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# Description of a new subspecies of *Salamandra algira* Bedriaga, 1883 (Amphibia: Salamandridae) from the Beni Snassen massif (Northeast Morocco)

Daniel Escoriza & María del Mar Comas

**Abstract.** A new subspecies of *Salamandra algira* is described from the Beni Snassen massif (Northeast Morocco). It is both genetically and morphologically distinct from all other *S. algira* populations and is geographically isolated. Morphologically, the new subspecies is characterized mainly by its large size, the presence of little red and white dots on the head, body and tail, rounded yellow blotches on head, body and tail, and black gular and ventral coloration. Aspects of ecology and conservation are discussed.

Keywords. Amphibia: Salamandridae: *Salamandra algira spelaea* spp. n., taxonomy, mitochondrial DNA, biogeography, endangered subspecies.

## Introduction

The genus *Salamandra* Laurenti, 1768 is widespread in the Western Palearctic where it occurs from the Iberian Peninsula and northern Africa to Asia Minor (Veith 1994), with several species described around the Mediterranean basin (Veith 1994, Steinfartz et al. 2000). The southernmost species, *Salamandra algira* Bedriaga, 1883 and *S. infraimmaculata* Mertens, 1948, have fragmented distributions, restricted to mountain regions (Bons & Geniez 1996, Degani 1996). *Salamandra algira* has a patchy distribution in Northeast Africa (Bons & Geniez 1996, Schleich et al. 1996, Donaire-Barroso & Bogaerts 2003), and several isolated populations show genetic divergence (Steinfartz et al. 2000, Donaire-Barroso & Bogaerts 2003, Escoriza et al. 2006).

In Morocco *S. algira* is confined to the relatively humid regions of the Rif, Middle Atlas (Bons & Geniez 1996) and the Beni Snassen mountains, where it has been recently rediscovered (Escoriza et al. 2006). According to mitochondrial DNA data (320 base pairs of cytochrome *b*, 370 base pairs of 12S rRNA) this population is genetically well differentiated from known populations of *S. algira* from Morocco and from the type locality of *S. algira* in Annaba, Northeast-Algeria (Escoriza et al. 2006). The specimens from the Beni Snassen also show distinctive morphological characters that suggest it deserves to be described as a different subspecies. The purpose of this paper is to formally describe and name it.

## Material and methods

All behavioural, ecological, climatological and distributional data were obtained during five field trips in November 2004, October-November 2005 and January-March 2006 to the Beni Snassen massif. In total, the study included 50 days of fieldwork during which time temperature and relative humidity were recorded when salamanders were found. Water temperature was recorded from a cattle watering place where salamander larvae were found every two hours with a Hobo pendant probe during a 20 days period. All captured individuals of the new subspecies (4 males, 2 females, 3 juveniles and 19 larvae) were photographed, measured with a digital calliper to the nearest 0.1 mm and weighed with a dynamometer. After these manipulations, for conservation reasons specimens were released at the place they were found, except for three voucher specimens (i.e. the type series). Sexes were determined by secondary sexual characters, as by the swollen cloaca of the male which is a constant criterion in several species of the genus (Griffiths 1996). Statistical tests were performed using STATGRAPHICS Plus (Statistical Graphics corp. 1994-1999). Data used to compare the different populations were obtained from museum specimens but measurements from Beni Snassen and Taza (Middle Atlas) specimens were obtained from live individuals directly in the field, and Rahr-el-Maden data was obtained from Doumergue (1901). Museum specimens deposited at Museu de Ciencies Naturals de Barcelona (MCNC) and The Natural History Museum (NHM), London, were studied. Collection numbers and locality of the specimens included in this study are given in Table 1.

Abbreviations used: TL: total length, taken from tip of snout to end of tail; SVL: snout-vent length, measured from tip of the snout the anterior edge of the vent; AL: anterior limb length, measured from the insertion into the body to the tip of the third toe; PL: posterior limb length, taken from the insertion to the tip of the third toe; HL: head length from posterior edge of parotid gland to tip of snout; HW: head width, measured at anterior edge of parotids; PAL: parotid length, i.e. from the anterior to the posterior edge; LJL: lower jaw length, measured from the corner of the mouth to the tip of the jaw; SL: snout length, taken from the anterior edge of the eye to the tip of the snout; TD: tail diameter in a horizontal plane measured at the posterior edge of the vent; BD: body diameter in a horizontal plane measured behind the anterior limbs; tail length, measured from the anterior edge of the vent to the tip of the tail; n.s. means not significant.

