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A new species of the genus *Aphanius* (Nardo, 1832) (Actinopterygii, Cyprinodontidae) from Algeria

JOSÉ L. BLANCO[1], TOMAS HRBEK[2] AND IGNACIO DOADRIO[1]

[1]*Deptamento de Biodiversidad, Museo Nacional de Ciencias Naturales. José Gutiérrez Abascal 2, 28006 Madrid. Spain. E-mail: mcnd147@mncn.csic.es*
[2]*Department of Biology, University of Puerto Rico, Río Piedras, San Juan, PR 00931, Puerto Rico. E-mail: hrbek@cnnet.upr.edu*

Abstract

A new species, *Aphanius saourensis* sp. nov., is described based on morphological and genetic characters. The new species comes from the Saoura Valley in northwestern Algeria. It differs from *Aphanius iberus*, in which the Saoura Valley populations have been included until now, by a combination of the following characters: 7–8 (9) branched rays in dorsal fin; greater preorbital length (PrOL/ED=0.9–1.3); and a narrower caudal peduncle (CPL/BLD=1.7–2.0). Body pigmentation in live males consists of a profusely mottled blue and silver pattern, without vertical bars. Females lack conspicuous black spots. The new species differs from both of its sister taxa (*Aphanius iberus* and *A. baeticus*) at 26 fixed nucleotide positions (molecular autapomorphies) in the cytochrome *b* gene. Genetic divergence between *Aphanius iberus* and *Aphanius saourensis* sp. nov. for the cytochrome *b* gene is 4.9–5.8 % and between *Aphanius baeticus* and *Aphanius saourensis* sp. nov. 6.3–6.5 %.

Key words: Algeria, *Aphanius saourensis* sp. nov., Cyprinodontidae, Cyprinodontiformes, Saoura, Taxonomy, Conservation, Endangered species

Resumen

Una nueva especie, *Aphanius saourensis* sp. nov., es descrita en base a caracteres morfológicos y genéticos. La nueva especie procede del Valle del Saoura, noroeste de Argelia. Esta se diferencia de *Aphanius iberus,* donde las poblaciones de la cuenca del valle del Saoura habían sido incluidas hasta ahora, por una combinación de los siguientes caracteres: 7–8 (9) radios blandos en la aleta dorsal; distancia preorbitaria larga (PrOL/ED= 0.9–1.3); pedúnculo caudal estrecho (CPL/BLD= 1.7–2.0). Los machos, en vivo, presentan el cuerpo densamente moteado de azul y plata sin barras verticales. Las hembras sin manchas negras muy marcadas. La nueva especie difiere de sus taxones hermanos (*Aphanius iberus* and *A. baeticus*) en 26 posiciones nucleótidicas fijadas, apomorfías moleculares, en el gen citocromo *b*. La divergencia genética entre *Aphanius iberus* y *Aphanius*

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saourensis sp. nov. para el gen citocromo *b* fue de 4.9–5.8% y entre *Aphanius baeticus* y *Aphanius saourensis* sp. nov. de 6.3–6.5%.

Introduction

The genus *Aphanius* is currently composed of approximately 16 species, and is distributed along the ancient coast of the Tethys Sea (Kosswig, 1967, Villwock, 1999), which closed at the Oligocene/Miocene boundary (Smith *et al.,* 1995); this area includes both coastal and interior bodies of water in the Mediterranean basin and the Persian Gulf as far as Iran and Pakistan (Kessel & Zee, 1984, Wildekamp, 1993). The greatest species diversity is considered to be in the Near East, especially in Anatolia (Wildekamp *et al.*, 1999), although recent studies suggest that this high diversity should also include Iran (Coad, 2000, Hrbek *et al.*, in press). Diversity is lower in western parts of the range of the genus, where only four species are found: *Aphanius apodus* (Gervais, 1853), *Aphanius baeticus* Doadrio, Carmona and Fernandez-Delgado, 2002, *Aphanius iberus* (Valenciennes, 1846), and *Aphanius fasciatus* (Valenciennes, 1821).

Genetically, *Aphanius* is divided into two major clades (eastern and western) (Hrbek & Meyer, 2003). All four species in the western Mediterranean region are members of the western clade, within which three lineages may be defined. *Aphanius apodus* was the earliest monotypic lineage to diverge from other members of the western *Aphanius* clade, whereas *Aphanius fasciatus* has a relatively nested position within the western *Aphanius* clade. The next lineage after *Aphanius apodus* to diverge from remaining western-clade *Aphanius* is the clade comprising *Aphanius iberus*, *A. baeticus,* and the new species described in the present study. Until recently this lineage was considered to be monotypic. However, molecular (Perdices *et al.*, 2001, Hrbek & Meyer, 2003) and morphological (Doadrio *et al.*, 2002) data clearly define two groups showing allopatric distributions, which support the hypothesis that they represent two distinct species, *Aphanius iberus* and *Aphanius baeticus* (Doadrio *et al.,* 2002). The species with the broadest distribution, *Aphanius iberus*, historically occurred in southern France and along the Mediterranean coast of Spain; populations considered to be this species were also known from Morocco and Algeria (Pellegrin, 1921, Kessel & Zee, 1984, Wildekamp, 1993, Doadrio, 1994, Villwock, 1999). *Aphanius iberus* is currently extant only in remnant populations in Spain. Many North African records are historical and possibly imprecise, since this species does not presently occur in these areas or is known to have become extinct. Other North African literature records are based on second-hand information or lack precise collection or locality data, and thus are highly questionable.

