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MORPHOLOGICAL AND MOLECULAR ANALYSES OF FRESHWATER BLENNIDS: A NEW SPECIES OF THE GENUS *SALARIA* FORSSKÅL, 1775 (ACTINOPTERYGII, BLENNIDAE) IN MOROCCO

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ABSTRACT

I. Doadrio, S. Perea & A. Yahyaoui. 2011. Morphological and molecular analyses of freshwater blennids: A new species of the genus *Salaria* Forsskål, 1775 (Actinopterygii, Blennidae) in Morocco. *Graellsia*, 67(2): 151-173.

The North African freshwater fish fauna is scarce compared to other regions of the world, probably due to historical and climatic factors. The western part of North Africa is more diverse than central or eastern areas. Populations of freshwater blennids that have been traditionally ascribed to the species *Salaria fluviatilis* nowadays inhabit the Sebou basin in Morocco. In this study we demonstrated morphologically and molecularly that these Moroccan populations constitute a new species, for which we provide a formal description. The following diagnostic characters distinguish the newly described species from other freshwater blennids: head without diagonal rows of dark dots or blotches on cheeks; supra-ocular tentacle thick and simple (not branched); 13-15 teeth on the upper jaw and 14-16 on the lower jaw; 16-17 soft anal fin rays; short pre-orbital distance (cephalic index: 2.9-3.8); height of the anterior (spiny) part of the dorsal fin short (dorsal fin index: 0.8-1.3) and 34 vertebrae. Mitochondrial and nuclear genetic distances between the new *Salaria* species and either *S. economidisi* or *S. fluviatilis* are high, especially in the mitochondrial control region (18%), while these genetic distances range from 2 to 3% when the nuclear S7 marker is compared. These morphological and molecular characters differentiate the new species from the remaining freshwater species of the genus (*Salaria economidisi* and *S. fluviatilis*). The new species is confined to a small restricted area of the Sebou basin in Morocco and should be considered endangered (EN) according to the IUCN Red List.

Key words: Perciformes; Blennidae; freshwater; *Salaria atlantica* sp. nov.; Sebou basin; Morocco.

RESUMEN

I. Doadrio, S. Perea & A. Yahyaoui. 2011. Análisis morfológico y molecular de los blenios de agua dulce: una nueva especie del género *Salaria* Forsskål, 1775 (Actinopterygii, Blennidae) en Marruecos. *Graellsia*, 67(2): 151-173 (en inglés).

La fauna de peces de agua dulce del norte de África es escasa comparada con otras regiones del mundo debido fundamentalmente a factores históricos y climáticos. El oeste del norte de África es más diverso que las regiones del centro y del este. En la cuenca del río Sebou habitan actualmente poblaciones de blenios de agua dulce que han sido tradicional-

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mente adscritas a la especie *Salaria fluviatilis*. En este estudio demostramos morfológica y molecularmente que estas poblaciones marroquíes constituyen una nueva especie, para la cual proveemos una descripción formal. Los siguientes caracteres diagnósticos distinguen a la nueva especie descrita del resto de especies de blenios de agua dulce: cabeza sin una fila diagonal de puntos oscuros o manchas en las mejillas; tentáculo supraocular grueso y simple (no ramificado); 13-15 dientes en la mandíbula superior y 14-16 en la inferior; 16-17 radios blandos en la aleta anal; distancia preorbital corta (índice cefálico: 2.9-3.8); altura de la region anterior de la aleta dorsal corta (índice dorsal: 0.8-1.3) y 34 vértebras. Las distancias genéticas mitocondriales y nuclear entre la nueva especie de *Salaria* y las otras dos especies son altas, especialmente en la región control (18%), mientras que estas distancias genéticas variaron entre 2-3% para el gen nuclear S7. Estos caracteres morfológicos y moleculares diferencian a la nueva especie del resto de blenios de agua dulce del género (*Salaria fluviatilis* y *S. economidisi*). La nueva especie está confinada a la cuenca del río Sebou en Marruecos y debería ser considerada como En Peligro (EN) de acuerdo a las categorías de la lista roja.

Palabras clave: Perciformes; Blennidae; agua dulce; *Salaria atlantica* sp. nov.; cuenca del Sebou; Marruecos.

Introduction

Compared to other regions of the world, North Africa has a depauperate freshwater fish fauna, probably because of climatic and historical factors. The influence of historical processes is evident when the freshwater fish fauna within the region is analyzed. The western North African fish fauna is more diverse, but this is not predicted by current climatology or size of the region compared to central or eastern North Africa (Doadrio, 1994). The fish fauna of North Africa was mainly described in the first half of the twentieth century when most of this area was under French colonial rule, and expeditions were carried out, mainly by the French Geographical Society (Société de Géographie) (Pellegrin, 1921). The description of new species was predominantly based on morphological traits, sometimes without considering geographic variation in populations. The result of these taxonomic studies was that many fish collected in the area were not ascribed to species already described and consequently many synonyms were generated (Eschmeyer & Fricke, 2011).

Molecular studies conducted in this geographical area since the 1990s, mainly supported by the Museo Nacional de Ciencias Naturales (Spain) in collaboration with the Faculty of Sciences of Rabat, found that most of the previously described species show a molecular structure that includes high genetic divergences and/or molecular autapomorphies (Machordom & Doadrio, 1993, 2001; Berrebi *et al*., 1995; Perdices *et al*., 1995; Doadrio *et al.*, 1998; Machordom *et al*., 1998; Delling & Doadrio, 2005; Doadrio & Perdices, 2005; Blanco *et al*., 2006).

In the course of our studies in North Africa we found two localities where *Salaria fluviatilis* occurs, one on the Mediterranean slope of Algeria and the other on the Atlantic slope of Morocco. This species belongs to the family Blennidae, a widespread family with almost 400 species within Percomorpha, closely related with the Gobiesocidae (Miya *et al.*, 2005). The family is morphologically characterized (except for some exceptions) by a scaleless body, a dorsal fin with spiny rays in the anterior part and soft rays in the posterior part, pelvic fins anterior to the pectorals and with one short embedded spine, anal fin with two spines, and one row of comblike teeth on each jaw, fixed or freely movable and generally with some canine teeth, and usually 28-44 vertebrae (Nelson, 2006; Kottelat & Freyhof, 2007). Gene-rally, blennids are benthic fish living in tropical and subtropical waters in the Atlantic, Pacific, and Indian Oceans. Some species are also found in brackish and even freshwater environments (Nelson, 2006). Within the blennids, the genus *Salaria* Forsskål, 1775 has an uncommon ecology in that the four species reported for this genus (Bath, 1986; Zander, 1986; Kottelat & Freyhof, 2007): *S. basilisca* (Valenciennes, 1836), *S. economidisi* Kottelat, 2004, *S. fluviatilis* (Asso, 1801), and *S. pavo* (Risso, 1810), show different ecological requirements. *Salaria fluviatilis* and *S. economidisi* inhabit freshwater habitats, *S. basilisca* lives among seagrass and sometimes on rocky bottoms in marine environments, and *S. pavo* is found in intertidal or in the first meters of subtidal areas, in addition to some populations in brackish waters.

We found adult specimens of *S. fluviatilis* in Moroccan freshwaters which were smaller and had a different head colouration pattern than that of

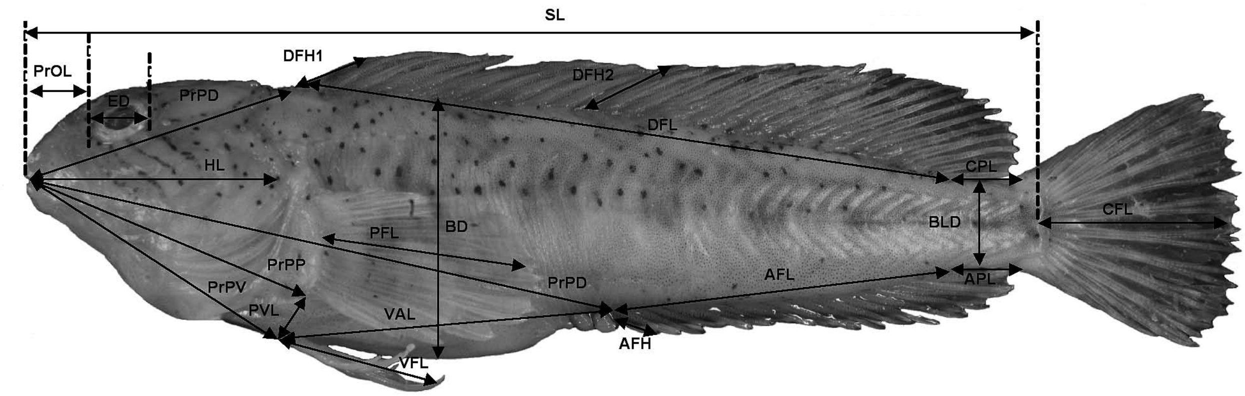

Fig. 1.– Morphometric measurements. Abbreviations correspond to those described in the Material and Methods epigraph.

Fig. 1.– Medidas morfométricas. Las abreviaturas corresponden con aquellas descritas en el epígrafe de Material y Métodos.

other *S. fluviatilis* populations. This Moroccan population, along with that inhabiting the Iberian Guadiana basin, constitute the only known Atlantic slope *Salaria* populations. Morphological and karyological studies over the past forty years have attempted to clarify the taxonomy of *S. fluviatilis* (Springer, 1968; Cataudella & Civitelli, 1975; Bath, 1977; Bock & Zander, 1986). Morphological studies identified Trichonis Lake (Greece) specimens as a separate species, *S. economidisi* Kottelat, 2004. Other approaches have attempted to analyse the molecular variation in *S. fluviatilis* populations (Perdices *et al.*, 2000; Almada *et al.*, 2009). Despite these efforts this variation remains unclear, and the diversity of *Salaria fluviatilis* is not currently well known. Within the molecular framework, a previous study of populations of *S. fluviatilis* throughout its distribution range concluded that "the Moroccan samples must have split more than 2 MYA, and their distinctiveness, both in the mitochondrial and nuclear genes analysed, argues in favour of the recognition of a new species in Morocco" (Almada *et al*., 2009). These authors were not able to separate *S. economidisi* from other populations of *S. fluviatilis*, making the latter species a paraphyletic entity. These were the reasons for conducting a morphological and molecular analysis of the Moroccan *Salaria* population.

The aim of the present study was to compare morphological and molecular aspects of populations of *S. economidisi* and *S. fluviatilis* with the Atlantic population from Morocco, and based on the differences found, to describe the latter as a new species.

Material and Methods

Morphological analyses

The description of the new *Salaria* species was based on 20 adult individuals (4 male and 16 female) from Ouerrha River, Sebou basin, Morocco (Voucher numbers: MNCN 279641-MNCN 279660). Twenty-three morphometric measurements and seven meristic variables were recorded. All morphometric measurements were in millimetres. For comparative purposes, we analyzed 22 individuals (9 male and 13 female) of *Salaria fluviatilis* from the Ebro basin in the Iberian Peninsula (Mediterranean slope) (voucher numbers: MNCN 74739-74758; Ebro R., Spain); 2 individuals (male) from the Guadiana basin in the Iberian Peninsula (Atlantic slope) (voucher numbers: MNCN 270686-270687; Ardila R., Spain); and 1 individual (female) from the Alphios River in Greece (MNCN 120533). Forty individuals (19 male and 21 female) of *S. economidisi* from Trichonis Lake (voucher numbers: MNCN 120747-120787; Greece) were included in the analysis.

