

Taxonomy, biogeography and evolution of *Euproctus* (Amphibia: Salamandridae), with the resurrection of the genus *Calotriton* and the description of a new endemic species from the Iberian Peninsula

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A phylogeny of West Palearctic Salamandridae based on 1208 bp of mtDNA sequences (300 bp of cytb, 346 bp of 12S rRNA and 562 bp of 16S rRNA) indicates the European brook newts (*Euproctus*) are polyphyletic. To reflect revised relationships, the Tyrrhenian species (*E. montanus* (Savi, 1838) and *E. platycephalus* (Gravenhorst, 1829)) are retained in *Euproctus* Genè, 1839, while the genus *Calotriton* Gray, 1858 is resurrected to include the Pyrenean brook newt (*Calotriton asper* (Dugès, 1852) **comb. nov.**) and a new species from the massif of El Montseny, Catalonia, Spain, described herein as *Calotriton arnoldi* **sp. nov.**, which is both morphologically and genetically distinct. Although according to the principle of priority *Megapterna* Savi, 1838 should take precedence over *Euproctus* Genè, 1839, for the sake of nomenclatural stability and in line with Art. 23.9.1 of the International Code of Zoological Nomenclature, *Megapterna* is considered a *nomen oblitum* and *Euproctus* a *nomen protectum*. The polyphyly of *Euproctus* (*s.l.*) contradicts previous, well-accepted, biogeographical hypotheses and represents a clear case of convergence, involving several morphological traits and a unique reproductive behaviour that is advantageous in stream situations. Molecular dating suggests the Western brook newt lineage (*C. asper* + *C. arnoldi*) originated towards the end of the Miocene (8.3 ± 0.11 Mya) and is part of a well-supported monophyletic assemblage, which also includes *Neurergus kaiseri* (Schmidt, 1952) and a clade formed by *Triturus karelinii* (Strauch, 1870), *T. carnifex* (Laurenti, 1768), *T. pygmaeus* (Wolterstorf, 1905) and *T. marmoratus* (Latreille, 1800). Speciation separating *E. montanus* and *E. platycephalus* might have coincided with the onset of the Messinian salinity crisis. © 2005 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2005, 145, 555–582.

ADDITIONAL KEYWORDS: 12S rRNA – 16S rRNA – adaptive convergence – *Calotriton arnoldi* – cytochrome *b* – external morphology – mtDNA – Montseny – osteology – phylogeny.

INTRODUCTION

The salamandrid genus *Euproctus* Genè, 1839 currently consists of three geographically isolated species that are confined to southern Europe: *E. montanus* (Savi, 1838) from Corsica, *E. platycephalus* (Gravenhorst, 1829) from Sardinia and *E. asper* (Dugès, 1852) from the Pyrenean region and adjacent areas (see Fig. 1). All three species are small- to medium-sized newts that usually live in cool well-oxygenated mountain streams (Thorn, 1968). Their distinctive features

include a strongly depressed head and body, reduction or even absence of lungs, and caudal capture of the female during copulation (Thorn, 1968; Clergue-Gazeau, 1971; Clergue-Gazeau & Martínez-Rica, 1978; Gasser & Clergue-Gazeau, 1981; Montori, 1988).

The present distribution of *Euproctus* has been associated with the disjunction and rotation of the Sardo-Corsican microplate from the French–Iberian massif (Accordi, Grassi-Milano & Gallo, 1984; Sbordoni *et al.*, 1982, 1985, 1990; Caccone *et al.*, 1994, 1997). According to this biogeographical scenario, which takes into account stratigraphic, palaeomag-

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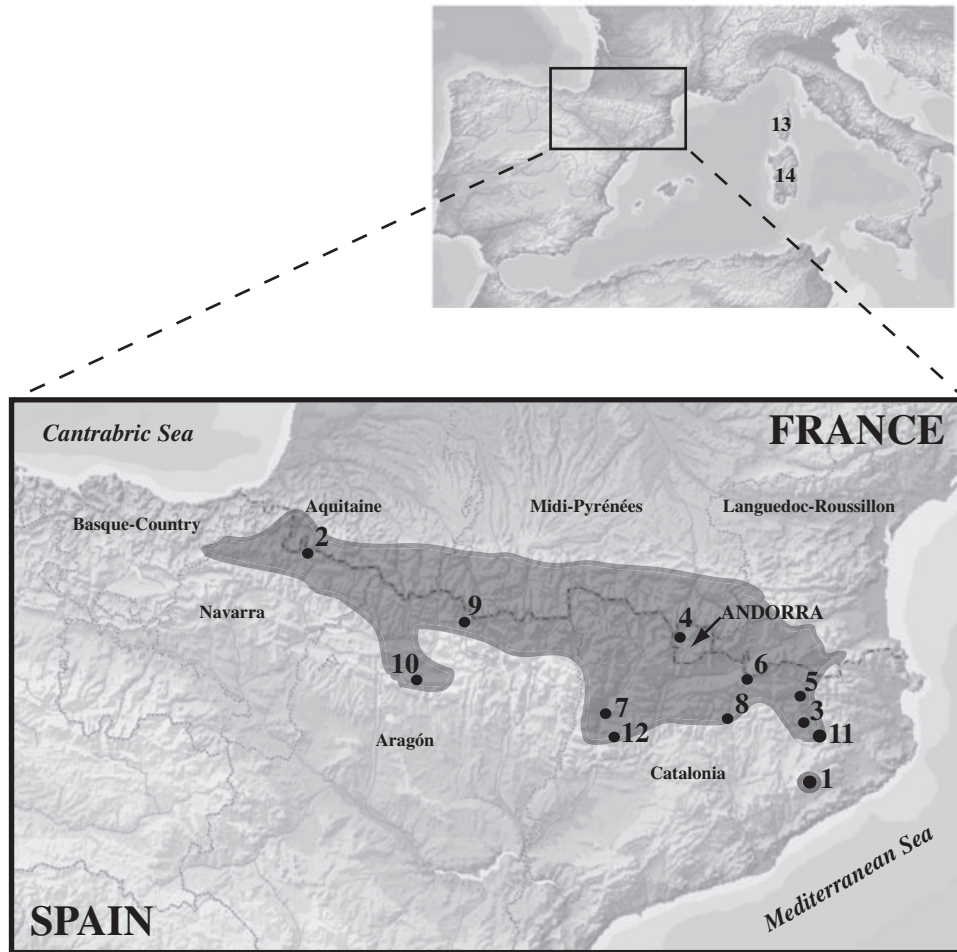


Figure 1. Map showing the distribution range of the Tyrrhenian brook newts and the Western brook newts (shaded areas). Numbers refer to the following localities: 1, El Montseny. 2, Irati. 3, Vidrà. 4, Xixarella. 5, Vall d'en Bac. 6, Collada de Tosses. 7, Font de l'Ús. 8, Berga. 9, Ordesa. 10, Monrepos. 11, Susqueda. 12, Vilanova de Meià. 13 Corsica. 14, Sardinia. Additional data are given in Table 1.

netic and geomorphological data (Alvarez, 1972; Alvarez, Franks & Nairn, 1973; Cherchi & Montadert, 1982), the Pyrenean brook newt (*E. asper*) split from the ancestor of the Tyrrhenian brook newts (*E. montanus* and *E. platycephalus*) approximately 29 Mya. After this, the ancestor of the Tyrrhenian forms drifted with the Sardo-Corsican microplate for approximately 13 Myr, until the separation of this block into Corsica and Sardinia produced the speciation event that resulted in *E. montanus* and *E. platycephalus*. The separation of Corsica and Sardinia started approximately 15 Mya, when the two islands were roughly at their present position, and was completed by 9 Mya. After this event, Corsica and Sardinia have been in contact intermittently as a result of the almost complete desiccation of the Mediterranean Sea during the Messinian salinity crisis (5.9–5.3 Mya), and sea-level oscillations occurred in

the area during the Pleistocene (Blondel & Aronson, 1999).

Despite the morphological similarities between all three species of brook newts and a widely accepted biogeographical scenario, the monophyletic status of *Euproctus* has been questioned several times on the basis of morphological (Boulenger, 1917; Despax, 1923) and molecular (Gasser, 1975) data. According to these authors, it is possible that the Pyrenean brook newt may not be related to the Tyrrhenian species and, therefore, the main characters that define *Euproctus* might be convergences, which originated independently as a result of the development of similar adaptations to the same lifestyle. Recent phylogenetic analyses carried out by Steinfartz *et al.* (2002) using partial sequences of the 12S and 16S rRNA mitochondrial genes support this hypothesis and suggest *E. asper* may be more closely related to some rep-

representatives of the genus *Triturus* and *Neurergus* than to *E. montanus* and *E. platycephalus*. Paraphyly of *Triturus* had been previously suggested in a landmark study based on mtDNA sequences from many key representatives of the Salamandridae (Titus & Larson, 1995), and more recently in a review of the Salamandridae (Larson, Weisrock & Kozak, 2003). As a result of these molecular analyses, there has been a recent taxonomic revision in which the genus *Triturus* has been split into three units: *Mesotriton*, *Lissotriton* and *Triturus* (García-París, Montori & Herrero, 2004).

The Pyrenean brook newt, *Euproctus asper*, is considered a relict species that originated in the early Tertiary (Herre, 1935; Steiner, 1950; Gasser & Clergue-Gazeau, 1981), although the only known fossil material that has tentatively been assigned to *Euproctus* are three vertebrae from the Upper Pleistocene of Cueva de las Hienas (Asturias), north-west Spain (Estes, 1981; Sanchiz, 1977a, b). *E. asper* is widely distributed across most of the Spanish, Andorran and French Pyrenees. It covers an area from Guipúzcoa-Navarra in the west to the Albera mountains in the east (see Fig. 1 and also Thorn, 1968; Clergue-Gazeau, 1971; Clergue-Gazeau & Martínez-Rica, 1978; Gasser & Clergue-Gazeau, 1981; Montori, 1988; Montori *et al.*, 2002). Here, it can be normally found in mountain streams but also in lakes and even caves, at altitudes ranging between 700 and 2500 m, but sometimes as far down as 175 m and up to 3000 m (Montori *et al.*, 2002). Apart from the many populations situated around the main Pyrenean axial ridge (localities 2, 9, 4, 6 in Fig. 1), several Spanish populations of *E. asper* are found at lower latitudes south of the main range, in the Prepyrenees (localities 5, 8, 7, 10 and 12 in Fig. 1; see also Martínez-Rica & Clergue-Gazeau, 1977; Montori *et al.*, 2002), the Catalan Transverse ridge (localities 3 and 11 in Fig. 1) and in the massif of El Montseny, which represents the southern limit of the distribution of *E. asper* (locality 1 in Fig. 1). Within the massif of El Montseny, the Pyrenean brook newt is rare and was thought to be restricted to just three mountain streams that run parallel and at a very short distance from each other (Montori & Pascual, 1981; Montori & Campeny, 1991). Following Montori & Campeny (1991), these populations will be referred to herein as A1, A2 and A3. In the course of a recent survey we found two new populations 0.5 km from each other on a different mountain within the limits of El Montseny Natural Park, at a relatively short distance from populations A1–A3. We will refer to these new populations as populations B1 and B2.

Despite populations from El Montseny being connected with the Prepyrenean ones by the Catalan Transverse ridge (consisting of the Guilleries, Puigscal and Collsacabra mountains), there is still a 30-km gap of relatively low terrain between populations from El Montseny and their nearest known popula-

tions situated north of the Guilleries mountains (locality 11 in Fig. 1) (Baucells, Camprodon & Ordeix, 1998). A preliminary morphological analysis by Montori & Campeny (1991) showed that both males and females of Pyrenean brook newt from populations A1 and A2 of the massif of El Montseny had a significantly lower body weight, shorter snout–vent length and total length than males and females of *E. asper* from a Pyrenean locality (Torrent del Pi, La Cerdanya, Spain. Close to locality 6 in Fig. 1).

Morphological, ecological and molecular differences have also been found between Pyrenean and Prepyrenean populations of *E. asper* (Martínez-Rica & Clergue-Gazeau, 1977; Gasser & Clergue-Gazeau, 1981; Serra-Cobo, Franks & Martínez-Rica, 2000a). In the molecular study, Gasser & Clergue-Gazeau (1981) showed that a population of *E. asper* from the Spanish Prepyrenees (Rio Flumen, Puerto de Monrepos, locality 10 in Fig. 1) has a polymorphism in the serum transferrin not detected in any of the five Pyrenean populations included in their study. Pyrenean populations also showed some differences with the Prepyrenean populations in the serum albumin and the enzyme alcohol dehydrogenase. These results contrast with the apparent lack of genetic diversity among all five Pyrenean populations included in their analysis, despite some of them being separated by more than 300 km of continuous mountainous terrain (Gasser & Clergue-Gazeau, 1981). According to Montori (1988), genetic uniformity of the Pyrenean populations of *E. asper* may be maintained by juveniles moving between different populations during the two years they spend on land after metamorphosis and before returning definitively to an aquatic lifestyle.

The molecular identity of all Pyrenean populations of *E. asper* contrasts with the relatively high degree of morphological variation in body size, colour pattern, skin granulation and ecology that exists among them (Thorn, 1968; Clergue-Gazeau & Martínez-Rica, 1978; Clergue-Gazeau & Bonnet, 1980; Serra-Cobo *et al.*, 2000a). As a result of these differences, many species, subspecies and forms of Pyrenean brook newt have been described in the past, all of which are now considered synonyms of *E. asper*.

In this paper, we use 1208 bp of mtDNA, external morphology and osteology to assess the taxonomic status, biogeography and evolution of the European brook newts.

MATERIAL AND METHODS

PHYLOGENETIC ANALYSIS

Samples and DNA extraction

A total of 54 specimens belonging to the family Salamandridae were used in the molecular study. These

included 29 individuals of *Euproctus* (27 specimens of Western brook newts including 20 *E. asper* and seven specimens of the new species, one *E. platycephalus* and one *E. montanus*), eight different species of *Triturus*, *Mesotriton* and *Lissotriton*, one *Neurergus kaiseri*, all three recognized species of *Pleurodeles* (see Carranza & Wade, 2004), one *Lyciasalamandra atifi* (Basoglu, 1967) and one *Salamandra salamandra bernardezi* Wolterstoff, 1928. The last two species were used as outgroups. Specimen data are given in Table 1 and Figure 1.