One North African population for which good geographic data exists, the Igli population, has been studied by Villwock & Scholl (1982). Those authors found morphological differences and differences in reproductive biology when compared to Spanish populations of *A. iberus* and *A. baeticus*. The absence of new material collected from northern

Africa since Dumont's (1980) and Morgan's (1981) collections, which are deposited at the Musée Royal de l'Afrique Centrale, Tervuren, Belgium (MRAC), has impeded further studies, including the use of new taxonomic tools such as molecular techniques. Lack of material has not resulted from lack of interest or lack of effort. Rather, adverse political conditions and extensive anthropogenically-induced environmental and faunal changes, with attendant habitat degradation, have impeded collection efforts. However, during an expedition to Algeria in 2004, new material of *Aphanius* from an oasis system to which Igli is connected (Fig. 2) was collected. This material forms the basis for the current study.

Materials and Methods

Material used in the present study is deposited in the Museo Nacional de Ciencias Naturales, Spain (MNCN) and Musée Royal de l'Afrique Centrale, Tervuren, Belgium (MRAC). Acronyms for the preceding collections follow Leviton *et al*. (1985).

Description of the new *Aphanius* species is based on five adult males and four adult females (holotype and eight paratypes) deposited in the MNCN collection, and three smaller individuals (paratypes) deposited at the MRAC (not measured). In addition, live individuals were brought back from the type locality; they are being raised at locations in Spain, Belgium, and the Netherlands as part of a species conservation program.

Twenty-three characters were included in the morphometric analysis. For comparative purposes we analyzed 20 individuals from an *Aphanius iberus* "Adra" population (Almeria, Spain) (MNCN 170799–170819) and 20 individuals of *Aphanius baeticus* from "Arroyo Salado" (Sevilla, Spain) (MNCN 170692–170712) (Table1). Each of these comparative series includes equal numbers of males and females.

Morphological and meristic information follows Doadrio *et al*. (2002): SL, standard length; HL, head length; PrOL, preorbital length; ED, eye diameter; IOW, interorbital width; HH head height; PrO, preorbital distance; 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; DFH dorsal fin height; DFL dorsal fin length; 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, branched dorsal fin rays; A, branched anal fin rays; P, branched pectoral fin rays; V, branched pelvic fin rays; C, branched caudal fin rays.

Nineteen samples from different populations of the genus *Aphanius* were analyzed; 16 correspond to different populations of *A. iberus* and *A. baeticus* throughout their geographic ranges (Table 1). One sample of *Orestias silustani* was analyzed as an outgroup. Total cellular DNA was extracted from tissues by the standard proteinase K and phenol/chloroform extraction method (Sambrook *et al.* 1989). Frozen and 70% ethanol-preserved specimens normally gave the same quality and quantity of DNA. Two overlapping fragments of the cytochrome *b* gene (total of 1041 bp) were amplified via polymerase chain reaction (PCR) from each individual DNA sample. The primers used for cytochrome *b* were those used by Machordom and Doadrio (2001). The amplification process was conducted as follows: 94ºC (2 min), 35 cycles at 94ºC (45 s), 48 ºC (1 min), 72ºC (5 min). PCR mixtures were prepared in 25 µl with a final concentration of 0.4 µM of each primer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, and 1 unit of Taq DNA polymerase (Biotools) and 1X of the provided buffer. After checking PCR products on 1.5% agarose gels, they were cloned using the pGEM-T vector (Promega) into *E. coli* JM109, and were sequenced using the FS-Taq Dye Deoxy Terminator cycle-sequencing kit (Applied Biosystems Inc.). DNA sequences of both strands were obtained using M13 universal (forward and reverse) sequencing primers.

TABLE 1. Samples, collection sites, number of sampled populations in the phylogenetic tree (Fig 8) and GenBank access numbers.

