When non-monophyly results in taxonomic consequences – the case of *Mertensiella* within the Salamandridae (Amphibia: Urodela)

MICHAEL VEITH*) & SEBASTIAN STEINFARTZ

Abstract

A dorsal tail projection is unique among the Urodela. It characterises *Mertensiella caucasica* and *M. luschani* and was "classically" seen a synapomorphy of both species. However, mitochondrial DNA sequence data has shed doubt on this view in that *Salamandra* appears to be the sister taxon of *M. luschani*, resulting in non-monophyly of *Mertensiella*. We present allozyme data that support the sister relationship of *Mertensiella luschani* and *Salamandra*. Since recent histological data indicate that the tail projection of *M. caucasica* and *M. luschani* may have evolved homoplastically, we consider non-monophyly of *Mertensiella* to be well established. Consequently, and based on levels of molecular divergence among "true" salamanders within the Salamandridae (*Mertensiella*, *Salamandra* and *Chioglossa*), we assign generic rank to the former *Mertensiella luschani* and describe a new genus, with *Molge luschani* STEINDACHNER, 1891, as the type species. In addition, we follow previous authors in assigning species rank to seven subspecies the of former *Mertensiella luschani*.

Key words: Taxonomy; allozymes; 16S rRNA; morphology; *Lyciasalamandra* g. nov.; *L. atifi* comb. nov.; *L. antalyana* comb. nov.; *L. billae* comb. nov.; *L. luschani* comb. nov.; *L. fazilae* comb. nov.; *L. flavimembris* comb. nov.; *L. helverseni* comb. nov.; Turkey; Greece.

1 Introduction

Every new taxon must be included in the LINNEAN hierarchy as soon as it has been properly delimited against other such taxa. LINNEAN hierarchy consists of a nested set of taxa of different categorical ranks, so-called classes. The reconstruction of classes in an upward classification involves a number of consecutive steps (MAYR & ASHLOCK 1991, MAYR & BOCK 2002):

(1) Entities to be classified are assembled into classes of similar entities that are as homogeneous as possible.
(2) A given entity is included in that class with the members of which it shares the greatest number of attributes.
(3) A separate class is established for any new item that is too different to be included in one of the previously established classes.
(4) The degree of difference among the classes is expressed by arranging them in a hierarchy of nested sets. Each categorical level (rank) in the hierarchy expresses a certain degree of distinctness.

Although not explicitly included in these rules, it is widely accepted that all taxa which are to be subsumed under the next higher-lever taxon should be descendants of the nearest common ancestor (the criterion of monophyly sensu HAECKEL 1866; see MAYR & ASHLOCK 1991). It is this view of 'phylogenetic realism of classification' that forms the major link between systematics and taxonomy. In turn, established taxonomy is repeatedly challenged whenever systematicists add new information (e. g., in terms of additional data/features such as molecular characteristics). When conflicting results emerge, taxonomy has to be adjusted to the phylogeny to meet the criterion of monophyly of classes.

*) Corresponding author

According to the approach of phylogenetic systematics sensu HENNIG (1950), shared attributes are given strong weight in tree reconstruction. Using derived (= apomorphic) characters that are shared by two or more taxa, HENNIG (1950) developed evolutionary trees that exclusively take the branching pattern (cladogenesis) into account (the amount of evolutionary change – the anagenesis – has no importance for the construction of such cladograms). Clades are justified using synapomorphies, which are derived characters that are shared by all members of the clade.

2 The genus *Mertensiella* WOLTERSTORFF, 1925 – a textbook example of monophyly?

WAGA (1876) described a new salamander genus of the family Salamandridae from the Caucasus, *Exaeretus caucasica*. Since *Exaeretus* is the junior homonym of *Exaeretus* FIBER, 1864, a group of insects, WOLTERSTORFF (1925) transferred *Exaeretus caucasica* to *Mertensiella caucasica*. WAGA (1876) noted a special morphological feature of *Mertensiella caucasica*: males are characterised by a conspicuous tubercle projecting from the skin of the dorsal surface of the tail base, which is used for courtship synchronisation and female stimulation (REHBERG 1981, MUDRACK 1984, KLEWEN 1991, SCHULTSCHIK 1994).

STEINDACHNER (1891) found a similar tail tubercle in males of another salamander species from southern Turkey, which he described as *Molge luschani*. WOLTERSTORFF (1925) included this species in the genus *Mertensiella.*

The dorsal tail projection is unique among the Urodela. Consequently, treating it as a synapomorphy was obvious and monophyly of *Mertensiella* was not in question (since monophyly sensu HENNIG (1950) is different from monophyly sensu HAECKEL (1866), it should more appropriately be called holophyly, according to ASHLOCK 1971). As a consequence, studies on the phylogeny of Salamandridae often included only one representative of the genus, either *M. caucasica* or *M. luschani* (e. g., HALLER-PROBST & SCHLEICH 1994). As such, doubts regarding the monophyly of *Mertensiella* simply did not arise prior to the application of molecular methods. Although striking morphological and ecological similarities exist between *M. caucasica* and the Iberian stream dwelling *Chioglossa lusitanica* BOCAGE, 1864 and between *M. luschani* and *Salamandra salamandra* LINNAEUS, 1758 (e. g., BOLKAY 1928, WOLTERSTORFF et al. 1936, ÖZETI 1967, ÖZETI & WAKE 1969, TARKHNISHVILLI 1994), monophyly of *Mertensiella* was never seriously questioned. There was common belief that such a unique feature as the dorsal tail projection of *Mertensiella* could not have evolved twice.

3 When molecules contradict morphology

The study of molecular data has changed the view of monophyly of *Mertensiella*. Sequences of mitochondrial genes (TITUS & LARSON 1995, VEITH et al. 1998) have provided strong evidence that *Mertensiella* is not monophyletic. In contrast, as predicted by morphology and ecology, sister relationships between *M. luschani* and *Salamandra* and between *M. caucasica* and *Chioglossa lusitanica* were supported by different tree building algorithms. In addition, a numerical-taxonomical re-examination of morphological data revealed striking evidence for non-monophyly of *Mertensiella* (TITUS & LARSON 1995). But what about the dorsal tail projection?

