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A new subspecies of the fire salamander, *Salamandra salamandra* (LINNAEUS, 1758) from the Tendi valley, Asturias, Spain

GUNTHER KÖHLER & SEBASTIAN STEINFARTZ

Abstract. The fire salamander of the Rio Tendi valley (Asturias, Spain) which have been so far considered as *Salamandra salamandra bernardezi* differs from individuals from the type locality Ovideo of *S. s. bernardezi* and all remaining recognized Iberian subspecies with regard to its colouration and mitochondrial sequence composition. It is therefore described as a new subspecies, *Salamandra salamandra alfredschmidti*. Morphologically, it is differentiated from all remaining subspecies of *Salamandra salamandra* by a lack of, or at least reduced, black dorsolateral stripes as well as a tendency towards a uniform dark colouration. Mitochondrial sequence analysis of the complete mitochondrial D-loop (753 base pairs) revealed five diagnostic nucleotide substitutions when compared with all Iberian subspecies (*S. s. almanzoris*, *S. s. bejarae*, *S. s. crespoi*, *S. s. fastuosa*, *S. s. gallaica*, *S. s. morenica*, and *S. s. longirostris*). The range of individual colour variation is wide and different colour morphs are typical for the new subspecies. To our present knowledge the geographical distribution of this subspecies is restricted to the Tendi and the Marea valleys in Asturias, Spain.

Key words: Amphibia: Caudata: Salamandridae: *Salamandra salamandra alfredschmidti* ssp. nov., Asturias, Spain, colouration, mitochondrial D-loop.

Introduction

The fire salamander, *Salamandra salamandra* (LINNAEUS, 1758) represents an outstanding and challenging species and subspecies complex with a high degree of geographical variation (EISELT 1958, STEINFARTZ et al. 2000). Within the Iberian Peninsula substantial regional differences in colouration and morphology can be found and are matched by genetic differentiation (JOGER & STEINFARTZ 1994, GARCIA-PARIS et al. 2003). As a result, the subspecific classification of the fire salamander is complex and has been discussed controversially (see overviews in THIESMEIER 2004, THIESMEIER & GROSSENBACHER 2004).

WOLTERSTORFF (1928) reported on fire salamanders from Oviedo (Asturias, Spain) that gave birth to fully developed juveniles, so called "Vollmolch-gebärende Feuersalamander" and described the respective population as a new local subspecies of *S. salamandra*, *Salamandra maculosa taeniata* forma *bernardezi* WOLTERSTORFF, 1928. Later, *S. s. bernardezi* was considered by EISELT (1958), MERTENS & WERMUTH (1960), and KLEWEN (1988) as a synonym of *S. s. fastuosa* SCHREIBER, 1912. At present, however, it is generally accepted that the name *bernardezi* refers to a valid subspecies that is represented by the populations of fire salamanders inhabiting north-eastern Galicia, Asturias and the eastern parts of Cantabria (THIESMEIER & GROSSENBACHER 2004). Spanish herpetologists first reported *S. s. bernardezi* fire salamanders from the Tendi to be quite unique due to their uniform dark colouration that misses the typical dual dorsally banding pattern (VILLANUEVA, 1993; BARRIO & FONOLL, 1997). Subsequent observations by GÜNTHER (1998) and PASMANS & KELLER (2000) underpinned the outstanding colouration of the Tendi population within *S. salamandra*. Furthermore, PASMANS et al. (2004) showed that the

morphometric characteristics of individuals from the Tendi valley matched those of *S. s. bernardezi*.

The main aim of the present study is therefore to investigate the extent of genetic differentiation between the fire salamanders of the Tendi Valley with regard to individuals of *S. s. bernardezi* from the type locality Oviedo and with regard to individuals representing all currently accepted Iberian subspecies of *S. salamandra*. Based on the unique colouration and the clear mitochondrial differentiation of the Tendi and Marea individuals within *S. salamandra* we separate these very locally distributed and therefore potentially endangered population as a new subspecies of *S. salamandra*.

