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TAXONOMIC REMARKS ON *BARBUS MOULOUYENSIS* PELLEGRIN, 1924 (ACTINOPTERYGII, CYPRINIDAE) WITH THE DESCRIPTION OF A NEW SPECIES OF *LUCIOBARBUS* HECKEL, 1843 FROM MOROCCO

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ABSTRACT

The taxonomy of *Barbus* Cuvier and Cloquet, 1816 has been reviewed in the last years and as consequence some species traditionally included in genus *Barbus sensu lato* have been assigned to different genera. In North Africa the species of the former genus *Barbus* have been included in the genera *Luciobarbus* Heckel, 1843, *Carasobarbus* Karaman, 1971 and *Enteromius* Cope, 1867. We studied populations of the former genus *Barbus* of the Moulouya river basin in Morocco through molecular, morphometric, and osteological data. Our data clearly showed that populations from Moulouya river basin described originally as *Barbus moulouyensis* Pellegrin, 1924 belong to the genus *Carasobarbus* and not to *Luciobarbus*. Moreover, populations of the genus *Luciobarbus* exist in the Moulouya river basin and could not be assigned to any previously described species. Consequently, we describe a new *Luciobarbus* species from the Moulouya river basin.

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Key words: North Africa; *Luciobarbus*; Systematics; mtDNA; morphology.

RESUMEN

Consideraciones taxonómicas sobre *Barbus moulouyensis* Pellegrin, 1924 (Actinopterygii, Cyprinidae) con la descripción de una especie nueva de *Luciobarbus* Heckel, 1843 de Marruecos

En los últimos años ha sido revisada la taxonomia de *Barbus* Cuvier and Cloquet, 1816 y como consecuencia de esta revisión algunas especies incluidas tradicionalmente en el género *Barbus sensu lato* han sido asignadas a otros géneros. En el Norte de África las especies del antiguo género *Barbus* han sido adscritas a los géneros *Luciobarbus* Heckel, 1843, *Carasobarbus* Karaman, 1971 y *Enteromius* Cope, 1867. Nosotros estudiamos las especies del antiguo género *Barbus* en la cuenca del río Moulouya en Marruecos a través de datos moleculares, morfométricos y osteológicos. Nuestros datos muestran claramente que las poblaciones de la cuenca del río Moulouya descritas como *Barbus moulouyensis* Pellegrin, 1924 pertenecen al género *Carasobarbus* y no al género *Luciobarbus*. Sin embargo, poblaciones del género *Luciobarbus* existen en la cuenca del río Moulouya y no pudieron ser asignadas a ninguna de las especies previamente descritas. En consecuencia nosotros describimos una nueva especie de la cuenca del río Moulouya.

Palabras clave: África del Norte; Sistemática; Morfología; mtDNA; *Luciobarbus*.

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Introduction

The freshwater fish fauna of North Africa is mainly characterized by the presence of barbel species inhabiting different habitats that drain to Mediterranean and Atlantic Sea or to endorheic lagoons (Doadrio, 1994). Those species can be grouped by their different levels of ploidy in diploids, tetraploids and hexaploids species. All of them were traditionally assigned to the genus *Barbus* Cuvier and Cloquet, 1816 (Pellegrin, 1921; Estève, 1947; Almaça, 1966, 1968, 1970).

Posterior phylogenetic studies based on morphological and molecular traits have placed the diploids, tetraploids and hexaploids barbel species from North Africa in different genera (Machordom & Doadrio, 2001a; Levin *et al.,* 2012; Borkenhagen & Krupp, 2013; Casal-López *et al*., 2015; Yang *et al*., 2015; Beshera *et al*., 2016). Thus, diploid species were placed in the genus *Enteromius* Cope, 1867, tetraploids in *Luciobarbus* Heckel, 1843 and all hexaploid species in *Carasobarbus* Karaman, 1971, except "*Barbus*" *reinii* Günther, 1874 that remains without a clear generic assignation.

In Morocco, the genera *Luciobarbus*, *Carasobarbus* and the enigmatic "*Barbus*" *reinii* are only present (Beshera *et al*., 2016). The genus *Luciobarbus* is composed of two different group of species: reophilic species of small body size and limnetic species of medium-large size (Doadrio, 1990; Doadrio *et al*., 2016). The genus *Carasobarbus* in Morocco is constituted by *C. fritschii* (Günther, 1874), a cosmopolitan species of small body size, and *C. harterti* (Günther, 1901), a species of large body size that inhabit only the large basins of the Atlantic slope from Morocco (Borkenhagen & Krupp, 2013).

The Moulouya River with 530 km in length and with a basin surface of 54,500 Km^2 represents the largest river of Morocco. Its sources are placed in the Atlas Mountains and flows into Mediterranean Sea near the Algerian border. In Moulouya Basin an endemic species, *Barbus moulouyensis* Pellegrin, 1924, was described on the basis of the morphological traits of one single individual of *Carasobarbus*. However some traits of "*Barbus*" *moulouyensis* as the morphology of the scales or the last dorsal fin ray denticulated were typical characters of genus *Luciobarbus* not of *Carasobarbus* (Doadrio, 1990; Borkenhagen & Krupp, 2013). Thus, the presence of traits of *Carasobarbus* and *Luciobarbus* placed "*Barbus*" *moulouyensis* in an uncertain taxonomic position.

Laterly, two varieties of "*Barbus*" *moulouyensis* were described, also with one single specimen for each variety: "*Barbus*" *moulouyensis* var. *grandisquamata* Pellegrin, 1930 from Tensift Basin and "*Barbus*" *moulouyensis* var. *bouramensis* Pellegrin, 1939 from Oum er Rbia Basin, both on the Atlantic slope of Morocco. Therefore, none of these varieties were found in the Moulouya River Basin. Currently, the fish fauna from Tensift and Oum er Rbia basins is considered to be comprised by the following barbel species: *Luciobarbus magniatlanti*s (Pellegrin, 1919); *Luciobarbus zayanensis* Doadrio, Casal-López and Yahyaoui, 2016; *Luciobarbus ksibii* (Boulanger, 1905); *Carasobarbus fritschii* (Günther, 1874) and *Carasobarbus harterti* (Günther, 1901) (Borkenhagen & Krupp, 2013; Geiger *et al.,* 2014; Doadrio *et al*., 2016). On the basis of three individuals, one of each variety, the differences found between typical "*Barbus*" *moulouyensis* from Moulouya Basin and its varieties "*Barbus*" *moulouyensis* var. *grandisquamata* and "*Barbus*" *moulouyensis* var. *bouramensis* are referred to small differences in the number of scales on the lateral line, barbels size and pectoral fin length (Pellegrin, 1930, 1939).

The first phylogenetic work on tetraploid barbel species from North Africa, based on molecular markers, named the specimens studied from Moulouya Basin as *Barbus cf. moulouyensis*, indicating the uncertainty in attributing the specimens to the species described by Pellegrin (1924) as *Barbus moulouyensis* (Machordom *et al.,* 1998). Subsequently, molecular works removed the expression "cf." referring to the same individuals, or individuals of the same population. In this direction *Barbus cf. moulouyensis* is referred in posterior molecular works as *Barbus moulouyensis* and currently as *Luciobarbus moulouyensis* (Machordom & Doadrio, 2001b; Tsigenopoulos *et al*., 2003; Berrebi *et al*., 2014, Geiger *et al*., 2014; Yang *et al*., 2015). Thus, the uncertainty on the generic assignation of "*Barbus*" *moulouyensis* and on the correct assignation of the individuals from Moulouya Basin, in phylogenetic studies, remains until present.

We hypothetized that "*Barbus*" *moulouyensis* should be assigned to genus *Carasobarbus* and that individuals present in former phylogenetic studies, from Moulouya Basin belong to one undescribed species of the genus *Luciobarbus*.

To test this hypothesis we analyzed the largest number of individuals from Moulouya Basin studied so far through molecular, morphometric and osteological traits.

Material and Methods

Our study of "*Barbus*" moulouyensis was based on populations of different localities along Moulouya River and its tributaries including the Terra Typica of "*Barbus*" *moulouyensis* in the Za River (Oued el Haï) in Guefait. The individuals collected in Moulouya Basin were identified as hexaploid *Carasobarbus* or tetraploid *Luciobarbus* species following the morphological traits established in taxonomic works (Doadrio, 1990; Borkenhagen & Krupp, 2013; Doadrio *et al.,* 2016) and lately confirmed by the sequencing of the mitochondrial cytochrome *b* gene.

We studied also Holotypes of *Barbus moulouyensis* Pellegrin, 1924, *Barbus moulouyensis var. bouramensis* Pellegrin, 1939 and the possible Holotype of *Barbus moulouyensis var. grandisquamata* Pellegrin, 1930, kept in the Museum of Comparative Zoology of Harvard. For comparative purpose we included the limnetic *Luciobarbus* species geographically closer to Moulouya Basin.