The possibility of convergence or parallelism in morphological phylogenetic analyses is often invoked to explain conflicts between trees derived from morphological and molecular data (WIENS et al. 2003). The indication that the tail tubercles in *M.*

luschani and *M. caucasica* may have evolved homoplastically came from histological studies. SEVER et al. (1997) found conspicuous anatomical differences between the tail tubercles of both species. However, they were not able to rule out that the interspecific differences in tubercle histology evolved after *M. caucasica* and *M. luschani* separated from a common ancestor (SEVER et al. 1997). Nevertheless, VEITH et al. (1998) hypothesised that an ancestor of all "true" salamanders (this clade comprises *Salamandra*, *Chioglossa* and both *Mertensiella* species) or at least an ancestor of a *Salamandra*/*M. caucasica*/*M. luschani*-clade may have evolved this tail tubercle. Striking similarities in the courtship behaviour of *Salamandra* and *M. luschani* support this view (VEITH et al. 1998). This would make the dorsal tail projection a synapomorphy not just for *M. caucasica* and *M. luschani*, but also for one or two additional lineages (*Salamandra* and/or *Chioglossa*) that subsequently had lost this feature (VEITH et al. 1998).

Support for the latter view comes from recent analyses of osteological characters of the Salamandridae. SCHOLZ (2002) showed that within the Salamandridae "true" salamanders exhibit the strongest tendency for paedomorphosis/neoteny, although they are not neotenic in a strict sense. Multiple homoplasies caused by non-independent evolution due to paedomorphosis may therefore account for misleading morphological phylogenies (EMERSON & HASTINGS 1998, WIENS et al. 2003).

What does paedomorphosis have to do with the evolution of the tail projection? In fact, paedomorphosis does not directly lead to an evolutionary loss of the tail tubercle. The final formation of the tail projection marks the end of sexual maturation in *M. luschani* (STEINFARTZ & MUTZ 1998, LESKOVAR 1998), and even adult females of *M. luschani* may show a tail tubercle of > 2 mm length (unpubl. observ.). Therefore, an ontogenetic developmental program seems to trigger the growth of the tail tubercle. Given that among true salamanders *Salamandra* shows the strongest tendency for paedomorphosis (SCHOLZ 2002), this developmental program may simply have been slowed down, finally leading to a loss of this feature in *Salamandra* (SCHOLZ 2002). However, this explanation will only hold if non-monophyly of *Mertensiella* is unambiguously proven (SCHOLZ 2002).

Molecular characters such as mitochondrial DNA data, which strongly support non-monophyly of *Mertensiella*, are often assumed to be selectively neutral (e. g., KIMURA 1983). What if incongruence between morphological and molecular data is caused by the failure of the mtDNA phylogeny to resolve the species phylogeny? An additional unlinked data set is therefore desirable to give further support to either hypothesis.

4 Allozyme data support non-monophyly of *Mertensiella*

In contrast to mitochondrial genes, nuclear coding genes provide information on the complete organismal gene pool. Allozymes are nuclear coded proteins. If several distinct loci are used, allozymes often result in new insights into amphibian evolution, including the detection of cryptic species (e. g., VEITH 1996). Here we present for the first time comparative allozyme data of 18 presumptive nuclear gene loci for *Mertensiella caucasica*, *M. luschani* (i. e., for seven out of nine subspecies currently recognised), *Salamandra salamandra* and *Chioglossa lusitanica* (see Appendices 1 and 2).

We calculated two different genetic distance estimates from the resulting allele frequency data (Appendix 3): the nonmetric standard genetic distance of NEI (1972), and the metric chord distance of CAVALLI-SFORZA & EDWARDS (1967). We transferred each

of the resulting distance matrices into phenograms using different algorithms for the construction of unrooted trees: UPGMA (SNEATH & SOKAL 1973), neighbor-joining (SAITOU & NEI 1987), and FITCH-MARGOLIASH (FITCH & MARGOLIASH 1967) using NTSYS (version 1.60; ROHLF 1990) and PHYLIP (version 3.5c; FELSENSTEIN 1993). Each 1000 bootstrap replicates were run by re-sampling loci (subroutine SEQBOOT of PHYLIP; FELSENSTEIN 1993).

A sister relationship of *Salamandra* and *M. luschani* was supported by all combinations of genetic distances and tree building approaches, with an average bootstrap support of 75 %. In contrast, but in concordance with mitochondrial DNA data, a monophyletic (*Chioglossa*/*M. caucasica*) clade was not always sufficiently supported, leaving open their relationship to *Salamandra* and *M. luschani*. Nevertheless, our allozyme data support previous mitochondrial DNA sequence data in that they prove non-monophyly of the genus *Mertensiella*.

5 Non-monophyly of *Mertensiella* claims for taxonomic consequences

Non-monophyly of *Mertensiella* is strongly supported, since – except for the males' tail projection – morphological, mitochondrial and nuclear data consistently support a sister relationship between *Salamandra* and *M. luschani*. Since increased paedomorphosis provides a plausible explanation for the secondary loss of the tail tubercle in *Salamandra* (SCHOLZ 2002), this feature can still be recognised as a synapomorphy, at least of *M. caucasica*, *M. luschani* and *Salamandra*. Two options emerge for a taxonomic revision:

(1) Inclusion of *M. luschani* in the genus *Salamandra*.
(2) Definition of a new genus for *M. luschani*.

The first view was proposed by ÖZETI (1967) on the basis of a morphological comparison of various Salamandridae species. WEISROCK et al. (2001) followed her opinion and also included the polytypic *M. luschani* in the genus *Salamandra*. However, PROBST & SCHLEICH (1994) favoured the second option, although emphasising the similarity of *Salamandra* and *M. luschani*. However, they suggested that the pronounced divergent evolution of *Mertensiella* and *Salamandra* should be mirrored by treatment as different genera.