Material and methods

Colour patterns of *S. salamandra* were compared between individuals from the Tendi/ Marea valley and individuals of all recognized Iberian subspecies of *S. salamandra* (see Appendix 1). Further data on total body length and colour patterns were extracted from PASMANS & KELLER (2000) and THIESMEIER & GROSSENBACHER (2004). In order to analyse the genetic differentiation between the Tendi population and all recognized Iberian subspecies, DNA sequences of the complete mitochondrial D-loop were compared between representative individuals. For this purpose, DNA sequences of the D-loop study by STEINFARTZ et al. (2000) of *S. s. longirostris* JOGER & STEINFARTZ 1994 (Sierra de Ronda; type locality), *S. s. morenica* JOGER & STEINFARTZ 1994 (Sierra Morena; type locality), *S. s. crespoi* MALKMUS 1983 (Serra de Monchique; type locality), *S. s. gallaica* SEOANE 1884 (Setubal, Portugal), *S. s. bejarae* WOLTERSTORFF 1934 (near Madrid, Spain), *S. s. fastuosa* SCHREIBER 1912 (from the Pyrenees), *S. s. bernardezi* WOLTERSTORFF 1928 (Oviedo; type locality) and *Salamandra salamandra* (Tendi Valley, Asturias; Genbank accession number DQ232892) were used.

Fig. 1. Holotype of *Salamandra salamandra alfredschmidti* (SMF 83023), SVL 78.0 mm. Bar = 1 cm.

Results

Fire salamanders of the Tendi valley exhibit a wide range of individual variability with regard to their body colouration. Ground colours vary amongst these specimens from dirty yellow through greyish yellow, olive, olive brown, greyish brown, greyish orange to chocolate-brown. A black dorsolateral stripe was absent in all specimens examined by us, but a few specimens showed a black pigmentation of the grooves. A dark brown to brownish black dorsal stripe was, on the other hand, expressed in several specimens, but usually this stripe was not sharply delimited. Such an expression and range of colour variation cannot be observed in fire salaman-

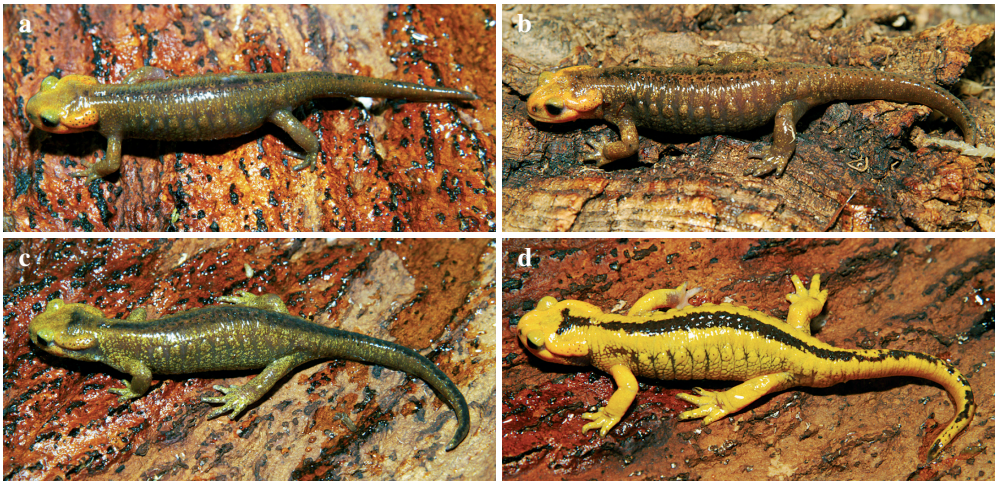

Fig. 2 a-f. *Salamandra salamandra alfredschmidti* (not preserved) from the Río Tendi Valley, Asturias, Spain.

ders from other parts of Asturias, including the Oviedo population (type locality of *Salamandra salamandra bernardezi*).

On the genetic level, the fire salamanders of the Tendi valley clearly differ not only from *S. s. bernardezi,* but also from all other recognized Iberian subspecies. The alignment of homologous DNA base pairs of the mitochondrial D-loop indicates that individuals of the Tendi population can be diagnostically distinguished from all other subspecies of Iberian *S. salamandra* by at least five base pair substitutions (see Appendix 2).