Species	Locality	Source	Population number
Orestias silustani	Puno, Laguna Ulmayo (Peru)	AY155565	
A. vladykovi	Zagros Mountains (Iran)	DQ367526	
A. fasciatus	Klisova Marshes (Greece)	AF299273	
A. saourensis	Mazzer (Algeria)	DQ367527	
A. baeticus	Salado River Conil (Spain)	AF299280	Conil-1
A. baeticus	Salado River Conil (Spain)	AF299281	Conil-2
A. baeticus	Lucio del Hondon (Spain)	AF299282	Doñana-1
A. baeticus	Lucio del Hondon (Spain)	AF299283	Doñana-2
A. baeticus	Salado River Iro (Spain)	AF299284	Iro-1
A. baeticus	Salado River Iro (Spain)	AF299285	Iro-2
A. iberus	Albuixec (Spain)	AF299286	Albuixec-1
A. iberus	Albuixec (Spain)	AF299287	Albuixec-2
A. iberus	Villena Lagoon (Spain)	AF299288	Villena
A. iberus	Adra (Spain)	AF299274	Adra-1
A. iberus	Adra (Spain)	AF299275	Adra-2
A. iberus	Aiguamolls (Spain)	AF299276	Aiguamolls-1
A. iberus	Aiguamolls (Spain)	AF299277	Aiguamolls-2
A. iberus	Santa Pola (Spain)	DQ367528	Santa Pola
A. iberus	San Pedro del Pinatar (Spain)	AF299278	Pinatar
A. iberus	Marchamalo (Spain)	DQ367529	Marchamalo

All samples were sequenced on an Applied Biosystems 3700 DNA sequencer following the manufacturer's instructions. Sequence analysis was performed with Sequencher ver. 3.0 (Gene Codes Corporation, Inc.). Chromatograms and alignments were visually checked and verified; there were no gaps in the resulting DNA sequences.

Nucleotide saturation was analyzed by plotting the absolute number of transitions and transversions against patristic distance values. Phylogenetic relationships were inferred using the Bayesian-likelihood approach implemented in MrBayes 3.04 (Ronquist & Huelsenbeck, 2003) by simulating one million Markov chain cycles; no burnin parameters were necessary. The model of molecular evolution selected in the program ModelTest 3.04 (Posada & Crandall, 1998) and used in the Bayesian-likelihood analyses was the GTR (Rodríguez *et al.*, 1990) model of molecular evolution.

Aphanius saourensis sp. nov.

Figs. 1, 4–5; Table 2.

Holotype. MNCN 246655 Male, 31.5 mm SL (Fig. 1). La tombe de Moché, Wadi (=Oued) Saoura, Mazzer, Algeria (Fig. 2). Geographic coordinates 30º19'N, 02º16'W. Coll. Nassim Louaileche, Dep. Herman Meeus; 19 October 2003.

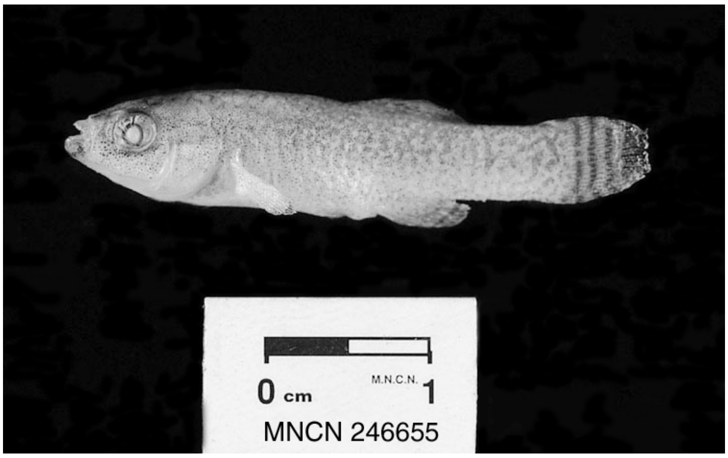

FIGURE 1. *Aphanius saourensis* sp. nov. Holotype, MNCN 246655, Male, 31.5 mm SL. Wadi (=Oued) Saoura, Mazzer. Algeria.

Paratypes. MNCN 246649–246654 (6); MNCN 246656–246657 (2); MRAC A4-24-8-1-3 (3) (measurements not taken). Same data as holotype.

Non-types. A series of live individuals, collected along with the types, have been brought back to the laboratory (and subsequently divided among facilities in Spain, Belgium, and the Netherlands) as stock for a species conservation program.

Diagnosis. *Aphanius saourensis* differs from its sister species, *A. iberus* and *A. baeticus,* by the following combination of characters: seven or eight (rarely nine) branched dorsal-fin rays (*vs*. eight or nine [rarely 10] branched dorsal rays in *A. iberus*); longer preorbital length (PrOL/ED = 0.9–1.3 *vs*. 0.5–0.7) (Table 3); and relatively longer and more narrow caudal peduncle (CPL/BLD = 1.7–2.0 *vs*. 1.3–1.9 [rarely 2.0]) (Table 4). Body pigmentation of live males a profusely mottled blue and silver, not forming vertical bars (Fig. 4) *vs*. alternating silver to silver-blue and blue-grey bars and small irregular silver spots in *A. iberus* (Fig. 6) and *A. baeticus* (Fig. 7). Females without conspicuous black spots (except a spot at the junction of the caudal peduncle and central caudal fin rays) (Fig. 5) *vs*. one row of large and two to three rows of small black spots in the posterior half of the body in *A. iberus* and *A. baeticus*, respectively. Twenty-six molecular autapomorphies in the cytochrome *b* gene (Table 6) also differentiate *A. saourensis* sp. nov. from *A. iberus* and *A. baeticus*. Divergence in the cytochrome *b* gene is 4.9–5.8% compared to *Aphanius iberus* and 6.3–6.5% compared to *Aphanius baeticus*. Summaries of differences among the three species appear in tables 5 and 6.