Biochemical and molecular levels of differentiation support the view of HALLER-PROBST & SCHLEICH (1994). We argue that accelerated evolution and evolutionary stasis are more likely to affect morphological rather than molecular characters (there is plenty of evidence that the latter often evolve clock-like). Therefore, we are inclined to rely more on molecular distances as an objective measure of evolutionary divergence. When comparing levels of allozyme differentiation (expressed by the standard genetic distance of NEI 1972) and the percent sequence divergence of 423 bp of the 16S rRNA gene, it became obvious that *M. luschani* and *Salamandra* are differentiated far above the level found within both clades and close to the level found among generic lineages of "true" salamanders (Tab. 1). Since "... the degree of difference among the classes is expressed by arranging them in a hierarchy of nested sets [and] each categorical level (rank) in the hierarchy [should] express[es] a certain degree of distinctness" (MAYR & BOCK 2002; see above), we establish a new genus for the polytypic *Mertensiella luschani* lineage for which no scientific name is available (*Mertensiella* has to be restricted to the *M. caucasica* lineage).

lineage	*Mertensiella luschani*	*Salamandra*	*Mertensiella caucasica*	*Chioglossa lusitanica*
M. luschani	**0.120**[1])	*9.7*	*12.3*	*14.2*
Salamandra	**1.189**	**0.523**[2]**)** / *4.5*	*11.4*	*12.2*
M. caucasica	**1.828**	**2.228**		*10.6*
C. lusitanica	**2.882**	**2.471**	**2.069**	

Tab. 1. Degrees of NEI's (1972) standard genetic distance based on 18 allozyme loci (printed in bold; this paper and VEITH 1994) and percent sequence divergence of 423 bp of the 16S rRNA gene (printed in italic; data from VEITH et al. 1998) within and among lineages of "true" salamanders. [1]) NEI's D among *Mertensiella luschani* lineages (this paper); [2]) NEI's D among *Salamandra* species (VEITH 1994, based on original data of OLIVIERI 1991).

Genetische Distanzen nach NEI (1972), basierend auf der Analyse von 18 Allozymgenorten (fett gedruckt; vorliegende Arbeit und VEITH 1994) sowie prozentualer Anteil der Sequenzdivergenz von 423 Basenpaaren des mitochondrialen 16S rRNA-Gens (kursiv gedruckt; Daten aus VEITH et al. 1998) innerhalb und zwischen Linien „Echter" Salamander. [1]) NEI's genetische Distanz zwischen Linien von *Mertensiella luschani* (vorliegende Arbeit); [2]) NEI's genetische Distanz zwischen Arten der Gattung *Salamandra* (VEITH 1994, basierend auf Originaldaten von OLIVIERI 1991).

6 Description of a new genus

Lyciasalamandra g. nov.

Mertensiella (non WOLTERSTORFF) – WOLTERSTORFF 1925, 4: 168.
Salamandra (Mertensiella [non WOLTERSTORFF]) – ÖZETI 1967: 287.
Salamandra (non LINNAEUS) – FROST 2003.

Type species: *Molge luschani* STEINDACHNER, 1891.

Diagnosis: *Lyciasalamandra* g. nov. can be distinguished unambiguously from all other Salamandridae by numerous apomorphic base substitutions in the mitochondrial genome (VEITH et al. 1998; GenBank accession number AF154051 accounts for the complete mitochondrial genome of *Lyciasalamandra* g. nov. [ZARDOYA & MAYER 2001 and ZARDOYA et al. 2003] as *Mertensiella luschani atifi*). Since the new genus contains only the former species *Mertensiella luschani* (STEINDACHNER, 1891), the formal description of its nominate subspecies *M. l. luschani* from Dodurga/Turkey (STEINDACHNER 1891) remains valid for the genus *Lyciasalamandra.* g. nov.

Several morphological features enable unambiguous differentiation of *Lyciasalamandra* g. nov. from other genera of "true" salamanders within the Salamandridae. Here we follow the genus diagnosis of FRANZEN & STEINFARTZ (1999): *Lyciasalamandra* g. nov. can be unambiguously distinguished from *Salamandra* and *Chioglossa* by an additional phalange at the first toe/finger of foreleg**s** and hind limbs. Accordingly, the number of phalanges is: 2, 2, 3, 2 (hand) and 2, 2, 3, 3, 2 (foot). Additionally, it can be distinguished from *Chioglossa* by its normal lungs, which are reduced in *Chioglossa*, and the missing tongue projection mechanism that is present in *Chioglossa*.