Based on the exclusive morphological as well as on the mitochondrial characteristics of fire salamanders from the Tendi population, we suggest that these locally restricted populations of *S. salamandra* should be considered as a separate subspecies of *S. salamandra*.

***Salamandra salamandra alfredschmidti* ssp. nov. (Figs. 2, 3)**

Salamandra salamandra bernardezi Villanueva 1993 (non Wolterstorff)

Salamandra salamandra cf *bernardezi* Pasmans et al. 2004 (non Wolterstorff)

Holotype: Forschungsinstitut und Naturmuseum Senckenberg (SMF) 83023 (Fig. 1), adult female, collected by M. Kettler 1996 in the Tendi valley, Asturias, Spain.

Paratypes: SMF 84975, Staatliches Naturhistorisches Museum Braunschweig (SNHM) N 39402-1 to -9, ZFMK 84033, all collected by S. Steinfartz and David Vieites (April 2000) at the type locality.

Diagnosis: A dwarfish (on average just over 100 mm in total length; Pasmans & Keller 2000) subspecies of *Salamandra salamandra* that differs from all other subspecies by the absence, or at least a greatly reduced, black dorsolateral stripe and a tendency towards a uniform colouration with no dark spots and stripes. The individual extent of colour variation is far-reaching, with the existence of various colour morphs being a characteristic trait of *S. s. alfredschmidti* whereas such is usually unknown from other subspecies of *Salamandra salamandra*.

Salamandra s. alfredschmidti (Figs. 2, 3) differs from all other subspecies of *Salamandra salamandra* of the Iberian Peninsula by the following traits (information on *S. s. alfredschmidti* in parentheses): *Salamandra*

s. almanzoris: a dorsal pattern of numerous small spots with black portions dominating (no such spots, at maximum some indistinct yellow speckling on a variety of ground colours, but never with black portions dominating); *Salamandra s. bejarae*: a dorsal pattern of hook- or horseshoe-shaped spots with black portions dominating (no such spots, at maximum some indistinct yellow speckling on a variety of ground colours, but never with black portions dominating); *Salamandra s. bernardezi* (Oviedo and vicinity; see also Fig. 4) and *S. s. fastuosa*: yellow ground colour with distinct, sharply delimited dorsal and dorsolateral stripes (ground colour ranging from dirty yellow through greyish yellow, olive, olive brown, greyish brown, greyish orange to chocolate-brown; dorsolateral stripes absent or substantially reduced; brownish black dorsal stripe, if at all present, obscurely delimited); *Salamandra s. crespoi*: dorsal pattern of numerous small spots with black portions dominating (no such spots, at maximum some indistinct yellow speckling on a variety of ground colours, but never with black portions dominating); large, 188 mm in total length on average (small, with just over 100 mm in total length on average); conspicuously long, flat head (head not conspicuously long and flat); *Salamandra s. gallaica*: a dorsal pattern of hook-, horseshoe-, ring-shaped or ocellate spots, rarely striped; the yellow spots often with red centres (no such spotted pattern, at maximum some indistinct yellow speckling on a variety of ground colours); robust build and large (delicately build and relatively small); *Salamandra s. longirostris*: a dorsal pattern of large, lemon-yellow spots (no such spotted pattern, at maximum some indistinct yellow speckling on a variety of ground colours); large, with 186 mm in total length on average (small, with just over 100 mm in total length on average); *Salamandra s. morenica*: a dorsal pattern of numerous small spots with black portions dominating (no such spots, at maximum some indistinct yellow speckling on a variety of ground colours, but never with black portions dominating); large, with 195 mm in total length on average (small, with just over 100 mm in total length on average).

