A new subspecies of *Lyciasalamandra antalyana* (Amphibia: Salamandridae) from the Lycian Coast, Turkey

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Abstract. A new subspecies of the Lycian salamander *Lyciasalamandra antalyana* is described from Yağcavillage (Antalya province) and Burdur province on the Lycian Coast, Turkey. It is distinguished from the nominotypical form by its dorsal colouration, multivariate morphometrics, and mitochondrial molecular markers.

Key words. Urodela, Lyciasalamandra antalyana gocmeni ssp. n., 16SrDNA gene, Turkey.

Introduction

Ten species and three subspecies have so far been described (Göçmen et al. 2013) in the genus *Lyciasalamandra* (Veith & Steinfartz 2004). Their occurrence is limited to a narrow area along the Lycian Coast in southern Turkey and some Aegean islands. All species and subspecies have relatively small and mostly allopatric distributions. In spite of their geographic proximity, species and subspecies differ significantly in colouration, and populations may exhibit substantial differences colour and pattern even within taxa (Veith & Steinfartz 2004).

During the last two decades, several new species of *Lyciasalamandra* and a large number of new populations have been recorded (e.g., Veith et al. 2001, Göçmen et al. 2011, Göçmen & Akman 2012). Especially in *Lyciasalamandra antalyana* (Basoglu & Baran, 1976), the known distribution was substantially extended towards the north. Just a small distance to the north from the type locality at Hurma village, recorded specimens are characterized by pronounced differences in terms of colouration (Veith et al. 2001). Specimens from these newly discovered populations (e.g., Yağcavillage and Thermessos) exhibit a fully striped yellow pattern, while topotypical animals from Hurma are mainly yellow in the region of the eyes, nostrils and parotoids (Fig. 1).

AKMAN et al. (2013) published a compilation of the known *L. antalyana* populations and discussed if an assignment to subspecific status of the newly discovered yellow-coloured populations might be warranted. Based on molecular data, multivariate analyses of morphometrics, and the striking colouration, we here suggest the popula-

tions from Yağca village and farther north in the Burdur province as a new subspecies.

Material and methods

Field trips took place between February of 2011 and November of 2013. Geographical coordinates for sample locations as given in Table 1 were computed with a Magellan XL GPS. Digital colour photos were taken of all live specimens. Specimens were euthanised by a 96% ethanol injection into their body cavity and subsequently fixed in 96% ethanol. Additional specimens were collected from the type locality of the species (Hurma/Antalya), as well as from Gökdere and Hacısekililer for comparison with the new populations from Yağca (Antalya) and Burdur. Specimens are deposited at The Zoology Museum of Harran University, Şanlıurfa, Turkey (ZMHRU; Tab. 1). All currently known localities of *L. antalyana* are illustrated in Figure 1.

We used the morphometric data published by Akman et al. (2013) to perform multiple discriminate analyses (DC) with PAST version 2.08 (Hammer et al. 2001). We prefer the DC to a principle component analysis (PCA) since DC is specifically designed for testing the power of characters to distinguish between pre-defined groups (here: potential subspecies; Sokal & Rohlf 1995). We studied males and females separately to make provision for potential differences among sexes as was previously described for other *Lyciasalamandra* species (e.g., Göçmen et al. 2011, Göçmen & Akman 2012). Obvious juveniles could easily be distinguished from adults by their smaller size and the lack of the hedonic gland; they were excluded from

analyses. We first used the raw measurements of Akman et al. (2013): rostrum—anus length (RA), tail length (TL), nostril—eye distance (NED), distance between nostrils (DBN), eye diameter (ED), head length (HL), head width (HW), parotoid length (PL), parotoid width (PW), fore limb length (FLL), hind limb length (HLL), and distance between fore- and hindlimbs (DFHL). In a second analysis, we employed ratios of these measurements calculated against RA (PERCRA values) to correct for the overall size of the specimens. We analysed a total of 11 and 10 males of *L. a. antalyana* and *L. a. gocmeni* ssp. n., respectively, and 12 females of each subspecies.