The material studied comprised the following specimens and localities: *Luciobarbus* specimens from Moulouya Basin: 11 specimens (3 females and 8 males) from Moulouya River in Ghafoula, Morocco (voucher numbers MNCN_ICTIO 290.951-290.961); 26 specimens (2 females, 23 males and 1 undet.) from Moulouya River in Ksabi, Morocco (voucher numbers MNCN: MNCN_ICTIO 290.864-290.878, 290.880-290.885, 290.887-290.991); 12 specimens (3 females, 8 males and 1 undet.) from Melloulou River in Guercif, Morocco (voucher numbers: MNCN_ ICTIO 290.995-290.997, 290.998-291.006); 27 specimens (11 females and 16 males) from Zobzite River in Berkine, Morocco (voucher numbers: MNCN_ ICTIO 290.910-290.936); 8 specimens (2 females and 6 males) from Za River in Guefait, Morocco (Type Locality of "*Barbus*" *moulouyensis*) (voucher numbers: MNCN_ICTIO 71.606-71.611, 71.613-71.614). *Carasobarbus cf. fritschii* specimens from Moulouya Basin: 6 males from Moulouya River in Ghafoula, Morocco (voucher numbers: MNCN_ICTIO 290.899-290.904); 2 males from Moulouya River in Ksabi, Morocco (voucher numbers: MNCN_ICTIO 290.897-290.898); 5 specimens (4 females and 1 male) from Melloulou River, in Guercif, Morocco (voucher numbers: MNCN_ICTIO 290.989-290.992, 290.994); 3 specimens from El Barred River in Asrire, Morocco (voucher numbers: MNCN_ICTIO 290.907-290.909). *Luciobarbus rifensis*: 47 specimens (17 females and 30 males) from Laou River (Laou Basin) in Derdara (voucher numbers: MNCN_ICTIO 290.639-290.652, 290.655, 290.657-663, 290.665-667) and Beni Fertan (Voucher numbers: MNCN_ICTIO 284.939-940, 284.942-945, 284.947-284.948, 284.950-951, 284.953-964), Morocco. *Luciobarbus maghrebensis*: 55 specimens (11 males and 44 females) from Ifrane River (Sebou Basin) in Ouad Ifrane (voucher numbers: MNCN_ICTIO 279.711-729, 290.731, 279.733-744) and Tizguit River (Sebou Basin) in Ifrane (Voucher numbers: MNCN_ICTIO 71.675-71.697), Morocco. *Luciobarbus setivimensis*. 15 specimens from Soummam River (Soummam Basin) in Takretz, Algeria (voucher numbers: MNCN_ICTIO 106.148-106.162). *Luciobarbus ksibi*: 28 specimens (20 males and 8 females) from Derna River (Oum er Rbia Basin) in Bounoval, Morocco (voucher numbers: MNCN 291.122-291.149). *Barbus moulouyensis*: Holotype Oued el Haï (Za River), in Guefait, Morocco (MNHN 1924-0167). *Barbus moulouyensis* var. *bouramensis*: Holotype Aïn Bouram in El ksiba- Tezghrit, Morocco (MNHN 1939-0121). *Barbus moulouyensis var. grandisquamata*. Holotype? Oued Tensift near of Marrakesh, Morocco (MCZ 32741) (Fig. 1; Table 1).

Twenty-four morphometric measurements were taken with digital callipers (0.01 mm), and ten meristic variables were counted with a stereoscopic microscope. The following acronyms were used for morphometric and meristic characters: A, number of anal fin rays; AFH, anal fin height; AFL, anal fin length; APL, anal peduncle length; BL1, first barbel length; BL2, second barbel length; BD, body depth; BLD, body least depth; C, central caudal fin rays; CFL, caudal fin length; CPL, caudal peduncle length; D, dorsal fin rays, DFL dorsal fin length; DFH dorsal fin height; ED, eye diameter; GR, gill rakers (number); HL, head length; IOW, interorbital width; LL lateral line scales; P, pectoral fin rays; PFL, pectoral fin length; PrAD, pre-anal distance; PrDD, pre-dorsal distance; PrOL, pre-orbital length; PrPD, pre-pectoral distance; PrVD, pre-ventral distance; PsOL, postorbital length; PVL, pectoral-ventral length; RSA, scale rows above lateral line; RSB scale rows below lateral line; SL, standard length; V, ventral fin rays; VFL, ventral fin length; VE, Number of vertebrae. The number of vertebrae was obtained by counting on X-ray images of specimens from all sampled populations. Osteological characteristics were investigated through computer tomography (CT) scan and digital dissection using VGStudio MAX v2.2 (Volume Graphics, http://www.volumegraphics.com).

After constructing the measurement matrix, Burnaby's method was used to correct size effect. The Burnaby method removes the effects of a within population size-factor from between-group morphometric analyses through an orthogonal projection procedure (Burnaby, 1966; Röhlf & Bookstein, 1987). All analyses were conducted with the corrected matrix. Morphometric and meristic characters were analysed independently. To identify the variables that contributed most to the variation among populations, two principal component analyses (PCA) were performed using the covariance matrix for morphometric characters. Statistical analyses were carried out using PAST software (Hammer *et al*., 2001).

MOLECULAR ANALYSES

For the molecular approach we analyzed all populations morphologically studied of the genus *Luciobarbus*. Also, all the other Moroccan species of genus *Luciobarbus* were added. The species *Aulopyge hueguelli* Heckel, 1843 and *Barbus meridionalis* Risso, 1827 were selected as outgroups based on previous phylogenetic analyses (Zardoya & Doadrio, 1999). Total genomic DNA was extracted from finclip tissue using the commercial kit Biosprint 15 for tissue and blood (Qiagen). For each specimen, the complete region (1140bp) of the mitochondrial

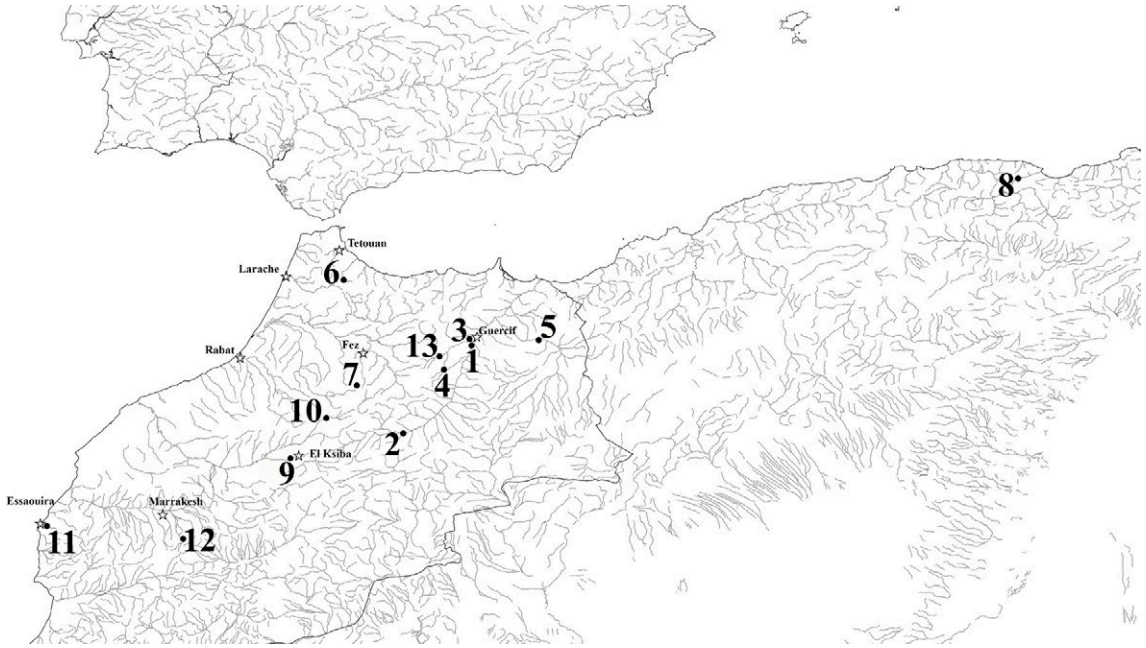

Fig. 1.— Sampling localities of *Luciobarbus* populations in northern Africa. Moulouya River, Ghafoula (1); Moulouya R., Ksabi (2); Melloulou R., Guercif (3); Zobzite R., Berkine (4); Za R., Guefait (5); Laou R., Derdara (6); Ifrane R., El Hamman (7); Soummam R., Takretz (8); Derna R., Bounoval (9); Chbouka R., El Herri (10); Kasab R., Essaouira (11); Reraia R., Asni (12); El Barred R., Asrire (13).

Fig. 1.— Localidades de las poblaciones del género *Luciobarbus* muestreadas en el Norte de África. Río Moulouya, Ghafoula (1); R. Moulouya, Ksabi (2); R. Melloulou, Guercif (3); R. Zobzite, Berkine (4); R. Za, Guefait (5); R. Laou, Derdara (6); R. Ifrane, El Hamman (7); R. Soummam, Takretz (8); R. Derna, Bounoval (9); R. Chbouka, El Herri (10); R. Kasab, Essaouira (11); R. Reraia, Asni (12); R. El Barred, Asrire (13).

Table 1.— Sampling localities for *Luciobarbus* and *Carasobarbus* and GenBank Accession numbers.

