

OSTEOLOGICAL DIFFERENTIATION AMONG IBERIAN *PELODYTES* (ANURA, PELODYTIDAE)

Sanchiz, B. *, Tejedo, M. ** and Sánchez-Herráiz, M. J. *,**

ABSTRACT

The morphological differentiation among *Pelodytes* species is analysed based on a sample of disarticulated bones from the main osteological regions of the male adult skeleton. A set of 35 interspecifically diagnostic characters, analysed under different outgroup hypotheses, clearly shows that *P. ibericus* and *P. punctatus* are a sister-group with respect to *P. caucasicus*. The Caucasian species retains a very primitive morphology, with only 17-23 % derived character-states, while both living Iberian species reach percentages of derivation over 68 %. There is little difference between *P. ibericus* and *P. punctatus* in their relative degree of evolutionary transformation, and when using *P. caucasicus* as the outgroup the percentage of derived character-states is 45 and 60 % respectively. Differentiation rates are calculated in darwin units for several characters, and we show that the skull components have higher rates than the traits directly related with locomotion. Several adult growth trajectories, different between species, are described and identified as diverse allometric heterochronies. Three factors have been detected that might have grouped several characters as coevolutionary units. These factors are: a) an ontogenetic factor, operating through heterochronic processes, expressed as a tendency to reduce ossification; b) a functional morphological integration, detected in the elements involved in skull size and proportions; and c) an ecomorphological factor, presumably an adaptive response, can be assumed for characters related to limb shape.

Key words: Osteology, Morphology, Phylogeny, Heterochrony, Divergence rates.

RESUMEN

Diferenciación osteológica entre los *Pelodytes* ibéricos (Anura, Pelodytidae)

Se analiza la diferenciación morfológica en *Pelodytes* mediante el análisis comparado de una muestra de elementos óseos desarticulados procedentes de las principales regiones del esqueleto macho adulto. Un conjunto de 35 rasgos interespecíficamente diferenciales, analizada bajo diversos grupos externos, permite inferir que *P. ibericus* y *P. punctatus* forman un grupo hermano frente a *P. caucasicus*. La especie caucásica mantiene una morfología muy primitiva, con sólo 17-23 % de estados derivados, en tanto que las especies ibéricas son ambas muy derivadas, con porcentajes superiores al 68 % de los rasgos. Hay poca diferencia entre *P. ibericus* y *P. punctatus* en cuanto a su grado relativo de transformación evolutiva, y utilizando a *P. caucasicus* como grupo externo presentan porcentajes de estados derivados del 45 y 60 % respectivamente. Se calculan las tasas de diferenciación de varios caracteres en darwines, presentando los componentes craneales tasas más altas que los rasgos directamente relacionados con la locomoción. Se describen e identifican en términos de alometrías heterocrónicas diversas trayectorias de crecimiento adulto, diferenciales entre especies. Se han detectado tres factores que permiten agrupar los caracteres en unidades de evolución conjunta. Estos factores son: a) un factor ontogenético producido mediante procesos heterocrónicos, apreciable en una tendencia a la disminución de la osificación; b) una integración morfológica funcional, detectable en los elementos implicados en el tamaño y proporciones del cráneo; y c) un factor ecomorfológico, presumiblemente una respuesta adaptativa, puede inferirse en rasgos relacionados con la robustez de las extremidades.

Palabras clave: Osteología, Morfología, Filogenia, Heterocronía, Tasas de divergencia.

* Museo Nacional de Ciencias Naturales, C.S.I.C., J. Gutiérrez Abascal 2. Madrid E-28006. Spain.

e-mail: mcnb105@mncn.csic.es

** Estación Biológica de Doñana, C.S.I.C., Pabellón del Perú. Parque María Luisa s/n. Sevilla E-41013. Spain.

e-mail (MT): tejedo@ebd.csic.es

Introduction

Pelobatoid frogs are one of the main primitive living anurans, with a complex fossil record and known since the late Jurassic of North America (Sanchiz, 1998). Four pelobatoid groups are currently considered monophyletic lineages, albeit with changing subfamiliar or familiar taxonomic status. These lineages are the western Palaearctic Pelobatidae sensu stricto (or Pelobatinae) and the Nearctic Scaphiopodidae (or Scaphiopodinae), both adapted to arid environments and often named together as Pelobatidae (sensu lato), the diverse eastern Palaearctic and Oriental Megophryidae, and the West Palaearctic Pelodytidae. The relationships among pelobatoids are still unsettled, with contradictory results in several recent studies. With morphological data, for instance, Henrici (1994) finds that Pelodytidae would be the sister group of pelobatids (sensu lato) plus megophryids, while according to Maglia (1998), pelobatids (sensu lato) and pelodytids are a sister group in relation to the megophryids. The most complete molecular study available (García-París *et al.*, in press) points to Pelobatidae (sensu stricto) as the sister group of megophryids, and this assemblage as the sister group for Pelodytidae.

The family Pelodytidae is currently composed by the living European *Pelodytes* Bonaparte, 1838, and by the extinct North American *Tephrodytes* Henrici, 1994, and *Miopelodytes* Taylor, 1941 (Henrici, 1994; Sanchiz, 1998). The genus *Pelodytes* presently includes three rare and poorly known living species, one Caucasian (*P. caucasicus* Boulenger, 1896), and two western European species (*P. punctatus* (Daudin, 1802) and *P. ibericus* Sánchez-Herráiz, Barbadillo, Machordom *et al.* 2000), and the extinct Miocene form *P. arevacus* Sanchiz, 1978 (Sánchez-Herráiz *et al.* 2000).

This article has several purposes. In the first place, a basic comparative description of the skeletal features of *Pelodytes* species has been never attempted, and their description will obviously facilitate the identification of these taxa for studies dealing with bone fragments and fossil remains. This comparative osteology will also allow a phylogenetic analysis that will serve as an independent test, based on morphology, for recent or future phylogenetic proposals relying on DNA sequences. Furthermore, Iberian *Pelodytes* have undergone a recent speciation process, well studied ecologically and genetically (Sánchez-Herráiz *et al.* 2000; Sánchez-Herráiz, *in litt.*), and their osteological comparison will provide a case study of morphological differentiation between

two anuran species. Finally, an integrative overview of the multiple minor differential features observed might contribute to the understanding of the causal biological factors underlying the process of osteological differentiation (Wake & Roth, 1989; Emerson & Hastings, 1998).

Material and methods

MATERIAL: The sample studied includes complete dry disarticulated (DS) male skeletons, but some females and whole cleared and stained (C&S) preparations (Dingerkus & Uhler, 1977; Wassersug, 1976), or mixed preparations (i.e. having both disarticulated and double-stained elements), have also been consulted. The material belongs to the collections of the Museum für Tierkunde (MTKD) in Dresden (Germany) and Museo Nacional de Ciencias Naturales, C.S.I.C. (MNCN) in Madrid (Spain). Their species, localities and catalogue numbers are as follows:

Megophrys montana: No locality data MNCN 20270-20278 (DS).

Pelobates cultripes: SPAIN: El Berrueco (Madrid) MNCN 18038 (DS), 18042-18045 (DS), 18048-18051 (DS). Paredes de Buitrago (Madrid) MNCN 18041 (DS).

Pelodytes caucasicus: GEORGIA: No locality data MNCN 18133 (DS), MTKD D9740 (DS).

Pelodytes ibericus: SPAIN: Alcalá de los Gazules (Cádiz) MNCN 18145-18148 (DS), 18150-18152 (DS). Medina Sidonia (Cádiz) MNCN 18487-18493 (DS). Facinas (Cádiz) MNCN 18159 (DS), 20141 (C&S), 20206-20220 (C&S). Córdoba (Córdoba) MNCN 20183-20188 (C&S), 20189 (DS), 20191-20198 (C&S). Aljaraque (Huelva) MNCN 18145 (DS). Almonte (Huelva) MNCN 20115-20119 (C&S). Los Corrales (Huelva) MNCN 18134-18144 (DS). Photographs here included are based on MNCN 18146 (squamosal, femur and tibiofibula) and MNCN 18152 (other bones).

Pelodytes punctatus: PORTUGAL: Porto Covo (Baixo Alentejo) MNCN 18153-18158 (DS). SPAIN: Garraf (Barcelona) MNCN 20176-20182 (C&S). Merindad del Río Ubierna (Burgos) MNCN 18495-18500 (DS), 20148-20165 (C&S), 20201-20205 (C&S). Embalse de La Toba (Cuenca) MNCN 19776 (C&S). Rivas del Jarama (Madrid) MNCN 18494 (DS). Valgañón (Rioja) MNCN 19775 (C&S). Montejo de la Vega de la Serrezuela (Segovia) MNCN 20120-20140 (C&S), 23692 (DS). Plá dels Corrals (Valencia) MNCN 20166-20175 (C&S). Photographs included in this article are based on MNCN 18499.

Scaphiopus couchii: U.S.A.: Oracle (County Pina, Arizona) MNCN 18164-18172.

Several minute elements (e.g. stapes, septomaxilla, quadratojugal, carpal, tarsals etc.), frequently lost during the preparation process, are rarely found in museum collections of dry skeletons. These elements, as well as others with low identification capabilities (e.g. mandible, phalanges etc.), will not

be considered here. The basic analyses of differences have been done using exclusively male specimens, in order to exclude any possible variation due to sexual dimorphism. Nevertheless, as will be discussed elsewhere, only the forelimbs present clear sexual dimorphism in this group, as the females do not develop the humeral distal crests present in males. Measurements have been taken with a digital Nikon Measurescope 10 to the nearest 0.01 mm and through camera lucida scaled drawings.

NOMENCLATURE. Osteological nomenclature basically follows Bolkay (1919) and Rocek (1980). Only well known Latin anatomical names are used, as indicated in Sanchiz (1998).

SELECTION OF CHARACTERS: There is an unlimited number of ways, virtually infinite, in which observed morphological differences can be defined ("coded") as "characters". This basic fact has been surprisingly overlooked in phylogenetic and taxonomic theory (e.g. Wiens, 2000), and it implies that the subjective selection of characters that we have made is just one among many other selections which allow to represent the same or similar morphological differences. Keeping that in mind, each character was selected so that its definition was as simple and unambiguous as possible, while integrating a maximum of the morphological variation detected by the human brain among the species involved. These characters apply to the present study and cannot be taken as "standards", because morphological differences are dependent of the set of morphologies analysed. Nevertheless, because of the relative homogeneity of anuran skeletons, some of these characters are likely to become useful in similar studies on other groups.

We have initially selected as many characters as possible for each individual bone, provided that they could be independently defined, and without involving other redundant possible ones. Character-states are here appropriately defined in order to provide unequivocal categorical ascription to each species. Nevertheless, in the cases in which quantitative definitions have been used to establish these categorical character-states, there could be outliers that might receive a wrong assignment. This possibility will not alter the results of our analyses on relative differentiation, but we warn that use of these character-states for identification purposes should be restricted to probabilistic levels. For the metric characters, we have set character-state boundaries only when the groups show statistically significant differences, and there is a region of minimal overlap, usually none, between the 95% confi-

dence intervals of their means. For only the purposes of character-state ascription, the mean of the two measured specimens of *Pelodytes caucasicus* is considered the mean of a hypothetical sample having a coefficient of variation equal to the mean one between those of *P. ibericus* and *P. punctatus*.

SELECTION OF OUTGROUPS: Within phylogenetic analyses based on parsimony, an outgroup makes possible the inference of polarities (Kitching *et al.*, 1998). In the case of Pelodytidae, the extinct fossil genera *Miopelodytes* and *Tephrodytes* are only incompletely known, and the genus *Pelodytes* itself has been recovered in fossil form at earlier ages (Rocek & Rage, 2000). Therefore, no reliable outgroup for *Pelodytes* is available in its own family, and we have to select it within other pelobatoids. Nevertheless, because the phylogenetic relationships of Pelodytidae are still not clear, representatives of all the other possible pelobatoid lineages and groups have been separately used as outgroups. As a consequence, each outgroup selected represents a different phylogenetic model as follows:

- Hypothesis " H_M ". Assumes a phylogeny in which Megophryidae is the sister group for Pelodytidae. This hypothesis is unlikely, as it has not been proposed in any of the available morphological, paleontological or molecular studies. *Megophrys montana* Kuhl et Van Hasselt, 1822, was selected as representative for Megophryidae.
- Hypothesis " H_p ". Assumes a closest relationship between Pelobatidae (sensu stricto, i.e. *Pelobates* Wagler, 1830, and related fossil forms) and Pelodytidae. *Pelobates cultripes* (Cuvier, 1829) was considered to be an appropriate pelobatid model.
- Hypothesis " H_s ". The sister group of Pelodytidae would be Scaphiopodidae (living *Scaphiopus* Holbrook, 1836, and *Spea* Cope, 1866). *Scaphiopus couchii* Baird, 1854, was the species considered representative of the group.
- Hypothesis " H_T ". It assumes that Pelodytidae is the sister group to a phylogenetic entity in which all the other pelobatoids are included. The results of this hypothesis are equivalent to the situation in which the polarities indicated by " H_M ", " H_p " and " H_s " agree, being uninformative otherwise.
- Hypothesis " H_{p+s} ". The sister group of Pelodytidae is the monophyletic lineage composed of pelobatids (sensu stricto) and scaphiopodids. This model is phylogenetically informative only when there is congruence in polarity between " H_p " and " H_s ".

- Hypotheses “H_{M+P}” and “H_{M+S}”. Pelodytidae would be sister group of a monophyletic lineage composed of megophryids and either pelobatids (*sensu stricto*; “H_{M+P}”) or scaphiopodids (“H_{M+S}”). These models can be deduced by the congruence between “H_M” and either “H_P” or “H_S”. The model “H_{M+P}” is suggested by some molecular data (García-París *et al.*, in press).
- Hypothesis “H_C”. Used only in the restricted analysis of *Pelodytes ibericus* versus *P. punctatus*. It considers that *Pelodytes caucasicus* shows the primitive features.

STATISTICAL ANALYSES: The lack of a statistical sample of *Pelodytes caucasicus* restricts our quantitative analysis of differentiation to the Iberian species. Several indexes are used for descriptive purposes and coding qualitative character-states, but they are not involved in further statistical analysis. Unless mentioned otherwise, the variables considered do not differ from normality (Kolmogorov-Smirnov tests). Non-parametric Mann-Whitney U-tests are used for mean comparisons. A few outliers with standarized values >3 were removed. The statistical package Statistica (Statsoft, 2001) have been used for the calculations.

Rates of evolution are measured in generalized darwin units, being one darwin an unit of evolutionary rate equivalent to a factor of *e* per million years (Haldane, 1949; Hendry & Kinnison, 1999; Kinnison & Hendry, 2001). Darwins were calculated for this study with the equation:

$$\text{darwin} = |\ln x_2 - \ln x_1| / (t_2 - t_1),$$

being $\ln x_1$ and $\ln x_2$ the natural logarithms of sample means in both Iberian *Pelodytes* species, and $t_2 - t_1$ the divergence time of 3.6 Ma.

For the study of heterochronies we have followed the allometric analysis of McKinney (1988) and the nomenclature of Reilly *et al.* (1997). We have considered as independent variables, denominator in the indexes, the more likely ones to represent “size”, usually the total lengths of the elements, and so the dependent variable would rather refer to “shape”. Natural logarithms have been used in order to minimize the general size differences between both Iberian species. For this study we have used a double analysis of covariance. In the fist place, the slopes of the *P. ibericus* and *P. punctatus* regressions are compared, being slope parallelism the null hypotheses. Afterwards, an intercept test is performed, and after rejection of the null hypotheses we proceed to infer the adjusted means of the dependent variables (“shape”) for equal

values of the independent variable (“size”). The resulting scenarios are: a) same slopes and intercepts would indicate allometric hypomorphosis / hypermorphosis; b) slopes not parallel is an indication of allometric acceleration / deceleration; c) slopes parallel and different intercepts would result in allometric pre- / postdisplacement. To assign polarities between both Iberian species we take as primitive the condition of the species more similar to *P. caucasicus* in morphology.

ABBREVIATIONS: In all figures and tables the *Pelodytes* species are abbreviated as follows: Pca: *P. caucasicus*, Pib: *P. ibericus*, Ppu: *P. punctatus*.

Description and character analysis

1- CRANIAL ARCHITECTURE AND COMPONENTS

The *Pelodytes* skull shows a generalized design, common to many primitive anurans, lacking clear specializations, such as multiple bone fusions, ornamentation, or a reduced number of elements (Trueb, 1973, 1993). General schematic figures of the *Pelodytes* skull are given by Sanchiz (1998) and Sánchez-Herráiz *et al.* (2000). Some basic features, common to all the species, are: premaxilla and maxilla dentate, having normal pedicellate teeth. Premaxillae, nasals, frontoparietals, and exoccipitals are paired bones, showing no medial fusion between right and left elements. Prootic and exoccipitals of each side are fused, as is the sphenethmoid medially. The unfused frontoparietals dorsally provide a quite variable cover to the cranial roof, but they always have complete lateral orbital margins. Stapes present and ossified. Orbita large and oval. Maxillary arch complete, having well developed pterygoids and squamosals, and maxilla reaches the quadratojugal. In ventral view the skull shows separate toothed vomers, small septomaxillae, and a well-developed free paraspheonoid.

PREMAXILLA. Within *Pelodytes* species there are differences in the number of teeth, and also in the shape of the *pars facialis* and symphysis (Figure 1). The premaxilla has 20-21 tooth positions in the available skeleton of *Pelodytes caucasicus*, while the number of positions (Table 1) reach a mean value of 15.5 in *P. punctatus*, and 14.4 in *P. ibericus*. The number of teeth per mm is about 6.6 in *P. caucasicus*, 7.1 in *P. punctatus* and 7.6 in *P. ibericus*, but these populations differ from normality, and the feature “tooth size” will be better considered in the maxilla. The intermaxillae symphysis

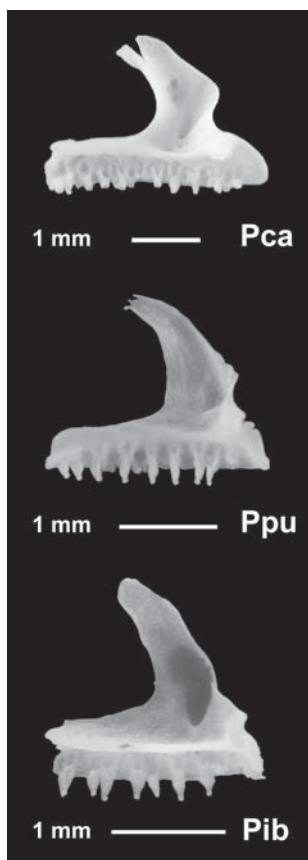


Fig. 1.— *Pelodytes* left premaxillae in inner (dental) view. For an easier comparison of shapes, equal bone widths are shown at different scales.

Fig. 1.— Premaxilares izquierdos de *Pelodytes* en vista interna (dental). Para facilitar la comparación, los huesos se muestran con anchuras iguales a diferentes escalas.

seems slightly more robust in *P. ibericus* than in *P. punctatus*. The *pars facialis*, measured as indicated in Figure 2, is proportionally somewhat wider in *P. ibericus* than in *P. punctatus*, the former of the

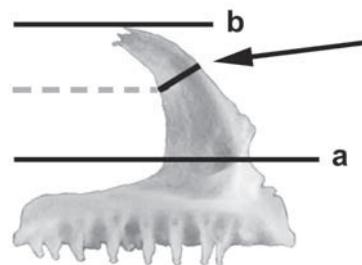


Fig. 2.— The diagram shows the place for measuring the minimum width of the *pars facialis* (indicated by an arrow), at the level of half the height between the base (a) and the end (b) of this process.

Fig. 2.— El diagrama muestra el lugar seleccionado para medir la anchura mínima de la *pars facialis*, a mitad de altura entre la base (a) y el final (b) del proceso.

same order as in *P. caucasicus*. Characters and character-states can be coded and defined as follows:

Character 01. Premaxilla. Relative width of *pars facialis*. According to the index Ifpremax (Table 1), the character-states can be coded (Figure 3) as: [a] broad, ratio >18.8 %, with mean values in the species included about 21.0 and 22.3; [b] narrow, with an index <18.8 % (mean approximately 16.8 %).