Genomic DNA was extracted from tissue samples following standard protocols described elsewhere (Carranza *et al.*, 1999, 2000). Primers used in both amplification and sequencing were cytochrome *b1* and cytochrome *b2* (Kocher *et al.*, 1989) for the cytochrome *b* (*cytb*) gene, 12Sa and 12Sb (Kocher *et al.*, 1989) for the 12S rRNA gene, and 16Sar and 16Sbr (Palumbi, 1996) for the 16S rRNA gene. Specific primers were designed to amplify the cytochrome *b* of the Pyrenean brook newts and the new species (Cytb1EuprF: 5'-CTAATGACCCACATYATACGAAAACTCACCCAC-3' and Cytb2EuprR: 5'-GAATGATATTTGTCTCAKGGCAGGACATATCCGAC-3'), and the 12S rRNA of some *Triturus*, *Mesotriton*, *Lissotriton* and *Euproctus* that could not be amplified with the standard primers (12StritF: 5'-AAACTGGGATTAGATACCCACTATGCC-3' and 12StritR: 5'-GAGGGTGACGGGCGGTGTGTGTGCGCGCT-3'). The three gene fragments were amplified by the polymerase chain reaction (PCR) and the resultant DNA was sequenced using the same standard protocols and conditions described by Carranza *et al.* (1999, 2000).

Phylogenetic analyses

DNA sequences were aligned using ClustalX (Thompson *et al.*, 1997) with default parameters (gap opening = 10; gap extension = 0.2). All the *cytb* sequences had the same length and therefore no gaps were postulated. These sequences were translated into amino acids using the vertebrate mitochondrial code and no stop codons were observed, suggesting they were probably all functional. Although some gaps were postulated in order to resolve length differences in the 12S rRNA and 16S rRNA gene fragments, all positions could be unambiguously aligned and were therefore included in the analyses.

Three methods of phylogenetic analysis were employed for all three independent partitions and the combined dataset and their results compared. These were: maximum likelihood (ML), Bayesian analysis and maximum parsimony (MP). Modeltest v.3.06 (Posada & Crandall, 1998) was used to select the most appropriate model of sequence evolution for the ML and Bayesian analyses of the independent partitions and the combined dataset, under the Akaike Informa-

tion Criterion. This was, in all four cases, the General Time Reversible model (GTR) taking into account the proportion of invariable sites (I) and the shape parameter alpha of the gamma distribution (G). For the MP analyses, apart from an unweighted analysis (ts = 1, tv = 1), independent analyses were also carried out for each dataset taking into account the observed transitions (ts)/transversions (tv) ratios and the presence of saturation in the *cytb* 3rd codon ts. These were: *cytb* (ts = 1, tv = 4); *cytb* (3rd codon ts = 0, tv = 1), 16S rRNA (ts = 1, tv = 2); 12S rRNA (ts = 1, tv = 2); combined analysis (ts = 1, tv = 4 and *cytb* 3rd codon ts = 0).

A second dataset including 354 bp of *cytb* sequence from *Triturus carnifex*, *T. marmoratus*, *T. pygmaeus* plus several representatives of the Western brook newts (including many populations of the Pyrenean brook newt and seven individuals of the new species from El Montseny) was also analysed. In this case, the most appropriate model of sequence evolution for the ML and Bayesian analyses selected by Modeltest v.3.06 (Posada & Crandall, 1998) under the Akaike Information Criterion was the Hishino–Kasegawa–Yano (HKY) model taking into account the shape parameter alpha of the gamma distribution (G). A saturation analysis clearly showed that, in this reduced dataset, not even the *cytb* 3rd codon transitions were saturated and, as a result therefore, all positions were included in the phylogenetic analyses. For the MP analyses of this reduced dataset, apart from an unweighted analysis (ts = 1, tv = 1) we also performed a weighted analysis (ts = 1, tv = 6) based on the observed ts/tv ratios.

Bayesian analyses were performed with MrBayes v.3.0b4 (Huelsenbeck & Ronquist, 2001). For the combined datasets (*cytb* + 12S + 16S), each partition had its own independent model of evolution (GTR + I + G in all three cases) and model parameters. Four incrementally heated Markov chains with default heating values were used. All analyses started with randomly generated trees and ran for 1.5×10^6 generations, with sampling occurring at intervals of 100 generations producing 15 000 trees. To ensure that the analyses were not trapped on local optima, the dataset was run three times independently, each run beginning with a different starting tree. For each independent analysis, the log-likelihood values of all trees saved were plotted against the generation time. After verifying that stationarity had been reached in terms of both likelihood scores and parameter estimation, the first 1000–1500 trees (depending on the dataset analysed) were discarded in all three runs and three independent majority-rule consensus trees were generated from the remaining (post-burnin) trees. The frequency of any particular clade of the consensus tree represents the posterior probability of that node (Huelsenbeck & Ronquist, 2001); only values above 95% were

Table 1. Details of material and sequences used in the present study. Numbers under Locality code refer to geographical localities given in Fig. 1.

Taxa	Locality code (see Fig. 1)	Locality	GenBank accession nos. cyt/b/16S/12S	Reference/Code
<i>Lyciasalamandra atifi</i>		Turkey	NC_002756 (complete mt genome)	Zardoya <i>et al.</i> 2003
<i>Salamandra s. bernardezi</i>		Oviedo (Spain)	DQ092219/DQ092260/DQ092285	E1712.19
<i>Pleurodeles waltl</i>		Perelló (Spain)	AY222531/DQ092261/AY222487	E1812.24
<i>Pleurodeles waltl</i>		Extremos (Portugal)	AY222515/DQ092262/AY222471	E1011.4
<i>Pleurodeles poireti</i>		Annaba (Algeria)	AY222507/DQ092263/AY222463	Anc13
<i>Pleurodeles nebulosus</i>		Tabarca (Tunisia)	AY222518/DQ092264/AY222474	E1812.10
<i>Pleurodeles nebulosus</i>		Larba (Algeria)	AY222504/DQ092265/AY222460	Anc10
<i>Pleurodeles nebulosus</i>		Constantine (Algeria)	AY222462/DQ092266/AY222506	Anc12
<i>Lissotriton helveticus</i>		Navarra (Spain)	DQ092220/DQ092267/DQ092286	E1806.17
<i>Lissotriton boscai</i>		Besullo (Spain)	DQ092221/DQ092268/DQ092287	E1806.12
<i>Lissotriton italicus</i>		Calabria (Italy)	DQ092222/DQ092269/DQ092288	E1806.18
<i>Triturus carnifex</i>		Latina (Italy)	U55949/U04703/U04702	Caccone <i>et al.</i> 1997
<i>Triturus karelinii</i> - 1		NE Greece (Greece)	DQ092223/DQ092270/DQ092289	E1806.19
<i>Triturus karelinii</i> - 2		NE Greece (Greece)	DQ092224/DQ092271/DQ092290	E1806.20
<i>Triturus pygmaeus</i>		Doñana (Spain)	DQ092225/DQ092272/DQ092291	E1806.29
<i>Triturus pygmaeus</i>		Cadiz (Spain)	DQ092226/DQ092273/DQ092292	E1806.28
<i>Triturus pygmaeus</i>		Sierra de Cazalla (Spain)	DQ092227/DQ092274/DQ092293	E1806.26
<i>Triturus pygmaeus</i>		Fuentes de León (Spain)	AY046082	Garcia-Paris <i>et al.</i> 2002
<i>Triturus marmoratus</i>		Busaco (Portugal)	DQ092228/DQ092275/DQ092294	E1806.24
<i>Triturus marmoratus</i>		Perelló (Spain)	DQ092229/DQ092276/DQ092295	E1806.27
<i>Triturus marmoratus</i>		Sanabria (Spain)	AY046081	Garcia-Paris <i>et al.</i> 2002
<i>Triturus marmoratus</i> - 1		Oporto (Portugal)	DQ092230/DQ092277/DQ092296	E1806.23
<i>Triturus marmoratus</i> - 2		Oporto (Portugal)	DQ092231/DQ092278/DQ092297	E1806.25
<i>Mesotriton alpestris inexpectatus</i>		Calabria (Italy)	DQ092232/DQ092279/DQ092298	E1806.1
<i>Neurergus kaiseri</i>		Iran	DQ092233/DQ092280/AY147250	Neurerg
<i>Euproctus montanus</i>	[13]	Corsica (France)	U55946/U04697/U04696	Caccone <i>et al.</i> 1997
<i>Euproctus platycephalus</i>	[14]	Sardinia (Italy)	U55947/U04699/U04698	Caccone <i>et al.</i> 1997
<i>Calotriton arnoldi</i> sp. nov. - 1	[1] (A2)	Montseny (Spain)	DQ092234/DQ092281/DQ092299	E1401.9
<i>Calotriton arnoldi</i> sp. nov. - 2	[1] (A2)	Montseny (Spain)	DQ092235/DQ092282/DQ092300	E1401.8
<i>Calotriton arnoldi</i> sp. nov. - 3	[1] (A2)	Montseny (Spain)	DQ092236	E1706.14
<i>Calotriton arnoldi</i> sp. nov. - 4	[1] (A2)	Montseny (Spain)	DQ092237	E1706.17
<i>Calotriton arnoldi</i> sp. nov. - 5	[1] (B1)	Montseny (Spain)	DQ092238	E1706.9
<i>Calotriton arnoldi</i> sp. nov. - 6	[1] (B1)	Montseny (Spain)	DQ092239	E1706.10
<i>Calotriton arnoldi</i> sp. nov. - 7	[1] (B2)	Montseny (Spain)	DQ092240	MontN
<i>Calotriton asper</i> - 1	[2]	Irati (Spain)	DQ092241	E209.4
<i>Calotriton asper</i> - 2	[2]	Irati (Spain)	DQ092242	E209.5
<i>Calotriton asper</i> - 3	[3]	Vidrà (Spain)	DQ092243	E1706.18
<i>Calotriton asper</i> - 4	[3]	Vidrà (Spain)	DQ092244	E1706.19
<i>Calotriton asper</i> - 5	[4]	Xixerella (Andorra)	DQ092245	E1706.7
<i>Calotriton asper</i> - 6	[4]	Xixerella (Andorra)	DQ092246	E1706.8
<i>Calotriton asper</i> - 7	[5]	Vall d'en Bac (Spain)	DQ092247	E209.2
<i>Calotriton asper</i> - 8	[5]	Vall d'en Bac (Spain)	DQ092248	E209.1
<i>Calotriton asper</i> - 9	[6]	Collada de Tosses (Spain)	DQ092249	E209.3
<i>Calotriton asper</i> - 10	[7]	Font de l'Ús (Spain)	DQ092250/DQ092283/DQ092301	E1401.7
<i>Calotriton asper</i> - 11	[8]	Berga (Spain)	DQ092251/DQ092284/DQ092302	E1401.5
<i>Calotriton asper</i> - 12	[8]	Berga (Spain)	DQ092252	E1706.11
<i>Calotriton asper</i> - 13	[9]	Ordesa (Spain)	DQ092253	E1706.16
<i>Calotriton asper</i> - 14	[9]	Ordesa (Spain)	DQ092254	E1706.15
<i>Calotriton asper</i> - 15	[10]	Pto. Monrepos (Spain)	DQ092255	E1706.12
<i>Calotriton asper</i> - 16	[10]	Pto. Monrepos (Spain)	DQ092256	E1706.13
<i>Calotriton asper</i> - 17	[11]	Susqueda (Spain)	DQ092257	Guilleri
<i>Calotriton asper</i> - 18	[12]	Vilanova de Meià (Spain)	DQ092258	Montsec1
<i>Calotriton asper</i> - 19	[12]	Vilanova de Meià (Spain)	DQ092259	Montsec2
<i>Calotriton asper</i> - 20		Pyrenees	U55945/U04695/U04694	Caccone <i>et al.</i> 1997

considered to indicate significant support (Wilcox *et al.*, 2002).

Both ML and MP analyses were performed in PAUP* v.4.0b10 (Swofford, 1998) and included heuristic searches involving tree bisection and reconnection (TBR) branch swapping with 10 and 100 random stepwise additions of taxa, respectively. In the MP analyses gaps were included as a fifth state. Reliability of the MP and ML trees was assessed by bootstrap analysis (Felsenstein, 1985), involving 1000 replications for the MP analyses and 100 replications for the ML analyses.

Topological incongruence among partitions was tested using the incongruence length difference (ILD) test (Mickevich & Farris, 1981; Farris *et al.*, 1994). In this test, 10 000 heuristic searches were performed after removing all invariable characters from the dataset (Cunningham, 1997). To test for incongruence among datasets we also used a reciprocal 70% bootstrap proportion (Mason-Gamer & Kellogg, 1996) or a 95% posterior-probability threshold. Topological conflicts were considered significant if two different relationships for the same set of taxa were both supported with bootstrap values $\geq 70\%$ or posterior-probability values $\geq 95\%$.

Topological constrains to test alternative topologies were constructed using MacClade v.4.0 (Maddison & Maddison, 1992) and compared with optimal topologies using the Shimodaira–Hasegawa (SH) (Shimodaira & Hasegawa, 1999) test implemented in PAUP*4.0b10 (Swofford, 1998).

ML estimates of divergence times for the combined dataset were obtained after discovery of lineage rate constancy across the tree using the likelihood ratio test (Huelsenbeck & Crandall, 1997). The error associated with finite sampling of nucleotides for recon-

structing branch lengths was calculated by a three-step non-parametric bootstrap procedure (Efron & Tibshirani, 1993): (1) 100 data matrices were generated using the SEQBOOT program in PHYLIP 3.57, (2) the matrices were imported into PAUP*4.0b10 and 100 trees with branch lengths were obtained using the GTR + I + G model of sequence evolution (see above) and the tree of Figure 2 as a constraint, and (3) trees with branch lengths were transformed into trees with node times using TREEEDIT v.1.0. The different values across the 100 trees were used to calculate the average and the standard deviation for the relevant nodes.

