

Geographical variation in the golden-striped salamander, *Chioglossa lusitanica* Bocage, 1864 and the description of a newly recognized subspecies

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Abstract

Morphological and genetic data for the Iberian golden-striped salamander, *Chioglossa lusitanica*, demonstrate the existence of two groups with southern and northern ranges, connected by a zone of intergradation in central Portugal. Because reproductive isolation between them is incomplete we consider the groups to be subspecies. The type locality of *C. lusitanica* (Buçaco near Lousã) is situated inside the mixed zone. This necessitates identification of the nominotypical subspecies. We sequenced a fragment of mitochondrial DNA from one of the species' syntypes and we determined what position over a latitudinal transect maximizes the morphological discrimination between the groups. Both approaches indicate that *C. lusitanica* from Buçaco represents the southern subspecies. A new subspecies of *C. lusitanica* is described from a northern locality (Valongo near Porto in north-western Portugal). A lectotype is designated for *Chioglossa lusitanica*.

Keywords: Ancient DNA, *Chioglossa lusitanica*, cytochrome-*b*, geographic variation, morphometrics, new subspecies, Portugal, salamander, Urodela

Introduction

The golden-striped salamander, *Chioglossa lusitanica* Bocage, 1864, occurs along mountain brooks of low and medium high altitudes in areas with high precipitation in the north-western corner of the Iberian Peninsula. *Chioglossa lusitanica* is the single extant representative of the genus *Chioglossa* that forms a monophyletic group with *Mertensiella*, another monotypical genus for *M. caucasica* (Waga, 1876) from the Caucasus. *Chioglossa lusitanica* and *M. caucasica* are streamside salamanders characterized by a slender build with

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short appendages and a long tail. They belong to the ‘true’ salamanders within the family Salamandridae together with the sister group composed of the more stoutly built and less aquatic species in the genera *Salamandra* and *Lyciasalamandra* (Veith et al. 1998; Sequeira et al. 2006; Steinfartz et al. 2007; Weisrock et al. 2006).

Studies on the ecology and natural history of *C. lusitanica* (Arntzen 1981; Vences 1990) were followed by the mapping and modelling of its distribution (Teixeira et al. 2001; Arntzen 2006; Arntzen & Teixeira 2006) and by studies on intraspecific variation of morphological and genetic characters (Alexandrino et al. 2005, 2007; Sequeira et al. 2005). This revealed the presence of two different forms of *C. lusitanica* that are roughly distributed to the south and the north of the river Mondego in central Portugal, respectively. At the enzyme locus *Pgm-1* different alleles predominate south and north of the river whereas the loci *Adh*, *Pep-C* and *Pep-D* show a full allelic transition at ca 10 km south of the Mondego (Alexandrino et al. 2000; Sequeira et al. 2005). Mitochondrial DNA (mtDNA) haplotypes show a full transition at ca 10 km north of the Mondego (Sequeira et al. 2005). The colour pattern of the salamander shows the tendency of a clinal variation, with the dorsal golden-striped aspect increasing from south to north. The variability in colour pattern is, however, higher in the Mondego area than at either side of the intergradation area, which has been associated with the merger of differentiated genotypes, i.e. intraspecific hybridization (Alexandrino et al. 2005, 2007). A morphometric transition takes the form of a stepped cline, with a complex pattern of variation around the western Mondego area (Alexandrino et al. 2005, 2007). Finally, an ecological analysis suggests slightly different habitat characteristics for the two forms, with a harder regime of lower annual temperature and lower humidity for the more mountainous southern form (Arntzen & Alexandrino 2004). Ecogeographical modelling indicates that the transition between the ecological regimes coincides with the river Mondego in western Portugal and is located ca 25 km north of the Mondego in central Portugal.

The data summary in Table I supports the recognition of a northern and a southern group of *C. lusitanica*. The substantial width of the intergradation zone for multiple

Table I. Discriminating character states in southern versus northern populations of *Chioglossa lusitanica* and the corresponding allocation of populations along a south to north axis.