Tabla 1.— Localidades de muestreo para *Luciobarbus* y *Carasobarbus* y números de acceso de GenBank.

Population assignment/ species	Locality	No. Individuals studied Morphology/ molecular	Code in phylogenetic tree	GenBank Accession Numbers	Number in Map
Moulouya population	Moulouya R. Ghafoula/Moulouya Basin	11/3	M4,M8-M9	KX681705, KX681704, KX681706	1
Moulouya population	Moulouya R. Ksabi/Moulouya Basin	26/-			2
Moulouya population	Melloulou R. Guercif/Moulouya Basin	12/3	M5-M7	KX681701-681703	3
Moulouya population	Zobzite R. Berkine/Moulouya Basin	27/3	M1-M3	KX681698-KX681700	4
Moulouya population	Za R. Guefait/ Moulouya Basin	8/-			5
Luciobarbus rifensis	Laou R. Derdara/ Laou Basin	47/4	R1-R4	KT003027-KT003930	6
Luciobarbus maghrebensis	Ifrane R. El Hamman/Sebou Basin	55/4	Ma1-Ma4	KT003941,KT003943-KT003945	7
Luciobarbus setivimensis	Soummam R. Takretz/ Soummam Basin	15/3	ST1-3	AY004748; KX681686-KX681687	8
Luciobarbus ksibi	Derna R. Bounoval/Oum er Rbia Basin	29/-			9
Luciobarbus ksibi	Chbouka R. El Herri/Oum er Rbia Basin	-/2	K5, K6	KU257529-30	10
Lucibarbus ksibi	Kasab R. Essaouira/ Kasab Basin	-/2	K1, K2	KU257523-24	11
Luciobarbus ksibi	Reraia R. Asni/Tensift Basin	-/2	K3,K4	KU257538-39	12

Table 1.— (*continued*)

Population assignment/ species	Locality	No. Individuals studied Morphology/ molecular	Code in phylogenetic tree	GenBank Accession Numbers	Number in Map
Carasobarbus cf. fritschii from Moulouya Basin	Moulouya R. Ghafoula	6/-			1
	Moulouya R. Ksabi	2/-			2
	Melloulou R. Guercif	6/-			3
	El Barred R. Asrire	3/-			13
Luciobarbus guercifensis	Moulouya R. Ghafoula/ Moulouya Basin	-/7	Ge1-Ge4, Ge6,Ge8-9 Ge5,Ge7,Ge10	KU257526, KX681697, KX681695, KX681696 KU257527, KX681693 KU257525 KU257528; KX681691-KX681692	1
	Melloulou R. Guercif/ Moulouya Basin	-/3			3
L. bocagei	Tajo R./Tajo Basin	-/1	B1	AF112125	
	Alberche R./Tajo Basin	-/1	B3	AF334054	
	Jerte R./ Tajo Basin	-/1	B2	AF334064	
L.comizo	Guadiana R./ Guadiana Basin	-/1	C2	AF334047	
	Tiétar R./ Tajo Basin	-/1	C3	AF334042	
	Tajo R. /Tajo Basin	-/1	C1	AY004735	
L. graellsii	Irati R./Ebro Basin	-/1	Gr2	AF334088	
	Cadagua R./ Nervión Basin	-/1	Gr3	JF798258	
	Mesa R./ Ebro Basin	-/1	Gr1	AF334089	
L. guiraonis	Turia R./Turia Basin	-/1	Gu2	AF334094	
	Júcar R./Júcar Basin	-/1	Gu3	AF334093	
	Palancia R/ Palancia Basin	-/1	Gu1	AF334097	
L. microcephalus	Guadiana R./ Guadiana Basin	-/2	Mc1	AF334085	
	Estena R./ Guadiana Basin	-/1	Mc2	AF334084	
		-/1	Mc3	AF045971	
L. sclateri	Segura R./Segura Basin	-/1	SC1	AF334071	
	Guadalquivir R./ Guadalquivir Basin.	-/1	SC2	AF334070	
	Guadiato R./ Guadalquivir Basin	-/1	SC3	AF334069	

cytochrome *b* (cyt*b*) was amplified. Primers and protocols used for PCR for cyt*b* followed Machordom & Doadrio (2001b). After checking PCR products on 1% agarose gels, they were purified by ExoSAP-IT™ (USB) and directly sequenced on MACROGEN service using a 3730XL DNA sequencer. All new sequences were deposited in the GenBank database (Accession Numbers: KX681686-KX681687, KX681691-KX681692, KX681695-KX681706).

PHYLOGENETIC ANALYSES

Phylogenetic analyses were performed using Bayesian inference (BI) implemented in MrBayes v.3.2 (Ronquist *et al*., 2012). The Akaike Information Criterion (Akaike, 1973) implemented in jModeltest (Posada, 2008) was used to determine the evolutionary model that best fit the data. In this case TIM1+G model was selected (R(a) [AC] =1.0000, R(b) [AG] = 29.9653, R(c) [AT] = 0.6120, R(d) [CG] = 0.6120, R(e) [CT] =11.9160, R(f) [GT] =1.0000, p-inv =0.1770). BI was performed using two independent runs of four Markov Montecarlo coupled chains (MCMC) of 5×10^6 generations each, to estimate the posterior probability distribution. Topologies were sampled every 100 generations, and majority-rule consensus tree was estimated after discarding the first 10% of generations. Robustness of clades was assessed using Bayesian posterior probabilities. The average genetic distances among populations were calculated for each gene using MEGA package v.6.0 (Tamura *et al*., 2013) according to the uncorrected-*p* distances.

Results and Discussion

COMPARISON OF MORPHOLOGY AMONG POPULATIONS

Two-way analysis of variance (ANOVA), testing for sexual dimorphism and differentiation among populations, showed significant differences ($p<0.01$) for sexual dimorphism only for the Standard length and Post-orbital distance (PsOL). To deal with the presence of sexual dimorphism we removed PsOL from posterior analyses. Most morphometric variables showed significant differences between populations in the two-way ANOVA analysis (Table 2).

Table 2.— Two-way analysis of variance (ANOVA) for sexual dimorphism, population variation, and their interaction. Significant differences p<0,01 (**). N= 156 males and N= 58 females. Abbreviations are described in the Material and Methods epigraph.

Tabla 2.— Análisis de la varianza (ANOVA) de dos vías para dimorfismo sexual, variación poblacional y su interacción. Diferencias significativas p<0,01 (**). N= 156 machos y 58 hembras. Las abreviaturas se describen en el epígrafe de Material y Métodos.

Variables	Sexual dimorphism (F/significance)	Population Variation (F/significance)	Sex/pop Variation (F/significance)
SL	53.58/**	2.948/	10.9/**
HL	0.3594/	34.05/**	0.8619/
PrOL	0.4139/	44.44/**	2.487/
ED	3.877/	15.55/**	3.664/**
PsOL	16.95/**	372.4/**	3.905/**
B1L	3.171/	70.95/**	1.229/
B2L	0.0254/	91.76/**	2.076/
PrDD	0.205/	75.83/**	1.915/
PrPD	0.5848/	21.88/**	2.85/**
PrVD	0.2496/	58.8/**	4.23/**
PrAD	0.0581/	58.32/**	3.601/**
CPL	2.246/	31.16/**	1.445/
APL	2.154/	22.49/**	1.77/
PVL	0.043/	23.38/**	1.371/
BD	3.927/	33.71/**	1.357/
BLD	2.752/	38.03/**	1.006/
DFL	0.1273/	7.027/**	0.9019/
DFH	0.5455/	24.14/**	1.674/
PFL	0.3356/	6.357/**	1.204/
VFL	5.781/	24.29/**	1.109/
AFL	1.275/	5.516/**	1.72/
AFH	6.088/	20.75/**	2.143/
CFL	0.374/	5.067/**	1.145/

A first morphometric analyses, through a Principal Component Analyses (PCA) was conducted to place the types of "*Barbus*" *moulouyensis* in tetraploid (*Luciobarbus*) or hexaploid (*Carasobarbus*) barbel specimens. The PCA clearly divided the barbel specimens from Moulouya Basin into two different groups, one corresponding to the genus *Carasobarbus* and the other to the genus *Luciobarbus* (Fig. 2).

On the basis of morphometric variables the type specimens corresponding to "*Barbus*" *moulouyensis* and "*Barbus*" *moulouyensis* var. *grandisquamata* were placed in *Carasobarbus* group (Fig. 2). In contrast, the type specimen of "*Barbus*" *moulouyensis* var. *bouramensis* from Oum er Rbia Basin was placed along with specimens of *Luciobarbus*. The variables that more contributed to PCA ordination of Moulouya specimens were barbels and fins size. The barbels were longer and the fins shorter in *Luciobarbus* than in *Carasobarbus* individuals (Table 3).