MORPHOLOGICAL AND OSTEOLOGICAL ANALYSES

Two hundred and fifty-nine alcohol-preserved adult specimens of Western brook newts were included in the morphological analyses. These were obtained from the following institutions: Museu de Zoologia de Barcelona (MZB), Instituto Pirenaico de Ecología de Jaca-CSIC (IPE), Museo Nacional de Ciencias Naturales-CSIC, Madrid (MNCN) and The Natural History Museum, London (BMNH) (see Appendix 1). In total, 16 variables related to body size, shape and skin granulation were selected (see Table 2). All linear measurements were taken to the nearest 0.01 mm by the same person using calipers. Variations in skin granulation were assessed with the aid of a binocular microscope by counting the number of keratinized protuberances within a 0.5×0.5 -cm square at several predetermined body areas (see Table 2).

Analyses of geographical patterns of morphological variation were performed in Statistica v.5.5 (Stat-Soft, Inc., Tulsa, OK, USA) and included a principal component analysis (PCA) and a canonical variate analysis

Table 2. Definition of the external measurements used in the multivariate morphological analysis

SVL	body length from snout to posterior side of the cloacal protuberance
HEADL	head length from snout to posterior margin of parotid area
DNAR	distance between narines
DORB	minimal distance between the inner angle of the eye orbits
DES	distance from the anterior angle of the eye orbit to snout
DEP	distance from the posterior angle of the eye orbit to posterior margin of parotid area
FLL	forelimb length from body insertion to tip of fourth digit
HLL	hindlimb length from body insertion to tip of fourth digit
AL	abdominal length between the closest insertion points of fore and hindlimb
TL	tail length from posterior angle of the cloacal protuberance to tip tail
TH	maximum tail height
TW	maximum tail width
HG	head granulation
DG	dorsal granulation
FG	flank granulation
TG	tail granulation

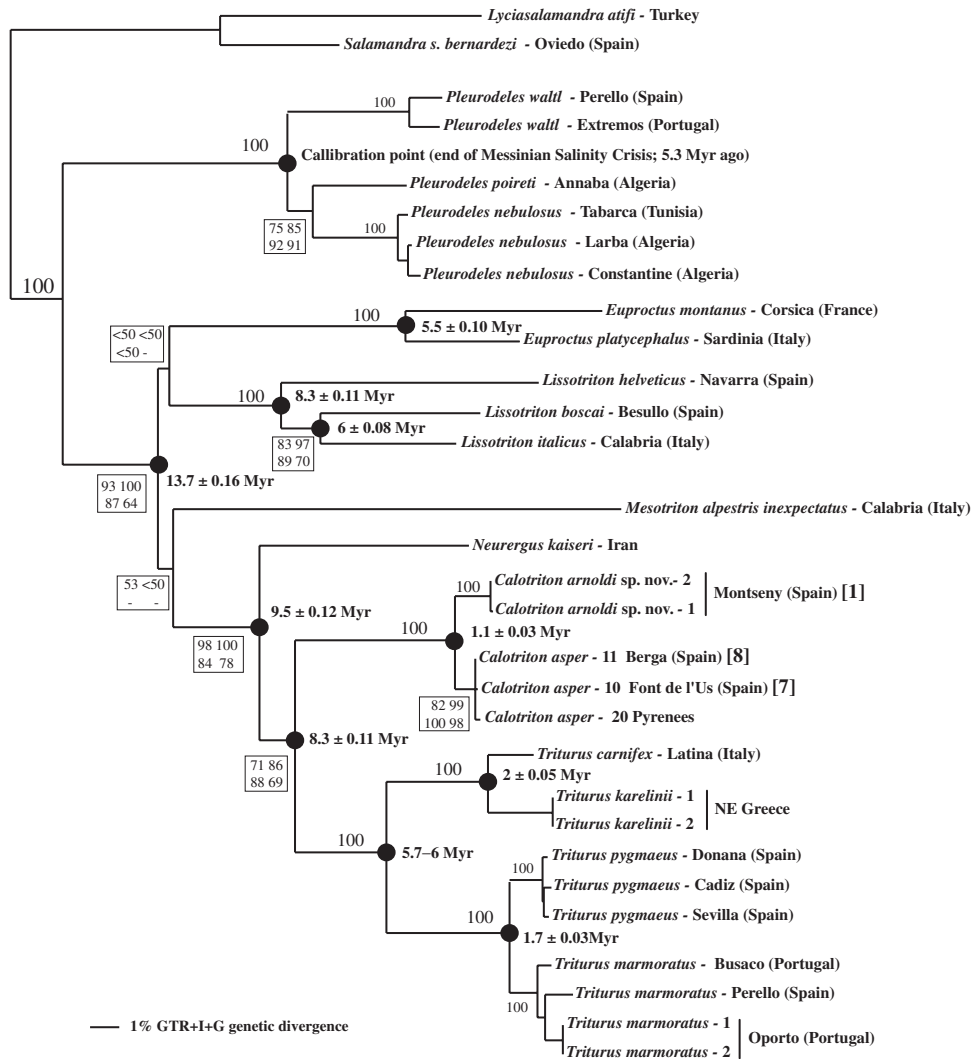


Figure 2. Maximum likelihood (ML) tree for some representatives of the Salamandridae (log likelihood -6882.66489, GTR + I + G model of sequence evolution) inferred from the combined dataset, which included cytb, 12S and 16S mtDNA sequences. Bootstrap support and Bayesian posterior probabilities for particular nodes are shown in the boxes with the figures indicating the percentage support for different analyses. Upper left, bootstrap support derived by ML (GTR + I + G). Upper right, posterior probability values from the Bayesian analysis (GTR + I + G). Lower left, maximum parsimony (MP) bootstrap support derived by MP (ts = 1; tv = 1). Lower right, bootstrap support derived by MP (ts = 1; tv = 4 and cytb 3rd codon ts = 0). When the difference between the four support values was < 5%, only the average value is shown. The ‘<’ symbol is used to show that the bootstrap/posterior probability value for that node is lower than 50% and the ‘-’ symbol indicates that a particular node is never recovered when using this method. Estimated ages are given for some bifurcations, which are marked by filled circles. Numbers in square brackets refer to localities shown in Fig. 1 and listed in Table 1.

(CVA) on log-transformed variables. CVA and the multivariate analysis of variance (MANOVA) require a previous definition of the operational taxonomic units (OTUs) used. In this intraspecific study, we considered the same OTUs all the specimens originating from geographically very close populations (usually inhabiting tributaries of the same river system within the same mountain valley). Geographically separated popula-

tions were considered different OTUs. In total, the sample comprised 157 males and 102 females assigned to 18 different OTUs covering most of the geographical distribution of the Pyrenean brook newt plus the new species from El Montseny: Aigüestortes, Ordesa, Ansó, Benasque, Jaca, Bigorre, Canfranch, Fanlo, Flumen, Gállego, Guarga, Oza, Navarra, Lac d’Oncet, Catalan Prepyrenees, Montseny, Cadí and Seros.

X-rays images used in the osteological comparisons were taken in a dedicated facility of the Natural History Museum, London following specific protocols optimized for urodeles. In total, 69 specimens belonging to the Salamandridae and covering the whole geographical distribution of the Western brook newts were X-rayed (see Appendix 2).

Two specimens of Pyrenean brook newt from Berga (locality 8 in Fig. 1) and one specimen of the new species from locality B1 from El Montseny were stained with alizarin red and cleared in KOH and glycerine.

CRITERIA FOR SPECIES RECOGNITION

The criteria for recognizing species used in this work was primarily based on that outlined by Good & Wake (1993), following Frost & Hillis (1990) and applied to other similar papers (García-París & Wake, 2000). In short, we consider as new species genetically cohesive units that are evolutionarily independent entities. Our main goal has been to be able to recognize and diagnose the taxa using morphological criteria, but we also present biochemical evidence that is diagnostic. When molecules suggest there is substantially more variation between samples than within accepted species, morphology was investigated and, if it also supported divergence, we recognized the unit as a new species. In the case of allopatric populations (evolutionarily independent at the present time), which may possibly resume interbreeding if they ever come into contact again, we also follow Good & Wake's (1993) criteria: 'Since knowledge of future events is impossible, inference about past events must suffice. The longer two populations have been isolated, and the more differences that have evolved between them in morphology, ecology, behavior, or biochemistry, the more likely it is that they will remain reproductively independent on recontact.'

RESULTS

PHYLOGENETIC RELATIONSHIPS

Independent ML, MP and Bayesian analyses of the three gene fragments (cytb, 12S and 16S rRNA) produced trees that differed in the position of some groups, such as *Neurergus kaiseri* and *Mesotriton alpestris* (Laurenti, 1768), as well as some other minor arrangements of taxa or individual samples. In all cases, these differences had low bootstrap and posterior-probability support, the respective values being less than 70% and less than 95%, respectively. Consequently, it was considered that there were no major topological conflicts between all three partitions (Mason-Gamer & Kellogg, 1996). The ILD test ($P > 0.20$) showed the three independent datasets

were not incongruent, and therefore we decided to combine them for further analyses. In total, the combined dataset included 1208 bp (300 bp of cytb, 346 bp of 12S rRNA and 562 bp of 16S rRNA). Of these, 427 were variable and 330 parsimony-informative, the respective numbers for each gene partition being as follows: cytb 141 and 115, 12S rRNA 100 and 75, 16S rRNA 186 and 140. A saturation analysis was carried out independently for each gene partition by plotting the uncorrected distances against the number of ts and tv. The results clearly showed that even when the uncorrected genetic divergence was more than 15%, only the cytb 3rd codon transitions were saturated. Consequently, the 3rd codon transitions of the cytb were given a weight of 0 in one of the MP analyses of the combined dataset and in an analysis involving only cytb but including all taxa.

To calibrate the phylogenetic trees, we used the methods described above (see Material and Methods) and an internal calibration point based on the assumption that divergence between *Pleurodeles waltl* Michahelles, 1830 and the ancestor of both north African *P. poireti* (Gervais, 1835) and *P. nebulosus* (Guichenot, 1850) was initiated by a vicariance event at the end of the Messinian salinity crisis, approximately 5.3 Mya, when the opening of the Strait of Gibraltar separated European and African populations of *Pleurodeles* (Carranza & Arnold, 2004; Carranza & Wade, 2004).

The results of the combined analyses for the combined dataset are presented in Figure 2 and all the different methods employed clearly indicate that *Euproctus* is polyphyletic. To test this result, the log likelihood of the ML tree presented in Figure 2 (-6882.664) was compared with the log likelihood of an ML tree constrained so that *Euproctus* was monophyletic (-6954.332). The results of the SH test showed that the constrained tree is significantly different, having a significantly worse log likelihood value than the unconstrained solution (Diff $-\ln L = 71.66744$; $P < 0.001$), and hence the tree in Figure 2, where *Euproctus* is polyphyletic, is consequently preferred.

Western brook newts are part of a very well-supported monophyletic group that began to diverge approximately 9.5 Mya and includes *Neurergus kaiseri*, *T. karelinii*, *T. carnifex*, *T. pygmaeus* (Wolterstoff, 1905) and *T. marmoratus*. All methods of analysis indicate *Neurergus kaiseri* is sister to the other representatives of this latter clade, although with a low bootstrap support and a posterior-probability value below 95. The same low bootstrap and posterior-probability values support the clade formed by the Western brook newts and a very well-supported (100% in all analyses) clade formed by two reciprocally monophyletic groups: the *T. cristatus* group, which includes *T. carnifex* and *T. karelinii*, and a clade formed by

T. marmoratus and *T. pygmaeus*. The molecular-clock analysis indicates that, contrary to previous suggestions (Herre, 1935; Steiner, 1950; Gasser & Clergue-Gazeau, 1981; Sbordoni *et al.*, 1985, 1990; Caccone *et al.*, 1994, 1997), the Western brook newts originated approximately 8 Mya, towards the end of the Miocene. Divergence between the *T. cristatus* group and *T. marmoratus* and *T. pygmaeus* was slightly later, at the end of the Miocene.

By contrast, the Tyrrhenian brook newts form a highly supported monophyletic group (100% in all analyses) with unresolved affinities to *Mesotriton* and *Lissotriton*. Our data support *E. montanus* and *E. platycephalus* having diverged from each other approximately 5.5 Mya, a date that coincides with the end of the Messinian salinity crisis and the refilling of the Mediterranean Sea (see Carranza & Arnold, 2004).

The phylogenetic analysis presented in Figure 2 also shows that variability within the Western brook newts is very high, with two clearly differentiated groups, one containing two specimens from El Montseny that will be described in this paper as a new taxon, and the other group including two populations from the Prepyrenees (populations 8 and 7; see Fig. 1 and Table 1) and one from the Pyrenees (specimen *E. asper* – 20 from Caccone *et al.*, 1997). The three Pyrenean–Prepyrenean specimens are very similar for all three gene fragments analysed here. The molecular-clock analysis indicates the Western brook newt population from El Montseny has been evolving independently from the rest of the Pyrenean and Prepyrenean populations analysed for more than 1 Myr. To explore this further, another analysis was carried out including 354 bp of cytb mtDNA (87 variable characters and 61 parsimony-informative characters) and more populations of Western brook newts from El Montseny (specimens from populations A2, B1 and B2), the Prepyrenees and the Pyrenees (see Table 1 and Fig. 1). The results of this phylogenetic analysis are shown in Figure 3 and support the hypothesis that populations from El Montseny are genetically different from the other populations of Western brook newts, including the closest populations from Susqueda, north of Les Guilleries Mountains (locality 11 in Fig. 1). This cytb mtDNA analysis also shows the degree of genetic divergence between El Montseny and the remaining populations of Western brook newts (4.1% average uncorrected genetic divergence) is similar to the genetic divergence that exists between *T. pygmaeus* and *T. marmoratus* (4.2% average uncorrected genetic divergence). Genetic divergence between populations outside El Montseny is almost non-existent, the most divergent ones being the Prepyrenean population from Ordesa and Puerto de

Monrepos (localities 9 and 10, respectively, in Fig. 1) and specimen 20 in Table 1 (from Caccone *et al.*, 1997), which differ from all other populations outside El Montseny in 0.56–0.84% of the cytb mtDNA fragment sequenced for this study. Genetic variability within El Montseny was found both between individuals inhabiting the same stream (population A2) and between the three populations sampled for the phylogenetic study (populations A2, B1 and B2). In population A2, one of the specimens presents two nucleotide differences (0.56% uncorrected genetic divergence) with the other two specimens analysed from the same population. The two specimens sequenced from population B1 and the single specimen sequenced from population B2 are identical in the 354 bp of cytb analysed. Genetic variability values between specimens from populations A2 and B range between 2 and 4 nucleotide changes in 354 bp (0.56 and 1.1% uncorrected genetic divergence, respectively).