A segment of 508 base pairs (bp) in length of the 16Sr-RNA gene was sequenced (for DNA extraction, primers, PCR conditions and sequencing details, see VEITH et al. 2008) for 16 specimens collected at new localities of the new subspecies and provided by AKMAN et al. (2013):

Kavacık (1 specimen), Kızılseki (5), Kocaaliler 1 (5), Kocaaliler 2 (1) and Kırkgözhan, Yağca (4) (GenBank accession number of the single new haplotype: KJ622364; www. ncbi.nlm.nih.gov). We aligned them with 17 homologous sequences from Veith et al. (2008) of additional Lyciasalamandra populations as well as Salamandra infraimmaculata, Mertensiella caucasica, Neurergus crocatus and Pleurodeles poireti as outgroup taxa (GenBank accession numbers: EU430952, EU430953, EU430954, EU430955, EU430956, EU430957, EU430958, EU430960, EU430964, EU430967, EU430970, EU430971, EU430976, EU430978, EU430979, and EU430980) using the clustal W option in Mega 6.06 (TAMURA et al. 2013). We used jModeltest, version 2.1.3 (DARRIBA et al. 2012), to select the best fit out of 56 nested substitution models. The HKY+G model (gamma shape parameter $\alpha = 0.178$) was selected based on the Bayesian Information Criterion (BIC). However, HKY is

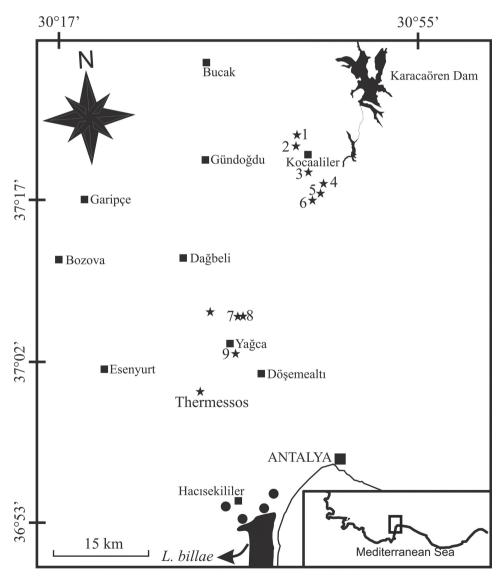


Figure 1. Map showing localities of *Lyciasalamandra antalyana antalyana* (solid circles) and *L. a. gocmeni* ssp. n. (asterisks); numbers are according to Table 1.

Table 1. Geographic and some climatic information on the localities of *Lyciasalamandra antalyana* antalyana and *L. a. gocmeni* ssp. n., as well as museum numbers of the specimens used. The numbers in brackets correspond to the localities shown in Figure 1. Asterisk: at the time of collecting.

Museum numbers (ZMHRU)	Locality	Altitude (m a.s.l.)	Latitude (DMS)	Longitude (DMS)	Collection date number of specimens
2012/6	North of Kocaaliler [1]	750	37°20'N	30°42'E	29.03.2012 1 juv.
2012/5	Hamartaşı/Kocaaliler [2]	720	37°20'N	30°42'E	01.04.2012 5 (2♂♂, 3♀♀)
2012/42	Hamartaşı/Kocaaliler [2]	720	37°20'N	30°42'E	10.04.2012 2 (1♂, 1 juv.)
2012/9	Kavacık [3]	653	37°17'N	30°44'E	01.04.2012 2 (1♂, 1♀)
2012/44	Kavacık [3]	653	37°17'N	30°44'E	10.04.2012 1 juv.
2012/8	North of Kızılseki [4]	400	37°16'N	30°45'E	01.04.2012 4 (2♂♂, 1♀, 1 juv.)
2012/7	West of Kızılseki [5]	438	37°15'N	30°44'E	01.04.2012 1♀
2012/43	Ortacamevki/Kızılseki [6]	541	37°15'N	30°44'E	10.04.2012 1 juv.
2012/2	Kırkgözhan, Yağca [7]	348	37°06'N	30°34'E	07.03.2012 10 (2♂♂, 6♀♀, 2 juv.)
2012/3	Kırkgözhan, Yağca [8]	350	37°06'N	30°34'E	12.03.2012 2 (1♂, 1 juv.)
2012/4	Çığlık [9]	313	37°03'N	30°33'E	12.03.2012 3 (1♂, 1♀, 1 juv.)
2011/82	Hurma	99	36°51'N	30°35'E	25.02.2011 12 (1♂, 3♀♀, 8 juv.)
2012/1	Hurma	99	36°51'N	30°35'E	06.03.2012 17 (8♂♂, 8♀♀, 1 juv.)
2013/173	Gökdere	50	36.82925°N	30.55599°E	24.11.2013 1 (1 juv.)
2013/175	Hacısekililer	581	36.80321°N	30.48953°E	26.11.2013 3 (1♂, 1♀, 1 juv.)