Description of the holotype: An adult female (Fig. 1; according to information received from the collector, the specimen had given birth to thirteen fully developed baby salamanders shortly after capture) with the following measurements and proportions: snout-vent length (SVL) 78.0 mm; tail length (TL): 56.0 mm; TL/SVL 0.72; axillar-inguinal distance (AI): 35.3 mm; AI/SVL 0.453; length of front leg (FL): 20.1 mm; FL/SVL: 0.258; length of hindleg (HL): 23.7 mm; HL/SVL: 0.304; length of head (LH): 13.0 mm; LH/SVL: 0.167; width of head (WH): 13.9 mm; WH/LH: 1.069; height of head (HH): 6.1 mm; HH/LH: 0.469; distance between anterior margin of eye and nostril (EN): 3.0; EN/LH: 0.231; internasal distance (IN): 4.5 mm; IN/LH: 0.346; interorbital distance (IO): 6.0 mm; IO/LH: 0.462.

Build rather delicate; head rounded, relatively compressed; eyes protruding, with round pupils and black irises; parotoid glands prominent, 10.1 mm in length and a maximum width of 4.1 mm, with distinctly visible, black glandular openings; neck not distinctly set off; gular cross-fold distinct; body roundish in cross section with a flattened bottom; nine distinct costal grooves, additional small grooves create an irregular reticulated pattern that is particularly distinct on the sides of the body; a row of glands extends on either side of the vertebral line ranging from the parotoid glands to the base of the tail; front and hindlegs sturdy, with four fingers on the front legs and five toes on the hindlegs; relative lengths of fingers 3>2>4>1; relative lengths of toes 3>4>2>5>1.

Ground colouration of the dorsal and lateral surfaces of head, body and limbs yellowish olive; an obscurely delimited, black dorsal stripe extends from the occiput to pelvic girdle and continues at a slightly narrower width to the tail tip; besides a cloudy black spot in the region of the shoulder

blades a diffuse black pigmentation is present beneath the parotoid glands and in the grooves; ventral side brownish olive (body and limbs), respectively yellowish olive (head and tail).

Variation: A detailed analysis of the morphological variation of *Salamandra s. alfredschmidti* from the Tendi Valley can be found in PASMANS & KELLER (2000). Following their study, the salamanders of the Tendi Valley have total lengths of 103 ± 11 mm (males), and 101 ± 15 mm (females), respectively, with masses of 4.7 ± 1.4 g (males), and 5.7 ± 2.4 g (females), respectively. The ground colouration varied from dirty to orange yellow (40% of the specimens), through brownish with yellow speckling (34%), chocolate-brown (13%), to greyish brown (13%). Black dorsolateral stripes were present in only one adult male out of 67 adult males examined and in none of the 51 females, whereas several juveniles had dorsolateral stripes (PASMANS & KELLER 2000).

Derivatio nominis: The academic career of both authors was substantially influenced by the Deutsche Gesellschaft für Herpetologie und Terrarienkunde (DGHT) wich unites several generations of amateur and professional herpetologists. It is a great pleasure for us to dedicate this new subspecies of *S. salamandra* to our friend, the honorary member of the DGHT, ALFRED A. SCHMIDT, in recognition of his achievements in furthering herpetology and captive breeding of amphibians with a special emphasis on salamanders and newts.

Distribution: *Salamandra s. alfredschmidti* is restricted to the Tendi and Marea valley in Asturias, Spain.

Discussion

The Iberian Peninsula has always been a hotspot of diversity of the fire salamander. This is reflected by a high number of eight distinct subspecies (THIESMEIER & GROSSENBACHER 2004) and a strong local differentiation of populations on the morphological and mitochondrial level (GARCIA-PARIS et al. 2003, MARTINEZ-SOLANO et al. 2005). Like all populations from the north-west of Spain, the fire salamanders of the Tendi valley have so far been taxonomically assigned to *Salamandra s. bernardezi*, which was originally described from Oviedo by WOLTERSTORFF in 1928. With regard to their colour pattern, the fire salamanders of the Tendi and adjacent Marea valleys (comp. Fig. 2) are special in their position not only amongst the fire salamanders of the Iberian Peninsula, but also within the whole *S. salamandra* species complex. Their distinct genetic differentiation from *S. s. bernardezi* from the type locality furthermore suggests that the nominal taxon *S. s. bernardezi* in fact comprises a genetic and morphological diversity of the fire salamander in northern Spain that has been so far underestimated. Although this genetic differentiation has so far been demonstrated mainly for mitochondrial genes that do not directly allow conclusions on possible gene flow, these differences clearly allow to differentiate fire salamanders from the Tendi valley from all remaining subspecies of *S. salamandra*. In our view, these molecular characters satisfy a taxonomic subdivision of the *S. s. bernradezi*-complex.