OSTEOLOGY OF WESTERN BROOK NEWTS

In all five specimens of brook newts from El Montseny included in this study, the number and general shape of presacral vertebrae and ribs is similar to those in several other populations of Western brook newts (see Appendix 2). However, animals from El Montseny differ in the form of the first three or four caudosacral vertebrae. In *E. asper*, these have prominent transverse processes projecting at approximately 90° to the vertebral axis (Fig. 4F–I, P–R). This character is far more conspicuous in adult male specimens than in females or young, where these transverse processes are in general less prominent. By contrast, in specimens from El Montseny these caudosacral vertebrae have short transverse processes that are directed obliquely backwards (Fig. 4A–E, S). Examination of other salamandrids in the context of a phylogeny of this group (Titus & Larson, 1995; Larson *et al.*, 2003) indicates that the situation in animals from El Montseny is the plesiomorphic state, and that in the remaining populations of Western brook newts it is derived and constitutes a synapomorphy for them (see Fig. 4 and Appendix 2).

MULTIVARIATE ANALYSIS OF THE MORPHOLOGICAL CHARACTERS

A preliminary analysis of Western brook newts shows there are significant differences between the sexes in eight biometrical variables (two-way ANOVA; OTU, sex and interaction significant, $P < 0.01$, results not shown). As a result, all subsequent analyses were carried out independently for males and females. Morphological differences between OTUs were detected using MANOVA in both males (Wilks

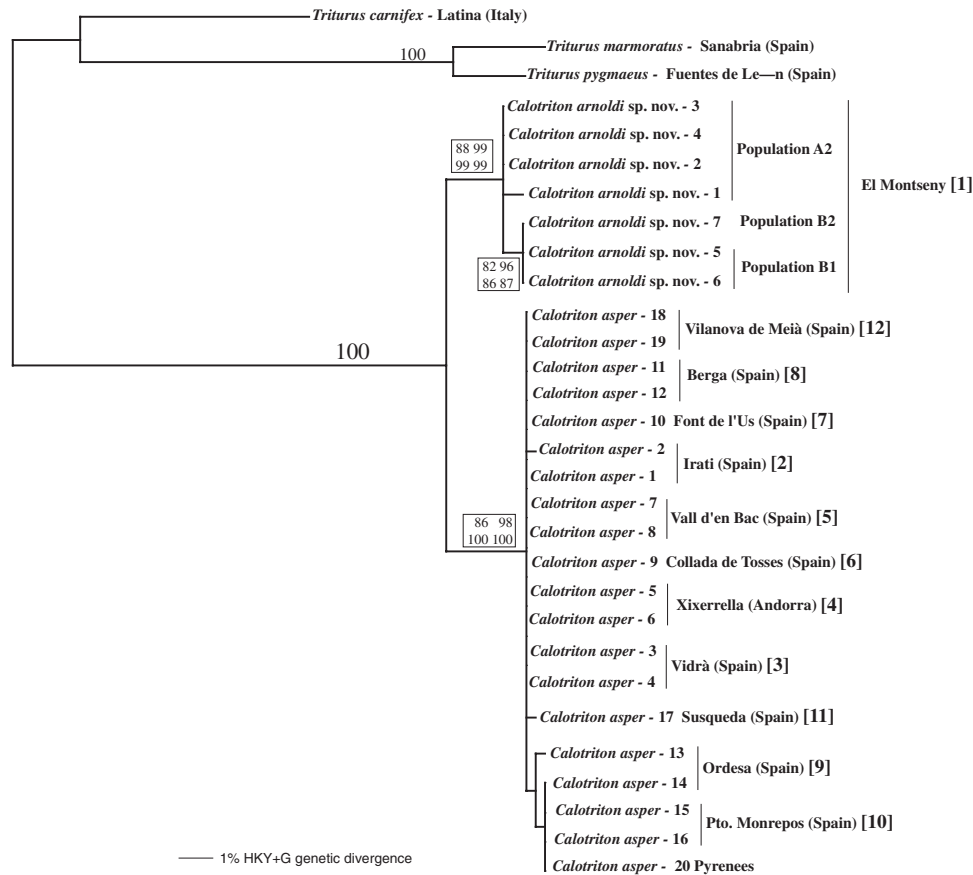


Figure 3. Maximum likelihood (ML) tree for some representatives of the Western brook newts (log likelihood -955.08162 , HKY + G model of sequence evolution) inferred from a reduced dataset, which included 354 bp of *cytb* mtDNA. Bootstrap support and Bayesian posterior probabilities for particular nodes are shown in the boxes with the figures indicating the percentage support for different analyses. Upper left, bootstrap support derived by ML (HKY + G). Upper right, posterior probability values from the Bayesian analysis (HKY + G). Lower left, maximum parsimony (MP) bootstrap support derived by MP ($ts = 1$; $tv = 1$). Lower right, bootstrap support derived by MP ($ts = 1$; $tv = 6$). When the difference between the four support values was $< 5\%$, only the average value is shown. Numbers in square brackets refer to localities shown in Fig. 1 and listed in Table 1.

Lambda = 0.041470, d.f. = 224, 1356, $P < 0.000001$) and females (Wilks Lambda = 0.006451, d.f. = 224, 763, $P < 0.000001$).

The first three principal components of the PCA of males and females represent 65.6 and 68.7% of the total variation, respectively (Table 3). PC1 shows positive loadings for both sexes in all four variables related to skin granulation, whereas all body measurements have higher values and are negatively loaded. In PC2, the situation is slightly different, with all variables having positive values and those related to skin granulation having much higher values than those related to overall body size, although two variables have slightly negative values in females. In PC1 there is a contrast between variables associated with overall size and those associated with skin granula-

tion. The large values of the component loadings of all 12 variables related to body size, in comparison with the much lower values of the component loadings of the four variables related to skin granulation, indicates that PC1 measures overall size. Although PC2 is mainly influenced by skin granulation, body size also has an influence. PC3 explains only 8.42% for males and 7.09% for females of the total information contained in the original variables and therefore it contributes little to providing a summary of the sample data. Interpretation of PC3 is more complicated than for PC1 and PC2.

A scattergram plot of the first three principal components for males and females independently produces a neat summary of the data and clearly identifies two groups that correspond to the individuals from El

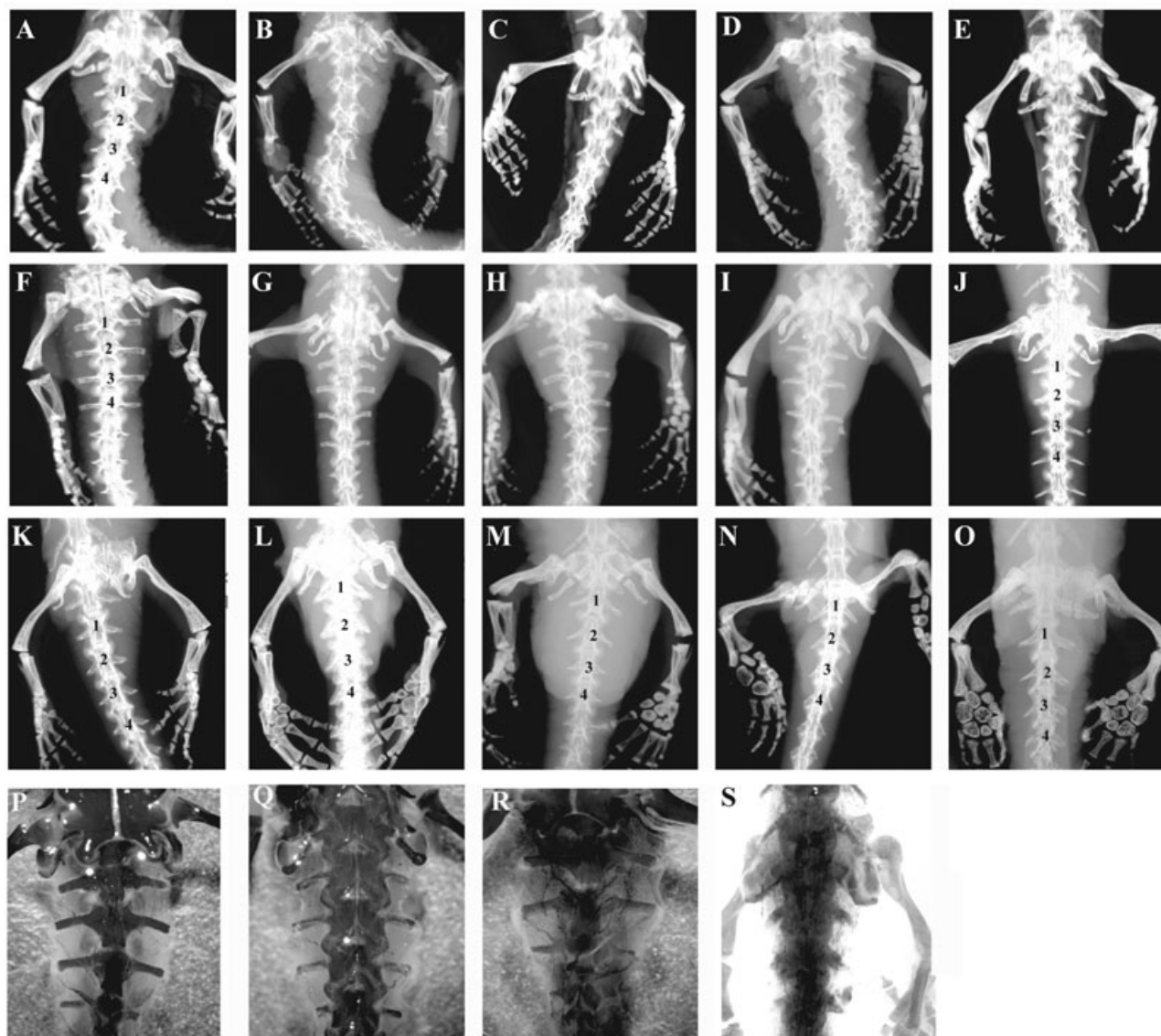


Figure 4. X-ray images of several species of newts and pictures of two clear-stained specimens showing a close-up of the caudosacral and caudal vertebrae. Numbers from 1 to 4 correspond to the first caudosacral vertebrae. A, *Calotriton arnoldi* sp. nov. female from El Montseny (MZB2004-0188). B, *C. arnoldi* male from El Montseny (MZB 82-8789). C, *C. arnoldi* male from El Montseny (MZB2004-0187). D, *C. arnoldi* male from El Montseny (MZB 82-8784). E, *C. arnoldi* female from El Montseny (MZB2004-0189). F, *C. asper* male from Baños de Benasque, Huesca, Spain (BMNH, 1970.2448). G, *C. asper* male from Torrent de Castelmouly, near Bagnères de Bigorre, France (BMNH, 1928.11.18-22). H, *C. asper* male from Pla de l'Estany, northern slope of the Maladeta, Spain (BMNH, 1928.11.22.13-14). I, *C. asper* female from Lac d'Oncet, French Pyrenees (BMNH, 1920.1.20.20). J, *Euproctus montanus* male (BMNH 82.11.15.50-55). K, *E. platycephalus* male (BMNH, 1947.1.4.4.x6). L, *Neurergus kaisseri* male (paratype – BMNH, 1952.4.2.85). M, *Triturus marmoratus* male (BMNH 86.6.29.52-56). N, *T. cristatus* male (BMNH, 1950.1.4.81-82). O, *T. karelinii* male (BMNH 96.3.28.18-19). P, ventral view of the caudosacral and caudal vertebrae of a clear-stained male of *C. asper* from Berga (locality 8 in Fig. 1). Q, dorsal view of the same *C. asper* specimen as in P. R, ventral view of male from Bergo. S, dorsal view of *C. arnoldi* from population B1 (locality 1 in Fig. 1).

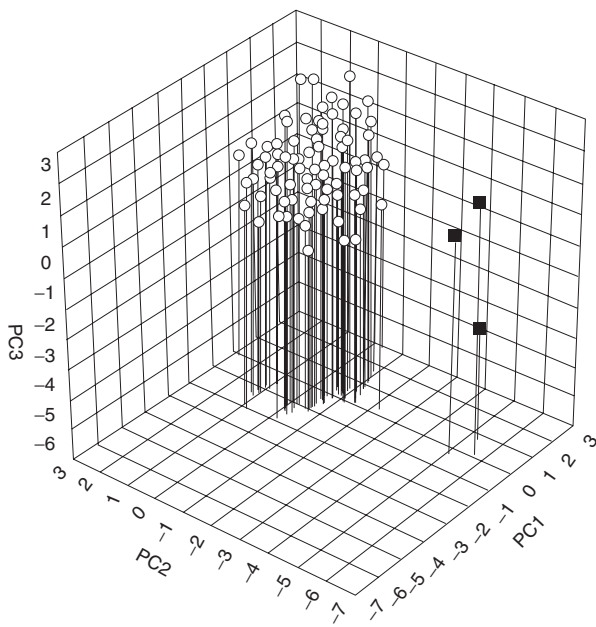
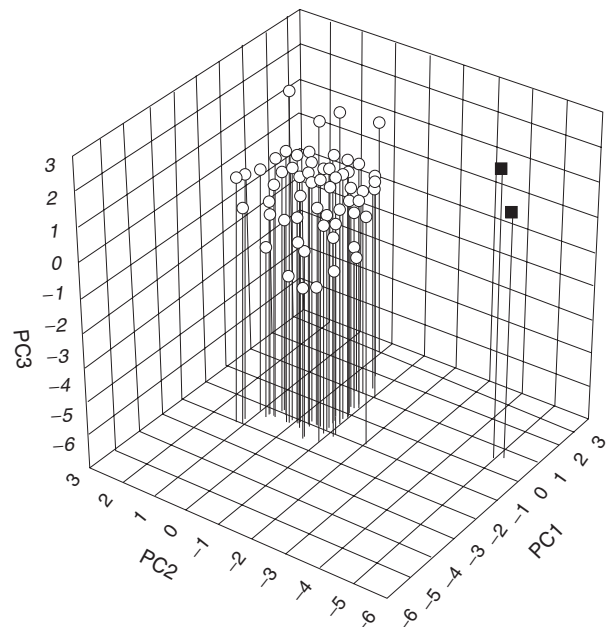
Montseny and to all the remaining individuals included in the multivariate analysis (see Figs 5, 6).