Measurements were taken with a digital calliper and follow Doadrio *et al.* (2002). The following abbreviations were used for morphometric and meristic characters (Fig. 1): A, anal fin rays; AFH, anal fin height; AFL, anal fin length; AIO, interorbital wide; APL, anal peduncle length; BD, body

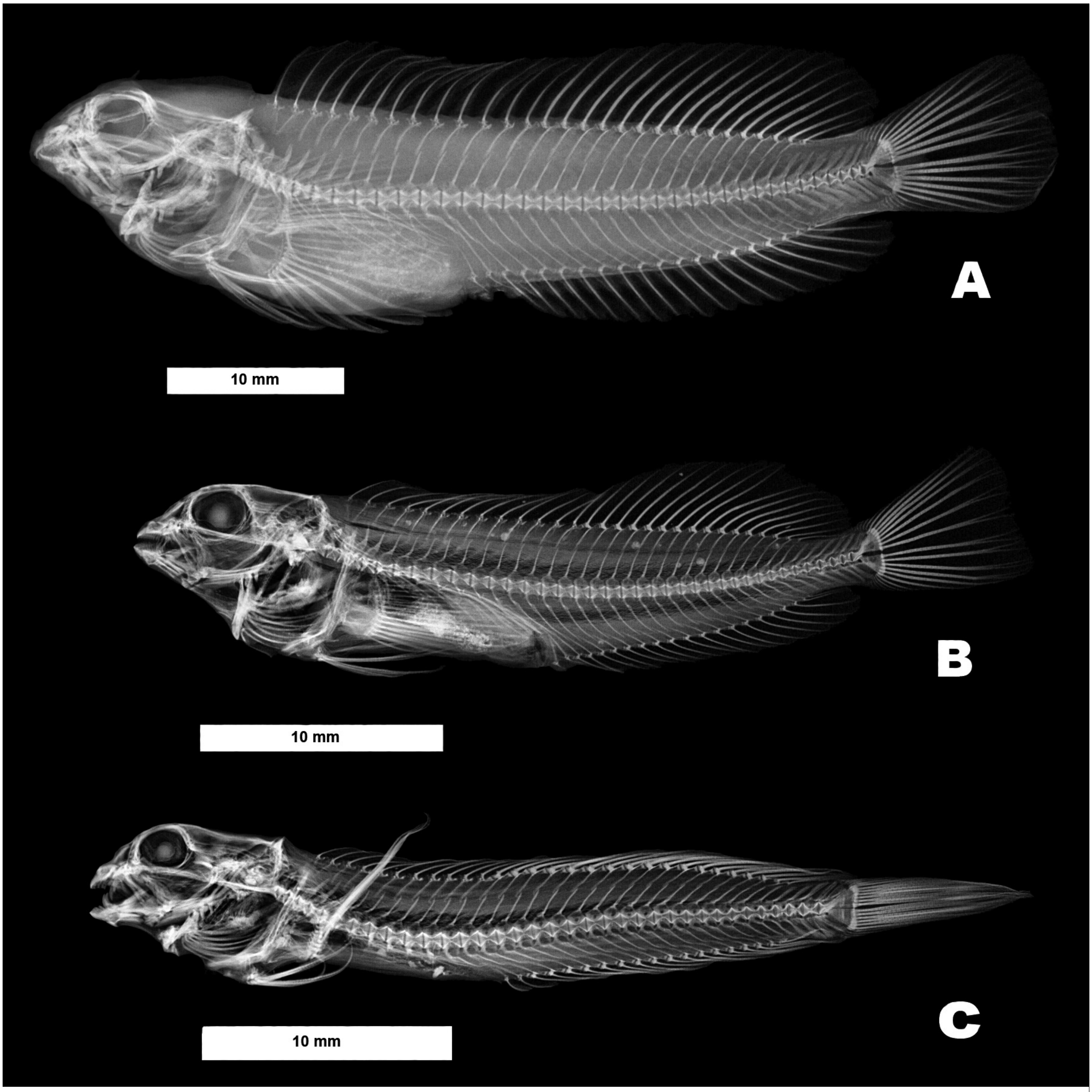

Fig. 2.– X-rays of the three *Salaria* populations analyzed.

Fig. 2.– Radiografías de las tres poblaciones de *Salaria* analizadas.

depth; BLD, body least depth; C, central caudal fin rays; CFL, caudal fin length; CPL, caudal peduncle length; D1, dorsal fin rays in the anterior region of dorsal fin (spiny rays); D2, dorsal fin rays in the posterior region of dorsal fin (soft rays); DFL dorsal fin length; DFH_1 dorsal fin height in the anterior region of dorsal fin (spiny rays); DFH_2 dorsal fin height in the posterior region of dorsal fin (soft rays); ED, eye diameter; HL, head length; P, pectoral fin rays; PFL, pectoral fin length; PrAD, preanal distance; PrDD, pre-dorsal distance; PrOL, pre-orbital length; PrPD, pre-pectoral distance;

Table 1.– Samples included in molecular phylogenetic analyses.

Tabla 1.– Muestras incluidas en los análisis filogenéticos.

Species	Label on phylogenetic trees	Genbank accession number 16S	D-loop	S7	Locality
Salaria pavo	Spavo	FJ465715	FJ465579	FJ465613	Galicia. Spain
Salaria sp.	Souerrha 1	FJ465736	FJ465527	FJ465591	Ouerrha R. Morocco
Salaria sp.	Souerrha 2	FJ465737	FJ465526	FJ465592	Ouerrha R. Morocco
Salaria economidisi	Secon 1	FJ465733	FJ465540	FJ465600	Trichonis L. Greece
Salaria economidisi	Secon 2	FJ465735	FJ465541	FJ465599	Trichonis L. Greece
Salaria fluviatilis	Sfluv 1	FJ465724	FJ465529	FJ465587	Verde R. Spain
Salaria fluviatilis	Sfluv 2	FJ465743	FJ465524	-	Verde R. Spain
Salaria fluviatilis	Sfluv 3	FJ465741	FJ465521	-	Noguera-Pallaresa R. Spain
Salaria fluviatilis	Sfluv 4	FJ465745	FJ465532	FJ465605	Noguera-Pallaresa R. Spain
Salaria fluviatilis	Sfluv 5	FJ465747	FJ465556	FJ465638	Bañolas L. Spain
Salaria fluviatilis	Sfluv 6	FJ465758	FJ465566	-	Bañolas L. Spain
Salaria fluviatilis	Sfluv 7	FJ465744	FJ465533	FJ465596	Matarraña R. Ebro basin. Spain
Salaria fluviatilis	Sfluv 8	FJ465740	FJ465522	-	Matarraña R. Ebro basin. Spain
Salaria fluviatilis	Sfluv 9	FJ465730	FJ465528	FJ465594	Calahorra L. Spain
Salaria fluviatilis	Sfluv 10	FJ465726	FJ465523	FJ465593	Calahorra L. Spain
Salaria fluviatilis	Sfluv 11	FJ465738	FJ465539	FJ465603	Dojranis L. Greece
Salaria fluviatilis	Sfluv 12	FJ465739	FJ465543	FJ465602	Dojranis L. Greece
Salaria fluviatilis	Sfluv 13	FJ465546	FJ465550	FJ465606	Croatia
Salaria fluviatilis	Sfluv 14	FJ465731	FJ465535	FJ465610	Iznik L. Turkey
Salaria fluviatilis	Sfluv 15	FJ465725	FJ465542	FJ465597	Miras R. Greece
Salaria fluviatilis	Sfluv 16	FJ465729	FJ465538	FJ465598	Miras R. Greece
Salaria fluviatilis	Sfluv 17	FJ465734	FJ465525	FJ465590	Zújar R. Guadiana basin. Spain
Salaria fluviatilis	Sfluv 18	FJ465732	FJ465531	FJ465589	Zújar R. Guadiana basin. Spain
Salaria fluviatilis	Sfluv 19	FJ465728	FJ465530	FJ465586	Esteras R. Guadiana basin. Spain
Salaria fluviatilis	Sfluv 20	FJ465718	FJ465567	FJ465625	Israel
Salaria fluviatilis	Sfluv 21	FJ465722	FJ465564	FJ465621	Israel
Salaria fluviatilis	Sfluv 22	FJ465537	FJ465723	-	Çatk t R. Turkey
Salaria fluviatilis	Sfluv 23	FJ465727	FJ465536	FJ465610	Ilica stream. Turkey
Salaria fluviatilis	Sfluv 24	-	-	FJ465585	Tahtal R. Turkey
Salaria fluviatilis	Sfluv 25	-	-	FJ465612	Çak rca R. Turkey

PrVD, pre-ventral distance; PVL, pectoral-ventral length; SL, standard length; V, ventral fin rays; VAL, ventral-anal length; VFL, ventral fin length; Ve, total number of vertebrae. Number of vertebrae was obtained by direct counting on X-rays images of individuals from all populations studied (Fig. 2). (Institutional acronyms: MNCN Museo Nacional de Ciencias Naturales. Madrid, Spain).

After constructing the measurement matrix, Burnaby's method was used to correct size effect (Burnaby, 1966; Rohlf & Bookstein, 1987). All analyses were conducted with the corrected matrix. Morphometric and meristic characters were analyzed independently. A two-way analysis of variance (ANOVA) comparing both morphometric and meristic characters was conducted to test for sexual dimorphism and variation among populations. Because sexual dimorphism analysis was significant, morphometric measurements were analysed separately for males and females. To assess significant population variation for each morphometric and meristic variable in each sex, Kruskal–Wallis analysis of variance (Kruskal-Wallis ANOVA) and Mann-Whitney *post hoc* pairwise comparisons were carried out. To identify the variables that contributed most to the variation among populations, a principal components analysis (PCA) was performed using the covariance matrix for morphometric characters and the correlation matrix for meristic ones. Canonical variate analysis (CVA) was used to maximize differences among populations. A classificatory hypothesis of the groups sug-

Table 2.– Phylogenetic performance for each gene and the combined data set.

Tabla 2.– Parámetros filogenéticos para cada gen y para el conjunto de datos.

Gene	Total characters (including gaps)	Parsimony informative characters (in%)	Ti/tv rate	Evolutionary model selected (AIC criterion)*
16S	600	87	1.98	Model: TVM+G I= 0 G= 0.188
Control Region	420	91	1.32	Model: HKY+G I= 0 G= 0.337
S7	662	22	1.04	Model: HKY I= 0 G= 0

* I = assumed proportion of invariable sites; G= alpha parameter of the gamma-shape distribution.

gested by the PCA was tested by a discriminant function analysis (DFA) with the Hotteling's test. All analyses were conducted with the statistics package PAST (Hammer *et al*., 2001).

Molecular analyses

For molecular approaches, we reanalyzed a subset of the samples published by Almada *et al*. (2009). These authors analyzed an exhaustive sampling from most of the distribution range of *Salaria* species, and suggested the distinctiveness of the Moroccan population. In our study, we analyzed specimens of *S. fluviatilis*, the Moroccan *Salaria* population, and *S. economidisi*. *Salaria pavo* was used as outgroup in all phylogenetic analyses. Samples included in molecular analyses are presented in Table 1.

Analyses included mitochondrial (16S + control region) and nuclear (S7 gene) to independently investigate mitochondrial and nuclear relationships. For all data sets, homologous regions were aligned with Clustal *W* (Thompson *et al*., 1994) implemented in the MEGA package v.4.0 (Tamura *et al*., 2007). Default parameters were allowed for gaps, but alignments obtained were visually checked and verified. In the S7 gene and the control region, unambiguous gaps were discarded in phylogenetic reconstructions.

