71.44	12.40	2.922	63	90.00	140.00	115.87	11.35	1.430				
	2	28	163.24	186.76	178.28	4.69	0.887	0.55	35	169.01	188.89	179.00	4.61	0.779	18	148.48	233.33	177.05	16.55	3.900	63	163.24	188.89	178.68	4.62	0.582				
RC	1	28	56.00	74.00	64.71	3.78	0.714	0.89	35	51.00	76.00	64.91	7.21	1.219	18	30.00	50.00	40.44	6.74	1.589	63	51.00	76.00	64.83	5.90	0.743				
LT	1	28	41.65	57.46	49.87	3.23	0.611	0.86	35	38.06	57.64	49.67	5.81	0.981	18	22.37	37.93	30.17	5.33	1.257	63	38.06	57.64	49.76	4.80	0.605				
	2	28	71.84	78.72	77.05	1.41	0.266	0.07	35	74.42	78.59	76.46	1.00	0.170	18	67.66	98.97	74.70	6.48	1.528	63	71.84	78.72	76.72	1.23	0.154				
TL	1	28	41.00	60.00	50.86	4.25	0.803	0.73	35	37.00	64.00	51.34	6.85	1.158	18	16.00	39.00	30.44	6.53	1.539	63	37.00	64.00	51.13	5.80	0.731				
	2	28	69.84	86.76	78.55	3.97	0.750	0.68	35	69.01	88.89	79.00	4.61	0.779	18	48.48	100.00	75.20	10.72	2.526	63	69.01	88.89	78.80	4.31	0.543				
NED	1	28	2.12	3.08	2.61	0.29	0.055	0.66	35	2.12	3.32	2.65	0.34	0.057	18	1.37	2.40	1.90	0.26	0.061	63	2.12	3.32	2.63	0.31	0.040				
	2	28	3.23	4.89	4.04	0.43	0.082	0.67	35	3.38	4.75	4.09	0.32	0.054	18	4.10	6.63	4.74	0.59	0.139	63	3.23	4.89	4.07	0.37	0.047				
DBN	1	28	3.83	5.28	4.46	0.31	0.058	0.44	35	3.61	5.28	4.53	0.44	0.075	18	2.53	4.02	3.20	0.48	0.113	63	3.61	5.28	4.50	0.39	0.049				
	2	28	6.01	7.40	6.89	0.35	0.066	0.27	35	5.84	7.84	7.01	0.48	0.081	18	6.93	10.07	7.96	0.67	0.157	63	5.84	7.84	6.96	0.43	0.054				
ED	1	28	3.79	4.78	4.35	0.26	0.050	0.74	35	3.47	4.98	4.32	0.39	0.066	18	2.46	4.01	3.13	0.38	0.090	63	3.47	4.98	4.33	0.34	0.043				
	2	28	5.83	7.35	6.73	0.42	0.079	0.67	35	5.79	7.92	6.68	0.44	0.074	18	6.74	9.97	7.83	0.78	0.184	63	5.79	7.92	6.70	0.42	0.053				
HL	1	28	13.04	16.54	14.66	0.75	0.141	<b>0.05</b>	35	12.20	18.34	15.25	1.54	0.260	18	9.55	12.63	10.68	1.04	0.245	63	12.20	18.34	14.99	1.27	0.161				
	2	28	20.81	25.63	22.69	1.04	0.196	<b>0.00</b>	35	21.41	25.58	23.55	1.00	0.169	18	24.14	34.37	26.75	2.66	0.627	63	20.81	25.63	23.17	1.10	0.138				
HW	1	28	9.30	11.71	10.64	0.57	0.107	0.29	35	8.64	12.76	10.88	1.15	0.194	18	6.22	9.06	7.62	0.88	0.207	63	8.64	12.76	10.77	0.94	0.118				