The results of the CVA analysis account for 75.9% of the variation in males and 66.6% of the variation in

females (Table 4). Graphical explorations of CVA specimen loadings for males and females independently are presented in Figures 7 and 8, respectively, and are congruent with results of the PCA (Figs 5, 6). Again,

Table 3. Factor loadings for the first three principal components (eigenvectors) for the principal components analysis on body-related and skin variables of *Calotriton*

Variable	Males			Females		
	1st PC	2nd PC	3rd PC	1st PC	2nd PC	3rd PC
SVL	-0.877	0.211	-0.105	-0.914	0.115	0.015
HEADL	-0.790	0.130	0.238	-0.794	-0.018	0.263
DNAR	-0.250	0.252	-0.619	-0.514	0.173	-0.621
DORB	-0.781	0.124	-0.063	-0.691	0.055	-0.110
DES	-0.722	0.178	0.091	-0.709	0.008	0.347
DEP	-0.698	0.139	0.191	-0.557	-0.046	-0.053
FLL	-0.747	0.126	-0.212	-0.740	0.257	0.355
HLL	-0.816	0.068	-0.015	-0.834	0.088	0.204
AL	-0.621	0.308	0.010	-0.770	0.160	-0.084
TL	-0.439	0.167	-0.656	-0.798	0.949	0.064
TH	-0.767	0.058	0.368	-0.752	0.147	-0.280
TW	-0.656	0.229	0.274	-0.700	0.249	-0.311
HG	0.357	0.793	-0.066	0.257	0.858	-0.001
DG	0.454	0.826	0.081	0.264	0.924	0.067
FG	0.404	0.838	0.109	0.264	0.924	0.067
TG	0.466	0.619	0.091	0.469	0.674	-0.001

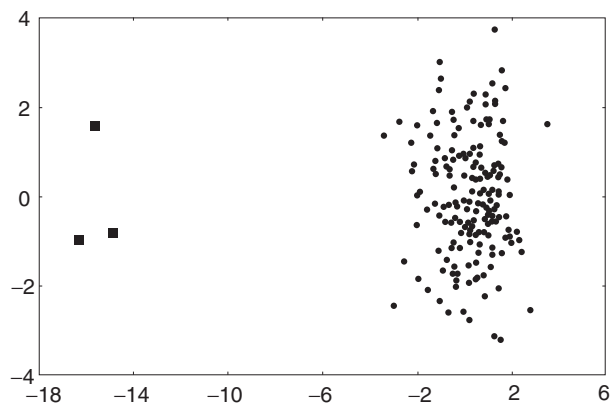
**Figure 5.** Scatter plot of principal component scores for the first three principal axes of the principal component analysis of male Western brook newts. Filled squares indicate specimens from the El Montseny massif and open circles the remaining specimens analysed.**Figure 6.** Scatter plot of principal component scores for the first three principal axes of the principal component analysis of female Western brook newts. Filled squares indicate specimens from the El Montseny massif and open circles the remaining specimens analysed.

the population from El Montseny forms an independent group from the remaining populations. A Student's *t*-test confirmed that, as already indicated by the PCA and CVA analyses, both male and female speci-

mens from El Montseny have a much lower number of granules in the head, dorsum, flank and tail than other Western brook newts included in the multivariate analysis (see Table 5). Descriptive statistics, of all

Table 4. Canonical coefficients for the first two canonical variables used in the morphometric analysis of *Calotriton*

Variable	Males		Females	
	1st CV	2nd CV	1st CV	2nd CV
SVL	0.075	0.672	-0.177	-0.386
HEADL	0.404	-0.021	0.272	0.100
DNAR	-0.097	0.248	0.037	-0.319
DORB	-0.075	-0.799	-0.068	0.328
DES	0.494	0.231	0.485	0.174
DEP	-0.315	0.227	-0.120	-0.695
FLL	0.150	-0.298	0.197	0.501
HLL	0.079	-0.313	0.064	-0.109
AL	0.031	-0.140	0.012	-0.314
TL	0.149	-0.582	0.084	-0.158
TH	-0.022	0.512	-0.024	0.001
TW	-0.294	-0.035	-0.055	0.554
HG	0.667	-0.020	0.830	-0.519
DG	-2.279	0.780	-1.802	-0.270
FG	2.740	-0.420	2.356	0.579
TG	-0.185	0.042	-0.387	-0.067

**Figure 7.** Plot of first and second canonical variables for male Western brook newts. Filled squares indicate specimens from the El Montseny massif and filled circles the remaining specimens analysed.

16 different values included in the morphometric analysis are presented in Tables 6–9.

SYSTEMATICS

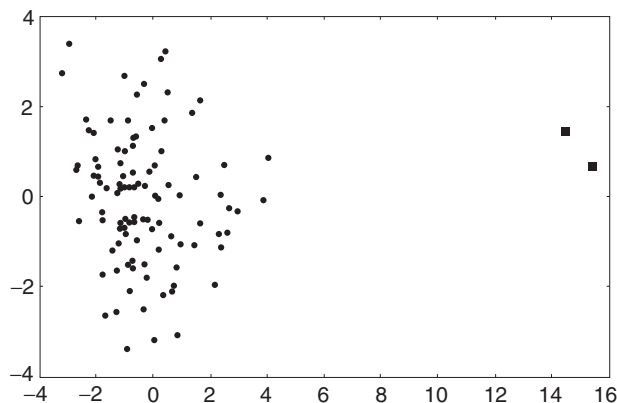
GENUS *EUPROCTUS* GENÈ 1839 *NOMEN PROTECTUM*
(SEE REMARKS BELOW)

Euproctus Genè, 1839: 281.

Type species: Euproctus rusconii Genè by monotypy.
Megapterna Savi, 1838: 211. *nomen oblitum* (see remarks below). Type species *Megapterna montana* Savi by monotypy.

Table 5. Results of Student's *t*-test analysis comparing the number of granules on the head (HG), dorsum (DG), flanks (FG) and tail (TG) between specimens from El Montseny and all remaining populations included in the multivariate analysis (see Appendix 1)

	<i>t</i>	d.f.	<i>P</i>
Males			
HG	4.866	155	0.00003
DG	4.536	155	0.00001
FG	2.617	155	0.00972
TG	2.960	155	0.00355
Females			
HG	3.661	100	0.0004
DG	3.409	100	0.00094
FG	2.447	100	0.01610
TG	3.428	100	0.00088

**Figure 8.** Plot of first and second canonical variables for female Western brook newts. Filled squares indicate specimens from the El Montseny massif and filled circles the remaining specimens analysed.

Phatnomatorhina Bibron, 1839: 131. Type species *Phatnomatorhina platycephalus* Bibron by monotypy.
Peleonectes Fitzinger, 1843: 33. Type species *Peleonectes platycephalus* Fitzinger by monotypy.

Remarks

Although the genus *Euproctus* Genè, 1839 was published a year later than *Megapterna* Savi, 1838, the latter was only a valid name for two years (synonymized by Bonaparte, 1840 2 : 405). Therefore, according to Article 23.9.1 of the International Code of Zoological Nomenclature, we consider *Megapterna* Savi a *nomen oblitum* because this name has not been used as a valid name after 1899 (Art. 23.9.1.1 of the ICZN) and we consider *Euproctus* Genè a *nomen protectum* because it has been mentioned in at least 25

Table 6. Morphometric data for male *Calotriton* excluding all specimens from El Montseny ($N = 176$, EE: standard deviation, all measurements are in millimetres). See Table 2 for abbreviations

Variable	Mean	EE	Maximum	Minimum
SVL	59.70	0.39	72.99	46.98
HEADL	15.72	0.12	20.13	10.70
DNAR	2.53	0.03	4.08	1.42
DORB	5.00	0.03	6.99	3.91
DES	4.80	0.03	6.76	3.63
DEP	8.53	0.09	11.44	4.11
FLL	15.55	0.13	19.77	9.76
HLL	17.98	0.14	22.44	13.08
AL	26.92	0.24	34.52	18.77
TL	41.30	0.30	54.60	24.68
TH	6.56	0.09	9.46	3.45
TW	4.46	0.05	6.15	2.35
HG	32.04	0.85	65	9
DG	22.37	0.62	51	6
FG	23.52	0.59	49	6
TG	26.76	0.62	49	11

Table 7. Morphometric data for male *Calotriton* from El Montseny ($N = 3$, EE: standard deviation, all measurements are in millimetres). See Table 2 for abbreviations

Variable	Mean	EE	Maximum	Minimum
SVL	57.73	0.72	58.64	56.30
HEADL	15.44	0.25	15.89	15.01
DNAR	2.67	0.42	3.51	2.13
DORB	4.77	0.13	5.02	4.55
DES	3.89	0.08	4.05	3.77
DEP	8.81	0.66	9.64	7.49
FLL	14.89	0.47	15.50	13.96
HLL	18.59	0.68	19.91	17.59
AL	25.37	1.19	27.75	23.94
TL	38.44	2.76	43.61	34.16
TH	8.01	0.83	9.61	6.78
TW	3.88	0.57	4.95	2.98
HG	5	2.08	8	1
DG	3	1.52	5	0
FG	11	3.21	16	5
TG	12	4.72	19	3

works in the last 50 years by a minimum of ten different authors (Art. 23.9.1.2 of the ICZN). As a result of this nomenclatorial act, *Euproctus* should be used to name the Tyrrhenian brook newts *E. montanus* and *E. platycephalus*. In compliance with Art. 23.9.1.2 of the ICZN the following list of works where *Euproctus* Genè has been used in the last 50 years is provided: Accordi *et al.* (1984: 1); Arnold (2002: 37); Bovero

Table 8. Morphometric data for female *Calotriton* excluding specimens from El Montseny ($N = 105$, EE: standard deviation, all measurements are in millimetres). See Table 2 for abbreviations

Variable	Mean	EE	Maximum	Minimum
SVL	60.81	0.64	78.16	47.15
HEADL	15.03	0.15	19.41	11.78
DNAR	2.50	0.04	3.59	1.37
DORB	4.84	0.04	6.30	3.17
DES	4.60	0.04	6.67	3.60
DEP	7.83	0.13	10.93	4.11
FLL	15.2	0.15	18.65	11.05
HLL	17.2	0.18	22.06	13.27
AL	27.44	0.41	37.01	17.82
TL	49.35	0.60	64.74	35.58
TH	5.20	0.08	6.90	3.37
TW	3.95	0.06	6.13	2.62
HG	35.70	1.33	69	8
DG	23.84	0.96	55	6
FG	23.43	0.82	46	8
TG	29.36	0.95	58	6

Table 9. Morphometric data for female *Calotriton* from El Montseny ($N = 2$, EE: standard deviation, all measurements are in millimetres). See Table 2 for abbreviations

Variable	Mean	EE	Maximum	Minimum
SVL	57.99	0.91	58.91	57.08
HEADL	14.46	0.23	14.69	14.23
DNAR	2.35	0.39	2.75	1.96
DORB	4.44	0.08	4.53	4.36
DES	4.17	0.27	4.44	3.90
DEP	8.30	0.08	8.38	8.22
FLL	11.82	0.10	11.93	11.72
HLL	15.81	0.78	16.60	15.03
AL	25.96	2.08	28.05	23.88
TL	42.02	1.69	43.71	40.33
TH	4.71	0.10	4.81	4.61
TW	3.19	0.25	3.45	2.94
HG	0.5	0.50	1	0
DG	0	0	0	0
FG	8.5	0.50	9	8
TG	5	1	6	4

et al. (2003: 1); Brizzi *et al.* (1995: 1); Caccone *et al.* (1994: 1, 1997: 1); Clergue-Gazeau (1971: 1); Clergue-Gazeau (1987: 1); Clergue-Gazeau & Bonnet (1980: 1); Clergue-Gazeau & Martínez-Rica (1978: 1); García-París (1985: 113); García-París *et al.* (2004: 110); Gasser (1975: 1); Guillaume (1999: 1); Guillaume (2002: 1); Hervant, Mathieu & Durand (2000: 1, 2001: 1); Lecis & Norris (2003: 1, 2004: 1); Llorente

et al. (1995: 34); Martínez-Rica & Clergue-Gazeau (1977: 1); Mertens & Wermuth (1960: 17); Montori (1988: 20); Montori (1991: 1); Montori & Campeny (1991: 1); Montori *et al.* (1997: 106); Montori *et al.* (2002: 48); Montori & Pascual (1981: 1); Read (1998: 1); Salvador & García-París (2001: 37); Salvidio, Sindaco & Emanuelli (1999: 1); Sbordoni *et al.* (1982: 1, 1985: 1); Schlegel (1997: 1); Serra-Cobo *et al.* (2000a: 1); Serra-Cobo, Marques-Bonet & Martínez-Rica (2000b: 1); Steinfartz *et al.* (2002: 1); Thiesmeier & Hornberg (1990: 1); Thiesmeier *et al.* (1997: 1); Thorn (1968: 253); Uiblein *et al.* (1992: 1); Uiblein, Engelke & Parzefall (1995: 1).

Diagnosis

Small- to medium-sized newts (70–150 mm including tail). Fronto-squamosal arch ligamentous or bony. Cadosacral vertebrae with short transverse processes that are directed obliquely backwards (Fig. 4J, K). Head rather large, depressed, longer than broad. Upper jaw slightly or completely overhanging. Paratoid glands more or less distinct. Gular fold absent. Limbs moderate in size, four fingers and five toes, all free. Skin finely tubercular on the flanks and head, smooth beneath. Body relatively flat or slightly rounded in cross-section, no dorsal crest even during the breeding season. Tail about as long as head and body and compressed laterally. Lungs absent or very reduced. Males have spurs on hind legs (see Fig. 9A, B). Caudal capture of female by male followed by direct transfer of spermatophores.

