Morphological and allozyme studies of midwife toads (genus *Alytes*), including the description of two new taxa from Spain

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Abstract

Allozyme variation in 31 to 50 presumptive loci of 12 populations of European midwife toads of the genus *Alytes* show appreciable genetic divergences (D_{Nei} from 0.29 to 0.72) among four groups. These groups correspond to *A. cisternasii* Boscá, 1879, *A. obstetricans* (Laurenti, 1768), *A. muletensis* (Sanchíz & Adrover, 1979), and a new species from the Betic mountains described here as *A. dickhilleni* n. sp. Smaller divergences among geographic groups of populations of *A. obstetricans* (up to D_{Nei} = 0.17) support the existence of three geographic units in Europe corresponding to the previously recognized subspecies *A. o. obstetricans* and *A. o. boscai* Lataste, 1879, plus a third one described here under the designation of *A. o. almogavarii* n. ssp. The analysis of morphological variation among taxa using principal component and discriminant analysis shows a remarkable similarity between *A. dickhilleni* and *A. obstetricans*; these are cryptic species. A phylogenetic analysis of allozyme data using *A. cisternasii* as the outgroup supports a sister taxon relationship between *A. muletensis* and *A. dickhilleni*, with *A. obstetricans* the sister taxon to this clade. The historical biogeography of the genus is discussed.

Resumen

El análisis de la variación genética en 12 poblaciones de sapos parteros europeos del género *Alytes*, basado en el estudio de 31 a 50 loci mediante electroforesis de proteinas, muestra la existencia de una pronunciada divergencia genética (D_{Nei} = 0.29–0.72) entre cuatro grupos de poblaciones. Estos grupos corresponden a las especies *Alytes cisternasii* Boscá, 1879, *A. obstetricans* (Laurenti, 1768), *A. muletensis* (Sanchíz & Adrover, 1979) y una especie nueva que se describe en este trabajo bajo el nombre de *A. dickhilleni* n. sp. Las diferencias genéticas observadas entre grupos de poblaciones geográficamente separadas dentro de la especie *A. obstetricans* (hasta D_{Nei} = 0.17) permite reconocer otros tres grupos correspondientes a las subespe-

cies *A. o. obstetricans* y *A. o. boscai* Lataste, 1879, descritas previamente, más una nueva subespecie del Noreste Ibérico descrita aquí como *A. o. almogavarii* n. ssp. El estudio de la variación morfológica entre especies y subespecies realizado mediante análisis de componentes principales y análisis discriminante pone de manifiesto la existencia de una destacada similitud morfológica entre *A. obstetricans* y *A. dickhilleni* que, por lo tanto, pueden considerarse como especies crípticas desde el punto de vista morfológico. El análisis filogenético realizado con datos electroforéticos, usando *A. cisternasii* como grupo externo, confirma la existencia de estrechas relaciones filogenéticas entre *A. dickhilleni* y *A. muletensis* que constituyen un grupo monofilético, mientras que *A. obstetricans* se sitúa como grupo hermano del par *A. dickhilleni–A. muletensis*. Adicionalmente se discute la biogeografía histórica del género.

Introduction

A population of midwife toads was recently discovered on Mallorca, the largest of the Balearic Islands (Alcover & Mayol, 1980; Mayol & Alcover, 1981). Surprisingly, these midwife toads appeared to be the survivors of the Mallorcan *Baleaphryne muletensis* described as fossil species by Sanchíz & Adrover (1979). The taxonomic status of the Mallorcan midwife toad as *Alytes muletensis* is now generally accepted (García-París, 1985; Salvador, 1985). Two continental species of midwife toads are currently recognized, the Iberian midwife toad *A. cisternasii* Boscá, 1879, from the southwestern region of the Iberian Peninsula, and the Common midwife toad *A. obstetricans* (Laurenti, 1768) with a wide distribution that ranges from the Middle Atlas in Morocco to the Harz Mountains in Ger-
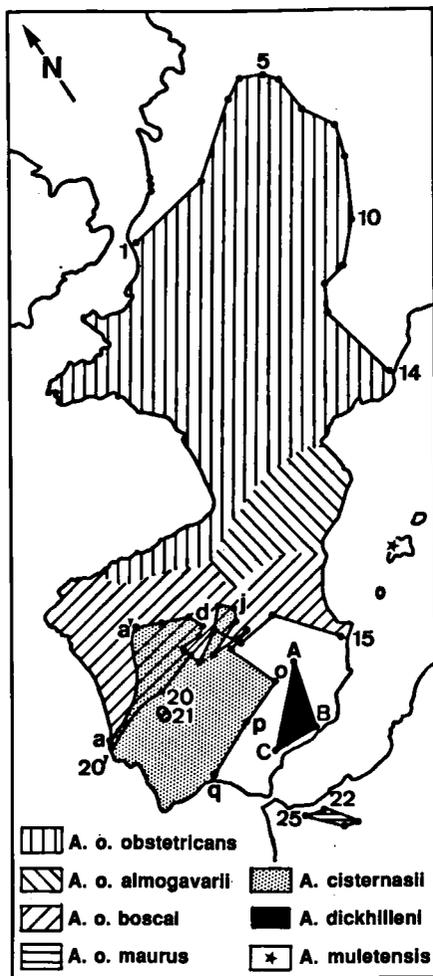
Fig. 1. Distribution of four species of midwife toads. Reliable records documenting the outer range of the species are as follows:

Alytes cisternasii. — Portugal: transect a-a' according to Rosa et al. (1990). Spain: b, Province of Zamora: UTM grid TL 69 (Pollo et al., 1988); c, Province of Valladolid: UTM grid UL 46 (García-París et al., 1990); d, Province of Segovia: UTM grid UL 71 (García-París et al., 1990); e, Avila (Arntzen, 1979); f, Province of Avila: UTM grid TK 88 (García-París et al., 1990); g, Province of Toledo: UTM grid UK 03 (García-París et al., 1990); h, Province of Madrid: UTM grid UK 99 (García-París et al., 1989); i, Province of Madrid: UTM grid VL 44 (García-París et al., 1989); j, Province of Guadalajara: UTM grid VL 81 (García-París et al., 1990); k, UTM grid VH 75 (Pleguezuelos & Moreno, 1990); l, Province of Toledo: UTM grid UK 42 (García-París et al., 1990); m, Province of Madrid: UTM grid VK 73 (García-París et al., 1990); n, Toledo (Boscá, 1880b); o, Province of Jaén: UTM grid VH 75 (Pleguezuelos & Moreno, 1990); p, Province of Córdoba: UTM grid UH 40 (López-Jurado et al., 1980); q, Province of Huelva: El Rocío (Collado et al., 1976).

many. In line with its wide distribution, *A. obstetricans* occupies a wide variety of habitats, ranging from rocky areas near mountain lakes, humid forests along coastal streams, to arid plateaus and, occasionally, xerophilous coastal scrub zones close to man-made ponds. In the southernmost part of the Iberian Peninsula, midwife toads appear to be restricted to the higher altitudes of mountain ranges (Antunez et al., 1982), where the toads may live under ecological conditions that are surprisingly similar to those of *A. muletensis* on Mallorca. Morphological and allozyme variation has been reported for *A. obstetricans* (Lataste, 1879a; Pasteur & Bons, 1962; Guyétant, 1977; Arntzen & Szymura, 1984; Viegas & Crespo, 1985; Rosa et al., 1990) and is reflected in the description of several subspecies. The known distributions of revised midwife toad species and subspecies are presented in Fig. 1.

Immunological, protein electrophoretic and mor-

A. dickhilleni. — Spain: A, Sierra de Alcaraz (present paper); B, Sierra de Gádor (Antúnez et al., 1988); C, Sierra Tejeda (present paper).

A. muletensis. — In its present-day distribution the species is only known from six localities in the Serra Tramuntana of Mallorca, Spain.

A. obstetricans. — France: 1, map of the "Institut Géographique National" (IGN) 1/50,000 XXII-2 (Calais) (Guyétant, 1989). The Netherlands: 2, Limburg, grid reference 60.42 (Bergmans & Zuiderwijk, 1986). Germany: 3, Westfalia, MTB grid reference 3819/4 (Feldmann, 1981); 4, Niedersachsen, MTB grid 3723/2 (Lemmel, 1977); 5, MTB grid 4132/2 (Schiemenz & Günther, 1994); 6, MTG grid 4434/1 (Schiemenz & Günther, 1994); 7, MTB grid 5330/4 (Schiemenz & Günther, 1994); 8, UTM grid QA 04 (Müller, 1976); 9, UTM grid PV 54 (Müller, 1976). Switzerland: 10, Berneck (Grossenbacher, 1988); 11, Grindelwald (Grossenbacher, 1988); 12, Vevey (Grossenbacher, 1988). France: 13, map IGN XXXIV-31 (Annecy-Ugine) (Guyétant, 1989); 14, map IGN XXXV-45 (St. Tropez) (Guyétant, 1989). Spain: 15, Province of Alicante: Alcoy (García-París et al., 1993); 16, Province of Cuenca: Uña (García-París, unpublished data); 17, Province of Guadelajara: Ciruelos (J. Prieto & G. Astudillo, pers. comm., 1990); 18, Province of Madrid: UTM grid UK 99 (García-París et al., 1989); 19, river Tormes, Sierra de Gredos (Arntzen, 1979). Portugal: transect 20-20' according to Rosa et al. (1990); 21, isolated occurrence on the Serra de S. Mamede (Rosa et al., 1990). Morocco: 22, Tleta Ketama (Pasteur & Bons, 1962; Arntzen & Szymura, 1984; cf. Bons, 1973); 23, Merhraoua (Libis, 1985; cf. Bons, 1973); 24, Djebel Tazekka, Taza (Mellado & Mateo, 1992; cf. Bons, 1973); 25, Chefchaouèn (García-París, pers. obs., 1993; cf. Bons, 1973).

phological studies identify *A. muletensis* and *A. obstetricans* as sister taxa (Clarke, 1984; Crespo et al., 1984; Hemmer, 1984; Maxson, 1984; Viertel, 1984). On the other hand, cytogenetic data suggest that *A. cisternasii* and *A. obstetricans* are a sister group (Herrero, 1984), while some morphological data ambiguously point to either *A. cisternasii* or *A. muletensis* as the sister taxon of *A. obstetricans* (Sanchíz, 1984). No support exists for grouping together *A. muletensis* and *A. cisternasii*.

In the present work we attempt to (1) elucidate the phylogenetic relationships of the species in the genus *Alytes*, (2) describe geographical variation in *A. obstetricans*, the most variable of midwife toad species, and (3) find readily applicable features in external morphology that allow correct identification of species and subspecies. As the result of the study we recognize two new taxa from Spain: *Alytes dickhilleni* n. sp. and *Alytes obstetricans almogavarii* n. ssp.

Materials and methods

Sampling

Material was obtained from the entire extant range of the genus, except for the northern African *A. o. maurus* Pasteur & Bons, 1962. Populations of *A. obstetricans* were selected for the present study on the basis of taxonomic, geographic, and ecological arguments. These included: (1) the type localities of described subspecies, (2) the potential contact zones between subspecies, and (3) geographically isolated mountain populations. The species *A. muletensis* is endangered and initially only two specimens were studied. Additional specimens later became available from the captive breeding program carried out at Jersey Zoo. Live specimens were available for morphometric analysis from the University of East Anglia. In the list presented below, these samples of *A. muletensis* are referred to as "Madrid", "Jersey", and "Norwich", although they are from the same Mallorcan locality. Designations between parentheses refer to protein electrophoresis (e) and external morphology (m); specimens with no collection reference were measured and released *in situ*. All sacrificed animals are stored as vouchers in the collections of the Zoological Museum of the University of Amsterdam (ZMA) and the Museo Nacional de Ciencias Naturales, Madrid (MNCN).

Alytes cisternasii: Portugal: Beja: Cuba, *N* = 5 (e) ZMA 8154; Viana do Alentejo, *N* = 9 (e) ZMA 8155. Spain: Badajoz: Alburquerque, *N* = 20 (m); Madrid: Villanueva de la Cañada, *N* = 2 (e) MNCN uncatalogued.

Alytes muletensis: Spain: Mallorca: "Madrid", *N* = 2 (e) MNCN 16728−29, "Jersey", *N* = 5 (e) MNCN uncatalogued, "Norwich", *N* = 30 (m).

Alytes obstetricans: France: Jublains, *N* = 5 (e, m) ZMA 8156, *N* = 5 (m) ZMA 7584, *N* = 2 (m) ZMA 7642. Germany: Köln, *N* = 4 (m) MNCN uncatalogued. Spain: Asturias: Picos de Europa, *N* = 1 (e) ZMA 8157; Asturias and León: Puerto de San Isidro, *N* = 7 (e, m) MNCN uncatalogued, *N* = 10 (m); Navarra: Ituren, *N* = 11 (m) MNCN uncatalogued; Irati, *N* = 5 (e, m) MNCN 16778 and uncatalogued, *N* = 5 (m) MNCN uncatalogued; Barcelona: Berga, *N* = 5 (e, m) MNCN 16774-79, *N* = 7 (e, m) MNCN 16743-45 and 16760-63, *N* = 3 (m) MNCN 16746-48, *N* = 10 (m); Barcelona, Universidad Central, *N* = 4 (m) MNCN 16749-52; Pontevedra: Tuy, *N* = 6 (e, m) MNCN 16780 and uncatalogued; Bayona, *N* = 9 (m); Castellón: Caudiel, *N* = 2 (e, m) MNCN uncatalogued; Benicassim, *N* = 2 (e, m) MNCN 16767 and 16773, *N* = 26 (m).

Midwife toads from the Betic mountains, Spain, are from: Albacete: Bienservida, *N* = 8 (e, m) MNCN 16768-72, MNCN 16739-41, *N* = 1 (m) MNCN 16742, *N* = 4 (m); Paterna del Madera, *N* = 2 (m) MNCN 16781-82, *N* = 17 (m); Málaga: Alcaucín, *N* = 4 (m) MNCN 16735−38; Canillas de Aceituno, *N* = 4 (e, m) ZMA 8156, *N* = 3 (m); Granada: La Alcauca, Ventas de Zafarraya, *N* = 5 (e, m) MNCN 16730-34, *N* = 5 (m) MNCN 16727, 16756-59.

Data derived from sites close together were pooled under the following locality designations: Mallorca ("Jersey", "Madrid", and "Norwich"), Picos de Europa (Puerto de San Isidro and Picos de Europa), Navarra (Irati and Ituren), Barcelona (Berga and Barcelona Universidad Central), Galicia (Bayona and Tuy), Valencia (Benicassim and Caudiel), Alcaraz (Bienservida and Paterna del Madera), and Sierra Tejeda (Alcaucín, Canillas de Aceituno, and Ventas de Zafarraya). The populations from Sierra Tejeda and Alcaraz, located near the two ends of the Betic mountains in the southern region of the Iberian Peninsula are together referred to as the Betic midwife toads or as *A. dickhilleni*.

Allozyme electrophoresis and phenetic analysis

Protein profiles were studied for a total of 78 individuals. Liver, heart, stomach, and muscle from the thigh were dissected from freshly sacrificed animals and stored in liquid nitrogen. Two specimens of *A. muletensis* were obtained in frozen condition. Tissues were ground in homogenizing buffer (100 mM Tris, 1 mM EDTA and 50 μM NADP, adjusted to pH 7.0 with HCl) and briefly centrifuged. The aqueous supernatant was decanted and stored at −70°C for future electrophoresis. Polyacrylamide slab gel electrophoresis of proteins was performed for some "general proteins" and enzyme precursor proteins according to Maurer (1971) and Taggart et al. (1978). Enzyme electrophoresis was performed using Connaught and Sigma starch in horizontal gels. Presumptive loci and alleles (electromorphs) were assigned numbers and letters, respectively, in sequence starting from the most anodally migrating forms. Proteins assayed and buffer systems used are presented in Table I.