not supported by Mega 6.06 and we therefore used the Tamura-Nei substitution model (Tamura & Nei 1993), since the HKY model is a special feature of the Tamura-Nei model that does not distinguish between substitution rates for the two types of transformations.

According to VEITH et al. (2008), a neighbour-joining (NJ) tree of *Lyciasalamandra* mitochondrial genes produced equivalent results of maximum likelihood, maximum parsimony, and Bayesian inference trees. We therefore only calculated an NJ tree from 2,000 bootstrap replicates using Mega 6.06 and applying the selected substitution model.

Results

In terms of colour and pattern, specimens from Yağca and Burdur populations differ from Hurma populations (Figs 2, 3). Yağca and Burdur populations have a more discontinuous pattern than Hurma populations. Additionally,

comparing the Yağca and Burdur populations, the discontinuous dorsal pattern of Burdur specimens is dominated by the dark background, while yellow is dominant in the Yağca population. The dorsal colouration patterns of either differ clearly from the nominotypical form. Yağca and Burdur specimens have yellow dorsal markings that also occur on the upper side of the tail. In contrast, specimens from Hurma are yellow only in the region of the eyes, nostrils and parotoids (Figs 2, 3). All other taxa of *Lyciasalamandra* do not exhibit any such yellow coloration.

Based on our raw data, the multiple discriminate function allowed to correctly classify both males and females from Yağca and Burdur versus other populations only at 81 and 54%, respectively (Fig. 4). The probability that males and females of both taxa belong to the same group was 0.1797 and 0.9068, respectively. However, when using ratios (Fig. 5), the percentages of correctly classified specimens of both sexes were 95% in males and 100% in females ($p_{\text{same group}} = 0.0447 + 0.0563$, respectively).

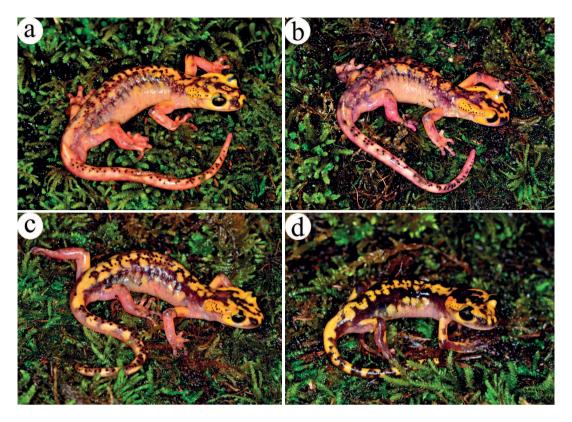


Figure 2. (a) Male of $Lyciasalamandra\ antalyana\ gocmeni\ ssp.\ n.$ from the type locality, Kırkgözhan, Yağca; (b) male, (c) female, and (d) juvenile of $L.\ a.\ gocmeni\ ssp.\ n.$ from Kızılseki.