Outgroups present broad indexes of the *pars facialis* width, either at the extreme of character-state [a], as *S. couchii* or *P. cultripes* (both in the order of 23 %), or broader (approximately 32 % in *M. montana*). Thus, polarities seem congruent for

Table 1.— Premaxilla measurements (Figure 2) and indexes. Lpremax: total width. Wfpremax: minimum width of *pars facialis* at the level of half its height. Teeth: number of teeth, or mean between right and left elements when both were available. Ipremax: index (%) between Wfpremax and Lpremax. Teeth/mm: index between number of teeth and Lpremax. max: maximum. min: minimum. P: probability after a Mann-Whitney U-test for an equal value of Pib and Ppu means. SD: standard deviation.

Tabla 1.— Medidas (Figura 2) e índices del premaxilar. Lpremax: anchura total. Wfpremax: anchura mínima de la *pars facialis* en la mitad de su altura. Teeth: número de dientes, o media entre elementos derecho e izquierdo cuando ambos estaban disponibles. Ipremax: índice (%) entre Wfpremax y Lpremax. Teeth/mm: índice entre número de dientes y Lpremax. max: máximo. min: mínimo. SD: desviación típica. P: probabilidad según el test U de Mann-Whitney para igualdad de medias entre Pib y Ppu.

	Pca				Ppu				Pib				U-test
	N	mean	N	mean	SD	max	min	N	mean	SD	max	min	P
Lpremax	2	2.96	11	2.25	0.145	2.43	2.02	12	1.91	0.219	2.45	1.60	0.001
Wfpremax	2	0.62	11	0.38	0.049	0.45	0.29	12	0.42	0.052	0.51	0.35	0.060
Teeth	1	20.50	13	15.50	1.275	18.00	13.50	25	14.40	1.429	17.50	12.00	0.026
INDEXES													
Ifpremax	2	20.98	11	16.81	2.304	18.63	12.63	12	22.26	3.052	26.63	18.23	<0.001
Teeth/mm	1	6.61	11	7.05	0.619	7.92	5.97	12	7.61	0.810	8.79	6.33	0.056

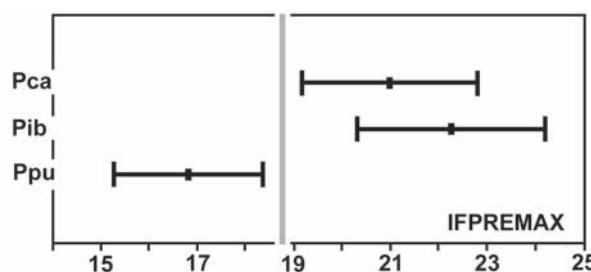


Fig. 3.— Means, confidence intervals (95 %), and character-state boundaries for the premaxillary index Ifpremax.

Fig. 3.— Medias, intervalos de confianza (95 %) y límites de los estados de carácter para el índice Ifpremax del premaxilar.

all the phylogenetic models, indicating a narrowing trend for the *pars facialis*. For only the Iberian species, “Hc” also points to [a] > [b].

MAXILLA. The most noticeable differences can be observed in the number of tooth positions, relative length of the toothed part, and *processus palatinus* (including in our case the *processus frontalis*), as can be seen in Figure 4. The number of tooth positions has mean values of 47 in *Pelodytes caucasicus*, 37.3 in *P. punctatus*, and 37.1 in *P. ibericus* (Table 2). With respect to the number of teeth per mm, the values are about 6.2 in *P. caucasicus*, 7.4 in *P. punctatus*, and 8.3 in *P. ibericus* (Table 2). The palatine process is well developed in *P. caucasicus*, reduced in *P. punctatus*, small and not well ossified in *P. ibericus*. Concerning this poorly ossified structure, we suppose that due to the techniques used to prepare the dry skeletons there might be instances in which the palatine process

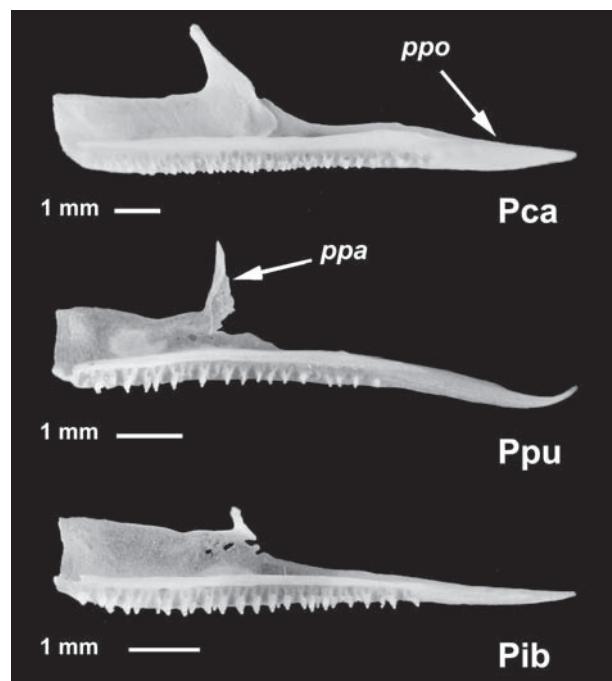


Fig. 4.— *Pelodytes* right maxillae in inner (dental) view. ppa: *pars palatina*. ppo: *pars posterior*. For an easier comparison of shapes, equal bone widths are shown at different scales.

Fig. 4.— Maxilares derechos de *Pelodytes* en vista interna (dentalaria). ppa: *pars palatina*. ppo: *pars posterior*. Para facilitar la comparación, los huesos se muestran con anchuras iguales a diferentes escalas.

could have been distally broken, and that such breakage would be difficult to detect. Nevertheless, there are several specimens of *P. ibericus* in the

Table 2.— Maxilla measurements and indexes. Lmax: total length. Lppmax: length of the *pars posterior*. Teeth: number of teeth, or the mean between right and left elements when both were available. Ippmax: index (%) between Lmax and Lppmax. Teeth/mm: index between number of teeth and Lmax minus Lppmax. Other abbreviations as in Table 1.

Tabla 2.— Medidas e índices del maxilar. Lmax: longitud total. Lppmax: longitud de la *pars posterior*. Teeth: número de dientes, o media entre elementos derecho e izquierdo cuando ambos estaban disponibles. Ippmax: índice (%) entre Lmax y Lppmax. Teeth/mm: índice entre número de dientes y Lmax menos Lppmax. Otras abreviaturas como en Tabla 1.

	Pca		Ppu				Pib				U-test		
	N	mean	N	mean	SD	max	min	N	mean	SD	max	min	P
Lmax	2	10.37	12	7.77	0.748	9.03	6.88	14	7.05	0.592	8.54	6.26	0.008
Lppmax	2	3.00	12	2.74	0.403	3.48	2.15	14	2.49	0.321	3.26	2.09	0.117
Teeth	1	47.00	12	37.25	4.475	48.00	32.50	25	37.14	3.309	43.50	29.00	0.446
INDEXES													
Ippmax	2	28.90	12	35.11	2.775	39.55	31.25	14	35.32	2.722	38.17	29.48	0.571
Teeth/mm	1	6.16	12	7.39	0.487	8.09	6.24	13	8.30	0.625	9.38	7.58	<0.001

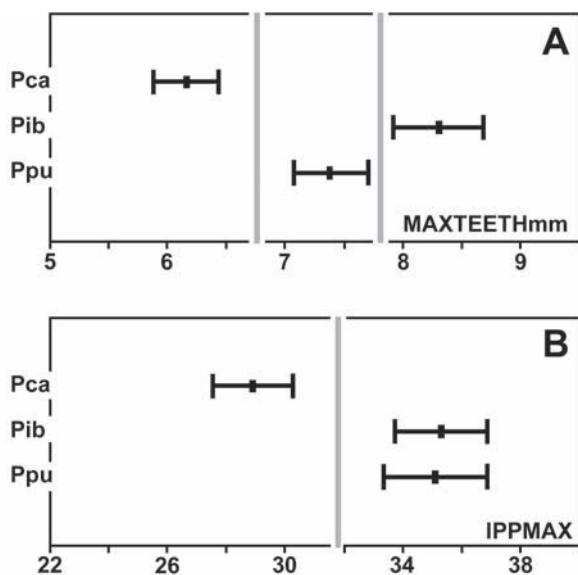


Fig. 5.— Means, confidence intervals (95 %) and character-state boundaries for the maxillary indexes Maxteethmm (A) and Ippmax (B).

Fig. 5.— Medias, intervalos de confianza (95 %) y límites de los estados de carácter para los índices maxilares Maxteethmm (A) e Ippmax (B).

collection in which these processes are so minimally connected by bone to the rest of the maxilla, that it can be suspected that there could be real instances in which the process might almost lack a complete osseous connection. The anterior margin of the maxilla is almost vertical in the Iberian species, while it is slightly anteriorly inclined in *P. caucasicus* (and in pelobatids sensu lato). The maxilla is longer in *P. punctatus* than in *P. ibericus*, but the ratio between the *crista dentalis* and the posterior process (i.e. part without teeth) is not significantly different between them (Table 2), while it seems different with respect to *P. caucasicus*. The selected characters are:

Character 02. Maxilla. Number of teeth (i.e. tooth positions) per mm (see Table 2 and Figure 5). Character-states are: [a] larger teeth, less than 6.8 per mm; [b] medium teeth, between 6.8-7.8 per mm, with mean values in the species included about 7.4; [c] smaller teeth, more than 7.8 per mm (mean about 8.3 in the species included). All the selected outgroups show a lower number of teeth per mm, less than 4.5 in all cases. Therefore, the polarity inferences would point to an increase in the number of teeth per mm. If *P. caucasicus* is consi-

dered the outgroup of the Iberian species, the polarity would also be [b] > [c].

Character 03. Maxilla. Development of the *pars palatina*. [a] the *pars palatina* is well developed; [b] the *pars palatina* is reduced and poorly ossified. This process is apparently more reduced in *P. ibericus* than in *P. punctatus*, but post-mortem breakages, in some instances almost impossible to detect, make it difficult to obtain reliable observations; thus it is preferable not to subdivide this character state. This process is well developed in all the outgroups, and the polarity assigned by the phylogenetic models is [a] > [b], a clear reduction trend.

Character 04. Maxillar. Relative length of the posterior process. The character is measured as an index (Ippmax) between the dentate and toothless portions of the maxilla (Table 2). The character-states (Figure 5) are: [a] short, having an index < 31.8 % (mean approximately 28.9 %); [b] long, index > 31.8 %, with means in the included species about 35 %.

All the outgroups have posterior processes proportionally shorter than *Pelodytes*, about 16 % in *M. montana* and 22 % in *P. cultripes* and as low as approximately 9 % in *S. couchii*, indicating an elongation trend for this process ([a] > [b]) under any phylogenetic model considered. There are no significant differences at this respect between both Iberian species.

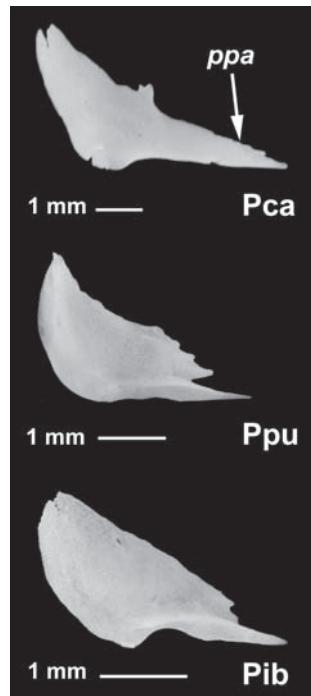


Fig. 6.— *Pelodytes* right nasals in dorsal view. ppa: *processus paraorbitalis*. For an easier comparison of shapes, equal bone sagittal lengths are shown at different scales.

Fig. 6.— Nasales derechos de *Pelodytes* en vista dorsal. ppa: *processus paraorbitalis*. Para facilitar la comparación, los huesos se muestran con longitudes sagitales iguales a diferentes escalas.

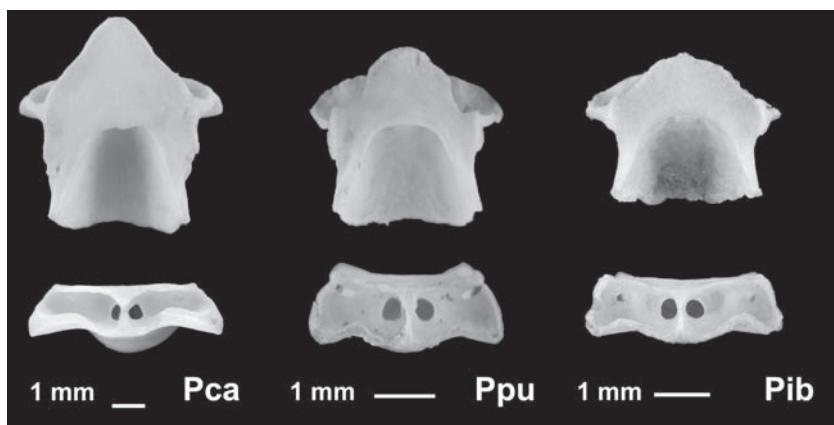


Fig. 7.— *Pelodytes* sphenethmoids in dorsal and anterior views. For an easier comparison of shapes, equal bone widths are shown at different scales.

Fig. 7.— Esfenetmoides de *Pelodytes* en vistas dorsales y anteriores. Para facilitar la comparación, los huesos se muestran con anchuras iguales a diferentes escalas.

NASAL. This bone is a fragile thin bone sheet, with a rather variable shape (Figure 6). Frequent breakages have been observed in the museological material, but most of them can be detected as such and not mistaken as real shape variants. The posterolateral process (*processus paraorbitalis*) seems somewhat more developed in *P. caucasicus* than in *P. ibericus* or *P. punctatus*, giving a more elongated and triangular aspect to the bone.

Character 05. Nasal. *Processus paraorbitalis*. The character-states are: [a] process well developed; [b] process reduced. The three selected out-groups present specialized morphologies, far different from the *Pelodytes* shape, and no reliable phylogenetic information could be obtained. Therefore, no polarity inference can be proposed.

SPHENETHMOID. Clear differences can be observed in this element among *Pelodytes* species (Figure 7). Structural proportions are different, in length, width and height (see Table 3 and Figure 8), and corresponds to different degrees of ossification of the *septum nasi*, dorsal *tectum*, and sizes of the cavities. In order to describe this variation, the following character-states have been selected:

Character 06. Sphenethmoid. Anterior development of *septum nasi* and relative width index (Iwsphen). The character-states (see Table 3, and Figures 7 and 9) are: [a] broad, having an index >114.7 (mean in the species included about 122%), with a reduced *septum nasi*; [b] moderately wide,

Table 3.— Sphenethmoid measurements (Figure 8) and indexes. Lsphen: maximal length. Wsphen: maximum width. Ltcsphen: minimum length of the dorsal *tectum*. Hsphen: maximum height. Iwsphen: index (%) between Wsphen and Lsphen. Itecsphen: index (%) between Ltcsphen and Lsphen. Ihspphen: index (%) between Hsphen and Lsphen. Ihwsphen: index (%) between Hsphen and Wsphen. Other abbreviations as in Table 1.

Tabla 3.— Medidas (Figura 8) e índices del esfenetmoids. Lsphen: longitud máxima. Wsphen: anchura máxima. Ltcsphen: longitud mínima del *tectum* dorsal. Hsphen: altura máxima. Iwsphen: índice (%) entre Wsphen y Lsphen. Itecsphen: índice (%) entre Ltcsphen y Lsphen. Ihspphen: índice (%) entre Hsphen y Lsphen. Ihwsphen: índice (%) entre Hsphen y Wsphen. Otras abreviaturas como en Tabla 1.

	Pca			Ppu				Pib				U-test	
	N	mean	N	mean	SD	max	min	N	mean	SD	max	min	P
Lsphen	2	6.36	12	3.15	0.388	3.70	2.64	14	2.65	0.342	3.56	2.16	0.002
Wsphen	2	5.80	12	3.37	0.337	3.92	2.90	14	3.22	0.341	3.88	2.70	0.341
Ltcsphen	2	3.30	12	1.42	0.210	1.69	1.05	14	1.19	0.172	1.43	0.88	0.016
Hsphen	2	1.65	12	1.29	0.147	1.46	1.04	12	1.07	0.066	1.17	0.93	<0.001
INDEXES													
Iwsphen	2	91.39	12	107.41	6.865	119.51	94.71	14	121.69	7.091	129.75	108.99	<0.001
Itecsphen	2	51.96	12	45.16	3.676	49.70	36.21	14	44.73	3.232	51.62	39.89	0.382
Ihspphen	2	26.01	12	41.07	3.136	47.99	37.36	12	41.30	4.455	49.07	32.87	0.773
Ihwsphen	2	28.45	12	38.38	3.822	45.11	32.36	12	33.70	3.381	39.26	28.41	0.007

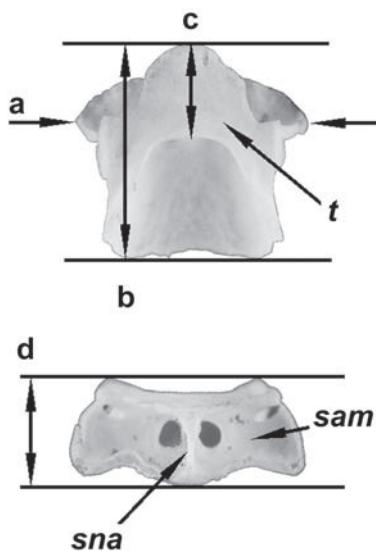


Fig. 8.— Diagram of sphenethmoid showing the measurements taken. a: Wsphen. b: Lsphen. c: Ltecsphen. d: Hsphen. sna: *septum nasi*. sam: *sella amplificans*. t: *tectum*.

Fig. 8.— Diagrama del esfenetmoids indicando las medidas tomadas. a: Wsphen. b: Lsphen. c: Ltecsphen. d: Hsphen. sna: *septum nasi*. sam: *sella amplificans*. t: *tectum*.

with index between 98.9 and 114.7 (mean about 107 %); [c] narrow, with an index <98.9 (mean approximately 91.4 % in the species included), and well developed *septum*.

Concerning the sphenethmoid, the outgroups are morphologically quite different one to another, but all are broad elements. If we assume that the character is homologous, the only inference of polarity would be a narrowing tendency ([a] > [b] > [c]). Nevertheless, there could be an alternative view if we suppose that a very complete degree of ossification, as in the outgroups, causes a broad shape, that a partial reduction in ossification originates a narrow shape (character-state [c]) because the *septum nasi* remains anteriorly elongated in the midline while the lateral sectors start to be proportionally reduced, and that a further reduction in ossification (which affects the *septum nasi*) converges to a broad shape index. In the latter possibility the polarity would be [a] > [c] > [b]. We lack information on how the shape of the anterior and lateral parts of this bone are affected by a reduction in ossification, and will preliminary use the polarity [a] > [b] > [c]. Concerning the Iberian species, the polarity [b] > [a] probably corresponds to a

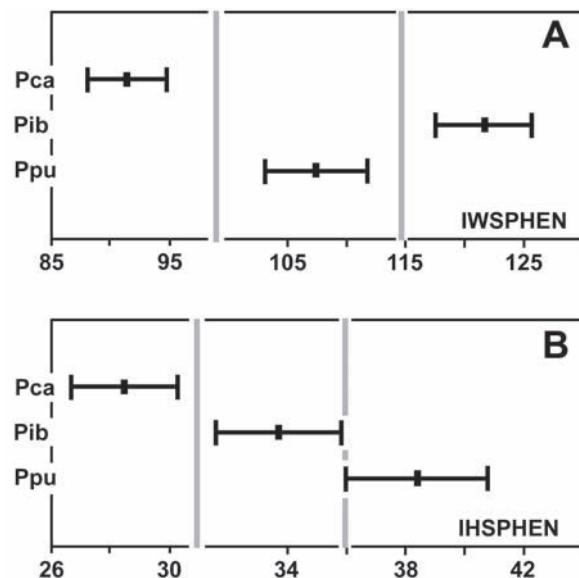


Fig. 9.— Means, confidence intervals (95 %), and character-state boundaries for the sphenethmoid indexes Iwsphen (A) and Ihsphen (B).