FIGURE 2. Type locality of *Aphanius saourensis* sp. nov. at La tombe de Moch, Wadi (=Oued) Saoura, Mazzer, Algeria.

Description. Morphometric data appear in Table 2. D. I–II, 7–8 (9) ($\bar{x}$=7.6), A. I,9-10 ($\bar{x}$=9.6), P. I (12) 13-15 ($\bar{x}$=13.7), V. I 4-5 ($\bar{x}$=4.9), C. 13-15 (16) ($\bar{x}$=14.6), LLS. 25-26 ($\bar{x}$=25.4). Body moderately elongated, maximum height 3.7 times in standard length in females and 4.1 times in males. Caudal peduncle narrow. Height of caudal peduncle 1.6 times the anal caudal peduncle length in females and 1.8 times in males. Height of caudal peduncle is 2.0 times in females and 1.9 times in males the dorsal caudal peduncle length. Head elongated, its length 3.6 times in standard length in females and 3.4 in males. Snout long, the preorbital length 3.0 times in head length in females and 4.2 times in males. Eye located in a forward position, its diameter 1.3 times in preorbital length in females and 1.0 time in males. Dorsal fin inserted before origin of anal fin, on the same axis. Preanal length 1.2 times longer than predorsal length in females and 1.1 times in males. Pectoral fin shorter than head length, its length 2.1 times in head length in females and 2.1 times in males. Ventral fin small, and in one individual (MNCN 246649) is absent. Tricuspid teeth in a single row.

TABLE 2. Morphometric and meristic characters for *Aphanius saourensis* sp. nov. type series. Variables are described in Methods. (SD= Standard deviation).

Aphanius saourensis **sp. nov.**							
	Males (n=5)				Females (n=4)		
Variable	Holotype	Range	Mean	SD	Range	Mean	SD
SL	31.5	31.5–23.8	28.3	2.9	33.8–31.6	32.8	0.9
HL	8.9	8.9–6.9	8.3	0.8	9.8–8.7	9.1	0.5
PRoL	2.1	2.1–1.6	2.0	0.2	3.2–2.9	3.0	0.2
ED	2.0	2.1–2.0	2.0	0.1	3.0–1.9	2.4	0.5
PrDD	16.8	16.9–13.9	15.9	1.2	20.0–19.2	19.6	0.3
PrPD	10.0	10.0–7.4	9.1	1.0	11.2–9.9	10.5	0.6
PRVD	16.4	16.4–12.2	14.0	1.6	19.2–17.8	18.5	0.7
PrAD	20.9	20.9–15.4	17.7	2.0	25.3–20.9	22.8	1.9
CPL	8.4	8.4–6.1	7.8	1.0	10.0–8.7	9.5	0.6
APL	7.2	8.6–6.3	7.3	1.0	8.9–6.7	7.7	0.9
PVL	5.8	5.8–4.1	5.1	0.7	9.0–5.7	7.1	1.7
VAL	2.1	3.0–1.9	2.3	0.4	5.7–3.3	4.2	1.3
DFL	4.5	4.5–2.2	3.3	0.9	3.9–3.0	3. 5	0.5
DFH	4.6	7.9–3.9	5.3	1.5	5.1–4.1	4.5	0.4
PFL	4.3	4.6–2.7	4.0	0.7	5.4–3.5	4.3	0.9
VFL	2.1	2.5–1.2	2.0	0.5	1.6–1.0	1.4	0.3
AFL	3.0	3.0–1.7	2.4	0.6	3.5–1.0	2.4	1.1
AFH	3.8	4.9–2.8	3.9	0.8	4.9–2.9	3.9	0.8
CFL	5.3	5.3–4.0	4.8	0.6	6.5–4.2	4.9	1.1
BD	7.6	7.6–5.3	6.9	0.9	11.7–7.7	8.9	1.9
BLD	4.9	4.9–3.5	4.1	0.5	5.4–4.3	4.8	0.5
AIO	2.9	3.5–2.8	3.1	0.3	4.5–3.7	4.0	0.4
HH	7.0	7.0–5.1	6.2	0.8	7.7–6.4	7.3	0.6
D	7	8–7	7.4	0.6	9–7	7.8	1.0
A	10	10–9	9.6	0.6	10–9	9.5	0.6
P	13	13–15	13.8	0.8	13–14	13.7	0.6
V	5	4–5	4.8	0.4	5	5.0	0.0
C	14	13–16	14.4	1.1	14–15	14.8	0.5