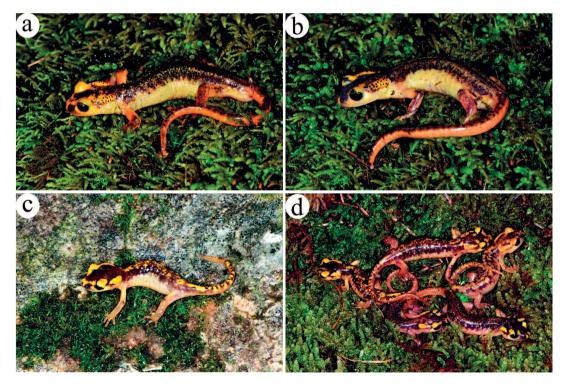


Figure 3. Male (a), female (b) and juvenile (c) of $Lyciasalamandra\ antalyana\ antalyana\ from the type locality, Hurma village; some variation in colour pattern is shown in (d).$

Of the 508 base pair positions, five were diagnostic between the two *L* .antalyana lineages (= 1% sequence divergence in uncorrected p-distances). Most of the newly sequenced specimens shared the haplotype found by Veith et al. (2008) in specimens from Yağca village. Only one additional haplotype with one mutation was found at Kirkgözhan, Yağca village. In the NJ tree, the haplotypes of the new subspecies form a clade of their own with 98% bootstrap support (Fig. 6).

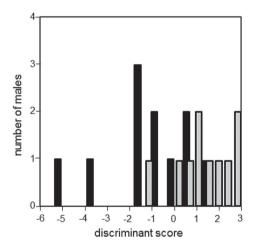
Five diagnostic base positions within the studied 508-bp fragment of the 16SrRNA mitochondrial gene, differences surfacing from a multivariate analysis of morphometric ratios, and a distinctive colouration clearly separate the suggested new subspecies from the nominotypical form. Based on these results, we describe the Yağca and Burdur populations as:

Lyciasalamandra antalyana gocmeni ssp. n.

Holotype and type locality (Figs 2a, 7): Adult male, ZMHRU 2012/2-1 from Kırkgözhan/Yağca, Antalya Province, Turkey, 348 m above sea level (37°03'17.21"N, 30°33'18.46"E). Leg. 07 March 2012 by B. GÖÇMEN, B. AKMAN, N. İĞCI, O. GODMANN and M. VEITH.

Paratypes: 31 specimens collected from Antalya (Yağca and Çığlık populations) and Burdur (Kavacık, Kızılseki, and Kocaaliler populations) were deposited as paratypes. For locality details and collection numbers see Table 1.

Diagnosis: *Lyciasalamandra antalyana gocmeni* ssp. n. differs from all other *Lyciasalamandra* species and subspecies by having yellow dorsal and supracaudal markings in life.



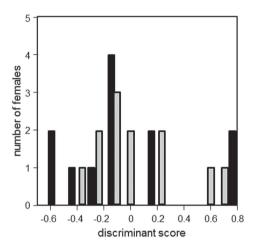
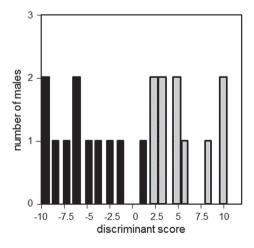


Figure 4. Multiple discriminate analysis of males and females of *L. a. antalyana* (black) and *L. a. gocmeni* ssp. n. (grey) based on raw data; males: Hotelling's $t^2 = 54.834$, F = 1.924; females: Hotelling's $t^2 = 10.81$, F = 0.4504.



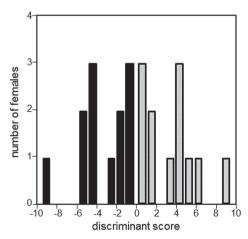


Figure 5. Multiple discriminate analysis of males and females of L. a. antalyana (black) and L. a. gocmeni ssp. n. (grey) based on ratios; males: Hotelling's $t^2 = 58.741$, F = 3.0916; females: Hotelling's $t^2 = 43.601$, F = 2.5764.