Four specimens of *Salamandra algira* from Beni Snassen were sequenced for the cytochrome *b* gene (cyt*b*), 12S rRNA and the D-loop mitochondrial genes and compared with other North African *S. algira* sequences available from GenBank (Table 2). DNA extractions and PCR amplifications of the desired mitochondrial fragments were carried out according to methods described in Carranza et al. (1999, 2000). Primers used in both amplification and sequencing of three mitochondrial genes were cytochrome *b*1 and cytochrome *b*2 (Kocher et al. 1989) for cyt*b*, 12Sa and 12Sb (Kocher et al. 1989) for the 12S rRNA gene and D-loopF (5'- CAT TTG TGT GGC GAC ATA TTA TGC T-3') and D-loopR (5'- GTC TTA ATA ATC GTA AGT AAA TAG GGC - 3') for a fragment of the control region (D-loop) homologous to the region between nucleotides 16,005 and 16,379 of the *Lyciasalamandra atifi* Basoglu, 1967 mitochondrial genome (GenBank accession number NC_002756).

Three hundred and twenty base pairs (bp) of cyt*b* and 370 bp of the 12S mitochondrial rRNA gene were sequenced for three specimens of *S. algira* from three different localities within the Beni Snassen massif (locality 5, Fig. 1) and compared with sequences from another individual from the Beni Snas-

Tab. 1. Specimens examined in this study (ind. = number of individual/s.).

| specimen | museum number | locality |
|---|---|---|
| holotype of the new species | MCNC 2005-0550 | Ouartass |
| paratype of the new species | MCNC 2006-0183 | Ouartass |
| paratype of the new species | MCNC 2006-0184 | Ouartass |
| *S. algira algira* (4 ind.) | NHM 1920.1.20.1194 | Mt. Edough |
| *S. algira algira* (2 ind.) | NHM 1889.12. 7.6-7 | Mt. Edough |
| *S. algira algira* (1 ind.) | NHM 1920.1.20.1094 | Larba |
| *S. algira tingitana* (8 ind.) | NHM 1889.3.12.9-16 | Benider Hills |

Tab. 2. Specimens and GenBank accession numbers for sequences referred to in Figure 9 (www.ncbi.nlm.nih.gov/Genbank). Numbers [1], [3] and [5] refer to localities shown in Figure 1.

| taxon | locality | accesion numbers: cyt *b* | accesion numbers: 12SrRNA | accesion numbers: D-loop | codes |
|---|---|---|---|---|---|
| *Salamandra algira spelaea* | Beni Snassen (Morocco) [5] | DQ221247 | DQ221231 | DQ221248 | E9035.1 |
| *Salamandra algira tingitana* | 1 Djebel El Fahies (Morocco) [1] | AY247732 | DQ221227 | | E1712.7 |
| *Salamandra algira tingitana* | 2 Tagramt (Morocco) [1] | AY247733 | DQ221228 | | E309.10 |
| *Salamandra algira* | 1 Askur near Talembot (Morocco) [3] | AY247734 | DQ221229 | | E1712.9 |
| *Salamandra algira* | 2 Chefchauen (Morocco) [3] | AY247735 | DQ221230 | | E1712.14 |
| *Salamandra infraimmaculata orientalis* | W. of Adana (Turkey) | DQ221242 | DQ221222 | | E3009.22 |
| *Salamandra salamandra longirostris* | Los Barrios (Spain) | DQ221243 | DQ221223 | | E1712.37 |
| *Salamandra atra aurorae* | Bosque del Dosso (Italy/ Austria) | DQ221246 | DQ221226 | | E3009.16 |
| *Salamandra lanzai* | Italy | DQ221244 | DQ221224 | | E3009.23 |
| *Salamandra corsica* | Cascade des Anglais Corsica (France) | DQ221245 | DQ221225 | | E3009.18 |
| *Salamandra salamandra almanzoris* | Sierra de Gredos (Spain) | DQ221239 | DQ221219 | | E1712.17 |
| *Salamandra salamandra crespoi* | Sta. Lucia, Algarve (Portugal) | DQ221237 | DQ221217 | | E1712.25 |
| *Salamandra salamandra morenica* | Villanueva del Rey (Spain) | DQ221238 | DQ221218 | | E1712.40 |
| *Salamandra salamandra alfredschmidti* | Valle del Río Tendi (Spain) | DQ221240 | DQ221220 | | E1712.20 |
| *Salamandra salamandra gigliolii* | Sierra de San Bruno (Italy) | DQ221241 | DQ221221 | | E1712.34 |
| *Salamandra salamandra bejarae* | San Pablo de los Montes (Spain) | DQ221236 | DQ221216 | | E1712.44 |
| *Salamandra salamandra gallaica* | Pto. Arrabida (Spain) | DQ221253 | DQ221215 | | E7110.11 |
| *Salamandra salamandra fastuosa* | Bagneres de Luchon (France) | DQ221234 | DQ221214 | | E1712.27 |
| *Salamandra salamandra salamandra* | Ukraine | DQ221232 | DQ221213 | | E7110.15 |
| *Salamandra salamandra europaea* | Montseny (Spain) | AY222503 | AY222459 | | E1712.49 |

sen massif and sequences from the recently described *S. algira tingitana* Donaire-Barroso & Bogaerts, 2003 from the Tangitanian district (locality 1, Fig. 1), *S. a. algira* from the Western Rif mountains (locality 2, Fig. 1), *S. a. algira* from the Central Rif region (lo-