Characters	Predominant character- state, or group		Population ^a								References ^b	
	South	North	1–5 (south of Mondego)	6 7 ^c 8 9 10 11						12–20 (north of Douro)		
Nuclear enzyme loci												
<i>Adh</i>	Allele 2	Allele 1	S	N	N	N	N	N	N	N	N	1, 2
<i>Pep-C</i>	Allele 2	Allele 1	S	N	N	N	N	N	N	N	N	1, 2
<i>Pep-D</i>	Allele 2	Allele 1	S	N	N	N	N	N	N	N	N	1, 2
<i>Pgm-1</i>	Allele 3F	Allele 1F	S	N	N	N	N	N	N	N	N	1, 2
Mitochondrial DNA												
Cytochrome-b	Clade 3-1	Clade 3-2	S	S	m	N	N	N	N	N	N	1, 3
Morphology												
Classification	Southern	Northern	S	S	S	a	N	N	N	N	N	4
Ecology												
Conditions	Harsh	Mild	S	S	a	N	N	N	N	N	N	5

^aS, southern; N, northern; a, ambiguous; m, mixed. ^b1, Alexandrino et al. (2000); 2, Sequeira et al. (2005); 3, Alexandrino et al. (2002); 4, present study; 5, Arntzen and Alexandrino (2004). ^cType locality of *Chioglossa lusitanica*.

character states and the absence of syntopic populations suggest that the forms are not, or not strongly, genetically isolated from one another. We, therefore, consider these groups not to be separate species, but subspecies of *C. lusitanica*. Before we can proceed with the formal description of the newly recognized subspecies the following has to be ascertained: (1) what is the type locality of *C. lusitanica* and (2) does the type material represent the northern or the southern subspecies? For a start, uncertainty exists about what constitutes the official description of *C. lusitanica*, because Bocage published two descriptions, both in 1864. As pointed out by Arntzen (1999), the description in the *Revue et Magazin de Zoologie Pure et Appliqué* (Bocage 1864a) has priority over that in the *Proceedings of the Zoological Society, London* (Bocage 1864b). Bocage's material was sent to him by a Mr Rosa in two shipments from: (1) the vicinity of Coimbra 'de Coimbra en mai de l'année dernière [1863] ... Ils ont été rencontrés aux environs de cette ville' and (2) from Buçaco 'dans les derniers jours de janvier [1864], sur la montagne du Bussaco [Buçaco], à 5 lieues de Coimbra'. So, whereas the origin of the second lot is known (namely Buçaco), the precise origin of the first shipment is uncertain and depends on what Bocage meant with 'the vicinity of Coimbra'. The known type material is limited to two adult syntypes that are kept at the Museum national d'Histoire naturelle, Paris (MNHNP 1182 and MNHNP 1182A). This material was in the first shipment and we follow Thireau (1986) in assuming that it originates from Buçaco that is, hence, the type locality.

By coincidence, Buçaco is located in the area where diagnostic character states provide contrasting signatures. In other words, whether the Buçaco population represents the southern or the northern subspecies depends on the data considered (Table I). Prior to the subspecies description by J.W.A. and J.A. in Appendices 1 and 2, we set out to answer the following questions: (1) where is the border between the subspecies located as seen from morphology, and (2) what are the morphological and molecular characteristics of the type material?

Materials and methods

Three hundred and fifty-eight adult *C. lusitanica* (183 males and 175 females) from 16 localities covering the entire range of the species were measured alive and released in the locality where they were found (Figure 1; Alexandrino et al. 2005). We also studied the two syntypes from the Museum national d'Histoire naturelle, Paris (MNHNP 1182 is a female and MNHNP 1182A is a male) and 54 ethanol-preserved specimens from Valongo near Porto in northwestern Portugal (localities 12 and 13; 30 males and 24 females). This material was available from the zoological museums in Amsterdam, Leiden, Lisbon and Madrid. The measurements taken were: snout-vent length (SVL), head length (HL), head width (HW), forelimb length (FLL), hind limb length (HLL), third finger length (TFL) and fourth toe length (FTL; see Alexandrino et al. 2005). The data were ln-transformed to increase statistical normality and to reduce distortion effects from allometric relationships. In a further treatment, the standardized residuals were calculated for the regression of ln<character> on ln<SVL> to reduce the impact of overall size in the analysis. Significant morphological differentiation between the sexes has already been demonstrated (Alexandrino et al. 2005) and because we are primarily interested in the spatial pattern of morphological variation (and not sexual dimorphism), data for males and females were analysed separately. Salamanders from Valongo that at time of measuring were alive (group a, $n=54$) or ethanol-preserved (group b, $n=54$), displayed significant differences between groups for ln-transformed data and not for size-adjusted data (results not shown).