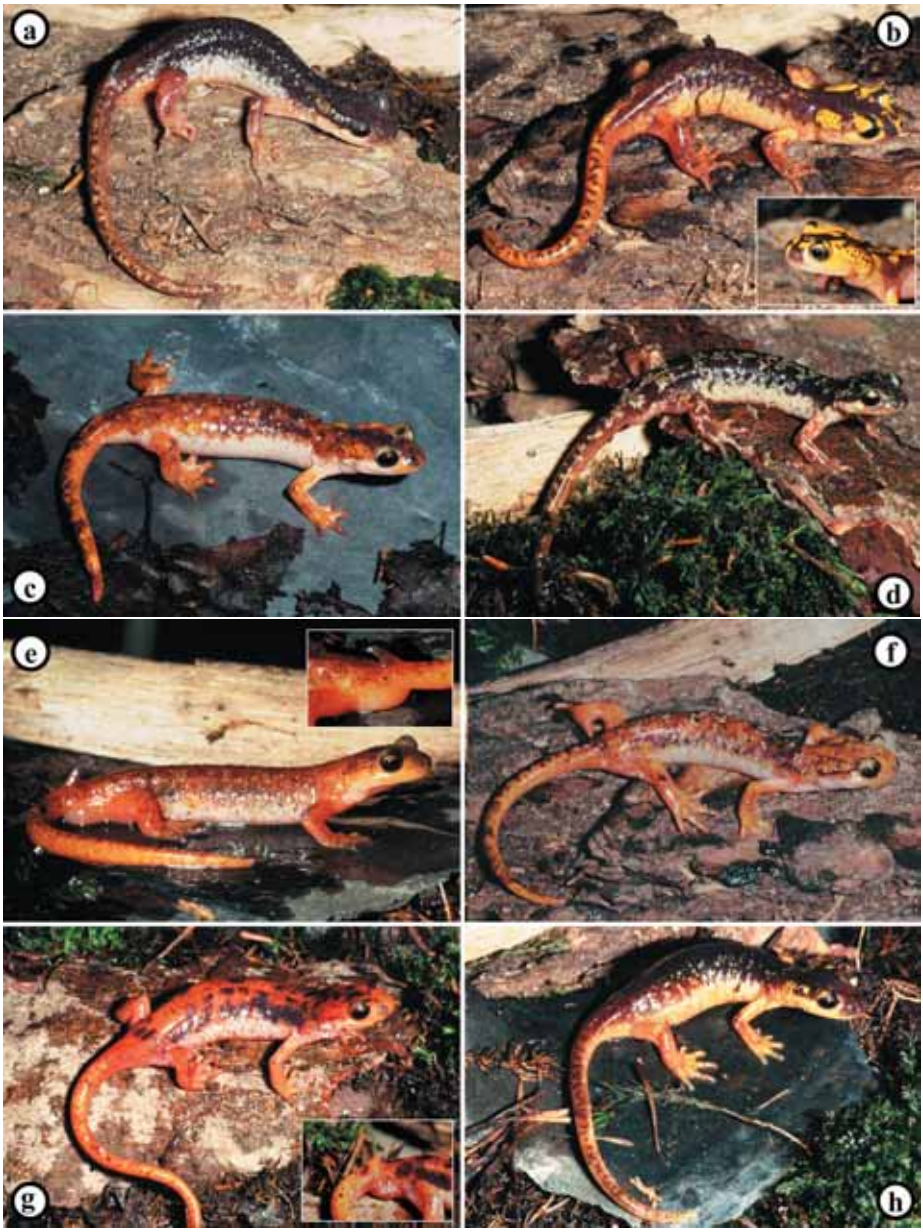
a
b
c
d
e
f
g
h

Fig. 1. (a) Male *Lyciasalamandra atifi* comb. nov. from Türbelinaz (type locality); (b) female *Lyciasalamandra antalyana* comb. nov. from Hurma (type locality); insert: portrait of a female *Lyciasalamandra antalyana* comb. nov. from Yagca Köyü (this population is characterised by a deficit of melanophores and a high proportion of yellow colour on the dorsum); (c) female *Lyciasalamandra billae* comb. nov. from Kale Tepe (type locality); (d) male *Lyciasalamandra luschani finikensis* comb. nov. from Finike (type locality); (e) male *Lyciasalamandra luschani basoglui* comb. nov. from Kastellorizon; insert: tail tubercle of another male *Lyciasalamandra luschani basoglui* comb. nov. from Kastellorizon; (f) female *Lyciasalamandra luschani luschani* comb. nov. from Dodurga (type locality); (g) male *Lyciasalamandra fazilae* comb. nov. from Gökceovacik (type locality); insert: tail tubercle of the same male; (h) female *Lyciasalamandra flavimembris* comb. nov. from Marmaris (type locality); (i) juvenile *Lyciasalamandra helverseni* comb. nov. from Pigadia (type locality); (k) *Mertensiella caucasica*; (l) *Chioglossa lusitanica*; (m) *Salamandra salamandra*; (all photographs by MIGUEL VENCES).

(a) Männchen von *Lyciasalamandra atifi* comb. nov. aus Türbelinaz (Typuslokalität); (b) Weibchen von *Lyciasalamandra antalyana* comb. nov. aus Hurma (Typuslokalität); kleines Foto: Portrait eines Weibchens von *Lyciasalamandra antalyana* comb. nov. aus Yagca Köyü (diese Population ist durch einen Mangel an Melanophoren und einen hohen Gelbanteil auf der Dorsalseite charakterisiert); (c) Weibchen von *Lyciasalamandra billae* comb. nov. aus Kale Tepe (Typuslokalität); (d) Männchen von *Lyciasalamandra luschani finikensis* comb. nov. aus Finike (Typuslokalität); (e) Männchen von *Lyciasalamandra luschani basoglui* comb. nov. aus Kastellorizon; kleines Foto: Schwanzwurzelhöcker eines Männchens von *Lyciasalamandra luschani basoglui* aus comb. nov. Kastellorizon; (f) Weibchen von *Lyciasalamandra luschani luschani* comb. nov. aus Dodurga (Typuslokalität); (g) Männchen von *Lyciasalamandra fazilae* comb. nov. aus Gökceovacik (Typuslokalität); kleines Foto: Schwanzwurzelhöcker des gleichen Tiers; (h) Weibchen von *Lyciasalamandra flavimembris* comb. nov. aus Marmaris (Typuslokalität); (i) Jungtier von *Lyciasalamandra helverseni* comb. nov. aus Pigadia (Typuslokalität); (k) *Mertensiella caucasica*; (l) *Chioglossa lusitanica*; (m) *Salamandra salamandra*.

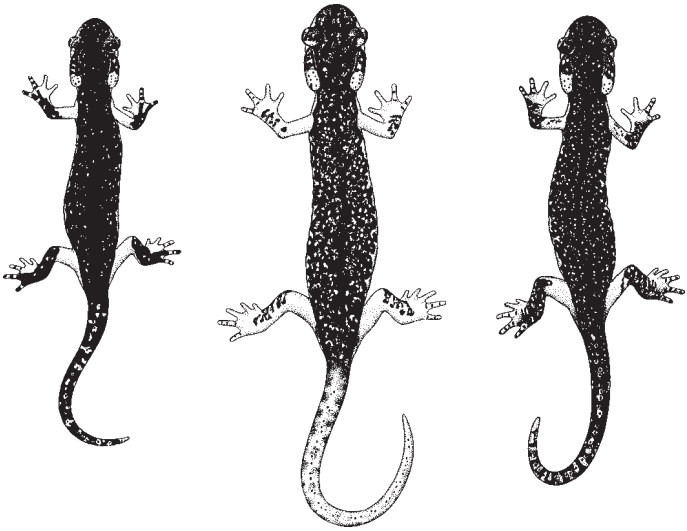

Fig. 2. Intraspecific pattern polymorphism of *Lyciasalamandra atifi* comb. nov. Left: female from Selge, which is characterised by a high amount of melanophores and only few iridophores; middle: female from Fersin, characterised by a lack of melanophores on tail and legs, but with iridophores densely distributed all over the dorsum; right: female from the type locality at Türbelinaz with its typical dense melanophore layer and an intermediate density of iridophores. All drawings by KÄTHE REHBINDER, Mainz.

Intraspezifische Variation des dorsalen Zeichnungsmusters von *Lyciasalamandra atifi* comb. nov. Links: typisches Weibchen aus Selge, das charakterisiert ist durch einen hohen Melaninanteil und nur wenige Iridophoren; Mitte: Weibchen aus Fersin mit dem für diese Population typischen Mangel an Melanophoren auf Schwanz und Beinen und der großen Zahl dorsaler Iridophoren; rechts: Weibchen von der Typuslokalität in Türbelinaz mit der typisch dichten Melanophorenschicht und der intermediären Dichte an Iridophoren.

