

Morphometric study of the Iberian *Aphanius* (Actinopterygii, Cyprinodontiformes), with description of a new species

Ignacio DOADRIO¹, José A. CARMONA¹ and Carlos FERNÁNDEZ-DELGADO²

¹ National Museum of Natural Sciences, Department of Biodiversity, c/José Gutiérrez Abascal 2, 28006 Madrid, Spain; e-mail: mcnd147@mncn.csic.es

² Faculty of Sciences, Department of Animal Biology, University of Córdoba, Av. San Alberto Magno s/n, 14004 Córdoba, Spain

Received 30 June 2000; Accepted 13 November 2001

A b s t r a c t . Morphometric variation in *Aphanius iberus* was analysed to demonstrate the remarkable genetic divergence between Mediterranean and Atlantic population of Iberia. Four discrete morphotypes in males and three in females were distinguished. Morphometric data discriminated the Atlantic and Mediterranean populations, but revealed the Villena population as the morphologically most differentiated. Atlantic populations were described as a new species, *Aphanius baeticus* sp. nov., which differs from *A. iberus* in the overall shape, coloration pattern and number of branched rays on the dorsal and anal fins. The Villena population was retained in *A. iberus* because, despite of its morphological differentiation, show high genetic introgression at the nuclear genome with neighbouring populations. The range of *A. baeticus* sp. nov. is restricted to the eight localities of the Atlantic slope of the Iberian Peninsula. This new species should be considered Critically Endangered (CR) according to the IUCN Red List Categories.

Key words: killifishes, taxonomy, *Lebias*

Introduction

The *Aphanius* Nardo, 1827 cyprinodontid killifishes are likely descendants of the Tethys fauna inhabiting coastal lagoons, marshes and salty rivers around the circum-Mediterranean area as well as the Arabian Peninsula as far as Iran and Pakistan (Villwock 1999). As a consequence of the paleogeographic history of the region, which supported dramatical changes during the Cenozoic period, species of *Aphanius* show an irregular distribution pattern with isolated populations. Hence, the genus *Aphanius* in the eastern Mediterranean basin and adjacent regions (the Middle and the Near East) is highly diverse, with at least ten different species (Huber 1996). In contrast, in the western Mediterranean area, only three species are known *Aphanius apodus* (Gervais, 1853), *A. fasciatus* (Valenciennes in Humboldt & Valenciennes, 1821) and *A. iberus* (Valenciennes in Cuvier & Valenciennes, 1846) (Villwock 1999, Doadrío 1994).

A. iberus has been reported in a wide area along the Mediterranean coast of southern France (Arnoult 1957), and Spain (Paracuellos & Nevado 1994, Fernández-Pedrosa et al. 1995), some localities of the Atlantic Iberian coast (Hernando 1975) and in North Africa in Algeria and Morocco (Pellegrin 1921, Villwock & Scholl 1982). *A. iberus* now appears to be extinct in France (Allardi & Keith 1991), and the African populations are both morphologically and genetically different from those of Iberia (Villwock & Scholl 1982) and might deserve distinct taxonomic recognition (Doadrío et al. 1996).

In the course of recent genetic studies of different populations of *A. iberus* using mitochondrial DNA and allozyme markers, the Atlantic populations were found to be genetically differentiated from the Mediterranean ones (D o a d r i o et al. 1996, P e r d i c e s et al. 2001). Similarly, restriction fragment length polymorphism analysis of mitochondrial DNA markers showed that the population of the endorrheic Villena Lagoon is highly differentiated within the Mediterranean populations (F e r n á n d e z - P e d r o s a et al. 1995). Since diagnostic loci and high genetic distances discriminate the Atlantic and Mediterranean populations, morphometric variation analysis is needed to characterise these two discrete gene pools.

In this paper, we conduct a morphometric study of several Atlantic and Mediterranean populations to characterise the genetically differentiated populations of *A. iberus*. Both genetic and morphological data are used in order to revise the taxonomic status of the populations of *A. iberus*.

Materials and Methods

The specimens originated from various locations in Spain and one location in Greece (Table 1). The samples housed in the Museo Nacional de Ciencias Naturales of Madrid (listed in the Appendix) were also revised as a comparative material. Twenty-two morphometric variables were measured. All measurements are in millimetres and were log-transformed for morphometric analysis. Only adult specimens of the 0+ and 1+ age groups were used for comparative morphometric analysis. The following abbreviations were used for morphometric and meristic characters (Tables 2, 3 and 6): SL, standard length; HL, head length; HD, head depth; PrOL, preorbital length; ED, eye diameter; PrDD, predorsal distance; PrPD, prepectoral distance; PrVD, preventral distance; PrAD, preanal distance; CPL, caudal peduncle length; APL, anal peduncle length; PVL, pectoral-ventral length; VAL, ventral-anal length; DFL, dorsal fin length; DFH, dorsal fin height; PFL, pectoral fin length; VFL, ventral fin length; AFL, anal fin length; AFH, anal fin height; CFL, caudal fin length; BD, body depth; BLD, body least depth; D, dorsal fin rays; A, anal fin rays; P, branched pectoral fin rays; V, branched ventral fin rays; C, branched caudal fin rays; LLS, lateral line scale rows; Abd. vert., abdominal vertebrae; Cau. vert., caudal vertebrae; GR, gill rakers. Only branched caudal, pectoral and ventral fins rays were counted. The osteological characters were studied

Table 1. Localities and sample size of *Aphanius* populations used for morphological, osteological and meristic analysis.