The general proteins were stained with Coomassie Blue. Enzymes, including Pepsin, were visualized on the gels using standard histochemical techniques (Harris & Hopkinson, 1976;

Table I. Electrophoretic conditions for 26 protein systems, corresponding to 50 loci, examined in midwife toads. Starch buffers are A: Tris-citrate pH 6.0 (XIII); B: Tris-citrate pH 7.0 (I); C: Tris-citrate pH 8.0 (V); D: Lithium-hydroxide-tris-citrate pH 8.1 (X); E: Tris-malate pH 7.4, electrode buffer is 0.22 M Tris, 0.10 M Maleic acid, 0.01 M EDTA and 0.01 M $MgCl_2$, gel buffer is electrode buffer diluted at 1 : 10; F: Tris-EDTA-borate pH 8.9 (Ayala et al., 1972); G: Histidine-sodium-citrate pH 8.0 (Brewer, 1970). Roman numerals refer to the buffer systems of Shaw & Prasad (1970). PAGE refers to acrylamide gels according to Maurer (1971: Table 4.1) for General Proteins and to Taggart et al. (1978) for Pepsinogen. Tissues used are: H = heart, L = liver, M = muscle, and S = stomach.

Protein	E. C. No.	Locus	Buffer system	Tissue extract
Acid phosphatase	3.1.3.2	*Acph-1, Acph-2*	A	L
Adenylate kinase	2.7.4.3	*Ak*	D, B	L, M
Aspartate transaminase	2.6.1.1	*Ata-1, Ata-2* *	D	L
Catalase	1.11.1.6	*Cat*	D	L
Creatine kinase	2.7.3.2	*Ck-1, Ck-2*	B	M
Esterase	3.1.1.1	*Est-1, Est-2*	D, E	L, H
Fucose dehydrogenase	1.1.1.122	*Fdh*	C	L
General Protein	-	*GP.M-1,2,4-7; GP.S-1-3*	PAGE	M, S
Glucose 6 phospate dehydrogenase	1.1.1.49	*G-6-pd*	C	L
Glucose dehydrogenase	1.1.1.47	*Gdh*	C	L
Glucose phosphate isomerase	5.3.1.9	*Gpi*	A	M
α-Glycerophosphate dehydrogenase	1.1.1.8	*α-Gly*	A	L
Isocitrate dehydrogenase	1.1.1.42	*Icd-1, Icd-2*	C	L
Lactate dehydrogenase	1.1.1.27	*Ldh-1, Ldh-2*	B	H, L
Leucine aminopeptidase	3.4.11	*Lap*	G	L
Malate dehydrogenase	1.1.1.37	*Mdh-1, Mdh-2*	E	H, M
Malic enzyme	1.1.1.40	*Me-1, Me-2*	C	L
Mannose phosphate isomerase	5.3.1.8	*Mpi-1, Mpi-2*	C	L
NADH dehydrogenase	1.6.99.2	*Nadhdh-1, Nadhdh-2*	D	L
Pepsine (Pepsinogen)	3.4.23.-	*Pg-1, Pg-2*	PAGE	S
Peptidase	3.1.1.-	*Pep-1, Pep-2*	D	S
Phosphoglucomutase	5.4.2.2.	*Pgm-1, Pgm-2*	A	L
Phosphogluconate dehydrogenase	1.1.1.44	*6-Pgd*	C	L
Sorbitol dehydrogenase	1.1.1.14	*Sdh-1, Sdh-2*	F	L
Superoxide dismutase	1.15.1.1	*Sod-1, Sod-2*	F	L
Unidentified Enzymatic Protein	?	*UEP-1, UEP-2*	B, E	M, L

* Formerly known as *Got* and *Aat*.

Shaw & Prasad, 1970; Taggart et al., 1978) with a few modifications. By exposing the acrylamide gel to the appropriate staining, the general protein with third highest migratory rate was identified as a Creatine kinase. On the starch gels, two enzymes that could not be properly identified (coined Unidentified Enzymatic Proteins, UEP-1 and UEP-2) showed up consistently in faint but perfectly scorable patterns when stained for GPI and LDH, respectively. In both cases these enzymes migrated with higher velocity than GPI and LDH. Two loci were also apparent on zymograms stained for MPI and SDH where a single locus was expected. Two enzymes with protein digesting properties were resolved, PEP-1 and PEP-2, that showed substrate specificity for Leucyl-tyrosine and Leucyl-glycyl-glycine, respectively. Zymograms were scored for 50 presumed loci in six populations of midwife toads. In a second survey, additional specimens were analyzed for 31 loci in conjunction with selected samples previously analyzed, for a total of 12 populations.

To assess the degree of genetic variability of populations we calculated the mean heterozygosity based on Hardy-Weinberg expectations (H_e) and the accompanying standard error, using the BIOSYS-1 computer program (Swofford & Selander, 1981). The degree of genetic differentiation across populations was calculated using the genetic distance measure of Nei (D_{Nei}) and its standard error (Nei, 1972, 1987). The reason for selecting Nei's distance is that (1) it is the most widely used measure of genetic distance, which allows comparison of the results with that of other studies, and (2) for low and intermediate values it may be linearly related to time (Nei, 1987). A matrix of distances was constructed for 12 populations over 50 to 31 loci. This composite matrix was converted into a phenogram using the unweighted pair-group method using arithmetic averages (UPGMA; Sokal & Sneath, 1963). A disadvantage of this clustering method is that the resulting phenogram can be interpreted in a phylogenetic sense only when rates of evolutionary change are homogeneous

across phyletic lines. In order to determine which branches of the dendrogram have ambiguous support we applied the jack-knife test as advocated by Lanyon (1985) for distance methods.

Outgroup designation and phylogenetic analysis

In midwife toads courtship and fertilization takes place on land. By putting the hind legs through the freshly deposited egg-clutch, the male picks up the eggs and carries them around for some weeks before depositing them in the water, where the embryos hatch and the free swimming larvae start their aquatic life. This etho-morphological feature, which is present in all midwife toads but in no other anurans, constitutes one of the synapomorphies for the genus, leaving no reasonable doubt about its monophyletic nature. To resolve the phylogenetic relationships of the species within the genus, one or more outgroups should be sought in related archeobatrachian genera such as *Barbouroula*, *Bombina*, and *Discoglossus*. Unfortunately, none of these taxa can profitably be used as an outgroup in our studies, because of the extensive morphological and genetic differentiation relative to *Alytes* (Lanza et al., 1976; Maxson & Szymura, 1984; Sanchíz, 1984).

To resolve the phylogenetic relationships within the *A. obstetricans* group of taxa (see below), *Alytes cisternasii* was taken as an outgroup *a priori* on the basis of a suite of morphological (Viertel, 1984), osteological (Clarke, 1984), immunological (Hemmer, 1984; Maxson, 1984) and protein electrophoretic characteristics (Arntzen & Szymura, 1984; Crespo et al., 1984; Hemmer, 1984; Viegas & Crespo, 1985; Rosa et al., 1990), suggesting that it is the sister taxon to the other members of the genus.

To study the phylogenetic relationships within the genus *Alytes*, matrices of Rogers' genetic distance (D_R, Rogers, 1972) were produced for individual loci with BIOSYS-1. These were interpreted as transformation matrices (for an early application of the use of this approach see Rafinski & Arntzen, 1986; cf. Mabee & Humphries, 1993: 172). Character states for internal nodes were not optimized simultaneously with the searching for the most parsimonious solution, as in some other software (Ellis, 1985, 1987) but this disadvantage was offset against the speed in which alternative trees could be analyzed which, with PAUP-3.1 (Swofford, 1993) on a Macintosh SE/30 desktop computer, allowed an exhaustive search. Bootstrap replication values (Felsenstein, 1985; Hillis & Bull, 1993) were calculated to gain an impression of the strength of support from the data to the phylogenetic tree showing maximum parsimony.

Morphological analysis

Thirteen external measurements were taken for 225 individuals from ten populations of *Alytes* with a precision vernier calliper (0.1 mm accuracy): snout-vent length (SVL), head width (HW), jaw length (JL), eye width (EW), horizontal diameter of the tympanum (HT), vertical diameter of the tympanum (VT), minimum distance between the eyes (ED), distance between the nostrils (ND), minimum distance of the eye to the nostril (END),

tibia-fibula length (TFL), hind limb length (HLL), distance between the tarsal tubercle and the tip of the third toe (TL), and the distance between the anterior end of the middle metacarpal tubercle and the tip of the third finger (FL). In all populations but the one from Jublains (France) live specimens were measured.

A principal component analysis (PCA) was performed on the residuals of the regression of log-transformed data for each of the variables versus log-transformed snout-vent length. The transformation was done in order to reduce the effect of variation in individual size and to increase the fit to the requirements for such analyses (Sokal & Rohlf, 1981; Thorpe & Leamy, 1983). All statistical analyses were carried out using SYSTAT-5 (Wilkinson, 1989). To compare the results of the morphological study with the results of the protein study, a Principal Coordinate Analysis was carried out for the genetic data using NTSYS-1.50 (Rohlf, 1988).

Following the allozyme survey, a phylogenetic interpretation of the results, and a taxonomic revision of the genus, a discriminant analysis to examine the degree of concordance between taxonomic and morphological units was carried out with newly established and re-established taxa as independent units. This was done in three rounds, at the species, subspecies, and population levels, respectively.

Results

Protein electrophoresis

The variability of 26 enzyme systems including "general" proteins and two unidentified enzymes was studied (Table I). Allele frequency data and sample sizes for 50 presumptive loci across 12 populations are listed in Tables II and III. Fourteen loci showed no variability in any of the assayed populations and five loci showed a maximum of five alleles across populations. The other loci showed intermediate levels of genetic variability. The maximum number of alleles segregating in a single population (three) was observed for *Est-1*, *Me-2*, *Mpi-1*, and *Sdh-1* in the populations from Mallorca, Cuba, Tuy, and Viana.

Using the UPGMA-method with the matrix of D_{Nei}, the 12 populations cluster into the following groups (Table IV, Fig. 2). Two main clusters, one corresponding to *A. cisternasii* populations, and the other to the remaining samples, cluster at $D = 0.72$. The populations of *A. cisternasii* show very little differentiation among themselves and are grouped at the level of $D \leq 0.02$. Within the other cluster, two main groups split at $D = 0.35$. One cor-
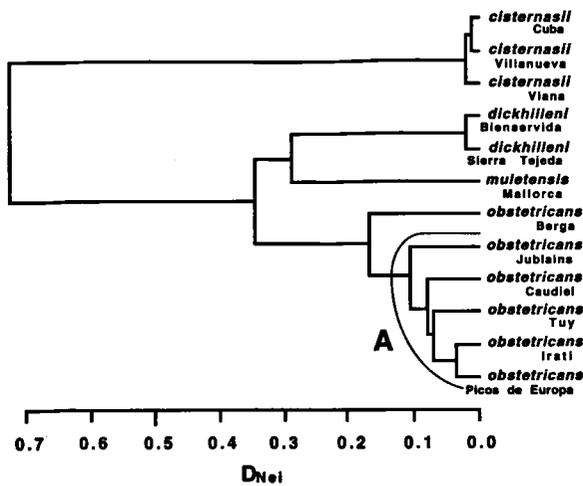
Fig. 2. Phenetic tree for four species (12 populations) of midwife toads, genus *Alytes*, on basis of Nei's genetic distance (D_{Nei}) over 31–50 protein loci. The branching order within the group designated "A" is not robust under the jack-knife test. For details see text.
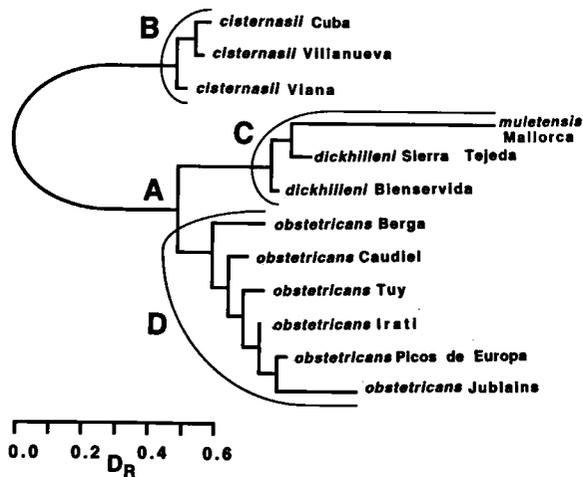

Fig. 3. Phylogenetic tree for four species (12 populations) of midwife toads, genus *Alytes*, on basis of Rogers' genetic distance (D_R) for 31–50 single protein loci. No strong bootstrap support (replication score $\leq$ 80%) exists for the branching order within the clades "B", "C", and "D". For details see text.

responds to all populations of *A. obstetricans*; it separates at the level of $D \leq 0.17$. The other corresponds to the populations of Betic and Balearic midwife toads (*A. dickhilleni* and *A. muletensis*), that separate at the level of $D = 0.29$. The Betic samples show limited genetic differentiation between them ($D = 0.02$). The results of the jack-knife test indicate that no unambiguous support exists for most of the branching patterns within the *A. obstetricans* group of populations (Fig. 2).

Table II. Allele frequencies for 50 gene loci, surveyed in four species of midwife toads from six localities. To the bottom: average sample size (N) and average heterozygosity estimated under the assumption of Hardy-Weinberg equilibrium (H_e), and standard error to heterozygosity (SE_H).

Taxon	*cisternasii*	*cisternasii*	*dickhilleni*	*muletensis*	*o. obstetricans*	*o. obstetricans*
Locality	Cuba	Viana	Sierra Tejeda	Mallorca	Jublains	Picos de Europa
	1	2	3	4	5	6
Locus						
N	5	9	5	5	5	1
Acph-1	a 1.00	a 1.00	a 0.70 b 0.30	b 1.00	b 1.00	b 1.00
N	5	9	5	5	5	1
Acph-2	a 0.20 b 0.80	a 0.72 b 0.28	d 1.00	d 1.00	a 0.90 b 0.10	a 1.00
N	5	9	5	5	5	1
Ak	b 1.00	b 0.94 c 0.06	a 1.00	a 1.00	c 0.10 b 0.90	b 1.00
N	5	9	5	6	5	1
Ata-1	c 0.30 d 0.70	c 0.11 d 0.89	a 0.60 c 0.40	c 1.00	a 0.80 b 0.20	a 1.00
N	5	9	5	4	5	1
Ata-2	b 1.00	b 1.00	b 1.00	a 1.00	a 1.00	a 1.00
N	5	9	5	2	5	1
Cat	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	5	9	5	6	5	1
Ck-1	b 1.00	b 1.00	a 1.00	b 1.00	a 1.00	a 1.00
N	5	9	5	6	5	1
Ck-2	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	5	9	4	2	5	1
Est-1	b 1.00	b 1.00	a 0.13 b 0.88	c 0.25 d 0.50 e 0.25	d 1.00	b 1.00
N	5	9	5	5	5	1
Est-2	b 1.00	b 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	5	9	4	2	5	1
Fdh	a 1.00	a 1.00	b 1.00	b 1.00	b 1.00	b 1.00
N	5	9	4	2	5	1
GP.M-1	b 1.00	b 1.00	a 0.50 c 0.50	c 1.00	c 1.00	a 0.50 c 0.50
N	5	9	4	2	5	1
GP.M-2	c 1.00	c 1.00	b 1.00	a 1.00	b 1.00	b 1.00
N	5	9	4	2	5	1
GP.M-4	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	5	9	4	2	5	1
GP.M-5	b 1.00	b 1.00	a 0.50 b 0.50	b 1.00	a 0.20 b 0.80	b 1.00

Table II cont.