Fig. 9.— Medias, intervalos de confianza (95 %) y límites de los estados de carácter para los índices Iwsphen (A) e Ihsphen (B) del esfenetmoids.

shape broadening through a reduction of the ossification degree.

Character 07. Sphenethmoid. Relative size of the *antrum olfactorium* and index between height and width (Ihsphen; see Table 3). Character-states (Figure 9) are: [a] low element, with an Ihsphen index <30.9 %, with a mean in the included species approximately 28.5 %; [b] medium high element, index between 30.9 and 35.9 % (mean about 33.7 %); [c] high element, with an index >35.9 (mean approximately 38.4 %). The outgroups are much different in shape, both among themselves and in relation to *Pelodytes*, but all of them are quite flat, with Ihsphen indexes <30 %, pointing to a polarity [a] > [b] > [c]. For the Iberian species alone the polarity is also [b] > [c], using *P. caucasicus* as an example of the primitive morphology.

FRONTOPARIETAL. The most noticeable variation in the frontoparietal concerns the degree of ossification of its medial margin. This region varies (Figure 10) from providing a complete cover, through the medial contact of both unfused frontoparietals, as in *P. caucasicus*, to remain mostly separated, with a small occasional medial contact (*P. punctatus*), or

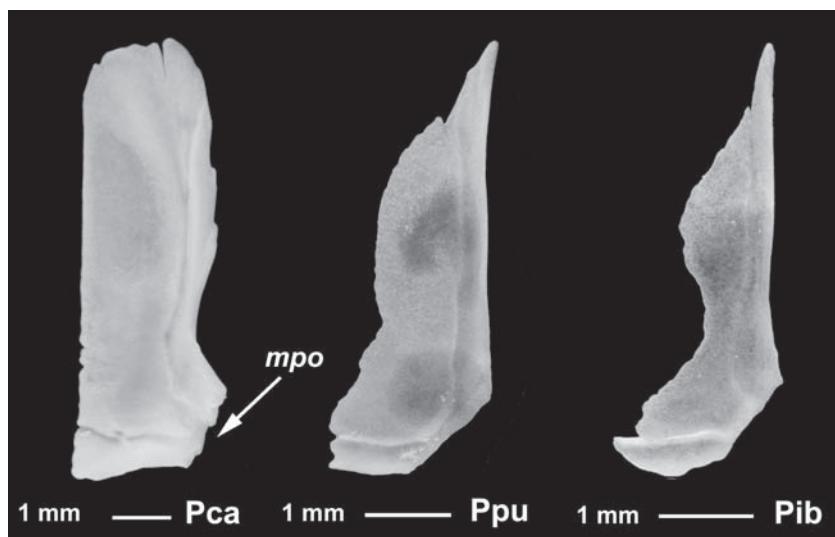


Fig. 10.— *Pelodytes* left frontoparietals in ventral view. mpo: *margo prootica*. For an easier comparison of shapes, equal bone lengths are shown at different scales.

Fig. 10.— Frontoparietales izquierdos de *Pelodytes* en vista ventral. mpo: *margo prootica*. Para facilitar la comparación, los huesos se muestran con longitudes iguales a diferentes escalas.

be widely separated as in *P. ibericus*. Within *P. punctatus* it has been observed that the medial development of this bone is slightly more complete in the available specimens from Barcelona than in the other populations studied, but our sample is not

the alternative Imfrpar index. Character-states are: [a] complete medial frontoparietal contact, in which there are no uncovered areas besides a small fontanel in the anterior margin; [b] no contact between the two bones, or they contact only in a small sector. The

Table 4.— Frontoparietal measurements (Figure 13) and indexes. Lfrpar: maximal length. Wfrpar: maximum width. Lofrpar: length of the orbital margin. Wmfrpar: width at the level of half the orbital margin. Iwfrpar: index (%) between Wfrpar and Lfrpar. Iofrpar: index (%) between Lofrpar and Lfrpar. Imfrpar: index (%) between Wmfrpar and Lfrpar. Iomfrpar: index (%) between Wmfrpar and Lofrpar. Other abbreviations as in Table 1.

Tabla 4.— Medidas (Figura 13) e índices del frontoparietal. Lfrpar: longitud máxima. Wfrpar: anchura máxima. Lofrpar: longitud del margen orbital. Wmfrpar: anchura a nivel de la mitad del margen orbital. Iwfrpar: índice (%) entre Wfrpar y Lfrpar. Iofrpar: índice (%) entre Lofrpar y Lfrpar. Imfrpar: índice (%) entre Wmfrpar y Lfrpar. Iomfrpar: índice (%) entre Wmfrpar y Lofrpar. Otras abreviaturas como en Tabla 1.

	Pca			Ppu				Pib				U-test	
	N	mean	N	mean	SD	max	min	N	mean	SD	max	min	P
Lfrpar	2	7.29	12	5.09	0.401	5.71	4.47	13	4.36	0.317	4.89	3.67	<0.001
Lofrpar	2	6.10	12	4.10	0.369	4.77	3.52	13	3.31	0.258	3.80	2.91	<0.001
Wfrpar	2	2.78	12	1.93	0.242	2.24	1.46	13	1.64	0.174	1.91	1.30	0.005
Wmfrpar	2	2.02	12	1.33	0.184	1.63	1.08	13	0.78	0.160	0.97	0.39	<0.001
INDEXES													
Iwfrpar	2	38.17	12	37.82	3.621	41.06	29.86	13	37.65	2.703	41.08	33.26	0.744
Iofrpar	2	83.72	12	80.50	1.850	83.54	77.70	13	75.87	2.491	79.57	72.20	<0.001
Imfrpar	2	27.57	12	26.07	2.683	30.74	22.82	13	17.74	3.269	21.98	10.63	<0.001
Iomfrpar	2	32.98	12	32.40	3.463	39.14	27.89	13	23.40	4.357	29.07	13.40	<0.001

numerous enough as to allow a quantification of this variation. The relative length of the orbital margin with respect to the total length seems also different among species (Table 4). The posterolateral margin (*margo prootica*) of the frontoparietal is well developed in *P. caucasicus*, but it is almost nonexistent in the Iberian species.

Character 08. Frontoparietal. Development of the *margo prootica*. Character-states: [a] present; [b] absent. The three outgroups have a well-developed *margo prootica*, and in consequence the polarity should be [a] > [b] under all phylogenetic models.

Character 09. Frontoparietal. Development of the medial margin (*margo sagittalis*). An approximate quantification of this variation can be done through index Iomfrpar (Table 4, Figures 11-12), congruent with

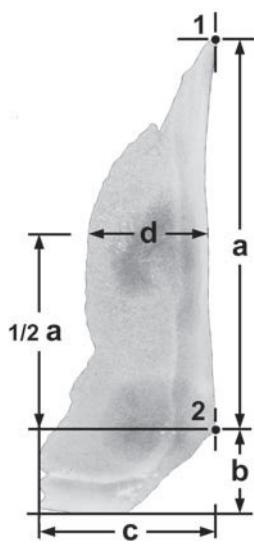


Fig. 11.— Frontoparietal diagram showing the measurements taken. a: Lofrpar. a+b: Lfrpar. c: Wfrpar. d: Wmfrpar.

Fig. 11.— Diagrama del frontoparietal indicando las medidas tomadas. a: Lofrpar. a+b: Lfrpar. c: Wfrpar. d: Wmfrpar.

index Iomfrpar have values >28.1 % and in the place where the uncovered area is maximal, fontanelle width is usually less than 50 % of the total bone width at this level. [c] Frontoparietals clearly separated, with an Iomfrpar index <28.1 and a maximal width of the uncovered area greater than 50 % of the bone width at this level. The three outgroups coincide in having frontoparietals providing a complete cover, thus indicating a decrease of ossification, and a polarity of [a] > [b] > [c].

Character 10. Frontoparietal. Relative development of the orbital margin (*margo orbitalis*). While the index relating total length and width (Iwfrpar) does not show significant differences among species, the index between orbital and total lengths (Iofrpar) does (Table 4). There is no obvious structural link between this variation and the other ones already coded for the frontoparietal, and therefore it will be separately included using the index Iofrpar (Table 4, Figure 12). The character-states are: [a] long orbital margin, with an Iofrpar index >82.0 %. The mean of the included species is 83.7 %. [b] Medium orbital margin, with indexes between 82.0 and 78.4 % (mean of the included species approximately 80.5 %). [c] Short orbital margin having an index <78.4 % (mean about 75.9 % in the included species). All the outgroups, if measured as indica-

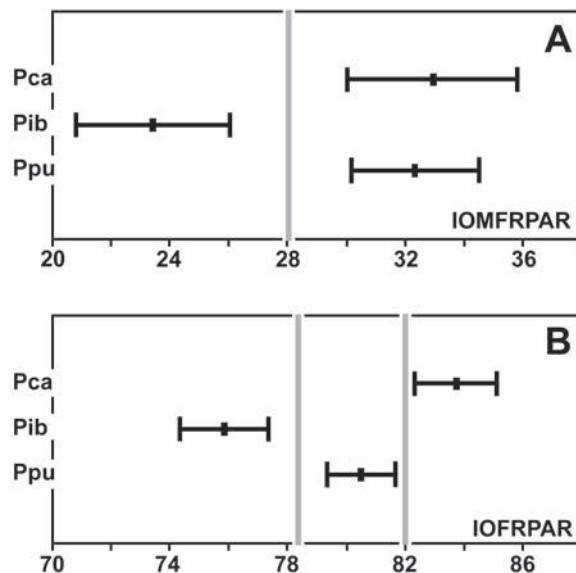


Fig. 12.— Means, confidence intervals (95 %), and character-state boundaries for the frontoparietal indexes Iomfrpar (A) and Iofrpar (B).

Fig. 12.— Medias, intervalos de confianza (95 %) y límites de los estados de carácter para los índices Iomfrpar (A) e Iofrpar (B) del frontoparietal.

ted in Figure 11, have indexes <76 %, pointing to a polarity [c] > [b] > [a].

PROOTIC-EXOCCIPITAL. This is a compound bone derived from the fusion of the prootic and exoccipital in all the *Pelodytes* species. The largest differences within *Pelodytes* (Figure 13) are in the dorsolateral sector of the prootic, which is relatively larger in *P. caucasicus* than in the other living species. Between both Iberian species, this feature seems slightly more developed in *P. punctatus* than in *P. ibericus*.

Character 11. Prootic. Relative development of the dorsolateral process (Figure 13). Character-states are: [a] process well developed, with a length subequal to the width of its anterior lateral extreme; [b] process developed with a length approximately half of its anterior distal width; [c] process underdeveloped, in which its length does not exceed one third of its anterior distal width. Within the outgroups, *M. montana* present prootics and exoccipitals that are unfused, but this species as well as *P. cultripes* and *S. couchii* have well developed dorsolateral sectors with projecting processes. The polarity is thus established as [a] > [b] > [c] for the genus *Pelodytes*, and [b] > [c] for the Iberian species.

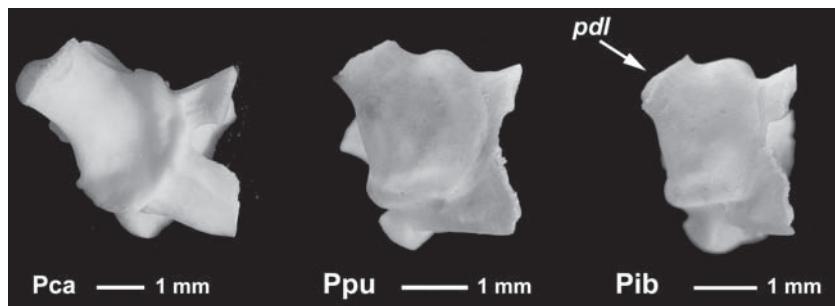


Fig. 13.—*Pelodytes* left prootic-exoccipitals in dorsal view. pdl: dorsolateral process. For an easier comparison of shapes, equal bone medial lengths are shown at different scales.

Fig. 13.—Proótico-exoccipitales de *Pelodytes* en vista dorsal. pdl: proceso dorso-lateral. Para facilitar la comparación, los huesos se muestran con longitudes mediales iguales a diferentes escalas.

SQUAMOSAL. The *ramus paroticus* of *Pelodytes caucasicus*, including an otic plate, is a well-developed structure, but the *ramus paroticus* of the two Iberian species is much reduced (Figure 14). To a lesser degree, the *ramus zygomaticus* of the Caucasian species is also better developed in proportion to those of the Iberian species. The differences between the squamosals of *P. ibericus* and *P. punctatus* are rather minor ones, difficult to code in any unequivocal way, and no differential character-states will be proposed.

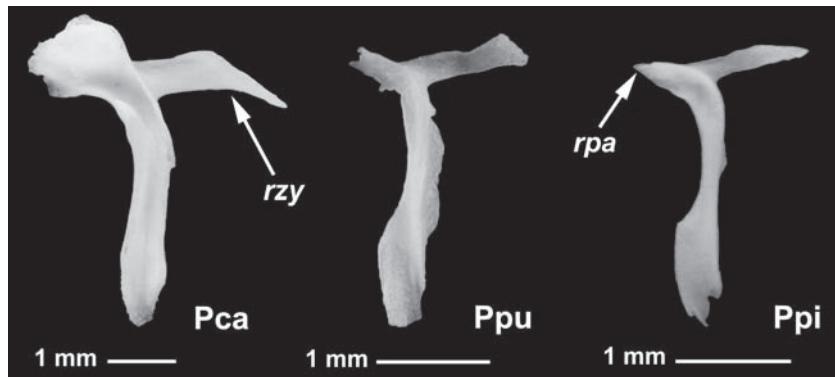


Fig. 14.—*Pelodytes* right squamosals. rpa: *ramus paroticus*. rzy: *ramus zygomaticus*. For an easier comparison of shapes, the lengths of the posterolateral processes are shown equal and vertical, with the bones at different scales.

Fig. 14.—Escamosos derechos de *Pelodytes*. rpa: *ramus paroticus*. rzy: *ramus zygomaticus*. Para facilitar la comparación, los huesos se muestran a diferentes escalas con longitudes de los procesos posterolaterales iguales y orientados verticalmente.

Character 12. Squamosal. Relative development of the *ramus paroticus*. The character-states (Figure 14) are: [a] *ramus paroticus* well developed, with an otic plate; [b] *ramus paroticus* reduced, without otic plate. The outgroups show quite different and specialized morphologies, but all of them present this process as well developed. The polarity is thus established as [a] > [b].

PTERYGOID. The pterygoid is a triradiate element in all cases, but in *P. caucasicus* the anterior branch (*ramus maxillaris*) is only slightly longer than the other branches, with a weak distal curvature. In both Iberian

species, similar in this respect, the *ramus maxillaris* is proportionally longer, bent distally, and forms a pointed distal end. (Figure 15).

Character 13. Pterygoid. Anterior branch. Character-states are: [a] the *ramus maxillaris* is not much longer than the other two *rami*, and is only slightly curved distally; [b] anterior branch more than twice the length of the other pterygoid processes and is clearly curved distally. All the selected outgroups, showing different morphologies, indicate a polarity [a] > [b], with the *P. caucasicus* morphotype as the primitive character-state.

VOMER. The morphology of the vomer is similar in the three living species of *Pelodytes*. Nevertheless, there are slight differences in the parachoanal processes (including the palatine), which delimit an opening with a diameter either similar or more open than the choanal notch (Figure 16). This element is dentate in the three species, with the number of tooth positions at about 7 in *P. caucasicus*, 3?–6 in *P. ibericus* (mode= 5, n= 19) and 4–9? in *P. punctatus* (mode= 5, n= 11).

Character 14. Vomer. Parachoanal processes. Character-states are: [a] choanal notch

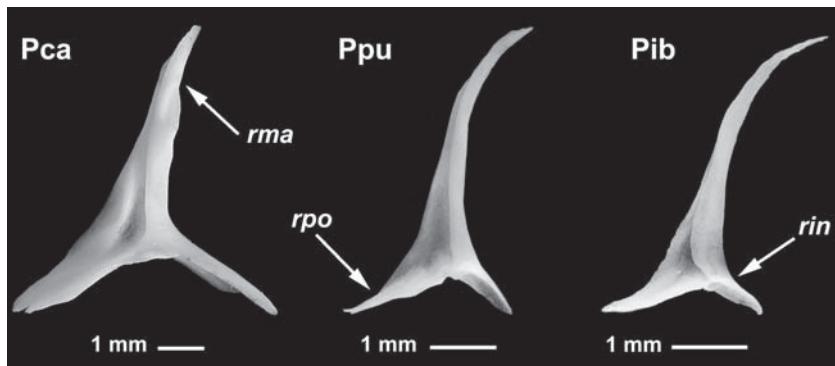


Fig. 15.— *Pelodytes* left pterygoids. rin: ramus interior. rma: ramus maxillaris. rpo: ramus posterior. For an easier comparison of shapes, equal bone lengths are shown at different scales in an artificial orientation.

Fig. 15.— Pterigoides izquierdos de *Pelodytes*. rin: ramus interior. rma: ramus maxillaris. rpo: ramus posterior. Para facilitar la comparación, los huesos se muestran a diferentes escalas con longitudes iguales en una orientación artificial.

with a wide opening, larger than any other part of the notch; [b] choanal notch with a narrow opening, similar to other parts of the notch. The outgroups are different among themselves, and the specialization of *M. montana* prevents any polarity inference under model “H_M”. *Pelobates cultripes* possesses the character-state [a] and *S. couchii* character-state [b].

PARASPHENOID. Given an equal width of the *pars medialis* in its most posterior sector (ventral view)

Fig. 16.— *Pelodytes* right vomers in ventral view. Arrow indicates the choanal opening. For an easier comparison of shapes, equal bone lengths are shown at different scales.

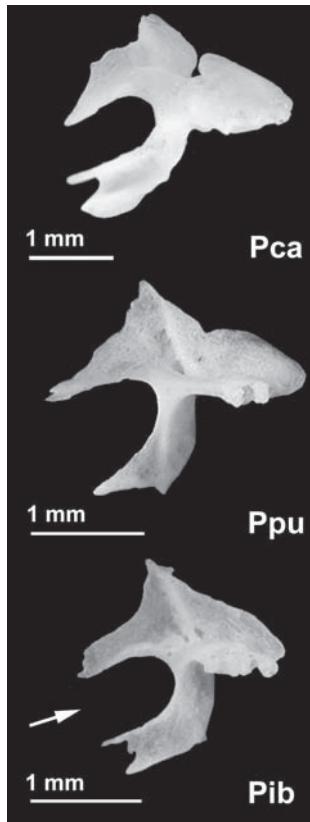


Fig. 16.— Vómeres derechos *Pelodytes* en vista ventral. La flecha indica la abertura coanal. Para facilitar la comparación, los huesos se muestran a diferentes escalas con longitudes iguales.

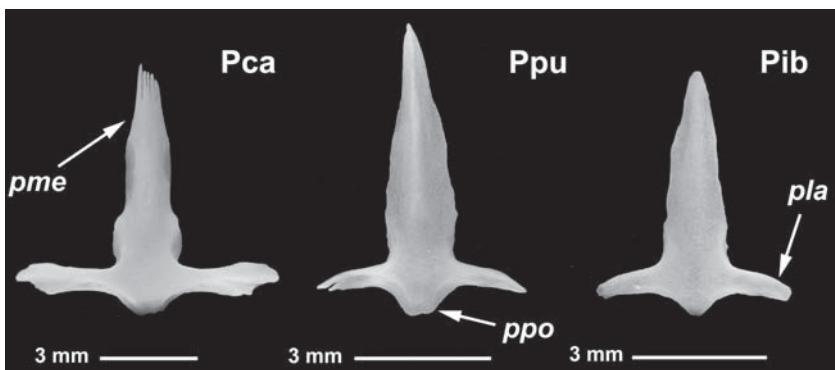


Fig. 17.— *Pelodytes* parasphenoids in ventral view. pla: processus lateralis. pme: pars medialis. ppo: processus posterior. For an easier comparison of shapes, equal widths at the base of the cultriform process are shown, with bones at different scales.