Locality	<i>Aphanius iberus</i>		morphometry		osteology & meristics	
	males	females	males	females	males	females
Salinas de San Pedro del Pinatar, Murcia, Spain	15	21	10	0		
Albufera de Valencia, Valencia, Spain	16	15	-	-		
River Adra, Adra, Almería, Spain	16	15	10	10		
Villena Lagoon, Villena, Alicante, Spain	14	19	10	10		
River Salado, Lebrija, Sevilla, Spain	19	13	10	10		
Iro River, Chiclana de la Frontera, Cádiz, Spain	11	8	-	-		
River San Pedro, Paterna de la Ribera, Cádiz, Spain	14	15	-	-		
	<i>Aphanius fasciatus</i>		morphometry		osteology & meristics	
	males	females	males	females	males	females
Messolongi Marshes, Greece	18	15	10	10		

Appendix

Aphanius iberus. France. MNHN 000-0185 Lectotype, MNHN 2000-5154 Syntypes. Spain. MNCN 14401-24, 14918-78, 15403-20, 15528-46, 16186-244, 16280-335, 136768-69 Albufera de Valencia, Valencia. MNCN 15289-91 15547-48, 16163-75, 28787, Albufera de Valencia, Silla, Valencia. MNCN 14355-97, 14979-98, 15343, Prat de Cabanes, Cabanes, Castellón. MNCN 15585-641, 15900-935 Albufera de Torreblanca, Torreblanca, Castellón. MNCN 14725-40, 14909-13, 15005-85, 15291-325, 15352-402, 15642-900, 15942-16144, 74888-96, 139550, 136742-54 Salinas de San Pedro del Pinatar, Murcia. MNCN 74823-5 Salinas de Marchamalo, Murcia. MNCN 14999-15002, 15575 Segura River, Guardamar de Segura, Alicante. MNCN 15549-71, 114033-564 Salinas de Santa Pola, Alicante. MNCN 16156-62 Albaterra, Alicante. MNCN 15572-74, 16247-76 La Cava, Tortosa, Tarragona. MNCN 56285-87 Pantano del Hondo, Elche, Alicante. MNCN 74846-62 Delta del Ebro, La Tancada, Tarragona. MNCN 74863-87 Aiguamolls del Empordá, Ampurias, Gerona. MNCN 120424-33, 120493-510 Albuixec, Valencia. MNCN170799-858 Balsas de riego, Adra, Almería. MNCN 170859-885 Adra River, Adra, Almería.

Aphanius baeticus sp. nov. MNCN 16244-46 Sevilla.

from cleared and stained specimens (W a s s e r s u g 1976). Caudal vertebres nomenclature follows C o e l h o et al. (1998).

To test for sexual dimorphism and population variation, we used analysis of variance (ANOVA) for unbalanced design to test for differences in means (for groups or variables) because it is more statistically powerful than the simple t test, permitting the test of each variable whilst controlling for all others as well as detection of interaction effects between variables. A multivariate analysis of variance (MANOVA) was performed to test for two dependent variables at a time (i.e. between species and sexes)

Differences in body shape among populations were analysed using Principal Component Analysis (PCA). Burnaby's method was used to correct for size effects (B u r n a b y 1966) using an 'iml' procedure written in SAS-pc by Dr. L.F. M a r c u s (corrected from version in Appendix in R h o l f & B o o k s t e i n 1987) (see B e k e l e et al. 1993). Canonical variate analyses (CVA) and Mahalanobis distances between group centroids were used to summarise differences among populations, which were clustered in a phenogram using the Unweighted Pair Group Method (UPGMA). All the computations were performed using SAS 11, (SAS I n s t i t u t e Inc. 1996) and NTSYS-pc version 1.8 (R o h l f 1993).

We maintain the use of *Aphanius* against *Lebias* until the International Commission on Zoological Nomenclature publishes the decision on the submitted application referring to Lazara's *Lebias* type species (see K o t t e l a t 1997). Institutional acronyms: MNHN Museum National d' Histoire Naturelle, Paris. MNCN Museo Nacional de Ciencias Naturales, Madrid. UCOBA Departamento de Biología Animal, Universidad de Córdoba, Córdoba.

Results

Significant differences (ANOVA) among populations for the morphometric variables were mainly due to differences in SL (Table 2), with sexual dimorphism statistically significant in practically all characters. These results suggest that females are larger than males and show overall smaller ventral fins.

There was interaction between sexes and populations for most of the variables, which should be due to size differences between sexes for each population. Significant differences (MANOVA) were found in all characters among populations and between sexes and between populations by sexes, justifying the separate treatment of males and females.

Table 2. Two-way analysis of variance testing for sexual dimorphism, population variation and their interaction (Pop*Sex). Mean squares from SAS type III 'glm' procedure for unbalanced designs. Except ventral fin length for sexual dimorphism and anal fin height for interaction between sexes and species, all variables were significant ($P < 0.01$). Variables are described in Methods. (n.s.= not significant).

Variable	Population	Sex	Pop*Sex
SL	1.0922	1.6202	0.1420
HL	0.9067	1.2304	0.1270
HH	1.0987	1.3388	0.1795
PrOL	2.4633	1.3235	0.2575
ED	0.5150	0.4538	0.0616
PrDD	1.1358	1.7330	0.1506
PrPD	0.9698	1.0853	0.1258
PrVD	1.1226	1.3139	0.1218
PrAD	1.1491	2.0540	0.1855
CPL	0.9729	1.0757	0.3377
APL	0.6940	0.2609	0.1720
PVL	1.6742	1.8573	0.2098
VAL	1.4774	6.0314	0.7012
DFL	2.5528	0.3862	0.3360
DFH	1.3895	0.1176	0.1363
PFL	1.7366	0.2190	0.1167
VFL	1.0114	0.0497 n.s.	0.0929
AFL	2.5875	0.6079	0.1325
AFH	1.3845	0.1502	0.0141 n.s.
CFL	0.8476	0.3677	0.0801
BD	1.3641	1.4431	0.2788
BLD	1.2926	0.7066	0.1514

Table 3. Eigenvectors and eigenvalues for the first four principal components for 22 variables for males and females. Variable codes are given in the Method section.