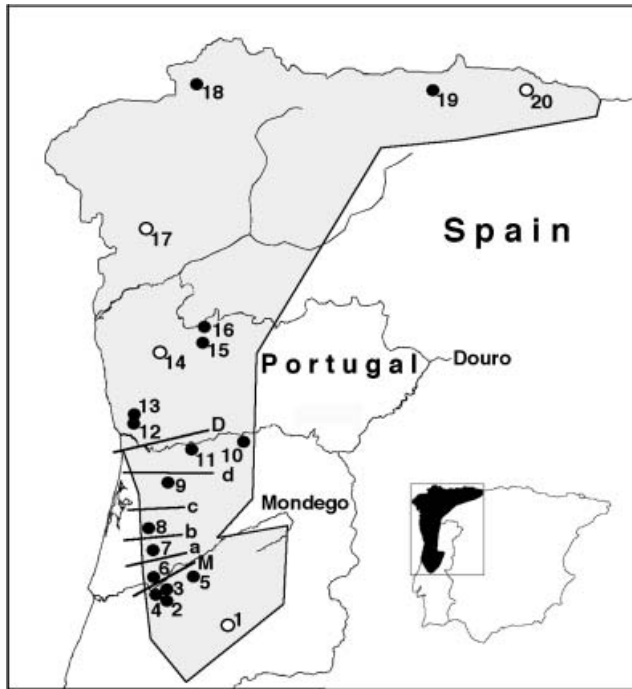


Figure 1. Approximate range of the golden-striped salamander, *Chioglossa lusitanica*, in the Iberian Peninsula (insert) with 16 populations that were subject to morphometric analysis (solid dots). Numbers 5–13 and letters a–d, D and M refer to putative subspecies borders that were designed to evaluate the congruence of range and subspecies morphology. Four populations with just molecular genetic data available are shown by open dots. Localities are as follows: 1, Muradal, Foz de Giraldo—40°0'23.6"N, 7°41'49.8"W; 2, Lousã, C. Pêra—40°5'28.7"N, 8°12'4.1"W; 3, Lousã, Fiscal—40°6'50.4"N, 8°13'27.6"W; 4, Lousã, Vilarinho—40°7'9.5"N, 8°12'32.5"W; 5, Açor, Margarça—40°13'14.0"N, 7°55'7.4"W; 6, Várzeas—40°14'53.7"N, 8°22'32.4"W; 7, Buçaco—40°21'51.6"N, 8°21'7.9"W; 8, Saide—40°26'46.2"N, 8°19'27.5"W; 9, Covelo—40°46'37.1"N, 8°12'47.3"W; 10, Tarouca—41°1'38.3"N, 7°47'12.1"W; 11, Montemuro—41°2'33.1"N, 8°3'57.6"W; 12, Valongo, Silveirinha—41°10'45.9"N, 8°29'57.3"W; 13, Valongo, Águas Férreas—41°11'18.3"N, 8°29'18.4"W; 14, Bom Jesus—41°33'25.7"N, 8°22'26.5"W; 15, Cabreira—41°39'39.6"N, 8°1'24.6"W; 16, Gerês—41°45'26.8"N, 8°8'45.4"W; 17, Pontevedra—42°30'5.7"N, 8°28'53.5"W; 18, Caaveiro—43°26'3.9"N, 8°2'54.9"W; 19, Salas—43°23'40.7"N, 6°15'22.8"W; 20, Cuera—43°17'45.7"N, 4°35'56.9"W.

Preserved specimens were therefore only included in the analysis of size-adjusted morphometric data. Statistical analyses were carried out with SPSS 14 (SPSS 2005).

To investigate whether the northern or the southern subspecies of *C. lusitanica* would incorporate the type locality we used three approaches. First, we investigated the syntype MNHNP 1182A for subspecies-specific character states at the mitochondrial cytochrome-b gene. A tiny tissue fragment was taken from the already broken tail of this syntype, with permission of the curator. The molecular work was carried out in a laboratory that is dedicated to the analysis of 'ancient' DNA (Leiden aDNA Facility, The Netherlands). Two primer pairs were developed that each would amplify short sections of the cytochrome-b gene with, respectively, three and two diagnostic characters discerning between the southern and the northern haplotypes (Alexandrino et al. 2002). Primers CB159_F (5'-AACCCCTCAAACATCTCATATT-3') and CB159_R (5'-TAATTAACGTCTCGGCAGATATGG-3') amplify a 159 bp fragment that starts at position 9 of known sequences (GenBank accession numbers AF329285–AF329314;

Alexandrino et al. 2002). Primers CB220_F (5'-CCACCGTAATCACAAATCTCCT-3') and CB220_R (5'-TCTGGGTTAGAGGGTAGTCCAG-3') amplify a 220 bp fragment, starting at position 371. The PCR reactions were done twice, in laboratories that are physically separated from one another as well as from the ancient DNA facility. None of these laboratories had previously seen work on *C. lusitanica* DNA.