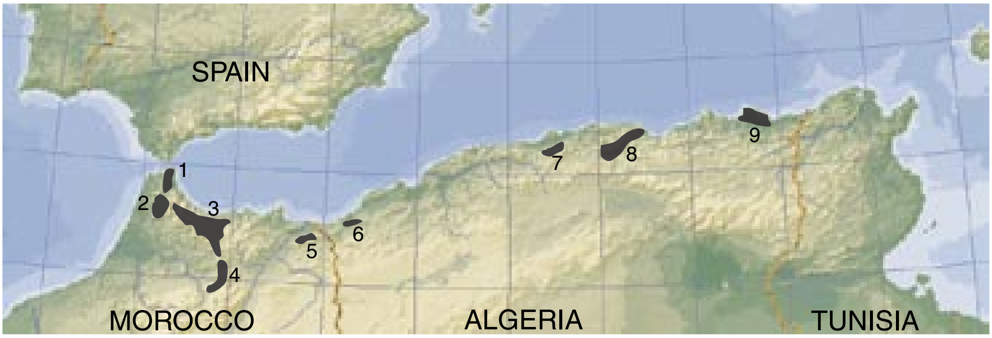


Fig. 1. Map showing the distribution of all known North African populations of *Salamandra algira*. Numbers beside black areas refer to geographical localities: 1 = Tangitanian district (North Jebala), *Salamandra algira tingitana;* 2 = Western Rif mountains, *Salamandra algira;* 3 = Central Rif mountains, *Salamandra a. algira;* 4 = Middle Atlas, *Salamandra a. algira;* 5 = Beni Snassen massif, *Salamandra algira spelaea;* 6 = Rahr-el Maden, *Salamandra a. algira;* 7 = Blida Atlas, *Salamandra a. algira;* 8 = Great and Small Kabylia, *Salamandra a. algira;* 9 = Edough peninsula, type locality of *Salamandra a. algira.*

cality 3, Fig. 1) and *S. a. algira* from the Middle Atlas (locality 4, Fig. 1). Moreover, 374 bp of the mitochondrial gene D-loop were also sequenced for three specimens of the new subspecies of *S. algira* described herein and

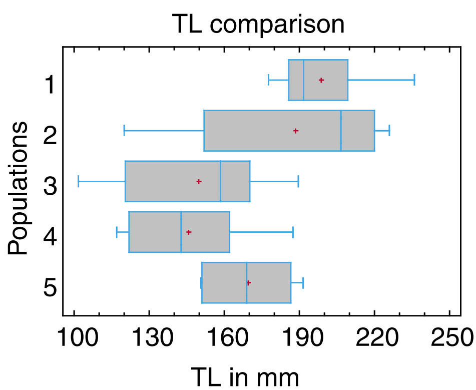


Fig. 2. Comparison of TL between the different studied populations of *Salamandra algira* by means of ANOVA. 1 = *S. algira spelaea;* 2 = *S. a. algira* Rahr-el-Maden; 3 = *S. a. algira* Annaba; 4 = *S. algira tingitana* Ceuta; 5 = *S. a. algira* Middle Atlas. Homogeneous groups at 95% confidence level: 1, 2, 5 and 5, 3, 4, using Duncan´s multiple comparison method of means. Contrast 1-2: 10.2 (n.s.); 1-3: 48.85; 1-4: 53; 1-5: 29.025 (n.s.); 2-3: 38.65; 2-4: 42.8: 2-5: 18.825 (n.s.); 3-4: 4.15 (n.s.); 3-5: -19.825 (n.s.); 4-5: -23.975 (n.s.).

compared with sequences of one specimen from the Central Rif region (locality 3, Fig. 1) and one specimen from the surroundings of the type locality of *S. a. algira* (Bona = Annaba) within the Massif of Edough in northeast Algeria (locality 9, Fig. 1), not available at GenBank but provided by Steinfartz et al. (2000).

All new sequences obtained for this work have been submitted to GenBank (www.ncbi.nlm.nih.gov/Genbank) and have the following accession numbers: 12S rRNA: E22036.2/DQ901439, E22036.3/DQ901440, E22036.4/DQ901441; cyt *b*: E22036.2/DQ901442, E22036.3/DQ901443, E22036.4/DQ901444; D-loop: E22036.2/DQ901445, E22036.3/DQ901446, E22036.4/DQ901447 (non collected specimens).

## ***Salamandra algira spelaea* spp. n.**

*Salamandra salamandra algira* (non Bedriaga, 1883): Nikolskii 1918: 187, Melhaoui & Chavanon 1989: 135, Bons & Geniez 1996: 47.