Eigenvectors	Males				Females			
	I	II	III	IV	I	II	III	IV
SL	-0.006	-0.008	0.006	0.031	0.006	0.042	-0.009	0.005
CFL	-0.011	-0.002	0.045	-0.137	0.001	-0.040	0.036	0.153
HL	-0.010	-0.033	-0.052	0.031	-0.010	-0.102	-0.087	-0.013
HH	-0.012	-0.109	-0.110	-0.047	-0.012	-0.112	0.028	-0.022
PROL	0.001	0.011	-0.160	0.358	0.039	-0.194	-0.062	-0.439
ED	0.001	-0.016	0.049	-0.049	-0.032	0.018	-0.005	0.066
PRDD	-0.011	-0.038	-0.012	0.051	0.001	-0.021	0.036	-0.035
PRPD	-0.010	-0.003	-0.089	0.043	-0.014	-0.119	-0.100	-0.082
PRVD	-0.019	-0.037	0.082	-0.017	0.001	-0.001	0.142	-0.033
PRAD	-0.009	-0.037	-0.014	0.019	0.001	0.024	0.016	-0.051
CPL	-0.150	0.271	0.131	0.216	-0.018	0.471	-0.110	0.014
APL	-0.009	-0.152	0.191	0.194	-0.051	0.202	0.158	-0.143
PVL	-0.041	-0.069	0.316	-0.047	0.034	0.148	0.385	0.010
VAL	0.016	-0.082	-0.411	0.163	-0.013	0.095	-0.398	-0.125
DFL	0.164	-0.203	0.104	-0.075	0.040	-0.263	0.069	0.085
DFH	0.044	-0.035	0.096	-0.222	-0.012	0.111	0.036	0.129
PFL	0.024	0.248	0.135	0.091	0.035	0.097	-0.186	0.058
VFL	-0.021	0.050	0.003	0.002	-0.012	-0.038	0.054	0.092
AFL	0.070	0.401	-0.221	-0.332	0.035	-0.139	-0.185	0.408
AFH	0.029	0.143	0.099	0.050	0.000	0.044	-0.011	0.142
BD	-0.005	-0.223	-0.113	-0.074	0.001	-0.051	0.078	-0.033
BLD	-0.002	-0.180	-0.058	-0.183	-0.022	-0.084	0.156	0.071
Eigenvalue	0.708 (53.35)	0.198 (68.24)	0.120 (77.30)	0.072 (82.75)	1.403 (71.09)	0.131 (77.74)	0.117 (83.69)	0.071 (87.29)

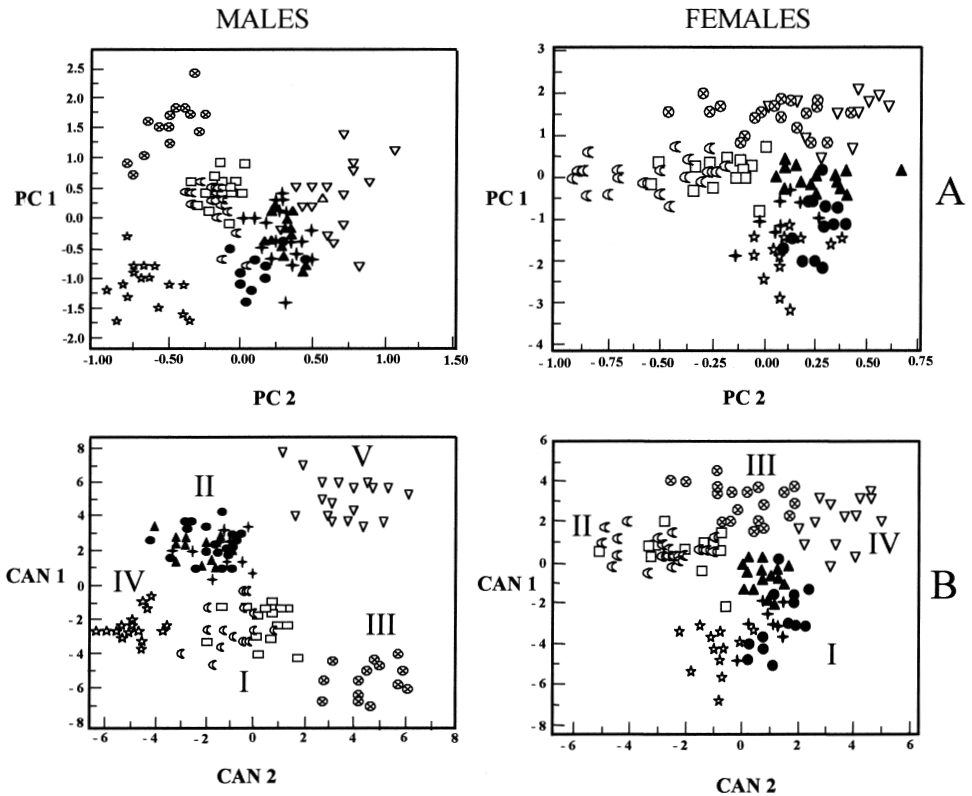


Fig. 1. A) Plots of first two principal components for 22 Burnaby corrected variables (males-left and females-right). B) Plots from canonical variates analysed (males-left and females-right). ● River Salado, Lebríja, Sevilla. ▲ River Iro, Chiclana de la Frontera, Cádiz. ◆ River San Pedro, Paterna de la Ribera, Cádiz. ☆ River Adra, Adra, Almería. □ Albufera de Valencia, Valencia. ⋈ Salinas de San Pedro del Pinatar, Mar Menor, Murcia. ⊗ Villena, Alicante, and ▽ *A. fasciatus* Messolongi Marshes, Greece.

The first four principal components accounted for 88.5% of variance in males and 92.6% in females. The areas of the scores of each component differed significantly (MANOVA) among populations for these first four components. Normalized eigenvectors on the first principal components showed close values for both sexes and with the same sign, suggesting an influence of body size. Variation in body size could be due to local adaptation to different habitats like marshes, irrigation channels, lagoons or salty springs.

A PCA with 22 Burnaby's corrected variables was carried out to remove size effect. The first two principal components accounted for 68.2% of variation in males and 77.7% in females (Table 3). For both sexes, the highest eigenvectors were dorsal fin length and caudal peduncle length with five different groups of males (Fig. 1). This five groups represent: Adra population, Villena population, the remaining Mediterranean populations, Atlantic populations and *A. fasciatus*. In females, this structure is less evident and the Adra population overlaps with Atlantic populations.

Significant differences in morphology were found between the populations (Fig. 1), but differentiation was more evident in males. Mahalanobis distances D^2 and its posterior cluster (Fig. 2) were congruent with previous genetic studies (Fernández-Pedrosa et al. 1995, Doadrió et al. 1996), although Adra population was not studied in there.