	1	2	3	4	5	6
N	5	9	4	2	5	1
GP.M-6	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	5	9	4	2	5	1
GP.M-7	a 1.00	a 1.00	a 0.75	b 1.00	a 0.90	a 1.00
			b 0.25		b 0.10	
N	5	9	4	2	5	1
GP.S-1	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	5	9	4	2	5	1
GP.S-2	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	5	9	4	2	5	1
GP.S-3	c 1.00	c 1.00	b 1.00	a 1.00	d 1.00	b 1.00
N	5	9	5	4	5	1
G-6-pd	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	5	9	5	4	5	1
Gdh	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	5	9	5	4	5	1
Gpi	a 0.70	a 0.78	a 0.80	a 0.75	a 1.00	a 1.00
	b 0.30	b 0.22	b 0.20	b 0.25		
N	5	9	5	6	5	1
α-Gly	a 1.00	a 1.00	b 1.00	b 1.00	a 1.00	a 1.00
N	5	9	5	5	5	1
Icd-1	a 0.10	a 0.06	a 0.20	b 0.80	b 0.20	d 1.00
	c 0.90	c 0.94	b 0.80	d 0.20	d 0.80	
N	5	9	5	4	5	7
Icd-2	b 1.00	b 1.00	b 1.00	a 0.25	b 1.00	b 1.00
				b 0.75		
N	5	9	7	6	5	7
Ldh-1	c 1.00	b 0.22	a 1.00	a 1.00	a 1.00	a 1.00
		c 0.78				
N	5	9	7	6	5	7
Ldh-2	b 1.00	b 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	5	9	5	4	5	1
Lap	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	5	9	4	2	5	1
Mdh-1	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	5	9	7	6	5	7
Mdh-2	d 0.90	d 1.00	c 1.00	c 1.00	c 1.00	b 0.07
	e 0.10					c 0.93
N	5	9	7	6	5	7
Me-1	a 1.00	a 1.00	b 1.00	c 1.00	b 1.00	b 1.00
N	5	9	7	6	5	7
Me-2	a 0.10	e 1.00	a 1.00	b 1.00	e 1.00	e 1.00
	d 0.20					
	e 0.70					

Table II cont.

	1	2	3	4	5	6
N	5	9	5	4	5	1
Mpi-1	e 1.00	e 1.00	a 1.00	b 1.00	d 1.00	d 1.00
N	5	9	5	4	5	1
Mpi-2	b 1.00	b 1.00	a 0.10	a 0.25	b 1.00	b 1.00
			b 0.90	b 0.75		
N	5	9	4	2	5	1
Nadhdh-1	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	5	9	4	2	5	1
Nadhdh-2	a 0.50	a 0.55	c 0.50	d 0.25	d 0.30	e 1.00
	b 0.50	b 0.44	e 0.50	e 0.75	e 0.70	
N	5	9	4	2	5	1
Pg-1	a 1.00	a 1.00	b 1.00	b 1.00	b 1.00	b 1.00
N	5	9	4	2	5	1
Pg-2	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	5	9	4	2	5	1
Pep-1	b 1.00	b 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	5	9	5	2	5	1
Pep-2	a 1.00	a 1.00	c 1.00	c 1.00	c 1.00	c 1.00
N	5	9	5	6	5	1
Pgm-1	b 1.00	b 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	5	9	5	4	5	1
Pgm-2	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	5	9	6	4	5	5
6-Pgd	c 1.00	c 1.00	b 0.92	b 1.00	c 1.00	c 1.00
			c 0.08			
N	5	9	5	2	5	1
Sdh-1	a 0.20	a 0.11	c 1.00	a 0.25	b 0.50	c 1.00
	c 0.80	b 0.11		c 0.75	c 0.50	
		c 0.78				
N	5	9	5	4	5	1
Sdh-2	b 1.00	a 0.33	a 0.80	a 1.00	a 1.00	a 1.00
		b 0.67	c 0.20			
N	5	9	7	6	5	7
Sod-1	c 1.00	c 1.00	b 1.00	b 1.00	a 1.00	b 1.00
N	5	9	5	4	5	1
Sod-2	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	5	9	4	2	5	1
UEP-1	c 1.00	c 1.00	b 0.50	a 0.75	c 1.00	c 1.00
			c 0.50	c 0.25		
N	5	9	4	2	5	1
UEP-2	a 0.80	a 0.94	a 1.00	a 1.00	a 1.00	a 1.00
	b 0.20	b 0.06				
N (average)	5	9	4.9	3.7	5	1.9
He (x 100)	6.9	6.2	10.8 *	7.9	5.4	2.3 *
SEH (x 100)	2.2	2.0	2.8	2.7	1.9	2.0

* Weighted mean of two populations.

In the most parsimonious phylogenetic tree (Fig. 3), the longest branch is the one connecting the designated outgroup (*A. cisternasii*) with the remaining populations. As in the UPGMA-phenogram, two main clades are resolved in the ingroup. One clade is composed of the Mallorcan and the Betic midwife toad populations. The other clade consists of the six populations of *A. obstetricans*. Bootstrapping indicates that no strong support (replication score ≤ 80%) exists for any pattern of cladogenesis within the groups (Fig. 3).

The levels of differentiation observed vary widely within and among clades. Twenty-three loci change in allele composition along the branch joining the

Table III. Allele frequencies for 31 gene loci in three species of midwife toads from six localities (same legend as Table II).

Taxon	*cisternasii*	*dickhilleni*	*o. almogavarii*	*o. boscai*	*o. boscai*	*o. obstetricans*
Locality	Villa-nueva	Bien-servida	Berga	Caudiel	Tuy	Irati
	1	2	3	4	5	6
Locus						
N	2	5	5	2	5	2
Acph-1	a 1.00	a 1.00	a 0.80 b 0.20	a 0.75 b 0.25	a 0.50 b 0.50	b 1.00
N	2	5	5	2	5	2
Acph-2	b 1.00	d 1.00	a 0.50 b 0.50	b 0.75 c 0.25	b 1.00	b 1.00
N	2	5	9	2	5	2
Ak	b 1.00	a 1.00	b 1.00	b 1.00	b 1.00	b 1.00
N	2	5	10	4	5	2
Ata-1	d 1.00	a 1.00	a 0.90 b 0.10	a 1.00	a 1.00	a 1.00
N	2	5	5	2	5	2
Ata-2	b 1.00	b 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	2	5	5	2	5	2
Cat	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	2	5	8	4	5	2
Ck-1	b 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	2	5	8	4	5	2
Ck-2	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	2	5	5	4	5	2
Est-2	b 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	2	5	10	2	5	2
G-6-pd	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	2	5	10	4	5	4
Gdh	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	2	5	5	4	5	2
Gpi	a 0.75 b 0.25	a 0.90 b 0.10	a 0.60 b 0.40	a 0.38 b 0.63	a 1.00	a 1.00
N	2	5	12	4	5	2
α-Gly	a 1.00	b 1.00	c 1.00	a 1.00	a 1.00	a 1.00
N	2	7	10	3	6	2
Icd-1	c 1.00	a 0.50 b 0.50	d 1.00	b 0.33 d 0.67	d 1.00	d 1.00
N	2	7	10	3	6	3
Icd-2	b 1.00	b 1.00	b 1.00	b 1.00	b 1.00	b 1.00

Table III cont.

	1	2	3	4	5	6
N	2	8	11	4	6	7
Ldh-1	c 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	2	8	12	4	6	7
Ldh-2	b 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	2	5	5	2	5	2
Lap	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	2	8	10	4	6	7
Mdh-2	d 1.00	c 1.00	a 0.40 c 0.60	c 1.00	c 1.00	c 1.00
N	2	8	10	4	6	7
Me-1	a 1.00	a 1.00	b 0.30 d 0.70	b 1.00	b 1.00	b 1.00
N	2	8	10	4	6	7
Me-2	e 1.00	b 1.00	c 1.00	e 1.00	e 1.00	e 1.00
N	2	5	5	2	5	2
Mpi-1	e 1.00	a 0.90 b 0.10	a 0.90 d 0.10	c 1.00	a 0.30 c 0.30 d 0.40	d 1.00
N	2	5	5	2	5	2
Mpi-2	b 1.00	b 1.00	b 1.00	b 1.00	b 1.00	b 1.00
N	2	5	4	2	3	2
Pep-2	a 1.00	c 1.00	c 1.00	c 1.00	b 1.00	c 1.00
N	2	5	8	4	5	2
Pgm-1	b 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	2	5	5	2	5	2
Pgm-2	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	2	8	12	3	6	6
6-Pgd	c 1.00	a 0.38 b 0.63	c 1.00	c 1.00	c 1.00	c 1.00
N	2	5	6	2	6	3
Sdh-1	c 1.00	c 1.00	c 1.00	c 1.00	b 0.08 c 0.92	c 1.00
N	2	5	6	2	6	3
Sdh-2	b 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N	2	8	12	4	6	7
Sod-1	c 1.00	b 1.00	b 1.00	b 1.00	b 1.00	b 1.00
N	2	5	5	2	5	2
Sod-2	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00	a 1.00
N (average)	2	5.8	7.8	3.0	5.3	3.3
He (x 100)	1.6	4.6	9.0	6.7	4.7	0.0
SEH (x 100)	1.6	2.4	3.3	3.2	2.9	0.0

outgroup and the ingroup (A−B in Fig. 3). Thirteen of these loci show alternative fixed alleles; the other ten loci change allele frequencies along that branch. The branch joining the nodes A−C is supported by nine loci, three of which show alternative fixed alleles. Finally, four loci change state between the nodes A−D and only one of these shows fixed differences. The latter branch is not strongly supported by the bootstrap test. Eight alleles over seven loci are found uniquely in *A. muletensis*, four of which are fixed for the sample. Five autapomorphic alleles were observed in the Betic midwife toad, all at low and intermediate frequencies (≤ 50%).

Table IV. Genetic distance (to the left of diagonal) and standard error (to the right of diagonal) (Nei, 1972, 1987) in 12 populations of midwife toads. Estimates are based on 50 loci (in italics) or on 31 loci.

Population	Locality	1	2	3	4	5	6	7	8	9	10	11	12
1 *A. cisternasii*	Cuba		*0.016*	*0.143*	*0.167*	*0.136*	*0.129*	0.016	0.206	0.188	0.170	0.169	0.179
2 *A. cisternasii*	Viana	*0.01*		*0.142*	*0.165*	*0.129*	*0.123*	0.028	0.202	0.184	0.168	0.168	0.177
3 *A. dickhilleni*	Sierra Tejeda	*0.70*	*0.70*		*0.078*	*0.083*	*0.070*	0.213	0.024	0.105	0.105	0.113	0.112
4 *A. muletensis*	Mallorca	*0.87*	*0.86*	*0.27*		*0.094*	*0.095*	0.246	0.108	0.136	0.134	0.142	0.130
5 *A. o. obstetricans*	Jublains	*0.66*	*0.61*	*0.29*	*0.37*		*0.041*	0.189	0.122	0.089	0.070	0.067	0.050
6 *A. o. obstetricans*	Picos de Europa	*0.61*	*0.56*	*0.22*	*0.37*	*0.08*		0.187	0.112	0.076	0.058	0.054	0.033
7 *A. cisternasii*	Villanueva	0.01	0.03	0.88	1.06	0.75	0.74		0.211	0.191	0.168	0.167	0.176
8 *A. dickhilleni*	Bienservida	0.84	0.82	0.02	0.31	0.38	0.33	0.86		0.102	0.102	0.110	0.111
9 *A. o. almogavarii*	Berga	0.74	0.72	0.30	0.45	0.22	0.16	0.76	0.28		0.071	0.076	0.076
10 *A. o. boscai*	Caudiel	0.64	0.63	0.29	0.44	0.14	0.10	0.63	0.28	0.15		0.049	0.049
11 *A. o. boscai*	Tuy	0.64	0.63	0.34	0.48	0.13	0.09	0.62	0.32	0.17	0.07		0.041
12 *A. o. obstetricans*	Irati	0.69	0.68	0.33	0.42	0.07	0.03	0.67	0.33	0.16	0.07	0.05	

Mean heterozygosity (H_e) varied widely across populations, exceeding the variation observed between species. No genetic variation across 31 loci was observed in a small sample ($N=2-7$) from Irati. The highest value of H_e ($H_e = 0.108 \pm 0.028$) was found in the sample from Sierra Tejeda with 13 polymorphic loci out of 50 loci surveyed in 4−7 specimens. For the remaining samples H_e varied from 0.016 ± 0.016 to 0.090 ± 0.033 with values mostly around 0.06 (Tables II and III). Heterozygosity values and the accompanying standard error over grouped populations, calculated by weighted means, are: *A. obstetricans*: 0.056 ± 0.024, *A. cisternasii*: 0.058 ± 0.020, *A. muletensis*: 0.079 ± 0.027, and for the Betic midwife toads: 0.074 ± 0.026.

Morphometric analysis

Mean, standard deviation and minimum and maximum values for untransformed morphometric variables for each population are shown in Table V. The smallest body size is found in the sample of *A. muletensis*. The individuals that make up this sample are bred in captivity which might explain their small size, but the mean SVL of 10 individuals collected in the field (measured by Mayol et al.,

1984) is not significantly different from that presented here.

The results of the PCA analysis demonstrate that midwife toads are a morphometrically homogeneous group, with within-group variability (*a posteriori* identification of group) largely exceeding between-group variability. Visual inspection of a three-dimensional plot shows that all populations are compactly clustered around y1 = y2 = y3 = 0. However, specimens from the Badajoz population (*A. cisternasii*) are mostly situated at the fringe of the main cluster. Morphometric characters with high loadings on the first PCA axis (Fig. 4B) are: (1) length of the tibia-fibula and length of the hind leg, (2) length of the third finger and third toe, and (3) length of the jaw. On the second axis the horizontal and vertical diameter of the tympanum have highest loadings. On the third axis the distance between the nostrils, eye-nostril distance, and head width have high loadings. The identification of the populations indicates that *A. cisternasii* differs from other midwife toads by its short extremities, finger and toes, a short jaw and a large tympanum, whereas *A. muletensis* differs from other midwife toads in the positioning of its narines and by a narrow head. The other midwife toads (six populations of *A. obstetricans* and two populations from the Betic mountains) are morphometrically very similar.

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Table V. Morphometric data for 13 external characters in midwife toads from 10 populations. Data include mean, standard deviation, and range in mm. Data are pooled for the sexes. For abbreviations used see text.