The adscription of type specimens of "*Barbus*" *moulouyensis* to tetraploid (*Barbus* and *Luciobarbus*) and not to hexaploid barbels (*Labeobarbus* or *Carasobarbus*) was mainly based on the presence of a weakly serrated last single ray in the dorsal fin. Until now a smooth last unbranched dorsal-fin ray was a diagnostic trait for *Carasobarbus* (Borkenhagen & Krupp, 2013) but we found in several individuals of *Carasobarbus* specimens from Moulouya Basin a serrated last unbranched dorsal-fin ray and 8 or 9 branched rays on the dorsal fin (Fig. 3). The denticulations found in individuals of *Carasobarbus* from Moulouya Basin were even weaker than those found in *Luciobarbus* specimens of the same localities (Fig 3). Some grade of introgression of *Luciobarbus* in those individuals of *Carasobarbus* with serrated ray could be claimed, but this is difficult to conclude without carrying out a molecular study of the nuclear genes.

The three type specimens of "*Barbus*" *moulouyensis* had less number of scales than all *Luciobarbus* samples studied and were in the rank of the samples of *Carasobarbus*, with the exception of the type of "*Barbus*" *moulouyensis* var. *bouramensis* which had the lowest number of scales on the lateral line of all the barbels (*Carasobarbus* and *Luciobarbus*) studied (Fig. 4).

In conclusion, the morphometric traits and scales counts as well as the morphology of the last unbranched dorsal-fin ray placed unequivocally the

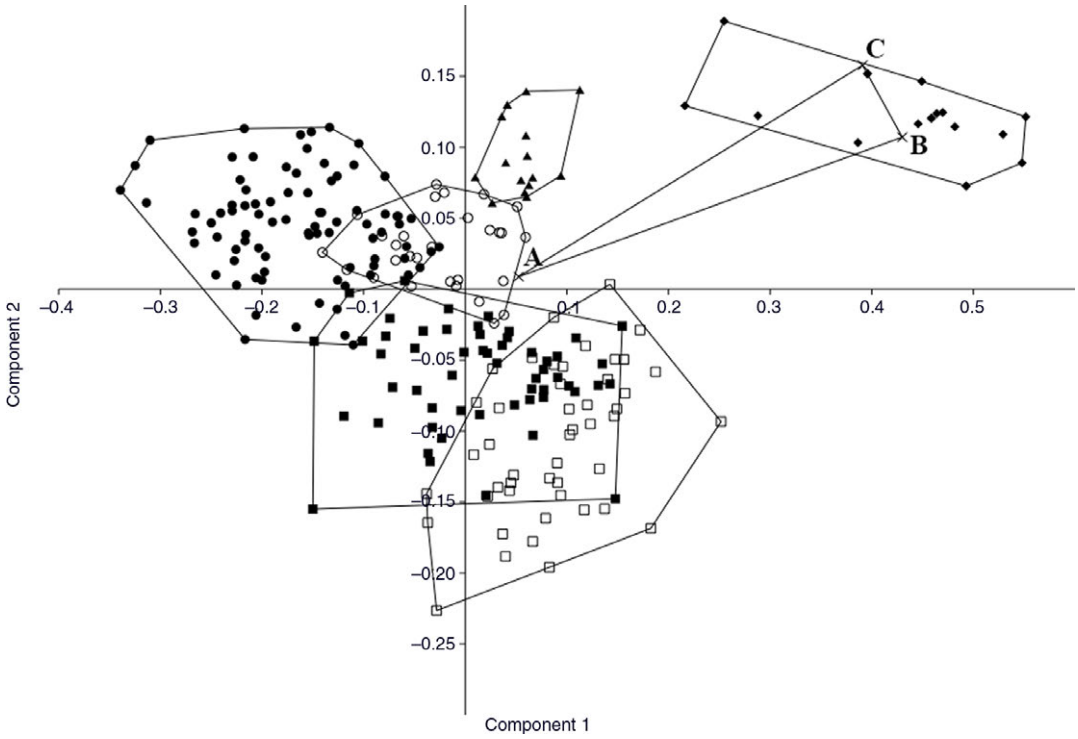

Fig. 2.— Variables that most contributed to the PCA analysis. Black dots all populations of tetraploids barbels from Moulouya Basin. Diamonds *Carasobarbus cf. fritschii*. Triangle: *L. setivimensis*. White square: *L. rifensis*. Black square: *L. maghrebensis*. White dots: *L. ksibi*. X: Types of "*Barbus*" *moulouyensis* A) "*Barbus*" *moulouyensis* var. *bouramensis*. B) "*Barbus*" *moulouyensis*. C) "*Barbus*" *moulouyensis* var. *grandisquamata*.

Fig. 2.— Variables que más contribuyeron al análisis de PCA. Puntos negros: Todas las poblaciones de barbos tetraploides de la cuenca del río Moulouya. Diamantes: *Carasobarbus cf. fritschii*. Triangulos: *L. setivimensis*. Cuadrados blancos: *L. rifensis*. Cuadrados negros: *L. maghrebensis*. Puntos blancos: *L. ksibi*. Equis: Tipos de "*Barbus*" *moulouyensis*. A) "*Barbus*" *moulouyensis* var. *bouramensis*. B) "*Barbus*" *moulouyensis*. C) "*Barbus*" *moulouyensis* var. *grandisquamata*.

Table 3.— Eigenvalues and eigenvectors for the first three principal components (PC1-PC3) of 22 morphometric variables for all populations studied. Acronyms are defined in the Material and Methods section. Variables with the highest eigenvalues for each PC are in bold.

Tabla 3.— Eigenvalores y eigenvectores para los tres primeros componentes principales (PC1-PC3) de 22 variables morfométricas para todas las poblaciones estudiadas. Los acrónimos están definidos en la sección de Material y Métodos. Las variables con los eigenvalores más altos para cada CP están en negrita.

Variables	PCI	PCII	PCIII
Eigenvalue	0.0302	0.0068	0.0023
% variance	61.04	13.8	4.74
	Eigenvectors		
SL	0.1111	−0.0401	−0.0049
PrDD	0.0662	−0.0965	−0.0875
PrPD	0.0194	−0.1596	0.1337
PrVD	0.0851	−0.1427	0.1660
PrAD	0.1023	−0.0862	0.0792
PVL	0.1392	−0.1184	0.2225

Table 3.— (*continued*)

Variables	PCI	PCII	PCIII
CPL	0.1540	0.0169	−0.1309
APL	0.1443	0.1092	−0.2711
BD	0.1387	−0.0621	−0.1026
BLD	0.0841	−0.1171	**−0.2636**
HL	0.0099	−0.2139	0.0025
PrOL	−0.0585	−0.0989	−0.0973
ED	0.0655	0.1750	**0.50343**
B1L	**−0.6846**	0.0213	−0.1304
B2L	**−0.5470**	0.2058	0.1866
PFL	0.1081	0.1914	**−0.3899**
VFL	0.0916	0.2711	**−0.2885**
AFL	0.1118	−0.1201	0.1650
AFH	0.0967	**0.2831**	−01986
DFL	0.1881	0.0720	0.0835
DFH	0.1152	**0.5012**	0.1299
CFL	0.0928	0.0598	0.2597

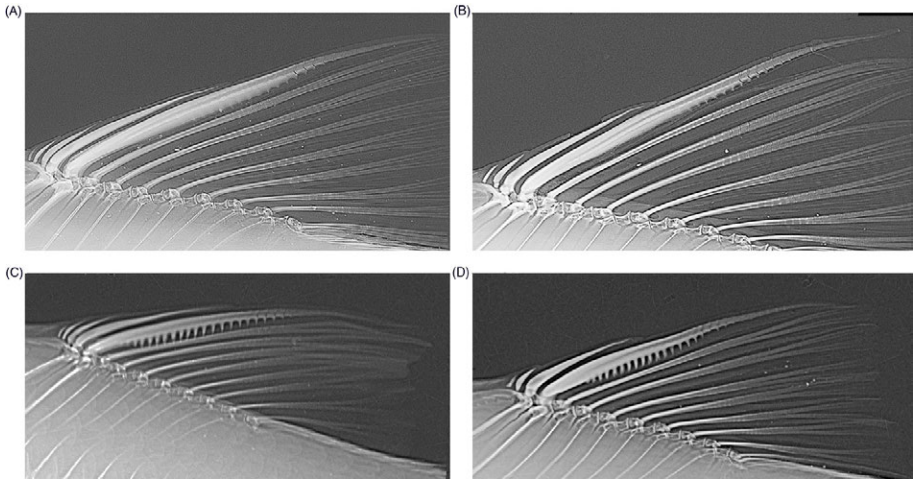

Fig. 3.— Serrated last unbranched dorsal-fin ray shown differences between *Carasobarbus* and *Luciobarbus* of similar size. A. *Carasobarbus cf. fritschii* from Moulouya River, Ghafoula (Moulouya Basin) (MNCN_ICTIO 290.906) 85.4 mm of SL. B *Carasobarbus cf. fritschii* from El Barred River, Asrire (Moulouya Basin) (MNCN_ICTIO 290.908) 94.1 mm of SL. C. Individual of the Moulouya population from Moulouya River, El ksabi, (Moulouya Basin) (MNCN_ICTIO 290.880) 94.7 mm of Standard Length. D. *Luciobarbus sp.* from Moulouya River, El ksabi, (Moulouya Basin) (MNCN_ICTIO 290.869) 117.4 mm of SL.