The recognition of diversity and their taxonomic "translation" is of broad scientific interest, especially if we consider that the conservation of populations is strongly influenced by the current taxonomy. For European amphibians, the Tendi and Marea populations of the fire salamander are a nice example for the manifestation of genetic and morphological local differentiation. The description of this subspecies represents a necessary taxonomical consequence of recognized biological diversity on different levels. It is interesting to note that the populations of the neighbouring Tendi and Marea valleys show the "normal" *bernardezi* colour pattern (GÜNTHER 1998). With the help of newly developed microsatellite loci for the fire salamander (STEINFARTZ et al. 2004) it

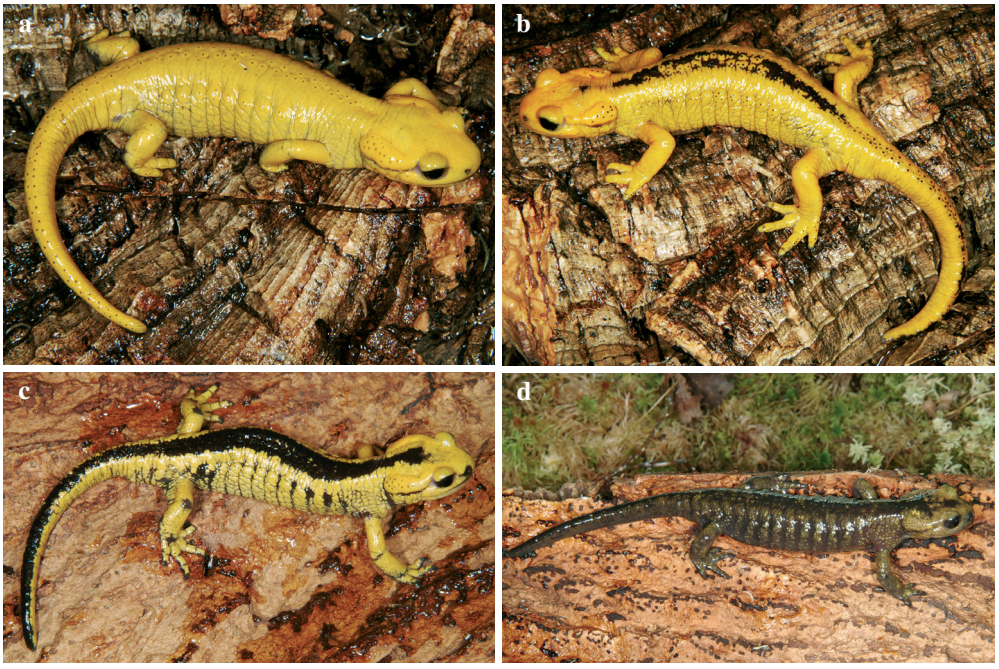

Fig. 3 a-d. *Salamandra salamandra alfredschmidti* (not preserved) from the Río Marea Valley, Asturias, Spain.

Fig. 4 a-b. *Salamandra salamandra bernardezi* (not preserved) from Oviedo, Asturias, Spain.

will be possible to estimate the degree of nuclear gene flow between these populations and the Tendi/Marea populations. Such investigations will help us to understand the phenomenon of local differentiation in the fire salamander.

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sches Museum Braunschweig, loaned us material from the collection in his custody.