Populations		SVL	HW	JL	EW	HT	VT	ED	ND	END	TFL	HLL	TL	FL
A. cisternasii														
Badajoz	mean	36.2	14.3	12.5	4.9	3.3	3.4	4.5	3.6	3.3	13.3	42.6	13.4	5.9
N=20	SD	2.75	1.01	0.78	0.37	0.29	0.31	0.29	0.24	0.34	0.70	2.88	1.26	0.58
	min	32.5	12.1	11.3	4.3	2.9	3.0	3.9	3.3	2.7	12.3	38.6	11.1	5.0
	max	43.3	16.6	14.1	5.7	3.9	4.3	4.9	4.3	4.0	14.8	49.9	15.5	7.1
A. dickhilleni														
Alcaraz	mean	44.2	16.2	14.6	5.4	3.7	3.9	4.6	3.8	4.2	17.7	54.9	16.0	7.8
N=32	SD	3.19	0.81	1.00	0.48	0.42	0.42	0.45	0.30	0.43	1.08	4.11	1.49	0.86
	min	36.7	14.4	12.4	4.2	2.9	2.8	3.7	3.0	3.4	14.7	42.8	12.8	5.9
	max	52.5	17.9	17.0	6.3	4.8	5.1	5.5	4.3	5.1	20.6	60.8	19.5	9.8
Sierra Tejeda	mean	42.3	16.5	14.4	5.2	3.6	4.0	4.5	3.6	3.9	17.5	52.2	16.4	7.6
N=21	SD	3.52	1.02	0.89	0.66	0.37	0.38	0.40	0.28	0.40	1.16	3.92	1.37	0.50
	min	32.8	14.2	11.9	4.0	3.0	3.3	3.8	3.1	3.1	14.3	42.0	13.6	6.7
	max	47.8	18.5	16.1	6.9	4.4	4.7	5.4	4.2	4.9	19.9	61.1	19.5	9.0
A. muletensis														
"Norwich"	mean	32.6	12.3	11.7	4.2	3.0	3.1	3.4	3.4	3.5	13.9	44.0	12.7	7.1
Mallorca	SD	2.17	0.59	0.70	0.31	0.33	0.26	0.45	0.28	0.32	0.63	2.14	0.91	0.41
N=30	min	28.7	10.9	10.0	3.6	2.5	2.6	2.6	2.9	2.8	12.7	40.5	11.1	6.4
	max	36.6	13.3	13.5	4.8	3.6	3.7	4.7	3.9	4.2	15.3	49.2	14.5	7.8
A. o. obstetricans														
Jublains	mean	41.0	16.4	14.5	5.0	3.5	3.6	4.2	3.5	3.8	16.5	52.6	15.7	7.7
N=11	SD	3.43	1.09	1.17	0.49	0.49	0.49	0.47	0.42	0.61	1.02	3.67	1.15	0.89
	min	35.0	14.8	12.6	4.4	4.4	3.0	3.4	3.0	2.6	14.6	46.9	13.2	6.5
	max	46.8	18.0	16.5	5.7	5.7	4.3	5.0	4.1	4.7	17.6	57.9	17.1	8.9
Navarra	mean	35.1	13.3	12.6	5.1	3.1	3.2	3.8	3.4	3.3	14.6	47.5	14.4	6.9
N=21	SD	2.29	0.76	0.50	0.41	0.26	0.26	0.37	0.22	0.30	0.89	3.60	1.42	0.83
	min	31.2	12.1	11.7	4.1	2.7	2.7	3.1	2.8	2.9	13.3	41.4	11.6	5.0
	max	38.6	14.7	13.4	5.8	3.6	3.5	4.7	3.6	4.2	16.1	54.7	17.1	8.1
Picos de Europa	mean	39.6	15.1	13.5	5.3	3.5	3.5	4.3	3.6	3.7	16.0	50.7	15.3	7.2
N=17	SD	4.16	1.48	1.21	0.69	0.37	0.33	0.50	0.27	0.44	1.20	4.82	1.50	0.65
	min	32.2	11.5	10.7	3.8	2.9	3.0	3.3	3.1	3.1	13.5	43.0	12.2	6.3
	max	45.4	17.1	15.0	6.5	4.4	4.2	5.3	4.1	4.3	18.0	59.7	17.7	8.9
A. o. almogavarii														
Barcelona	mean	39.1	15.1	13.6	5.1	3.5	3.5	4.1	3.6	4.0	16.3	50.6	15.3	7.5
N=29	SD	5.63	2.03	1.65	0.63	0.60	0.52	0.56	0.45	0.49	1.71	5.66	2.12	0.84
	min	29.5	11.5	11.1	4.1	2.3	2.7	2.9	2.9	2.9	12.8	40.1	11.4	5.9
	max	47.5	18.7	17.8	6.7	4.5	4.4	5.2	4.9	4.9	18.9	60.3	19.9	8.8
A. o. boscai														
Galicia	mean	38.0	14.5	12.9	5.2	3.3	3.5	4.3	3.3	3.2	14.9	46.7	14.6	6.8
N=14	SD	2.35	1.54	0.98	0.37	0.45	0.41	0.40	0.39	0.28	0.86	2.55	1.03	0.48
	min	34.4	12.0	10.8	4.4	2.6	2.7	3.4	2.5	2.6	13.5	43.9	13.1	6.1
	max	42.3	17.7	14.1	5.7	4.1	4.2	4.9	4.0	3.7	16.6	52.0	16.3	7.8
Valencia	mean	42.1	16.0	14.1	5.2	3.5	3.7	4.4	3.6	3.9	16.6	50.0	14.7	7.1
N=30	SD	2.99	0.93	0.86	0.68	0.49	0.53	0.49	0.37	0.47	1.00	2.93	1.16	0.73
	min	37.4	14.6	12.3	4.1	2.8	2.9	3.3	3.2	3.0	15.3	45.5	12.7	5.3
	max	48.6	17.8	15.9	6.7	4.6	4.9	5.8	4.7	4.9	19.5	55.9	17.0	8.4

Redefinition of taxonomic units

Marked differences and similarities are apparent for the multivariate ordination of genetic and morphological data. The populations from the Betic mountains are characterized by high genetic distances to all other continental populations, though morphologically they are similar to *A. obstetricans*, and though genetically similar to the insular *A. muletensis* (Fig. 4A) these forms differ markedly in morphology (Fig. 4B). These results indicate that a taxonomic revision of the genus *Alytes* is in order. In line with arguments discussed below we propose to recognize the Betic midwife toad as a species, the formal description of which is included in the Appendix.

Within *A. obstetricans* a genetically distinct group is represented by the samples from Barcelona.

Midwife toads with a morphology and allozyme profile as in Barcelona are found in the central and eastern Pyrenees north to the Ebro River (Catalonia and northern Aragón) (García-París, 1992). This group does not correspond to any previously recognized taxonomic unit. This, and arguments discussed below, lead us to propose the recognition of the *A. obstetricans* populations from northeastern Spain as a subspecies, the formal description of which is included in the Appendix. The remaining populations of *A. obstetricans*, according to the morphologic and genetic data presented here, can be accommodated in the subspecies recognized previously.

The genus *Alytes* is thus composed of four extant specific taxa: *A. cisternasii*, *A. muletensis*, *A. obstetricans*, and *A. dickhilleni* n. sp. Of these species, *A. obstetricans* shows marked geographical variation warranting the recognition of four subspecies: *A. o. obstetricans*, *A. o. boscai* Lataste, 1879, *A. o. maurus*, and *A. o. almogavarii* n. ssp.

Discriminant analysis

Correct classifications of specimens to their respective groups on the basis of morphometric characteristics was achieved to varying degrees in the discriminant analysis (Table VIa). The level of correct classification (Geisser, 1977) was lowest for *A. dickhilleni* (51%), with misclassifications equally attributed to *A. muletensis* and *A. obstetricans*. *A. obstetricans* was frequently wrongly classified either as *A. dickhilleni* (25%), *A. muletensis* (7%), or *A. cisternasii* (4%). All specimens of *A. cisternasii* and *A. muletensis* were correctly classified (Table VIa).

A relatively high degree of discrimination is obtained for the three studied subspecies of *A. obstetricans*, with 69% of *A. o. obstetricans*, 72% of *A. o. almogavarii* and 75% of *A. o. boscai* individuals correctly classified (Table VIb). Erroneous classifications are more or less homogeneously distributed among groups.

At the population level the degree of discrimination is surprisingly high. Excluding the population from Jublains in which preserved specimens were
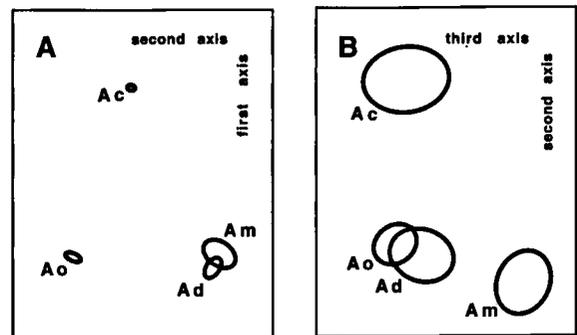
Fig. 4. Bivariate plot of the (ellipsoid) 95% confidence interval of the mean scores along two multivariate axes, for four species of midwife toads with *a posteriori* taxonomic allocation: (A) Principal Coordinate Analysis of electrophoretic data, (B) Principal Component Analysis of morphometric data. Note that three distinct, non-overlapping groups are recognized for either data set. The plots differ in the positioning of *Alytes dickhilleni* (Ad) that in the electrophoretic data set is grouped with *A. muletensis* (Am), and with *A. obstetricans* (Ao) in the morphometric data set. *A. cisternasii* (Ac) is separated from all other taxa in either data set. In (B) the second axis is plotted versus the third axis; the first axis, expressing a combination of morphometric parameters not on the other axes, also separates *A. cisternasii* from the other taxa (for details see text).

measured, the level of correct allocation to populations averages at 76% for *A. o. obstetricans* and at 89% for *A. o. boscai* (Table VIc).

Discussion

Genetic distance and taxonomic units

Several published estimates are available for Nei's genetic distance between *A. cisternasii* and *A. obstetricans*: 0.73 (Viegas & Crespo, 1985), 0.99 (Rosa et al., 1990) and 1.6 (Arntzen & Szymura, 1984); on the basis of studies involving 17, 17, and 15 loci, respectively. Considering that the statistical error of the estimate decreases with increasing number of loci, the value of 0.72, determined in the present study, may be more accurate than the previous estimates. However, different proteins accumulate electrophoretically detectable substitutions at unequal rates, so the choice of proteins may affect the outcome. The comparison of single locus heterozygosity values may provide an indication for the

Table VI. Classification of midwife toads by means of discriminant analysis of morphometric data at three taxonomic levels: a) species, b) subspecies of Alytes obstetricans, and c) populations within subspecies (for details see text).

a) species		N	classified as				classified correctly
			Ac	Ad	Am	Ao	
A. cisternasii	(Ac)	20	20	0	0	0	100%
A. dickhilleni	(Ad)	53	0	27	13	13	51%
A. muletensis	(Am)	30	0	0	30	0	100%
A. obstetricans	(Ao)	122	5	30	9	78	64%

b) subspecies		N	classified as			classified correctly
			Aoo	Aoa	Aob	
A. o. obstetricans	(Aoo)	49	34	8	7	69%
A. o. almogavarii	(Aoa)	29	5	21	3	72%
A. o. boscai	(Aob)	44	6	5	33	75%

c) populations A. o. obstetricans		N	classified as			classified correctly
			J	N	PE	
Jublains	(J)	11	10	1	0	91%
Navarra	(N)	21	3	15	3	71%
Picos de Europa	(PE)	17	0	3	14	82%

A. o. boscai		N	classified as		classified correctly
			G	V	
Galicia	(G)	14	13	1	93%
Valencia	(V)	30	4	26	87%

strength of this effect. Average H_e values for individual proteins are listed by Ward et al. (1992: table VIII). Some proteins were studied both by Rosa et al. (1990) and in the present study. Comparing these with the ones unique to the present study, it is observed that the first set of proteins on average shows higher levels of heterozygosity in amphibians than the latter group ($H_e \pm SE = 0.113 \pm 0.045$ and 0.086 ± 0.065, respectively). This suggests that the difference between the observed genetic distances, at least in part, can be attributed to the particular set of protein loci studied.

The local existence of areas of sympatry of A. cisternasii and A. obstetricans is well documented (Malkmus, 1983; Perez Mellado, 1983; García-París et al., 1990; Pleguezuelos & Moreno, 1990; Rosa et al., 1990), while evidence for interspecific hybridization is absent. Mutually incompatible gene complexes precluding successful development of hybrids appear to have evolved between the species over the long period of evolutionary independence suggested by the large genetic distance between them. However, a genetic distance similar to the one observed here was reported for the hybridizing toads Bufo boreas Baird & Girard, 1852 and B. punctatus Baird & Girard, 1852 ($D_{Nei} = 0.91$; Feder, 1979). These toads are the most genetically distinct pair of terrestrial vertebrate species known to hybridize in nature. Much smaller genetic distances are observed between the other species of midwife toads and, in sympatry, hybridization between them may be anticipated. Studying the contact zones would help in assessing the taxonomic status of the taxa involved. Unfortunately, except for the pair A. cisternasii–A. obstetricans, all species of midwife toads have allopatric distributions (Fig. 1). In southeastern Spain the southern border of the range of A. obstetricans has not been determined precisely. A geographic contact with northern A. dickhilleni, for example along the Almansa plains in the province of Albacete, seems unlikely given the topography of this region.

The recognition of the midwife toad from the Betic mountains as a distinct taxonomic unit poses a classificatory problem. One possible solution, considering Betic midwife toads conspecific with A. obstetricans, which effectively represents the current situation, can be ruled out immediately on the basis of the long-standing independent evolutionary history of both groups, and the paraphyletic origin the single species would have. The remaining options are to distinguish, besides A. cisternasii: (1) one species – the toads of Mallorca and the Betic mountains are conspecific with A. obstetricans, (2) two species – the toads from the Betic mountains are conspecific with A. muletensis, or, (3) three species – the toads from the Betic mountains are considered a new species, distinct from A. obstetricans and A. muletensis. In

our discussion of these options we rely mainly on the phylogenetic relationships, the presence of gene flow among contiguous populations, and the levels of genetic and morphological differentiation between the taxa.

The first of the three options outlined above has to be challenged because the Balearic population is genetically and morphologically distinct from all other midwife toads. The second option in which the Balearic and Betic toads are conspecific gains some support from the phylogenetic reconstruction, because the monophyly of this group is supported. However, the Betic and Balearic toads differ in a suite of genetic and morphological characters, and gene flow between them is absent. In consequence we accept the third option: the Betic midwife toads represent a species (*A. dickhilleni*) separate from *A. obstetricans* and *A. muletensis*, to which solution we see no valid objections. Morphologically *A. dickhilleni* is a cryptic species, almost indistinguishable from *A. obstetricans*, although its sister species is not *A. obstetricans* but *A. muletensis*.

Geographic variation

No evidence for geographical substructuring is available for within *A. cisternasii*, *A. dickhilleni*, or *A. muletensis*. However, groups of populations within *A. obstetricans* differ markedly from one another. Currently three subspecies of *A. obstetricans* are recognized, including the European *A. o. obstetricans* and *A. o. boscai*. On the basis of the high level of genetic differentiation of midwife toads from Barcelona as compared with other populations of *A. obstetricans*, that is paralleled by a marked morphological differentiation, we recognize them as representing a (new) subspecies, *A. o. almogavarii*. The high genetic distance between *A. o. almogavarii* and all other *A. obstetricans* and the apparent absence of gene flow between the populations from Barcelona (*A. o. almogavarii*) and Valencia (*A. o. boscai*) might be considered to support even the *specific* status of *A. o. almogavarii*. However, a zone of introgression between *A. o. almogavarii* and *A. o. obstetricans* in the

western Pyrenees (García-París, 1992) challenges such a view.