*Salamandra algira* (non Bedriaga, 1883): Veith 1996: 174-177, Mateo et al. 2003: 73.

*Salamandra algira algira* (non Bedriaga, 1883): Donaire & Bogaerts 2003: 88 (by implication), Bogaerts & Donaire 2003: 49.

Fig. 3. Holotype of *Salamandra algira spelaea* from Ouartass in life.

Holotype: MCNC 2005-0550, adult male, collected at Ouartass, Beni Snassen massif, Northeast-Morocco (Locality 5, at approximately 1,300 m above sea level), on 24 November 2004 by Daniel Escoriza and María del Mar Comas.

Paratypes: MCNC 2006-0183 and MCNC 2006-0184, two juveniles, collected at the type locality, November 2005, by Daniel Escoriza and María del Mar Comas.

Diagnosis: *Salamandra algira spelaea* is a salamander with a slender body and tail and moderate size (177-236 mm TL). Head flat, longer than wide, with prominent eyes having a black iris. Snout rounded, limbs large, tail large and cylindrical. Jet black ground coloration with scattered rounded or elongated yellow blotches, not arranged in bands, located at head, dorsum and tail; predominance of black over yellow. Large parotid glands with reddish or some black glandular pores. Tiny red spots around parotids, eyes, flanks, and on limbs and tail, sometimes as independent spots or fused to the yellow or white ones. Occasionally little white spots in lateral, gular and ventral region. Ventral and gular region black. Four fingers and five toes, all without membranes. Eight to eleven lateral tubercles. Tail a little shorter than head plus body. Ovoviviparous. Juveniles with similar color pattern as adults, with many white spots located on both sides of the body and without ventral spots.

Fig. 4. Adult male of *Salamandra algira spelaea* from Beni Snassen massif.

Fig. 5. Paratype of *Salamandra algira spelaea* from the type locality.

*Salamandra algira spelaea* differs from *S. a. algira* from Jebel Edough (Algeria, locality 9, Fig. 1) by the combination of several morphological characters: maximum TL *S. a. algira* 198 mm versus *S. algira spelaea* 236 mm (TL comparison using Mann-Whitney test: $U = 34$, $P = 0.01$, $n = 6$ of each subspecies); the presence of red spots in *S. algira spelaea* [red spots are used as morphological criterion to distinguish other salamander subspecies such as *S. salamandra bejarae* Mertens & Müller, 1940 and *S. salamandra almanzoris* Müller & Hellmich, 1935 (Martinez Solano et al. 2005)]. *Salamandra a. algira* from Middle Atlas and Rif (Morocco, localities 3 and 4, Fig. 1) has reddish gular coloration (versus black with occasional little white spots in *S. algira spelaea*), wider red


Fig. 9. Phylogenetic relationships of *Salamandra* species and subspecies treated here A: ML tree (inferred using PHYML and the HKY+I+G model of sequence evolution) combining 320 bp of *cyt*b and 370 bp of 12S mitochondrial sequences (690 bp with 159 variable positions). B: ML tree (inferred using PAUP and the HKY+G model of sequence evolution) using 374 bp (78 variable positions) of mitochondrial DNA sequences from the control region (D-loop). Values by the nodes indicate bootstrap values above 50. Numbers between square brackets after locality names refer to locality numbers in Figure1.

spots in head, body and tail and the males have swollen forearms in the reproductive season (pers. obs.). *Salamandra algira tingitana* from Northwest-Morocco (locality 1, Fig. 1) is smaller, mainly viviparous, without red spots and with a tendency to the hypoluteism (Donaire-Barroso & Bogaerts 2003). *Salamandra a. algira* from Rahr-el-Maden (Algeria, locality 6, Fig. 1), the geographically nearest known population to the Beni Snassen massif, are large salamanders like the new subspecies (TL comparison with

Mann-Whitney test: n.s.; U = 21, P = 0.94, n = 7 and 6 specimens), with a color pattern of alternating yellow blotches and little red dots along the head, body and tail. But one individual with red dots in the gular region has been described (Doumergue 1901) whereas all examined *S. algira spelaea* never had red gular coloration. Comparisons of TL running an ANOVA among the five different studied populations (see also Table 3 for morphometric data of the different studied populations) showed significant differences (n = 6 for each population, F-ratio 3.68, P = 0.01, Fig. 2).