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Appendix 1

Salamander specimens examined (SMF = Forschungsinstitut und Naturmuseum Senckenberg)

Salamandra salamandra almanzoris: Spain: Laguna Grande de Gredos, Sierra de Gredos, 2027 m: SMF 45423, 49730-36, 51765, 54121, 55011, 71015, 73397; Cinco Lagunas, Sierra de Gredos: SMF 51766-74, 54491-95, 54756. *Salamandra salamandra bejarae*: Spain: Lagunilla at Bejar, prov. Salamanca: SMF 29387 (neotype); Andújar, Sierra Morena, prov. De Jaén: SMF 35737-38, 37125-26; Linares de Ríofrio: SMF 40066; Baños de Benasque, prov. Huesca, 1600-1700 m: SMF 137; bog Rossinyol, San Miguel del Fay, prov. Barcelona, 550 m: SMF 138-141; between Peralba and Figuerola, Montsech de Rubris, prov. Lérida: SMF 142; Barcelona: SMF 21321-23; Montseny, prov. Barcelona: SMF 21754. *Salamandra salamandra bernardezi*: Spain: Oviedo: SMF 29390 (neotype), 29388-89, 70875-76, 71987, 73961, 83003; Ribadesella: SMF 83101-02; Barruelo de Santullan, Picos de Europa (Palencia): SMF 68919; Picos de Europa (western slopes), above Covadonga, Asturias, 1600 m: SMF 29391; Lugo: SMF 71277-78. *Salamandra salamandra crespoi*: Portugal: Serra do Caldeirão, Algarve: SMF 82938; Bordeira, Alfambras: SMF 82949. *Salamandra salamandra gallaica*: Portugal: Corni´a: SMF 164; Gandara de Espariz, western edge of the Serra de Estela, road Coimbra-Guarda, prov. Beira Alta:

SMF 67868-70; Serra de Sintra at Lisbon: SMF 70877, 71296-97; Serra de Sintra, west of Lisbon, Estremadura: SMF 77643-46, 78483-84; north and east of Marvão, east of Castelo de Vide, district Portalegre: SMF 81694-97; east of São Luís, Monte da Estrada-Lameiro, district Beja: SMF 81699; south-east of Vila Vicosa, west of Mina do Bugalho: district Évora: SMF 81700; north-west of Évora, south of Valeira, district Évora: SMF 82950. Spain: Galicia: SMF 70968.

	11111111122222333455566 77
	22359055555668456880142355 4523
	47016213489467435390410934 5685
S.s.longirostris	GTTAAACCCAGCCAAAATTGCGCCTGATTT
S.s.morenica	A.....A.....T..T..A
S.s.crespoi	A.....GG.A.A...T..T..A
S.s.bernadezi	AC.G.....GAT.....AA..A.T.T.C.A
S.s.alfredschmidti	A..G..TTTGATT..GTAA..AGTCT...A
S.s.alfredschmidti	A..G..TTTGATA..G.AA..AGTCT...A
S.s.alfredschmidti	A..G..TTTGATA..G.AA..AGTCT...A
S.s.gallaica	A..G.....G...AAA...T.T...A
S.s.almanzoris	...G.....G...AAA...T....CC
S.s.bejarae	A..GGG.....G...AAA...T.T..CC
S.s.fastuosa	A.CG.....G...AAAT..T.TT..A

Appendix 2

Alignment of the variable nucleotide positions of the complete mitochondrial D-loop (753 base pairs, after exclusion of insertions and deletions) for all Iberian subspecies of the fire salamander and the new subspecies *Salamandra s. alfredschmidti* from the Tendi valley. Dots in the alignment indicate an identical character condition of a homologous base pair position. Grey print highlights nucleotide positions of *S. s. alfredschmidti* that are diagnostic in comparison with all other Iberian subspecies of the fire salamander. Nucleotide positions that refer to the respective alignment of the listed subspecies are printed in bold typeface for the respective position in their vertical alignment; for example, the first variable base pair of the alignment is position 24 and the last variable base pair of the alignment is position 735.

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Authors' addresses: GUNTHER KÖHLER, Forschungsinstitut und Naturmuseum Senckenberg, Senckenberganlage 25, D-60325 Frankfurt am Main, Germany; E-Mail: gkoehler@senckenberg.de; SEBASTIAN STEINFARTZ, Universitaet Bielefeld, Lehrstuhl für Verhaltensforschung, Morgenbreede 45, D-33615 Bielefeld, Germany; E-Mail: sebastian.steinfartz@uni-bielefeld.de.