The tissue was cut as small as possible to enlarge the tissue-solution contact surface. DNA was extracted using the DNeasy[®] Tissue Kit (Qiagen). Tissue incubation time was 14 h in buffer ATL and 20 µl of proteinase K were added twice (once at the start and once after 4 h of incubation). DNA was eluted in 40 µl of buffer AE. PCR reactions were done using a standard Taq DNA polymerase kit from Qiagen. Reaction volume was 25 µl and PCR conditions were 0.2 mM dNTPs, 0.4 µM of each primer, 5 U of Taq DNA polymerase (Qiagen) and no additional MgCl₂ (1.5 mM MgCl₂ is present in PCR buffer). Thermocycling conditions were 3 min at 94°C for initial denaturation followed by 40 cycles (15 s at 94°C, 30 s at 50°C, and 40 s at 72°C) and final extension 5 min at 72°C. PCR products were cleaned using a Nucleospin[®] kit (Macherey-Nagel) and cleaned products were cloned using the Topo TA Cloning[®] system (Invitrogen). White colonies were picked and used to initiate reamplifications with primers 21M13_F 5'-TGTAACGACGGCCAGT-3' and 21M13_R 5'-CAGGAAACAGCTATGACC-3'.

PCR, thermocycling and product cleaning were as described above, except that the annealing temperature was raised to 57°C. Sequencing was performed on a Megabace[™] 1000 DNA Analysis System (Amersham).

Secondly, we addressed the question of what position of the subspecies border, from a morphological perspective, would yield the most convincing classification between the northern and the southern subspecies. We varied the position of the putative range border across central and northern Portugal from positions 5, M (=Mondego river), 6, a, 7, b, 8, c, 9, d, 10, 11, D (=river Douro), 12 and 13 (see Figure 1) and subjected the data with these a priori defined groups to discriminant analysis. If population localities were taken as the subspecies border, local individuals were excluded and subjected to an a posteriori classification. Following this approach we determined what subspecies the two syntype specimens would represent. The congruence among the morphology- and geography-based classifications was expressed by kappa (κ ; Cohen 1960) and its asymptotic standard error. All analyses and calculations were performed in SPSS. Following Altman (1991), the strength of agreement is classified as "fair" for $0.2 < \kappa < 0.4$, "moderate" for $0.4 < \kappa < 0.6$, and "good" for $0.6 < \kappa < 0.8$.

Results

The 159 bp fragment of the cytochrome-b gene was successfully amplified while amplification of the 220 bp fragment failed, possibly due to DNA degradation. Two subsamples of the 159 bp fragment were sequenced in both directions and three subsamples were sequenced using only primer 21M13_F (GenBank EF122233–EF122237). Unexpected character states found are a T for a C at position 71 in one clone (GenBank EF122236) and a C for a T at position 124 in another (GenBank EF122235), possibly due to postmortem damage (Lindahl 1993; Hofreiter et al. 2001; Gilbert et al. 2003; Stiller et al. 2006). Both substitutions would in vivo represent amino acid replacements. Character states at the subspecies diagnostic positions 54, 102, and 114 are T, A, and T, respectively. The mtDNA of the type specimen MNHNP 1182A is the one otherwise found over the southern part of the *C. lusitanica* range.

The raw morphometric data are summarized in Appendix 2 and in Alexandrino et al. (2005). For males the congruence between the morphometry- and geography-based subspecies classifications is mostly moderate ($0.4 < \kappa < 0.6$; Figure 2). A bell-shaped curve is obtained with lower values at the fringes than in the middle. In females the congruence is consistently higher than for males and the bell-shaped aspect of the graph is less prominent. To investigate the possibility that the bell shapes would represent a mid-domain effect operating through unbalanced sample sizes, we repeated the analysis with a weighing scheme in place, such that sample sizes for the northern and southern groups were effectively equal. Results obtained were similar to those in Figure 2. Considering both sexes together κ is highest if locality 8 is taken as the subspecies border.