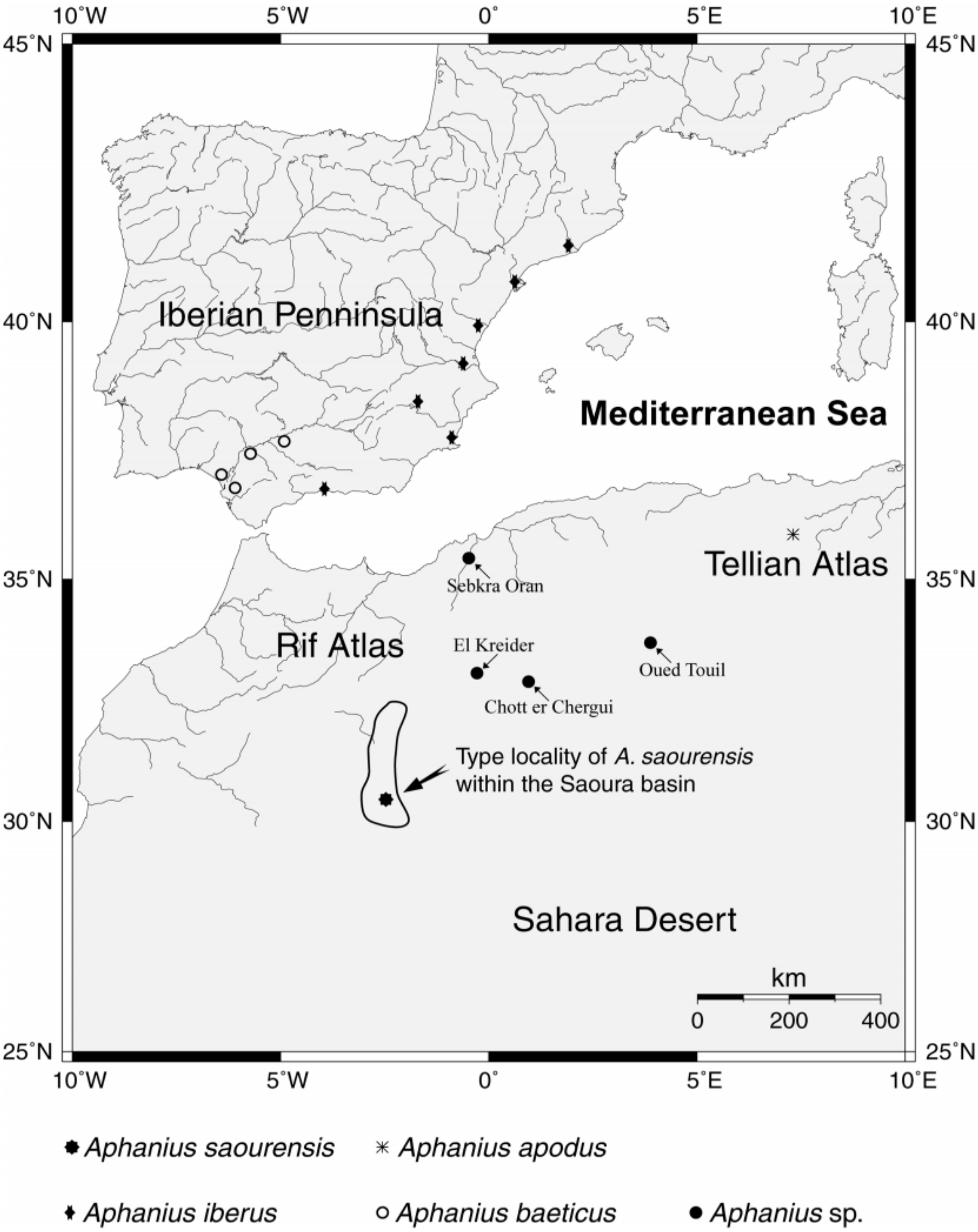

FIGURE 3. Distribution map of western Mediterranean species of *Aphanius* including *A. iberus* and *A. baeticus* from Spain, *A. apodus* from the Tel Atlas in eastern Algeria, and the type locality of *A. saourensis* sp. nov. within the Saoura basin in eastern Algeria. Full circles represent localities cited in the literature for which no museum material exits, and which appear to be extinct. It is uncertain if *Aphanius* from these localities represent other undescribed species, or if these *Aphanius* populations belong to *A. iberus*, *A. baeticus* or *A. saourensis* sp. nov. Base map was created at Online Map Creations http://www.aquarius.geomar.de/omc/.

Pigmentation Pattern. Live Males (Fig. 4): Mottled light blue and silver pattern without bars, with a tendency in some individuals to form bars in the area of the caudal peduncle. Dorsal, anal, and caudal fins with black spots forming vertical bars. Five bars on caudal fin, four on dorsal fin, and three to four on anal fin. Black line under lower lip. Iris silvery, pupil black. Paired fins hyaline, unpaired fins hyaline to opaque bluish-white.

FIGURE 4. Male specimen of *Aphanius saourensis* sp. nov., collected with the type series but not preserved.

TABLE 3. Frequency distribution of orbital indexes (PrOL/ED) in *Aphanius iberus*, *A. baeticus* and *A. saourensis* sp. nov.