Description of holotype (Fig. 3): Third and fourth fingers of the left posterior leg missing. Nine lateral tubercles on both sides of the body. Rounded snout. Parotids without marked black glandular pores. Ground color black with yellow blotches arranged irregularly on head, dorsum, limbs and tail. Ventral region black without spotting. Little red spots in dorsolateral region, under both parotids and at the base of left forelimb. Four yellow blotches on head: each two above eyes and on parotids. Between the head and the base of the hind limbs, there are ten yellow spots of variable size, not arranged in bands, two at the base of the tail and eight along the tail. Yellow spot at base of each limb. On right forelimb this spot faces the external edge of the forearm; on this limb there are two more yellow spots on the internal edge of the arm and another at the base of first and second fingers. On left forelimb, besides the yellow spot at the base, there is another one on the internal edge of the arm. On right hind limb there is a yellow spot at the base and three additional yellow spots, each one on the internal edge of the thigh, on the internal edge of the leg and at the base of the second and third fingers. On left hind limb there is a yellow spot at the base, on the internal edge of the leg and at the base of the first finger.

For dimensions see Table 4.

Variation: Data used here refer to the type series and five non-collected specimens. For measurements see Table 4.

The black background always has yellow blotches (Figs. 3-5). Numerous circular or elongated yellow-orange spots are distributed along the body, usually two above both eyes and parotids (being circular or elongated too), at the base of each limb, on dorsal region (six to ten) and on the tail (seven to eleven) (Fig. 6). Furthermore, this subspecies always presents an irregular number of tiny red spots (maximum measured diameter 5.9 x 2.3 mm), around eyes, parotids, limbs, in lateroventral region and at lateral tubercles as well as on dorsum and tail alone or even joint with yellow blotches. Some individuals show tiny white gular and lateral spots. One specimen found had one very little white spot in the ventral region between both forelimbs.

Three juveniles (Fig. 5) had a similar number and arrangement of yellow blotches

Tab. 3. Comparison of morphometric data of *Salamandra algira* subspecies and populations: A = *S. algira spelaea* Beni Snassen massif, locality 5; B = *S. algira algira* Annaba, locality 9; C = *S. algira algira* Middle Atlas, locality 4; D = *S. algira tingitana* Tangier, locality 1; E = *Salamandra algira algira* Larba, locality 7. Data are in mm; for their abbreviations see material and Methods. Mean ± one standard deviation are given.

| | A | B | C | D | E |
|---|---|---|---|---|---|
| | N = 6 | N = 6 | N = 8 | N = 2 | N = 1 |
| LT | 198.7 ± 21 | 151.5 ± 30.6 | 164.3 ± 18.8 | 142.8 ± 14.3 | 164 |
| SVL | 116.7 ± 9.3 | 80.2 ± 15 | 91 ± 9.8 | 77.9 ± 7 | 90.8 |
| AL | 41.4 ± 6 | 26.2 ± 6.6 | 27.6 ± 4.2 | 22.2 ± 2.4 | 26 |
| PL | 42.5 ± 5.8 | 27.9 ± 6.9 | 29.6 ± 5.8 | 25.6 ± 3.7 | 27.7 |
| HL | 26 ± 2 | 20.8 ± 4.2 | 19.3 ± 3.2 | 20.4 ± 1.6 | 22.7 |
| HW | 18 ± 1.9 | 14.3 ± 2.7 | 13.5 ± 1.9 | 14.6 ± 1 | 16.3 |

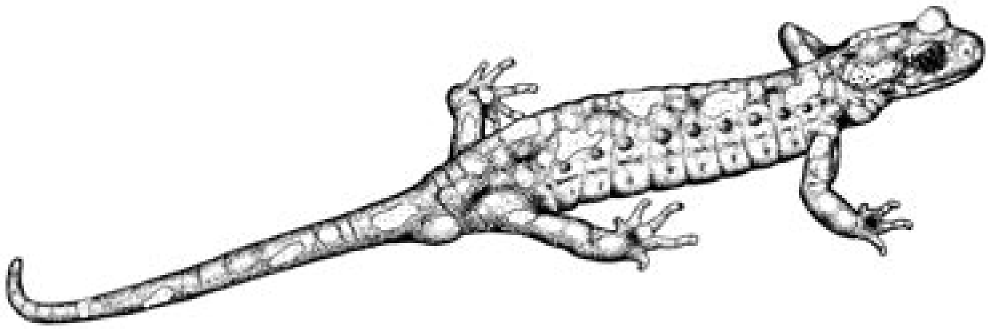

Fig. 6. Illustration based on an adult male observed in Ouartass.

Fig. 7. *Salamandra algira spelaea* larva feeding on a *Discoglossus pictus* tadpole.

like the adults over a jet black ground color. But they lacked red spots and all had numerous white dots of variable size in the dorsoventral but not so in the ventral region.

Nineteen larvae found were dull brown with four yellow spots at the base of each limb and with a series of black dots along the tail and body. Larvae were found in November (n = 13, TL values between 39.5-30 mm, mean = 33.4 mm) and January (n = 6, TL values 46.7-34.8 mm, mean = 41.7 mm). Fully metamorphosed individuals were observed in mid February (n = 3, TL values 48.7-43.3 mm, mean = 46.6 mm).