	2	28	14.75	17.32	16.46	0.57	0.107	<b>0.05</b>	35	15.74	18.76	16.79	0.71	0.121	18	16.79	23.80	19.02	1.53	0.362	63	14.75	18.76	16.64	0.67	0.084				
PL	1	28	6.35	8.88	7.52	0.67	0.127	0.18	35	5.41	9.65	7.82	1.05	0.178	18	4.18	6.85	5.30	0.86	0.202	63	5.41	9.65	7.69	0.91	0.114				
	2	28	10.35	13.32	11.62	0.72	0.135	<b>0.03</b>	35	10.40	13.59	12.03	0.75	0.127	18	11.76	17.37	13.19	1.51	0.355	63	10.35	13.59	11.85	0.76	0.096				
PW	1	28	1.71	3.07	2.40	0.32	0.061	0.47	35	1.73	3.51	2.47	0.44	0.074	18	1.25	2.37	1.72	0.34	0.081	63	1.71	3.51	2.44	0.39	0.049				
	2	28	2.51	4.58	3.71	0.49	0.092	0.45	35	2.79	4.88	3.81	0.56	0.095	18	3.28	5.90	4.30	0.74	0.173	63	2.51	4.88	3.77	0.53	0.067				
FLL	1	28	16.49	22.24	19.44	1.23	0.233	0.71	35	15.17	23.20	19.30	1.82	0.308	18	10.84	16.31	13.28	1.73	0.407	63	15.17	23.20	19.36	1.58	0.199				
	2	28	28.00	33.50	30.06	1.20	0.226	0.43	35	27.07	32.10	29.81	1.25	0.212	18	28.63	40.43	33.09	2.64	0.621	63	27.07	33.50	29.92	1.23	0.154				
HLL	1	28	20.13	24.86	22.30	1.07	0.202	0.48	35	17.92	26.57	22.60	2.12	0.358	18	11.45	18.63	15.21	2.22	0.523	63	17.92	26.57	22.47	1.72	0.217				
	2	28	32.43	37.52	34.51	1.37	0.258	0.28	35	32.40	37.72	34.91	1.49	0.251	18	33.03	50.77	37.88	3.77	0.889	63	32.40	37.72	34.73	1.44	0.181				
DFHL	1	28	25.31	38.43	31.86	2.88	0.544	0.74	35	24.04	38.04	32.16	4.45	0.752	18	14.75	25.25	19.88	3.12	0.735	63	24.04	38.43	32.02	3.80	0.479				
	2	28	41.75	52.63	49.17	2.48	0.468	0.68	35	41.08	52.85	49.43	2.41	0.407	18	43.50	67.97	49.46	5.30	1.248	63	41.08	52.85	49.31	2.42	0.305				
HDPBT	1	28	1.06	2.27	1.66	0.35	0.067																							
	2	28	1.78	3.44	2.56	0.53	0.099																							
HW/HL	1	28	0.65	0.78	0.73	0.03	0.006	0.08	35	0.67	0.80	0.71	0.03	0.005	18	0.65	0.76	0.71	0.03	0.007	63	0.65	0.80	0.72	0.03	0.004				
TL/TBL	1	28	0.41	0.46	0.44	0.01	0.002	0.84	35	0.41	0.47	0.44	0.01	0.002	18	0.33	0.47	0.42	0.03	0.007	63	0.41	0.47	0.44	0.01	0.002				
PW/PL	1	28	0.22	0.39	0.32	0.04	0.008	0.80	35	0.23	0.39	0.32	0.05	0.008	18	0.25	0.41	0.33	0.04	0.011	63	0.22	0.39	0.32	0.04	0.006				
NED/HL	1	28	0.15	0.21	0.18	0.02	0.004	0.27	35	0.15	0.20	0.17	0.01	0.002	18	0.14	0.20	0.18	0.02	0.004	63	0.15	0.21	0.18	0.02	0.002				