Species	0.5–0.7	0.7–0.9	0.9–1.1	1.1–1.3
Aphanius iberus	19	1		
Aphanius baeticus	19	1		
Aphanius saourensis sp. nov.			5	4

TABLE 4. Frequency distribution of caudal peduncle heights (CPL/BLD) in *Aphanius iberus*, *A. baeticus* and *A. saourensis* sp. nov.

Species	1.3–1.5	1.5–1.7	1.7–1.9	1.9–2.0
Aphanius iberus	7	8	5	
Aphanius baeticus	3	5	7	5
Aphanius saourensis sp. nov.			4	5

TABLE 5. Summary of diagnostic morphological and meristic characters.

Characters	***A. iberus***		***A. saourensis* sp. nov.**		***A. baeticus***
Caudal peduncle height	Deep		Narrow		Medium
Number of branched rays in dorsal fin	8–9 (10)		7–8 (9)		8 (9)
Orbital index	Low		High		Low
Genetic divergence		4.9–5.8%		6.3–6.5%	

Live Females (Fig. 5): Light brown body with irregular darker brown mottling tending to darken along the posterior third of lateral line, lighter on ventral region. Most individuals with a well defined dark spot at junction of caudal peduncle and central rays of the caudal fin. All fins colorless. Horizontal line under lower lip similar to males, but less conspicuous. Iris silvery, pupil black.

FIGURE 5. Female specimen of *Aphanius saourensis* sp. nov., collected with the type series but not preserved.

Sexual Dimorphism. There is clear sexual dimorphism in pigmentation pattern (Figs. 4 and 5). Females also appear to have a greater body size than males, although our small sample size precludes unequivocal confirmation of this.

Distribution. Only individuals of the Algerian population of Mazzer (30º 19'N, 2º 16'W; Fig. 2) inside the Oued Saoura basin could be collected and analyzed in this study. Villwock and Scholl (1982) analyzed specimens from Igli, Oued Zousfana basin, a tributary basin to the Saoura. Specimens from El Ouata (MRAC 83-002-P-0082-0111) and Kerzaz (MRAC A3-045-P-0906) are deposited in Royal African Museum in Tervuren, both of which are within the greater Oued Saoura basin (R. Wildekamp, com. pers.) and in geographic proximity to Mazzer; Kerzaz is the most distant locality, approximately 120 km away. Despite several attempts to collect at these localities, no *Aphanius* were found, and it can be assumed that the genus has been extirpated from these areas. However, morphological characters suggest these specimens are *Aphanius saourensis*. The distribution of *Aphanius saourensis* is therefore restricted to the Oued Saoura basin, and is presumed to have originally occurred throughout the entire region (Fig. 3). It is unlikely that other populations from Algeria ascribed to *Aphanius iberus,* such as those from Sebkra Oran, El Kreider, Oued Touil and Chott er Chergui (Pellegrin 1921; see also Fig. 3) belong to the new species or to *A. iberus*. Material from these areas has either not been deposited in a museum or has been lost. Recent efforts to recollect in these areas were unsuccessful.

Etymology. The species name "*saourensis*" comes from the geographical toponym "Saoura", the name of the valley from which the type series was collected, and the only known locality with an extant population.

Proposed Common Names. Sahara Aphanius (GB/English); Afanius sahariano (E/Spanish); Aphanius saharien (F/French); Saharischer Aphanius (D/German).

Conservation. This species should be considered critically endangered following IUCN criteria. The species is only known from one remnant population in the Sahara desert, having disappeared from numerous other localities of this same spring system (Kessel & Zee, 1984). The presence of introduced North American *Gambusia* sp. poses a serious threat, with current densities of *Gambusia* to *Aphanius* being more than 100 to one. Excessive ground water withdrawal for agricultural purposes, the drying of wetlands, and water pollution are, along with the introduced *Gambusia,* the major threats to the survival of this species. Its survival is unlikely in the wild, but a small captive breeding program is underway.

Comparative Data and Discussion

As discussed above, specimens of *Aphanius* from Mazzer, Algeria (Figs. 4–5) show many diagnostic genetic and morphological differences when compared to Spanish specimens of *A. iberus* (Fig. 6) and *A. baeticus* (Fig. 7), and are thus considered to represent a new species. Tables 5 and 6 provide summaries of differences between the three species.

Bayesian analysis of phylogenetic data supports the hypothesis that *A. saourensis* forms a well supported clade together with *A. iberus* and *A. baeticus* (see Fig. 8; Table 6). However, relationships among these three species are not well resolved. Assuming a molecular clock of 1% pair-wise sequence divergence per million year in cytochrome *b* (Dowling *et al.,* 2002; Doadrio & Carmona, 2004), separation among the three species would coincide with the upper Miocene period and the Messinnian crisis. The actual distribution of *A. iberus*, *A. baeticus*, and *A. saourensis* occurs along the old coastline existing in the Miocene before to separation of the Betic-Rif Massif at the Miocene-Pliocene boundary 5.3 million years ago (Doadrio, 1994, Krijgsman *et al.,* 1999a, Krijgsman *et al.,* 1999b).