Fig. 3.— Último radio no ramificado de la aleta dorsal mostrando las diferencias entre *Luciobarbus* y *Carasobarbus*. A. *Carasobarbus cf. fritschii* del río Moulouya, Ghafoula (Cuenca del Moulouya) (MNCN_ICTIO 290.906) 85.4 mm de longitud estándar. B *Carasobarbus cf. fritschii* del río El Barred, Asrire (Cuenca del Moulouya) (MNCN_ICTIO 290.908) 94.1 mm de longitud estándar. C. Ejemplar de la población del Moulouya colectado en el río Moulouya, El ksabi, (Cuenca del Moulouya) (MNCN_ICTIO 290.880) 94.7 mm de longitud estándar. D. *Luciobarbus sp.* del río Moulouya, El ksabi, (Cuenca del Moulouya) (MNCN_ICTIO 290.869) 117.4 mm de LS.

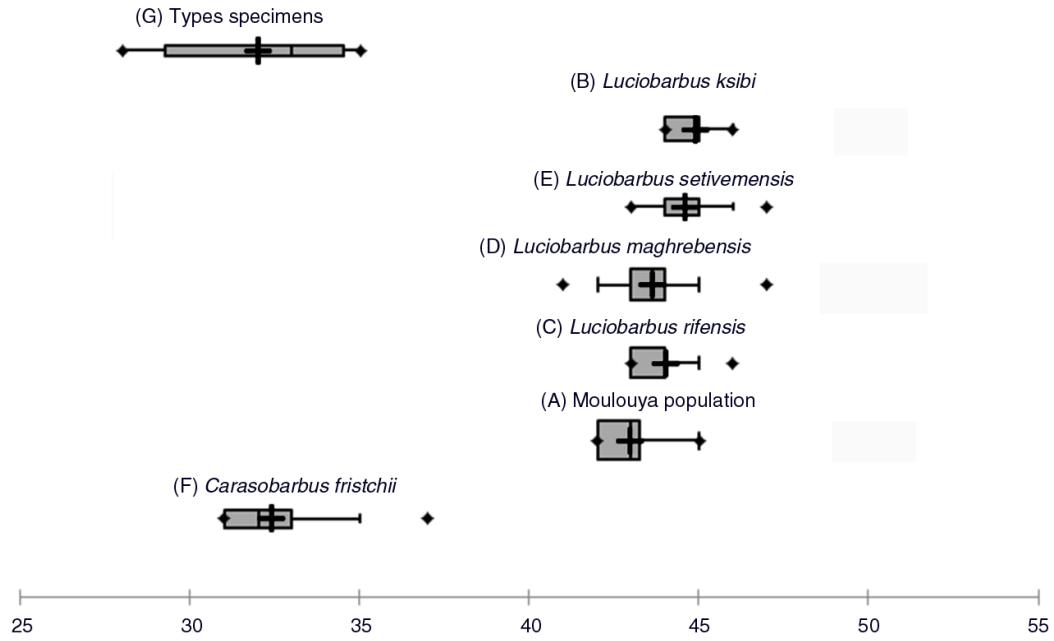

Fig. 4.— Box-plots of lateral line scale numbers. A, Moulouya Population; B, *Luciobarbus ksibi*; C, *Luciobarbus rifensis*: D, *Luciobarbus maghrebensis*; E, *Luciobarbus setivimensis*; F, *Carasobarbus cf. fritschii*; G. types specimens: Holotype of "*Barbus*" *moulouyensis*; Holotype of "*Barbus*" *moulouyensis* var *grandisquamata*; Holotype of "*Barbus*" *moulouyensis* var. *bouramensis*.

Fig. 4.— Box-plots para el número de escamas en la línea lateral. A, Población del Moulouya; B, *Luciobarbus ksibi*; C, *Luciobarbus rifensis*: D, *Luciobarbus maghrebensis*; E, *Luciobarbus setivimensis*; F, *Carasobarbus cf. fritschii*; G, ejemplares tipo: Holotipo of "*Barbus*" *moulouyensis*; Holotipo of "*Barbus*" *moulouyensis* var *grandisquamata*; Holotipo of "*Barbus*" *moulouyensis* var. *bouramensis*.

types of "*Barbus*" *moulouyensis* from Moulouya Basin and "*Barbus*" *moulouyensis* var. *grandisquamata* from Tensift Basin in the genus *Carasobarbus*. The ascription to *Carasobarbus fritschii,* a species widely distributed in Morocco (Doadrio, 1994) or to another different species, which could be named *Carasobarbus moulouyensis* (Pellegrin, 1924), is not the focus of this work and should be addressed taking into account populations of all the distribution range of *Carasobarbus*.

Different is the case of "*Barbus*" *moulouyensis* var. *bouramensis* that was described on the basis of one individual from Aïn Bouram, (Bouram Spring), 300 meters from the Ksiba to Taghzirt trail, Morocco. Bouram Spring could not be found despite of our sampling efforts from Ksiba to Taghzirt trail. The only river with fishes was Derna River (Oum er Rbia Basin), which flows through the Bouhzam Mountains. In this river we only sampled *Luciobarbus ksibi*, *Carasobarbus fritschii*, *Luciobarbus zayanensis* and *Pterocapoeta maroccana* Günther, 1902. The morphometric data placed "*Barbus*" *moulouyensis* var. *bouramensis* within *Luciobarbus* populations but the number of scales on the lateral line (28) was surprisingly low in comparison to all the species of *Luciobarbus* that were studied which had more than 40 scales on the lateral line. On the basis of the number of scales and the morphometric variables (Figs. 2 and 4) the Holotype of "*Barbus*" *moulouyensis* var. *bouramensis* could not be assigned to any known population of the genus *Luciobarbus* and therefore it could be a valid species.

The assignment of the types "*Barbus*" *moulouyensis* and "*Barbus*" *moulouyensis var. grandisquamata* to genus *Carasobarbus* resulted in the need of classifying correctly those samples from the Moulouya Basin named in previous molecular works as *Barbus* cf. *moulouyensis* or *Luciobarbus moulouyensis* and that were clustered with other African tetraploid barbels in phylogenetic trees (Machordom *et al.,* 1998; Machordom & Doadrio, 2001a; Tsigenopoulos *et al*., 2003; Berrebi *et al*., 2014; Geiger *et al*., 2014; Yang *et al*., 2015).

An analysis of body proportions based on Kruskal-Wallis and Mann-Whitney *post hoc* comparisons was used to detect differences in body shape of the tetraploid populations studied (*Luciobarbus sp*.) from Moulouya Basin with respect to the species *L. maghrebensis*, *L. rifensis*, *L. ksibi* and *L. setivimensis* from the nearest basins (Appendix I). No differences in SL were found among *Luciobarbus* populations. However we found significant differences in all morphometric and meristic variables studied.

The population from Moulouya Basin had the longest barbels of all populations studied, a fact that could be habitat-related. Most rivers from Moulouya Basin have muddy bottoms in contrast to the stony or sand bottoms present in the rivers inhabited by *L. rifensis*, *L. ksibi* or *L. setivimensis*. Our samples of the Zobzite River from Moulouya Basin were an exception, because there is no muddy bottom. However, no significant differences were found in the length of the barbels between samples of Zobzite and the rest of samples of Moulouya Basin.

The samples of *L. ksibi* and *L. setivimensis* had the length of the head significantly smaller than *L. rifensis*, *L. maghrebensis* and samples of Moulouya Basin as consequence of a shorter snout. In this way, our samples of *L. setivimensis* and *L. ksibi* from Oum er Rbia Basin showed a head conspicuously smaller and rounded than the rest. The eye diameter was significantly longer in Moulouya Basin population with respect to other *Luciobarbus* species. Due to allometry in fishes, juvenile specimens present an eye bigger in proportion to body size. Nonetheless, all our samples were mature individuals and no differences in body size among the populations studied were found. The longest barbels were found in Moulouya Basin population and usually the first barbel reached the half of the eye diameter and the second barbel reached the preopercular (Fig. 5). The caudal peduncle was lower in *L. moulouyensis* and *L. ksibi* than in other *Luciobarbus* species and shortest in *L. setivimensis*.

Number of scales on the lateral line were less numerous in *Luciobarbus moulouyensis* (Median=43), meanwhile *L. ksibi* showed more scales in the lateral line (Median=45) and transversal rows (Median RSA=9.48 and Median RSB=6.35) than other *Luciobarbus*.

The PCA separated the specimens of *L. setivimensis* of other *Luciobarbus* species. The remaining populations showed some overlap between them but could be clearly identified in the PCA as differentiated populations. *Luciobarbus moulouyensis* overlapped *L. ksibi* and *L. maghrebensis* (Fig. 6). The eigenvalues of the three first principal components, with the Burnaby-corrected matrix, explained most of the variance (Table 4). The highest eigenvector values (barbels length, size of the fins and body least deep) were in agreement with results of Kruskal–Wallis and Mann–Whitney analyses (Table 4, Appendix 1).