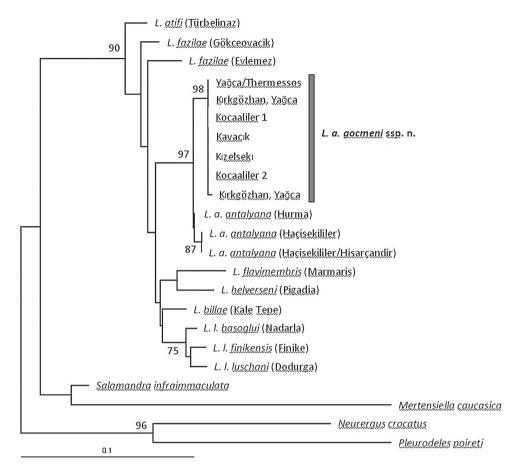


Figure 6. NJ tree of the Tamura-Nei distances of *Lyciasalamandra antalyana*, showing the monophyletic position of *L. a. gocmeni*; numbers at branches indicate bootstrap support \geq 70% for 2,000 replicates.

In contrast, specimens of L . a. antalyana are yellow only in the region of the eyes, nostrils and parotoids. No other taxon of Lyciasalamandra shows any such yellow colouration.



Figure 7. Type locality of *Lyciasalamandra antalyana gocmeni* ssp. n. at Kırkgözhan, Yağca.

Description of the holotype: The body shape is equivalent to other species of *Lyciasalamandra*. Head flat, longer than broad (HW/HL 0.67). Snout rounded. Parotoids long and narrow (PW/PL 0.39), the posterior part broader than the anterior part. Gular fold distinct. The cloacal region shows a very slight swelling, and the pads on the upper arm are quite developed. In profile, the tiny thorns on the dorsal side are visible. The finger-like projection above the base of the tail measures about 2.44 mm tall; it is pointed and curved forward at its free end.

In life, the colouration of the dorsum including the head and upper jaws was brownish-red, especially on the parotoids that were dotted with 15 black dermal pores on each side. On each upper eyelid, there was a thin black cross bar. The interparotoid, interorbital and internasal spaces were yellow with brown flecks (Fig. 2a).

The ground colour of the dorsal side of the trunk had an interrupted light yellow pattern. This pattern extended backwards into the median region of the trunk. Two broad brown stripes that were intersected by transverse brown bars extended along the dorsolateral sides of the trunk. Yellow was dominant on the dorsum. The legs and the tail were flesh-coloured. The proximal half of each leg had a

Table 2. Some morphometric characters (in mm) and ratios of *Lyciasalamandra antalyana gocmeni* ssp. n. 1: Values in raw data; 2: Values in PERCRA; N: number of specimens; SD: Standard deviation; the other abbreviations of characters are given in Material and methods.