Lyciasalamandra g. nov. differs from *Salamandra* in the lack of the Processus ascendentes of the Intermaxillaria (present in *Salamandra*) and that the Nasalia are medially connected (separated in *Salamandra* by the Processus ascendentes). *Lyciasalamandra* g. nov. also have keratinised epidermal projections, which cover the whole dorsal part of the body (keratinised epidermal projections are lacking in *Salamandra* and *Chioglossa*). Diagnostic differences between *Lyciasalamandra* g. nov. and *Mertensiella caucasica* are tail length and colouration. In *Lyciasalamandra* g. nov., the tail length equals 80-90 % of the head-body length (120-200 % in *Mertensiella caucasica*). The ground colour of metamorphosed *Mertensiella caucasica* is always dark. Dorsal pigmental ornaments are always surrounded by yellow or gold-orange and are never resolved; they are irregularly dispersed over the whole dorsal side as in *Lyciasalamandra* g. nov. (FRANZEN 1999). See also STEINDACHNER (1891), BOLKAY (1928), ÖZETI (1967), HALLER-PROBST & SCHLEICH (1994) and SCHOLZ (2002).

Distribution: *Lyciasalamandra* g. nov. is restricted to the south-western coast of Turkey and several Aegean islands. Its distribution ranges along the Turkish coast, from Marmaris in the West to the vicinity of Alanya (Cebireis Mountains) in the East

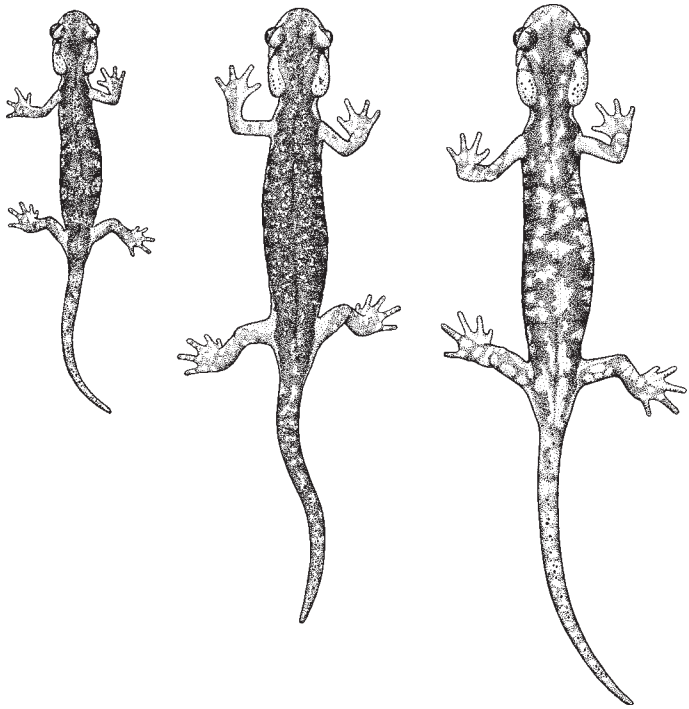

Fig. 3. Intra-subspecific pattern polymorphism of *Lyciasalamandra luschani finikensis* comb. nov. Left: juvenile from south Finike with iridophores grouped together; middle: female from Finike (type locality); its iridophores are scattered across the whole dorsum; right; female from Arif, with iridophores forming large silverish blotches. All drawings by KÄTHE REHBINDER, Mainz.

Intra-subspezifische Variation des dorsalen Zeichnungsmusters von *Lyciasalamandra luschani finikensis* comb. nov. Links: Jungtier aus einer Population südlich von Finike; die Iridophoren sind gruppiert; Mitte: Weibchen aus Finike (Typuslokalität); die Iridophoren stehen einzeln und sind über den ganzen Rücken verstreut; rechts: Weibchen aus Arif; die Iridophoren verschmelzen zu großen silbernen Flecken.

(see VEITH et al. 2001 for a recent compilation). Inhabited islands are Bogazic Adasi (now connected with the mainland by a dam), Tersane Adasi, Domuz Adasi, Kekova Adasi, Kastellorizon (Turkish: Meis) and Karpathos with its two satellite islands Saria and Kasos. With the exception of Kastellorizon, Karpathos, Saria and Kasos (Greek islands), the distribution of *Lyciasalamandra* g. nov. is restricted to Turkey.

Derivatio nominis: The name *Lyciasalamandra* g. nov. refers to both its major geographic distribution and its close phylogenetic affinity to the sister genus *Salamandra*. It combines *Lycia*, the name of the ancient Roman province in southern Turkey, and *Salamandra*, forming the name *Lyciasalamandra* g. nov.

Remarks: *Lyciasalamandra* g. nov. *luschani* was originally described as *Molge luschani* by STEINDACHNER in 1891. However, the name *Molge* MERREM 1820 is a synonym of *Triturus* RAFINESQUE, 1815 (FROST 2003).

Referred species and subspecies: *Lyciasalamandra* g. nov. is a polytypic genus. On the basis of a large comprehensive mitochondrial dataset WEISROCK et al. (2002) emphasised the high degree of intrageneric differentiation among most of the subspecies of the former *Mertensiella luschani*. They regard this differentiation as species-specific. According to WEISROCK et al. (2002) and our own unpublished data we recognise six monotypic and one polytypic species within *Lyciasalamandra* g. nov.:

(1) *Lyciasalamandra luschani* comb. nov. (*Molge luschani* STEINDACHNER, 1891) with three subspecies: *Lyciasalamandra luschani luschani* comb. nov. (*Molge luschani* STEINDACHNER, 1891), *Lyciasalamandra luschani finikensis* comb. nov. (*Mertensiella luschani finikensis* BASOGLU & ATATÜR, 1975) and *Lyciasalamandra luschani basoglui* comb. nov. (*Mertensiella luschani basoglui* BARAN & ATATÜR, 1980).
(2) *Lyciasalamandra helverseni* comb. nov. (*Mertensiella luschani helverseni* PIEPER, 1963).
(3) *Lyciasalamandra atifi* comb. nov. (*Mertensiella luschani atifi* BASOGLU, 1967).
(4) *Lyciasalamandra fazilae* comb. nov. (*Mertensiella luschani fazilae* BASOGLU & ATATÜR, 1974).
(5) *Lyciasalamandra antalyana* comb. nov. (*Mertensiella luschani antalyana* BASOGLU & BARAN, 1976).
(6) *Lyciasalamandra billae* comb. nov. (*Mertensiella luschani billae* FRANZEN & KLEWEN, 1987).
(7) *Lyciasalamandra flavimembris* comb. nov. (*Mertensiella luschani flavimembris* MUTZ & STEINFARTZ, 1995).