The male and female syntypes had a posteriori probability for representing the southern subspecies of 0.95 and 0.36, respectively (Figure 3).

Discussion

The level of genetic differentiation at enzyme and mtDNA loci, combined with the observed mtDNA phylogeographic pattern over the species range, support the largely independent evolution of two forms of *Chioglossa lusitanica*. Average genetic distance between the northern and the southern form is $D_{Nci} = 0.15$, measured over a set of 22 enzyme electrophoretic loci. The average cytochrome-b sequence divergence is $d = 1.4\%$. Following a 'molecular clock' argument these data place the onset of the divergence at 1.5–3 million years (Alexandrino et al. 2000, 2002). Despite past geographical isolation—probably lasting throughout most of the Pleistocene—and a full transition over four enzyme genetic markers (Table I), *C. lusitanica* populations show amixture within the contact zone. This suggests (1) the absence of genetic barriers to neutral diffusion of alleles across the zone; (2) the absence of strong selection against hybrid genotypes (Sequeira et al. 2005); and (3) that the genetic cohesiveness between the two forms of *C. lusitanica* is maintained

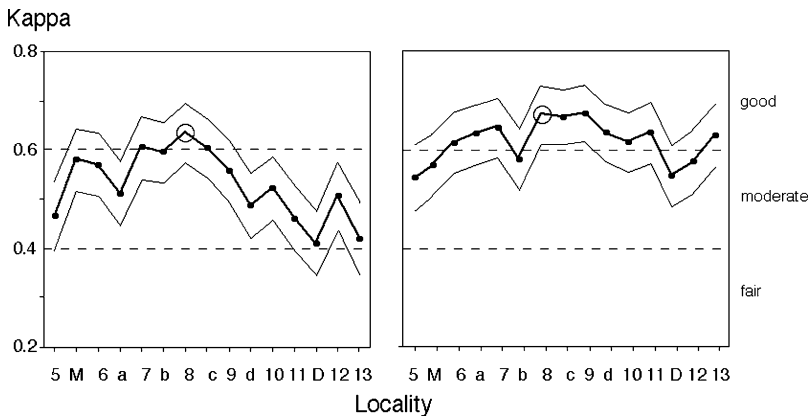


Figure 2. Congruence between morphometry- and geography-based classifications of the southern and northern subspecies of *Chioglossa lusitanica* across a latitudinal transect in central and northern Portugal, for males (left) and females (right). Numbers 5–13 and letters a–d, M and D refer to putative subspecies borders (see Figure 1). Congruence is expressed by kappa (κ) \pm asymptotic standard error. Following Altman (1991) κ -scores of < 0.4 indicate a 'fair' congruence between classifications; with $0.4 < \kappa < 0.6$ congruence is 'moderate' and with $\kappa > 0.6$ congruence is 'good'. The most convincing discrimination from morphology is achieved when the border between the southern and the northern population is set at population 8 (open round symbol).

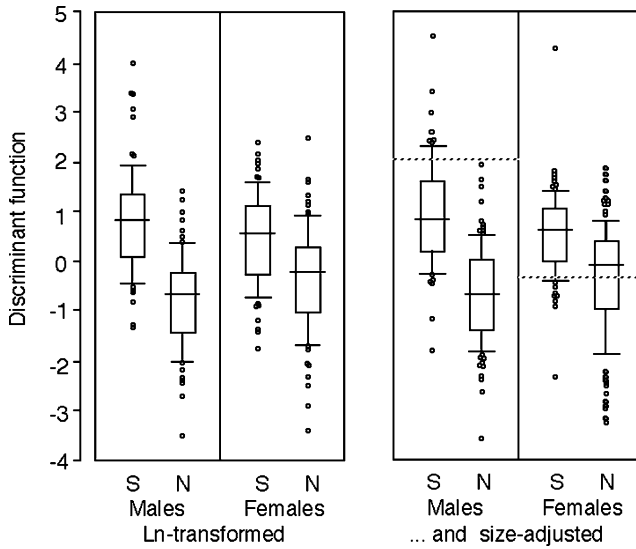


Figure 3. Discriminant function scores for the southern (S) and northern (N) subspecies in males and females of *Chioglossa lusitanica* with the range border at locality 8. The boxes and bars represent 50% and 80% of the data, respectively, and round symbols are outliers, for ln-transformed (left panel) and ln-transformed, size-adjusted data (right panel). Interrupted lines represent the discriminant functions for the two *C. lusitanica* syntypes.

by population-level processes associated with restricted gene flow across the Mondego river valley.