TABLE 6. Summary of diagnostic molecular characters that differentiate *A. saouensis* sp. nov. from *A. iberus* and *A. baeticus*. Numbers above autapomorphic characters represent relative 5' to 3' position in the complete cytochrome *b* gene.

Position	8	72	102	120	156	177	228	279	312	420	552	564	573	621	657	738	744	795	945	966	999	1020	1086	1092	1114	1118
A. saourensis	G	G	G	C	T	T	A	C	C	C	T	T	C	C	A	T	T	T	G	G	C	C	C	C	C	A
A. iberus	A	A	C	T	C	C	T	T	T	T	C	C	T	T	G	C	C	C	C	A	A	G	T	T	T	G
A. baeticus	A	A	C	T	C	C	T	T	T	T	C	C	T	T	G	C	C	C	C	A	T	A	T	T	T	G

FIGURE 6. Male specimen of *Aphanius iberus*.

FIGURE 7. Male specimen of *Aphanius baeticus*.

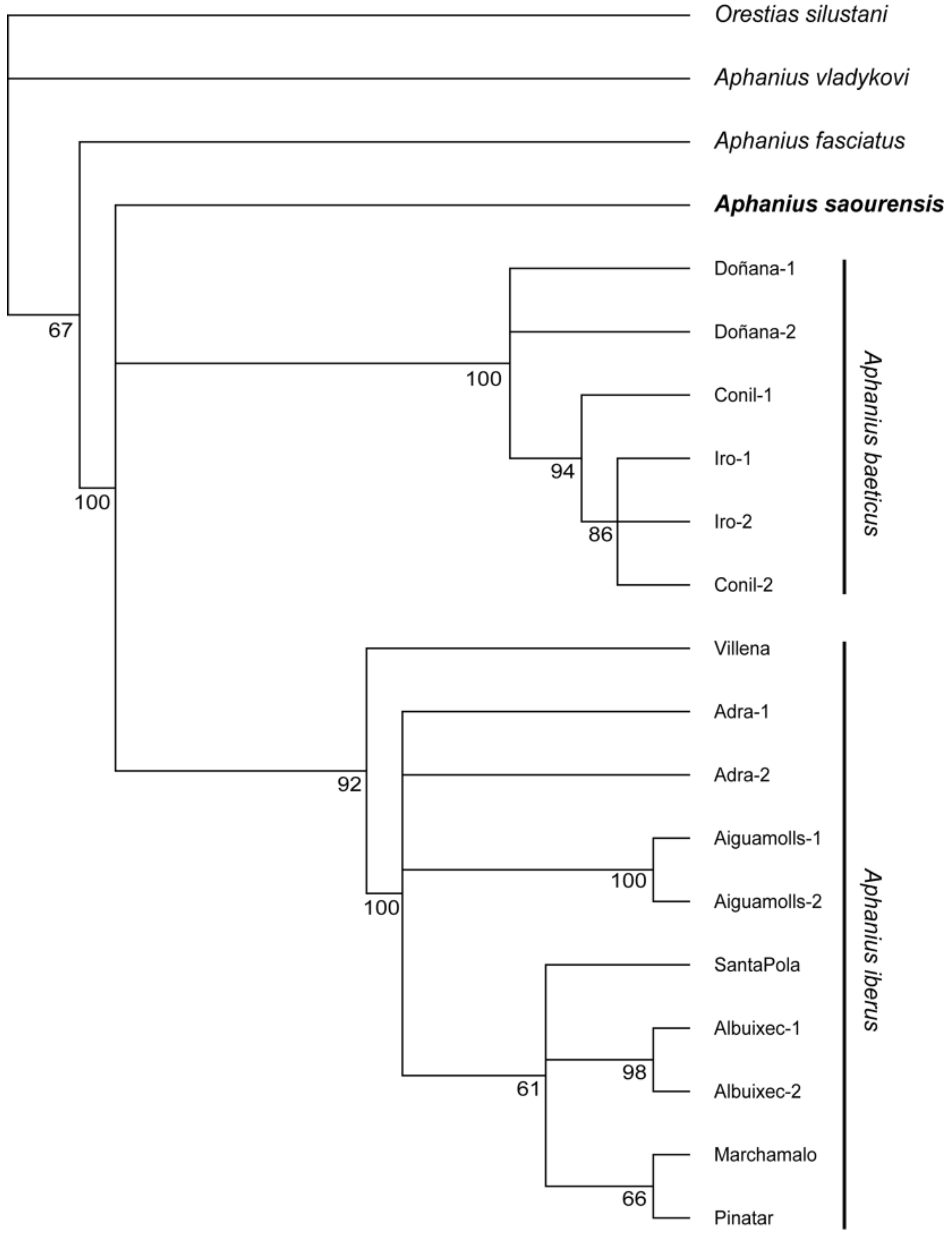

FIGURE 8. Phylogenetic relationships among the four species of *Aphanius* from western Mediterranean basin, based on the complete cytochrome *b* gene. Numbers on the tree are posterior probability values from the Bayesian analysis. Populations labeled the same as in Table 1.