All species are well distinguishable on biochemical (allozymes), molecular (mitochondrial DNA sequences) and morphological (body size and colouration; for reviews see KLEWEN 1991 and STEINFARTZ & MUTZ 1998; see Fig. 1) grounds. Intraspecific and intra-subspecific differentiation is strong in all species and subspecies, respectively (unpubl. observ.), as is exemplified by pattern polymorphisms of *Lyciasalamandra atifi* comb. nov. (Fig. 2) and *Lyciasalamandra luschani finikensis* comb. nov. (Fig. 3).

Acknowledgements

Some samples studied in this paper came from BURKHARD THIESMEIER, THOMAS MUTZ and WOLFRAM MISSLER. We are indebted to JOCHEN MARTENS, WOLFGANG BÖHME and STEFAN LÖTTERS for taxonomic advice. Photographs were kindly provided by MIGUEL VENCES. The English was improved by RENATA PLATENBERG.

Wenn Nicht-Monophylie taxonomische Konsequenzen erfordert – das Beispiel der Gattung *Mertensiella* innerhalb der Salamandridae (Amphibia: Urodela)

Der Besitz eines Schwanzwurzelhöckers ist innerhalb der Urodelen einmalig. Er charakterisiert lediglich die Arten *Mertensiella caucasica* und *M. luschani*. Daher wurde sein Besitz stets als klassischer Fall einer Synapomorphie beider Gattungen angesehen. Mitochondriale DNA-Sequenzen zeigten jedoch, dass die Gattung *Salamandra* und nicht *M. caucasica* das Geschwistertaxon von *M. luschani* darstellt, wodurch die Monophylie der Gattung *Mertensiella* in Frage gestellt wurde. Wir präsentieren hier Allozym-Daten, welche die Nicht-Monophylie der Gattung *Mertensiella* weiter untermauern. Da zudem neuere histologische Daten darauf hinweisen, dass der vermeintlich synapomorphe Schwanzwurzelhöcker bei *M. caucasica* und *M. luschani* homoplastisch evolviert sein könnte, sehen wir die Nicht-Monophylie der Gattung *Mertensiella* als gesichert an. Gestützt auf Daten zur genetischen Differenzierung innerhalb der Echten Salamander (*Mertensiella, Salamandra* und *Chioglossa*) weisen wir daher der früheren Art *Mertensiella*

luschani Gattungsrang zu und beschreiben für diese eine neue Gattung mit *Molge luschani* STEINDACHNER, 1891 als Typusart. Wir schließen uns zudem der Meinung früherer Autoren an und weisen sieben der Unterarten der ehemaligen Art *Mertensiella luschani* Artrang zu.

Schlagwörter: Taxonomie; Allozyme; 16S rRNA; Morphologie; *Lyciasalamandra* g. nov.; *L. atifi* comb. nov.; *L. antalyana* comb. nov.; *L. billae* comb. nov.; *L. luschani* comb. nov.; *L. fazilae* comb. nov.; *L. flavimembris* comb. nov.; *L. helverseni* comb. nov.; Türkei; Griechenland.

References

ASHLOCK, P.D. (1971): Monophyly and associated terms. – Syst. Zool., **20**: 63-69

BOLKAY, S.J. (1928): Die Schädel der Salamandrinen, mit besonderer Rücksicht auf ihre systematische Bedeutung. – Z. Anat. Enwicklungsgesch., **86**: 259-319.

CAVALLI-SFORZA, L.L. & A.W.F. EDWARDS (1967): Phylogenetic analysis: Models and estimation procedures. – Evolution, **32**: 550-570.

EMERSON, S.B. & P.A. HASTINGS (1998): Morphological correlations in evolution: consequences for phylogenetic analysis. – Q. Rev. Biol., **73**: 141-162.

FELSENSTEIN, J. (1993): PHYLIP: Phylogenetic Inference Package, 3.5. – Washington.

FITCH, W.M. & E. MARGOLIASH (1967): Construction of phylogenetic trees. – Science, **155**: 279-284.

FRANZEN, M. (1999): *Mertensiella caucasica* (Waga, 1876) – Kaukasus-Salamander. – pp. 329-366 in GROSSENBACHER, K. & B. THIESMEIER (eds.): Handbuch der Reptilien und Amphibien Europas, Vol. 4/I: Schwanzlurche. – Wiesbaden.

— & S. STEINFARTZ (1999): *Mertensiella* (WOLTERSTORFF, 1925) – Kleinasiatische Salamander. – pp. 322-328 in GROSSENBACHER, K. & B. THIESMEIER (eds.): Handbuch der Reptilien und Amphibien Europas, Vol. 4/I: Schwanzlurche. – Wiesbaden.

FROST, D.R. (2003): Amphibian Species of the World: an online reference. V2.21. – New York. Electronic database available at: http://research.amnh.org/herpetology/amphibia/index.html (inquiry: 13 October 2003)

HAECKEL, E. (1866): Generelle Morphologie der Organismen. – Berlin.

HALLER-PROBST, M. & H.H. SCHLEICH (1994): Vergleichende osteologische Untersuchungen an einigen Urodelen Eurasiens (Amphibia: Urodela, Salamandridae, Proteidae). – Cour. Forschungsinst. Senkenberg, **173**: 23 - 77.

HENNIG, W. (1950): Grundzüge einer Theorie der phylogenetischen Systematik. – Berlin.

KLEWEN, R. (1991): Die Landsalamander Europas, Teil 1., 2nd. Edition. – Wittenberg Lutherstadt.

KIMURA, M. (1983): The neutral theory of molecular evolution. – New York.

LESKOVAR, C. (1998): Skelettochronologische Untersuchungen zur Altersstruktur und des alterspezifischen Wachstums bei einer Hoch- und einer Tieflandpopulation von *Mertensiella luschani* (STEINDACHNER 1891). – Unpubl. diploma thesis, Mainz Univ.

MAYR, E. & P.D. ASHLOCK (1991): Principles of zoological systematics. – 2nd Edition, New York.

— & W.J. BOCK (2002): Classifications and other ordering systems. – J. Zool. Syst. Evolut. Res., **40**: 169-194.

MUDRACK, W. (1984): *Mertensiella luschani antalyana* BASOGLU und BARAN, 1976. – Sauria, **6**: 19-21.

ÖZETI, N. (1967): The morphology of the salamander *Mertensiella luschani* (STEINDACHNER) and the relationships of *Mertensiella* and *Salamandra*. – Copeia, **1967**: 287-297.