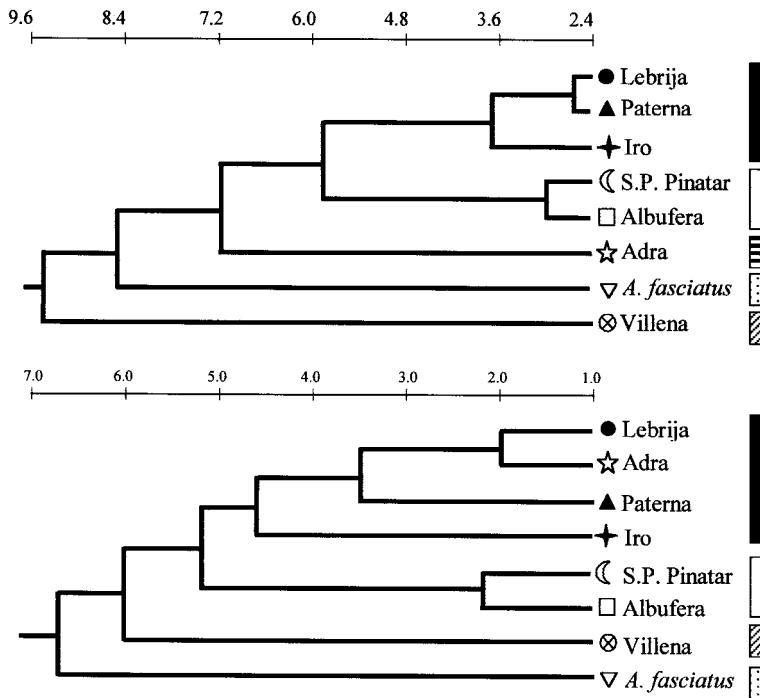


Fig. 2. UPGMA clusters on Mahalanobis distances between groups of centroids. Top – Tree for males: ■ *A. baeticus* sp. nov. □ *A. iberus*. ▤ Adra population. ▥ *A. fasciatus*. ▧ Villena population. Bottom – Tree for females: ■ *A. baeticus* sp. nov. and Adra population. □ *A. iberus*. ▧ Villena population. ▥ *A. fasciatus*.

Discussion

Taxonomic considerations

Morphological analyses revealed the existence of four morphotypes in the Iberian populations. Eventhough morphotypes have previously been used as the base of species description, many species show a high degree of phenotypic plasticity and therefore it is necessary to corroborate the presence of a well-differentiated gene pool for taxonomical purposes. Hence, we consider the presence of diagnostic loci as a strong criterion for species recognition (Beerli et al. 1995, Mendoza-Qijano et al. 1998) since, in a concordant geographic framework, we can distinguish populations that may be isolated ephemerally only from those that have been isolated long enough to differentiate unlinked characters (Baum & Donoghue 1995). Therefore, the two morphotypes corresponding to the Villena and Adra populations can not be considered as new species due to the lack of diagnostic loci. The Adra population showed a different morphotype in males and currently is isolated from other *Aphanius* populations by at least for 350 kilometers of coastal line, nevertheless, its low genetic divergence seems to indicate a recent speciation of this populations. On the contrary, the drainage channel in Villena Lagoon connected this population with the neighbouring Mediterranean populations favouring a nuclear DNA introgression in this century. The introgression process and the persistent small population size could have propitiated the high morphological divergence of this population.

The Atlantic populations showed such genetic and morphological divergence with respect to other populations that merit to be distinguished at the species level. No available name can be applied to the Atlantic populations, and therefore, we choose to designate these populations *A. baeticus* sp. nov. Hence, *A. iberus* is maintained for the Mediterranean populations.

Aphanius baeticus sp. nov. is characterized by morphological characters that refer to mensural and meristic characters (Tables 4 and 5). Moreover, using molecular markers *A. baeticus* sp. nov. showed diagnostic loci in different enzymatic systems (D o a d r i o et al. 1996), and highly differentiated haplotypes of mitochondrial DNA (P e r d i c e s et al. 2001). An interesting finding was that males showed a higher number of differentiated morphotypes than females, indicating that sexual dimorphism varies from one species to another.

Table 4. Frequency distribution of Peduncle indeces (CPL/BLD) in *Aphanius* from Iberia.

Species	Caudal peduncle index								
	0.6-0.8	0.8-1	1-1.2	1.2-1.4	1.4-1.6	1.6-1.8	1.8-2	2-2.2	2.2-2.4
male									
<i>A. iberus</i> (Villena).	10	4							
<i>A. iberus</i>					3	15	12	1	
<i>A. baeticus</i> sp. nov.					2	20	20	2	
females									
<i>A. iberus</i> (Villena).		5	7	5	2				
<i>A. iberus</i>					2	7	18	7	3
<i>A. baeticus</i> sp. nov.					2	18	15	1	

Table 5. Summary of diagnostic characters of Iberian *Aphanius*.

Characters	<i>A. iberus</i>	<i>A. baeticus</i> sp. nov.
Number of branched rays on dorsal fin	8-9(10)	8(9)
Number of branched rays on anal fin	8-9	(9)10(11)
Preorbital distance	long	short
Coloration pattern in males	narrow silver transversal bars	wide silver transversal bars
Coloration pattern in females	numerous and small black spots	few large black spots in flanks
Unique alleles in allozymes	<i>IDHP-1*100</i> <i>IDHP-2*100</i> <i>sMDH-2*100</i>	<i>IDHP-1*73</i> <i>IDHP-2*110</i> <i>sMDH-2*110</i>

The high degree of differentiation among populations of *A. iberus* is similar to that detected in the closely related species *A. fasciatus*. In the latter species, population differentiation corresponds to its naturally fragmented distribution and to a restricted gene flow among populations, which favours a genetic structure through isolation by distance (M a l t a g l i a t i 1998,1999, M a l t a g l i a t i & C a m i l l i 2000).

The main divergence between the Atlantic and Mediterranean populations of the Iberian *Aphanius* probably occurred after the Mediterranean dessication and subsequent interruption of water communication during the Lower Pliocene (D o a d r i o et al. 1996). The same pattern of speciation, based on a paleogeographical event, was proposed for the Anatolian species *Aphanius asquamatus* (Sözer, 1942) which probably diverged from the central

Anatolian cyprinodonts during the Upper Miocene-Lower Pliocene (see Villwock 1999). During this period, Anatolia was raised several meters above sea level whilst the entire region was separated in two large systems. Afterwards, the central Anatolian cyprinodonts gradually split during the Pliocene and Pleistocene periods, rendering distinguishable species, such as *A. chantrei* Gaillard, 1895 and *A. anatoliae* Leidenfrost, 1912, besides many subspecies and micro populations (Villwock 1963).

Although *Aphanius* are euryhaline and a coastal route of colonization should be used, the strait of Gibraltar appears to act as an important barrier. Thus, for instance, the exotic species *Fundulus heteroclitus*, which quickly colonized the southern Atlantic area of the Iberian Peninsula, has not been able to colonize the Mediterranean basin through the strait of Gibraltar. Natural geographic barriers and life-history traits, which are similar to those observed in *A. fasciatus* e. g. benthic eggs and absence of larval stages (Maltagliati & Camilli 2000), should prevent gene flow between Atlantic and Mediterranean populations.