The moderate level of genetic differentiation and the lack of genetic isolation justify the recognition of the northern and the southern form of *C. lusitanica* at the level of subspecies. The population of Buçaco is located within the hybrid zone, right in the middle of a steep mtDNA transition and somewhere near the centre of smooth morphological and ecological transitions. We therefore consider the Buçaco population of mixed origin, even though diagnostic enzyme loci would classify it unambiguously with the northern subspecies. The mtDNA sequence data show that the syntype MNHNP 1182A from Buçaco has the character states that are found in—and hence represents—the southern form. The morphological analysis also indicates that *C. lusitanica* from Buçaco represents the southern form. Consequently, the new taxon will be a subspecies for which the name-bearing type should be sought in the northern part of the species range and preferably well away from the zone of subspecific intergradation. The formal description of this taxon with type locality Valongo near Porto in northwestern Portugal is presented in Appendices 1 and 2. The nominotypical subspecies (with the trinomial '*lusitanica*') will hence have its range within the boundaries of historical Lusitania, i.e. the province of the Roman empire with that name, whereas the new subspecies with a range over northern Portugal, Galicia and Asturias, and largely outside Lusitania, will carry a different trinomial.

It is fortunate that the one syntype for which we obtained permission to use a tiny fragment of tissue for molecular genetic analysis—the one with a tail already broken—was a male, because morphometric discrimination of the subspecies is more apparent in males than in females (Figure 2). This is reflected in the more clear-cut discriminant scores at the population level and at the level of the individual syntypes also (Figure 3). It must be noted that the northern and southern haplotypes occur in approximately the same frequency in the Buçaco population (Alexandrino et al. 2002). It is therefore not inconceivable that the

syntype that we did not study for its mtDNA carries not the southern but the northern haplotype. In order to prevent future destabilization of the nomenclature, we designate syntype MNHNP 1182A as lectotype and MNHNP 1182 as paralectotype of *C. lusitanica*.

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Appendices by J.W. Arntzen and J. Alexandrino.

Appendix 1. Description of a new subspecies of *Chioglossa lusitanica* Bocage, 1864 *Chioglossa lusitanica* Bocage, 1864

Material

MNHNP 1182A is designated lectotype and MNHNP 1182 is designated paralectotype. For morphometric data see Appendix 2.

Type locality

Buçaco, Beira Litoral, Portugal, by implication (see Thireau 1986).

Species description

Head depressed, longer than wide with a rounded snout and with large protruding eyes. Paratoids small. The body and the tail are almost cylindrical. In adults the tail is approximately 1.9–2.0 times the length of the body (modal value; maximum observed value is 2.5). Towards its posterior part the tail becomes progressively laterally compressed. The sides of the body bear a series of, usually 11, costal grooves. The sides of the tail are likewise marked with grooves that are smaller and shallower. The feet are small but well developed, with four toes at the slender forefeet and five toes at the more robust hindfeet. The inner fingers and toes are very small. The skin is smooth and a skin fold is found across the throat. The basic colour of the salamander is black. On the back the salamander normally has two broad copper-coloured bands that join over the groin and continue as a single band over the tail. The copper coloration fades towards the tip of the snout and the tip of the tail. The dark areas of the body and the tail are star-spangled with many minute azure dots. Altogether, the salamander has an almost metallic appearance. Due to its elongated shape

this salamander appears smaller than stouter species of similar size as for instance *Salamandra salamandra* (Linnaeus, 1758).