OSTEOLOGICAL FEATURES

The shape of the last unbranched dorsal-fin ray (DFR) has been considered an important diagnostic trait in barbel taxonomy (Doadrio, 1990). A smooth

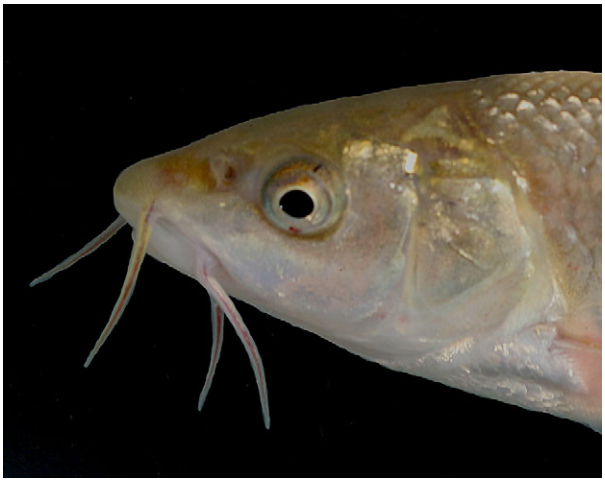

Fig. 5.— Head details of one specimen of Moulouya population of 136 mm SL showing the big develop of the barbels.

Fig. 5.— Detalles de la cabeza de un ejemplar de 136 mm de SL de la población del Moulouya mostrando el gran desarrollo de las barbillas.

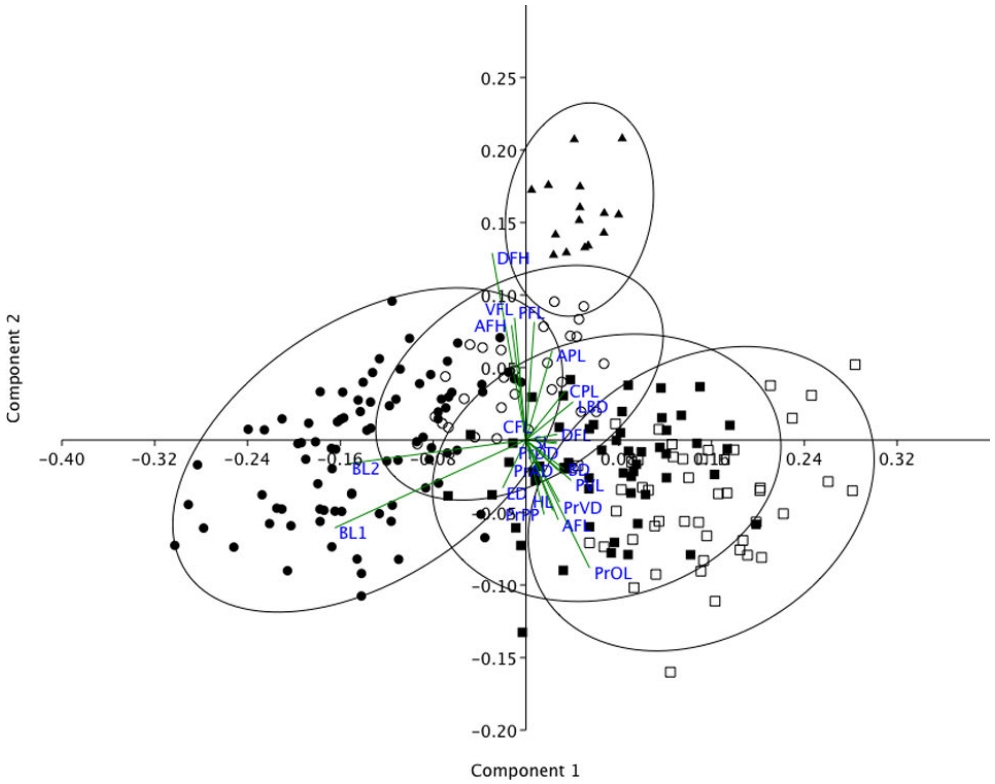

Fig. 6.— Variables that most contributed to the PCA analysis. Black dots all populations of tetraploids barbels from Moulouya Basin. Triangle: *L. setivimensis*. White square: *L. rifensis*. Black square: *L. maghrebensis*. White dots: *L. ksibi*.

Fig. 6.— Variables que más contribuyeron al análisis de PCA. Puntos negros: Todas las poblaciones de barbos tetraploides de la cuenca del río Moulouya. Triangulos: *L. setivimensis*. Cuadrados blancos: *L. rifensis*. Cuadrados negros: *L. maghrebensis*. Puntos blancos: *L. ksibi*.

Table 4.— Eigenvalues and eigenvectors for the first three principal components (PC1-PC3) of 22 morphometric variables for all populations studied of *Luciobarbus* in North Africa. Acronyms are defined in the Material and Methods section. Variables with the highest eigenvalues for each PC are in bold.

Tabla 4.— Eigenvalores y eigenvectores para los tres primeros componentes principales (PC1-PC3) de 22 variables morfométricas para todas las poblaciones estudiadas del género *Luciobarbus* en el Norte de África. Los acrónimos están definidos en la sección de Material y Métodos. Las variables con los eigenvalores más altos para cada CP están en negrita.

Variables	PCI	PCII	PCIII
Eigenvalue	0.0162	0.0038	0.0019
% variance	52.46	12.33	6.04
	Eigenvectors		
SL	0.1031	−0.0042	0.0614
PrDD	0.1034	−0.0092	0.0524
PrPD	0.0606	−0.1989	0.0779
PrVD	0.1118	−0.1656	0.1403
PrAD	0.1081	−0.0813	0.0949

Table 4.— (*continued*)

Variables	PCI	PCII	PCIII
PVL	0.1509	−0.1076	0.1799
CPL	0.1316	0.1312	0.0237
APL	0.0874	0.2385	0.0545
BD	0.1277	−0.0779	0.1051
BLD	0.1580	0.1021	−0.0592
HL	0.0903	−0.1888	−0.0359
PrOL	0.2125	−0.3416	−0.0266
ED	−0.0774	−0.1279	0.2710
B1L	**−0.6381**	−0.2345	**−0.4552**
B2L	**−0.5950**	−0.0632	**0.4363**
PFL	0.0277	0.3152	−0.3151
VFL	−0.0382	0.3267	−0.2215
AFL	0.1079	−0.2131	−0.2332
AFH	−0.0478	0.3067	−0.1997
DFL	0.1049	0.0153	−0.1932
DFH	−0.1128	**0.4994**	0.3868
CFL	0.0371	0.0354	−0.0746

DFR was characteristic of *Carasobarbus* but as we have previously pointed out, some specimens from Moulouya population had denticulations but in all cases those denticulations were very small and could be a signal of genetic introgression with *Luciobarbus*. Reophilic and limnetic barbels can be recognized for the hardness of the DFR. Reophilic barbels have a stronger DFR than limnetic kind and this can be measured (Doadrio *et al*., 2016).

Within limnetic barbels *L. rifensis* and *L. maghrebensis* had denticulations in practically all the length of the DFR, in contrast to the species *L. setivimensis*, *L. ksibi* and the population of Moulouya Basin that had denticulations only in 2/3 of the length of DFR. The number of denticulations, in the DFR from Moulouya Basin was always less than twenty-one (16-20, n=10) but longer than in the other species studied (Appendix 2.1).

The skull of *L. setivimensis*, *L. ksibi* and of Moulouya population was wider than that of *L. rifensis* and *L. maghrebensis* and this was especially remarked in the interorbital distance (Appendix 2.2). The species *L. maghrebensis* showed narrower ethmoids than any other species (Appendix 2.2). In a lateral view, the skull was placed higher with respect to its length in Moulouya Basin population with respect to the rest of populations (Appendix 2.3). The opercular was small and was slightly anteriorly projected (Appendix 2.3). The infraorbital bones were wide as in *Luciobarbus ksibi* and the lachrymal was poorly notched (Appendix 2.4). The dorsal branch of the pharyngeal bone was the longest of all studied populations and scarcely curved (Appendix 2.5). The maxilla and the dentary in Moulouya Basin population showed longer anterior process than the rest of populations (Appendix 2.6). The basioccipital had a triangular and small basal plate (Appendix 2.6). Kinethmoides was as small and wide as in other limnophilic *Luciobarbus* (Appendix 2.6).