Natural History: The Beni Snassen massif is a moderately high mountain range (reaching 1532 m above sea level at its highest point, Jebel Fourhal) near the Mediterranean coast. The climate is of the thermo-mediterranean type at lower altitudes with mean annual rainfall of 350 mm (Raynal 1961), mainly in winter-early spring (December-April) and with a xerothermic index of $\bar{x}$ = 100-150 days (Despois & Raynal 1967). The maximum average temperature in August is 31 °C and the minimum average temperature in January is 5 °C at lower altitudes (Dakki et al. 2003). However, as the altitude increases, a mesomediterranean climate with marine influence appears, marked by an increase in the amount of rainfall (> 500-600 mm/year) (Despois & Raynal 1967) and by a shorter dry season ($\bar{x}$ < 90 days). The vegetation from Beni Snassen massif is typically Mediterranean, the domain of the phytocenologic order *Quercetalia ilicis*. An altitudinal gradient in vegetation is observed between mountain holm oak forest (mountain stage) and thermic vegetation in basal stage. The former is characterized by elements that need greater soil moisture and the latter is made up by species more adapted to thermomediterranean conditions with marked dry season such as *Pistacia atlantica* located at lower altitudes and on the southern slopes.

*Salamandra algira* in the Beni Snassen massif has been observed from 600 to 1300 m above sea level, occupying the diverse types of forests that appear between these altitudinal ranges. Specimens of *S. algira spelaea* have been found on limestone outcrops and granite soils, in the same way as the *S. a. algira* from Middle Atlas (pers. obs.). Adult individuals have been found within a maximum 50 m radius of the several water bodies where larvae were observed.

"Begleitherpetofauna" and "Begleitflora". Plant, reptile and amphibians species recorded in addition to the new subspecies during the present study. Holm oak montane forest (1300-1200 m above sea level): flora: *Juniperus oxycedrus*, *Pinus halepensis*, *Cistus laurifolius*, *Salix* sp., *Quercus ilex*, *Rubus* sp., *Rosa* sp., *Crataegus monogyna*, *Nerium oleander*, *Ruscus aculeatus*; herps: *Discoglossus pictus, Bufo mauritanicus, Bufo viridis*, *Rana saharica, Tarentola mauritanica*, *Scelarcis perspicillata*. Thermic forest with continental tendency (1000-600 m above sea level): flora: *Tetraclinis articulata*, *Pinus halepensis*, *Cistus laurifolius*, *Rhamnus* sp., *Salix* sp., *Quercus coccifera*, *Q. ilex*, *Rubus* sp., *Rosa* sp., *Prunus dulcis*, *Ceratonia siliqua*, *Genista* sp., *Pistacia lentiscus*, *Erica multiflora*, *Olea europaea*, *Nerium oleander, Rosmarinus officinalis*, *Ruscus hypophyllum*; herps: *Discoglossus pictus, Bufo mauritanicus, B. viridis, Rana saharica, Mauremys leprosa, Testudo graeca, Saurodactylus mauritanicus, Tarentola mauritanica, Chamaeleo chamaeleon, Scelarcis perspicillata*, *Hemorrhois hippocrepis.*

Several breeding localities were located between 600 and 1300 m above sea level, most of them were cattle watering places or man-made springs but larvae were also observed in small shaded pools. The average water temperature in one of the sites (at 1200 m) was 14 °C in November. Larvae prey upon invertebrates of suitable size and *Discoglossus* larvae (Fig. 7). Predation of *S. algira spelaea* larvae by *Nepa cinerea* was observed under natural conditions. Active adults have been located during November, usually during wet weather, in late afternoon and during the night, on the forest floor and inside cavities (Fig. 8). Air temperatures have ranged between 5.8 and 7° C, the latter being the air temperature one meter inside the entrance of the cavity where one female was observed, a full 2° C higher than outside. Humidity was around 75% or higher (values ranging 75-85%). As a defensive mechanism, some individuals expelled water through the vent when they were handled. One female, with an initial weight of 49.5 g lost 13.5 g (27%), one male of 34 g fell to 24 g (29%) and other male of 24.4 g lost just 1.9 g (8%).

Fig. 8. Rock cavities where several adult *Salamandra algira spelaea* were found (Ouartass, 1200 m above sea level).

Distribution: The new subspecies is limited to the Beni Snassen massif, Northeast-Morocco. It has been observed in a restricted area of less than 45 km$^2$ and most likely should be considered an endangered subspecies.

Etymology: *Salamandra algira spelaea* was named after the sites where several individuals have been observed, i.e. inside or in the vicinity of rock cavities.