Fig. 17.— Parasfenoides de *Pelodytes* en vista ventral. pla: processus lateralis. pme: pars medialis. ppo: processus posterior. Para facilitar la comparación, los huesos muestran la misma anchura de la base del proceso cultriforme, a escalas diferentes.

to all the species, differences in proportions are observable (Figure 17), both with respect to the length of the cultriform process, and also with respect to the total width of the lateral processes (*processus lateralis*). The cultriform process of the *pars medialis* shows shape differences among *Pelodytes* species. *Pelodytes caucasicus* has a cultriform process with a wide posterior quadrangular zone, continued by a medial zone with parallel borders, but in the parasphenoid of the Iberian species the process has the shape of a blade with borders convergent in most of its length, sometimes

Table 5.— Parasphenoid measurements and indexes. Lparas: maximum length. Lcultrif: length of the cultriform process, starting at the inflexion level of the lateral curvatures joining this process with the lateral processes. Wparas: maximum width. Iwparas: index (%) between Wparas and Lparas. Icultrif: index (%) between Lcultrif and Lparas. Other abbreviations as in Table 1.

Tabla 5.— Medidas e índices del parasfenoides. Lparasf: longitud máxima. Lcultrif: longitud del proceso cultriforme, comenzando en el nivel de inflexión de las curvaturas laterales que unen este proceso con los procesos laterales. Wparasf: anchura máxima. Iwparasf: índice (%) entre Wparasf y Lparasf. Icultrif: índice (%) entre Lcultrif y Lparasf. Otras abreviaturas como en Tabla 1.

	Pca			Ppu				Pib				U-test	
	N	mean	N	mean	SD	max	min	N	mean	SD	max	min	P
Lparas	2	7.53	13	6.15	0.627	6.97	5.08	13	5.18	0.396	5.98	4.68	<0.001
Lcultrif	2	6.04	13	4.88	0.502	5.50	3.99	13	4.08	0.317	4.71	3.74	<0.001
Wparas	2	7.79	13	4.58	0.411	5.17	3.83	13	4.17	0.436	4.98	3.52	0.021
INDEXES													
Iwparas	2	103.54	13	74.59	4.699	82.02	63.72	13	80.43	5.073	90.47	73.20	0.008
Icultrif	2	80.15	13	79.30	0.974	80.90	76.97	13	78.68	1.429	80.65	76.92	0.397

including a small basal narrowing. Lateral processes are perpendicular to the sagittal axis, with wide extremities in *P. caucasicus* instead of the slightly backward, curved and sharpened extremities observable in the Iberian species, particularly in *P. punctatus*. Table 5 gives some basic measurements (Figure 18) of this bone.

Character 15. Parasphenoid. Shape of the *pars medialis*. Character-states are: [a] parallel edges in their middle part, with a quadrangular broadening in the posterior zone of the process; [b] in the medial sector the edges of the process are conver-

gent. Among the outgroups, the edges are semi parallel in *P. cultripes*, and slightly divergent, with maximal width of the process in its middle part, in *M. montana* and *S. couchii*. The polarity for *Pelodytes* is thus established as [a] > [b].

Character 16. Parasphenoid. Global relative proportions (index Iwparas in Table 5). Character-states (Figure 19) are: [a] parasphenoid relatively wide, with an index >91.5 %, and a mean of the included species approximately 103.5 %; [b] parasphenoid relatively narrow, with an Iwparas index between 91.5 and 77.4 % (mean approximately 80.4 %); [c] parasphenoid very narrow, with an index <77.4 % (mean about 74.6 %). The outgroups have relatively wide parasphenoids, thus providing a common polarity of [a] > [b] > [c], and for the Iberian species ([b] > [c]) if *P. caucasicus* is considered representative of the primitive morphology.

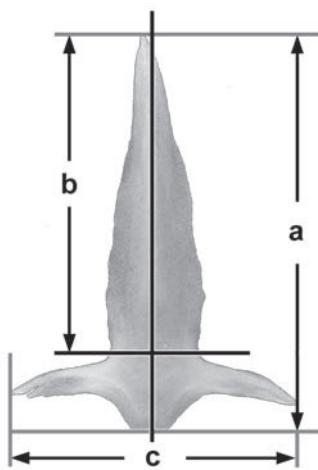


Fig. 18.— Parasphenoid diagram showing the measurements taken. a: Lparas. b: Lcultrif. c: Wparas.

Fig. 18.— Diagrama del paraesfenoides indicando las medidas tomadas. a: Lparas. b: Lcultrif. c: Wparas.

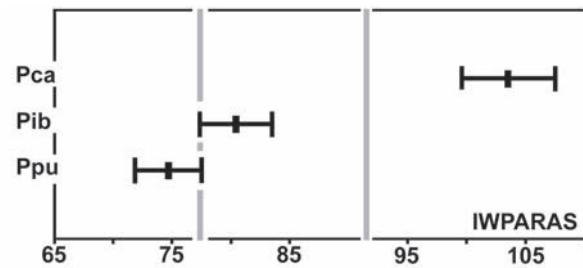


Fig. 19.— Means, confidence intervals (95 %), and character-state boundaries for the parasphenoid index Iwparas.

Fig. 19.— Medias, intervalos de confianza (95 %) y límites de los estados de carácter para el índice Iwparas del paraesfenoides.

Character 17. Parasphenoid.

Shape of the lateral processes. Character-states are: [a] lateral processes oriented perpendicular to medial process, with terminal extremities blunt; [b] processes curved slightly backwards, with pointed extremities. All outgroups show a polarity [a] > [b].

2- VERTEBRAL COLUMN

The vertebral column has eight presacral vertebrae. In normal conditions the atlas (V_1) is not fused to V_2 , nor sacrum to the urostyle. Nevertheless, as in other anurans, there are some anomalous exceptions, which in the case of the fusion V_1-V_2 have been considered even the normal situation (e.g. Sanchiz, 1978). This observation should be corrected, because in our sample the fusion V_1-V_2 occurs (both sexes) only twice in *P. punctatus* ($n=15$) and never in the *P. caucasicus* ($n=2$) or *P. ibericus* ($n=25$) samples. Free ribs are not present in *Pelodytes* (Blanco & Sanchiz, 2000).

Concerning the atlas of *Pelodytes* (Figure 20), specific differences are observed in the intercotylar region. The cotyles of *P. caucasicus* are tangent one to another in the midline, typologically representing an intermediate stage between types II and III of Lynch (1971). The Iberian species, on the other hand, present well separated cotyles, without any medial notch, and they can be placed between Lynch's (1971) types I and II, closer to type I. Concerning other features, the atlas seems rather similar for *Pelodytes* species, and only some differences have been detected in the neurapophysary apophysis. The atlantal neurapophysis, rather variable in its development, is better developed in *P. caucasicus*, usually smaller in *P. punctatus*, and almost lacking in *P. ibericus*, but being a general feature in the column, it will be coded jointly with the neurapophysis of vertebrae V_2-V_5 .

Character 18. Atlas. Intercotylar relationship. Character-states are: [a] cotyles tangent in the midline; [b] well separated cotyles, without a medial notch. Both *Pelobates cultripes* and *Scaphiopus couchii* have a characteristic type II cotyle relationship of Lynch (1971), with slightly separated cotyles and a medial notch. Therefore, the polarity inference for *Pelodytes* under the phylogenetic models " H_p " and

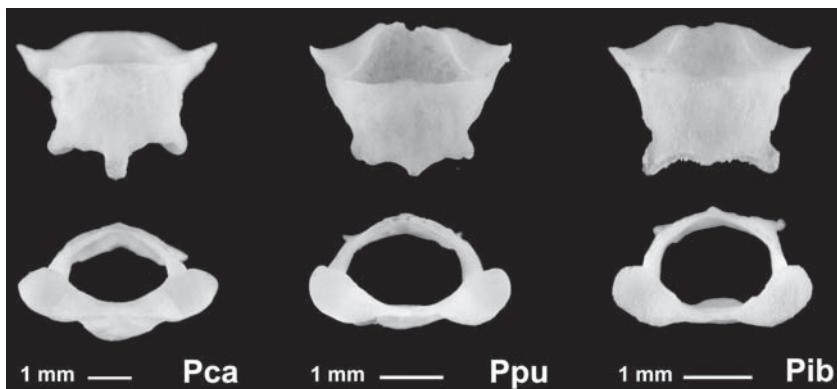


Fig. 20.— *Pelodytes* atlas in dorsal and anterior views. For an easier comparison of shapes, equal widths are shown, with bones at different scales.

Fig. 20.— Atlas de *Pelodytes* en vista anterior. Para facilitar la comparación, los huesos se muestran con la misma anchura, a escalas diferentes.

" H_s " would require a bifurcation from the common ancestor to either a near type I (character-state [b]) or to a near type III (character-state [a]). *Megophrys montana* shows a clear type III of Lynch (1971), with a complete cotylar fusion, indicating that under model " H_m " the polarity would be [a] > [b].

Vertebrae V_2-V_5 present in *P. caucasicus* a higher degree of imbrication than in the Iberian species, having neurapophyses that surpass the postzygapophyseal level and contact the following vertebra (Figure 21). The transverse processes of V_4 are straight in *P. caucasicus* and bent in *P. punctatus* and *P. ibericus*. In *P. caucasicus* vertebrae V_5-V_8 show transverse processes oriented at angles about 90° (V_5) and 45° (V_6-V_8) with respect to the sagittal axis, while in the Iberian species this angle is <45°, with the exception of V_5 in *P. punctatus* (not in *P. ibericus*) in which it is close to 45°. In both western species the transverse processes are thin cylinders, somewhat smaller in *P. ibericus*.

Character 19. Vertebral column. Imbrication in vertebrae V_4 . Character-states are: [a] vertebrae clearly imbricate, with neurapophyses projecting posteriorly beyond the postzygapophyses; [b] nonimbricating vertebrae, with neurapophyses reaching at most the posterior postzygapophyseal level; [c] nonimbricating vertebrae with a reduced neural spine. All the outgroups show imbrication to some extent, and the polarity is consequently established as [a] > [b] > [c].

Character 20. Vertebral column. Transverse processes of V_4 . Character-states are: [a] transverse

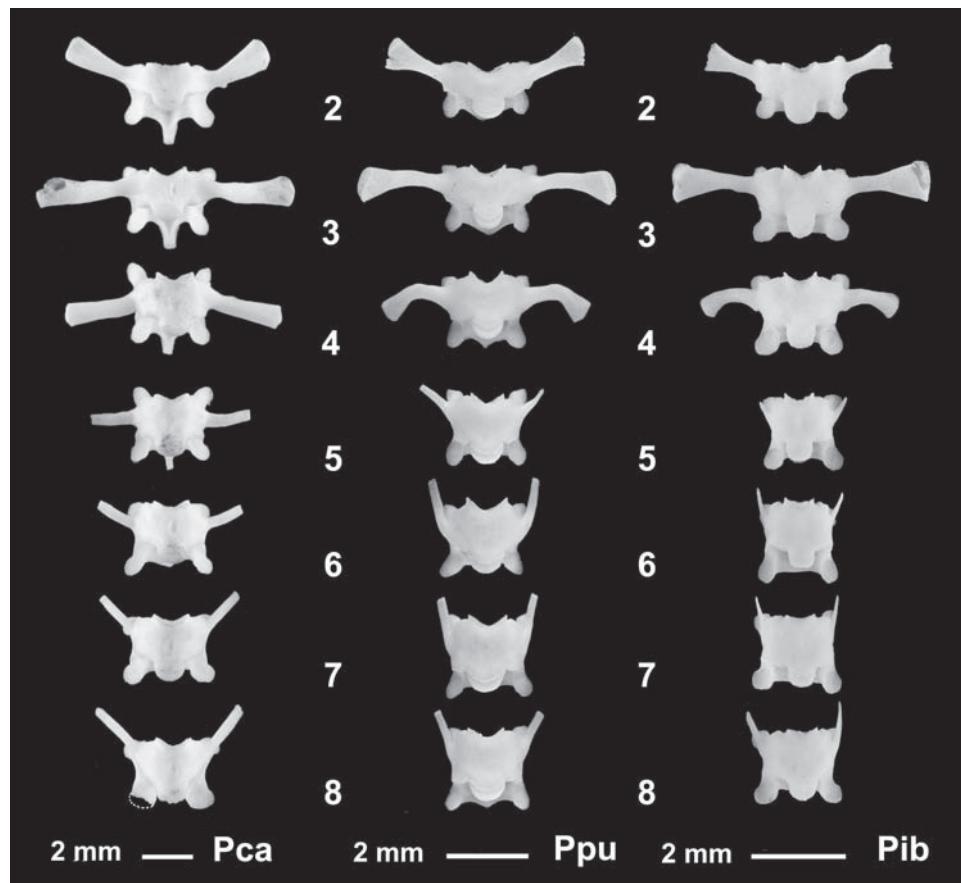


Fig. 21.—*Pelodytes* vertebral columns (V_2 - V_8) in ventral view. Numbers indicate the vertebral rank order. For an easier comparison of shapes, similar central lengths are shown for each vertebrae, with columns at different scales.

Fig. 21.—Columnas vertebrales (V_2 - V_8) en vista ventral. Los números indican el rango ordinal de cada vértebra. Para facilitar la comparación entre especies, se muestra cada vértebra con una longitud similar del centrum, en columnas a diferentes escalas.

processes straight; [b] transverse processes bent. Outgroups differ in the polarity inference. Under the phylogenetic inference “ H_M ” the polarity is clearly [a] > [b]. Both *Pelobates cultripes* and *Scaphiopus couchii* show transverse processes very weakly bent, and the polarity inferred requires a dichotomy from the common ancestor to the straight morphology, and from the common ancestor to the clearly bent morphotype.

Character 21. Vertebral column. Transverse processes of V_5 - V_8 . The character-states are: [a] transverse processes at an angle of about 90° (V_5) and 45° (V_6 - V_8) with respect to the sagittal axis; [b] angle about 45° or less (V_5), and clearly $<45^\circ$ (V_6 - V_8). Within the outgroups, *P. cultripes* shows similar features to character-state [b], while the

transverse processes of *S. couchii* and *M. montana* in this columnar segment are oriented approximately perpendicular to the longitudinal axis. Polarities are thus established as [a] > [b] under hypotheses “ H_M ”, and “ H_S ”, and as [b] > [a]) under hypothesis “ H_P ”.

Sacral vertebrae (Figure 22) are similar among *Pelodytes* species, only the diapophyses are proportionately longer in *P. ibericus* and *P. punctatus* than in *P. caucasicus*. In the two available skeletons of *P. caucasicus*, the sacral wing edges are straight and divergent one to another in their medial sector, while in *P. punctatus* and *P. ibericus*, with some variation, this margin is not straight but oval or bell shaped, more acute in its end, and proportionately slightly longer.

Character 22. Sacrum. Post-central medial edge of the transverse processes. The character-states are: [a] straight divergent edges; [b] slightly curved edges, typically having a concave and a small convex segment. All the outgroups show these edges basically straight, giving a polarity inference of [a] > [b].

The morphology of the sacro-urostyle articulation, monocondylar in *Pelodytes*, shows a high degree of intraspecific variability, with frequent presence of ossified hypochordal remains, even in large adults of *P. caucasicus*. The urostyle is also quite variable, and in the absence of large samples and ontogenetic studies, it is preferable to delay coding of interspecific differential features for this element.

3- PECTORAL GIRDLE AND FORELIMB

SCAPULA. Sánchez-Herráiz *et al.* (2000) provide a detailed discussion and illustrations of this element. The scapular morphology allows a quite clear distinction between *Pelodytes caucasicus* and the Iberian species, in which this element is quite narrow (Figure 23). Between *P. ibericus* and *P. punctatus* the differences are subtle and difficult to delimit, but there are some that seem to be consistent enough for coding, as for example the *crista anterior*, which is more developed in *P. ibericus*. Other differences can be observed (*pars acromialis*, proportions), but they show larger interspecific overlap, and will not be considered in this study.

Character 23. Scapula. Width and proportions. Character-states are: [a] wide scapula, in which its width is greater or equal to 2 times the length of the *margo suprascapularis*; [b] narrow scapula, in which its width is less than twice the length of the suprascapular margin. All of the outgroups indicate a polarity [a] > [b].

Character 24. Scapula. Development of the *crista anterior*. Character-states are: [a] *crista anterior* lacking; [b] *crista anterior* moderately developed in its whole length, typically with a straight anterior margin; [c] *crista anterior* well developed in all its length, with a convex anterior margin. All the outgroups lack any *crista anterior*, and conse-

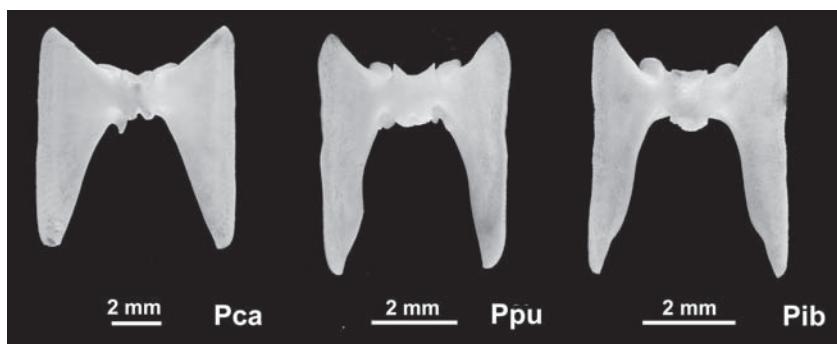


Fig. 22.— *Pelodytes* sacral vertebrae in ventral view. For an easier comparison of shapes, equal bone widths are shown at different scales.

Fig. 22.— Sacros de *Pelodytes* en vista ventral. Para facilitar la comparación, los huesos se muestran con anchuras iguales, a diferentes escalas.

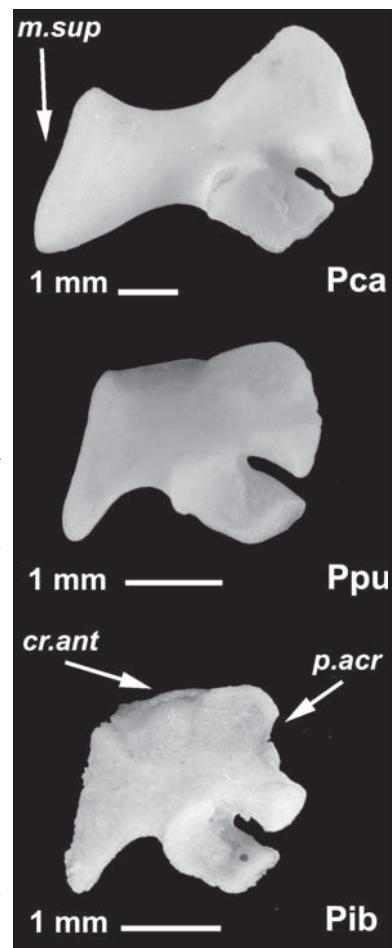


Fig. 23.— *Pelodytes* right scapulae in external view. cr.ant: crista anterior. m.sup: margo suprascapularis. p.acr: pars acromialis. For an easier comparison of shapes, equal lengths of the suprascapular margin are shown, with bones at different scales.

Fig. 23.— Escábulas derechas de *Pelodytes* en vista externa. cr.ant: crista anterior. m.sup: margo suprascapularis. p.acr: pars acromialis. Para facilitar la comparación, se muestran con iguales longitudes del margen suprascapular, a diferentes escalas.