Species

Euproctus montanus (Savi, 1838) and *E. platycephalus* (Gravenhorst, 1829).

Distribution

Restricted to the Mediterranean islands of Corsica and Sardinia.

EUPROCTUS MONTANUS (SAVI, 1838) (FIGS 4J, 9B, D, F)

Megapterna montana Savi, 1838: 211

Triturus (Euproctus) montanus Boulenger, 1878: 308

Euproctus montanus Giglioli, 1878: 97; Despax, 1923: 11; Mertens & Muller, 1928: 10; Mertens & Muller, 1940: 8; Wolterstoff & Herre, 1935: 224; Mertens & Wermuth, 1960: 18; Thorn, 1968: 260

Molge montana Boulenger, 1882: 23

Triton montanus Schultze, 1891: 170; Schreiber, 1912: 53

Triton (Euproctus) montanus Wolterstoff, 1900: 6

Diagnosis

Adults usually between 80 and 100 mm including the tail with a maximum of 130 mm. Fronto-squamosal arch ligamentous. Frontal bones with distinct orbital processes. Body relatively flat, no dorsal crest. Dorsal skin smooth in aquatic phase and finely tubercular in terrestrial phase, smooth beneath in both aquatic and terrestrial phases. Brown or olive above, sometimes with lighter yellowish, reddish or greenish markings that may form a thin vertebral line. Venter yellowish, grey or brown, speckled with whitish. Snout rounded, upper jaw slightly overhanging, absence of labial folds and gular fold. Large well-defined paratoid glands present on each side of the neck. Tongue rather large, subcircular, attached along the median line, slightly free behind, protractile. Spurs on hind legs of males laterally flattened and not strongly protruding (Fig. 9B, D). Fingers and toes short and depressed (Fig. 9B, D). Tail a little shorter than head and body, compressed laterally and pointed; without crests. Cloacal swelling of males conical and projecting backwards. Cloacal chamber containing a pseudopenis, which may protrude from the cloacal cavity during amplexus (Fig. 9D). Cloacal swelling hemispherical in females with a vertical slit, the borders of which are scarcely swollen (Fig. 9F).

Material examined

BMNH 1882.11.15.51–55; BMNH 1882.4.12.2–3; BMNH 1891.6.15.6–13; BMNH 1928.12.20.378–397; BMNH 1952.1.4.35–47; BMNH 1955.1.1.6–11. Specimens included in the X-ray osteological analysis are listed in Appendix 2.

Distribution

Euproctus montanus is restricted to the Mediterranean island of Corsica (France), where it is found from sea level up to 2260 m, being more abundant in still or running waters in mountainous areas situated between 600 and 1500 m.

EUPROCTUS PLATYCEPHALUS (GRAVENHORST, 1829) (FIGS 4K, 9A, C, E)

Molge platycephala Gravenhorst (part) 1829: 84.

Euproctus rusconni Genè, 1839: 28, pl. 1, figs 3, 4; Gray, 1858: 139; Duméril, Bibron & Duméril (part) 1854: 158 (under *Euproctus rusconi*).

Euproctus platycephalus Bonaparte, 1832–1841: vol 26; Lataste, 1878: 495; Mertens & Muller, 1928: 10; Wolterstoff & Herre, 1935: 224; Mertens & Muller, 1940: 8; Mertens & Wermuth, 1960: 18; Thorn, 1968: 265.

Phatnomatorhina platycephalus Bibron, 1839: sign 131.

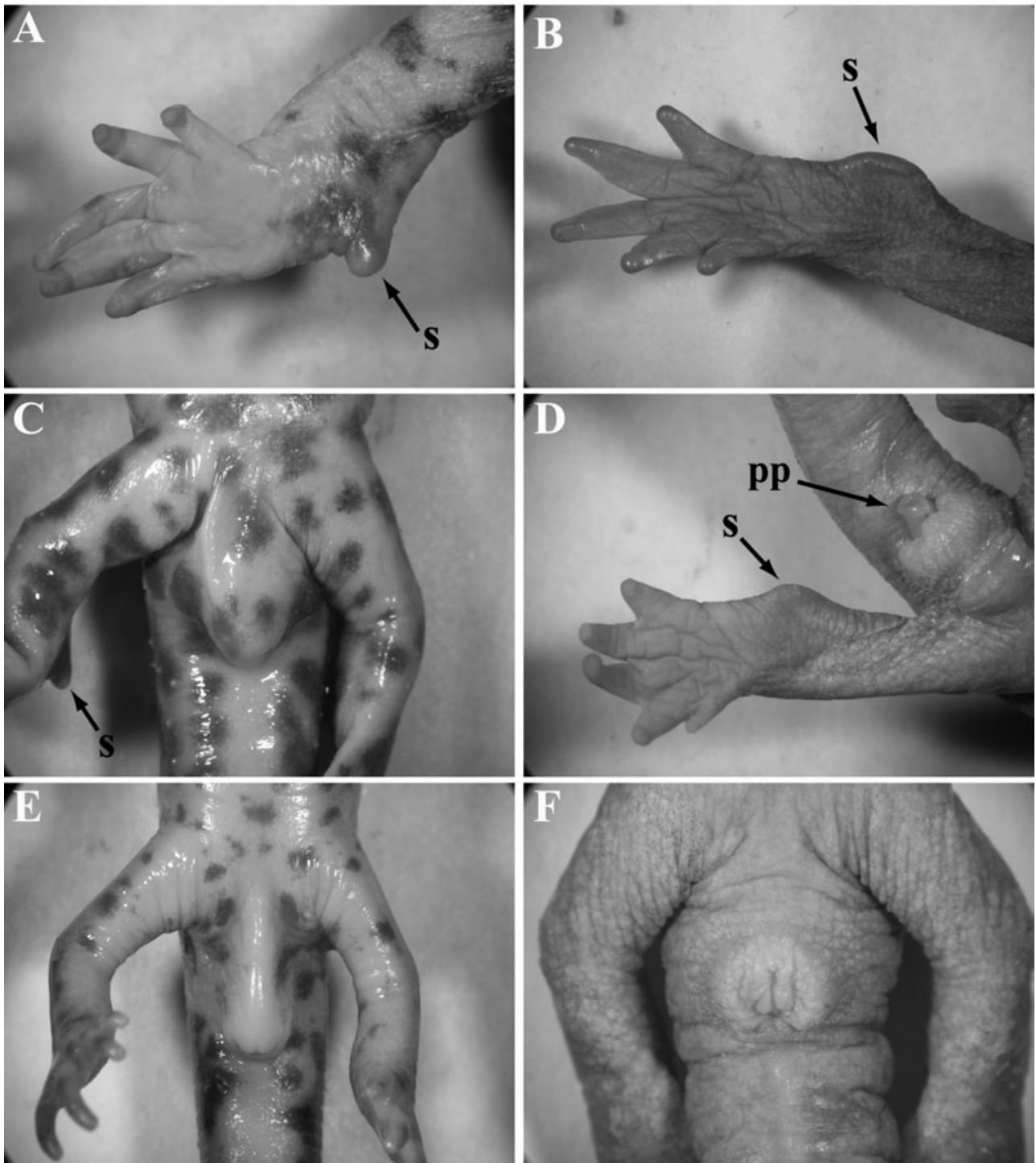


Figure 9. A, detail of a hind leg of a male *Euproctus platycephalus* showing the spur (s) that characterizes the Tyrrhenian brook newts. B, detail of hind leg of a male *E. montanus* showing the spur (s). C, detail of a male cloaca of *E. platycephalus*; the spur on the right hind leg is also visible. D, detail of a male cloaca of *E. montanus* showing the pseudopenis (pp) and the spur. E, detail of a female cloaca of *E. platycephalus*. F, detail of a female cloaca of *E. montanus*.

Pelonectes platycephalus Fitzinger, 1843: 33.
Triton rusconii Bonaparte, 1832–1841: 453; Gray, 1850: 24.
Triton platycephalus Strauch, 1870: 50.
Triturus (Euproctus) platycephalus Boulenger, 1878: 307.
Triturus (Euproctus) rusconii Boulenger, 1878: 308; Wolterstoff, 1900: 5.
Molge rusconii Boulenger, 1882: 24.
Triton rusconii Schultze, 1891: 170; Schreiber, 1912: 58.

Diagnosis

Adult males approximately 127 mm including tail, adult females up to 112 mm with a maximum of 150 mm for both sexes. Fronto-squamosal arch bony. Body relatively flat, absence of dorsal crest. Dorsal skin smooth or finely tubercular above, venter completely smooth. Typically brown or olive above, variegated with greenish, yellowish or light grey with an orange-reddish vertebral stripe. The venter is whitish on the sides and yellowish or reddish along the centre, with irregularly distributed black spots on the belly and throat, especially in males. Head depressed, much longer than broad, snout semi-elliptical, upper jaw overhanging, upper lips with well-developed labial folds. No gular fold. Paratoid glands not very well defined. Tongue small, subelliptical, free at the sides. Spurs on hind legs of males strongly protruding (like a fifth finger), especially in older specimens (Fig. 9A). Fingers rather elongate and slender (Fig. 9A). Tail about as long as snout–vent length, broad at the base and increasingly compressed laterally, ending in an obtuse point, with small upper and lower crests, the latter beginning half way along the tail. Cloacal swelling conical in both sexes with a circular opening (Fig. 9C, E).

Material examined

BMNH 1882.12.15.56–59; BMNH 1886.10.19.2–3; BMNH 1895.4.16.2–10; BMNH 1885.4.16.11 (skeleton); BMNH 1895.5.14.7–9; BMNH 1928.12.20.365–373; BMNH 1903.1.25.14–16; BMNH 1920.1.20.2055. Specimens included in the X-ray osteological analysis are listed in Appendix 2.

Distribution

Euproctus platycephalus is restricted to the Mediterranean island of Sardinia, Italy, where it inhabits still or more usually running waters in mountainous areas situated between 50 and 1800 m altitude, being more abundant above 600 m.

GENUS *CALOTRITON* GRAY, 1858

Calotriton Gray, 1858: 139

Type species: Hemitriton punctulatus Dugès, 1852 by monotypy (= *Calotriton asper* (Dugès, 1852))
Hemitriton Dugès, 1852: 266. Type species not designated. Name already occupied by *Hemitriton* Hoeven, 1833: 305.

Remarks

The use of *Hemitriton* Dugès, 1852 for the Western brook newts pre-dates *Calotriton* Gray, 1858, but it is already occupied by *Hemitriton* Hoeven, 1833, so *Calotriton* is the first available name for the group.

Diagnosis

Small- to medium-sized newts (70–167 mm including tail). Fronto-squamosal arch bony. Palatine teeth in two series in contact anteriorly, diverging posteriorly, therefore forming an inverted Y-shaped figure. The series starts on a line with the choanae. Tongue small, elliptical, the sides slightly free. Head depressed, longer than wider, fairly robust (especially males), with the intranasal cavity elongated and well developed. The greatest head width is at the posterior corners of eyes. Prominent swellings on the posterior sides of the head (see detail in Fig. 10A, C). Upper jaw overhanging with well-developed lateral upper labial folds extending over the edge of the lower jaw. Paratoid glands absent. Gular fold present. Skin covered with tubercles bearing horny tips, more or less strongly tuberculate above, less tuberculate or completely smooth beneath. Limbs moderate, four fingers and five toes, all depressed and free, with the tips covered by a black corneous nail-like sheath. No distinct spurs on male or female hind legs. Body rounded or slightly depressed, absence of cutaneous dorsal and caudal crest even during the breeding season. Tail ending in an obtuse point and about as long as head and body (longer in females) and compressed from side, deeper in males. Lungs very reduced. Cloacal swelling rounded and with a vertical slit in males, and conical or cylindrical, directed backwards, in females. Caudal capture of female to facilitate direct transfer of spermatophores.

Species

Calotriton asper (Dugès, 1852) and *C. arnoldi* sp. nov.

Distribution

The distribution range includes most of the Pyrenees, the Massif of El Montseny and some intermediate areas (see shadowed area in Fig. 1).



Figure 10. A, female *Calotriton arnoldi* sp. nov. from population A2 with uniform chocolate coloration. B, male specimen of *C. arnoldi* from population A2 showing several yellowish blotches on the sides of the tail and body. C, close up of same female as in A. D, female *C. arnoldi* from population B2 showing the typical uniform chocolate coloration of this population. E, larvae of *C. arnoldi* from population B1. F, same female as in A in ventral view. G–H, juvenile of *C. arnoldi* from population A2 with several yellowish blotches on the sides of the tail and body (note the absence of the vertebral line that is typical of *C. asper*). I–J, details of the female cloaca of the same specimen as in A. K–L, detail of the female cloaca of a living specimen of *C. asper* from Berga, Spain (K) and Ordesa, Spain (L).