Nucleotide composition was examined, and the χ^2 test for base homogeneity, carried out in Paup *4.0b10 (Swofford, 2002), indicated that base frequency distribution was always homogeneous among taxa (16S: χ^2 = 3.209864 (df = 87), p = 1.000; Control region: χ^2 = 13.041157 (df = 93), p = 1.000; S7: χ^2 = 3.257056 (df = 75), p = 1.000). The transition/transversion (ti/tv) rate was estimated using a maximum-likelihood approach (Table 2). The Akaike Information Criterion (Akaike, 1973) implemented in jModeltest (Posada, 2008, 2009) was used to determine the evolutionary model that best fit each data set. Phylogenetic ML parameters estimated are summarized in Table 2 and were used for subsequent analyses. In the combined mitochondrial data set each gene partition was allowed to follow its own model of evolution, and congruence between partitions was tested by the Partition Homogeneity test (ILD test, Farris *et al.*, 1994) in PAUP* 4.0b10 (Swofford, 2002), showing non-significant heterogeneity among pairwise partitions (p>0.05). The maximum likelihood method implemented in Phyml (Guindon & Gascuel, 2003) was used to reconstruct phylogenetic relationships between *Salaria* spp. Robustness of the likelihood analysis was assessed by a non-parametric bootstrap test (Felsenstein, 1985) with 1000 replicates. Likewise, Bayesian inference was carried out with MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). For each data set, tree topology was obtained by simulating two simultaneous Markov chain analyses (MCMC) for 1 000 000 generations each to estimate the posterior probabilities distribution. Topologies were sampled every 100 generations, and a majority-rule consensus tree

Table 3.– Two-way analysis of variance (ANOVA) for sexual dimorphism, population variation, and their interaction. Significant differences (*); highly significant differences (**). Abbreviations are described in the Material and Methods epigraph.

Tabla 3.– Análisis de la varianza (ANOVA) de dos vías para dimorfismo sexual, variación poblacional y su interacción. Diferencias significativas (*); diferencias muy significativas (**). Las abreviaturas se describen en el epígrafe de Material y Métodos.

	Sexual dimorphism (f/p-value)	Population variation (f/p-value)	Sex-pop variation (f/p-value)
		Morphometric variables	
SL	25.45/**	21.22/**	2.403/-
HL	0.834/-	15.57/**	1.027/-
PrOL	0.117/-	5.666/**	1.813/-
ED	1.96/-	2.813/-	1.14/-
PrDD	4.919/*	7.168/*	2.102/-
PrPD	11.01/-	18.71/-	0.464/-
PrVD	0.806/-	15.97/**	0.494/-
PrAD	0.0002/-	5.059/**	0.874/-
CPL	0.364/-	2.029/-	1.74/-
APL	0.975/-	0.917/-	0.147/-
PVL	0.003/-	2.072/-	1.514/-
VAL	14.24/**	0.676/-	1.446/-
DFL	2.266/-	7.363/**	0.361/-
DFH_1	8.414/**	6.43/**	3.534/*
DFH_2	3.869/-	0.599/-	0.489/-
PFL	3.781/-	20.03/**	1.884/-
VFL	0.744/-	5.731/**	1.846/-
AFL	2.899/-	22.57/**	0.815/-
AFH	0.804/-	5.799/**	1.799/-
CFL	7.508/**	56.76/**	4.358/*
BD	0.831/-	14.97/**	0.982/-
BLD	0.007/-	22.76/**	2.017/-
AIO	0.012/-	1.781/-	2.298/-
		Meristic characters	
D_1	0.143/-	3.24/*	0.934/-
D_2	0.210/-	0.407/-	1.29/-
A	4.053/*	29.85/**	1.36/-

was estimated after eliminating the first 10% of generations in each analysis.

The average genetic distances among *Salaria* spp. and populations were calculated for each gene using MEGA package v.4.0 (Tamura *et al*., 2007) according to the uncorrected-*p* distances. To estimate genetic distances, gaps and missing data were eliminated from the data set.

Results and discussion

Morphological comparison among populations

Analysis of variance (ANOVA) for sexual dimorphism showed significant differences ($p<0.05$) for several morphometric variables but not for meristic ones (Table 3). These results justify the separation of subsequent morphological analyses by sex. ANOVA analyses showed significant differences ($p<0.05$) among populations in both morphometric and meristic variables (Table 3).

Significant differences between males and females were marked by the following morphometric variables: standard length, pre-dorsal distance, ventral-anal distance, height of the anterior region (spiny) of dorsal fin, and caudal fin length. All these variables were greater for males than for females (Table 4). None of the meristic variables showed significant difference between sexes.

Almost all the morphometric variables showed significant differences between populations in the two-way ANOVA analysis (Table 3): standard and head lengths, pre-orbital distance, pre-dorsal distance, pre-ventral distance, pre-anal distance, the

Table 4.– Morphometric variables showing significant sexual dimorphism ($p < 0.05$). Abbreviations are described in the Material and Methods epigraph.

Tabla 4.– Variables morfométricas que muestran dimorfismo sexual significativo ($p < 0.05$). Las abreviaturas están descritas en el epígrafe de Material y Métodos.

Morphometric variable	Males (mm)	Females (mm)
SL	49.2 (33.6-90.7±15.4)	43.97 (32.9-72.4±10.2)
PrDD	14.1 (8.8-28.2 ± 4.8)	12.5 (8.9-21.7 ± 3.1)
DFH_1	5.1 (2.6-00.7 ± 1.3)	3.8 (2.1-8.3 ± 1.4)
CFL	10.3 (5.8-19.7 ± 3.6)	9.2 (5.9-14.2 ± 2.1)

length of all fins (dorsal, pectoral, ventral, anal and caudal), height of the anterior part (spiny) of dorsal fin, height of anal fin, body depth, and body least depth. Thus, males of the Moroccan *Salaria* population showed a range of standard length (43.8-50.1, mean = 46.7) intermediate between *S. economidisi* (33.6-53.0, mean = 41.9) and *S. fluviatilis* (35.8-90.7, mean = 64.2), but more similar to the former. In females, smaller than males, the range of body size between the Moroccan *Salaria* population and *S. economidisi* overlapped (35.7-46.2, mean = 40.6 and 32.9-45.9, mean = 39.5, respectively) and was smaller than in *S. fluviatilis* (33.8-72.4, mean = 55.7). Similar patterns of sizes ranges for all linear measurements of morphometric variables were found, being greater in *S. fluviatilis* than in the other two taxa examined.

A more fine-scale analysis based on univariate non-parametric Kruskal-Wallis ANOVA and Mann-Whitney *post hoc* comparisons for both sexes (Table 5) demonstrated that significant differences between the three taxa were more pronounced when the proportion of those morphometric variables relative to standard length or other variables such as head length or body depth are analysed independently in the sexes (Table 5). These morphological differences were found even between *S. economidisi* and the Moroccan *Salaria* populations, which are similar in body size and consequently most of the ranges of the linear measurements overlapped, as mentioned.

In body proportions, males of *S. economidisi* presented a higher ratio of body depth to standard length (21.7-24.7% SL) than did *S. fluviatilis* (17.3-22.9% SL) or the Moroccan population (21.5-22.6% SL). The former showed greater head length relative to standard length (HL/SL) compared to that of the other two taxa, being 27.5-27.9% SL in *S. economidisi*, while in *S. fluviatilis* and the Moroccan population, HL/SL was similar, 25.3-26.9% and 25.1-26.5%, respectively. The pre-orbital distance was shorter in the Moroccan *Salaria* population (6.8-8.3% SL and 29.1-31.6% HL) than in either *S. economidisi* (8.9-9.8% SL and 31.9-35.6% HL) or *S. fluviatilis* (8.6-9.7% SL and 34.0-36.2% HL). The head was wider in the Moroccan population and *S. economidisi* than in *S. fluviatilis*, which is reflected by the broader inter-orbital width of the first two taxa relative to HL (20.9-23.3%, 21.3-22.6% and 15.6-21.9%, respectively). The Moroccan population and *S. economidisi* possessed larger eyes relative to HL (27.2-27.8, 25.5-26.7% and 25.3-26.4% respectively) than *S. fluviatilis*. *Salaria economidisi* showed the pectoral and ventral fins in a more posterior position compared to the other two taxa in which the position of these fins, relative to SL, were analogous. Thus, pre-pectoral distance in proportion to SL was 34.2-35.1%, in *S. economidisi*, 29.0-30.2% in *S. fluviatilis*, and 29.7-30.3% in the Moroccan population. The ratio of pre-ventral distance to SL was 30.9-32.4% in *S. economidisi*, 22.2-25.7% in *S. fluviatilis*, and 24.4-27.7% in the Moroccan population. The position of the anal fin was similar in *S. economidisi* and the Moroccan *Salaria* population and in a more anterior position relative to *S. fluviatilis*, being 56.8-61.1%, 57.9-59.1%, and 49.7-56.9%, respectively, in the three taxa. The Moroccan *Salaria* population showed a longer pectoral fin (23.9-24.1% SL) compared to either *S. economidisi* (22.6-22.6% SL) or *S. fluviatilis* (22.3-23.9% SL). *Salaria economidisi* showed longer ventral and shorter caudal fins (ventral: 17.5-19.9% SL; caudal: 17.5-21.3% SL) than *S. fluviatilis* (ventral: 14.2-17.7% SL; caudal: 21.8-22.6% SL) or the Moroccan population (ventral: 14.6-15.5% SL; caudal: 20.7-21.9% SL). *Salaria fluviatilis* (41.7-43.2% SL) presented a longer anal fin than *S. economidisi* (35.7-39.2% SL) or the Moroccan population (36.7-36.9% SL). The anterior region of the dorsal fin was much shorter in the Moroccan population (5.5-8.0% SL and 9.1-11.7% DFL) than in *S. economidisi* (8.6-11.3% SL and 13.7-18.0% DFL) or *S. fluviatilis* (10.6-11.7% SL and 16.9-18.6% DFL). The caudal peduncle was longer in the Moroccan *Salaria* population (BLD: 10.9-12.4% SL) in comparison to *S. economidisi* (11.0-11.1% SL) and *S. fluviatilis* (9.6-9.8% SL) and

Table 5.– Non-parametric one-way analysis of variance (Kruskal-Wallis) and Mann-Whitney's pairwise comparions for both sexes. [1]: significant differences; [2]: very significant differences. Abbreviations are described in the Material and Methods epigraph.

Tabla 5.– Análisis de la varianza no paramétrico (Kruskal-Wallis) y comparaciones dos a dos de Mann-Whitney para ambos sexos. [1]: diferencias significativas; [2]: diferencias muy significativas. Las abreviaturas están descritas en el epígrafe de Material y Métodos.