Aphanius iberus is critically endangered (Blanco & González 1992, Maitland & Crivelli 1996, Council Directive 92/43/ECC). The distribution range of *A. baeticus* sp. nov. is relatively narrow and pollution, exotic species and hydraulic alterations render the species critically endangered too.

Description of new species

Aphanius baeticus sp. nov.

Diagnosis. Differs from all other known species of *Aphanius* in the combination of: ten branched rays in the anal fin, eight branched rays in the dorsal fin, long and low caudal peduncle, short preorbital length, males show light wide transversal bars and females large black spots. Presence of unique alleles at *IDHP-1**, *IDHP-2** and *sMDH-2** (Doadrio et al. 1996).

Holotype. (Fig. 1). MNCN 170691 Male, 31.3 mm SL. Salado River, Lebrija, Sevilla. Guadalquivir basin, Spain. 20 m altitude. Leg. C. Fernández-Delgado. 6. VI. 1995.

Paratypes. MNCN 170692-719, 29 individuals. Salado River, Lebrija, Sevilla. Guadalquivir basin, Spain. Leg. C. Fernández-Delgado. 6.VI.1995. MNCN 170720-764, 45 individuals. Salado River, Lebrija, Sevilla. Guadalquivir basin, Spain. Leg. C. Fernández-Delgado. 17.VII.1995. MNCN 170765-798, 34 individuals. Salado River, Lebrija, Sevilla. Guadalquivir basin, Spain. Leg. C. García Utrilla. 25.I.1999. UCOBA 147-351, 55 individuals. Salado River, Lebrija, Lebrija, Sevilla, Spain. Guadalquivir basin. Leg. Fernández-Delgado. MNCN 114693-728, 65 individuals. Hondón Lagoon, Parque Nacional de Doñana, Huelva, Spain. Leg. L. Dominguez. 5.XI.1994. MNCN 16145-155, 12 individuals. Salinas de San Carlos, Sanlúcar de Barrameda, Cádiz, Spain. Leg. J.A. Hernando. 15.VI.1975. MNCN 170886-913, 28 individuals. Salado de San Pedro River, Paterna de la Ribera, Cádiz, Spain. Leg. C. Fernández-Delgado. UCOBA 1-96, 96 individuals. Salado de San Pedro River, Paterna de la Ribera, Cádiz, Spain. Leg. C. Fernández-Delgado. UCOBA 97-126, 30 individuals. Roche River, Roche, Cádiz, Spain. Leg. C. Fernández-Delgado. MNCN 170914-948, 35 individuals. Iro River, Chiclana de la Frontera, Cádiz, Spain. Leg. Fernández-Delgado. 21.IX.1995. UCOBA 127-146, 50 individuals. Iro River, Chiclana de la Frontera, Cádiz, Spain. Leg. C. Fernández-Delgado.

Description. D I-II 8(9), A I-(9)10(11), P (8)9-10(11), V (3)4-5, C (13)14-16. LLS (24)25(26), Abd. vert. (10)11, Cau. vert. (14,15)16(17), G. R. 8-10(11). The body form of

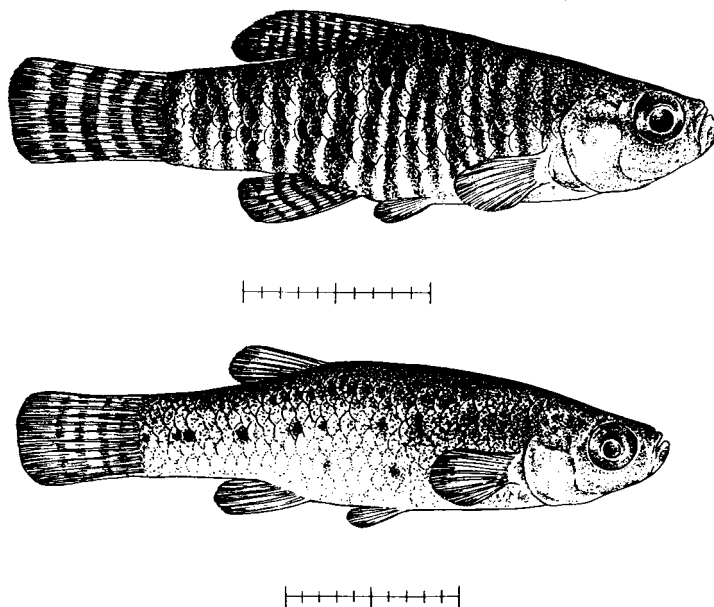


Fig. 3. *Aphanius baeticus* sp. nov. Top: Holotype, male, MNCN 170691 Bottom. Paratype, female, MNCN 170692. Salado River, Lebrija, Sevilla. Spain. Scale 1 cm. Drawing by M. Merino.

Aphanius baeticus sp. nov. (Fig. 3, Table 6) is characterised by a deep but more elongated body than in *A. iberus*. Minimal body depth is 1.4-2.1 (1.7) times in the caudal peduncle in females and 1.4-2.1 (1.8) in males. Minimum body depth 0.9-1.5 (1.2) times in the anal peduncle in females and 0.8-1.7 (1.2) in males. Short head comprising 3.1-5.4 (3.5) times the standard length in females and 3-3.9 (3.4) in males. Preorbital length is small, reaching 1-2.5 (1.7) times the eye diameter in females and 1.2-2.6 (1.8) in males. Insertion of the ventral fin is anterior to dorsal fin origin. Preventral length is 1-1.2 (1.1) times the predorsal length in females and 1-1.2 (1.2) in males. Fin size moderately large. Robust premaxille (maximum length / maximum high = 2-2.6 (2.3)) with (7)8-10(11) teeth. Dentary with (7)8-11(12,13) teeth.

Pigmentation pattern. Males. Light wide vertical bars along the body side, being more evident in juveniles (Fig. 4). The number of bars on the flanks is typically 14-17 and 4-5 on the caudal fin. Four rows of dark spots extend onto the dorsal and anal fins. Pectoral and ventral fins are light orange.

Females. Light brown with few and large dark spots on the body flanks. Two rows of spots are shown, one of them along the lateral line and another in the ventral side. Frequently, the spots are arranged as bars on the middle of the body. An additional row of spots is shown on the caudal peduncle. Caudal fin has 4-6 dark vertical bars.

Sexual dimorphism. Males are smaller overall than females with proportionally larger ventral fins.