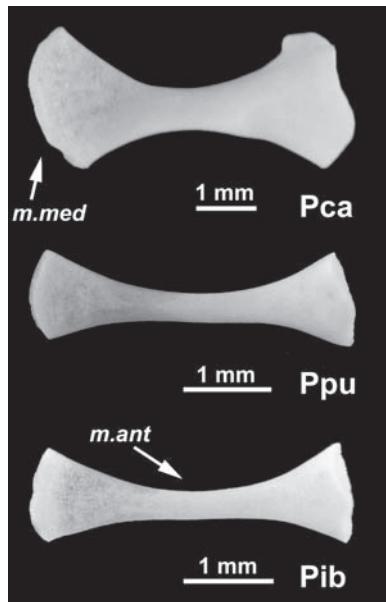


Fig. 24.— *Pelodytes* right coracoids in inner view. m.ant: *margo anterior*. m.med: *margo medialis*. For an easier comparison of shapes, equal coracoid widths are shown, with bones at different scales.

Fig. 24.— Coracoides derechos de *Pelodytes* en vista interna. m.ant: *margo anterior*. m.med: *margo medialis*. Para facilitar la comparación, los coracoides se muestran con anchuras iguales, a diferentes escalas.

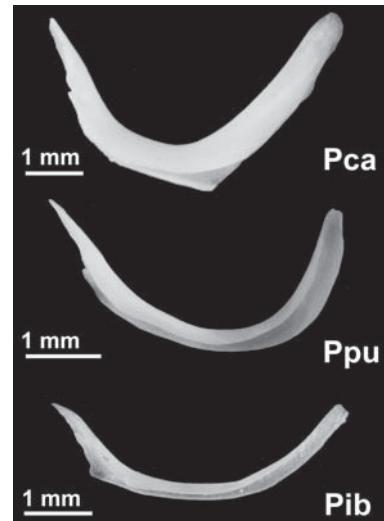


Fig. 25.— *Pelodytes* left clavicules in inner view. For an easier comparison of shapes, equal clavicle widths are shown, with bones at different scales.

Fig. 25.— Clavículas izquierdas de *Pelodytes* en vista interna. Para facilitar la comparación, las clavículas se muestran con anchuras iguales, a diferentes escalas.

quently the inference of polarity would be [a] > [b] > [c]. Concerning the Iberian species, using *P. caucasicus* as the outgroup, the polarity is [b] > [c]. This character has an added value since the scapular morphology of *Pelodytes arevacus*, an extinct species from the Iberian Middle Miocene is known. *Pelodytes arevacus*, as illustrated by Sanchiz (1978) and Sánchez-Herráiz *et al.* (2000), presents a narrow scapula with a *crista anterior*, but in which this crest is not developed in the lateral suprascapular sector. It seems a somewhat more primitive morphology than the one observed in both living Iberian species, but clearly different from the structure of *P. caucasicus*.

CORACOID. The different species have similar coracoid morphologies, but the Caucasian species has a better developed medial margin and anterior scapu-

lar process (Figure 24). These features lead to a more pronounced curvature of the anterior margin in *P. caucasicus* than in *P. ibericus* or *P. punctatus*.

Character 25. Coracoid. Development of the medial margin and anterior curvature. The character-states are: [a] medial margin expanded, with a pronounced anterior curvature of the bone; [b] medial margin only slightly expanded, in which the anterior curvature of the bone is quite open. All of the outgroups have coracoid morphologies similar to character-state [a], thus indicating a polarity [a] > [b].

CLAVICLE. The proportion between length and width is different for *Pelodytes* species (Figure 25), highest in *P. caucasicus* and lowest in *P. ibericus*. The same feature can be seen in the curvature of the bone in its anterior margin.

Character 26. Clavicle. General proportions and anterior curvature. The index Iclav (Table 6, Figures 26-27) allows quantification of the following cha-

Table 6.— Clavicle measurements (Figure 26) and indexes. Lclav: maximum length. Wclav: maximum width. Iclav: index (%) between Lclav and Wclav. Other abbreviations as in Table 1.

Tabla 6.— Medidas (Figura 26) e índices de la clavícula. Lclav: longitud máxima. Wclav: anchura máxima. Iclav: índice (%) entre Lclav y Wclav. Otras abreviaturas como en Tabla 1.

	Pca			Ppu				Pib				U-test	
	N	mean	N	mean	SD	max	min	N	mean	SD	max	min	P
Lclav	1	2.94	10	1.89	0.265	2.23	1.44	11	1.40	0.166	1.72	1.19	<0.001
Wclav	1	4.97	10	4.21	0.391	4.82	3.72	11	3.70	0.377	4.48	3.18	0.009
INDEX													
Iclav	1	59.15	10	44.75	4.026	50.39	37.70	11	37.87	3.335	42.57	33.04	0.002

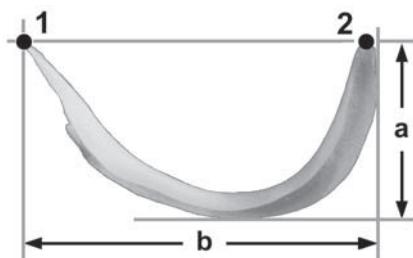


Fig. 26.— Clavicle diagram showing the measurements taken. a: Lclav. b: Wclav.

Fig. 26.— Diagrama de la clavícula indicando las medidas tomadas. a: Lclav. b: Wclav.

racter-states: [a] anterior curvature pronounced, the bone has a length more than 51.6 % of its width (mean 59.1 % in the species included); [b] moderate anterior curvature, clavicle length between 41.0 and 51.6 % of its width (mean about 44.8 %); [c] clavicle slightly curved, with a length <41.0 % of its width (mean approximately 37.9 % in the species included). Among the outgroups, *P. cultripes* and *S. couchii* have clavicles that would correspond to character-state [a], while *M. montana* could be included in character-state [b]. Therefore, the polarity under the phylogenetic models “ H_p ” and “ H_s ” is [a] > [b] > [c], and under model “ H_m ” is [a] < [b] > [c].

HUMERUS. Similar morphologies are present in all the species of *Pelodytes* (Figure 28). The bone is more robust in *P. caucasicus*, which has a stronger ventral crest. The relative robustness of this element is indicated in Table 7 through indexes relating the humeral length with the proximal and distal

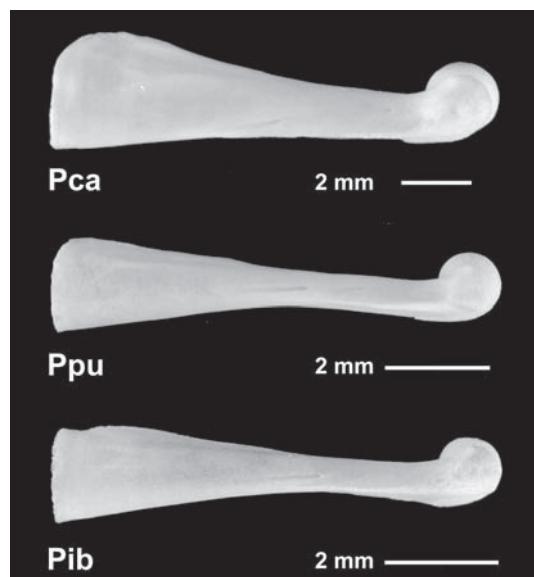


Fig. 28.— *Pelodytes* left humeri in lateral view. For an easier comparison of shapes, equal humeral lengths are shown, with bones at different scales.

Fig. 28.— Humeros izquierdos de *Pelodytes* en vista lateral. Para facilitar la comparación, se muestran húmeros de igual longitud, a diferentes escalas.

widths, and to the diameter of the *eminentia capitata*. Definition of these variables follows Sanchiz (1984).

Differences in the humeral robustness of *P. ibericus* and *P. punctatus* have not been found for the relative size of the *eminentia capitata*, are marginally significant for the proximal width index, and are very clear for the distal one (Table 7). The differences at this respect between *P. caucasicus* and the Iberian species would also be clearly significant for all the indexes if we assume for the Caucasian species a similar variability than the mean one of *P. ibericus* and *P. punctatus*. For the Iberian species, taking *P. caucasicus* as an outgroup, there would be a tendency towards slender humeral morphologies.

Character 27. Humerus. Distal robustness (index Idhumer). Character-states (Figure 29) are: [a] more robust, with an index >22.1 (mean of included species about 23 %); [b] medium robustness, index between 22.1 and 20.3 % (mean approximately 21 %); [c] minor robustness, index <20.3 % (mean about 19.7 %). Among the outgroups, *Megophrys montana* (Idhumer about 21.5 %) would correspond to character-state [b], and the polarity

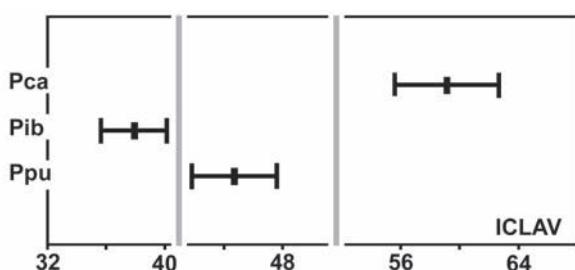


Fig. 27.— Means, confidence intervals (95 %) and character-state boundaries for the clavicle index Iclav.

Fig. 27.— Medias, intervalos de confianza (95 %) y límites de los estados de carácter para el índice Iclav de la clavícula.

Table 7.— Humeral measurements and indexes. Lhumer: maximum length. Wphumer: proximal diaphysary width. Lecapita: diameter of the *eminentia capitata*. Wdhumer: maximum distal width. Iphumer: index (%) between Wphumer and Lhumer. Iechumer: index (%) between Lecapita and Lhumer. Idhumer: index (%) between Wdhumer and Lhumer. Other abbreviations as in Table 1.

Tabla 7.— Medidas e índices humerales. Lhumer: longitud máxima. Wphumer: anchura diafisaria proximal. Lecapita: diámetro de la *eminentia capitata*. Wdhumer: anchura distal máxima. Iphumer: índice (%) entre Wphumer y Lhumer. Iechumer: índice (%) entre Lecapita y Lhumer. Idhumer: índice (%) entre Wdhumer y Lhumer. Otras abreviaturas como en Tabla 1.

	Pca			Ppu				Pib				U-test	
	N	mean	N	mean	SD	max	min	N	mean	SD	max	min	P
Lhumer	2	12.99	34	8.98	0.810	10.59	7.30	34	7.76	0.899	9.42	6.13	<0.001
Wphumer	2	1.79	34	1.11	0.140	1.39	0.81	34	0.93	0.109	1.12	0.69	<0.001
Lecapita	2	2.09	34	1.29	0.090	1.45	1.07	34	1.13	0.119	1.29	0.87	<0.001
Wdhumer	2	3.02	34	1.77	0.154	2.04	1.47	34	1.63	0.199	2.09	1.23	0.002
INDEXES													
Iphumer	2	13.78	34	12.39	0.797	14.11	10.73	34	12.07	0.747	14.04	10.64	0.053
Iechumer	2	16.14	34	14.43	0.730	15.98	13.12	34	14.57	0.673	15.77	13.30	0.404
Idhumer	2	23.26	34	19.71	0.748	21.20	18.51	34	21.01	0.969	23.19	19.56	<0.001

under the hypothesis “H_M” would be [a] < [b] > [c]. *Pelobates cultripes* (index approximately 22.9 %) and *Scaphiopus couchii* (index about 24.7 %) correspond to character-state [a], suggesting a polarity [a] > [b] > [c]. The polarity for the Iberian living species, with *P. caucasicus* as outgroup, is [b] > [c].

RADIOULNA. The Iberian species are similarly robust concerning the radioulna (Figure 30), as indicated by the indexes between length and distal or proximal widths (Table 8), with the exception of the central width index (Icradul) for which a significant difference has been found. Nevertheless, the robustness values for all these indexes are clearly higher in *P. caucasicus* than in any of the Iberian species, if we assume that the two available specimens are representative. Definition of measurements follows Sanchiz (1984).

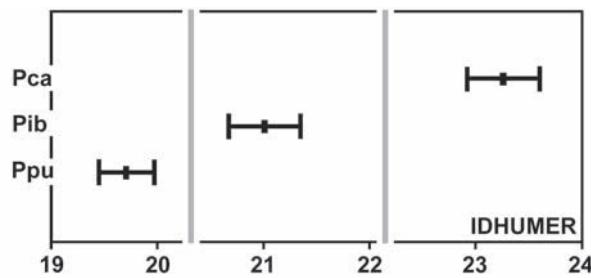


Fig. 29.— Means, confidence intervals (95 %), and character-state boundaries for the humeral index Idhumer.

Fig. 29.— Medias, intervalos de confianza (95 %) y límites de los estados de carácter para el índice humeral Idhumer.

Character 28. Radioulna. Robustness (index Icradul, Table 8). There is congruence among the different indexes concerning the relationships between *P. caucasicus* and the Iberian species. We have selec-

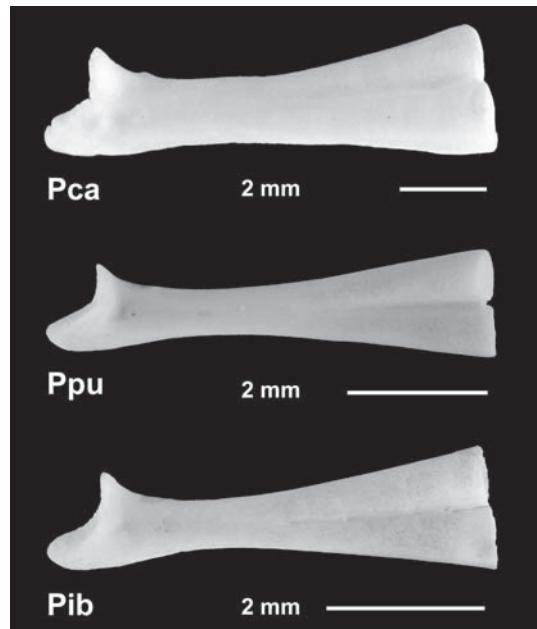


Fig. 30.— *Pelodytes* left radioulnae in lateral view. For an easier comparison of shapes, equal radioulna lengths are shown, with bones at different scales.

Fig. 30.— Radioulnas izquierdas de *Pelodytes* en vista lateral. Para facilitar la comparación, se muestran radioulnas de igual longitud, a diferentes escalas.

Table 8.— Radioulna measurements and indexes. Lradul: maximum length. Wpradul: proximal width. Wcradul: minimum central width. Wdradul: maximum distal width. Ipradul: index (%) between Wpradul and Lradul. Icradul: index (%) between Wcradul and Lradul. Idradul: index (%) between Wdradul and Lradul. Other abbreviations as in Table 1.

Tabla 8.— Medidas e índices de la radioulna. Lradul: longitud máxima. Wpradul: anchura proximal. Wcradul: anchura mínima central. Wdradul: anchura máxima distal. Ipradul: índice (%) entre Wpradul y Lradul. Icradul: índice (%) entre Wcradul y Lradul. Idradul: índice (%) entre Wdradul y Lradul. Otras abreviaturas como en Tabla 1.

	Pca			Ppu				Pib				U-test	
	N	mean	N	mean	SD	max	min	N	mean	SD	max	min	
Lradul	2	9.92	35	6.70	0.533	7.62	5.23	36	5.57	0.637	6.68	4.14	<0.001
Wpradul	2	2.36	35	1.48	0.130	1.74	1.16	36	1.21	0.136	1.45	0.90	<0.001
Wcradul	2	1.35	35	0.85	0.113	1.07	0.62	36	0.67	0.095	0.86	0.52	<0.001
Wdradul	2	3.00	35	1.89	0.226	2.26	1.43	36	1.57	0.175	1.85	1.23	<0.001
INDEXES													
Ipradul	2	23.79	35	22.06	0.986	24.58	20.14	36	21.72	0.823	23.50	19.63	0.161
Icradul	2	13.55	35	12.66	1.162	15.27	10.58	36	12.04	0.758	13.57	10.82	0.021
Idradul	2	30.24	35	28.22	2.031	32.47	23.89	36	28.14	1.176	30.04	25.80	0.713

ted Icradul for coding character-states (Figure 31) because it is the only one showing a small but significant difference between *P. ibericus* and *P. punctatus*. The character-states are: [a] more robust, with an index >13.1 % (mean of the included species approximately 13.5 %); [b] medium, with an index between 13.1 and 12.3 % (mean about 12.7 % in the included species); [c] more slender, with an Icradul index below 12.3 % (mean in the species included about 12.0 %). The selected outgroups, with indexes >16.5, all suggest a polarity towards the slender radioulna morphotype. The same polarity ([b] > [c]) can be applied to the Iberian species differentiation.

4- PELVIC GIRDLE AND HINDLIMB

ILIUM. This element is very similar in all *Pelodytes* species (Figure 32). The presence of a much redu-

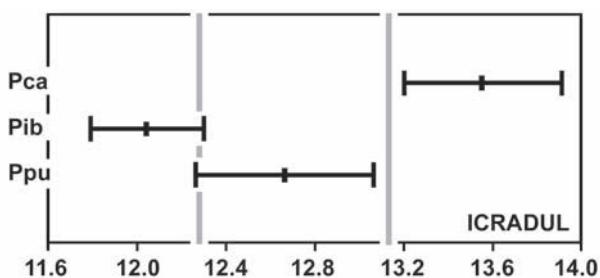
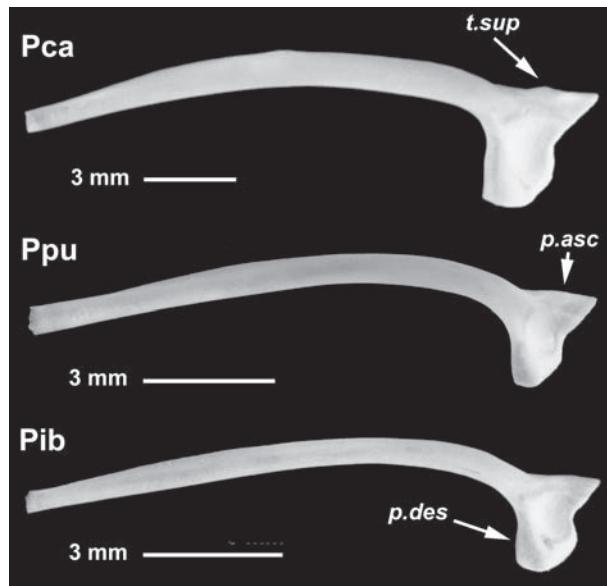


Fig. 31.— Means, confidence intervals (95 %), and character-state boundaries for the radioulna index Icradul.

Fig. 31.— Medias, intervalos de confianza (95 %) y límites de los estados de carácter para el índice radioulnar Icradul.

Fig. 32.— *Pelodytes* left ilia in external lateral view. p.asc: pars ascendens. p.des: pars descendens. t.sup: tuber superior. For an easier comparison of shapes, equal heights of the acetabulum (along the acetabular symphysis) are shown, with bones at different scales. The linear measurement Lilium is taken from the upper end point of the iliac acetabular margin to the central anterior point of the ilium shaft.

Fig. 32.— Iliones izquierdos de *Pelodytes* en vista lateral externa. p.asc: pars ascendens. p.des: pars descendens. t.sup: tuber superior. Para facilitar la comparación, se muestran acetábulos de igual altura (a lo largo de la síntesis acetabular), con huesos a diferentes escalas. La longitud Lilium se toma desde el punto superior final del margen acetabular ilíaco hasta el extremo central anterior del hueso.

Table 9.— Ilium measurements and index. Lilium: maximum length. Acetab: acetabular length measured along the ilio-ischiatic symphysis. Iailium: index (%) between Acetab and Lilium. Other abbreviations as in Table 1.

Tabla 9.— Medidas e índice ilíaco. Lilium: longitud máxima. Acetab: longitud acetabular medida a lo largo de la sínfisis ilio-isquiática. Iailium: índice (%) entre Acetab y Lilium. Otras abreviaturas como en Tabla 1.