CALOTRITON ASPER (DUGÈS, 1852)

(FIGS 4F–I, P–R, 10K–L)

- Triton glacialis* Philippe 1847 : 20 (*nomen nudum*)
Hemitriton repandus Duméril & Valenciennes, 1852.
 In Dugès, 1852: 256 (*nomen nudum*)
Hemitriton cinereus Dugès, 1852: 263, pl. 1, figs 14, 15.
Hemitriton rugosus Dugès, 1852: 264, pl. 1, figs 16, 17.
Hemitriton puncticulatus Duméril 1852. In Dugès, 1852: 265 (*nomen nudum*)
Hemitriton punctulatus Dugès, 1852: 265, pl. 1, fig. 18.
Hemitriton asper Dugès, 1852: 266 (*nomen conservandum*)
Hemitriton bibronii Dugès, 1852: 266, pl. 1, figs 19, 20.
Triton Pyrenaeus Duméril, Bibron & Duméril, 1854: 139.
Triton rugosus Duméril, Bibron & Duméril, 1854: 150.
Triton cinereus Duméril, Bibron & Duméril, 1854: 151.
Triton repandus Duméril, Bibron & Duméril, 1854: 151, ATLAS, pl. 106, fig. 2.
Triton puncticulatus Duméril, Bibron & Duméril, 1854: 152. Incorrect spelling of *punctulatus*.
Triton Bibroni (incorrect spelling of *bibronii* Dugès). Duméril, Bibron & Duméril, 1854: 153 (*nomen nudum*).
Calotriton punctulatus Gray, 1858: 139.
Euproctus Pyrenaeus Lataste, 1878: 495.
Molge aspera Boulenger, 1882: 24; Maluquer, 1916: 58.
Euproctus aspera Camerano, 1885: 419 (lapsus).
Euproctus asper var. *rugosa* Bedriaga, 1895: 150.
Triton asper Wolterstoff, 1900: 31; Wolterstoff, 1902: 12.
Molge bolivari Bosca, 1918: 58.
Euproctus asper Wolterstoff, 1925: 61; Wolterstoff & Herre, 1935: 224; Thorn, 1968: 253.
Euproctus asper var. *peyreladensis* Wolterstoff, 1925: 71.
Euproctus asper var. *d'Onceti* Wolterstoff, 1925: 66.
Euproctus asper var. *castelmouliensis* Wolterstoff, 1925: 66; Mertens & Wermuth, 1960: 17.
Euproctus (Hemitriton) asper Wolterstoff, 1925: 296.
Euproctus asper asper Mertens & Muller, 1928: 9; Mertens & Wermuth, 1960: 17.
Euproctus asper castelmouliensis Mertens & Muller, 1940: 8; Mertens & Wermuth, 1960: 17.

Diagnosis

Maximum total length of preserved males included in our study 127.5 mm, females 143 mm. Measurements for 14 other morphometric characters are presented in Tables 6, 8 for males and females, respectively. First 3–4 caudosacral vertebrae with prominent transverse

processes at approximately 90° to the vertebral axis (Fig. 4F–I, P–R). This character is very clear in adult male specimens (Fig. 4F–H, P–R) and less evident in females and juvenile specimens (Fig. 4I, our pers. observ.). Skin usually very rough, covered with a high proportion of tubercles bearing horny tips (including the venter and underside of the tail; see Fig. 10K, L). Usually greyish, olive, or blackish above, uniform or with light yellowish spots, which often are confluent into a broad vertebral line, especially in subadults and younger adults. Venter opaque with a yellow, orange or red central band usually unspotted with large dark markings at sides or with few spots. Cloacal swelling bulbous-conical in females, usually relatively broad at the base (frequently broader than the tail) (Fig. 10K, L).

Material examined

Museum specimens used in the multivariate morphological analysis are listed in Appendix 1. Specimens included in the X-ray osteological analysis are listed in Appendix 2. Observations on live material include specimens from the whole distribution range of the species (see Fig. 1).

CALOTRITON ARNOLDI SP. NOV.

(FIGS 4A–E, S, 10A–J)

Euproctus asper (Dugès, 1852: 1) (part); Llorente *et al.*, 1995: 34; Montori, 1988: 20; Montori & Campeny, 1991: 1; Montori *et al.*, 1997: 106; Montori *et al.*, 2002: 48; Montori & Pascual, 1981: 1; Salvador & García-París, 2001: 35; García-París *et al.*, 2004: 120.

Diagnosis

Similar to *Calotriton asper* but differs in the following features: mitochondrial DNA sequences different (divergence: cytb, 4.1%; 12S rRNA, 1.2%; 16S rRNA, 0.7%; GenBank accession numbers: DQ092234–40, DQ092281–82, DQ092299–300). First caudosacral vertebrae with short transverse processes that are directed obliquely backwards (Fig. 4A–E, S) (not elongate and at approximately right angles to the vertebral axis as in *C. asper*; Fig. 4F–I, P–R). In each sex, spiny-tipped tubercles on the dorsum smaller and fewer, so surface generally much smoother than in *C. asper* (Fig. 10A–D). Tubercles never present in the venter or underside of the tail (Fig. 10F, I–J) (can be present in *C. asper* Fig. 10K, L). Maximum body-size of male and female alcohol-preserved specimens less (maximum females 102.62 mm, maximum males 102.25 mm), compared with 143 mm and 127.5 mm of maximum total length of adult female and male

C. asper, respectively. Dorsum dark, chocolate-coloured (Fig. 10A–D), sometimes with light silvery-gold stippling on the sides. Adult and young *C. arnoldi* from populations B1 and B2 always uniform dorsally, with a light and very thin brownish-orange stripe from the base to the tip of the tail, sometimes extending further into the body (see Fig. 10D). Some adult and young specimens from populations A1–A3, apart from the thin orange stripe mentioned above, also present light greenish or yellowish blotches on the sides of the tail and body (Fig. 10B), these being more obvious when young (Fig. 10G, H). Complete absence in both adults and young of the broad yellow or light-coloured vertebral stripe common in *C. asper* (especially in young *C. asper*). Throat very light, of a pink-ivory colour, largely unspotted or with a lightly dark stippling (Fig. 10F). Venter translucent (follicles can be clearly seen by transparency in adult females), of a light ochre-brown colour, with dark markings of approximately the same colour as the dorsum consisting of dark stippling that are best developed at the sides (Fig. 10F) (belly opaque with large dark spots or blotches usual in *C. asper*). Throat posterior to gular region with a relatively large whitish-ivory immaculate blotch (see Fig. 10F). Female cloaca cylindrical, relatively narrow and of a contrasted bright reddish-orange colour at the tip (Fig. 10F, I–J) (usually bulbous-conical, broader at the base in *C. asper*; see Fig. 10K, L). When manipulated, adult males, females and young specimens quickly release a whitish, noxious, sticky and very odorous skin secretion, probably used as defence mechanism against predators (under similar conditions and much longer manipulation times, adult *C. asper* secrete much less quantities of a much less odorous substance).

Material examined

Type material. HOLOTYPE of *C. arnoldi* deposited at the Museu de Zoologia de Barcelona (MZB), Spain, with the following accession number and data: MZB 82-8784. Adult male preserved in alcohol; Fogars de Montclús, Montseny, Spain, collected 2 May 1980. Collectors Xavier Pascual and Albert Montori. PARATYPES: MZB 82-8789 adult male preserved in alcohol; Fogars de Montclús, Montseny collected 2 May 1980. Collectors Xavier Pascual & Albert Montori. MZB 2004-0187 adult male preserved in alcohol, Fogars de Montclús, Montseny, April 1980; MZB 2004-0188 adult female preserved in alcohol, Fogars del Montclús, Montseny, April 1980; MZB 2004-0189, adult female preserved in alcohol, Fogars de Montclús, Montseny, April 1980.

Additional material examined. Apart from the type material preserved in alcohol listed above, morpholog-

ical observations were based on 59 live specimens from populations A2, A3, B1 and B2. All observations were carried out during the course of an amphibian study from the Natural Park of El Montseny. No additional specimens were preserved for conservation reasons. Instead, data on the morphological appearance of the live specimens was carefully recorded by S.C. and F.A. directly in the field. In total, 20 specimens from locality A2 (10 females 6 males and 4 juveniles), 4 specimens from locality A3 (3 females and 1 male), 32 specimens from population B1 (15 females, 12 males, 3 juveniles and 2 larvae) and 3 specimens from population B2 (2 females and 1 male) were observed and used as a reference for the diagnosis of *C. arnoldi* (see above).

Description of the holotype (MZB 82-8784)

Measurements: SVL 58.64 mm, HEADL 15.42 mm, DNAR 3.51 mm, DORB 5.02 mm, DES 4.05 mm, DEP 9.32 mm, FLL 15.22 mm, HLL 18.27 mm, AL 24.44 mm, TL 37.56 mm, TH 9.61 mm, TW 3.71 mm.

An adult male. Head strongly flattened, broadest at level of eyes; snout blunt with convex sides in dorsal view, canthus rostralis marked; areas above eyes raised, their width about a third of the distance between them; prominent swellings on the posterior sides of the head present. Body with oval cross-section and some dorsoventral compression; tubercles tipped with hard blunt spines widely distributed on dorsum, becoming smaller and more scattered on head and, on flanks, mainly distributed between the vertical grooves. Cloacal swelling hemispherical with a sagittally elongate opening from which grooves extend laterally. Digits on fore and hind limbs 4:5, not elongate, unwebbed. Tail short (about 64% of snout–vent distance) and strongly compressed laterally, very deep basally (maximum depth 25.5% of length) and tapering gradually to a blunt point.

Colour in alcohol

Dark chocolate brown above with greyish tinge, flanks and sides of tail with scattered irregular, pale grey spots. Underside dark cream, brighter under tail; belly partly translucent with obscure dark markings consisting of dark stippling that are best developed at the sides; throat posterior to gular region with a large dark blotch (immaculate ivory in live specimens, see above). Throat, upper lip margins, underside of tail, and ulnar and palmar aspects of limbs pale and immaculate; tips of digits dark brown.

Other distinguishing features of holotype

A transverse tear across posterior belly extending on to the sides of the body, and a shallow notch on upper border of tail near its tip.

Variation

All living and preserved specimens show the same distinctive traits of the species. The only variation observed is in the pattern of coloration. Some *Calotriton arnoldi* from populations A1–A3 have irregular pale yellow spots on the tail and body flanks (Fig. 10B), usually more numerous and conspicuous in post-metamorphic newts than in adults (see Fig. 10G–H). The extension of pigmentation on the belly is also slightly variable, this being the result of individual variation rather than seasonal variation (our pers. observ.).

Distribution, habitat and conservation

Calotriton arnoldi has only been found in five mountain streams (populations A1–A3, B1 and B2), all within the boundaries of El Montseny Natural Park. *Calotriton arnoldi* is found between 600 and 1200 m in oligotrophic, cold (below 15 °C) fast running waters preferentially in beech forest (*Fagus sylvatica*) but also in Holm oak forest (*Quercus ilex*), with patches of *Alnus glutinosa*. In fact, dorsal coloration in all populations of *C. arnoldi* strikingly resembles that of the submerged leaves of *Fagus sylvatica*, suggesting it may have a mimetic function.

Although extensive demographic studies have not yet been carried out, preliminary analyses indicate populations A2, B1 and B2 may have very low densities and populations A2 and A3 extremely low densities. Apart from drying out of the mountain streams, other consequences for the low numbers of both specimens and populations observed may be related to reductions in habitable patches as a result of human alteration of its original habitat and replacement of the beech forests by Holm oak forests at medium altitudes (800–1400 m) in response to global warming (Peñuelas & Boada, 2003). Appropriate conservation measures should be adopted immediately to preserve this interesting Iberian endemic.

Etymology

This new species of newt (*Calotriton arnoldi*) is named after the British herpetologist Dr E. N. Arnold (Department of Zoology, The Natural History Museum, London), for his life-long dedication and contribution to herpetology, and especially to European herpetology. This dedication is also a personal recognition for the 6 years he spent guiding the senior author through his herpetological research while at the Natural History Museum, London.

Proposal of common names in different languages

The following common names for *Calotriton arnoldi* are suggested:

Catalan: Tritó del Montseny
Spanish: Tritón del Montseny
English: Montseny brook newt

DISCUSSION

The phylogenetic analyses presented in Figure 2 and previously published works (Steinfartz *et al.*, 2002) indicate *Euproctus* and *Calotriton* have independent evolutionary origins. This contradicts a previous hypothesis that these two taxa formed a clade that was divided by the Oligocene separation of Corsica and Sardinia from north-eastern Iberia (Sbordoni *et al.*, 1982, 1985, 1990; Caccone *et al.*, 1994, 1997).

Our clock estimates suggest *E. montanus* and *E. platycephalus* colonized the Sardo-Corsican block approximately 13 Mya, during the mid-Miocene. It is possible that the ancestor of *Euproctus* (*s.s.*) moved into the present range of the genus via a land bridge that formed around 12 Mya and connected, for an unspecified period of time, the Sardo-Corsican block with the continent (Orsag-Sperber *et al.*, 1993; Fromhage, Vences & Veith, 2004). Other palaeogeographical scenarios include the possibility that *Euproctus* moved into Corsica and Sardinia during the Messinian salinity crisis, and that colonization occurred across a bridge that included the Argentario promontory and the Tuscan archipelago about 1–2 Mya (Nascetti, 1996). However, neither of these scenarios is supported by our data and clock calibrations and are unlikely to be so even if more taxa are added. Although very unlikely, there is the possibility that the ancestor of the Tyrrhenian brook newts reached Corsica and Sardinia by oversea dispersal. Although there is no evidence for transmarine dispersal in urodeles, recent research has shown that at least two groups of tree frogs have colonized the volcanic island of Mayotte, in the Comoros, by this means (Vences *et al.*, 2003). According to our data (Fig. 2), the split between *E. montanus* and *E. platycephalus* occurred 5.5 Mya, towards the end of the Messinian salinity crisis. At that time, the Mediterranean basin, which was largely emptied during the crisis, refilled isolating many animal groups (including several amphibians), which, for more than 600 000 years, had been able to disperse across land between the continents and islands of the Mediterranean basin (Arntzen & García-París, 1995; García-París & Jockusch, 1999; Carranza & Arnold, 2004; Fromhage *et al.*, 2004; Veith *et al.*, 2004).

Our results suggest the west Asian genus *Neuregus* split approximately 9.5 Mya from the clade formed by *Calotriton* and the four representatives of *Triturus* sampled for our study. Palaeotectonic reconstructions of the Mediterranean basin show this event might have coincided with the end of the trans-Mediterra-

nean connection between Italy and Asia Minor resulting from the uplift of the Alps (Dercourt *et al.*, 1986). The later split between *Triturus* and *Calotriton* occurred approximately 8 Mya, and may have been triggered by an ecological shift in *Calotriton*, which probably changed from being a pond-breeding lineage adapted to lentic aquatic habitats to a lineage adapted to live in fast-running, well-oxygenated mountain streams. This may have been promoted by the most recent uplift and subsequent formation of the Neopyrenees approximately 10 Mya.

Our hypothesis of a recent origin of *Calotriton* argues against the previously proposed Eocene origin for this group of newts (Herre, 1935; Steiner, 1950; Gasser & Clergue-Gazeau, 1981; Sbordoni *et al.*, 1982, 1985, 1990; Accordi *et al.*, 1984; Caccone *et al.*, 1994, 1997), and is in accordance with the known fossil record of this taxon, of which the oldest known fossil dates only from the Upper Pleistocene of Cueva de las Hienas (Asturias), Spain (Estes, 1981; Sanchiz, 1977a, b).