***Chioglossa lusitanica longipes* subsp. nov.**

Material

Holotype: adult male from Valongo, Douro Litoral in northwestern Portugal, leg. F. Sequeira and J. W. Arntzen, 18 May 2006, RMNH R and A 40113. Locality specification: Mina das Águas Férreas, Serra de Santa Justa, Valongo, 41°11'18.3"N, 8°29'18.4"W (locality 13 in Figure 1) at 120 m above sea level. Paratypes: 61 specimens. MB-06-450-01–MB-06-450-04 from type locality, Mina das Águas Férreas, Serra de Santa Justa, Valongo, leg. F. Sequeira and J. W. Arntzen, 18 May 2006; MNCN 43641–43644 from type locality, Mina das Águas Férreas, Serra de Santa Justa, Valongo, leg. F. Sequeira and J. W. Arntzen, 18 May 2006; RMNH R and A 18796–18805 from type locality, Ribeira da Silveirinha, Serra de Santa Justa, Valongo, 41°10'45.9"N, 8°29'57.3"W (locality 12 in Figure 1) at 220 m above sea level, leg. J. W. Arntzen, 22 October 1978; RMNH R and A 40114–40116 from type locality, Mina das Águas Férreas, Valongo, leg. F. Sequeira and J. W. Arntzen, 18 May 2006; ZMA 7307a–e, from type locality, leg. J. W. Arntzen, 8 October 1978; ZMA 7340a–j, from type locality, Ribeira da Silveirinha, Serra de Santa Justa, Valongo, leg. J. W. Arntzen, 22 October 1978; ZMA 7358a–d (two metamorphs and two juveniles), from type locality, leg. J. W. Arntzen, October 1978; ZMA 7387a–d (one sub-adult and three juveniles), from type locality, leg. J. W. Arntzen and A. G. M. Gerats, autumn 1977; ZMA 7402, from type locality, leg. J. W. Arntzen and A. G. M. Gerats, 6 April 1977; ZMA 7482, from type locality, leg. J. W. Arntzen, 15 October 1981; ZMA 7588a–c, from type locality, leg. J. W. Arntzen, 18 September 1983; ZMA 7669, from type locality, leg. G. Veenstra, 26 August 1984; ZMA 8086a–k from type locality, Ribeira da Silveirinha, Serra de Santa Justa, Valongo, leg. J. W. Arntzen, 22 October 1978.

Description of holotype

Adult male of 146 mm total length, SVL 44.9 mm, HL 10.4 mm, HW 7.4 mm, FLL 11.1 mm, HLL 13.1 mm, TFL 2.2 mm, and FTL 3.4 mm.

Description of paratypes

For morphometric data and remarks see Appendix 2.

Differential diagnosis

Subspecies with longer arms and fingers, legs and toes than the nominotypical subspecies, stronger so in the northern than in the southern part of the range. With diagnostic allelic profiles at the enzyme loci *Adh*, *Pgm-1*, *Pep-C*, and *Pep-D*, except at the southernmost part of its range. Characterized by cytochrome-b mtDNA haplotypes of its own group with diagnostic properties except at the southern range limit.

Derivatio nominis

The name of this newly recognized subspecies refers to the extremities that are longer than in the nominotypical subspecies (see Alexandrino et al. 2005).

Appendix 2. Morphometric data for ethanol-preserved specimens in the museum collections of Amsterdam (ZMA), Leiden (RMNH), Lisbon (MB), and Madrid (MNCN)

Museum	Collection number	Sex	SVL	HL	HW	FLL	HLL	TFL	FTL	Remarks ^a
RMNH R and A, Leiden ZMA, Amsterdam	40113	Male	44.9	10.4	7.4	11.1	13.1	2.2	3.4	
	7307a	Female	42.9	10.0	6.3	10.1	11.6	2.5	3.5	
	7307b	Female	41.9	10.1	6.0	9.3	11.9	2.2	3.1	
	7307c	Female	43.3	9.3	6.2	9.6	12.3	2.6	3.2	
	7307d	Male	44.6	10.7	7.0	10.9	13.5	2.8	3.7	
	7307e	Female	41.2	10.1	6.3	10.2	12.3	2.5	3.1	
	7340a	Male	43.0	9.9	7.1	10.9	13.0	2.6	3.3	
	7340b	Female	43.1	10.3	7.0	10.0	12.1	2.8	3.2	
	7340c	Female	44.1	10.1	6.9	8.6	12.2	2.2	3.0	
	7340d	Male	42.5	10.3	6.9	10.1	12.4	2.4	3.3	
	7340e	Female	43.8	9.5	6.8	9.8	12.5	3.0	3.2	
	7340f	Male	42.0	9.5	6.7	9.7	12.2	2.9	3.4	
	7340g	Male	43.7	9.8	6.8	11.5	12.8	2.7	3.8	Toe 20 missing
	7340h	Male	40.4	9.2	6.6	9.4	12.6	2.6	3.5	
	7340i	Female	44.1	10.4	6.9	10.0	13.6	2.7	3.4	
	7340j	Female	45.6	10.9	7.4	9.8	13.2	2.3	3.5	
	7402	Female	44.7	10.3	6.7	11.1	13.1	2.6	3.2	
	7482	Male	40.6	9.5	6.9	9.7	12.2	2.6	2.9	Toe 70 missing
	7588a	Male	44.4	9.9	7.0	11.2	13.4	3.0	3.5	Toe 20 missing
	7588b	Female	45.6	9.9	6.9	10.1	13.2	2.4	3.1	
	7588c	Female	43.7	9.8	6.9	9.6	13.0	2.6	3.3	
	7669	Male	41.6	9.2	6.1	11.0	12.6	2.4	3.6	Tail half broken, toe 6 missing
	8086a	Female	46.0	10.8	6.9	10.8	12.7	3.1	3.7	Autotomized tail
	8086b	Female	42.8	10.1	7.0	10.6	12.1	2.8	3.7	
	8086c	Male	41.4	9.9	6.8	10.5	11.9	2.4	3.0	
	8086d	Male	43.4	10.6	7.0	9.9	11.6	2.5	3.3	
	8086e	Male	42.4	10.1	7.0	10.7	12.4	2.5	3.7	
8086f	Male	41.4	9.6	6.6	10.4	12.3	2.6	3.4		
8086g	Male	40.4	9.4	6.4	9.6	12.9	2.5	3.0		
8086h	Male	42.1	9.0	6.2	11.2	12.7	2.5	3.2		
8086i	Female	41.9	9.9	6.7	10.3	12.5	2.8	3.6		
8086j	Male	43.0	10.3	7.0	10.1	12.7	2.9	3.3		
8086k	Male	41.2	9.4	6.6	9.6	12.3	2.9	3.1		