MOLECULAR DATA

Phylogenetic analyses based on the *cytb* gene supported two main clades in the tree, one corresponding to the populations of Iberian Peninsula, *L. setivimensis* from Algeria and *L. guercifensis* from Morocco together; the other one, comprised by the remaining populations of North African (Fig. 7). These phylogenetic relationships are in agreement with previous works (Machordom & Doadrio, 2001b; Doadrio *et al.,* 2016). In the second group comprised by the rest of

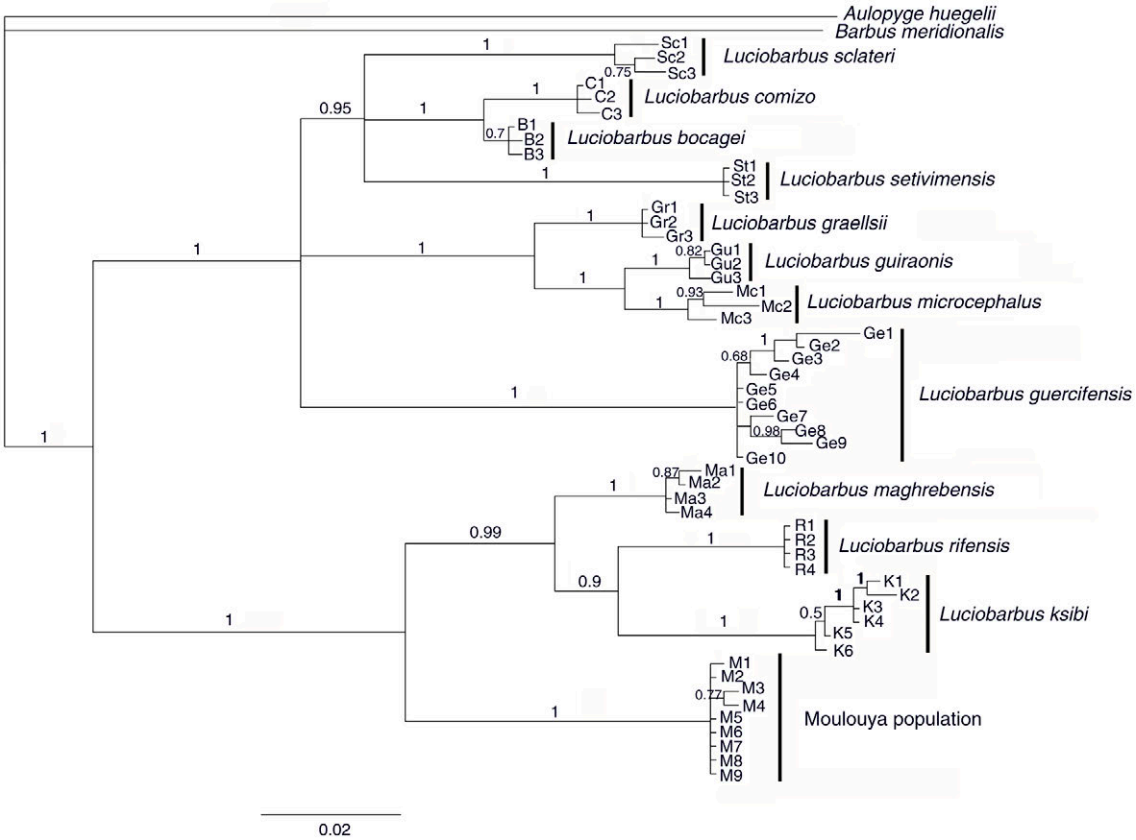

Fig. 7.— Phylogenetic tree rendered by Bayesian Inference of the mitochondrial cytochrome *b* gene. Numbers on branches indicate posterior probability values. Identification of individuals and localities is defined in Table 1.

Fig. 7.— Árbol filogenético del gen mitochondrial citocromo *b* obtenido a partir de Inferencia Bayesiana. Los números sobre las ramas indican valores de probabilidad posterior. La identificación de las localidades está definida en la Tabla 1.

North African species, *L. moulouyensis* was basal and the most differentiated from a genetic point of view. The species L. *maghrebensis* and *L. ksibi* were sister group to *L. rifensis*. Uncorrected-*p* genetic distances of *L. moulouyensis* with respect to *L. maghrebensis*, *L. rifensis* and *L. ksibi* were greater than 5% (Table 5). With respect to reophilic *L. guercifensis* inhabiting the same basin, the uncorrected-*p* genetic distances were $\bar{x}$ = 8.8%. These distances were of similar range as those between well-recognized species of cyprinid fishes (Doadrio *et al*., 2002; Doadrio & Carmona, 2003, 2006; Doadrio & Madeira, 2004; Robalo *et al*., 2005; Doadrio & Elvira, 2007; Doadrio *et al*., 2007; Domínguez-Domínguez *et al*., 2007, 2009; Casal-López *et al*., 2015; Doadrio *et al*., 2016). These results confirmed the differences found with allozyme analyses (Machordom *et al*., 1998). Allozyme studies have been useful to distinguish *Luciobarbus* species especially when diagnostic loci are present (Machordom *et al*., 1995) and also to describe hybridization between barbel species (Machordom *et al*., 1990). In a previous work based on 23 polymorphic loci in *Luciobarbus* species of North Africa, one diagnostic locus (LDH-4*) was found in populations of *Luciobarbus* from Moulouya Basin with respect to other species or populations from North Africa (Machordom *et al*., 1998).

DESCRIPTION OF *LUCIOBARBUS* POPULATIONS

The high degree of morphological and genetic differentiation of limnetic populations of the genus *Luciobarbus* from Moulouya Basin in North Africa justifies the consideration of these populations as a distinct species. No available name for these populations exists, and therefore one new species is described in this study.

Luciobarbus yahyaouii Doadrio, Casal-López & Perea, **sp. nov**.

http://urn:lsid:zoobank.org:act:60BBBFE5-4D66-4B34-BF0F-F0B7A7D77CF1

TYPE MATERIAL: Holotype: Fig. 8, Table 6. MNCN_ICTIO 290.958 male, 119.8 mm (SL); Moulouya River, Moulouya Basin, Ghafoula, Mediterranean slope in Morocco (34.13502°N, 3.39653°O) (Fig. 1); 2/V/2015. Collected by (Coll.) Doadrio, I.; Garzón, P.; Yahyaoui, A; Perea, S.

Paratypes: Table 6. MNCN_ICTIO 290.951-290.957, 290.959-290.961: 10 specimens from the Moulouya River, Moulouya Basin, Ghafoula, Mediterranean slope in Morocco (34.133318, -3.391995), 2/V/2015, Coll. Doadrio, I; Yahyaoui, A.; Garzón, P.; Perea, S. MNCN_ICTIO 290.864-290.878, 290.880-290.885, 290.887-290.991); 26 specimens from Moulouya River, Moulouya Basin, El ksabi, Atlantic slope in Morocco (32.834840, -4.405431), 19.X.2014, coll. Doadrio, I; Yahyaoui, A.; Garzón, P.; Perea, S. MNCN_ICTIO 290.995-290.997, 290.998-291.006: 12 specimens from the Melloulou River, Moulouya Basin, Guercif, Mediterranean slope in Morocco (34.21526 -3.37568), 2.V.2015, coll. Doadrio, I; Yahyaoui, A.; Garzón, P.; Perea, S. MNCN_ICTIO 290.910-290.936); 27 specimens from Zobzite River, Moulouya Basin, Berkine, Atlantic slope in Morocco (33.78631, -3.79980), 19.X.2014, coll. Doadrio, I; Yahyaoui, A.; Garzón, P.; Perea, S. MNCN_ICTIO 71606-71.611, 71.613-71.614: 8 specimens from Za River, Moulouya Basin, Guefait (type locality of "*Barbus*" *moulouyensis*), Atlantic slope in Morocco (33.78631, -3.79980), 21/4/1991, Coll. Doadrio, I; Cubo, J.; Perdices, A.
Holotype and a series of paratypes (83 specimens) have been deposited at the Fish Collection of the Museo Nacional de Ciencias Naturales, CSIC (Madrid, Spain).

DIAGNOSIS: Differs from other known species of *Luciobarbus* by the following combination of characters: 42-45 scales on the lateral line ($\bar{x}$ =43, Median=43; 8-9 ($\bar{x}$ =8.1 Median=8) above lateral line and 5-6 ($\bar{x}$ =5.3, Median=5) below lateral line. Insertion of the ventral fin is placed in the same edge of the dorsal fin origin. The last single fin ray is ossified in two/thirds of its length, deeply serrated with teeth in the middle part longer than the wide of the ray (Fig. 6). Barbels longer than in other studied populations, the first barbel overpasses the preorbital distance ($\bar{x}$ =1.1 Median=1.1; range=0.7-1.4) while in other species it does not reach the preorbital distance (*L. magrebensis* Median= 0.7; *L. ksibi* Median= 0.8; *L. rifensis* Median= 0.6; *L. setivimensis* Median= 0.8). The second barbel usually has the same length that the postorbital distance ($\bar{x}$ =1 Median=1;

Table 5.— Genetic distances for the complete mitocondrial *cytb* gene. Uncorrected-*p* genetic distances between species are below the diagonal. Uncorrected-*p* genetic distances within species are shown in the diagonal.

Table 5.— Distancias genéticas para el gen mitocondrial citocromo *b* completo. Las distancias genéticas no corregidas entre especies están debajo de la diagonal. Las distancias genéticas no corregidas dentro de las especies se muestran en la diagonal.