Distribution. *A. baeticus* sp. nov. occurs in the lower reaches of the River Guadalquivir and streams located on the southern Atlantic slope of Spain (Fig. 5). The species is currently found in the following areas: River Santiago, El Palmar de Troya, Sevilla. River Salado, Lebrija, Sevilla. Parque Nacional de Doñana, Huelva. Salinas de Sanlúcar de Barrameda, Sanlúcar de Barrameda, Cádiz. River Salado de San Pedro, Paterna de la Ribera, Cádiz.

Table 6. Statistical parameters for the morphometric characters of *A. baeticus* sp. nov. Second row (SL/) represents standard length / each variable. Variables are described in Methods. (SD = Standard deviation).

<i>Aphanius baeticus</i> sp. nov.						
Variable	males (n = 44)			females (n = 36)		
	range	mean	SD	range	mean	SD
SL	16.3-25.8	20.9	2.3	17.7-29.1	24.2	3.8
CFL	4.3-6.1	4.9	0.5	3.7-6.2	5.2	0.7
SL/	3.7-5.2	4.2	0.4	4.0-5.3	4.7	0.4
HL	4.6-7.9	6.2	0.7	4.2-8.4	6.9	1.0
SL/	3.0-3.9	3.4	0.2	3.1-5.5	3.5	0.4
HH	3.3-5.4	4.4	0.5	3.6-6.9	4.9	0.8
SL/	4.3-5.4	4.8	0.2	4.1-5.7	4.9	0.3
PROL	0.8-1.7	1.2	0.2	0.8-2.1	1.4	0.3
SL/	12.7-25.7	17.3	2.7	10.6-28.1	17.9	2.7
ED	1.7-2.8	2.1	0.2	1.7-2.8	2.3	0.2
SL/	8.6-11.4	9.9	0.8	8.2-13.1	10.6	1.3
PRDD	9.4-15.5	12.5	1.3	10.1-19.5	14.7	2.5
SL/	1.5-1.8	1.7	0.1	1.4-1.9	1.7	0.1
PRPD	4.9-8.5	6.7	0.8	5.3-10.2	7.5	1.1
SL/	2.8-3.8	3.1	0.2	2.5-4.1	3.2	0.3
PRVD	8.4-13.6	11.1	1.2	9.1-16.4	12.7	2.0
SL/	1.7-1.9	1.9	0.1	1.7-2.2	1.9	0.1
PRAD	10.6-17.3	13.6	1.5	1.3-21.5	16.3	2.9
SL/	1.4-1.6	1.5	0.1	1.3-1.7	1.5	0.1
CPL	4.1-7.5	5.6	0.9	4.4-6.9	5.8	0.7
SL/	3.0-4.9	3.8	0.5	3.6-4.8	4.2	0.3
APL	2.8-5.5	4.2	0.6	3.3-6.4	4.7	0.8
SL/	3.9-7.2	5.1	0.7	4.2-6.5	5.2	0.6
PVL	2.9-5.47	4.5	0.6	3.5-8.5	5.4	1.2
SL/	3.7-5.6	4.6	0.3	3.4-6.1	4.6	0.6
VAL	1.9-3.7	2.6	0.4	1.6-5.5	3.7	1.0
SL/	6.6-9.5	8.0	0.7	5.0-11.2	6.8	1.2
DFL	2.3-4.6	3.2	0.5	2.0-4.2	3.2	0.6
SL/	5.3-10.0	6.7	0.8	6.7-9.7	7.7	0.7
DFH	3.4-7.4	5.3	0.7	3.7-7.2	5.5	0.9
SL/	3.3-4.9	3.9	0.4	3.8-5.1	4.4	0.4
PFL	3.0-5.4	4.1	0.5	2.7-5.2	3.9	0.6
SL/	4.2-6.7	5.1	0.5	5.2-8.4	6.2	0.7
VFL	2.1-4.3	2.9	0.4	1.9-3.6	2.9	0.5
SL/	5.2-8.3	7.4	0.6	7.4-9.8	8.5	0.6
AFL	1.6-3.9	2.6	0.5	1.5-3.7	2.7	0.6
SL/	6.2-11.1	8.1	1.2	5.5-12.3	9.2	1.6
AFH	3.6-5.9	4.5	0.5	3.4-6.6	4.8	0.7
SL/	4.1-5.4	4.6	0.3	3.9-6.1	5.1	0.5
BD	4.0-7.5	5.9	0.8	4.5-9.8	6.9	1.4
SL/	3.1-4.4	3.6	0.2	2.9-4.1	3.6	0.3
BLD	2.5-4.6	3.5	0.5	2.7-5.0	3.9	0.7
SL/	5.2-6.8	6.0	0.4	5.5-7.1	6.1	0.4

River Iro, Chiclana de la Frontera, Cádiz. River Roche, Roche, Cádiz. River Salado de Conil, Conil, Cádiz (Moreno-Amich et al. 1999).

Etymology. The species name “*baeticus*” derives from the roman name of the Guadalquivir River valley. The range of the species overlaps almost exactly with this area.

Remarks. *A. baeticus* sp. nov. has been found in three different types of habitat: lagoons (one population), tidal channels (one population) and small streams (six populations). The

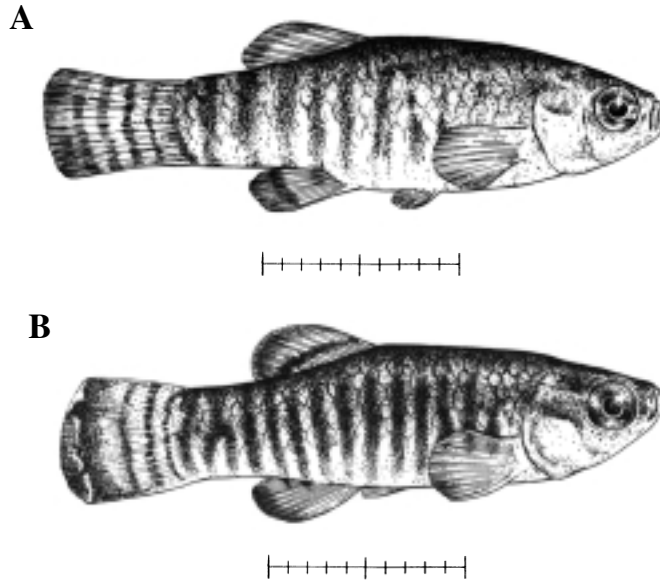


Fig. 4. Vertical bar and shape patterns of juvenile males in Iberian *Aphanius*. (A) *A. baeticus* sp. nov. (B) *A. iberus*.