On the basis of genetic distances and the colouration pattern observed in the field, the geographic distribution of *A. o. obstetricans* stretches across the Pyrenees all along the Cantabrian mountains to the contact zone with *A. o. boscai* in Galicia. The distribution of *A. o. boscai* appears to be limited to the western and central parts of the Iberian Peninsula, and in the east to the south of the Ebro river. Unfortunately, the distributional boundaries of the various subspecies are as yet not well documented. Wide zones of intergradation may exist between them and these in particular require additional investigation.

Genetic variation

Genetic distances obtained among populations of *A. cisternasii* are close to zero and nominally higher than the ones obtained by Rosa et al. (1990). The divergence among the two Portuguese populations is similar to that measured for each of them versus the population from central Spain. The observed lack of differentiation in *A. cisternasii* seems compatible with the species' ecological characteristics and pattern of distribution. The area of the Iberian Peninsula where *A. cisternasii* predominates over *A. obstetricans* consists of a remarkable monotonous environment of plains and rolling hills (Rosa et al., 1990). *A. cisternasii* is distributed in small, local populations (demes) that are associated with sandy and loamy deposits of rivers and streams (Crespo, 1979). A network of demes follows the fluvial systems on granite soils (see García-París et al., 1990). The reproductive period of *A. cisternasii* coincides with the annual highest fluvial instability (Márquez, 1990), favouring the dispersal of larvae along streams and rivers.

A small genetic distance is also observed between the populations of *A. dickhilleni* from different mountain ranges (Sierra Tejeda and Sierra de Alcaraz). In contrast to *A. cisternasii*, *A. dickhilleni* is probably restricted to places with water present the year round, suggesting a fragmented distribution for this species, with limited gene flow among

demes. The observed lack of genetic differentiation in *A. dickhilleni* may indicate a distribution that only recently became fragmented. Future studies on this species should include many more populations, especially some of the seemingly isolated ones, such as those found at the top of high arid plateaus in the Sierra Nevada, Sierra de Baza, and Sierra de Filabres in southeastern Spain (Pleguezuelos & Moreno, 1990; García-París et al., 1993). At present six natural populations of *A. muletensis* are known, all located in and around small streams in the limestone mountains of northern Mallorca. Given the similarities of habitat and population distribution, the metapopulation structure of this species and *A. dickhilleni* is likely to be similar.

In contrast to other species of midwife toads, *A. obstetricans* shows substantial genetic variation. On the Iberian Peninsula *A. obstetricans* lives in mountainous areas, perhaps involving stronger fragmentation than is encountered by *A. cisternasii*. Within *A. o. obstetricans* the genetic distance between a population from central France (Jublains) to populations from Navarra ($D_{Nei} = 0.07$) and Picos de Europa ($D_{Nei} = 0.08$) is more pronounced than the distance between the latter two populations ($D_{Nei} = 0.03$) that are geographically closer. Similar results suggesting an isolation-by-distance effect, involving five populations, are presented by Rosa et al. (1990) for *A. o. boscai*.

Alytes o. almogavarii from the northeastern corner of the Iberian Peninsula has reached a high degree of genetic differentiation relative to the other subspecies. No introgression has been documented with eastern *A. o. boscai* with which its distribution is contiguous along the eastern part of the Ebro river but limited introgression with *A. o. obstetricans* from the western Pyrenees may exist (García-París, 1992).

The existence of a smooth cline between *A. o. obstetricans* and *A. o. boscai* is suggested by the remarkable decrease in genetic distances when geographically intermediate populations are included in the analysis (García-París, 1992). Similarly, Arntzen & Szymura (1984) noted a cline for two loci, *Sod* and *6-Pgd*, centered, however, in the Pyrenees rather than farther southwest.

Average heterozygosities in midwife toad spe-

cies, ranging from 0.056 to 0.079, are lower than the value reported for amphibians in general ($H_e = 0.109$; Ward et al., 1992). As in most cases, it is almost impossible to decide to what extent differences reflect historical factors such as population size reduction, gene flow, fragmentation or fusion of formerly isolated populations, and to what extent they are influenced by natural selection. It is surprising though that the highest values are observed for the sister species *A. dickhilleni* and *A. muletensis* which have small and, perhaps, strongly bottle-necked populations.

Alytes cisternasii is clearly distinct from other species of midwife toads in morphological and genetic characteristics. The use of *A. cisternasii* as an outgroup for reconstructing the phylogenetic relationships of *A. dickhilleni*, *A. muletensis*, and *A. obstetricans* appears to be justified. Placing the root of the phylogenetic tree on any other branch than the one between points C and D of Fig. 3 would point to highly uneven rates of protein evolution among lineages, for which there is little support.

Morphological variation

Compared with other anurans that show similar levels of genetic differentiation, *A. cisternasii* does not show dramatic changes in morphology (Crespo, 1979) or in behavioural aspects such as mating call (Márquez, 1990) relative to other midwife toad species. Despite the general homogeneity in midwife toads, three different morphotypes can be distinguished (Sanchíz, 1984). *A. cisternasii* shows structural pecularities which are related to a fossorial mode of life (Crespo, 1979; Sanchíz, 1984). *A. muletensis*, which lives in wall crevices in deep limestone canyons, is characterized by adaptations for climbing (Sanchíz, 1984). Thirdly, *A. obstetricans* and *A. dickhilleni* constitute a generalist group (*sensu* Crespo, 1979) which species commonly inhabit eroded soils in rocky areas. The generalist morphotype is inferred to be basal to the *A. obstetricans—A. muletensis—A. dickhilleni* clade. Otherwise, the generalist morphotype evolved twice (along the lineages leading to *A. dickhilleni* and
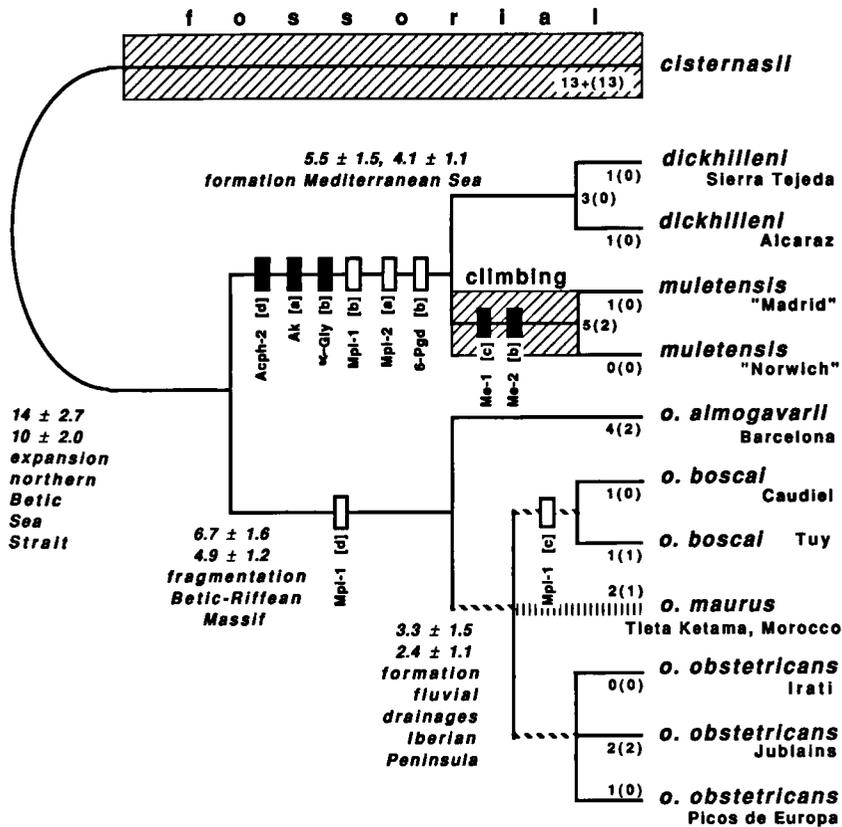
Fig. 5. Evolutionary classification of the genus *Alytes*. Interrupted lines describe "hierarchical" relationships below the species level. Loci describing particular clades are indicated along the branches (alleles shown in brackets, fixed alleles indicated by solid symbols). Along terminal branches only the number of informative alleles is indicated with, in parentheses, the number of alleles at particular loci observed to be fixed. The positioning of *A. o. maurus* (wide interrupted line) is according to Arntzen & Szymura (1984). Indicated are estimates of diverging time of lineages (in mY), according to calibrations of the "molecular clock" by Thorpe (1982) and Maxson & Maxson (1979), respectively. Geological events thought to be associated with speciation in *Alytes* are described. Hypothesized changes in mode of life are indicated by shading. For further details see text.

A. obstetricans, respectively) which does not represent a parsimonious scenario of evolutionary change. Consequently, the climbing morphotype is considered an evolutionary innovation for the *A. muletensis* lineage (Fig. 5). Without an adequate outgroup it is not possible to determine whether the "fossorial" or the "generalist" morphotype is basal to the genus *Alytes*. The more likely hypothesis is that proto-*Alytes* possessed the "generalist" morphotype, because it is present in the most basal of archeobatrachian branches, such as the ones leading to *Ascaphus* and *Leiopelma* (Duellman & Trueb, 1985). This makes the fossorial morphotype an innovation on the *A. cisternasii* lineage (Fig. 5).

Alytes obstetricans and *A. dickhilleni* show marked genetic differences but morphologically they are cryptic species. Following the consensus classification presented in Fig. 5, six different alleles are found to be characteristic of the lineage leading to the *A. dickhilleni−A. muletensis* clade. The loci *Acph-2*, *Ak*, and α-*Gly* show alternative fixed alleles. In *A. muletensis* both *Me* loci have fixed alleles that are unique to the species. For *A. obstetricans*, *Mpi-1* is the most informative locus with the *d*-allele unique to the species, the *c*-allele only found in *boscai*, and another allele at this locus fixed in *A. o. maurus* (Arntzen & Szymura, 1984). Finally, some alleles at the loci α-*Gly*, *Mdh-2*, *Me-1* and *Me-2* are uniquely found in *A. o. almogavarii*.

We have not found clear external qualitative or

morphometric diagnostic characters other than the pattern of colouration and skin structure to distinguish some taxa. *A. muletensis* and *A. dickhilleni* do not possess the marked dorsal warts and dorsal orange or red spots that are characteristic of *A. obstetricans*. *A. muletensis* can further be distinguished by the generally brownish or greenish dorsal colour with metallic reflections (see Plate I and Appendix). It might be concluded that the morphometric data are more useful in characterizing populations and subspecies of *A. obstetricans* than in characterizing the full species *A. obstetricans* and *A. dickhilleni*. *A. cisternasii* can be distinguished from other midwife toad species by a suite of morphological and genetic characters. In the field, one of the most practical and reliable features to identify *A. cisternasii* is the presence of two metacarpal tubercles where other species have three.

Historical biogeography of midwife toads

Among the different calibrations that relate genetic distance of species with the date of their speciation, the one in which one unit of D_{Nei} reflects 18.9 mY of lineage independence (Thorpe, 1982) fits the observed genetic differentiation of African and European amphibian populations in with the minimum time of separation by the Strait of Gibraltar (Busack, 1986). Applying this calibration to the presented genetic data estimates the age of *A. cisternasii* as 14 ± 2.7 mY. To some degree independent estimates, on the basis of immunological distance (Maxson, 1984) and genetic distance (Rosa et al., 1990) give estimates of 16–14 mY. Applying the calibration of Maxson & Maxson (1979) gives slightly lower estimates (10±2.0 mY). Accepting the Middle Miocene as the date of differentiation seems reasonable. This also fits in with the Paleogeographic model of the western Mediterranean region put forward by López Martínez (1989).

According to this scenario, the ancestral stock of *Alytes* settled in Iberia in the Lower Miocene, reaching the Balearic-Betic-Riffean Massif (Giusti & Manganelli, 1984). In this epoch a faunistic exchange among Asian, European, and African taxa may have been widespread (Aguirre & Morales,

1980). During the Middle Miocene the *Alytes* populations of the Balearic-Betic-Riffean Massif became isolated from the continental populations as a result of the expansion of the northern Betic Sea Strait. The continental stock gave rise to *A. cisternasii* and the island stock to a proto-*A. obstetricans*. *A. cisternasii* may have become adapted to the arid sandy soils of the western Iberian Peninsula, as was suggested for *Bufo calamita* Laurenti, 1768 by Hemmer et al. (1981). The sympatric area between *A. cisternasii* and *A. obstetricans* is probably secondary, and a consequence of range expansion after initial separation.

An alternative scenario would refer to the uprising of the Neo-Pyrenees at 10 mY as the vicariant event giving rise to *A. cisternasii* on the Iberian Peninsula versus the *A. obstetricans* group to the north. The uprising of the Pyrenees is thought to have played a major role in the vicariance biogeography of several insect and amphibian taxa (Oosterbroek & Arntzen, 1992) but seems less likely to have been of importance in *Alytes*. This scenario would require a recolonization by *A. obstetricans* of the Iberian Peninsula from the north. We consider this unlikely because most genetic and morphological variation of *A. obstetricans* is in the Iberian Peninsula, not to the north of it.

The fragmentation of the Betic-Riffean Massif and the associated formation of an archipelago during the Upper Miocene at some 5–6 mY ago may have caused the differentiation of *A. obstetricans* as being distinct from the lineage leading to *A. muletensis* and *A. dickhilleni* (Fig. 5). Indications are that during the Messinian Crisis, connections between the islands of the former Balearic-Betic-Riffean Massif and the mainland came into existence prior to the complete desiccation of the Mediterranean Sea. This would allow the expansion of *A. obstetricans* across the continent. The, as yet, undifferentiated *A. muletensis-dickhilleni* clade remained isolated in the Betic mountains and further dispersion of *Alytes* may have been limited, particularly since this was a period of extreme aridity (Crowley & North, 1991). We further hypothesize that the opening of the Strait of Gibraltar at the end of the Miocene (5 mY) and the formation of the Mediterranean Sea isolated the Balearic Islands

from the mainland. In line with their estimated age of 4–5 mY, this separated the existing stock into lineages leading to extant *A. muletensis* and *A. dickhilleni*. Further contact between *A. obstetricans* and *A. dickhilleni* was prevented by the inundation of the Guadalquivir valley and the Almansa plains (López Martínez, 1989).

The survival of *A. muletensis* on Mallorca and the presence of the – now subfossil – *A. talaioticus* (Sanchíz & Alcover, 1982) on Menorca required a steady supply of fresh water on calcareous islands. The marked morphological differentiation of *A. muletensis* may be the result of a rapid adaptation to deep canyons, the only places with water available throughout the year.

The expansion of *A. obstetricans* to the north was probably followed by steps of regression and transgression during the climate fluctuations of the Pleistocene. The formation of the main fluvial drainages of the Iberian Peninsula about 4 mY ago (Doadrio, 1988) may have caused the isolation of *A. o. boscai* in the west and *A. o. almogavarii* in the east. The differentiation of *A. o. obstetricans* and *A. o. boscai* seems to have happened during the Pleistocene. Further analysis is required to examine the extent of differentiation reached between populations of *A. o. boscai*.