	Non-parametric Kruskal-Wallis Anova		Mann-Whitney's pairwise comparisons					
	Males H/p	Females H/p	*Salaria* sp. nov.- *S. fluviatilis*		*Salaria* sp. nov.- *S. economidisi*		*S. fluviatilis*- *S. economidisi*	
			Male p	Female p	Male p	Female p	Male p	Female p
			Morphometric variables					
SL	**9.03/0.020[1]**	**9.08/0.010[1]**	**0.016[1]**	**0.010[1]**	0.443	0.493	**0.002[2]**	**0.005[2]**
SL/HL	**22.56/0.0001[2]**	**25.72/0.0001[2]**	0.436	0.318	**0.003[2]**	**0.0001[2]**	**0.0001[2]**	**0.0001[2]**
SL/PrOL	**6.99/0.03[1]**	**21.31/0.0002[2]**	0.179	**0.0002[2]**	**0.010[1]**	**0.0001[2]**	0.224	0.891
HL/PrOL	4.70/0.095	**15.21/0.0004[2]**	0.056	**0.0003[2]**	0.200	0.082	0.160	**0.004[2]**
SL/ED	**17.16/0.0003[2]**	**15.40/0.0004[2]**	0.040[1]	0.090	0.155	**0.007[2]**	**0.0001[2]**	**0.0004[2]**
HL/ED	**7.77/0.020[1]**	**15.21/0.0004[2]**	0.103	**0.0003[2]**	0.839	0.08	**0.007[2]**	**0.0004[2]**
ED/PrOL	**9.85/0.007[2]**	**16.92/0.0002[2]**	**0.028[1]**	**0.0005[2]**	0.598	0.331	**0.004[2]**	**0.0002[2]**
ED/AIO	0.49/0.785	0.13/0.937	0.943	0.834	0.490	0.738	0.730	0.891
HL/AIO	**7.37/0.025[1]**	1.51/0.468	**0.040[1]**	0.500	0.394	0.577	**0.020[1]**	0.235
SL/PrDD	2.24/0.326	0.92/0.630	0.943	0.357	0.180	0.514	0.301	0.782
SL/PrPD	**23.04/0.0001[2]**	**21.70/0.0001[2]**	0.229	**0.013[1]**	**0.0002[2]**	**0.001[2]**	**0.0001[2]**	**0.0001[2]**
SL/PrVD	**23.47/0.0001[2]**	**25.46/0.0001[2]**	0.179	**0.001[2]**	**0.002[2]**	**0.0004[2]**	**0.0001[2]**	**0.0001[2]**
SL/PrAD	**17.66/0.0001[2]**	**18.62/0.0001[2]**	**0.005[2]**	**0.002[2]**	0.113	**0.007[2]**	**0.0001[2]**	**0.0003[2]**
SL/CPL	**11.18/0.003[1]**	3.50/0.173	0.524	0.561	0.113	0.094	**0.001[2]**	0.192
SL/APL	1.04/0.595	0.95/0.620	0.723	0.443	0.903	0.987	0.301	0.360
SL/PVL	**8.58/0.013[1]**	**15.24/0.0005[2]**	0.943	**0.0003[2]**	0.130	0.555	**0.005[2]**	**0.001[2]**
SL/VAL	5.39/0.067	2.38/0.303	0.229	0.693	0.715	0.331	**0.023[1]**	0.134
SL/DFL	1.36/0.506	3.75/0.153	0.943	0.625	0.394	0.115	0.346	0.114
SL/ DFH_1	**14.79/0.0006[2]**	**29.65/0.0001[2]**	**0.005[2]**	**0.0001[2]**	**0.004[2]**	**0.0001[2]**	**0.010[1]**	0.205
DFL/ DFH_1	**12.37/0.002[2]**	**28.69/0.0005[2]**	**0.005[2]**	**0.0001[2]**	**0.005[2]**	**0.0001[2]**	0.051	0.572
SL/ DFH_2	2.95/0.228	**9.02/0.010[1]**	0.179	**0.006[2]**	0.655	0.780	0.148	**0.009[2]**
DFL/ DFH_2	1.89/0.388	4.50/0.105	0.288	**0.030[1]**	0.655	0.961	0.261	0.106
SL/PFL	1.77/0.412	12.52/0.002[2]	0.358	**0.043[1]**	0.208	**0.0002[2]**	0.696	0.891
SL/VFL	**15.34/0.0004[2]**	**10.40/0.005[2]**	0.229	0.561	**0.003[2]**	**0.003[2]**	**0.002[2]**	**0.018[1]**
SL/AFL	**17.95/0.0001[2]**	**14.10/0.0008[2]**	0.028[1]	0.081	0.068	**0.005[2]**	**0.0001[2]**	**0.001[2]**
SL/AFH	**10.59/0.005[2]**	**22.80/0.0001[2]**	0.103	**0.0007[2]**	**0.967**	**0.347**	**0.001[2]**	**0.0001[2]**
AFL/AFH	1.53/0.464	18.18/0.0001[2]	0.358	0.0001[2]	0.490	**0.007[2]**	0.370	**0.009[2]**
SL/CFL	**16.15/0.0003[2]**	**23.95/0.0006[2]**	0.229	0.170	**0.016[1]**	**0.0001[2]**	0.0003[2]	**0.004[2]**
SL/BD	**7.927/0.019[1]**	**17.60/0.0001[2]**	0.943	0.390	**0.038[1]**	**0.0001[2]**	0.002[2]	**0.002[2]**
HL/BD	0.40/0.819	0.12/0.937	0.723	0.944	0.967	0.762	0.566	0.800
SL/BLD	**6.98/0.030[1]**	**11.20/0.003[2]**	0.077	**0.027[1]**	**0.1016[2]**	**0.001[2]**	0.224	0.342
BLD/CPL	**12.24/0.0002[2]**	**9.30/0.009[2]**	0.943	0.090	**0.016[1]**	**0.005[2]**	**0.002[2]**	0.106
BLD/APL	2.69/0.260	2.15/0.341	0.723	0.907	0.394	0.300	0.124	0.167

Meristic characters

	Males + Females H/p	*Salaria* sp. nov.- *S. fluviatilis*	*Salaria* sp. nov.- *S. economidisi*	*S. fluviatilis* - *S. economidisi*
D1	2.49/0.287	0.140	0.643	0.214
D2	0.44/0.800	0.520	0.643	0.785
A	**32.84/0.0001[2]**	**0.0001[2]**	**0.0001[2]**	**0.002[2]**
V	**44.82/0.0001[2]**	**0.0001[2]**	**0.0001[2]**	1

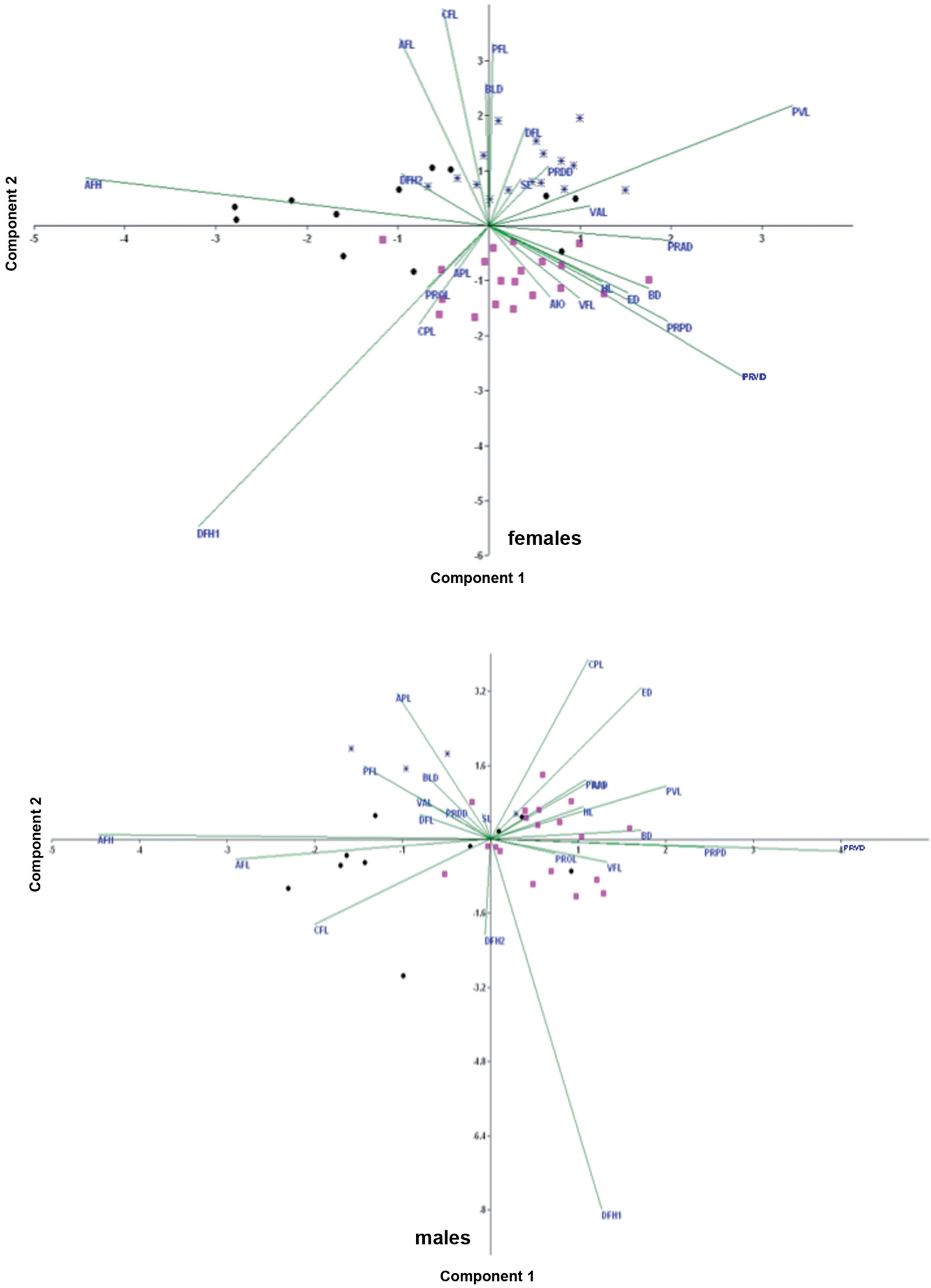

Fig. 3.– Variables that most contributed to the PCA analysis for females (top) and males (bottom). Stars, *Salaria* sp. nov. Squares, *S. economidisi*. Circles, *S. fluviatilis*.

Fig. 3.– Variables que más contribuyen al ordenamiento en el PCA para hembras (arriba) y machos (abajo). Estrellas, *Salaria* sp. nov. Cuadrados, *S. economidisi*. Círculos, *S. fluviatilis*.

Table 6.– Eigenvalues and eigenvectors for the first third principal components (PC1-PC3) from 23 morphometric variables for all *Salaria* species in independent male and female data sets. Abbreviations are described in the Material and Methods epigraph.

Tabla 6.– Eigenvalores y eigenvectores para los tres primeros componentes principales (PC1-PC3) de 23 variables morfométricas para todas las especies de *Salaria* para machos y hembras. Las abreviaturas están descritas en el epígrafe de Material y Métodos.

	Males			Females		
	PC I	**PC II**	**PC III**	**PC I**	**PC II**	**PC III**
Eigenvalue	0.017	0.015	0.011	0.009	0.009	0.008
Percent variation explained	25.42	22.58	16.73	13.65	13.23	12.53
Eigenvectors						
SL	0.01343	0.07098	-0.0219	0.04225	0.08089	-0.03378
HL	0.08117	-0.008743	0.06496	0.1473	-0.09784	0.01654
PrOL	0.06426	-0.03215	-0.00452	-0.08255	-0.1094	-0.2038
ED	0.1272	0.06524	0.3367	0.1819	-0.,1169	-0.1313
AIO	-0.02247	0.0871	-0.03812	0.07752	0.1033	-0.005765
PrDD	0.1505	-0.1589	0.1504	0.2334	-0.1667	-0.05698
PrPD	0.2535	-0.3453	0.2614	0.3394	-0.2683	0.1404
PrVD	0.0828	0.002606	0.1149	0.2336	-0.02614	0.008241
PrAD	0.1327	0.2049	0.3246	-0.09225	-0.1722	0.5427
CPL	-0.06845	0,1646	0.1713	-0.04565	-0.07091	0.6599
APL	0.7577	0.2255	-0,3797	0,3969	0,2073	-0,09112
PVL	-0.2735	-0.06541	0.306	0.1322	0.0333	-0.07835
VAL	-0.04195	0.1198	-0.01997	0.04816	0.1717	-0.1978
DFL	-0.1159	-0.6193	-0.4013	-0.3803	-0.523	-0.3043
DFH_1	-0.1253	-0.2223	-0.05963	-0.1157	0.08867	0.02867
DFH_2	-0.1219	0.1522	0.04835	0.004895	0.3165	0.01882
PFL	0.04271	-0.1511	0.1276	0.1176	-0.1266	-0.1884
VFL	-0.1635	0.2322	-0.2936	-0.1177	0.3239	-0.03549
AFL	-0.2958	0.3585	-0.06883	-0.5281	0.08163	-0.02266
AFH	-0.1279	0.0966	-0.293	-0.06137	0.3761	0.004637
CFL	0.1202	-0.07412	0.09418	0.2091	-0.1098	0.004207
BD	-0.01571	0.1504	-0.07497	-0.003915	0.2463	0.04555
BLD	0.1207	0.000534	0.1701	0.07928	-0.1244	-0.00601

shorter in *S. economidisi* (CPL: 90-98% body least depth [BLD]), being 77.1-79% BLD in the Moroccan population and 67.5-73% BLD in *S. fluviatilis*.