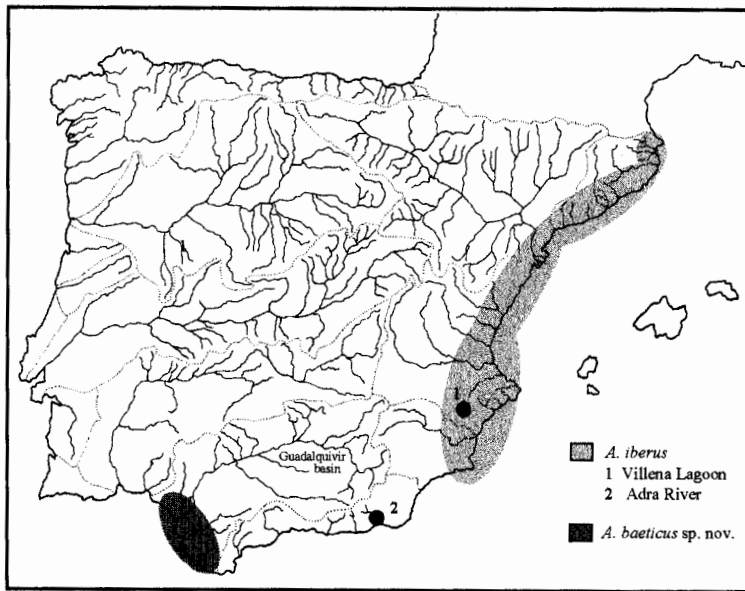


Fig. 5. Geographical distribution of Iberian species of the genus *Aphanius*.

lagoon has permanent, very shallow (30 cm mean deep), freshwater with a silty clay bottom and scarce aquatic vegetation (*Juncus* sp., *Phragmites* sp.). The most common habitats of *A. baeticus* sp. nov. are first order streams (2-3 m width, 0.5 m deep), close to the sea with a silty clay bottom and scarce bank vegetation dominated by *Salicornia* sp., *Tamarix* sp. and/or *Juncus* sp. (Fernández-Delgado et al. 1999). Water salinity is highly variable, two populations are located in freshwaters and the rest are living in hyperhaline

water (reaching up to 110%). Aquatic vegetation is dominated by *Cladophora* sp. In the hyperhaline small streams, *A. baeticus* sp. nov. does not coexist with any other fish species and occurs in high densities. At the remaining locations, the species coexists with one or another of the introduced fish species, the mosquito-fish (*Gambusia holbrooki* Baird et Girard, 1853) and the salt-marsh killifish (*Fundulus heteroclitus* L.), when this occurs, the density of *Aphanius* is very low (FERNÁNDEZ-DELAGADO et al. 1999).

Aphanius baeticus sp. nov. shows a very short life-span, with a winter age-structure of 95.9% 0+, 3.9% 1+ and 0.2% 2+ fish. The growth period is from April to September and the annulus on the scales is formed in April. The species spawns intermittently during the reproductive period. From April to July, 1+ specimens (7 to 12 months old) reproduce. Their offspring (0+ group; 3-4 months old) reproduce from July to September. Very few of the 1+ group specimens survive to spawn the following year. Males mature before females, but females are larger than males. The overall sex ratio does not differ from 1:1, but males outnumber females considerably during the spawning period (FERNÁNDEZ-DELAGADO et al. 1988).

Conservation. Four of the eight populations reported are in a critical situation. This is due to the fact that the species occurs in habitats with *G. holbrooki* and/or *F. heteroclitus*. Apparently, the other four populations are well protected, occurring in high densities. The most serious threat to this new species is that all the populations are isolated, without any connection, and hence genetic flow between them. In our opinion, given the restricted number of known populations and their actual status, this species should be catalogued as critically endangered (CR) according to the IUCN Red List Categories.

Acknowledgements

Part of this study was financially sponsored by the Consejería de Medio Ambiente (Autonomous Government of Andalusia) under the "Plan de recuperación de las poblaciones del género *Lebias* en Andalucía" and DGES project PB97-1178 to I. Doadrio. For the assistance with the field collections, we thank L. Domínguez, P. Garzón, F. Gomez-Carwana, A. Machordom, R. Moreno-Amich, J. C. Nevado, S. Peiró, M. Planelles, P. Risueño and A. Sostoa. We are grateful to J. Castroviejo, T. Chapa, A. García-Valdecasas, M. Kottelat, A. Perdices, A. Machordom, M. Merino, S. Reig, R. Márquez and D. Buckley for suggestions and improve the English. We are also grateful to Prof. Guy Duhamel (MNHN-Paris) for facilities in the designation of a lectotype of *A. iberus* from the syntype series. Special thanks is to Palmira Guarnizo for her invaluable field and laboratory assistance.

LITERATURE

- ALLARDI, J. & KEITH, P., 1991: Atlas préliminaire des poissons d'eau douce de France. *Muséum National d'Histoire Naturelle, Paris*, 232 pp.
- ARNOULT, J., 1957: Sur quelques poissons rares et peu connus dans les eaux douces de France. *Bull. Museum Natl. d'Histoire Nat.*, 6: 464-466.
- BAUM, D. A. & DONOGHUE, M. J., 1995: Choosing among alternative "phylogenetic" species concepts. *System. Biol.*, 20: 560-573.
- BEERLI, P., HOTZ, H. & UZZELL, T., 1995: Geologically dated sea barriers calibrate a protein clock for Aegean water frogs. *Evolution*, 50: 1676-1687.
- BEKELE, A., CAPANNA, E., CORTI, M., MARCUS, L. F. & SCHLITTER, D. A., 1993: Systematics and geographic variation of Ethiopian *Arvicanthis* (Rodentia, Muridae). *J. Zool. London*, 230: 117-134.
- BLANCO, J.C. & GONZÁLEZ, J.L., 1992: Libro rojo de los vertebrados de España. *ICONA, colección técnica, Madrid*.
- BURNABY, T. P., 1966: Growth-invariant discriminant functions and generalized distances. *Biometrics*, 22: 96-110.
- COELHO, M. M., BOGUTSKAYA, N. G., RODRIGUES, J. A. & COLLARES-PEREIRA, M. J., 1998: *Leuciscus torgalensis* and *Leuciscus aradensis*, two new cyprinids for Portuguese fresh waters. *J. Fish Biol.*, 5: 937-950.