Speciation between *Calotriton asper* and *C. arnoldi* occurred relatively recently, approximately 1.1 Mya, during the Pleistocene. This date coincides roughly with the speciation event that separated *Triturus marmoratus* from *T. pygmaeus*, which according to our dataset occurred approximately 1.7 Mya. This small difference in the estimated ages of the speciation events in these two pairs of taxa is because that in *C. asper* and *C. arnoldi* involves a much lower uncorrected genetic divergence for the 16S rRNA mitochondrial gene than that in *T. marmoratus* and *T. pygmaeus* (0.7 and 2.1%, respectively). Uncorrected genetic divergence values for the cytb gene only (see Fig. 3) and the 12S rRNA gene only are very similar for the two pairs of taxa: *T. marmoratus*–*T. pygmaeus* (4.2% for the cytb and 1.3% for the 12S rRNA gene) and *C. asper*–*C. arnoldi* (4.1% for the cytb and 1.2% for the 12S rRNA gene). If only cytb were used for the calibration of the molecular clock, the date of separation between *C. asper* and *C. arnoldi* would be approximately 2 Mya instead of the value of 1.1 Mya calculated using the three mitochondrial genes together (see Fig. 2).

Divergence between *Triturus marmoratus* and *T. pygmaeus* also occurred during the Pleistocene (approximately 1.7 Mya). The present distributions of these two Iberian newts seem to indicate that either the mountains of the Sistema Central or the River Tajo (or both) may have acted as a barrier for some time, interrupting gene flow between the two forms and allowing speciation to take place, although limited gene flow still occurs between them. Other speciation events that took place in Iberia towards the end of the Pliocene and in the early Pleistocene involve several amphibian and reptile populations most

probably separated as a result of the formation of the Guadalquivir River basin, e.g. for: *Salamandra* (García-París, Alcobendas & Alberch, 1998), *Discoglossus* (García-París & Jockusch, 1999; Fromhage *et al.*, 2004); *Alytes* (Arntzen & García-París, 1995; Fromhage *et al.*, 2004; Martínez-Solano *et al.*, 2004), *Pleurodeles* (Carranza & Arnold, 2004; Carranza & Wade, 2004), *Lacerta lepida* (Paulo, 2001) and *Psammotromus* (S. Carranza, unpubl. data).

In the phylogeny presented in Figure 2, the branch that leads to the two species of *Calotriton* is relatively long and 'bald' before diversifying into *C. asper* and *C. arnoldi*. This may be because no speciation events occurred, which would suggest *Calotriton* had a continuously small range since its divergence from *Triturus*. Alternatively, external basal branches may have existed but have been pruned by extinction. Either possibility would fit with long-term persistence only in a small montane area and the lack of success in any long-term colonization beyond these habitats.

A northward–westward rapid range extension from Catalonia is in agreement with the greatest genetic diversity in *Calotriton* being around this area, where both species are found less than 30 km apart (see Figs 1, 2). Speciation within *Calotriton* may have been caused by a geographical barrier or may have resulted from one or more of the climatic fluctuations frequent in the Western Palearctic during the Pleistocene. These promoted genetic and morphological differentiation by changing the distribution and demography of many species (Hewitt, 1996, 2000; Veith, Kosuch & Vences, 2003). This hypothesis of a south-eastern origin of *Calotriton* followed by recent expansions to the north-west through the Pyrenees and the Prepyrenees is also supported by harsh climatic conditions in these latter massifs during the Pleistocene and especially during the glacial maximum approximately 50 000–45 000 years ago (Jalut *et al.*, 1992; Montserrat-Martí, 1992), which would have made it impossible for *C. asper* to inhabit most of its present range in the Pyrenees. The fact that *C. asper* can be found at very high altitude inhabiting lakes in places that were permanently covered by ice not long ago is an indication of the high dispersal capabilities of the Pyrenean brook newt and helps to explain its rapid dispersion through the Pyrenean axial chain and the Prepyrenees. There is also the possibility that *C. asper* moved to the west soon after it diverged from *C. arnoldi* and, as mountain lizards of the genus *Iberolacerta*, persisted through the Pleistocene in the lower southern slopes of the Pyrenean mountains and valleys, or in the Prepyrenees (see Carranza *et al.*, 2004). Genetic divergence between Pyrenean and Prepyrenean populations at Puerto de Monrepos (Figs 1, 3; Gasser & Clergue-Gazeau, 1981) suggests the latter

may have acted as a Pleistocene refuge for *C. asper*, although further analyses are necessary to confirm this. A genetic analysis including many individuals from more than 20 different populations covering the whole distribution range of both *C. asper* and *C. arnoldi* and also including information from nuclear genes indicates that gene flow between these two species has been interrupted for a long time (S. Carranza, unpubl. data).

Gene flow within *C. arnoldi* may be lower than in *C. asper* as a result of a unique behaviour observed in the former. Contrary to what occurs in *C. asper*, neither juvenile nor adult *C. arnoldi* have been found on land (S. Carranza & F. Amat, pers. observ.; A. Montori, pers. comm.). If this behaviour is confirmed, the only way that contact among populations could occur is by moving down one watercourse and then up other tributaries of the same river system.

Such movement may have been relatively easy during colder periods, for instance during the Pleistocene, but it may be more difficult at the present time as progressive replacement of cold-temperate ecosystems by Mediterranean ecosystems is taking place in the El Montseny massif in response to global warming. For instance, the beech (*Fagus sylvatica*) forest, an excellent habitat for *C. arnoldi*, has shifted upwards by 70 m at the highest altitudes (1600–1700 m) since 1945, and is being replaced by Holm oak (*Quercus ilex*) forest at lower altitudes (800–1400 m) (Peñuelas & Boada, 2003).

European brook newts represent a marked case of convergence within salamanders. *Euproctus* and *Calotriton* share at least three morphological and behavioural characters that could at first sight be regarded as synapomorphies. As noted, both genera have a broad and flattened head and relatively depressed body which enables them get into crevices and under stones, avoiding strong currents. Reduction or even absence of lungs may be advantageous in lowering buoyancy, reducing the likelihood of the newts being carried away by such currents. Reduction may be permitted by the high oxygen levels in the water in which they live, enabling them to depend almost entirely on cutaneous respiration and removing the necessity of coming to the surface to breathe. Finally, these newts exhibit a modified courtship behaviour so that males can capture and subdue the females with their tails while transferring one or several spermatozoa directly into her cloaca.

Although there are striking similarities between the Tyrrhenian and Western brook newts, there are also differences that result from the long independent histories of these groups. They include morphological osteological features and behavioural features (see above), including some related to mating behaviour (Thiesmeier & Hornberg, 1990). Among these are the

presence of spurs on the hind legs of the Tyrrhenian brook newts, which facilitate the transfer of spermatozoa (Fig. 9A, B; Thorn, 1968) and, in *E. montanus*, the presence of a conspicuous pseudopenis that protrudes during amplexus to facilitate sperm transfer (Fig. 9D).

The two species of Western brook newts also share some morphological adaptations to life in fast-running waters and mountain streams. For instance, they have depressed fingers with the tips covered by black corneous nail-like sheaths, most probably to increase the grip on slippery rocky surfaces. In addition, *Calotriton* has the skin above covered with tubercles bearing horny tips, which may increase the grip while moving around or sitting in rock crevices in streams with strong currents. These tubercles are much more pronounced in *C. asper*, which sometimes has a very rough skin with horny tips beneath as well as above (Fig. 10K, L). Presence of smooth skin during the aquatic phase of the sister group of *Calotriton* (see Fig. 2) indicates this character has probably developed secondarily in the Western brook newts, and especially in *C. asper*. This latter species also presents a unique osteological autapomorphy. In most cases analysed (see Appendix 2 and Fig. 4) and especially in males, the first caudosacral vertebrae of *C. asper* have transverse processes at approximately 90° to the vertebral axis, a situation not found in any other representatives of the Salamandridae analysed for this study (see Appendix 2 and Fig. 4). Until a more careful morphological, osteological and functional analysis of the caudosacral vertebrae of *C. asper* is carried out, any hypothesis related to the possible function of this structure is tentative.

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APPENDIX 1

Material of Western brook newts (*Calotriton asper* and *Calotriton arnoldi*) used in the multivariate morphological analysis. Museu de Zoologia de Barcelona (MZB), Instituto Pirenaico de Ecología de Jaca-CSIC (IPE), Museo Nacional de Ciencias Naturales-CSIC (MNCN) and the Natural History Museum, London (BMNH)

Calotriton asper

MNCN: 1923, 1926, 1930, 1923, 1925, 1929, 1928, 1927, 1922, 1940, 1938, 1939, 1935, 1934, 1937, 1932, 1936, 1941, 1949, 1943, 1947, 1944, 1945, 1946, 1951, 1950, 1948, 17025, 9798, 9692, 9794, 9795, 17026, 17027, 9799, 9804, 21682, 1907, 1910, 1908, 1906, 1909, 1911, 1902, 1903, 1901, 1917, 1918, 1913, 1915,

1914, 1916, 1912, 15726, 15727, 15725, 15724, 9802, 9800, 9803, 9797, 15754, 15755, 15752, 15756, 1900, 1899 and 1898.

MZB: 83-0401, 83-0399, 83-0397, 83-0398, 83-0398, 83-0397, 83-0339, 83-0320, 83-0340, 82-8772, 82-8774, 82-8769, 82-8763, 82-8765, 82-8770, 82-771, 82-8775, 82-8777, 82-8755, 82-8776, 82-8766, 82-8759, 82-8778, 83-0405, 83-0436, 83-0404, 83-0405, 83-0404, 83-0433, 83-0442, 83-0434, 83-0433, 82-8787, 82-8782, 83-0315, 83-0310, 83-0316, 01-0267, 86-1769, 00-0606, 82-8780, 82-8779, 82-8784, 82-8789, 86-1710, 82-8773, 83-0449, 83-0445, 83-445, 86-2118, 86-2119, 82-8767, 82-8760, 83-0435, 83-0435.

BMNH: 1928.12.20.377, 1970.2446–2451, 1920.1.20.20, 1920.1.20.696–698, 1894.11.10.3–5, 1951.1.5.71, 1928.12.20.373–376.

IPE: 2196, 2261, 2240, 2241, 1663, 1664, 1628, 1653, 1652, 1700, 2190, 1687, 2177, 1655, 1669, 1656, 1680, 1672, 2181, 2179, 1651, 2175, 2176, 2180, 1657, 1667, 1631, 1674, 1670, 1678, 1676, 1671, 1668, 3313, 3316, 3315, 3317, 3314, 3312, 2246, 2248, 2245, 2249, 2244, 3857, 3858, 3855, 3856, 3850, 3854, 3849, 3853, 3852, 3851, 3829, 2217, 2216, 2201, 2219, 2209, 2213, 2205, 2207, 2208, 2200, 2210, 2203, 2211, 2202, 2215, 2214, 2218, 2204, 2206, 2909, 2222, 2235, 2224, 2278, 2234, 2254, 2231, 2278, 2221, 2225, 2255, 2226, 2220, 3816, 3817, 3818, 3819, 3814, 3848, 1712, 2272, 3357, 3353, 3356, 3354, 3355, 2273, 2273, 2193, 2298, 2297, 2924, 2286, 2269, 2907, 2931, 2299, 2932, 2910, 2934, 2930, 2908, 2285, 2252, 2242, 2243, 2276, 1701, 1703, 1704, 2274, 3806, 3807, 2282, 2284, 2281, 2280 and 2283.

Calotriton arnoldi

MZB: 82-8785, 82-8786, 2004-0187, 2004-0188, 2004-0189.

APPENDIX 2

List of museum specimens used in the X-ray osteological analysis. Museu de Zoologia de Barcelona (MZB), The Natural History Museum, London (BMNH)

BMNH:

Calotriton asper: 1951.1.5.69, 1951.1.5.70, 1951.1.5.60, 1928.11.22.11–22, 1976.1248, 1920.1.20.20, 1928.11.22.13–14, 1928.11.22.18–22, 94.11.10.3–5, 1951.1.5.72, 1928.12.20.377, 1970.2446, 1970.2449, 91.12.29.7, 1970.2450, 1970.2448, 1976.1248–50. *Euproctus montanus*: 82.11.15.51–55, 82.11.15.51–55, 1947.1.4.8–10. *Euproctus platycephalus*: 1947.1.4.4-x6, 82.11.15.56–59. *Cynops pyrogaster*: 70.12.15.1. *Cynops orientalis*: 1959.1.5.2–3. *Notophthalmus viridescens*: 1983.888–893. *Notophthalmus meridionalis*: 89.9.25.10–12. *Neurergus kaisseri*: 1952.4.2.85. *Taricha rivularis*: 1957.1.7.43–44. *Pachytriton brevipes*: 1933.12.4.34, 1904.6.8.3. *Neurergus crocatus*: 1917.5.3.8–10, 1946.9.5.90–97, 1940.1.26.1–20, 1961.1563.4. *Triturus cristatus*: 1914.6.9.6–7, 1956.1.4.81–82. *Triturus dobrogicus*: 1911.2.28.11–13. *Lissotriton vulgaris*: 1969.768, 1969.761, 1969.764, 1968.765, 94.4.6.5–8. *Lissotriton helveticus*: 97.5.17.1–4. *Mesotriton alpestris*: 94.12.31.48–49.36–37. *Lissotriton montandoni*: 1958.1.6.64–65. *Triturus marmoratus*: 86.6.29.52–56, 96.3.26.6–8, 1928.12.20.29. *Triturus carnifex*: 1902.5.9.4–8. *Triturus vittatus*: 96.3.28.20–24, 86.11.19.18–1985.8.81.58. *Triturus karellini*: 1903.6.8.1–2, 96.3.28.16–19. *Tylotriton verrucosus*: 93.1.9.51–52. *Salamandra s. fastuosa*: 1972. 1746, 1972. 1747. *Lyciasalamandra atifi*: 1964.353–370.

MZB:

Calotriton arnoldi: 82-8789, 82-8784, 2004-0187, 2004-0188, 2004-0189.