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Appendix 2. (Continued.)

Museum	Collection number	Sex	SVL	HL	HW	FLL	HLL	TFL	FTL	Remarks ^a
RMNH R and A, Leiden	18796	Male	44.9	10.0	7.3	10.0	13.7	2.4	3.3	Tail damaged at base
	18797	Male	44.7	10.5	7.6	10.9	13.0	2.8	3.3	
	18798	Male	44.6	10.2	7.5	11.0	12.5	2.9	3.6	
	18799	Male	42.9	9.4	7.2	10.9	11.9	2.9	3.3	Toe 8 missing, toe 2 short
	18800	Female	48.7	11.4	7.7	10.7	12.7	2.9	3.4	Toe 10 missing, toes 2, 3, and 7 short
	18801	Male	41.6	9.7	6.5	10.6	11.9	3.4	3.2	Toe 40 short
	18802	Female	44.0	10.1	7.0	9.6	12.4	3.3	3.7	Autotomized tail
	18803	Male	43.8	9.9	7.3	11.4	12.8	2.7	3.4	Toe 2 missing
	18804	Female	43.9	9.8	7.1	9.7	12.1	2.5	3.4	
	18805	Male	44.0	10.5	7.2	10.5	12.3	2.8	3.3	
	40114	Male	46.0	10.7	7.0	11.1	13.1	2.7	3.7	
	40115	Female	46.5	10.5	7.6	10.2	12.6	2.5	3.2	
	40116	Female	45.4	9.9	7.2	10.0	11.9	2.1	2.8	
	MB, Lisbon	06-450-01	Male	45.9	9.9	7.4	11.0	12.6	3.0	3.2
06-450-02		Female	47.3	10.7	7.3	11.0	12.7	2.3	3.5	
06-450-03		Female	45.8	10.7	6.8	11.1	13.8	2.8	3.5	
06-450-04		Male	45.6	10.2	7.3	10.3	12.4	2.8	2.8	
MNCN, Madrid	43641	Male	47.0	11.0	7.6	10.2	13.0	2.3	3.3	
	43642	Male	47.2	11.1	7.7	10.0	12.7	2.7	2.8	Several toes at forefeet incomplete
	43643	Female	47.7	10.8	7.3	11.0	12.6	2.4	3.1	
	43644	Female	44.2	10.2	6.9	11.2	13.4	2.8	3.4	

The averages of three measurements by a single observer (J.W.A.) of snout-vent length (SVL), head length (HL), head width (HW), forelimb length (FLL), hind limb length (HLL), third finger length (TFL), and length of the fourth toe (FTL) are presented.

^aFingers and toes are numbered as in the Hero (1989) scheme. Missing fingers or toes may be due to marking for ecological research. Some specimens are slit open at ventral side for the purpose of stomach content and female fecundity analyses (Arntzen 1979, 1981).