The existence of *A. obstetricans* in northern Africa poses a difficult question. One would expect midwife toads in this region to belong to the *A. muletensis–A. dickhilleni* clade of the Balearic-Betic-Riffean Massif and to be of the same age as the Strait of Gibraltar, or older. This is not reflected in the genetic differentiation of African and European populations. Close similarity between the populations from the Rif to *A. obstetricans* from the Iberian Peninsula is indicated by the works of Arntzen & Szymura (1984) and Maxson & Szymura (1984). The level of differentiation is similar to the level reached between two geographically distant populations within *A. o. obstetricans*. An anthropogenic introduction in the Rif has been considered (Arntzen & Szymura, 1984). This hypothesis is conceivable because, unlike in most other anurans, the introduction of a single male with eggs can generate a new population. Similarly, dispersal across the Strait has been already proposed for *Pleurodeles*

waltl Michahelles, 1830 (Busack, 1986), an urodele species with internal fertilization that is common in southern Spain. However, for *Alytes* such a hypothesis has become unlikely with the discovery in Morocco of a population on the northern slopes of the Middle Atlas (Libis, 1985; assuming that Rif and Atlas populations both belong to *A. o. maurus*). The hypothesis of active dispersal from the Sierra Tejeda or Sierra Nevada to the Rif, across the Strait of Gibraltar (Arntzen & Szymura, 1984) must be rejected, since it is now understood that populations from these areas belong to different species. The scenario that we see as the least unlikely couples dispersal across the Strait of Gibraltar with the fragmentation of a previously continuous distribution of *A. obstetricans* ranging from central Portugal to southern Spain. This hypothesis would be supported if the similarity between Moroccan and Portuguese populations of *A. obstetricans* were particularly marked (Rosa et al., 1990). This line of research could prove especially rewarding if populations of *A. obstetricans* in the Serra de Monchique, southern Portugal (Malkmus, 1983) were to be rediscovered.

Acknowledgements

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Appendix

Taxonomic revision of the genus *Alytes*, including the description of *Alytes dickhilleni* n. sp. and *Alytes obstetricans almogavarii* n. ssp., with notes on their distribution, habitat and conservation status of species and subspecies, and a key to the identification of adults.

Alytes Wagler, 1830

Alytes Wagler, 1830: 206.
   Type species: *Bufo obstetricans* Laurenti, 1768.
Obstetricans Dugès, 1839: 7.
   Type species: *Obstetricans vulgaris* Dugès, 1839.
Ammoryctis Lataste, 1879b: 983.
   Type species: *Alytes cisternasii* Boscá, 1879.
Baleaphryne Sanchíz & Adrover, 1979: 6.
   Type species: *Baleaphryne muletensis* Sanchíz & Adrover, 1979.

Diagnosis. – Adult: Pupil vertical. Vomerine teeth in short transverse series behind choanae. Tongue entire, circular, slightly free behind. Tympanum distinct. Fingers free, toes webbed. Diapophyses of sacral vertebrae strongly dilated. Intercotyle ratio of atlas as type III of Lynch (1971). Absence of a ventral longitudinal crest in atlas. Sacro-urostyle with bicondylar articulation. Cresta dorsalis in urostyle and ilium absent. Tuber superius iliacus present. Columella absent.

   Larvae: Larvae of type III of Orton (1953, 1957). Eyes on upper surface of body. Spiraculum in mid-ventral line on anterior half of body. Anal tube medial. Lip entirely surrounded by a series of papillae. Upper lip with two series of labial teeth. Lower lip with three series of labial teeth. All series continuous, or inner series of lower lip narrowly interrupted in the middle. Outer series of each lip composed of one or two rows of teeth, other series of two or three rows.

Geographical distribution. – Western Europe: The Netherlands, Belgium, Luxemburg, Germany, Switzerland, France, Andorra, Spain, Portugal. Northwestern Africa: Morocco.

Taxonomic comments. – The genus *Alytes* encompasses four extant species, *A. cisternasii* Boscá, 1879, *A. dickhilleni* n. sp., *A. muletensis* (Sanchíz & Adrover, 1979), *A. obstetricans* (Laurenti, 1768), and additionally one species only known as a subfossil, *Alytes talaioticus* (Sanchíz & Alcover, 1982) from the Holocene of Menorca (Balearic Islands, Spain). The latter taxon was previously included in the genus *Baleaphryne*. *Alytes grandis* is a junior synonym of *Rana temporaria* (Sanchíz & Schleich, 1986).
   Some marked ontogenetic variation present in several

osteological characters (Sanchíz, 1984) makes these less useful for identification purposes and therefore they have been excluded from the descriptions (see instead Clarke, 1984; and Sanchíz, 1984).
   The body and tail proportions of larvae, by some considered to be reliable characters for distinguishing species, have been shown to be taxonomically uninformative. For example, local variation between stream and pond populations may exceed variation among species (M. García-París, unpublished data). Therefore, these characters have not been taken into account.
   We consider *Ammoryctis* and *Baleaphryne* as junior synonyms of *Alytes*, but it is not inconceivable that they should be classified at the subgeneric level. At present, a subgeneric structure of *Alytes*, composite of two (*Alytes* and *Ammoryctis*) or three subgenera (*Alytes*, *Ammoryctis*, and *Baleaphryne*) is supported. In the first case, *Ammoryctis* would be monotypic, including *A. cisternasii* only; in the other case two subgenera, *Alytes* and *Ammoryctis* would be monotypic, while the third one, *Baleaphryne*, would include the species *A. muletensis* and *A. dickhilleni* described below. It should be noted that a revision of the osteological features that define *Baleaphryne* is required (M. García-París & B. Sanchíz, in prep.).

Alytes cisternasii Boscá, 1879
(Pls. Ib, IIa–b)

Alytes cisternasii Boscá, 1879a: 217.

Terra typica. – Mérida (province of Badajoz, Spain).

Diagnosis. – Adult: Two metacarpal tubercles. Presence of red-orange dorsal glandular spots. Second through fourth vertebrae with marked neural apophyses; pars acromialis and processus glenoidalis of the scapula joined, leaving an open foramen at the suture line; short and wide phalanges, the terminal ones wide with blunt tips.
   Larvae: External skin of atriopore slightly surpassing inner edges. Characteristic pattern of spots on dorsal part of muscular portion of tail in the form of a large subrectangular block of black or brown spots that can be fused, then forming a wide dorsal band. Another series of spots usually runs along lower part of muscular portion of tail.

Ventral colouration almost silvery, sometimes with a diffuse metallic median line running from atriopore towards anus.

Geographical distribution (Fig. 1). — Western Spain and Portugal, in the basins of the rivers Douro, Tajo, Guadiana, and the northern part of the Guadalquivir.

Taxonomic comments. — The material cited in the original description of the species (Boscá, 1879a), originates from Mérida and Alange (both in the province of Badajoz), from El Pardo, in the province of Madrid, and from near Toledo in the province of Toledo. The individuals from Mérida, Alange, and El Pardo, recorded in the collections of the MNCN, appear to be lost. According to Boulenger (1897), at least one specimen of the typical series was in the collection of F. Lataste. Mertens & Müller (1928) designated Mérida in the province of Badajoz as the terra typica restricta for *A. cisternasii*.

Alytes dickhilleni n. sp.
(Pls. Ia, IIIc—d)

Material. — Holotype: Adult specimen, probably of the male sex. Locality: Canillas de Aceituno in the Sierra Tejeda, Provincia de Málaga, Spain. Leg.: J.W. Arntzen and M. García-París, 16-V-1992, MNCN 16662.

Paratypes: Paratype 1 from type locality, leg. J.W. Arntzen and M. García-París, 18-V-1992, ZMA Herp. 9227; paratypes 2 and 3 same date and place, ZMA Herp. 9228; paratypes 4 to 7 from the same place, tissues removed for electrophoretic studies, leg. J.W. Arntzen, 24-V-1989, ZMA Herp. 8156; paratype 8 from Barranco de los Cazadores, Sierra de Almijara, Nerja, provincia de Málaga, Spain, leg. A. Antúnez, June 1991, ZMA Herp. 9229; paratypes 9 to 14 from La Alcauca, Ventas de Zafarraya, Sierra Tejeda, Provincia de Granada, Spain, cleared and stained, leg. G. Astudillo, J. Prieto and M. García-París, 24-III-1990, MNCN 16727, 16730-34; paratypes 15 to 18 from Alcaucín, Sierra Tejeda, Provincia de Málaga, Spain, cleared and stained, leg. C. Martín and M. García-París, 26-V-1990, MNCN 16735-38.

Description of holotype. — Measurements: snout-vent length 35.1 mm, head width 14.5 mm, distance between nostrils 3.6 mm, eye diameter 3.7 mm, diameter tympanum 3.7 mm, tibia length 15.0 mm, and distance from metatarsal tubercle to tip of fourth toe 14.7 mm.

Head massive, progressively wider from snout to scapular region. Snout short and high, obtusely pointed, with a shallow but distinct nasolacrimal groove. Upper jaw markedly prognathous. Tympanum round.

Hind limbs longer than body with tibiotarsal articulation reaching halfway tympanum. Three carpal tubercles, central one smallest and in contact with external one. Some small tubercles at base of fingers. Fingers

without interdigital membranes, decreasing in size as $3 > 2 > 4 > 1$. Marked inner rounded metatarsal tubercle. Incipient subarticular tubercles at base of three inner toes. Dispersed granules on sole. Interdigital membrane on fourth toe does not surpass distal end of second basal phalanx. Toes presenting a flattened aspect due to existence of a short cutaneous fringe extending from interdigital membrane. Decreasing toe length as $4 > 3 > 2 = 5 > 1$.

Smooth dorsal skin covered with tiny glandular granulations all over back. Glandular tubercles localized in dorsolateral area and posterior parts of body. Short and thin but well-marked paratoid glands. Throat skin and ventral skin of anterior portion of trunk smooth, becoming granulated towards hind limbs. Gular skin fold not pronounced but clearly visible.

Background dorsal colouration grey with a marked light gray zone between eyes, on snout, scapular region, flanks, lateral portions of head and limbs. Head and anterior portion of trunk sprinkled with many gray and blackish fine dots equally mixed with pale ones, giving a general dusty appearance to dorsal skin; rounded dark spots irregularly distributed over posterior side of dorsal surface, with a brownish hue and a high density of fine black dots. Distinct whitish marking present between eyes, forming posterior end of a facial triangle that is bordered laterally by two dark lines that come from nostrils and almost reach eyes. An irregular whitish spot marks centre of scapular region. Dorsal spots decreasing in size at flanks, background colouring becoming pale towards belly. Sides of head and tympanic region show pale colouration and small irregular blackish spots. Tympanum brown.

Marked line of small white glandular tubercles running along sides of trunk, starting at paratoid region and ending towards urostyle. Dorsal side of limbs showing a pale gray hue, with big brownish spots that decrease in size towards end. Toes and fingers pale with spots forming a ring on outer toes. Fingertips distally padded. Iris lead gray, densely reticulated or marbled with black lines, especially fused in lower half. Ventral colouration whitish. Throat with submandibular and subgular pigmentation. Ventral side of limbs almost unpigmented at humeral-femoral and radioulnar-tibial regions, but densely covered by a blackish pigmented layer in carpal and tarsal regions, while small granules of these regions are white. Large tarsal and carpal tubercles unpigmented.

Description of variation. — Material: Riopar Viejo, Sierra de Alcaraz, Provincia de Albacete, Spain, 21-V-1992, leg. J.W. Arntzen, $N = 9$, ZMA Herp. 9231; Paterna del Madera, Provincia de Albacete, 27-III-1990, leg. G. Astudillo, J. Prieto and M. García-París, $N = 4$, MNCN 16682—16685; Bienservida, provincia de Albacete, 20-IX-1990, leg. C. Martín and M. García-París, $N = 4$, MNCN 16739—16742. Comparing the holotype and paratypes with this material we observed some variation in the following features:

Shape of head from tympanum to axillary region can be quadrangular rather than triangular, which is common in populations from Sierra de Alcaraz. Ventral skin normally more strongly granulated at posterior part of body. Fingers decreasing in size as 3>4>2>1. Pads on finger tips commonly absent, perhaps due to seasonal variation. Metatarsal tubercle egg-shaped. Small tubercles at base of digits may be absent. White glandular tubercles may extend from urostyle all way up lumbar region and may also be found on dorsal surfaces of limbs.

Background colouration varies from general gray to white or from gray to chestnut brown; in dark coloured animals, lightly coloured hand and feet may show up as if toads were "white gloved". Rounded thick spots on dorsum more frequently green than brownish, with or without metallic reflection, although sometimes obscured by dark background colouration. Background colour especially intense in a triangular-shaped area behind eyes. The white markings between eyes are frequently fused to form an uninterrupted line. The colouration of facial triangle may be obscured in middle. Whitish mark in scapular region frequently appears in triangular shape and may be accompanied by similar markings on upper flanks. Pigmentation on submandibular and subgular regions ranges from almost absent to densely spread all over throat. Tympanum more often than not gray brown with marbled spots. Colour of iris varies from described lead gray to a dark copper colouration.

Description of larvae. − Material: type locality, leg. J.W. Arntzen and M. García-París, 18-V-1992, $N = 5$, ZMA Herp. 9230; Calar del Mundo, Riopar, Sierra de Alcaraz, Provincia de Albacete, Spain, $N = 15$, MNCN uncatalogued; La Alcauca, Ventas de Zafarraya, Sierra Tejeda, Provincia de Granada, Spain, leg. G. Astudillo, J. Prieto and M. García-París, 24-III-1990, $N = 2$, MNCN 16765; Sierra de Gádor, Provincia de Almería, Spain, leg. R. Márquez, 5-VI-1992, $N = 4$, MNCN 16764.

Large larvae with long tail (1.75 to 2.1 times length of body) covered all around by a low fin. Height of tail including its fin usually a little more than body height (1: 1.1). Dorsal fin starts at end of body and ventral fin at level of anus. Hypognathous wide oral disc wider than half of maximum width of body. External narines and small eyes placed in dorsal and dorsolateral position, respectively. External skin of atriopore ending before inner edges. Larvae are of type III of Orton (1953, 1957), with medioventral position of spiracle and anal tube, keratinized upper and lower jaws, and oral disc with rows of horny denticles.

Oral disc bordered by a fringe of marginal papillae. Anterior labium with one single or double row and another double row of conspicuous black horny labial teeth. Posterior labium with three double or partially triple rows of teeth, upper one interrupted in middle. Partially black-rimmed horny beak situated inside oral disc.

Dorsal colouration of body may vary from pale gray to dark brown. Ventral side with multicoloured white, yellow, or brown metallic spots. A distinct metallic track links spiraculum with anal region. Muscular portions of tail with marked upper black stripe formed by thick spots usually fused, ending well before tip of tail. A series of dispersed black spots present along lower half portion of muscular region. Tail fin covered with black rounded or quadrangular spots, formed by aggregates of dermal melanophores, varying in size and density. Epidermal melanophores dispersed.

Diagnosis. − Adult: Three metacarpal tubercles. Absence of red-orange dorsal glandular spots. Second to fourth vertebrae without marked neural apophyses. Pars acromialis and processus glenoidalis of scapula separated. Short and wide phalanges, terminal ones wide with blunt, slightly spatulated tips.

Larvae: External skin of atriopore ending before inner edges. Characteristic colouration pattern in dorsal part of muscular portion of tail in shape of long black or dark brown band at each side of tail fusing dorsally and forming a typical dorsal band bifurcating towards body. This band may be interrupted, then forming a series of blotches. Ventral colouration marbled with irregular silver spots with marked silvery median line.