Female *S. economidisi* also showed a greater relative body depth (21.4-23.5% SL) and a longer head (24.9-28.1% SL) than that of *S. fluviatilis* (BD: 16.9-22.5% SL; HL: 21.6-24.8% SL) or the Moroccan *Salaria* population (BD: 19.6-21.1% SL; 24.6-27.1% SL). The Moroccan population presented a shorter pre-orbital length, being 7.5-8.3% SL and 29.5-30.7% HL in the Moroccan population; 8.9-9.4% SL and 29.5-30.7% HL in *S. economidisi* and 8.9-14.7% SL and 37.2-41.1% HL in *S. fluviatilis*.

Unlike males, females of different populations did not show significant differences in head width. Also in contrast to males, females of *S. fluviatilis* presented a bigger eye (25.0-26.0% HL) than either the Moroccan population (26.1-26.7% HL) or *S. economidisi* (29.6-29.6% HL). The position of the fins in females was analogous to that found in males, with pectoral and ventral fins in the most posterior position in *S. economidisi*. Pre-pectoral distance was 27.2-31.4% SL in the Moroccan population, 27.8-30.6% SL in *S. fluviatilis,* and 28.1-33.8% SL in *S. economidisi*, and pre-ventral distance was 24.6-27.5% SL in the Moroccan population, 22.2-25.7% SL in *S. fluviatilis*, and 22.2-33.8% SL in *S. economidisi*.

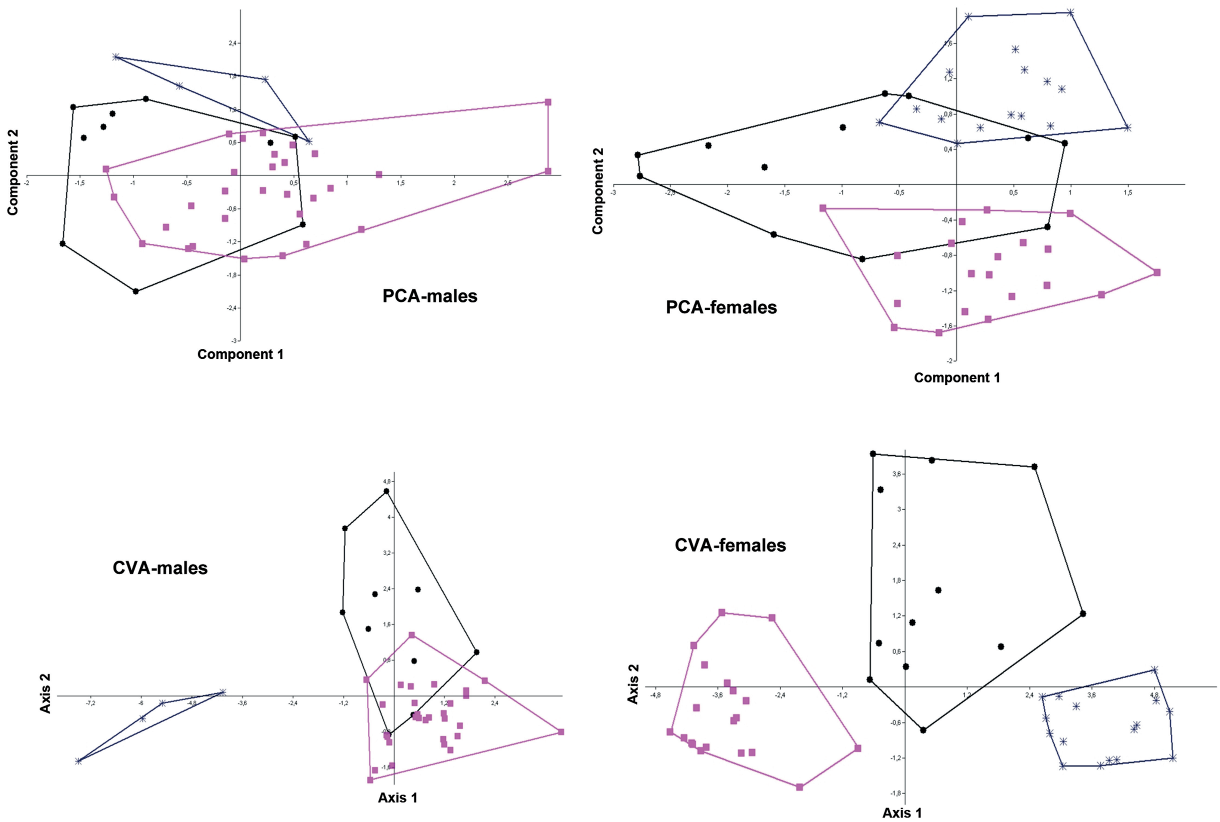

Fig. 4.– Principal component analysis (PCA, top) and Canonical variate analysis (CVA, bottom) for morphometric characters. Males on the left and females on the right. Stars, *Salaria* sp. nov. Squares, *S. economidisi*. Circles, *S. fluviatilis*.

Fig. 4.– Análisis de componentes principales (PCA, arriba) y análisis canónico de la varianza (CVA, abajo) para los caracteres morfométricos. Machos a la izquierda y hembras a la derecha. Estrellas, *Salaria* sp. nov. Cuadrados, *S. economidisi*. Círculos, *S. fluviatilis*.

Similar to males, the anal fin was located in a more posterior position in *S. fluviatilis* (49.7-56.69% SL) than in the two other taxa (54.6-57.9% SL and 53.2-61.0% SL in the Moroccan population and *S. economidisi,* respectively). Females from the Moroccan population also showed longer pectoral fins (24.9-26.5% SL) than either *S. economidisi* (20.1-22.4% SL) or *S. fluviatilis* (22.3-23.9% SL). Ventral fins were shorter in the Moroccan *Salaria* population (15.1-19.2% SL) than either *S. economidisi* (17.0-21.6% SL) or *S. fluviatilis* (17.0-25.7% SL), although ranges overlapped. Also similar to males, anal fin length showed significant differences between *S. fluviatilis* (41.3-43.4% SL) and the Moroccan population (34.4-40.2% SL) and was longer in these two species than in *S. economidisi* (34.6-35.3% SL). The height of the spiny part of the dorsal fin was substantially shorter in the Moroccan population (5.8-7.0% SL and 10.5-10.7% DFL) than in *S. economidisi* (7.3-9.8% SL and 13.0-14.8% DFL) or *S. fluviatilis* (10.0-11.4% SL and 16.3-17.4% DFL). The caudal fin was longer in the Moroccan population females (22.6-23.5% SL) in comparison to the other two species, *S. fluviatilis* (19.6-22.7% SL) and *S. economidisi* (18.3-19.4% SL). Finally, as in males, the caudal peduncle was longer in the Moroccan population (BLD: 10.9-11.0% SL) than in either *S. fluviatilis* (10.3-10.3% SL) or *S. economidisi* (10.2-10.3% SL).

Two meristic variables confirmed the morphological differentiation between populations: number of soft anal fin rays (A) and number of vertebrae (Ve). Thus, the number of soft anal rays and vertebrae was lower in the Moroccan *Salaria*

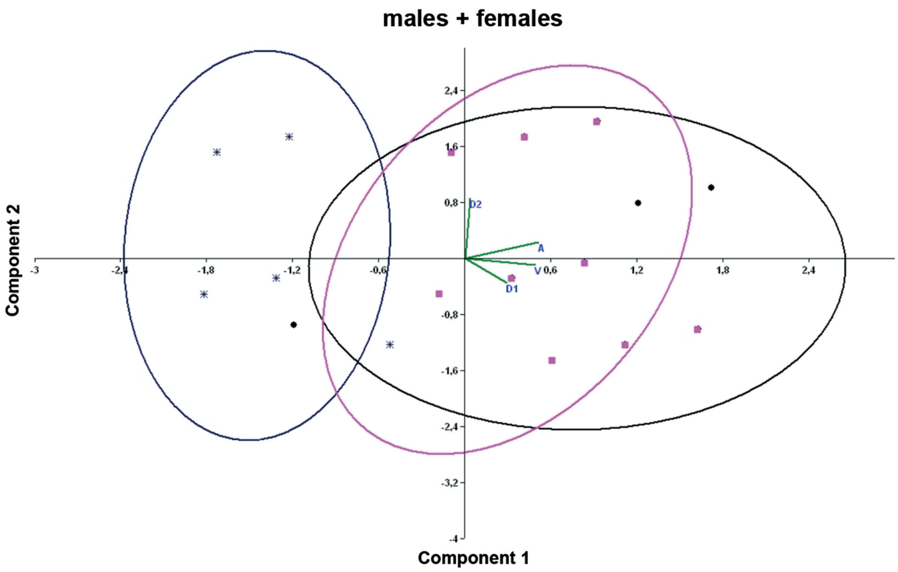

Fig. 5.– Principal component analysis (PCA) for meristic variables in males and females. *Stars, Salaria* sp. nov. Squares, *S. economidisi*. Circles, *S. fluviatilis*.

Fig. 5.– Análisis de componentes principales (PCA) para las variables merísticas en machos y hembras. Estrellas, *Salaria* sp. nov. Cuadrados, *S. economidisi*. Círculos, *S. fluviatilis*.

population (16-17 and 34 respectively) than in the two other species, in which these characters completely overlapped (16-19 and 36). The number of jaw teeth also differed between the Moroccan *Salaria* population (upper jaw: 13-15; lower jaw: 14-16) and the two other freshwater *Salaria* spp. (*S. economidisi*: upper jaw: 25-30, lower jaw: 20-27; *S. fluviatilis*: upper jaw: 16-24, lower jaw: 16-20). Cephalic pores were more conspicuous in the Moroccan *Salaria* population than in the other two *Salaria* species, especially the infra-orbital ones, with 3 in the supra-temporal canal, 7-9 in the pre-opercular canal, and 8-9 in the circum-orbital canal.

With regards to PCA analysis of morphometric measurements, in an initial exploratory analysis, PCI explained 95% of variance in males and 90% in females. This outcome may have been attributed to the influence of fish size (standard length) on the results (Bookstein *et al.*, 1985; Doadrio *et al.*, 2002). After eliminating size, a second PCA with the Burnaby-corrected matrix, attributed 25% of the variance in males to PCI, 17% to PCII, and 13% to PCIII (Fig. 3, Table 6). For females, the percentages of variance explained by the first three PCs are PCI 22%, PCII 14%, and PCIII 12% (Fig. 3, Table 6). The highest values for eigenvectors in both males and females, and, consequently, the variables that contributed most to the ordination in the PCA analysis were anal fin height (AFH), the anterior part of the dorsal fin height, (DFH_1), the pre-ventral

Table 7.– Eigenvalues and eigenvectors for the first third principal components (PC1-PC3) from 4 meristic variables for all *Salaria* species. Abbreviations are described in the Material and Methods epigraph.

Tabla 7.– Eigenvalores y eigenvectores para los tres primeros componentes principales (PC1-PC3) de 4 variables merísticas para todas las especies de *Salaria*. Las abreviaturas están descritas en el epígrafe de Material y Métodos.

	PC I	PC II	PC III
Eigenvalue	1.693	1.060	0.871
Percent variation explained	42.32	26.50	9.38
Eigenvectors			
D1	0.4068	-0.09976	0.8975
D2	0.05485	0.9516	0.0347
A	0.6665	0.1889	-0.1736
V	0.6223	-0.221	-0.4038

Table 8.– Uncorrected-*p* genetic distances between *Salaria* spp. based on mitochondrial 16S gene (1), control region (2) and nuclear S7 gene (3). Between groups (below diagonal) and within group (diagonal).