	Pca			Ppu				Pib				U-test	
	N	mean	N	mean	SD	max	min	N	mean	SD	max	min	P
Lilium	2	19.13	33	14.03	1.250	17.32	11.07	32	11.36	1.189	13.72	8.79	<0.001
Acetab	2	2.52	33	1.65	0.243	2.04	0.77	33	1.53	0.181	2.05	1.22	0.013
INDEX													
Iailium	2	13.13	32	11.88	0.948	14.68	10.54	32	13.50	1.212	16.84	11.30	<0.001

ced *tuber superior* can be noticed in one of the available specimens of *P. caucasicus*, but not in the other. This tubercle is absent in *P. ibericus* and in the Middle Miocene *P. arevacus*. Oddly, the presence of a developed *tuber superior* seems to vary at the population level in *P. punctatus*, a species in which this structure is usually absent, but has been clearly detected in several specimens from a single population in the Segovia province. The acetabulum is relatively better developed in *P. caucasicus* and *P. ibericus* than in *P. punctatus*, being well delimited and showing a larger symphysis (Table 9). The *pars descendens* seems relatively smaller in *P. punctatus* than in *P. ibericus*, but this feature has not been successfully coded.

Character 29. Ilium. Development of acetabulum and symphysis (index Iailium in Table 9). Character-states (Figure 33) are: [a] acetabulum more developed, with a relatively stronger symphysis, delimited by an Iailion index >12.5 % (mean of the included species about 13.1-13.5 %); [b] acetabulum and symphysis less developed, delimited by

Iailion < 12.5 % (mean approximately 11.9 %). Within the outgroups, *M. montana* presents an index (about 12.3 %) that corresponds to character-state [b], while *P. cultripes* (about 13 %) and *S. couchii* (over 19 %) would be included in character-state [a].

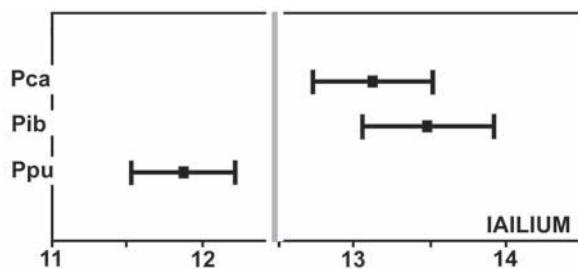


Fig. 33.— Means, confidence intervals (95 %), and character-state boundaries for the iliac index Iailium.

Fig. 33.— Medias, intervalos de confianza (95 %) y límites de los estados de carácter para el índice ilíaco Iailium.



Fig. 34.— *Pelodytes* ischia in lateral left view. For an easier comparison of shapes, equal heights of the acetabulum (along the acetabular symphysis) are shown, with bones at different scales.

Fig. 34.— Isquiones de *Pelodytes* en vista lateral izquierda. Para facilitar la comparación, se muestran acetábulos de igual altura (a lo largo de la sínfisis acetabular), con huesos a diferentes escalas.

Table 10.— Ischium measurements (Figure 35) and indexes (symphysial view). Lischia: maximum height. Wischia: maximum width. Lpaischia: dorsal height, over the acetabular level. Iwischia: index (%) between Wischia and Lischia. Ipaischia: index (%) between Lpaischia and Lischia. Other abbreviations as in Table 1.

Tabla 10.— Medidas (Figura 35) e índices isquiáticos (vista sinfisaria). Lischia: altura máxima. Wischia: anchura máxima. Lpaischia: altura dorsal, por encima del nivel acetabular. Iwischia: índice (%) entre Wischia y Lischia. Ipaischia: índice (%) entre Lpaischia y Lischia. Otras abreviaturas como en Tabla 1.

	Pca			Ppu				Pib			U-test		
	N	mean	N	mean	SD	max	min	N	mean	SD	max	min	P
Lischia	2	4.10	12	2.32	0.367	3.02	1.82	12	1.85	0.249	2.48	1.57	0.001
Wischia	2	2.86	12	1.56	0.152	1.83	1.36	12	1.31	0.143	1.62	1.09	0.002
Lpaischia	2	1.23	12	0.84	0.093	1.01	0.74	12	0.65	0.127	0.93	0.52	0.001
INDEXES													
Iwischia	2	69.99	12	67.72	5.546	77.47	60.60	12	71.21	3.489	79.21	65.32	0.057
Ipaischia	2	29.73	12	36.57	3.774	43.41	31.10	12	35.12	3.180	40.28	30.81	0.356

ISCHIUM. The ischium of *Pelodytes* is similar among species, differing mostly in the relative length of the dorsal sector that corresponds to the *pars ascendens* in the ilium (Table 10 and Figure 34). The difference in general proportions between both Iberian species is marginally significant (Table 10), as measured in symphysial view (Figure 35), in which *P. caucasicus* has an intermediate shape. Also from a symphysial view, the sector below the acetabular area seems somewhat shorter in *P. ibericus* in relation to the acetabular length, but this is a quite variable area that is in contact with the pubic cartilages and will not be quantified.

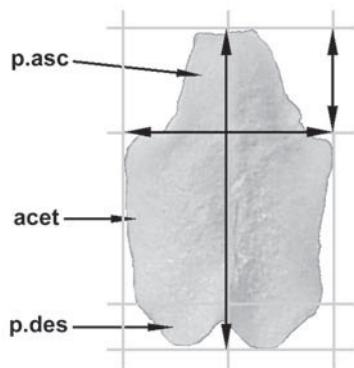


Fig. 35.— Ischium in diagrammatic symphysial view, showing by arrows the measurements taken. acet: acetabulum. p.asc: dorsal sector (*pars ascendens*). p.des: posterior sector (*pars descendens*).

Fig. 35.— Diagrama del isquion en vista sinfisaria. Las flechas muestran las medidas efectuadas. acet: acetáculo. p.asc: sector dorsal (*pars ascendens*). p.des: sector posterior (*pars descendens*).

Character 30. Ischium. Relative size of the *pars ascendens* (index Ipaischia, see Table 10). Character-states (Figure 36) are: [a] *pars ascendens* relatively short, with an index <32.3 %; [b] *pars ascendens* relatively long, with indexes >32.3 %. All the outgroups are concordant in having indexes greater than character-state [b], and the common polarity inference would be [b] > [a].

FEMUR. Interspecific differences in this element (Figure 37) refer to their proportions (Table 11). *Pelodytes punctatus* is significantly less robust than *P. ibericus* for both proximal and distal indexes, and *Pelodytes caucasicus* has index values equal (Idfemur) or only slightly larger (Ipifemur) than *P. ibericus*. Definition of measurements follows Sanchiz (1984).

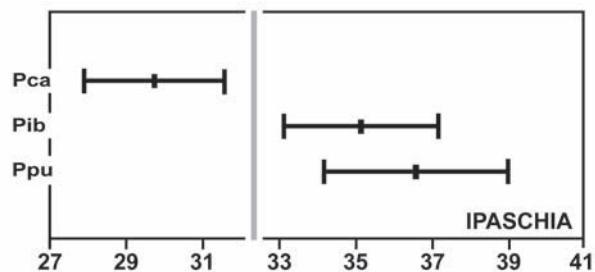


Fig. 36.— Means, confidence intervals (95 %), and character-state boundaries for the ischiatic index Ipaschia.

Fig. 36.— Medias, intervalos de confianza (95 %) y límites de los estados de carácter para el índice isquiático Ipaschia.

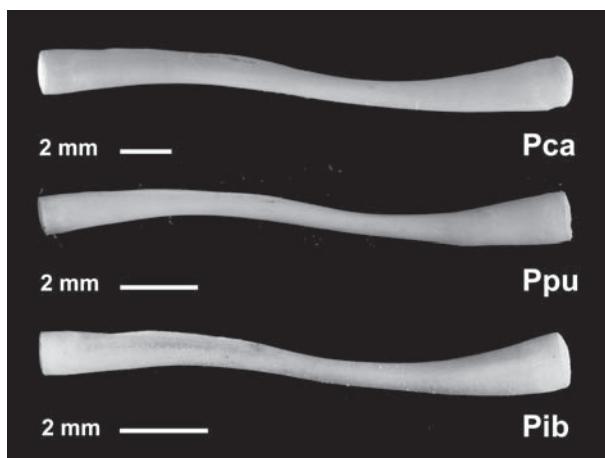


Fig. 37.— *Pelodytes* right femora. For an easier comparison of shapes, equal femoral lengths are shown, with bones at different scales.

Fig. 37.— Fémures derechos de *Pelodytes*. Para facilitar la comparación, se muestran fémures de igual longitud, a diferentes escalas.

Character 31. Femur. Proximal width index (Ipfemur, see Table 11). The character-states (Figure 38) are: [a] more robust, with an index >8.4 % (means about 8.7 and 9.0 % in the species included); [b] more slender, with an index <8.4 % (mean approximately 8.1 %). All the outgroups have indexes over 9.5 and thus are more robust than *Pelodytes*, indicating a polarity of [a] $>$ [b]. Concerning the living Iberian species, with *P. caucasicus* as the outgroup, the polarity is the same ([a] $>$ [b]).

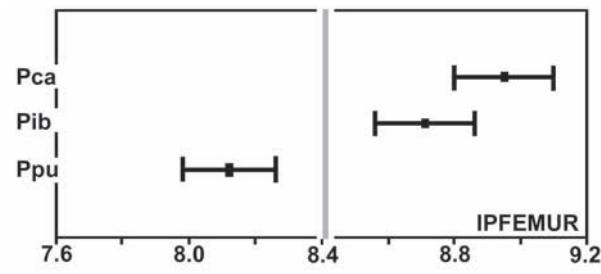


Fig. 38.— Means, confidence intervals (95 %), and character-state boundaries for the femoral index IpFEMUR.

Fig. 38.— Medias, intervalos de confianza (95 %) y límites de los estados de carácter para el índice femoral IpFemur.

TIBIOFIBULA. Interspecific differences within Iberian *Pelodytes* for the tibiofibula (Figure 39) have been found for both the proximal and distal robustness, but not for the central diaphysary region (Table 12). *Pelodytes caucasicus* presents diverse relations with respect to the Iberian species, being more robust than either of them in the central sector, similar to *P. punctatus* in the proximal index, and similar to *P. ibericus* in the distal one (Table 12, Figure 40). Considering the latter situation, it seems preferable to code two different character-states.

Character 32. Tibiofibula. Proximal width index. The index Iptibia (Table 12) allows the following character-state diagnoses (Figure 40): [a] robust element, with Iptibia >12.2 % (mean about 12.5 % in the included species); [b] bone more slender, with index <12.2 % (means of the two included species approximately 11.8 %). All the

Table 11.— Femur measurements and indexes. Lfemur: maximum length. Wpfemur: maximum proximal width. Wdfemur: maximum distal width. Ipfemur: index (%) between Wpfemur and Lfemur. Idfemur: index (%) between Wdfemur and Lfemur. Other abbreviations as in Table 1.

Tabla 11.— Medidas e índices del fémur. Lfemur: longitud máxima. Wpfemur: anchura proximal máxima. Wdfemur: anchura distal máxima. Ipfemur: índice (%) entre Wpfemur y Lfemur. Idfemur: índice (%) entre Wdfemur y Lfemur. Otras abreviaturas como en la Tabla 1.

	Pca			Ppu				Pib				U-test	
	N	mean	N	mean	SD	max	min	N	mean	SD	max	min	
Lfemur	2	21.06	35	14.49	1.223	16.61	11.46	34	11.60	1.393	14.07	8.35	<0.001
Wpfemur	2	1.89	35	1.18	0.115	1.40	0.95	34	1.01	0.107	1.20	0.78	<0.001
Wdfemur	2	2.36	35	1.50	0.205	1.88	1.09	34	1.31	0.139	1.71	1.02	<0.001
INDEXES													
Ipfemur	2	8.95	35	8.12	0.405	9.17	7.18	34	8.71	0.432	9.62	7.84	<0.001
Idfemur	2	11.24	35	10.31	0.944	12.16	9.02	34	11.37	1.124	13.29	9.24	<0.001

Table 12.— Tibiofibula measurements and indexes. Ltibia: maximum length. Wptibia: maximum proximal width. Wctibia: minimum central width. Wdtibia: maximum distal width. Iptibia: index (%) between Wptibia and Ltibia. Ictibia: index (%) between Wctibia and Ltibia. Idtibia: index (%) between Wdtibia and Ltibia. Other abbreviations as in Table 1.

Tabla 12.— Medidas e índices de la tibiofibula. Ltibia: longitud máxima. Wptibia: anchura proximal máxima. Wctibia: anchura central mínima. Wdtibia: anchura distal máxima. Iptibia: índice (%) entre Wptibia y Ltibia. Ictibia: índice (%) entre Wctibia y Ltibia. Idtibia: índice (%) entre Wdtibia y Ltibia. Otras abreviaturas como en Tabla 1.

	Pca			Ppu				Pib			U-test		
	N	mean	N	mean	SD	max	min	N	mean	SD	max	min	P
Ltibia	2	23.78	34	15.56	1.217	17.81	12.01	34	12.72	1.351	15.22	9.41	<0.001
Wptibia	2	2.81	34	1.83	0.206	2.33	1.46	34	1.59	0.165	1.86	1.25	<0.001
Wctibia	2	1.13	34	0.68	0.079	0.85	0.51	34	0.57	0.067	0.69	0.44	<0.001
Wdtibia	2	3.17	34	2.00	0.216	2.43	1.56	34	1.73	0.186	2.05	1.29	<0.001
INDEXES													
Iptibia	2	11.81	34	11.79	0.894	13.91	10.38	34	12.52	0.472	13.57	11.55	<0.001
Ictibia	2	4.76	34	4.40	0.380	5.31	3.83	34	4.51	0.241	5.00	3.96	0.162
Idtibia	2	13.32	34	12.87	0.837	14.39	1.45	34	13.58	0.493	14.72	12.50	<0.001

outgroups are more robust, showing clearly much larger indexes (at least >15 %). The common polarity would be [a] > [b].

Character 33. Tibiofibula. Distal width index. Character-state diagnoses using the index Idtibia (Table 12, Figure 40) are: [a] robust element, with Idtibia >13.1 % (means values about 13.3-13.6 % in the included species); [b] bone more slender, with index <13.1 % (mean approximately 12.9 %). All the outgroups are more robust, showing clearly

much larger indexes (at least >16 %). The common polarity would be [a] > [b].

TIBIALE-FIBULARE. The family Pelodytidae, with the only parallel of some centrolenids within anurans, have completely fused tibiale and fibulare (“calca-

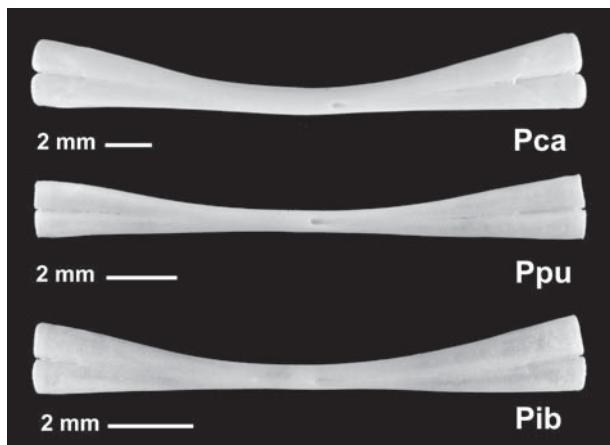


Fig. 39.— *Pelodytes* left tibiofibulae. For an easier comparison of shapes, equal tibiofibula lengths are shown, with bones at different scales.

Fig. 39.— Tibiofibulas izquierdas de *Pelodytes*. Para facilitar la comparación, se muestran con iguales longitudes, a diferentes escalas.

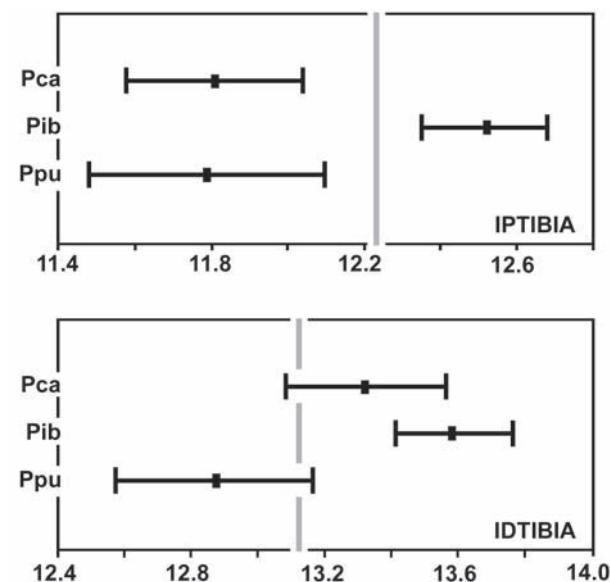


Fig. 40.— Means, confidence intervals (95 %), and character-state boundaries for the tibiofibula indexes Iptibia (A) and Idtibia (B).

Fig. 40.— Medias, intervalos de confianza (95 %) y límites de los estados de carácter para los índices Iptibia (A) e Idtibia (B) de la tibiofibula.

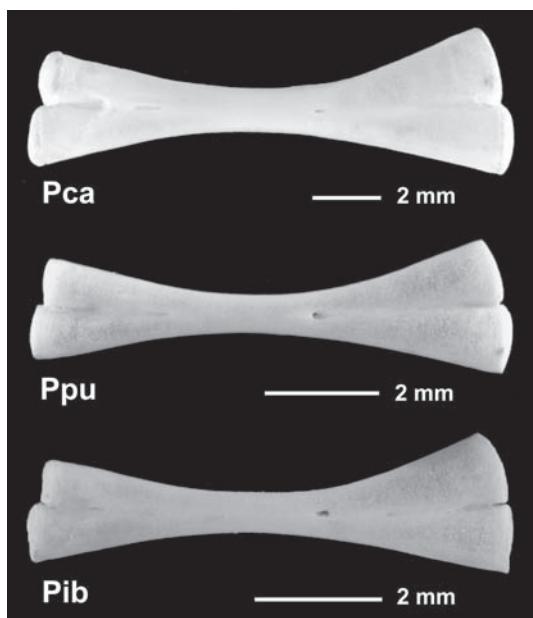


Fig. 41.— *Pelodytes* left tibiale-fibulare. For an easier comparison of shapes, equal lengths are shown, with bones at different scales.

Fig. 41.— Tibial-fibulares izquierdos de *Pelodytes*. Para facilitar la comparación, se muestran los huesos de igual longitud, a diferentes escalas.

neum” and “astragalus”) along its entire length. These elements consequently are transformed in a biomechanical element similar to the tibiofibula. The interspecific differences within *Pelodytes* (Figure 41) rely in the robustness of this element,

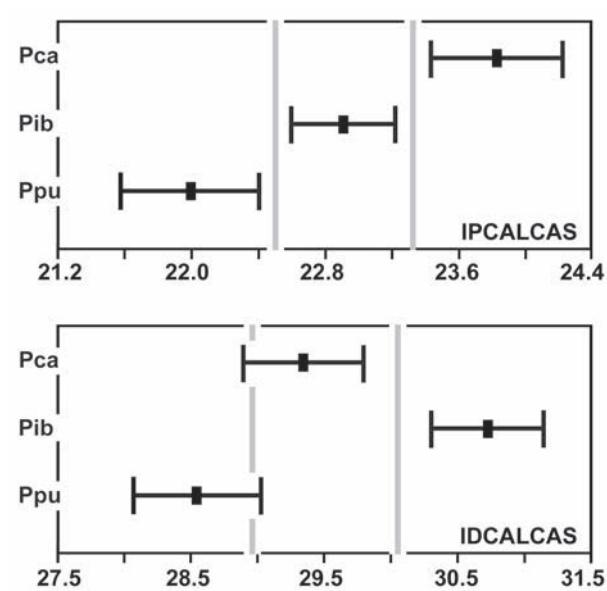


Fig. 42.— Means, confidence intervals (95 %), and character-state boundaries for the tibiale-fibulare indexes Ipcalcas and Idcalcas.

Fig. 42.— Medias, intervalos de confianza (95 %) y límites de los estados de carácter para los índices Ipcalcas e Idcalcas del tibial-fibular.

slender in *P. punctatus* and more robust in the other species, as indicated by proximal, central and distal width indexes (Table 13). *Pelodytes caucasicus* is more robust than either Iberian species for the proximal index, similar to *P. ibericus* in the central

Table 13.— Tibiale-fibulare measurements and indexes. Lcalcas: maximum length. Wpcalcas: maximal proximal width. Wccalcas: minimum central width. Wdcalcas: maximum distal width. Ipcalcas: index (%) between Wpcalcas and Lcalcas. Iccalcas: index (%) between Wccalcas and Lcalcas. Idcalcas: index (%) between Wdcalcas and Lcalcas. Other abbreviations as in Table 1.