Derivatio nominis. − We dedicate the newly recognized species of midwife toad from southern Spain to the memory of Dr. D. Hillenius, biologist / literator, or more aptly polymath, living and working in Amsterdam (1927−1987). His fascination with both Spain and midwife toads is expressed in his book "Het romantisch mechaniek":

I set foot for the first time in Spain in 1947. By chance. The freighter with which I had been able to travel received a last minute change in orders with the result that I arrived not in Italy, about which I had exhaustively researched, but in the South of Spain about which I knew nothing ... The country was poor. The people were often clothed in rags and at that time there were many blind people and other sorts of beggars. The recent experience of our own "hongerwinter" [winter of famine, 1944−1945] meant however that I did not notice the poverty so much; I saw only the merits of life in the South and after spending a day at the Feria in Sevilla, with seldom a tourist to be seen, Spain had won my heart.

D. Hillenius (1969: 183)

The sound of small toads
together, apart, with yet another somewhat farther
somewhat lower, together in tilting accord
untraceable at every turn
because of other toads just farther, together
halting, apart, together
while those close to my foot
briefly hold their breath

D. Hillenius (1969: 105)

To arrive at the species' name we fused Hillenius' Christian and family names, referring to the authentic form of the latter. Its latinized form therefore, carries a single *i* at the end. As vernacular name we suggest "Midwife toad of Hillenius" or alternatively "Betic midwife toad".

Geographical distribution (Fig. 1). – *A. dickhilleni* is endemic to southeastern Spain. Its distribution appears to be limited to several mountain ranges including the Sierra Tejeda and Sierra Almijara (provinces of Málaga and Granada), the Sierra Nevada (province of Granada), the Sierra de Gádor (province of Almería), the Sierra de Baza (province of Granada), the Sierra Mágina (province of Jaén), the Sierra de Cazorla and the Sierra de Segura (province of Jaén), and the Sierra de Alcaraz (province of Albacete).

Habitat. – *A. dickhilleni* can be found at altitudes of approximately 700 to 2000 m, in pine forests, oak forests and in open rocky landscapes. Adults are usually observed on eroded soils near the water, or found under stones. The substrate varies from slates and schist to limestone. The larvae live in clean mountain streams and in man-made reservoirs that have water all year round. The larvae commonly pass one winter in the water.

Conservation aspects. – The restricted range occupied by *A. dickhilleni* and the apparent scarcity of the species at many of the places where it has been observed, lead us to recommend its immediate legal protection. Although the conservation status of the species might be not as delicate as that of *A. muletensis*, some similarity is apparent, with many of the populations confined to deep valleys that are probably isolated from one another. Biotopes seem to be disappearing, mainly as a consequence of water withdrawal and droughts. With the decline of traditional cattle raising practices, drinking troughs that serve as habitats for the tadpoles are disappearing. Fortunately, the Spanish Institute for Nature Conservation ICONA quickly recognized the need for research and commissioned a study to assess the conservation status of *A. dickhilleni* (García-París et al., 1993).

Alytes muletensis (Sanchíz & Adrover, 1979)
(Pls. Ic, IIIe–f)

Baleaphryne muletensis Sanchíz & Adrover, 1979: 6.

Terra typica. – Cova de Muleta, Upper Pleistocene, Mallorca, Spain.

Diagnosis. – Adult: Three metacarpal tubercles. Absence of red-orange dorsal glandular spots. Second to fourth vertebrae without neural apophyses. Pars acromi-

alis and processus glenoidalis of scapula separated. Long and narrow phalanges, terminal ones slightly spatulated.
   Larvae: External skin of atriopore ending before inner edges, describing a pronounced arc. Narines placed close to median line of body. Body usually as high or slightly higher than tail including its fin. Dorsal part of muscular portion of tail with variable colour pattern, viz. large pale brown spots, sometimes fused or almost absent. Ventral colouration with metallic spots and a median track from atriopore to anus.

Geographical distribution (Fig. 1). – Limited to the Serra Tramuntana of northern Mallorca, Balearic Islands.

Taxonomic comments. – The species was described in 1977 on the basis of fossil material. Living specimens were discovered three years later (Alcover & Mayol, 1980). The taxonomic position of *A. talaioticus* (Sanchíz & Alcover, 1982) that recently became extinct (Sanders, 1984) is unclear. Alcover et al. (1984) pointed out that the taxon might be conspecific with *A. muletensis*, but insufficient data are available to re-evaluate its taxonomic status.

Alytes obstetricans (Laurenti, 1768)

Bufo obstetricans Laurenti, 1768: 28.
Rana campanisona Laurenti, 1768: 30.
Obstetricans vulgaris Dugès, 1834: 7.

Terra typica. – France (terra typica restricta by Mertens & Müller, 1928).

Diagnosis. – Adult: Three metacarpal tubercles. Presence of orange-red dorsal glandular spots. Second to fourth vertebrae without marked neural apophyses. Pars acromialis and processus glenoidalis of scapula separated. Phalanges short and wide, terminal ones wide with blunt tips.
   Larvae: External skin of atriopore ending before or at level of inner edges. Dorsal part of muscular portion of tail with small black, sometimes fused, spots. Ventral colouration marbled with silver, irregular spots, and a marked silvery median line.

Geographical distribution. – Western Europe: The Netherlands, Belgium, Luxemburg, Germany, Switzerland, France, Andorra, Spain, Portugal. Northwestern Africa: Morocco.

Taxonomic comments. – This species is composed of four subspecies: *A. o. obstetricans* (Laurenti, 1768), *A. o. almogavarii* n. ssp., *A. o. boscai* Lataste, 1879, and *A. o. maurus* Pasteur & Bons, 1962.

Alytes obstetricans obstetricans (Laurenti, 1768)
(Pls. If, IIa–b)

Bufo obstetricans Laurenti, 1768: 28.
Alytes obstetricans delislei Lataste, 1879a: 543.

Terra typica. – As for the species.

Diagnosis. – Adult: Thick and warty dorsal skin. Dorsal colouration greyish or brownish all over, or with diffuse dark spots. Ventral skin gray-whitish with lower jaw and throat covered more or less densely by dark spots.

Larvae: External skin of atriopore ending before inner edges. Dorsal part of muscular portion of tail with small black spots, sometimes forming small aggregates. Ventral colouration marbled with silver, irregular spots, and a marked silvery median line.

Geographical distribution (Fig. 1). – Western Europe: The Netherlands, Belgium, Luxemburg, Germany, Switzerland, France, and northern Spain. In Spain the range of this subspecies is restricted to Cantabria, from Asturias to Navarra, with apparently a transitional zone with *A. o. boscai* to the west and the south, and with *A. o. almogavarii* to the east of its distribution (García-París, 1992).

Taxonomic comments. – The genetic distance between *A. o. obstetricans* and *A. o. boscai* is small, but distinctive morphological traits exposed by Lataste (1879a), and the typical dorsal glandular pattern of *A. o. obstetricans* that is absent in *A. o. boscai*, suggest the retention of a subspecific status.

Alytes obstetricans almogavarii n. ssp.
(Pls. Id, IIe)

Material. – Holotype: Adult specimen, probably of the male sex. Locality: Rasos de Peguera, Berga, Provincia de Barcelona, Spain. Leg.: M. García-París and J.W. Arntzen, 6-IX-1992, MNCN 16663.

Paratypes: Paratypes 1 to 6 from type locality, leg. M. García-París and J.W. Arntzen, 6-IX-1992, MNCN 16675–16680; paratypes 7 to 12 from type locality, leg. M. García-París and J.W. Arntzen, 6-IX-1992, ZMA Herp. 9232; paratypes 13 to 18 from type locality, leg. M. García-París, 22-VI-1990, MNCN 16743–16748 (tissues removed for electrophoretic studies, then cleared and stained for osteological studies).

Description of holotype. – Measurements: snout-vent length 35.1 mm, head width 14.9 mm, distance between nostrils 3.8 mm, eye diameter 4.7 mm, diameter tympanum 3.7 mm, tibia length 15.6 mm, and distance from metatarsal tubercle to tip of fourth toe 13.8 mm (left), 14.3 mm (right).

Head massive, becoming progressively wider from snout to ocular region, from where head width widens less quickly to scapular region. Snout short and high, obtusely pointed, with a marked naso-lacrimal groove. Upper jaw prognathous. Tympanum round. Hind limbs longer than body, with tibiotarsal articulation reaching halfway to tympanum. Three carpal tubercles, central one smallest and in contact with external one. Some small tubercles at base of fingers. Fingers without interdigital membranes, decreasing in size as $3>2=4>1$. Inner metatarsal tubercle well-marked and of ellipsoid shape. Subarticular tubercles absent. Granules on sole scarce and dispersed. Interdigital membrane not surpassing distal end of second basal phalanx of fourth toe. Toes decreasing in length as $4>3>2=5>1$.

Dorsal skin covered with glandular granulations, especially well marked on scapular regions, posterior part of dorsum and on flanks. Glandular tubercles densely distributed over dorsolateral area and posterior part of body. Thick and well-marked paratoid glands in shape of an arc, each bordering tympanum along its dorsal and posterior edges. Skin of throat and belly smooth, granular on flanks and posterior part of trunk. Gular skin fold conspicuous.

Background dorsal colouration yellowish-brown with many irregular olive green spots. Posterior part of head and anterior part of trunk gray brown with many small dark marks, varying in colour from olive green to gray and brown, giving rise to a mosaic appearance. Background colouration pale towards belly. Distinct beige spot present between scapulae and at both sides between eyes and tympanum. Dorsal parts of limbs yellowish with many small dark spots, those on hind limbs distally arranged in bands. Sides of head and tympanum region with pale colouration and some small irregular blackish spots. Tympanum marbled with brown and beige.

Distinct line of orange dorsolateral tubercles running from paratoid gland to inguinal region. Orange tubercles present on posterior part of back, on flanks, on dorsal surface of limbs, and on eyelid. Toes and fingers pale with a few dark spots. Iris golden, densely reticulated with black lines, especially fused in lower half. Ventral colouration whitish, without dark pigmentation. Ventral side of limbs yellowish, without dark pigmentation except for outer toes that ventrally are slightly pigmented.

Description of variation. – Material: Barcelona (University campus), province of Barcelona, Spain, leg. A. Montori & N. García, 15-IV-1991, $N = 4$, MNCN 16749–16752; Ibón de Piedrafita, province of Huesca, Spain, leg. M. García-París, 3-VII-1990, $N = 3$, MNCN 16753–16755. Comparing holotype and paratypes with this material, some variation was observed in the following features:

Shape of head from tympanum to axillary region can be quadrangular rather than triangular. Limb size variable, sometimes only just reaching tympanum. Tubercles on base of fingers may

be completely absent. Either second or fourth finger could be the longer one. Subarticular tubercles at base of toes more often present than not. In some specimens sole densely granulated. A well-developed cutaneous fringe on toes may give them a flattened aspect; perhaps a characteristic of males.

Extensive variation present in extent to which dorsal skin is granulated, ranging from very smooth with few granulations to rugose with many well-developed tubercles. Paratoid gland, rather than forming an arc, frequently is straight, pointing to dorsolateral line of tubercles.

Background colouration shows little variation but its appearance may vary drastically due to presence or absence of dorsal spots. Spot shape may be irregular, rounded, or angular, with a colour ranging from ochre and green to dark brown. Interscapular spot usually takes a triangular or V-shape but may be absent. Similar spots of irregular shape are occasionally present on scapula. In about half of the specimens a distinct whitish marking appears between eyes, forming posterior end of a facial triangle. Triangle bordered laterally by two dark lines that, coming from nostrils, reach eyes. Light spots between tympanum and eye mostly small or absent.

Dorsal parts of limbs occasionally with large spots, usually not forming bands but a marbled pattern. Orange tubercles are invariably present but their density varies.

Toes and fingers usually covered with spots giving a marbled or reticulate aspect. Iris may be copper coloured.

Ventral colouration of throat whitish, often with dark spots. These spots can be spread out all over throat or be almost absent. In submandibular region these spots tend to be large. The dark pigmentation of ventral side of foot may run from external toes onto sole.

Description of larvae. – Material: type locality, leg. J.W. Arntzen and M. García-París, 6-IX-1992, $N = 21$, MNCN 16681, ZMA Herp. 9233 (1 larva, 7 juveniles just metamorphosed and 13 metamorphosing larvae; some material fixed upon metamorphosis); type locality, leg. J.W. Arntzen and M. García-París, 6-IX-1992, $N = 19$, MNCN 16682.

Large larvae with long tail (at least 1.5 times length of body). Dorsal fin relatively high, starting near end of body; ventral fin starting at level of anus. Hypognathous wide oral disc varying in size but usually narrower than half of maximum width of body. External narines and small eyes placed in a dorsal and dorsolateral position, respectively. External skin of atriopore ending at level of inner edges.

Oral disc bordered by a fringe of marginal papillae. Anterior labium with one external double row and an internal triple row of conspicuous black horny labial teeth. Posterior labium with three double, triple, or partially quadruple rows of teeth, upper one slightly interrupted in middle. Partially black rimmed horny beak situated inside oral disc.

Dorsal colouration of body may vary from pale to dark brown, sometimes with a pale mark on dorsum. Ventral side with metallic and brown spots. A distinct metallic track links spiraculum with anal region. Muscular portions of upper part of tail with a marked black stripe, ending well before tip of tail.

Stripe formed by thick spots that usually are clearly fused. Tail fin covered with black rounded spots, formed by aggregates of dermal melanophores, varying in size and density. Epidermal melanophores dispersed.

Diagnosis. – Adult: Thick dorsal skin granulated or with small tubercles. Dorsal colouration brownish or yellowish with a marbled pattern of marked green or brown spots. Ventral skin whitish with lower jaw and throat sometimes more or less densely covered with dark spots.

Larvae: External skin of atriopore ending almost at level of inner edges. Dorsal part of muscular portion of tail with black spots that sometimes are fused, forming thin dorsal band. Ventral colouration marbled with metallic irregular spots and a marked silvery median line.

Derivatio nominis. – The name of this newly recognized subspecies is derived from the "Almogávares", medieval warriors from the kingdom of Aragón in the eastern Pyrenees, covering the region where this taxon occurs. They were famous and dreaded across the Mediterranean region for their brave and fierce behaviour.

Geographical distribution (Fig. 1). – Northeastern Spain north of the Ebro valley along the eastern Pyrenees, in Catalonia and Aragón (provinces of Barcelona, Girona, Lleida, and Huesca). The limits of the range of this subspecies are not yet well defined. A transitional zone between *A. o. obstetricans* and *A. o. almogavarii* appears to be located in eastern Navarra. The distribution of this subspecies probably ranges all over Tarragona and Zaragoza, the northern part of the Ebro valley, and may extend into the French eastern Pyrenees and Andorra (cf. Veith, 1991). The populations south of the Ebro River in the province of Castellón are referred to as *A. o. boscai*.

Habitat. – The altitudinal distribution of *A. o. almogavarii* ranges from sea level to 2200 m altitude. It can be found in a variety of landscapes, from rivers, man-made ponds and reservoirs in the lowlands to high mountain lakes. Adults frequently can be observed on eroded soils near the water, or sheltering under stones. The substrate varies from granite to limestone. The larvae live in clean mountain streams, in ponds and in man-made reservoirs that have water all year round. They commonly pass one winter in the water, or more than one in high mountain lakes.

Conservation aspects. – In the eastern Pyrenees, populations appear to be numerous and thriving. However, at lower altitudes the situation is different and populations may be affected adversely due to changes in land use, especially in coastal regions where the urban development is extensive. The conservation status of this taxon remains to be evaluated more precisely.