Tabla 8.– Distancias genéticas no corregidas entre las especies de *Salaria* basadas en el gen mitocondrial 16S (1), la región control (2) y el gen nuclear S7 (3). Entre grupos (debajo de la diagonal) y dentro de grupos (encima de la diagonal).

	***Salaria* sp. nov.**	***S. economidisi***	***S. fluviatilis* (Israeli population)**	***S. fluviatilis* (excluding Israeli population)**	***S. fluviatilis* (including Israeli population)**
***Salaria* sp. nov.**	0.000 (1) 0.000 (2) 0.000 (3)				
S. economidisi	0.051 (1) 0.180 (2) 0.030 (3)	0.000 (1) 0.000 (2) 0.000 (3)			
***S. fluviatilis* (Israeli population)**	0.053 (1) 0.171 (2) 0.033 (3)	0.022 (1) 0.091 (2) 0.011 (2)	0.001 (1) 0.011 (3) 0.000 (3)		
***S. fluviatilis* (excluding Israeli population)**	0.048 (1) 0.180 (2) 0.028 (3)	0.014 (1) 0.057 (2) 0.002 (3)	0.014 (1) 0.089 (2) 0.009 (3)	0.003 (1) 0.019 (2) 0.000 (3)	
***S. fluviatilis* (including Israeli population)**	0.055 (1) 0.179 (2) 0.030 (3)	0.015 (1) 0.062 (2) 0.003 (3)	–	–	0.006 (1) 0.037 (2) 0.014 (3)

distance (PRVD), and the pectoral-ventral length (PVL) (Fig. 4, Table 6).

Meristic characters did not show sexual dimorphism. The variables that most contributed to the ordination in PCA analysis were the number of vertebrae (Ve) and the number of anal fin rays (A). These variables explained 42% of variance in PCI, 26% in PCII, and 22% in PCIII (Fig. 5, Table 7).

Canonical variate analysis (CVA) for each sex (Fig. 3) and the Hotteling's test showed highly significant differences ($p<0.01$) in all the compared groups for both males and females of each species for morphometric characters (data not shown), with the exception of Moroccan males for which Hotteling's test failed, due to the low number of samples. These results corroborate the variation pattern found in the PCA.

Molecular data and remarks on *Salaria* phylogeny

Salaria is characterized by species inhabiting both marine and freshwater environments. Freshwater species have been shown to form a monophyletic entity (Almada *et al.*, 2009) in the absence of a phylogenetic analysis of *S. basilisca*, a marine species not yet molecularly analyzed. In our analysis, of a total of 1713 bp analyzed, 1020 corresponded to mitochondrial DNA (16S gene + D-loop) and 693 to nuclear DNA (S7 gene). Independent phylogenetic analyses rendered congruent topologies demonstrating the molecular differentiation of the Atlantic Moroccan population of *Salaria*, which appeared as a basal group to the other freshwater *Salaria* populations (Fig. 6), and revealing an early divergence of this Moroccan population from the others, as has been previously established (Almada *et al.*, 2009). The high genetic distances demonstrated between the Moroccan population and the other analyzed *Salaria* populations corroborate this statement (Table 8) and support the description of a new species. The greater genetic distances between populations, based on the control region, was 18% between the Moroccan population and both *S. fluviatilis* and *S. economidisi*, whereas genetic distance between *S. economidisi* and *S. fluviatilis,* based on this marker, was 6%. Based on the 16S gene, the values of genetic distances were 5% for the Moroccan *Salaria* population and the other two species and 1% between *S. economidisi* and *S. fluviatilis*. Even the nuclear gene S7 showed comparatively high genetic distances between the Moroccan population and the

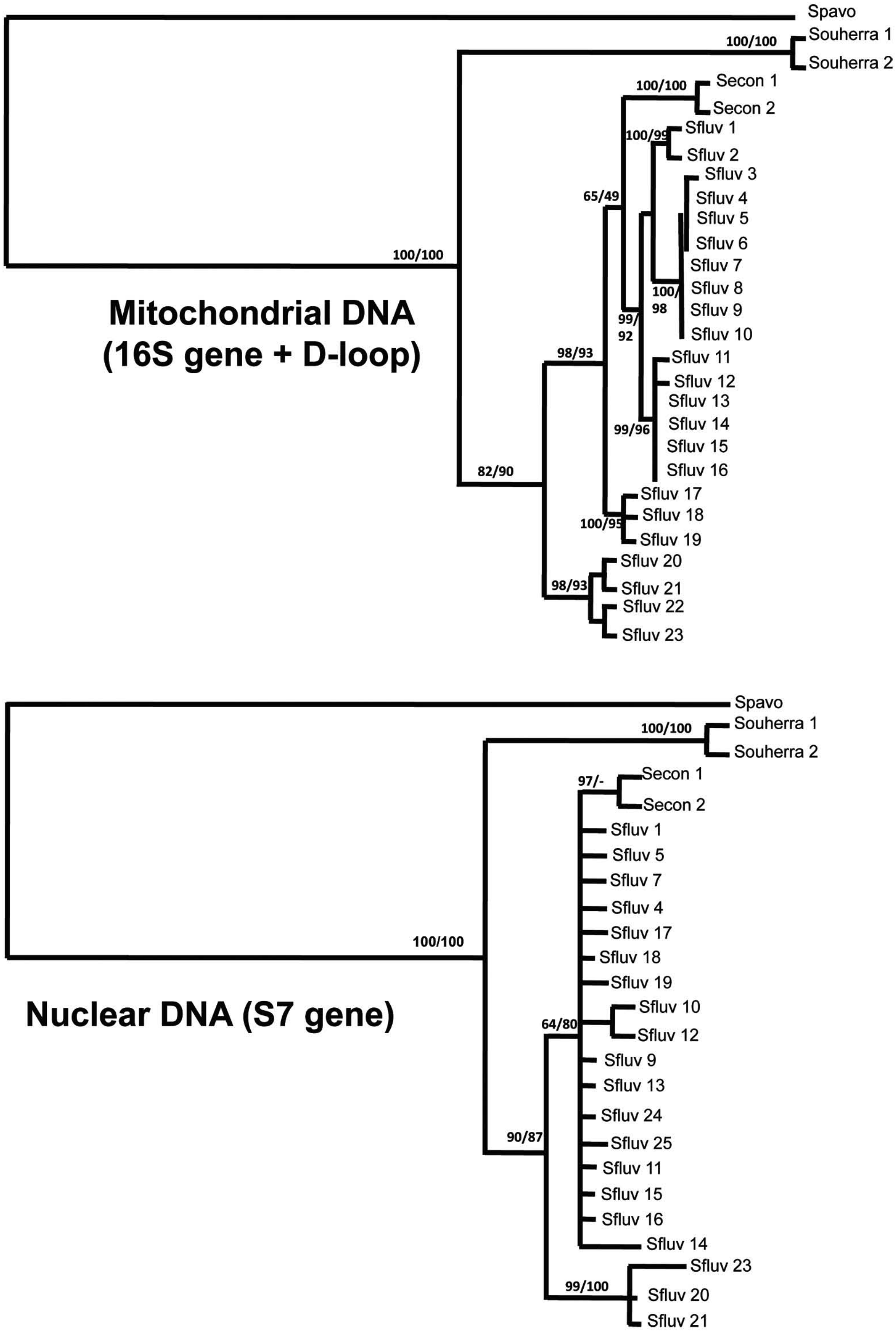

Fig. 6.– Phylogenetic tree rendered by Bayesian Inference of the mitochondrial genes 16S plus control region (above) and nuclear (below) data sets. Numbers on branches indicate posterior probabilities values (before slash) and bootstrap values (after slash) for Bayesian Inference and Maximum Likelihood respectively.

Fig. 6.– Árbol filogenético obtenido por Inferencia Bayesiana para los conjuntos de datos mitochondrial (16S y control region) (arriba) y nuclear (abajo). Los números en las ramas indican los valores de probabilidad posterior (antes de la barra oblicua) y de bootstrap (después de la barra oblicua) para Inferencia Bayesiana y Maximum Likekihood respectivamente.

Table 9.– Uncorrected-*p* genetic distances between *Salaria fluviatilis* populations based on mitochondrial 16S gene (1), control region (2) and nuclear S7 gene (3). Between groups (below diagonal) and within group (diagonal).

Tabla 9.– Distancias genéticas no corregidas entre las poblaciones de *Salaria fluviatilis* basadas en el gen mitocondrial 16S (1), la región control (2) y el gen nuclear S7 (3). Entre grupos (debajo de la diagonal) y dentro de grupos (encima de la diagonal).

	Israeli/some Turkish populations	Zujar and Esteras Rivers	Verde River	Iberian Mediterranean basins	Mediterranean non Iberian populations	*Salaria economidisi*
Israeli/some Turkish populations	0.001 (1) 0.011 (2) 0.000 (3)					
Zujar and Esteras Rivers	0.013 (1) 0.074 (2) 0.010 (3)	0.000 (1) 0.002 (2) 0.000 (3)				
Verde River	0.013 (1) 0.087 (2) 0.010 (3)	0.007 (1) 0.033 (2) 0.000 (3)	0.000 (1) 0.000 (2) - (3)			
Iberian Mediterranean basins	0.015 (1) 0.092 (2) 0.010 (3)	0.009 (1) 0.046 (2) 0.000 (3)	0.002 (1) 0.022 (2) 0.000 (3)	0.000 (1) 0.000 (2) 0.000 (3)		
Mediterranean non Iberian populations	0.013 (1) 0.090 (2) 0.010 (3)	0.007 (1) 0.034 (2) 0.000 (3)	0.000 (1) 0.003 (2) 0.000 (3)	0.002 (1) 0.021 (2) 0.000 (3)	0.000 (1) 0.002 (2) 0.000 (3)	
Salaria economidisi	0.022 (1) 0.091 (2) 0.011 (3)	0.016 (1) 0.057 (2) 0.009 (3)	0.013 (1) 0.056 (2) 0.009 (3)	0.014 (1) 0.064 (2) 0.009 (3)	0.013 (1) 0.051 (2) 0.009 (3)	0.000 (1) 0.000 (2) 0.000 (3)

other two taxa at approximately 2-3% for both pairwise comparisons, while the genetic distance based on this marker between *S. economidisi* and *S. fluviatilis* was 0.3%. Although there is no consensus on the level of genetic distance between populations necessary to consider them as separate species, this level of mitochondrial genetic distance has been found in other freshwater fish only at the species level (Ornelas-García *et al*., 2008; Doadrio *et al.*, 2009).

Our molecular results confirm that the population of *Salaria* from the Sebou basin in Morocco constitutes a different species. Other divergent populations from Israel and some streams in Turkey, which showed a range of 7 to 9% genetic distance from other *S. fluviatilis* populations, should be morphologically studied. *Salaria fluviatilis* populations showed a high genetic distance range within group (0.6% in 16S gene, 3.7% in control region and 1.4% in S7 gene), if we include the Israeli/Turkish stream population. Almada *et al*. (2009) estimated the divergence time of this latter population from the remaining *S. fluviatilis* populations and *S. economidisi* to be in the Lower Pleistocene (1.2-2.4 MYA). These authors have suggested that the *Salaria* population from Kinneret Lake (Israel) could be specifically distinct. This points to the need to investigate the diversity of *S. fluviatilis*. In the present study, *S. fluviatilis* populations showed a paraphyletic status due to the phylogenetic position of *S. economidisi*, nested within the *S. fluviatilis* clade and corroborate the results found in a previous molecular study (Almada *et al*., 2009), which questioned the taxonomical level of *S. economidisi*. In the current study, genetic distances between *S. economidisi* and other *S. fluviatilis* populations (excluding that from Israel and some Turkish streams) were found to be 0.9-1.1% for S7 nuclear gene and close to 1% for all mitochondrial pairwise comparisons (Table 9).