Supplementary Table S3. Samples used for the molecular analysis with haplotypes, GenBank Accession Numbers and populations. N = sample size.

Haplotype	GenBank Acc. Nr.	Population (n)
H1	MH721922/ EU430975	Gökböl (7), Okçular (3), Kargıcak cove (3), Aşı cove-Mergenli (5), South of Mergenli/ Ortaca (3)
H2	MH721923	Manastır-Kleopatra cove (Kapıdağ peninsula) (1), Göbüñ cove (Kapıdağ peninsula) (3), Çataldağ-Darboğaz (Kapıdağ peninsula) (5)
H3	MH721924/ EU430973	Tersane Adası (2), Manastır-Kleopatra cove (Kapıdağ peninsula) (4), Göbüñ cove (Kapıdağ peninsula) (1), Domuz island-Darboğaz (2)
H4	MH721925/ EU430976	Uelemez (3) North Ekincik-South Sultaniye (5)
H5	MH721926	Güzelyurt/ Ortaca (1)
H6	MH721927	Kargıcak cove (2)
H7	MH721928	South of Mergenli/ Ortaca (2)
H8	MH721929	Okçular (2)
H9	MH721930/ EU430974	Sarsila (9), Şerefler (2)
H10	MH721931	Şerefler (2)
H11	MH721932	Cöymen (3)
H12	MH721933/ EU430971	Cöymen (1), Goekceovacık (5), Üzümlü (1)
H13	MH721934	Cöymen (1)
H14	MH721935	Gökböl (1)
H15	MH721936	Gökböl (1)
H16	EU430972	Tersane Adası (2), Domuz Adası (5)
outgroup species	KY342278	<i>Lyciasalamandra antalyana</i>
outgroup species	KY342274	<i>Lyciasalamandra arikani</i>
outgroup species	KY342280	<i>Lyciasalamandra atifi</i>
outgroup species	KY342275	<i>Lyciasalamandra billae</i>
outgroup species	KY342285	<i>Lyciasalamandra flavimembris</i>
outgroup species	KY342287	<i>Lyciasalamandra helverseni</i>
outgroup species	KY342276	<i>Lyciasalamandra irfani</i>
outgroup species	KY342291	<i>Lyciasalamandra luschani</i>
outgroup species	KY342277	<i>Lyciasalamandra yehudai</i>
outgroup species	EU880331	<i>Salamandra salamandra</i>
outgroup species	EU880308	<i>Chioglossa lusitanica</i>

Supplementary Table S4. Populations included in the molecular analysis with population numbers according to Figure 4, sample sizes and haplotypes. N = sample size.

Nr.	Population	N	Haplotypes (n)
1	Cöymen	5	H11 (3), H12 (1), H13 (1)
2	Sarsila	9	H9 (9)
3	Uelemez	3	H4 (3)
4	Gökcceovacık	5	H12 (5)
5	Üzümlü	1	H12 (1)
6	Gökbel	9	H1 (7), H14 (1), H15 (1)
7	Tersane Adası	4	H16 (2), H3 (2)
8	Domuz Adası	5	H16 (5)
9	North Ekincik-South Sultaniye	5	H4 (5)
10	Okçular	5	H1 (3), H8 (2)
11	Kargıcak cove	5	H1 (3), H6 (2)
12	Aşı cove-Mergenli	5	H1 (5)
13	South of Mergenli/Ortaca	5	H1 (3), H7 (2)
14	Şerefler	4	H9 (2), H10 (2)
15	Manastır-Kleopatra cove (Kapıdağ peninsula)	5	H2 (1), H3 (4)
16	Göbüñ cove (Kapıdağ peninsula)	4	H2 (3), H3 (1)
17	Çataldağ-Darboğaz (Kapıdağ peninsula)	5	H2 (5)
18	Domuz island-Darboğaz	2	H3 (2)
19	Güzelyurt/Ortaca	1	H5 (1)



Supplementary Figure S1. Type locality [Ülemez-Horozlar (ca. 2 km NE of Çandır), Köyceğiz district, Muğla province] of *L. f. ulfetae* ssp. n.



Supplementary Figure S2. *Potamon rhodium*, a natural predator of the *L. f. ulfetae* ssp. n., preying on a male specimen from SW Güzelyurt, Dalaman population.