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Literature Cited

Coad, B.W. (2000) Distribution of *Aphanius* species in Iran. *Journal of the American Killifish Association*, 33, 183–191.

Doadrio, I. (1994) Freshwater fish fauna of North Africa and its biogeography. *Annales Musée royal de l'Afrique centrale, Sciences Zoologiques*, 274, 21–34.

Doadrio, I. & Carmona, J.A. (2004) Phylogenetic relationships and biogeography of the genus *Chondrostoma* inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 33, 802–815.

Doadrio, I., Carmona, J.A. & Fernandez-Delgado, C. (2002) Morphometric study of the Iberian *Aphanius* (Actinopterygii, Cyprinodontiformes), with description of a new species. *Folia Zoologica*, 51, 67–79.

Dowling, T.E., Tibbets, C.A., Minckley, W.L. & Smith, G.R. (2002) Evolutionary relationships of the plagopterins (Teleostei: Cyprinidae) from cytochrome *b* sequences. *Copeia*, 2002(3), 665–678.

Hrbek, T., Keivany, Y. & Coad, B.W. (in press) New species of *Aphanius* (Teleostei, Cyprinodontidae) from Isfahan province of Iran and a reanalysis of other Iranian species. *Copeia*, 2006.

Hrbek, T. & Meyer, A. (2003) Closing of the Tethys Sea and the phylogeny of Eurasian killifishes (Cyprinodontiformes: Cyprinodontidae). *Journal of Evolutionary Biology*, 16, 17–36.

Kessel, C.M. & Zee, J.R. (1984) The creation of sanctuaries for the endangered fish species *Aphanius apodus, A. iberus* and *Valencia hispanica* (Cyprinodontidae). *In:* the western Mediterranean: Research proposal. Agricultural University, Nature Conservation Department, Groningen, The Netherlands.

Kosswig, C. (1967) Tethys and its relation to the peri-Mediterranean faunas of freshwater fishes. *In*: C.G. Adams & Ager, D.V., (Eds.), *Aspects of Tethyan biogeography.* Systematics Association Publication, pp. 313–324.

Krijgsman, W., Hilgen, F.J., Raffi, I., Sierro, F.J. & Wilson, D.S. (1999a) Chronology, causes and progression of the Messinian salinity crisis. *Nature*, 400, 652–655.

Krijgsman, W., Langereis, C.G., Zachariasse, W.J., Boccaletti, M., Moratti, G., Gelati, R., Iaccarino, S., Papani, G. & Villa, G. (1999b) Late Neogene evolution of the Taza-Guercif Basin (Rifian Corridor, Morocco) and implications for the Messinian salinity crisis. *Marine Geology*, 153, 147–160.

Leviton, A.E., Gibbs Jr., R.H., Heal, E. & Dawson, C.E. (1985) Standards in herpetology and ichthyology. Part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. *Copeia*, 1985, 802–832.

Machordom, A. & Doadrio, I. (2001) Evidence of a Cenozoic Bertic-Kabilian connection based on a freshwater fish phylogeography (*Luciobarbus*, Cyprinidae). *Molecular Phylogenetics and Evolution*, 18, 252–263.

Pellegrin, J. (1921) Les poissons des eaux douces de l'Afrique du Nord française. *Mémoires de la Société des Sciences Naturelles du Maroc*, 1, 1–216.

Perdices, A., Carmona, J.A., Fernández-Delgado, C. & 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.

Posada, D. & Crandall, K.A. (1998) MODELTEST: Testing the model of DNA substitution. *Bioinformatics*, 14, 817–818.

Rodríguez, F.J., Oliver, J.L., Marín, A. & Medina, J.R. (1990) The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology*, 142, 485–501.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual, second edition*. Cold Spring Harbor Laboratory Press, Cold Springs Harbor, NY

Smith, A.G., Smith, D.G. & Funnell, B.M. (1995) *Atlas of Mesozoic and Cenozoic Coastlines*. Cambridge University Press, New York

Villwock, W. (1999) Biogeography of cyprinodontiform fishes (Teleostei: Cyprinodontidae) of the Mediterranean region. *In*: M. Planelles Gomis, (Ed.), *Peces ciprinodóntidos Ibéricos Fartet y Samaruc*. Generalitat Valenciana Conselleria de Medio Ambiente, Valencia, Spain, pp. 13–31.

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. *Mitteilungen aus dem hamburgischen Zoologischen Museum und Institut*, 79, 267–271.

Wildekamp, R.H. (1993) *A World of Killies*. American Killifish Association, Mishawaka, IN

Wildekamp, R.H., Küçük, F., Ünlüsayin, M. & Neer, W.V. (1999) Species and subspecies of the genus *Aphanius* Nardo 1897 (Pisces: Cyprinodontiformes) in Turkey. *Turkish Journal of Zoology*, 23, 23–44.