Excluding the clade formed by the Israeli and some Turkish stream populations of *S. fluviatilis*, the remaining populations showed a well-defined mitochondrial, but not nuclear, genetic structure. Within the mitochondrial topology, populations from the Zujar and Esteras rivers (Guadiana basin) constitute a separate group from the remaining pop-

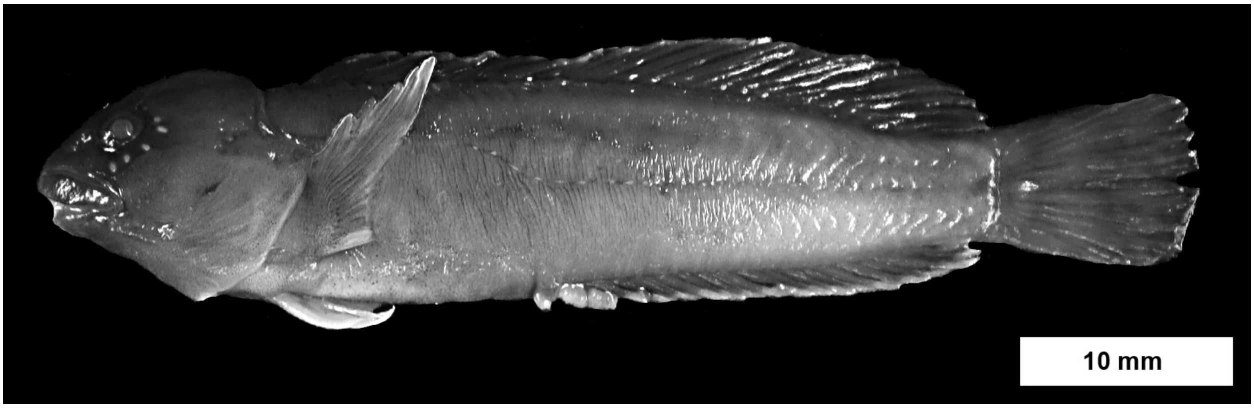

Fig. 7.– Holotype for *Salaria atlantica* sp. nov. Holotype (MNCN 279641). Ouerrha river (Sebou basin, Northern Morocco).

Fig. 7.– Holotipo de *Salaria atlantica* sp. nov. Holotipo (MNCN 279641). Río Ouerrha (cuenca del Sebou, Norte de Marruecos).

ulations of *S. fluviatilis* (Mediterranean Iberian and Mediterranean non Iberian populations) and *S. economidisi*. The basal relationship of these two clades was not resolved and formed a polytomy along with *S. economidisi*. This politomy was more evident within the nuclear topology. The highest mitochondrial genetic distances between *S. fluviatilis* populations were those found between the Guadiana populations and the others (3-4% for control region and 0.7-0.8% for 16S; Table 9); in contrast, nuclear genetic distances were equal to zero between these populations.

DESCRIPTION OF THE MOROCCAN *SALARIA* POPULATION

The high degree of morphological and genetic differentiation of the Moroccan *Salaria* specimens from the Sebou basin justifies the consideration of this population as a distinct species. No available name for this population exists, and therefore a new species is described.

***Salaria atlantica* sp. nov.**

Holotype: Fig. 7, Table 1 and 10. MNCN 279641; male, 45.31 mm (SL); Ouerrha R. Sebou basin, Khénichèt, Morocco; IV/07/2007. Holotype has been deposited at the Fish Collection of the Museo Nacional de Ciencias Naturales (Madrid, Spain).

Paratypes: Table 10. MNCN 279642-MNCN 279660. Ouerrha R. Sebou basin, Khénichèt, Morocco. IV/07/2007. The series of paratypes (19 specimens) has been deposited at the Fish Collection of the Museo Nacional de Ciencias Naturales (Madrid, Spain).

DIAGNOSIS: Differs from other known species of *Salaria* by the following combination of characters: head without diagonal rows of black dots on the cheeks running backwards and downwards from the lower edge of the eye as in *S. economidisi* and *S. fluviatilis* (Fig. 8); number of teeth 13-15 on upper jaw and 14-16 on lower; supra-temporal canal with 3 cephalic pores; pre-opercular canal with 7-9 cephalic pores; circum-orbital canal with 8-9 cephalic pores (Fig. 9). Supra-ocular tentacle thick and simple; 16-17 branched rays in the anal fin (16-19 in both *S. fluviatilis* and *S. economidisi*); short pre-orbital distance (cephalic index (PrOL/HL x10): 3.2-3.4 in males and 2.9-3.8 in females); short anterior region (spiny) of the dorsal fin (dorsal fin index (DFH_1/DFL x10) (0.8-1.2 in males and 0.9-1.3 in females); 34 vertebrae. Differences of diagnostic characters between all *Salaria* populations analyzed are represented in Table 11.

DESCRIPTION: D1 XII-XIII, D2 16-17 A II 16-17, P I 10(11), V II 2-4, C 12. A small species that rarely reaches 60 mm. Maximum body depth is 21.7-24.7% SL in males and 19.6-21.1% SL in females. Head is wider in males, head length is similar in sexes, reaching 25.1-26.5% SL in males and 24.6-27.1% SL in females. The head length is greater than maximum body depth. Pre-orbital distance is less relative to the other freshwater *Salaria* spp., reaching 29.1-31.6% HL in males and 29.5-30.7% HL in females. Simple (unbranched) thick supra-ocular tentacle shorter than eye diameter.

Table 10.– Morphometric and meristic characters of *Salaria atlantica* sp nov. type series. Measurements are presented as raw data (mm). Abbreviations are described in the Material and Methods epigraph.

Tabla 10.– Caracteres morfométricos y merísticos de la serie tipo de *Salaria atlantica* sp. nov. Las medidas se presentan como datos directos (mm). Las abreviaturas se describen en el epígrafe de Material y Métodos.

	***Salaria atlantica* sp. nov.**			
	Holotype	**Paratypes (n= 19)**		
Morphometric variables	**Measurement (mm)**	***Range***	***Mean***	***Standard Deviation***
SL	50.11	35.73-47.42	41.44	3.60
HL	13.36	8.84-12.77	10.44	1.02
PrOL	4.21	2.67-4.24	3.39	0.42
ED	3.07	2.37-3.45	2.94	0.32
PrPP	14.79	10.06-14.32	11.92	1.17
PrPD	15.22	9.73-14.75	12.36	1.63
PrVD	13.9	8.8-12.95	10.98	1.43
PrAD	26.02	19.29-27.26	24.15	2.22
CPL	4.99	2.43-5.2	3.51	0.84
APL	5.08	2.3-5.18	3.52	0.87
PVL	3.65	2.63-4.45	3.76	0.52
VAL	17.04	7.22-18.32	14.74	2.40
DFL	34.19	20.09-30.65	26.67	2.84
DFH_1	3.96	2.11-3.68	2.78	0.38
DFH_2	8.39	4.16-7.38	5.96	0.82
PFL	12.14	8.9-10.42	10.41	1.07
VFL	7.58	5.37-8.97	7.02	1.01
AFL	18.46	12.3-18.8	15.7	1.84
AFH	5.54	1.8-5.51	3.08	0.86
CFL	10.2	8.06-11.02	9.30	0.87
BD	10.79	7-10.44	8.82	1.08
BLD	6.2	3.93-5.38	4.53	0.47
AIO	3.11	1.33-3.08	2.22	0.41
Meristic characters	**Number**	***Range***	***Median***	***Standard Deviation***
Anterior dorsal fin rays	12	12-13	12	0.22
Posterior dorsal fin rays	16	16-17	16	0.47
Anal dorsal fin rays	16	16-17	17	0.34

Conspicuous cephalic pores on sensory canal, especially infra-orbital. Supra-temporal canal with 3 cephalic pores; pre-operculo-mandibular canal with 7-9 cephalic pores; orbital canal with 8-9 cephalic pores (Fig. 9). Blue cephalic pores and blue blotches in gular region. 19-22 teeth on upper jaw and 13-15 teeth on lower jaw. Ventral fins are inserted anterior to origin of the dorsal fin. Pre-ventral length is 86.3-92.7% pre-dorsal length in males and 87.1-95.5% in females. Pectoral fin is 23.9-24.1% SL in males and 24.9-26.5% in females. Anterior dorsal fin height is 9.1-11.7% DFL in males and 10.5-10.7% in females. High caudal penduncle (minimum body depth 10.9-12.4% SL in males and 10.9-11.0% SL in females). 34 vertebrae. Morphometric and meristic measurements for the holotype and the paratypes of the newly described species are represented in Table 10.

DISTRIBUTION: the new species is endemic to Morocco, inhabiting the Ouerrha River in the Sebou River basin (Fig. 10).

ETYMOLOGY: The species name “*atlantica*” has been selected because its range covers the Sebou River basin in Morocco that drains into the Atlantic slope.

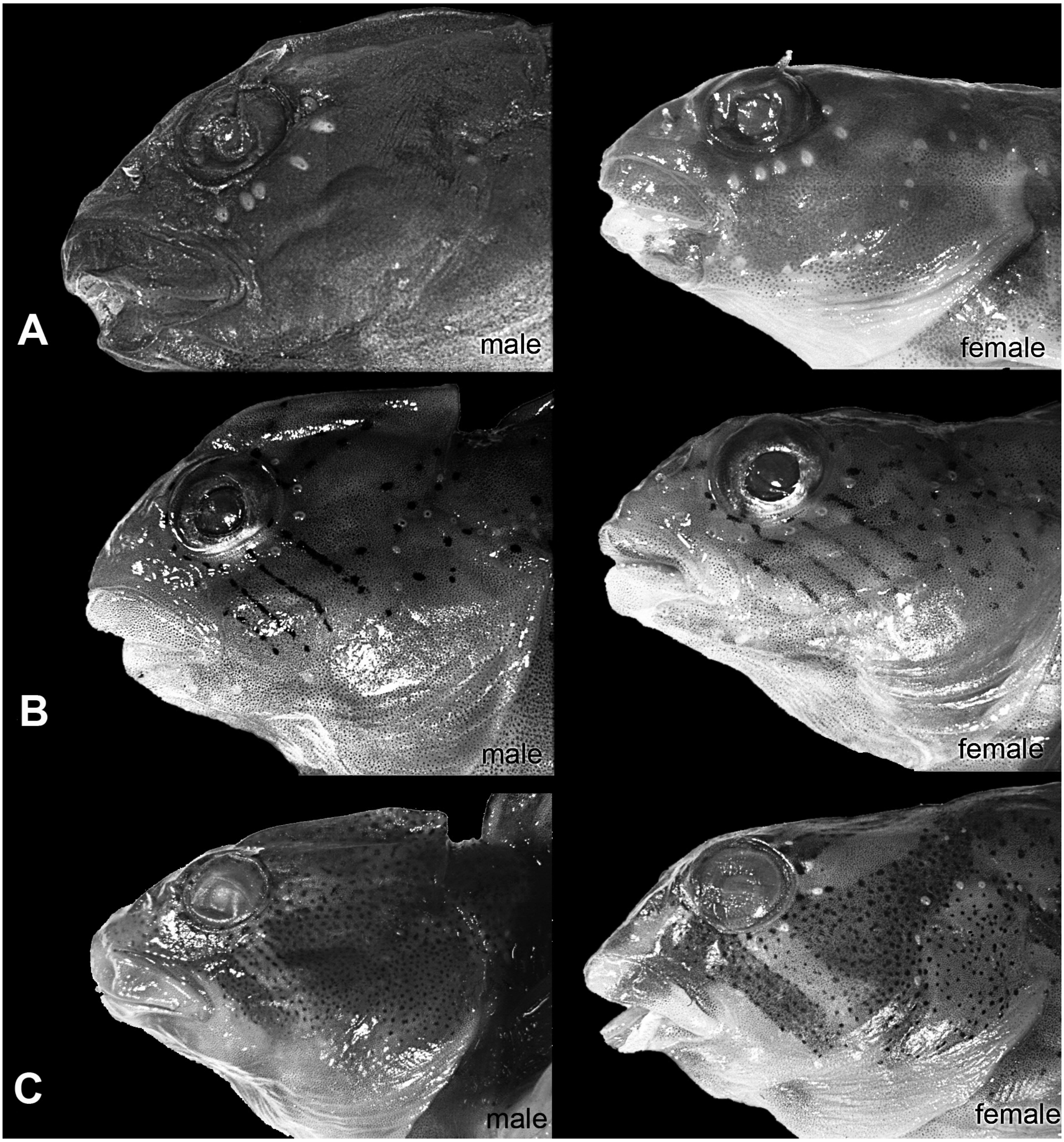

Fig. 8.– Colouration patterns of the head of the three freshwater blennid species. A. *Salaria atlantica* sp. nov.; B. *Salaria economidisi*; C. *Salaria fluviatilis*.