Tabla 13.— Medidas e índices del tibial-fibular. Lcalcas: longitud máxima. Wpcalcas: anchura proximal máxima. Wccalcas: anchura central mínima. Wdcalcas: anchura distal máxima. Ipcalcas: índice (%) entre Wpcalcas y Lcalcas. Iccalcas: índice (%) entre Wccalcas y Lcalcas. Idcalcas: índice (%) entre Wdcalcas y Lcalcas. Otras abreviaturas como en Tabla 1.

	Pca			Ppu				Pib				U-test	
	N	mean	N	mean	SD	max	min	N	mean	SD	max	min	
Lcalcas	2	14.07	34	9.17	0.759	10.92	7.28	34	7.39	0.833	8.91	5.40	<0.001
Wpcalcas	2	3.35	34	2.02	0.223	2.56	1.59	34	1.69	0.195	2.09	1.28	<0.001
Wccalcas	2	1.25	34	0.75	0.078	0.91	0.59	34	0.67	0.079	0.82	0.53	<0.001
Wdcalcas	2	4.13	34	2.62	0.253	3.11	2.10	34	2.27	0.291	2.87	1.61	<0.001
INDEXES													
Ipcalcas	2	23.82	34	21.99	1.187	24.13	19.64	34	22.90	0.887	24.73	21.23	<0.001
Iccalcas	2	8.92	34	8.14	0.434	9.03	7.22	34	9.04	0.486	10.19	7.94	<0.001
Idcalcas	2	29.34	34	28.54	1.374	31.32	26.04	34	30.73	1.227	33.96	28.74	<0.001

one, and intermediate between *P. ibericus* and *P. punctatus* concerning the distal index (Table 13).

Character 34. Tibiale-fibulare. Relative proximal width. The index Ipcalcas (Table 13) allows coding for the following character-states (Figure 42): [a] more robust element, with an index >23.3 % (mean of the species included about 23.8 %); [b] intermediate element, with an index between 23.3 and 22.5 % (mean about 22.9 in the included species); [c] more slender, with Ipcalcas <22.5 % (mean approximately 22.0 %). All the outgroups have unfused tibiale and fibulare, and they provide no phylogenetic information on polarity for *Pelodytes*. For the restricted analysis of the living Iberian species, with *P. caucasicus* as the outgroup, the polarity would be [b] > [c].

Character 35. Tibiale-fibulare. Relative distal width. The index Idcalcas (Table 13) gives the following character-state diagnoses (Figure 42): [a] more robust element, with an index >30.1 % (mean of the species included about 30.7 %); [b] intermediate element, with index between 30.1 and 29.0 % (mean approximately 29.3 %); [c] element more slender, with Idcalcas <29.0 % (mean approximately 28.5 %). The outgroups do not provide any information on polarity. For the restricted analysis of the living Iberian species, showing *P. caucasicus* an intermediate morphology, the polarity would be a dichotomy [a] v [b].

Results and discussion

EVOLUTIONARY RELATIONSHIPS

In the previous section, using only isolated bones, we have considered a large set of osteological features that show constant differences among *Pelodytes* species. Even if the sample is not exhaustive and includes only males, we think that it is representative enough to analyse the osteological differentiation between the living Iberian species. Table 14 presents the character-state exhibited by each species in the 35 characters. Using the various phylogenetic models derived from our outgroup selection, Table 15 summarizes the polarity inferences for character-state transformation.

If we preliminarily assign an equal weight to all the characters, the results summarized in Table 15 are quite clear concerning the relative degree of derivation of each *Pelodytes* species. Under any acceptable phylogenetic hypothesis about the morphology of the common *Pelodytes* ancestor, it seems clear that *Pelodytes caucasicus* has retained

by far the most primitive morphology, only having percentages of derivation about 17 to 23 %. On the contrary, the percentages of derived traits for *P. ibericus* or *P. punctatus*, under different phylogenetic possibilities, are always over the 68 % level. Thus, the living Caucasian species seems morphologically much closer to the common *Pelodytes* ancestor than any of the living Iberian species.

Concerning the conservation of primitive morphological traits, under any polarity model, there is little difference between *P. ibericus* and *P. punctatus* in their relative degree of evolutionary transformation. Using *Pelodytes caucasicus* as an outgroup, the percentage of derived character-states is also similar, 45 % for *P. ibericus* and 60 % for *P. punctatus* (Table 15).

Both the qualitative and metric characters that we have used provide phenetic similarity estimates, which would be also cladistic when the characters are analysed under outgroup polarity models (Kitching *et al.*, 1998). The Manhattan distances (Sneath & Sokal, 1973) for all the values given in Table 15 (equally weighted), as well as the Euclidean distances (Sneath & Sokal, 1973) based on metric characters, are indicated in Table 16 for all the phylogenetic hypotheses.

The diverse analyses and distances obtained with the osteological data indicate quite clearly that the living *Pelodytes ibericus* and *P. punctatus* are sister species in relation to *P. caucasicus*. The few comparable elements of the Middle Miocene *Pelodytes arevacus*, in particular the scapula, place this extinct species in the Iberian lineage, as the sister group of the combined lineage *P. ibericus* + *P. punctatus*, and not closely related to *P. caucasicus*. The results of this osteological data set agree with the phylogenetic inferences derived from molecular sequence data (mDNA genes 16SARNr and cytochrome *b*, García-París *et al.*, in press), which are also quite conclusive. As a consequence, if this temporal scheme is accepted, the cladistic relationships among *Pelodytes* would require consideration of the extinct *P. arevacus* as a valid species, and not as a synonym of either *P. punctatus* or *P. ibericus*, even if they were morphologically identical (which is not the case). A paleontological study of the new available pelodytid fossil record will probably contribute to a better understanding of their Miocene evolutionary and faunistic history.

EVOLUTIONARY DIVERGENCE RATES

Both the osteological (paleontological) and molecular data sets (molecular clocks) suggest a

Table 14.— Character-state distribution for *Pelodytes* species and their polarities under different phylogenetic models. The Pelodytidae sister group is: “H_M”: Megophryidae, “H_P”: Pelobatidae (sensu stricto), “H_S”: Scaphiopodidae and “H_T”: a monophyletic lineage containing Megophryidae, Pelobatidae and Scaphiopodidae. Hypotheses “H_{M+P}”, “H_{M+S}”, and “H_{P+S}” can be deduced after the congruence between “H_M” and “H_P”, “H_M” and “H_S”, or “H_P” and “H_S” respectively. “H_C”: *P. caucasicus* as outgroup for Iberian *Pelodytes* species. Both ">" and "<" indicate linear polarity changes. "v": transformation requires a dicotomy of two lineages from the common ancestor.

Tabla 14.— Distribución de estados de carácter en las especies de *Pelodytes* y su polaridad según diferentes modelos filogenéticos. El grupo hermano de Pelodytidae es: “H_M”: Megophryidae, “H_P”: Pelobatidae (sensu stricto), “H_S”: Scaphiopodidae, y “H_T”: un linaje monofilético compuesto de Megophryidae, Pelobatidae y Scaphiopodidae. “H_C”: *P. caucasicus* como grupo externo para las especies ibéricas de *Pelodytes*. ">" y "<" indican una polaridad lineal. "v": la transformación requiere una dicotomía de dos linajes a partir del ancestro común.

CNr	Element	Feature	Pca	Pib	Ppu	“H _M ”	“H _P ”	“H _S ”	“H _T ”	“H _C ”
01	Premaxilla	<i>pars facialis</i>	[a]	[a]	[b]	a>b	a>b	a>b	a>b	a>b
02	Maxilla	teeth per mm	[a]	[c]	[b]	a>b>c	a>b>c	a>b>c	a>b>c	b>c
03	Maxilla	<i>pars palatina</i>	[a]	[b]	[b]	a>b	a>b	a>b	a>b	=
04	Maxilla	<i>pr. posterior</i>	[a]	[b]	[b]	a>b	a>b	a>b	a>b	=
05	Nasal	<i>pr. paraorbitalis</i>	[a]	[b]	[b]	---	---	---	---	=
06	Sphenethmoid	width index	[c]	[a]	[b]	a>b>c	a>b>c	a>b>c	a>b>c	a>b
07	Sphenethmoid	height index	[a]	[b]	[c]	a>b>c	a>b>c	a>b>c	a>b>c	b>c
08	Frontoparietal	<i>margo prootica</i>	[a]	[b]	[b]	a>b	a>b	a>b	a>b	=
09	Frontoparietal	<i>margo sagittalis</i>	[a]	[c]	[b]	a>b>c	a>b>c	a>b>c	a>b>c	b>c
10	Frontoparietal	<i>margo orbitalis</i>	[a]	[c]	[b]	c>b>a	c>b>a	c>b>a	c>b>a	b>c
11	Prootic	dorsolateral	[a]	[c]	[b]	a>b>c	a>b>c	a>b>c	a>b>c	b>c
12	Squamosal	<i>ramus paroticus</i>	[a]	[b]	[b]	a>b	a>b	a>b	a>b	=
13	Pterygoid	<i>ramus anterior</i>	[a]	[b]	[b]	a>b	a>b	a>b	a>b	=
14	Vomer	parachaoanal pr.	[b]	[b]	[a]	---	a>b	b>a	---	b>a
15	Parasphenoid	<i>pars medialis</i>	[a]	[b]	[b]	a>b	a>b	a>b	a>b	=
16	Parasphenoid	width index	[a]	[b]	[c]	a>b>c	a>b>c	a>b>c	a>b>c	b>c
17	Parasphenoid	lateral pr.	[a]	[b]	[b]	a>b	a>b	a>b	a>b	=
18	Atlas	intercotyle relation	[a]	[b]	[b]	a>b	avb	avb	---	=
19	V1-V5	imbrication	[a]	[c]	[b]	a>b>c	a>b>c	a>b>c	a>b>c	b>c
20	V4	transverse pr.	[a]	[b]	[b]	a>b	avb	avb	---	=
21	V5-V8	transverse pr.	[a]	[b]	[b]	a>b	b>a	a>b	---	=
22	Sacrum	<i>margo medialis</i>	[a]	[b]	[b]	a>b	a>b	a>b	a>b	=
23	Scapula	width	[a]	[b]	[b]	a>b	a>b	a>b	a>b	=
24	Scapula	<i>crista anterior</i>	[a]	[c]	[b]	a>b>c	a>b>c	a>b>c	a>b>c	b>c
25	Coracoid	anterior curvature	[a]	[b]	[b]	a>b	a>b	a>b	a>b	=
26	Clavicle	curvature	[a]	[c]	[b]	ac	a>b>c	a>b>c	---	b>c
27	Humerus	distal width index	[a]	[b]	[c]	ac	a>b>c	a>b>c	---	b>c
28	Radioulna	central width index	[a]	[c]	[b]	a>b>c	a>b>c	a>b>c	a>b>c	b>c
29	Ilium	acetabulum	[a]	[a]	[b]	b>a	a>b	a>b	---	a>b
30	Ischium	<i>pars ascendens</i>	[a]	[b]	[b]	b>a	b>a	b>a	b>a	=
31	Femur	proximal width index	[a]	[a]	[b]	a>b	a>b	a>b	a>b	a>b
32	Tibiofibula	proximal width index	[b]	[a]	[b]	a>b	a>b	a>b	a>b	a>b
33	Tibiofibula	distal width index	[a]	[a]	[b]	a>b	a>b	a>b	a>b	a>b
34	Tibiale-fibulare	proximal width index	[a]	[b]	[c]	---	---	---	---	b>c
35	Tibiale-fibulare	distal width index	[b]	[a]	[c]	---	---	---	---	avc

basic time frame for *Pelodytes* differentiation. The electrophoretic allozyme studies on Iberian *Pelodytes* (Sánchez-Herráiz *et al.*, 2000) have found a maximum unbiased Nei genetic distance of 0.192, but unpublished results using more populations indicate a Nei distance approximately 0.245. Application of a molecular clock with a calibration of about 14 Ma per Nei unit, which is almost a mean between the general calibration of 18.9 Ma/D_{NEI} suggested by Thorpe (1982) and the value

8.1 Ma/D_{NEI} given by Beerli *et al.* (1996) for green frogs, would date the differentiation between the Iberian species at 3.4 Ma, in the boundary between Lower and Upper Pliocene. The sequence of the cytochrome *b* mDNA gene gives an uncorrected distance of 16.07-18.53 % between the Caucasian and Iberian lineages (García-París *et al.*, in press), and assuming a clock calibration of 0.8 % per Ma (Tan & Wake, 1995), it would indicate that they differentiated about 12.9-14.4 Ma ago, in the Middle

Table 15.— Polarity assignment to character-states under different phylogenetic hypotheses. MAX: theoretical maximum sum of evolutionary steps. SUM: real sum of evolutionary steps. % D: percentage of derivation over the theoretical maximum. 0: primitive character-state. 1: derivation by one step. 2: lineal derivation by two steps. 2*: bifurcating transformation, counts as one step. X: polarity unknown. =: no differences between Iberian species. CNr: character number. Other abbreviations as in Table 12.

Tabla 15.— Asignación de polaridad a estados de carácter según diferentes hipótesis filogenéticas. MAX: suma teórica máxima de pasos evolutivos. SUM: suma real de pasos evolutivos. % D: porcentaje de derivación sobre el máximo teórico. 0: estado primitivo. 1: derivado, un paso evolutivo. 2: transformación lineal, dos pasos evolutivos. 2*: derivación por bifurcación, un paso evolutivo. X: polaridad desconocida. =: sin diferencia entre las especies ibéricas. CNr: número del carácter. Otras abreviaturas como en Tabla 12.

CNr	“H _M ”			“H _P ”			“H _S ”			“H _T ”			“H _{P+S} ”			“H _{M+P} ”			“H _{M+S} ”			“H _C ”		
	Pca	Pib	Ppu	Pca	Pib	Ppu	Pca	Pib	Ppu	Pca	Pib	Ppu	Pib	Ppu										
01	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	1	
02	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	1	0	
03	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	=	=	
04	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	=	=	
05	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	=	=	
06	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	0	1	
07	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	
08	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	=	=	
09	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	1	0	
10	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	1	0	
11	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	1	0	
12	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	=	=	
13	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	=	=	
14	x	x	x	1	1	0	0	0	1	x	x	x	x	x	x	x	x	x	x	x	x	0	1	
15	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	=	=	
16	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	2	0	1	
17	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	=	=	
18	0	1	1	1	2*	2*	1	2*	2*	x	x	x	1	2*	2*	x	x	x	x	x	x	x	=	=
19	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	1	0	
20	0	1	1	1	2*	2*	1	2*	2*	x	x	x	1	2*	2*	x	x	x	x	x	x	x	=	=
21	0	1	1	1	0	0	0	1	1	x	x	x	x	x	x	x	x	x	0	1	1	=	=	
22	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	=	=	
23	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	=	=	
24	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	1	0	
25	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	=	=	
26	1	2*	0	0	2	1	0	2	1	x	x	x	0	2	1	x	x	x	x	x	x	1	0	
27	1	0	2*	0	1	2	0	1	2	x	x	x	0	1	2	x	x	x	x	x	x	0	1	
28	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	0	2	1	1	0	
29	1	1	0	0	0	1	0	0	1	x	x	x	0	0	1	x	x	x	x	x	x	0	1	
30	1	0	0	1	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	=	=		
31	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	1	
32	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	1	1	0	1	1	0	1	0	
33	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	1	
34	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	0	1	
35	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	2*	1	
MAX	41	41	41	44	44	44	44	44	44	35	35	35	42	42	42	35	35	35	36	36	36	20	20	
SUM	9	29	30	10	30	32	8	30	34	6	24	26	8	29	32	6	24	26	6	25	27	9	12	
% D	22.0	70.7	73.2	22.7	68.2	72.7	18.2	68.2	77.3	17.1	68.6	74.3	19.0	69.0	76.2	17.1	68.6	74.3	16.7	69.4	75.0	45.0	60.0	

Miocene. With the same gene and calibration, a 4.1-4.8 % uncorrected distance between the Iberian *Pelodytes* species indicates that their most recent common ancestor would have lived 3.3-3.8 Ma ago (García-París *et al.*, in press).

Assuming a speciation date of 3.6 Ma for *Pelodytes ibericus* and *P. punctatus*, the evolutio-

nary rates of morphological differentiation can be estimated using the procedure suggested by Haldane (1949), and measured in darwin units, being one darwin an unit of evolutionary rate equivalent to a factor of *e* per million years (Haldane, 1949). The values obtained (Table 17) show in general rates about 11-90 millidarwins (mDw) for

Table 16.— Similarity among *Pelodytes* species. Cladistic Manhattan distances are used for all characters under different outgroup hypotheses, and phenetic Euclidean distances for the metric ones. Abbreviations as in Table 14.

Tabla 16.— Similitud entre especies de *Pelodytes*. Se utilizan distancias cladísticas Manhattan con todos los caracteres bajo distintos grupos externos, y distancias fenéticas Euclídeas para aquellos cualitativos. Abreviaturas como en Tabla 14.

Model	Pca	Pib	Model	Pca	Pib
Phenetic					
Pca	----		"H _T "		
Pib	6.32	----	Pca	----	
Ppu	6.20	5.45	Pib	1.20	----
"H _M "			Ppu	1.04	0.56
Pca	----		"H _{p+s} "		
Pib	1.16	----	Pca	----	
Ppu	1.06	0.53	Pib	1.21	----
"H _p "			Ppu	1.14	0.55
Pca	----		"H _{M+p} "		
Pib	1.19	----	Pca	----	
Ppu	1.13	0.56	Pib	1.20	----
"H _s "			Ppu	1.04	0.56
Pca	----		"H _{M+s} "		
Pib	1.19	----	Pca	----	
Ppu	1.13	0.56	Pib	1.22	----
			Ppu	1.04	0.56

the indexes involved. Interestingly, the skull seems to have been differentiating at higher rates (17-90 mDw) than the limb elements (all < 21 mDw). The two shoulder bones considered, clavicle and ilium, also show faster rates than the appendages. This result is here considered as an indication that the skull and appendicular skeletons are probably different co-evolutionary complexes.

HETEROCHRONY

Several of the characters that we have used, and particularly those coded as indexes, can be described in terms of their allometric relations. Even if we are concerned only with adult specimens, there are instances in which the adult development does incorporate ontogenetic trajectories, different for each species. In this respect, the description of the osteological divergence between the Iberian *Pelodytes* species would be more complete if it includes, within the heterochronic paradigm, the differential trends observable in the growth process. Using the nomenclature established by Reilly *et al.* (1997) and the allometric analytical procedure formulated by McKinney (1988), Table 18 presents the characters in which

Table 17.— Quantitative differential features among *Pelodytes* species, as defined by mean indexes (%), rates of evolutionary change per Ma, and their equivalent in millidarwins (mDw). CNr: Character number. Rates are calculated by dating at 3.6 Ma the Iberian speciation event. Pca is considered primitive and the closest Iberian species is taken as the starting point. In the cases in which Pca shows intermediate values, rates are calculated in the two bifurcating directions.

Tabla 17.— Diferencias en rasgos cuantitativos entre especies de *Pelodytes*, definidas mediante índices medios (%), tasas de cambio evolutivo por Ma, y su equivalente en milidárwin (mDw). CNr: Número de carácter. Las tasas se calculan datando en 3.6 Ma el evento de especiación ibérica. Se considera Pca como primitivo y la especie ibérica más próxima se toma como punto de inicio. En los casos en que Pca presenta valores intermedios, las tasas se calculan para las dos direcciones de bifurcación.