— & D.B. WAKE (1969): The morphology and evolution of the tongue and associated structures in salamanders and newts (family Salamandridae). – Copeia, **1969**: 91-123.

OLIVIERI, L. (1991): Differenziamento genetico nel genera *Salamandra* (Amphibia, Urodela, Salamandridae): Aspetti tassonomici ed evolutivi. – Unpubl. Master thesis, Rome Univ.

REHBERG, F. (1981): Nachtrag zu „*Mertensiella luschani*, der Lykische Salamander“ in herpeto-

fauna 9, Seite 16. – Herpetofauna, **10**: 23.

RICHARDSON, B.J., P.R. BAVERSTOCK & M. ADAMS (1986): Allozyme electrophoresis. A handbook for animal systematics and population structure. – Sydney.

ROHLF, F.J. (1990): NTSYS: Numerical taxonomy and multivariate analysis system, Version 1.60. – New York.

SAITOU, N. & M. NEI (1987): The neighbor-joining method: a new approach for reconstructing phylogenetic trees. – Mol. Biol. Evol., **4**: 406-425.

SCHOLZ, K.P. (2002): Pädomorphe Merkmale im Kopfskelett von Salamandriden: Heterochronie als Folge von Lebenszyklusstrategien. – Bielefeld.

SCHULTSCHIK, G. (1994): Ergebnisse einer urodelenkundlichen Exkursion an die lykische Küste (Südwesttürkei). – Herpetozoa, **7**: 75-77.

SEVER, D.M., M. SPARREBOOM & G. SCHULTSCHIK (1997): The dorsal tail tubercle of *Mertensiella caucasica* and *M. luschani* (Amphibia: Salamandridae). – J. Morph., **232**: 93-105.

SNEATH, P.H.A. & R.R. SOKAL (1973): Numerical taxonomy. – San Francisco.

STEINDACHNER, F. (1891): Über einige neue und seltene Reptilien- und Amphibienarten. – Sitzsber. Akad. Wissensch. Wien., math.-natwiss. Kl. 1, **100**: 289-314.

STEINFARTZ, S. & T. MUTZ (1998): *Mertensiella luschani* (STEINDACHNER, 1891) – Lykischer Salamander, Kleinasiatischer Salamander. – pp. 367-397 in GROSSENBACHER, K. & B. THIESMEIER (eds.): Handbuch der Reptilien und Amphibien Europas, Vol. 4/I: Schwanzlurche. – Wiesbaden.

TARKHNISHVILI, D.N. (1994): Interdependence between populational, developmental and morphological features of the Caucasian Salamander, *Mertensiella caucasica*. – pp. 315-325 in GREVEN, H. & B. THIESMEIER (eds): Biology of *Salamandra* and *Mertensiella*. – Mertensiella, Rheinbach, **4**.

TITUS, T.A. & A. LARSON (1995): A molecular phylogenetic perspective on the evolutionary radiation of the salamander family Salamandridae. – Syst. Biol., **44**: 125-151.

VEITH, M. (1994): Morphological, molecular and life history variation in *Salamandra salamandra* (L.). – pp. 355-397 in GREVEN, H. & B. THIESMEIER (eds): Biology of *Salamandra* and *Mertensiella*. – Mertensiella, Rheinbach, **4**.

— (1996): Molecular markers and species delimitation – examples from the European batrachofauna. – Amph.-Rept., **17**: 303-314.

—, S. STEINFARTZ, R. ZARDOYA, A. SEITZ & A. MEYER (1998): A molecular phylogeny of "true" salamanders (family Salamandridae) and the evolution of terrestriality of reproductive modes. – J. Zool. Syst. Evol. Res., **36**: 7-16.

—, I. BARAN, O. GODMANN, A. KIEFER, M. ÖZ & M.R. TUNC (2001): A revision of population designation and geographic distribution of *Mertensiella luschani* (STEINDACHNER, 1891). – Fauna in the Middle East, **22**: 67-82.

WAGA (1876): Nouvelle espèce de salamandride. – Rev. Mag. Zool. (Ser. 3): 326-328.

WEISROCK, D.W., J.R. MACEY, I. UGURTAS, A. LARSON & T.J. PAPENFUSS (2001): Molecular phylogenetics and historical biogeography among salamandrids of the "true" salamander clade: Rapid branching of numerous highly divergent lineages in *Mertensiella luschani* associated with the rise of Anatolia. – Mol. Phyl. Evol., **18**: 434-448.

WIENS, J.J., P.T. CHIPPINDALE & D.M. HILLIS (2003): When are phylogenetic analyses misled by convergence? A case study in Texas cave salamanders. – Syst. Biol., **52**: 501-514.

WOLTERSTORFF, A. (1925): Katalog der Amphibien-Sammlung im Museum für Natur- und Heimatkunde. – Abh. Ber. Mus. Nat. Heim. Magdeburg, **4**: 155-310.

—, L.A. LANTZ & W. HERRE (1936): Beiträge zur Kenntnis des Kaukasussalamanders (*Mertensiella caucasica* WAGA). – Zool. Anz., **116**:1-13.

ZARDOYA, R. & A. MEYER (2001): On the origin of and phylogenetic relationships among living amphibians. – Proc. Natl. Acad. Sci. USA, **98**: 7380-7383.

—, E. MALAGA-TRILLO, M. VEITH & A. MEYER (2003): Complete nucleotide sequence of the mitochondrial genome of a salamander, *Mertensiella luschani*. – Gene, in press.

Appendix 1

Samples, sample localities, number of individuals (n) and tissues used for allozyme electrophoresis (*tt* = terra typica).

Untersuchte Populationen, Fundorte, Zahl der Individuen (n) und für die Allozymelektrophorese verwendete Gewebe (*tt* = Typuslokalität).

sample	locality	n	tissue
(1) *M. l .atifi*	Türbelinaz, Turkey (*tt*)	4	blood
(2) *M. l. antalyana*	Hurma, Turkey (*tt*)	5	blood
(3) *M. l. billae*	Kale Tepe, Turkey (*tt*)	4	blood
(4) *M. l. fazilae*	Gögceovacik, Turkey (*tt*)	5	blood
(5) *M. l. finikensis*	Finike, Turkey (*tt*)	4	muscle
(6) *M. l. flavimembris*	Marmaris, Turkey (*tt*)	5	blood
(7) *M. l. luschani*	Dodurga, Turkey (*tt*)	5	blood
(8) *M. caucasica*	Caucasus, Georgia	3	blood
(9) *S. salamandra*	Paikon Mts., Greece	5	blood
(10) *C. lusitanica*	Spain	2	muscle

Appendix 2

Presumptive allozyme loci studied; electrophoresis was carried out on cellulose-acetate gels using standard protocols (RICHARDSON et al. 1986); we stained 16 enzyme systems, altogether providing information on 18 presumptive gene loci.