Fig. 8.– Patrón de coloración de la cabeza de las tres especies de blenios de agua dulce. A. *Salaria atlantica* sp. nov.; B. *Salaria economidisi*; C. *Salaria fluviatilis*.

Table 11.– Summary of diagnostic morphometric and meristic characters of *Salaria atlantica* sp. nov. and differences with *S. economidisi* and *S. fluviatilis*.

Tabla 11.– Resumen de los caracteres diagnósticos morfométricos y merísticos de *Salaria atlantica* sp. nov. y diferencias con *S. economidisi* y *S. fluviatilis*.

Characters	*Salaria atlantica* sp. nov.	*Salaria economidisi*	*Salaria fluviatilis*
Teeth jaw	Upper jaw: 13-15 Lower jaw: 14-16	Upper jaw: 25-30 Lower jaw: 20-27	Upper jaw: 16-24 Lower jaw: 16-20
Pre-opercular pores	8-9	(9)10-11	9-10
Colouration pattern	Without dark dots or blotches on cheeks. Blue cephalic pores and blue blotches in gular region	From three to five row of bold black dots on cheeks. Without blue cephalic pores and blue blotches in gular region	A broad diagonal band of tiny dots on cheeks. Without blue cephalic pores and blue blotches in gular region
Circum-orbital pores	8-9	8	8
Pre-orbital distance (cephalic index)	Males: 3.2-3.4 Females: 2.9-3.8	Males: 2.9-4.0 Females: 2.9-4.0	Males: 3.4-4.8 Females: 3.2-4.0
Height of the anterior (spiny) part of the dorsal fin (dorsal fin index)	Males: 0.9-1.3 Females: 0.8-1.2	Males: 1.2-2.0 Females: 0.1-1.7	Males: 1.2-2.1 Females: 1.5-2.0
Anal soft rays	16-17	16-19	16-19
Vertebrae	34	36	36

COMMON NAME: we propose using the English common name “Moroccan blenny” for this new species.

HABITAT: The species inhabits streams with shallow and running waters and stony substrata.

CONSERVATION: *Salaria atlantica* sp. nov. is restricted to a small area in the Sebou River basin in Morocco. The singularity of this species, as one of the two known Atlantic populations within the freshwater representatives of the genus *Salaria*, and the fact that it is restricted to a single basin, confers to *Salaria atlantica* sp. nov. the need of effective protection. For this reason, we consider that this species should be included in the IUCN category Endangered (EN), based on IUCN criteria (IUCN, 2001).

GENETICS: Genetic distance between *Salaria atlantica* sp. nov. and both *S. economidisi* and *S. fluviatilis* for each gene analyzed were 18% based on the control region, 5% based on 16S gene, and 2-3% based on S7 gene. These values support early divergence of *Salaria atlantica* sp. nov.

Key to species of freshwater blennids

1.1. No rows of bold or tiny black dots on cheeks; supra-ocular tentacle thick and simple above eye; blue cephalic pores and blue blotches in the gular region; less than 16 teeth on upper jaw .... ***Salaria atlantica***
1.2. Rows of small black dots on cheeks; no blue cephalic pores; no blue blotches in the gular region; more than 16 teeth on upper jaw 2

2.1. 3-5 diagonal rows of bold black dots on cheeks; supra-ocular tentacle above eye simple (sometimes branched); 25-30 teeth on upper jaw .... ***Salaria economidisi***
2.2. A diagonal row of small black dots on cheeks; supra-ocular tentacle above eye branched; 16-24 teeth on upper jaw .... ***Salaria fluviatilis***

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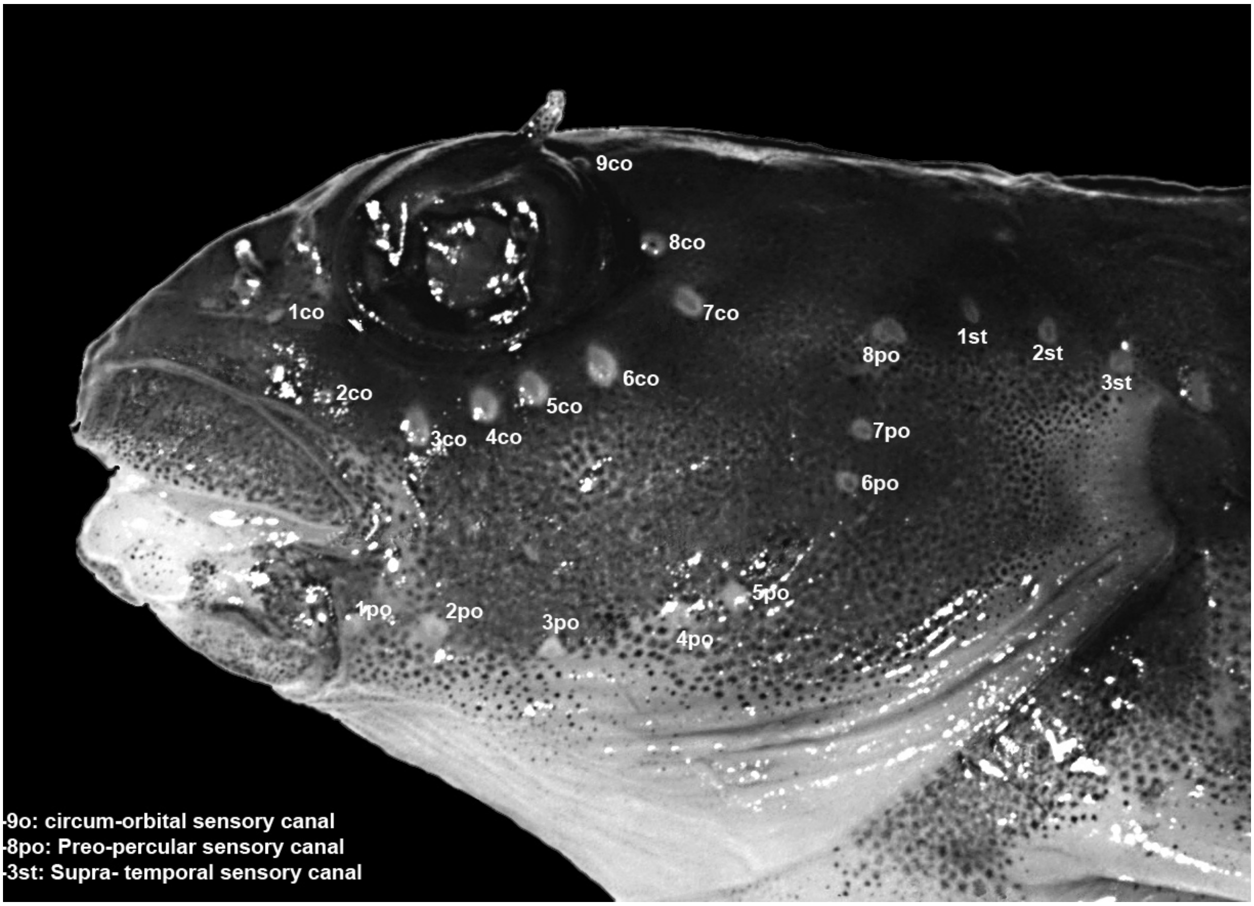

Fig. 9.– Disposition of cephalic pores on sensory canals in *Salaria atlantica* sp. nov.

Fig. 9.– Disposición de los poros sobre los canales sensoriales cefálicos en *Salaria atlantica* sp. nov.

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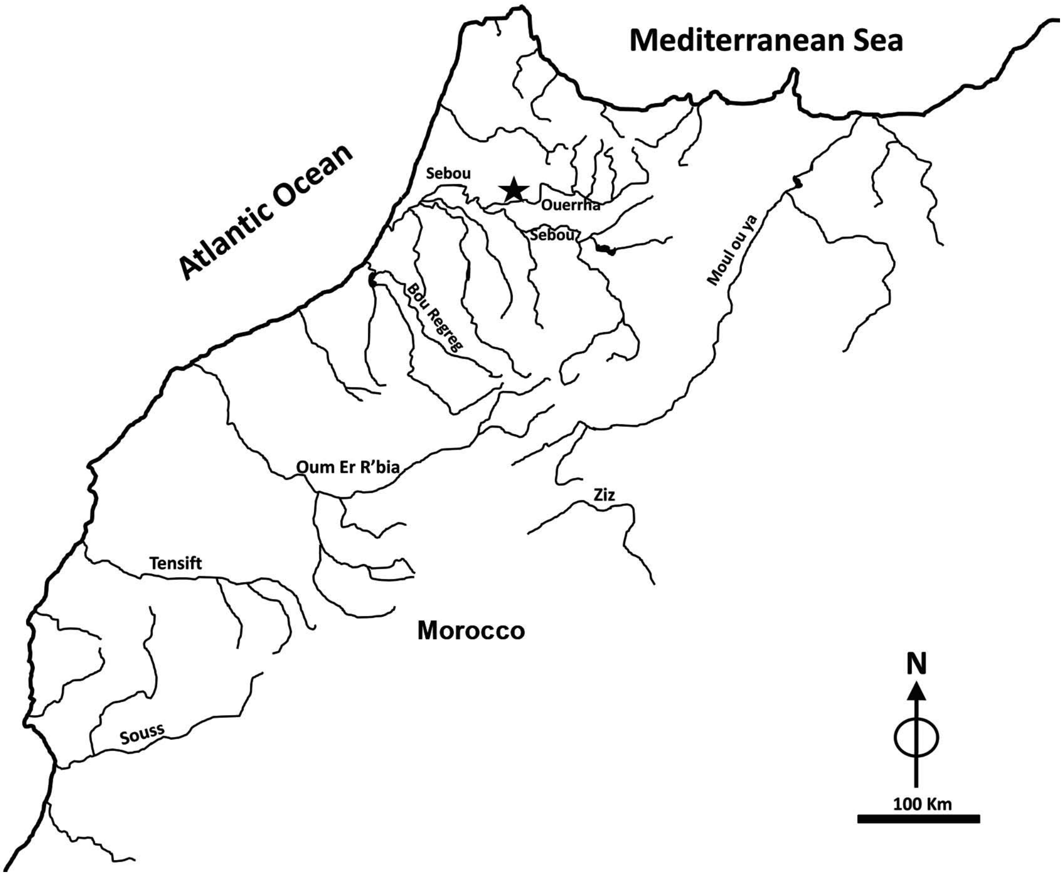

Fig. 10.– Distribution of *Salaria atlantica* sp. nov. in Ouerrha river (Sebou basin, Northern Morocco).

Fig. 10.– Distribución de *Salaria atlantica* sp. nov. en el río Ouerrha (cuenca del Sebou, Norte de Marruecos).

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