Alytes obstetricans boscai Lataste, 1879
(Pls. Ie, IIc–d)

Alytes obstetricans boscai Lataste, 1879a: 543.

Terra typica. – Tuy, province of Pontevedra, Spain (see taxonomic comments).

Diagnosis. – Adult: Smooth or granulated dorsal skin without warts or tubercles. Dorsal colouration whitish with marked and well-defined green or brown spots. Ventral skin white and bright. Lower jaw and throat white (eastern populations) or with dark marks (western populations).
   Larvae: External skin of atriopore ending before inner edges. Dorsal part of muscular portion of tail with small black spots, usually fused, forming dense but small subquadrangular aggregates. In eastern populations this colouration may be different and spots can be fused forming bands. Ventral colouration marbled with red, brown, and metallic irregular spots and with a marked metallic median line.

Geographical distribution. – Northwestern, central and eastern Spain, northern and central Portugal. In northern Spain distributional limits of this subspecies are unclear. A transitional area with *A. o. obstetricans* south to the Cantabrian mountains appears to exist. The southernmost known populations of *A. o. boscai* are located in the mountains of Alcoy and Petrel in the province of Alicante (García-París et al., 1993).

Taxonomic comments. – Lataste (1879a) specifies that the material used for the description of *A. o. boscai* originates from Fuy (sic) in the province of Pontevedra and from Valencia. Only five specimens from the former locality are kept at the Museums of Bordeaux, Brussels, and Paris, and hence these five constitute the typical series. However, a locality of spelling "Fuy" does not exist in Pontevedra, nor elsewhere on the Iberian Peninsula. Assuming a simple typographical error we consider Tuy in the province of Pontevedra to be the type locality for *A. o. boscai*. Facts supporting this interpretation are that E. Boscá: (1) provided F. Lataste with midwife toads, *prior* to the original description (Boscá, 1879b), (2) collected in Tuy (Boscá, 1879b), and (3) sent specimens from this locality to Lataste (Boscá, 1880a). Moreover, two individuals from the type series kept in Brussels do show all the characters described by Lataste (1879a), and do not differ statistically in morphometric aspects from a sample of Galicia. The locality Tuy then is to be considered the "terra typica" of *A. o. boscai*, contrary to the indications of Mertens & Müller (1928) who designated Valencia as terra typica. This observation is not completely trivial, because an osteological analysis suggests the existence of marked differences between populations from the Mediterranean and Atlantic coasts (M. García-París & B. Sanchíz, in prep.). The observation that individuals from central Spain (Guadalajara) possess some alleles characteristic of *A. o. almogavarii* (García-París, 1992) points to the existence of a transitional zone in the western Ebro Valley.

Alytes obstetricans maurus Pasteur & Bons, 1962

Alytes [obstetricans] maurus Pasteur & Bons, 1962: 71.

Terra typica. – Massif du Talasse n'Tane, Rif Mts., Bab-Taza, Morocco.

Diagnosis. – No morphological characters, alone or in combination, are known to be diagnostic for *A. o. maurus*. The range of variation is within that shown by the Iberian *A. o. boscai* and *A. o. almogavarii*. In a small sample, an autapomorphic allele was found to be fixed on the *Mpi* locus (Arntzen & Szymura, 1984).
   Adult: Dorsal skin granulated, usually with small tubercles. Dorsal colouration brownish or whitish with well-defined green or brown spots. Ventral skin white. Lower jaw and throat with more or less extensive dark marks.
   Larvae: External skin of atriopore ending before inner edges. Dorsal part of muscular portion of tail with large black spots, usually fused, forming dense subquadrangular aggregations or bands. According to Pasteur & Bons (1962), tail fin with well-developed pigmentary net, lower part of mouth with three rows of denticles, and distance between eyes shorter than mouth width.

Geographical distribution. – Rif mountains and Middle Atlas in Morocco (Pasteur & Bons, 1962; Bons, 1973; Arntzen & Szymura, 1984; Libis, 1985; Mellado & Mateo, 1992).

Taxonomic comments. – Described ambiguously at the specific level and placed as subspecies on the basis of an allozyme study (Arntzen & Szymura, 1984).

Key to the identification of adult live midwife toads

1. Two metacarpal tubercles .... *A. cisternasii*
 — Three metacarpal tubercles 2
2. Dorsal red or orange spots present, or with marked dorsal
   warts .... *A. obstetricans*–4
 — No dorsal red or orange spots nor marked dorsal warts
   present 3
3. Elongated body shape, dorsal spotted pattern on a yellowish
   or ochraeous background, usually with metallic reflections
   .... *A. muletensis*
 — Body shape massif, dorsal spotted pattern on a brownish or
   greenish background, without metallic reflections ....
   .... *A. dickhilleni*
4. No dorsal tubercles or marked round warts, dorsal pattern
   with dark spots or a dark marbling on a pale whitish or
   brownish background .... *A. o. boscai*
 — Dorsal tubercles or warts more or less well-developed, dorsal
   pattern uniform or with dark spots, usually on a brownish or
   greyish background 5
5. Uniform grey general colour pattern, with marked round
   dorsal warts .... *A. o. obstetricans*
 — Spotted or brown colour pattern, with dorsal granules or
   small tubercles 6
6. Marbled colour pattern, usually with green spots (south-
   western Europe) .... *A. o. almogavarii*
 — Colour pattern uniformly brown or spotted, usually with
   brown spots (northwestern Africa) .... *A. o. maurus*
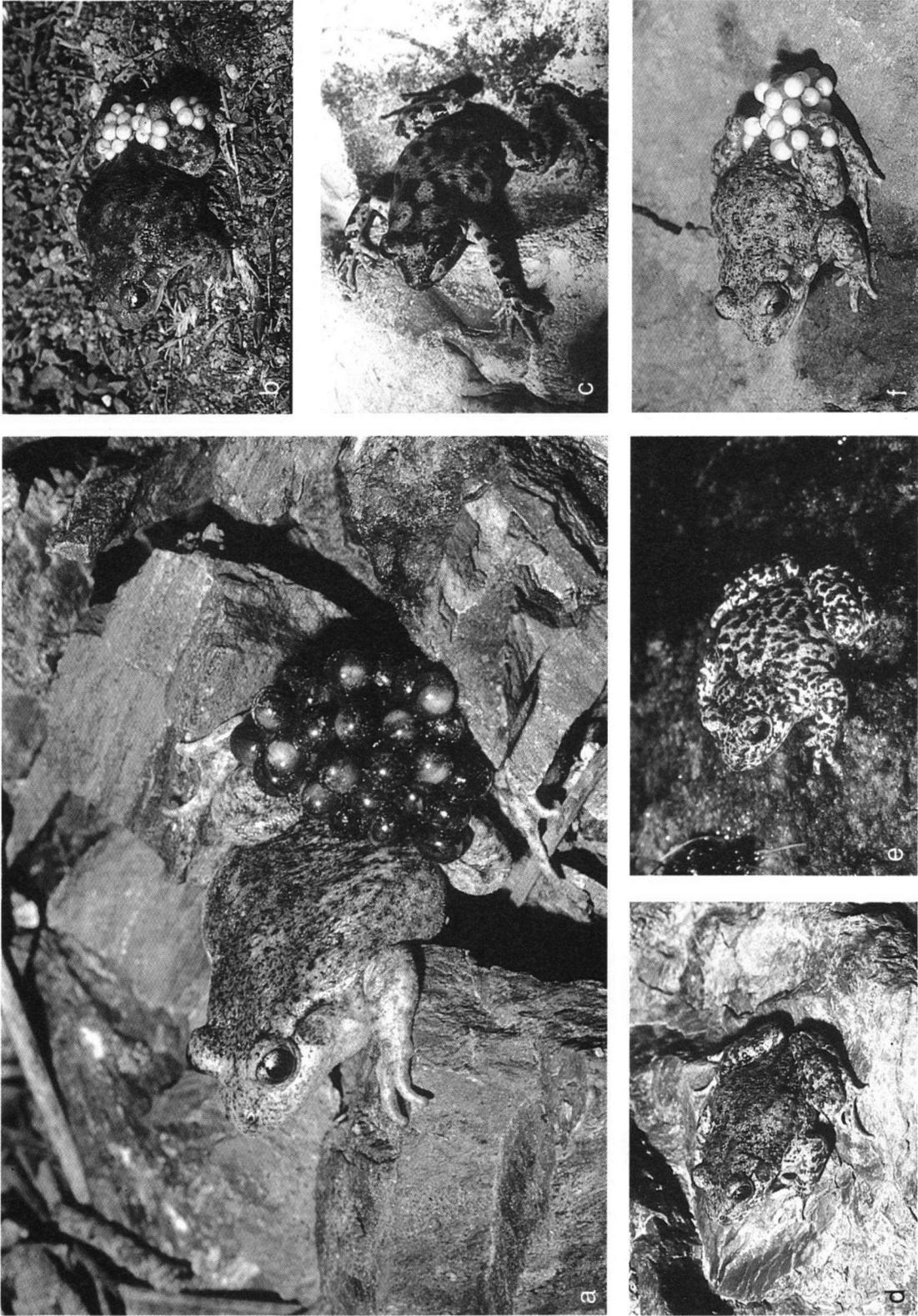
Plate I. Adult midwife toads: a, *Alytes dickhilleni* from Canillas de Aceituno, Sierra Tejeda, Málaga, Spain (type locality); b, *A. cisternasii* from Guadalix de la Sierra, Madrid, Spain; c, *A. muletensis* from the Serra Tramuntana, Mallorca, Balearic Islands, Spain; d, *A. obstetricans almogavarii* from Berga, Barcelona, Spain (type locality); e, *A. o boscai* from Tuy, Pontevedra, Spain; and f, *A. o. obstetricans* from Picos de Europa, León, Spain.
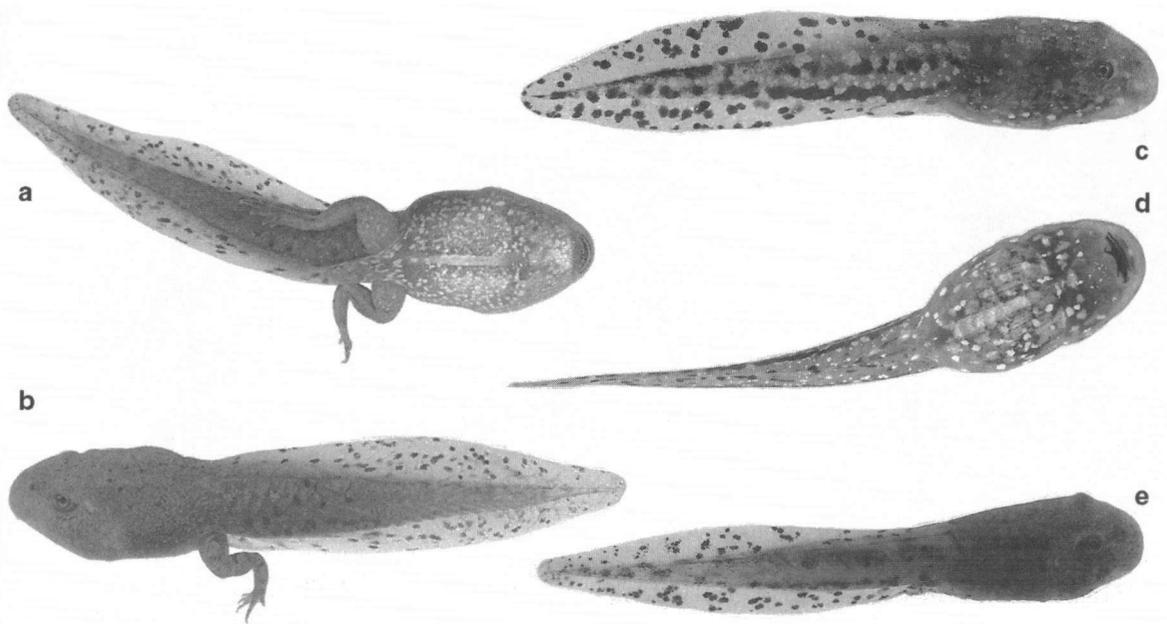
Plate II. Larval midwife toads: a–b, *Alytes obstetricans obstetricans* from Marquise, Pas-de-Calais, France; c–d, *A. o. boscai* from Valongo, Douro Litoral, Portugal; e, *A. o. almogavarii* from Berga, Barcelona, Spain (type locality).
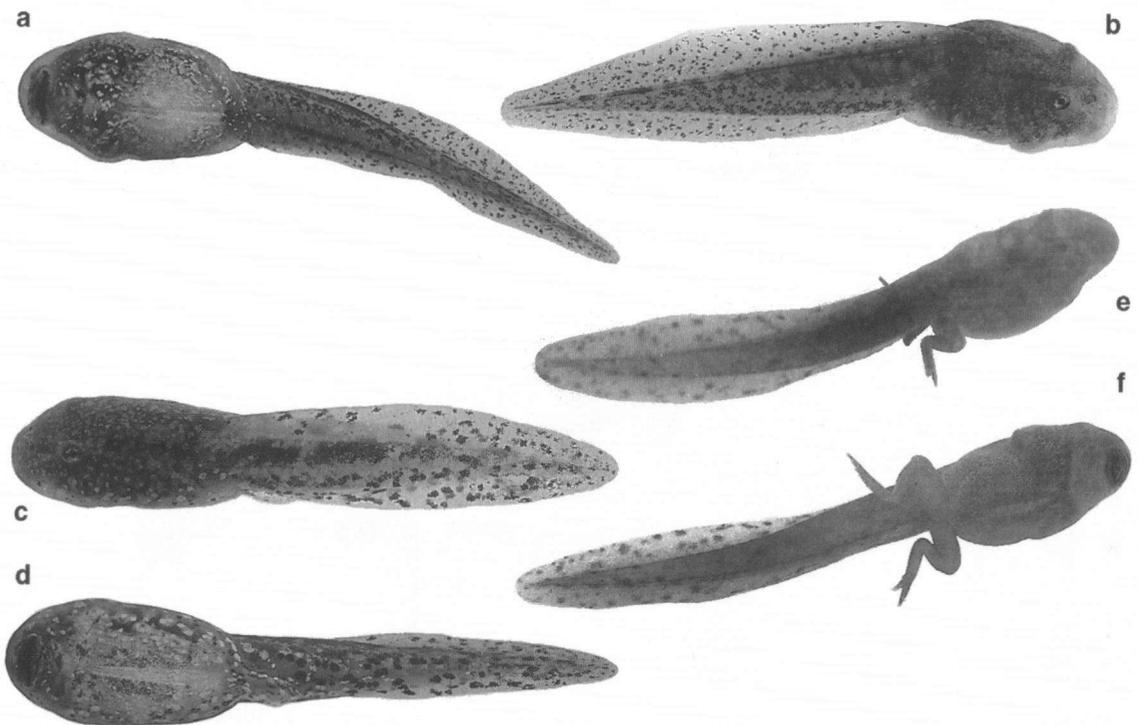
Plate III. Larval midwife toads: a–b, *Alytes cisternasii* from Andujar, Jaén, Spain; c–d, *A. dickhilleni* from Canillas de Aceituno, Sierra Tejeda, Málaga, Spain (type locality); e–f, *A. muletensis* from Serra Tramuntana, Mallorca, Balearic Islands, Spain (raised in captive breeding programme Norwich).