		Juveniles (N = 10) Mean±SD (Range)	Adults (N = 22) Mean±SD (Range)
ToL	1	86.50±9.76 (77.00-108.00)	130.68±15.65 (101.00-153.00)
	2	1.79±0.08 (1.72-1.93)	1.79±0.06 (1.66-1.89)
RA	1	48.40±5.52 (42.00-60.00)	72.95±7.67 (60.00-86.00)
LT	1	33.07±3.36 (29.13-40.12)	48.25±5.39 (39.50-58.17)
	2	0.69±0.05 (0.61-0.76)	0.66±0.02 (0.61-0.69)
TL	1	38.10±5.02 (33.00-48.00)	57.73±8.49 (40.00-68.00)
	2	$0.79\pm0.08~(0.72-0.93)$	0.79±0.06 (0.66-0.89)
NED	1	2.27±0.32 (1.67-2.92)	2.92±0.41 (2.22-3.68)
	2	0.05±0.01 (0.04-0.06)	0.04±0.00 (0.03-0.05)
DBN	1	3.72±0.32 (3.24-4.26)	5.09±0.54 (4.13-5.92)
	2	$0.08\pm0.00~(0.07-0.08)$	0.07±0.01 (0.06-0.08)
ED	1	3.28±0.42 (2.84-4.14)	4.27±1.27 (3.26-9.78)
	2	0.07±0.01 (0.05-0.09)	0.06±0.02 (0.05-0.16)
HL	1	13.58±0.87 (12.41-15.37)	17.61±1.49 (15.12-20.94)
	2	0.28±0.02 (0.26-0.32)	0.24±0.01 (0.22-0.26)
HW	1	8.75±0.74 (7.61-9.89)	11.42±0.88 (9.88-12.84)
	2	0.18±0.02 (0.16-0.21)	0.16±0.01 (0.14-0.18)
PL	1	6.37±0.77 (5.28-7.25)	8.59±0.78 (7.31-10.29)
	2	0.13±0.02 (0.11-0.16)	0.12±0.01 (0.10-0.14)
PW	1	2.24±0.39 (1.68-2.77)	3.22±0.46 (2.61-4.11)
	2	0.05±0.01 (0.04-0.06)	0.04±0.00 (0.04-0.05)
FLL	1	15.94±1.87 (13.82-19.49)	21.72±1.65 (18.20-24.75)
	2	0.33±0.02 (0.31-0.36)	0.30±0.02 (0.26-0.34)
HLL	1	18.10±2.75 (15.00-23.47)	26.19±2.16 (21.79–28.92)
	2	0.37±0.03 (0.34-0.41)	0.36±0.02 (0.34-0.40)
DFHL	1	26.83±2.86 (22.23-31.52)	39.91±4.84 (31.54-47.10)
	2	0.56±0.05 (0.50-0.65)	0.55±0.03 (0.49-0.58)
HW/HL	1	0.64±0.04 (0.56-0.71)	0.65±0.04 (0.59-0.74)
TL/TBL	1	0.44±0.02 (0.42-0.48)	0.44±0.02 (0.40-0.47)
PW/PL	1	0.35±0.03 (0.28-0.38)	0.37±0.04 (0.30-0.44)
NED/HL	1	0.17±0.02 (0.13-0.19)	0.17±0.02 (0.13-0.20)
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light yellow maculation, and a light brown reticulation was found on elbows and knees. Tiny flecks were present on the dorsal side of some finger joints. On the dorsal side of the tail, which had rows of black dermal pores, there were a light brown and yellow maculation. The lower parts of the trunk, tail and legs were flesh-coloured. The throat region had a yellow tinge.

Measurements of the holotype (in mm): Total length (ToL) 135.00; RA and length of trunk (LT) 77.00 and 50.11, respectively; TL 58.00; NED 3.09; DBN 4.67; ED 3.79; HL 16.57; HW 11.05; PL 8.33; PW 3.29; FLL 22.92; HLL 26.09; DFHL 37.70

Paratype variation: Variation observed in some morphometric characters and ratios vis-à-vis the holotype are summarized separately for adults and juveniles in Table 2. Sexual dimorphism was observed within the population $(p \le 0.05)$ regarding HL, PL, PW and FLL in raw data or PERCRA values. In addition, the projection at the base of the tail in nine male specimens ranged between 1.45 and 3.34 mm with an average of 2.70 mm. As far as the colour pattern is concerned, the description given for the holotype largely applies to the other males and females as well (Fig. 2). The discontinuous light yellow parts in the pattern of adult females are broader and more pronounced than that of adult males. Juveniles sport a darker ground colour with a more intensely yellow and brown pattern on their dorsum. From this series and the specimens observed in the field, it would appear that the pattern as well as the dorsal background colour change with age and depending on sex. Both females and juveniles lack any protuberance at their tail bases and have smooth or less swollen cloacae.

Habitat, geographic distribution, and ecology: We found *Lyciasalamandra antalyana gocmeni* ssp. n. under rocks in a karst-dominated landscape. In the dry months of summer, the animals use hollow spaces in the karst or heaps of rock for sheltering. The hillside is usually vegetated with plane (*Platanus orientalis*) and pine trees (*Pinus brutia*). Activity outside of the shelters is highly dependent on temperatures and humidity. All localities recorded lie within the potential distribution predicted by RÖDDER et al. (2011). The habitat of the new subspecies extends into the Taurus mountain range at the western border of the Antalya lowlands. In the east, the area is bordered by the Aksu valley. In the west and north, the forest-free tablelands of the Taurus do not allow further expansion.

Derivatio nominis: The name of the new subspecies has been chosen in honour of the Turkish herpetologist, Professor Dr. Bayram Göçmen. Besides his contributions to herpetology, he is a role model and a good friend.

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