Untersuchte Genorte; die Elektrophorese wurde gemäß Standardprotokoll (RICHARDSON et al. 1986) auf Zellulose-Acetat-Platten durchgeführt; es wurden 16 Enzymsysteme mit zusammen 18 Genorten gefärbt.

TC = tris-citric acid, pH 8.2; TG = tris-glycin, pH 8.5; TM = tris-maleic acid, pH 7.0; PP = phosphate, pH 7.0.

locus	E.C. number	buffer system
ak	2.7.4.3	TC
apk	2.7.3.3	PP
ck-1	2.7.3.2	PP
ck-2	2.7.3.2	PP
est	3.1.1.-	PP
fum	4.2.1.2	TM
gpi	5.3.1.9	PP
idh	1.1.1.42	TM
ldh-1	1.1.1.27	TM
ldh-2	1.1.1.27	TM
mdh	1.1.1.37	TM
me	1.1.1.40	TM
pep-B	3.4.-.-	TG
pep-D	3.4.-.-	TM
6pgd	1.1.1.44	TC
pgm	5.4.2.2	TG
pk	2.7.1.40	PP
tre	3.2.1.28	PP

Appendix 3

Allele frequencies; sample numbers refer to Appendix 1.

Allelfrequenzen; die Populationsnummern beziehen sich auf Anhang 1.

locus/ sample	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
ak b	(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	a(1.00)	c(1.00)	d(1.00)
*apk*b	(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	a(1.00)	c(1.00)	d(1.00)
ck-1	d(1.00)	d(1.00)	d(1.00)	c(0.20) d(0.80)	e(1.00)	d(1.00)	e(1.00)	a(1.00)	d(1.00)	b(1.00)
ck-2	c(0.50) d(0.50)	c(0.90) d(0.10)	c(1.00)	c(1.00)	c(1.00)	c(1.00)	c(1.00)	b(1.00)	e(1.00)	a(1.00)
*est*b	(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	a(1.00)	c(1.00)
fum	c(1.00)	c(1.00)	c(1.00)	c(1.00)	c(1.00)	c(1.00)	c(1.00)	b(1.00)	c(1.00)	a(1.00)
gpi	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	a(1.00)	b(1.00)	a(1.00)
idh	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	b(1.00)
ldh-1	c(1.00)	g(1.00)	g(1.00)	d(1.00)	g(1.00)	a(1.00)	f(1.00)	e(1.00)	b(1.00)	h(1.00)
ldh-2	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	a(1.00)	b(1.00)	c(1.00)
mdh	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	c(1.00)	d(1.00)	a(1.00)
me	c(1.00)	c(1.00)	c(1.00)	c(1.00)	c(1.00)	c(1.00)	c(1.00)	d(1.00)	a(1.00)	b(1.00)
pep-b	c(1.00)	c(1.00)	c(1.00)	c(1.00)	c(1.00)	c(1.00)	c(1.00)	b(1.00)	a(1.00)	d(0.50) d(0.50)
pep-d	c(1.00)	c(1.00)	c(1.00)	a(0.10) c(0.90)	c(1.00)	c(1.00)	c(0.20) d(0.80)	b(1.00)	e(1.00)	f(1.00)
6pgd	d(0.90) e(0.10)	d(1.00)	d(1.00)	d(1.00)	e(1.00)	d(1.00)	e(1.00)	a(1.00)	c(1.00)	b(1.00)
pgm	a(0.20) b(0.80)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	d(0.25) e(0.75)	c(1.00)	d(1.00)
pk	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	b(1.00)	a(1.00)	c(1.00)	d(1.00)
tre	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)	a(1.00)

Appendix 4

NEI's (1972) standard genetic (below diagonal) and CAVALLI-SFORZA & EDWARDS' (1967) chord distance (above diagonal) between lineages of "true" salamanders, calculated from allele frequencies of 18 presumptive allozyme loci (see Appendix 3).

Genetische Distanzen nach NEI (1972) (unterhalb der Diagonalen) und CHORD-DISTANZEN nach CAVALLI-SFORZA & EDWARDS (1967) (oberhalb der Diagonalen) zwischen Linien „Echter" Salamander, berechnet auf der Basis der Allelfrequenzen von 18 Allozymgenorten (siehe Anhang 3).

sample	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
(1) *M. l. atifi*	–	0.094	0.107	0.119	0.107	0.228	0.269	1.111	0.889	1.259
(2) *M. l. antalyana*	0.068	–	0.004	0.090	0.078	0.152	0.267	1.111	0.889	1.259
(3) *M. l. billae*	0.072	0.001	–	0.086	0.074	0.148	0.263	1.111	0.889	1.259
(4) *M. l. fazilae*	0.077	0.058	0.058	–	0.058	0.226	0.265	1.111	0.897	1.259
(5) *M. l. flavimembris*	0.072	0.055	0.054	0.086	–	0.222	0.263	1.111	0.889	1.259
(6) *M. l. finikensis*	0.183	0.112	0.111	0.165	0.172	–	0.115	1.111	0.963	1.259
(7) *M. l. luschani*	0.228	0.216	0.215	0.203	0.215	0.091	–	1.111	0.963	1.259
(8) *M. caucasica*	1.809	1.831	1.836	1.823	1.836	1.836	1.827	–	1.185	1.148
(9) *S. salamandra*	1.112	1.135	1.139	1.160	1.139	1.322	1.313	2.228	–	1.207
(10) *C. lusitanica*	2.862	2.885	2.890	2.876	2.890	2.890	2.881	2.069	2.471	–

Manuscript received: 20 October 2003

Authors: MICHAEL VEITH, Institut für Zoologie, Universität Mainz, Saarstraße 21, D-55099 Mainz, Germany; E-Mail: mveith@uni-mainz.de; SEBASTIAN STEINFARTZ, Institut für Genetik, Universität zu Köln, Weyertal 121, D-50931 Köln, Germany.