Molecular analyses: Results indicate that all four specimens from the Beni Snassen massif are genetically identical in the 1064 bp of mitochondrial DNA sequenced and different from all other North African populations of *Salamandra algira*, highlighting the uniqueness of this isolated population and justifying its recognition as a different subspecies. The phylogenetic analyses were carried out following exactly the same methodology as in Escoriza et al. (2006) and are presented in Figure 9. These clearly show that the population from the Beni Snassen massif is an independent evolutionary lineage. Genetic divergence values (uncorrected p-distance) between the Beni Snassen specimens (locality 5, Fig. 1) and all the rest of Moroccan populations analyzed (localities 1–4, Fig. 1) range between 7.2% and 9% for the cyt*b* fragment and 2.4% and 2.7% for the 12S mitochondrial

Tab. 4. Morphometric data (mm and g) of *Salamandra algira spelaea* specimens studied. For abbreviations see Material and Methods.

| | individual 1 | individual 2 | individual 3 | individual 4 | individual 5 | holotype | paratype | paratype |
|---|---|---|---|---|---|---|---|---|
| sex | male | female | male | male | female | male | | |
| weight | 20.5 | 33.5 | 24.5 | 34.5 | 49.5 | 34 | 1 | 2 |
| LT | 193.5 | 209. 4 | 177.7 | 185.7 | 235.9 | 190 | 61.4 | 73.8 |
| LSV | 112.3 | 122.9 | 104.7 | 108.9 | 127.7 | 124 | 36.5 | 41 |
| AL | 41.3 | 42.8 | 34.4 | 42.1 | 51.6 | 36.4 | 10.2 | 10.8 |
| PL | 40.7 | 50.8 | 37.4 | 42.3 | 47.8 | 35.8 | 11.5 | 12.8 |
| HL | 25 | 27.9 | 23.6 | 24.8 | 29.2 | 25.9 | 9.6 | 10 |
| HW | 16.8 | 18.6 | 17.2 | 15.6 | 21.03 | 18.9 | 7.4 | 7.8 |
| PAL | 13.6 | 16 | 13.2 | 15.1 | 16.4 | 14 | 4.9 | 5.4 |
| LJL | 15.2 | 18.5 | 17.3 | 17.1 | 19.2 | 16.3 | 6.2 | 6.4 |
| SL | 7.1 | 8.9 | 5.8 | 7.7 | 8 | 7.7 | 3.1 | 2.6 |
| TD | 6.1 | 6.1 | 5.5 | 6 | 6.9 | 7.1 | 3 | 3 |
| BD | 14.5 | 15.1 | 16 | 14.3 | 21 | 14.8 | 5.5 | 6.8 |

rRNA gene. These values almost double the divergence found between *S. algira* from the Central Rif region (locality 3, Fig. 1) and *S. a. tingitana* (locality 1, Fig. 1), which differ by 4.6% to 5% of the cyt*b* sequenced and in 1% of the 12S mitochondrial rRNA gene. Divergence values between specimens from Beni Snassen and remaining Moroccan samples are similar to the genetic divergence found among all recognized species of Eurasian *Salamandra* (4.3-7.5% for the cyt*b* gene and 1-4% for the 12S mitochondrial rRNA gene). Alignment of the diagnostic nucleotide positions of the Cyt *b*, 12SrRNA and D-loop for the the new subspecies compared with other *S. algira* complex populations is showed in the Appendix.

A phylogenetic tree using partial sequences of the D Loop mitochondrial region including specimens of *S. algira spelaea* from the Beni Snassen and *S. algira* from the type locality and from the Central Rif region was also performed and is presented in Figure 9. As with the cyt*b* and 12S rRNA sequences, the results also confirm that *S. a. spelaea* is a distinct evolutionary lineage. Uncorrected genetic divergence values between *S. a. spelaea* and *S. a. algira* from the type locality (locality 9, Fig. 1) and from the Central Rif region (locality 3, Fig. 1) are 2.8% and 4.3%, respectively. These results indicate that *S. a. spelaea* is more closely related to *S. a. algira* from the type locality (approximately 800 km to the east) than to the nearby population from the Central Rif mountains (200 km to the west).

Remarks: The transition populations of *S. a. algira* recorded from Algeria in Rahr-el-Maden, Blida Atlas and Great Kabylia (see Fig. 1) requires further studies to unravel their relationship with *S. a. spelaea* or the nominal subspecies. The morphological resemblance of the Rahr-el-Maden specimens to the Beni Snassen individuals and the geographical proximity between them suggest that Rahr-el-Maden and Beni Snassen populations might be closely related. Blida Atlas populations have red spots in different parts of the head and the body but no data exist about the morphological characters for the Kabylia population (Bogaerts & Donaire-barroso 2003, Escoriza et al. 2006).