CNr	Element	Feature	Pca	Pib	Ppu	Rate (x10 ⁻⁶)	mDw
01	Premaxilla	<i>pars facialis</i>	20.98	22.26	16.81	0.078	78.0
02	Maxilla	teeth per mm	6.16	8.30	7.39	0.032	32.3
06	Sphenetmoid	width index	91.39	121.69	107.41	0.034	34.7
07	Sphenetmoid	height index	28.45	33.70	38.38	0.036	36.1
09	Frontoparietal	<i>margo sagittalis</i>	32.98	23.40	32.40	0.090	90.4
10	Frontoparietal	<i>margo orbitalis</i>	83.72	75.87	80.50	0.016	16.5
16	Parasphenoid	width index	103.54	80.43	74.59	0.021	20.9
26	Clavicle	width index	59.15	37.87	44.75	0.046	46.4
27	Humerus	distal width index	23.26	21.01	19.71	0.018	17.7
28	Radioulna	central width index	13.55	12.04	12.66	0.014	14.0
29	Ilium	acetabulum	13.13	13.50	11.88	0.008	7.7
						0.028	27.8
						total CNr29:	35.5
31	Femur	proximal width index	8.95	8.71	8.12	0.020	19.5
32	Tibiofibula	proximal width index	11.81	12.52	11.79	0.017	16.7
33	Tibiofibula	distal width index	13.32	13.58	12.87	0.015	14.9
34	Tibiale-fibulare	proximal width index	23.82	22.90	21.99	0.011	11.3
35	Tibiale-fibulare	distal width index	29.34	30.73	28.54	0.013	7.7
						0.008	12.9
						total CNr35:	20.5

Table 18.— Adscription to heterochronic basic perturbations of the differential adult growth trajectories between Pib and Ppu. CNr: Character number. Cases in which there is no significant developmental adult trend in one of the species are not included.

Tabla 18.— Adscripción de las trayectorias de crecimiento adulto, diferenciales entre Pib y Ppu, a perturbaciones heterocrónicas básicas. CNr: número de carácter. No se indican los casos en los que no hay tendencia significativa de desarrollo adulto en una de las especies.

CNr	Element	Slope Test	Intercept Test Adjusted means	Polarity	Heterochronic Process
06	Sphenethmoid	$F_{1,22}=0.419$ $P=0.524$	$F_{1,23}=8.094$, $P=0.009$ Pib= 3.414; Ppu= 3.169	Pib>Ppu	Postdisplacement Ppu
09	Frontoparietal	$F_{1,21}=1.258$ $P=0.275$	$F_{1,22}=10.265$, $P=0.004$ Pib= 0.906; Ppu= 1.198	Ppu>Pib	Postdisplacement Pib
10	Frontoparietal	$F_{1,21}=1.013$ $P=0.326$	$F_{1,22}=10.947$, $P=0.003$ Pib= 3.610; Ppu= 3.796	Ppu>Pib	Postdisplacement Pib
16	Parasphenoid	$F_{1,22}=2.156$ $P=0.156$	$F_{1,23}=1.895$, $P=0.188$ means not different	Pib>Ppu	Hypomorphosis Ppu
26	Clavicle	$F_{1,17}=1.038$ $P=0.322$	$F_{1,18}=11.642$, $P=0.003$ Pib= 1.504; Ppu= 1.782	Ppu>Pib	Postdisplacement Pib
27	Humerus	$F_{1,64}=1.220$ $P=0.273$	$F_{1,65}=19.972$, $P<0.001$ Pib= 1.746; Ppu= 1.650	Pib>Ppu	Postdisplacement Ppu
28	Radioulna	$F_{1,67}=0.711$ $P=0.402$	$F_{1,68}=1.066$, $P=0.305$ means not different	Ppu>Pib	Hypomorphosis Pib
29	Ilium	$F_{1,61}=2.258$ $P=0.138$	$F_{1,62}=17.422$, $P<0.001$ Pib= 1.704; Ppu= 1.476	Pib>Ppu?	Postdisplacement Ppu
31	Femur	$F_{1,65}=2.335$ $P=0.131$	$F_{1,66}=5.218$, $P=0.025$ Pib= 1.116; Ppu= 1.068	Pib>Ppu	Postdisplacement Ppu
32	Tibiofibula	$F_{1,64}=0.272$ $P=0.603$	$F_{1,65}=5.491$, $P=0.022$ Pib= 1.759; Ppu= 1.666	Pib>Ppu	Postdisplacement Ppu
33	Tibiofibula	$F_{1,64}=0.661$ $P=0.419$	$F_{1,65}=8.147$, $P=0.006$ Pib= 1.918; Ppu= 1.811	Pib>Ppu	Postdisplacement Ppu
34	Tibiale-fibulare	$F_{1,64}=2.271$ $P=0.137$	$F_{1,65}=6.682$, $P=0.012$ Pib= 1.903; Ppu= 1.808	Pib>Ppu	Postdisplacement Ppu
35	Tibiale-fibulare	$F_{1,64}=0.481$ $P=0.490$	$F_{1,65}=28.231$, $P<0.001$ Pib= 2.551; Ppu= 2.341	Pib v Ppu	Displacement Pib-Ppu?

both Iberian species differ, and the heterochronic processes presumably involved are identified, assigning polarities that consider *P. caucasicus* as primitive. A general heterochronic process, involving all the features, derives from the fact that *P. ibericus* has a smaller general adult size than the other species, and has been minimized by the use of natural logarithms. This size reduction probably can be explained in part because *P. ibericus* attains sexual maturity within one year, while *P. punctatus* is variable in this respect, with at least some individuals (not all) reaching maturity in the second year (Esteban *et al.*, 2002).

The list of characters given in Table 18 does not include several skull elements that do not show significant regressions, and no heterochronic perturbation can be observed, as their shape is

not directionally changed during the adult growth trajectory. Other skull, column and girdle elements, although not quantified allometrically, show a clear hypomorphosis in *P. ibericus*. Differences in the appendicular skeleton are mainly based in pre- / postdisplacement perturbations (Figure 43), usually a postdisplacement of the larger *P. punctatus*. Since the age of the specimens studied is not known, and being perhaps generation times slightly different between both species, we lack a reliable time component to back the heterochronic analysis. Its substitution by allometric heterochronies should be considered only as a convenient descriptive way to document the evolutionary divergence between both species, and to provide comparative data for future studies in other anuran speciation cases.

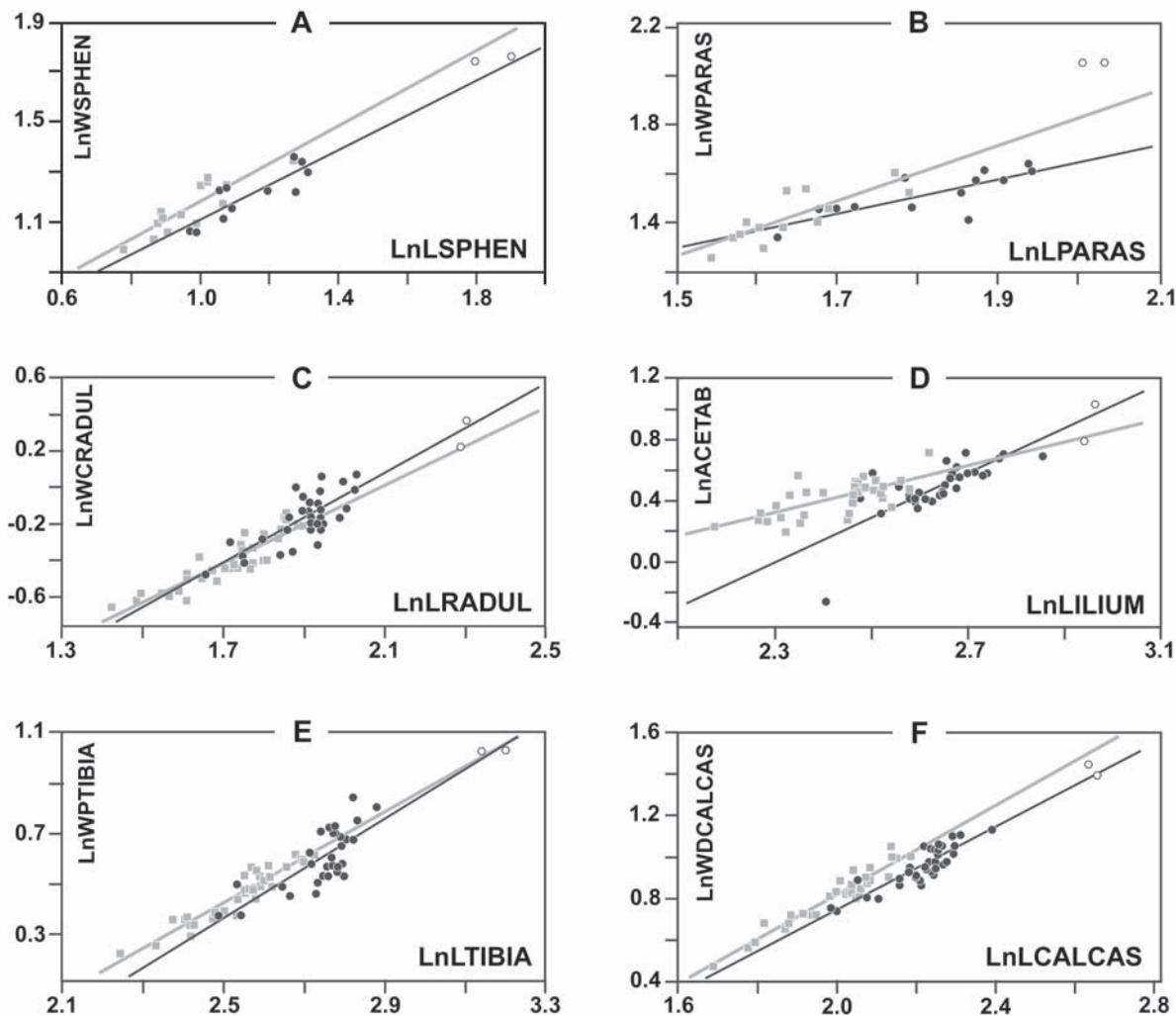


Fig. 43.— Scatterplots in natural logarithms corresponding to indexes selected as examples of different adult growth trajectories. (A) Iwsphen. (B) Iwparas. (C) Icradul. (D) Iailium. (E) Iptibia. (F) Idcalcas. See variable definitions in Tables 3, 5, 8, 9, 12 and 13 respectively. Pca: white circles. Pib: grey squares. Ppu: black circles.

Fig. 43.— Diagramas de puntos en logaritmos naturales correspondientes a índices seleccionados como ejemplos de trayectorias de crecimiento adulto. (A) Iwsphen. (B) Iwparas. (C) Icradul. (D) Iailium. (E) Iptibia. (F) Idcalcas. Ver definiciones de variables en las Tablas 3, 5, 8, 9, 12 y 13 respectivamente. Pca: círculos blancos. Pib: cuadrados grises. Ppu: círculos negros.

FACTORS OF TRANSFORMATION

The supposition that all the characters we have analysed, taken from many different bone elements, represent independently evolving traits is probably unrealistic. Following the review by Emerson & Hastings (1998), there are four basic and nonexcluding factors which may have caused these traits to cluster in groups evolving together: genetic, ontogenetic, functional, and adaptative. The genetic factor,

mostly relying on pleiotropy, would require QTL-mapping approaches (Cheverud, 2001) and will not be considered as we are not aware of any approach by which it could be detected with the kind of data we have used. Concerning the other factors, probable groups of jointly evolving features can be suggested within the osteological context of this study. These groups are the following:

- a) Ontogenetic factor. There might be a common biological basis to diminish skull and columnar

ossification, particularly between the Iberian and Caucasian species, but also in *P. ibericus* with respect to *P. punctatus*, for most of the traits analysed. This type of change is usually related to a truncation in the most derived species relative to the plesiomorphic one (Reilly *et al.*, 1997), and in our case it can be in general associated with hypomorphosis, directly linked with the smaller size and precocious sexual maturity of *P. ibericus*.

- b) Morphological integration. The presence of a morphological functional complex, probably incorporating an epigenetic component, can be detected in our case in the general skull shape, which is proportionally shorter and broader in *P. ibericus*. The different elements of such a morphological complex are likely to evolve together, and we can detect this situation in our data because several of those elements show comparable evolutionary differentiation rates (Table 17), and do not show significant heterochronic change in the adult growth period.
- c) Ecomorphological integration. In our analysis, a genetic-adaptive morphological factor can be suspected for the appendicular system. There seems to be a more slender derived morphology in the appendages of *P. punctatus* than in *P. ibericus*, the latter being more robust for most index shapes, in spite of being smaller. From a heterochronic perspective, there is a derived postdisplacement in *P. punctatus*. Nevertheless, the biological basis and potential adaptive role of this morphological differentiation cannot be studied using only adult specimens, but a common genetic basis is to be expected for this interrelationship.

We are not aware of similar osteological or paleontological studies using other anuran cases, and consequently we cannot provide a comparative view on the osteological differentiation of this highly specialized Iberian group.

ACKNOWLEDGEMENTS

We have received helpful assistance from several staff members of the Museo Nacional de Ciencias Naturales, in particular from J. E. González, curator of the Herpetological collection, and from the expert photographers J. Muñoz and R. Sánchez. Daniel Ruiz measured some of the specimens, and M. París prepared the samples for photography. M. García-París and A. Machordom gave us advice and information on several unpublished molecular studies on *Pelodytes*. G. Böhme facilitated the study of the Dresden specimen of *P. caucasicus*. A. Henrici, R. Márquez and an anonymous reviewer improved earlier drafts of this article.

References

- BEERLI, P.; HOTZ, H. & UZZELL, T., 1996. Geologically dated sea barriers calibrate a protein clock for Aegean water frogs. *Evolution*, 50: 1676-1687.
- BLANCO, M. J. & SANCHIZ, B., 2000. Evolutionary mechanisms of rib loss in anurans: a comparative developmental approach. *Journal of Morphology*, 244: 57-67.
- BOLKAY, S. J., 1919. Osnove uporedne osteologije anurskih batrahija sa dodatkom o porijeklu Anura i sa skicom naravnoga sistema istih. *Glasnik Zemaljskog Muzeja Bosni Herzegovini*, 31(4): 277-353. (Plates published by R. Zaplata in 1933 in the same journal).
- CHEVERUD, J. M., 2001. The genetic architecture of pleiotropic relations and differential epistasis. In: G.P. Wagner (ed.), *The character concept in evolutionary Biology*. Academic Press. San Diego: 411-434.
- DINGERKUS, G. & UHLER, L. D., 1977. Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. *Stain Technology*, 52: 229-232.
- EMERSON, S. B. & HASTINGS, P. A., 1998. Morphological correlations in evolution: Consequences for phylogenetic analysis. *Quarterly Review of Biology*, 73(2): 141-162.
- ESTEBAN, M.; SÁNCHEZ-HERRÁIZ, M. J.; BARBADILLO, L. J.; CASTANET, J. & MÁRQUEZ, R., 2002. Effects of age, size and temperature on the advertisement calls of two Spanish populations of *Pelodytes punctatus*. *Amphibia-Reptilia*, 23(3): 249-258.
- GARCÍA-PARÍS, M.; BUCHHOLZ, D. & PARRA-OLEA, G., in press. Phylogenetic relationships of Pelobatoidea re-examined using mtDNA. *Molecular Phylogenetics and Evolution*.
- HALDANE, J. B. S., 1949. Suggestions as to quantitative measurement of rates of evolution. *Evolution*, 3: 51-56.
- HENDRY, A. P. & KINNISON, M. T., 1999. Perspective: the pace of modern life: measuring rates of contemporary microevolution. *Evolution*, 53: 1637-1653.
- KINNISON, M. T. & HENDRY, A. P., 2001. The pace of modern life. II: from rates to pattern and process. *Genetica*, 112-113: 145-164.
- HENRICI, A. C., 1994. *Tephrodytes brassicarvalis*, new genus and species (Anura: Pelodytidae), from the Arikareean Cabbage Patch Beds of Montana, USA, and Pelodytid-Pelobatid relationships. *Annals of Carnegie Museum*, 63(2): 155-183.
- KITCHING, I. J.; FOREY, P. L.; HUMPHRIES, C. J. & WILLIAMS, D. M., 1998. *Cladistics. The theory and practice of Parsimony Analysis*. Oxford University Press. Oxford. 228 pp.
- LYNCH, J. D., 1971. Evolutionary relationships, osteology and zoogeography of leptodactyloid frogs. *University of Kansas Miscellaneous Publications*, 53: 1-238.

- MAGLIA, A. M., 1998. Phylogenetic relationships of extant pelobatoid frogs (Anura: Pelobatoidea): Evidence from adult morphology. *Scientific Papers of the Natural History Museum, University of Kansas*, 10: 1-19.
- McKINNEY, M. L., 1988. Classifying heterochrony: allometry, size and time. In: M. L. McKinney (ed.) *Heterochrony in evolution: a multidisciplinary approach*. Plenum Press. New York: 17-34.
- REILLY, S. M.; WILEY, E. O. & MEINHARDT, D. J., 1997. An integrative approach to heterochrony: the distinction between interspecific and intraspecific phenomena. *Biological Journal of the Linnean Society*, 60: 119-143.
- ROCEK, Z., 1980. Cranial anatomy of frogs of the family Pelobatidae Stannius, 1856, with outlines of their phylogeny and systematics. *Acta Universitatis Carolinae Biologica*, (1-2): 1-164.
- ROCEK, Z. & RAGE, J. C., 2000. Tertiary Anura of Europe, Africa, Asia, North America, and Australia. In: H. Heatwole & R. L. Carroll (eds.). *Amphibian Biology. Volume 4. Palaeontology. The evolutionary history of amphibians*. Surrey Beatty. Chipping Norton: 1332-1387.
- SÁNCHEZ-HERRÁIZ, M. J.; BARBADILLO, L. J.; MACHORDOM, A. & SANCHIZ, B., 2000. A new species of Pelodytid frog from the Iberian Peninsula. *Herpetologica*, 56(1): 105-118.
- SANCHIZ, B., 1978. Nuevos restos fósiles de la familia Pelodytidae (Amphibia, Anura). *Estudios Geológicos*, 34: 9-27.
- SANCHIZ, B., 1984. Análisis filogenético de la tribu Alytini (Anura, Discoglossidae) mediante el estudio de su morfoestructura ósea. In: H. Hemmer & J. A. Alcover (eds.), *Història biològica del Ferreret. Life history of the Mallorcan Midwife toad*. Moll. Palma de Mallorca: 61-108.
- SANCHIZ, B., 1998. *Salientia. Handbuch der Paläoherpelologie Pars 4*. Dr. F. Pfeil. Munich. 275 pp.
- SNEATH, P. H. A. & SOKAL, R. R., 1973. *Numerical Taxonomy*. W.H. Freeman. San Francisco. 573 pp.
- STATSOFT INC., 2001. *STATISTICA for Windows. Computer Program Manual*. Statsoft Inc. Tulsa, Oklahoma.
- TAN, A. M. & WAKE, D. B., 1995. MtDNA phyogeography of the California newt, *Taricha torosa* (Caudata, Salamandridae). *Molecular Phylogenetics and Evolution*, 4: 383-394.
- THORPE, J. P., 1982. The molecular clock hypothesis: Biochemical evolution, genetic differentiation and systematics. *Annual Review of Ecology and Systematics*, 13: 139-168.
- TRUEB, L., 1973. Bones, frogs, and evolution. In: J.L. Vial (ed.). *Evolutionary Biology of anurans*. University of Missouri Press. Columbia: 65-132.
- TRUEB, L., 1993. Patterns of cranial diversity among the Lissamphibia. In: J. Hanken & B.K. Hall (eds.). *The skull. Patterns of structural and systematic diversity*. University of Chicago Press. Chicago: 255-343.
- WAKE, D. B. & ROTH, G., 1989. *Complex organismal functions: Integration and evolution in vertebrates*. John Wiley. New York. 451 pp.
- WASSERSUG, R. J., 1976. A procedure for differential staining of cartilage and bone in whole formalin-fixed vertebrates. *Stain Technology*, 51: 131-134.
- WIENS, J. J. (ed.), 2000. *Phylogenetic analysis of morphological data*. Smithsonian Institution Press, Washington & London. 220 pp.

Recibido, el 17-X-2002

Aceptado, el 17-XII-2002

Publicado, el 31-XII-2002