- DOADRIO, I., 1994: Freshwater fish fauna of North Africa and its biogeography. *Konink. Mus. Midd. Afri. Terr. Belg. Annal. Zool. Wetenschap.*, 275: 21-34.
- DOADRIO, I., PERDICES, A. & MACHORDOM, A., 1996: Allozyme variation of the endangered killifish *Aphanius iberus* and its application to conservation. *Environ. Biol. Fish.*, 45: 259-271.
- FERNÁNDEZ-DELGADO, C., HERNANDO, J. A., HERRERA, M. & BELLIDO, M., 1988: Age, growth and reproduction of *Aphanius iberus* (Cuv. & Val., 1846) in the lower reaches of the Guadalquivir River (south-west Spain). *Freshwat. Biol.*, 20: 227-234.
- FERNÁNDEZ-DELGADO, C., TORRALVA, M. M., OLIVA, F. & PINTOS, R., 1999: Caracterización ecológica del hábitat del fartet (*Lebias iberica* Valenciennes, 1846) en una pequeña cuenca hidrográfica del bajo Guadalquivir. In: Generalitat Valenciana (ed.), Peces cyprinodontidos ibéricos: Fartet y Samaruc. *Conselleria de Medio Ambiente, Valencia*: 99-114.
- FERNANDEZ-PEDROSA, V., GONZÁLEZ, A., PLANELLES, M., MOYA, A. & LATORRE, A., 1995: Mitochondrial DNA variability in three Mediterranean populations of *Aphanius iberus*. *Biol. Conserv.*, 72: 251-256.
- HERNANDO, J. A., 1975: Notas sobre la distribución de los peces fluviales en el Suroeste de España. *Doñ. Acta Verteb.*, 2: 263-264.
- HUBER, J. H., 1996: Killi-Data 1996. Liste actualisée des noms taxonomiques, des localites de pêche et des références bibliographiques des poissons cyprinodontes ovipares (Atherinomorpha, Pisces). *Société Française d'Ichtyologie, Paris*, 399 pp.
- KOTTELAT, M., 1997: European freshwater fishes. An heuristic checklist of the freshwater fishes of Europe (exclusive of former USSR), with an introduction for non-systematics and comments on nomenclature and conservation. *Biologia (Bratislava)*, 52 (suppl. 5): 1-271.
- MAITLAND, P. S. & CRIVELLI, A. J., 1996: Conservation of freshwater fish. *Conserv. Mediter. Wetl.*, 7: 1-94.
- MALTAGLIATI, F., 1998: A preliminary investigation of allozyme genetic variation and population geographical structure in *Aphanius fasciatus* from Italian brackish-water habitats. *J. Fish Biol.*, 52: 1130-1140.
- MALTAGLIATI, F., 1999: Genetic divergence in natural populations of the Mediterranean brackish-water killifish *Aphanius fasciatus*. *Mar. Ecol. Prog. Ser.*, 179: 155-162.
- MALTAGLIATI, F. & CAMILLI, L., 2000: Temporal genetic variation in a population of *Aphanius fasciatus* (Cyprinodontidae) from a brackish-water habitat at Elba Island (Italy). *Environ. Biol. Fish.*, 57: 107-112.
- MENDOZA-QIJANO, F., FLORES-VILLELA, O. & SITES, J. W., 1998: Genetic variation, species status and phylogenetic relationships in rose-bellied lizards (*Variabilis* group) of the genus *Sceloporus* (Squamata: Phrynosomatidae). *Copeia*, 2: 354-366.
- MORENO-AMICH, R., PLANELLES-GOMIS, M., FERNÁNDEZ-DELGADO, C. & GARCÍA-BERTHOUE, E., 1999: Distribución geográfica de los ciprinodontiformes en la Península Ibérica. In: Generalitat Valenciana (ed.), Peces cyprinodontidos ibéricos: Fartet y Samaruc. *Conselleria de Medio Ambiente, Valencia*: 33-57.
- PARACUELLOS, M. & NEVADO, J. C., 1994: Localización del Fartet, *Aphanius iberus*, en la cuenca del río Adra (Almería, sudeste ibérico). *Doñ. Acta Verteb.*, 21 (2): 199-204.
- PELLEGRIN, J., 1921: Les poissons des eaux douces de l'Afrique du Nord française, Maroc, Algérie, Tunisie, Sahara. *Mem. Soc. Sci. Nat. Maroc*, 1: 1-216.
- PERDICES, A., CARMONA, J. A. & DOADRIO, I., 2001: Nuclear and mitochondrial data reveal high genetic divergence among Atlantic and Mediterranean populations of the Iberian killifish *Aphanius iberus* (Teleostei: Cyprinodontidae). *Heredity*, 87: 314-324.
- ROHLF, F. J., 1993: NTSYS-pc. Numerical taxonomy and multivariate analysis system, Version 1.80. *Setauket, New York*.
- ROHLF, F. J. & BOOKSTEIN, F. L., 1987: A comment on shearing as a method for "size correction". *Sys. Zool.*, 36: 356-367.
- SAS Institute Inc., 1996: SAS, User's guide statistics, Version 11.1. *Ed. Cary, North Carolina, SAS Institute, USA*.
- VILLWOCK, W., 1963: Genetische Analyse des Merkmals "Beschuppung" bei anatolischen Zahnkarpfen (Pisces, Cyprinodontidae) im Auflöserversuch. *Zool. Anz.*, 170: 23-45.
- VILLWOCK, W., 1999: Biogeography of the cyprinodontiform fishes (Teleostei: Cyprinodontidae) of the mediterranean region. In: Generalitat Valenciana (ed.), Peces cyprinodontidos ibéricos: Fartet y Samaruc. *Conselleria de Medio Ambiente, Valencia*: 13-33.
- VILLWOCK, W. & SCHOLL, A., 1982: Ergänzende Mitteilungen über *Aphanius* aus der Oase Azraq/Jordanien sowie Betrachtungen zum taxonomischen Status eines neuen *A. iberus* (Cyprinodontidae: Pisces) aus dem Oued Zousfana, Igli/Nordwest-Algerien. *Mitteil. Hamburg. Zool. Mus. Zool. Inst.*, 79: 267-271.
- WASSERSUG, R. J., 1976: A procedure for differential staining of cartilage and bone in whole formalin-fixed vertebrates. *Stain Technol.*, 51: 131-134.