There is a correlation between the climatic parameters and the distribution pattern of the different forms of *S. algira* in Northern Africa; the species are present in mesomediterranean climate above the 500 mm of mean annual precipitation. Therefore the different populations are isolated by the line delimiting the thermomediterranean climate (see Despois & Raynal 1967 for xerothermic index values map) and, in the present climate conditions, this prevents gene flow among populations. The humid/subhumid regions of Algeria and Morocco are clearly isolated by an expansion of the arid inland climate, the subdesertic Moulouya basin, where several Saharan reptile species approach the Mediterranean coast (Bons & Geniez 1996). This plain that might have acted as a barrier for several Mediterranean amphibians and reptiles (Wade 2001, Zangari et al. 2006), has promoted differentiation between populations located on either side of the plain. The phylogenetic analysis shows that *Salamandra a. spelaea* is more closely related to the nominal *S. a. algira* from Annaba (Northeast Algeria) than to the geographically nearby population of *S. algira* from the Middle Atlas, placed on the western side of the Moulouya basin. The high genetic divergence among studied populations (Fig. 9) shows that genetic isolation between them is moderately old and occurred approximately during the Pliocene (Escoriza et al. 2006). Allopatric speciation in *Salamandra* has been promoted by the drastic climatic fluctuations during the Pliocene-Pleistocene periods in the Western Paleartic, with successive waves of contraction/expansion of the species' ranges, isolating the different populations in favorable refugia and consequently promoting genetic differentiation, as probably happened in several amphibian species (Riberon et al. 2001, Carranza & Amat 2005). The isolated populations of the *S. algira* complex show marked morphological differences between them as the presence of red spots, body size, reproductive strategies (*S. algira tingitana* is mainly viviparous, Donaire-Barroso & Bogaerts 2003) or some secondary sexual characters, as the swollen forearms of the males of the Middle Atlas (pers. obs.) and of the Tangitanian district (Donaire-Barroso and Bogaerts 2003) in the reproductive season.

*Salamandra algira* has been considered a relict species with reduced and fragmented populations, some of which are highly threatened by deforestation (Schleich et al. 1996, Fahd et al. 2004). Although more field data is required, *Salamamandra algira spelaea* spp. n. appears to be very localised and adult individuals are very difficult to find in their habitat compared with other *S. algira* populations from Taza or the Rif mountains (pers. obs.). Moreover, preliminary molecular analyses (see above) suggest the genetic variability of within the Beni Snassen massif is very low, wich might also have a negative effect on the long-term survival of this population (Újvári et al. 2002). *S. a. spelaea* spp. n. seems to be very fragile due to the isolation of this population and the limited chances of expansion in this dry environment, and for this reason urgent conservation measures for this subspecies are suggested.

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## Appendix

Alignment of the variable nucleotide positions of the Cyt *b* (320 base pairs), 12S rRNA (321-690 bp excluding deletions) for 5 studied Moroccan *Salamandra algira* complex populations and D-loop (374 bp excluding deletions) for the nominotypical form from Annaba (**Algeria**), from the central Rif region (Morocco) and for the *S. algira spelaea*. Locality numbers are as indicated in Figure 1; codes given are after locality numbers. Underlined and printed in bold nucleotides in the alignment mark diagnostic nucleotide positions for the new subspecies. Dots show an identical nucleotide for the same bp position compared with the previous specimen nucleotide sequence.

**cyt*b***

```
                111111111112222222233
       1223445678001123577780245678 00
       2574651408702434247678531599 2125
```

**11111111111222222233**
**1223445678001123577780245678 00**
**25746514087024342476785315992125**

1. AACTTCTTAATAGACATAAGACCTTGTCTCGT *Salamandra algira spelaea* Beni Snassen Locality 5 E9035.1
2. CCTA..AC..C.ATTCCCG.G.TC.A.TCTAC *Salamandra algira tingitana* Djebel El Fahies Locality 1 E1712.7
3. ................................ *Salamandra algira tingitana* Tagramt Locality 1 E309.10
4. .TC.CTCAGGTGG....T.A.T..C.C..... *Salamandra algira* Askur near Talembot Locality 3 E1712.9
5. .......CA....................G. *Salamandra algira* Chefchauen Locality 3 E1712.14

**12SrRNA**

**34455566666**
**55503922225**
**04741023582**

1. GTAGTAATTAA *Salamandra algira spelaea* Beni Snassen Locality 5 E9035.1
2. ACTAACGACG. *Salamandra algira tingitana* Djebel El Fahies Locality 1 E1712.7
3. ........... *Salamandra algira tingitana* Tagramt Locality 1 E309.10
4. ....T..GT.G *Salamandra algira* Askur near Talembot Locality 3 E1712.9
5. ........... *Salamandra algira* Chefchauen Locality 3 E1712.14

**D-Loop**

```
       11111112222222233333
    226794566799134455603446
   4497624345056663458028693
1. AACTAGAGGCTCCGTCTCGTTTGTA Salamandra algira spelaea Beni Snassen
                                                          Locality 5
2. ..TCGCT.AT.......A..A..CC Salamandra algira algira Annaba Local-
                                                               ity 9
3. TGC.AGATGCAATAATC.ACTCATA Salamandra algira Rif mountains Local-
                                                               ity 3
```

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