	L. maghrebensis	*L. rifensis*	*L. ksibi*	*L. setivimensis*	*L. guercifensis*	Moulouya Basin
L. maghrebensis	**0.2**					
L. rifensis	3.6	**0**				
L. ksibi	3.9	4.0	**0.4**			
L. setivimensis	9.5	9.5	11.2	**0**		
L. guercifensis	9.3	9.1	10.5	7.6	**0.6**	
Moulouya Basin	5.6	5.7	6.8	9.6	8.8	**0.1**

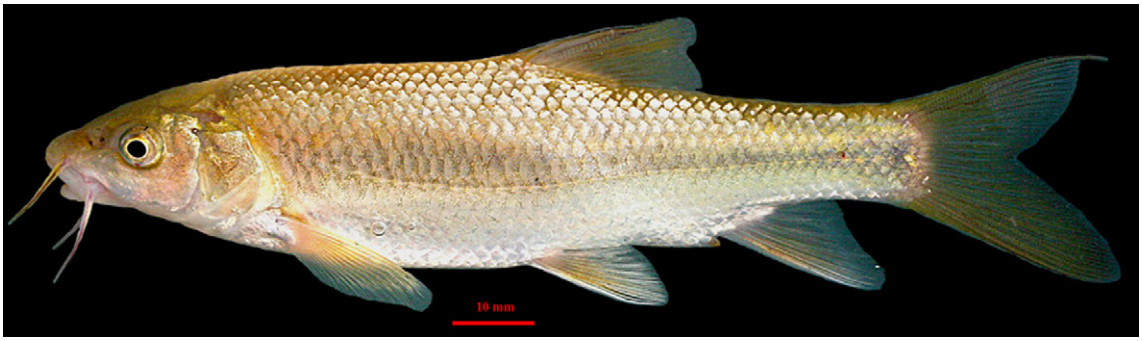

Fig. 8.— Holotype of *Luciobarbus yahyaouii* from the Molouya River, Molouya Basin, Ghafoula, Morocco. MNCN_ICTIO 290.958.

Fig. 8.— Holotipo de *Luciobarbus yahyaouii* del río Moulouya, cuenca del Moulouya, en Ghafoula, Marruecos. MNCN_ICTIO 290.958.

Table 6.— Morphometric and meristic measurements of the holotype and paratypes of *Luciobarbus yahyaouii*.

Tabla 6.— Medidas morfométricas y merísticas del holotipo y paratipos de *Luciobarbus yahyaouii*.

Morphometric variables	Holotype MNCN_ICTIO 290.958	Paratypes n= 83		
	Measurements (mm)	Mean	Range	Standard deviation
SL	119.8	110.7	182.7–64.3	27.8
HL	30.2	27.3	44.3–16.3	6.6
PrOL	9.6	9.1	16.1–4.6	2.6
ED	6.5	6	8.4–4.2	1.1
PsOL	13.7	12.4	19.7–7.2	3
BL1	12	9.9	17.8–3.7	3.4
BL2	15.2	12.7	20.7–6.1	3.5
PrDD	63.2	58.6	95.6–34.7	14.1
PrPD	33.1	29.1	47.5–16.4	7.5
PrVD	65.8	59.8	104.2–33.8	15.7
PrAD	90.5	83.9	143.6–47.3	21.9
CPL	43.5	40	65.6–22.8	10.3
APL	22.4	20.9	34.1–12.9	5
PVL	34.7	31	54.1–16.5	8.3
BD	28.3	27.4	45.6–15.6	6.8
BLD	12.7	12.3	19.5–7.5	2.8
DFL	15.8	15.3	23.8–9.1	3.7
DFH	25.5	21.3	34–12.3	5.1
PFL	23.5	20.9	33.3–9.8	5.5
VFL	20.3	18.9	30.1–11.7	4.5
AFL	8.8	9	15.7–4.8	2.6
AFH	23.6	20.7	33.1–12.5	5.3
CFL	25.6	25.5	40.6–15.2	5.9
LL	43	43	45–42	0.8
RSA	8	8.1	9–8	0.3
RSB	5	5.3	6–5	0.5

range=0.8-1.4) while in other species it does not reach the postorbital distance (*L. magrebensis* Median= 0.5; *L. ksibi* Median= 0.8; *L. rifensis* Median= 0.6; *L. setivimensis* Median= 0.7). The ethmoid bone is wider than its length. The dorsal branch of the pharyngeal bone is long and forms an open angle with respect to the inferior branch. Vertebrae 41-43 ($\bar{x}$ =41.6, n=10), Gill Rakers (GR) 14-17 ($\bar{x}$ =15.9 Median=16).

DESCRIPTION: D IV 8, A III 5, P I 15-16, V I 8, C 18; LL 42-45 ($\bar{x}$ =43, Median=43), RSA 8-9 ($\bar{x}$ =8.1, Median=8), RSB 5-6 ($\bar{x}$ =5.3, Median=5). Pharyngeal teeth in adults 4.3.2/4.3.2., GR 14-17 ($\bar{x}$ =15.9, Median=16), VE 41-43 ($\bar{x}$ =41.6, n=10). A medium-sized species, rarely reaching 500 mm, females are larger than males. The body is elongated in relation to maximum body depth with BD 22-28% of SL. The head is short with respect to the body with HL 22-24% of SL. Infraorbital bones are narrow. The first barbel reaches the rim of the eye and in several specimens it is extended to half the width of the eye. The second barbel usually extends beyond the posterior rim of the eye, usually reaching the preopercule. The anterior barbel is 22-48% (Median=36%) and the second 36-62% (Median=45% of HL). The lips are thick and exhibit a well-developed medial lobe. The lacrimal bone has a medium-sized manubrium. The snout is prominent, but less marked than in *L. maghrebensis* and *L. rifensis*, with preorbital length 6-9% of SL. The iris, as in other Moroccan species of *Luciobarbus,* is yellowish pigmented at the superior border but less conspicuously than in *L. ksibi*. The profile of the dorsal fin is straight, with the last single ray ossified in two-thirds of its length and deeply serrated (Appendix 2-1). The caudal peduncle is less deep than in other species studied, with the exception of *L. ksibi*, and the BLD is 10-12.8% SL. The height of the caudal peduncle is 1.5 to 1.9 times (Median=1.7) the length of the anal peduncle. The pectoral and ventral fins are longer in males, and the anal fin is longer in females. Males exhibit nuptial tubercles in the preorbital space. Ventral fins are inserted on the edge of the dorsal fin insertion. The caudal fin is 19.8-26.7% SL. Morphometric and meristic measurements for the holotype and paratypes of *Luciobarbus yahyaouii* are represented in Table 6. The coloration of *L. maghrebensis* is silver or silver-yellowish in fins (Fig. 8). Some specimens exhibit a mid-flank dark stripe. Juveniles present blotches, as in other *Luciobarbus* species. The skull is wide with a wide ethmoid bone; the pharyngeal bone has a long dorsal process. The lacrimal bone is well developed, and infraorbital bones are wide. The dentary and the maxilla have long anterior processes. The basioccipital plate is small and triangular.

ETYMOLOGY: The species is named after Dr. Ahmed Yahyaoui, who contributed to the knowledge of the fish fauna of Morocco and North Africa.

DISTRIBUTION: This species is endemic to east part of Morocco, inhabiting Moulouya Basin in the Mediterranean slope (Fig. 1).

COMMON NAME: We propose using the English common name "Yahyaoui barbel" for this new species.

HABITAT AND BIOLOGY: The species is ubiquist generally inhabiting rivers with sandy and gravel substrates and in downstream muddy substrates. *Luciobarbus yahyaouii* is the most common fish species in the Moulouya Basin where are also present other species as the trout (*Salmo* sp.) in upperstream currents, the North African shad (*Alosa algeriensis* Regan, 1916) and the sea lamprey (*Petromyzon marinus* Linnaeus, 1758) in the lower courses of rivers. Another two cyprinid species are known in Moulouya Basin; the scarce and rare species *Luciobarbus guercifensis* that inhabits riffle areas; and *Carasobarbus cf. fritschii* that shows habitat requeriments similar to *L. yahyaouii* but are less abundant than the new described species. From April to May the species migrates upstream for spawning. The species is also present in reservoirs.

CONSERVATION: *Luciobarbus yahyaouii* is a common species in Moulouya Basin but the populations are declining due to the development of agricultural activity in the area. As consequence, the Upper Moulouya River has a low regime and poor water quality linked to agriculture. Besides, the river is regulated downstream where there are both a dam and a reservoir. Therefore our suggestion would be to include this species in the IUCN category of Near Threatened (NT).

GENETICS: Uncorrected-*p* distances of mitocondrial gene *cytb* between *L. yahyaouii* and the other analysed species are presented in Table 5. *Luciobarbus yahyaouii* shows one diagnostic locus to (LDH-4*) and 12 diagnostic nucleotide positions to Cytochrome b.

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Appendix 1.— Kruskal-Wallis test and Non-parametric Mann-Whitney's pairwise comparisons for all populations. Values of Kruskal-Wallis test (H) below variables. Values of Mann–Whitney test are below the diagonal. Median in the diagonal of each variable. Significant differences p<0.05 (*); p<0.01 (**). Acronyms are defined in the Material and Methods.

Apéndice 1.— Test de Kruskal-Wallis y análisis no paramétrico de Mann-Whitney para todas las poblaciones. Valores para el test de Kruskal-Wallis (H) debajo de las variables. Valores de Mann-Whitney por debajo de la diagonal. Valor de la Mediana en la diagonal de cada variable. Diferencias significativas p<0,05 (*); p<0,01 (**). Las abreviaturas están descritas en el epígrafe de Material y Métodos.

Variables	**Populations**	**Moulouya Basin (n=12)**	***L. rifensis* (n=12)**	***L. maghrebensis* (n= 27)**	***L. setivimensis* (n=8)**	***L. ksibi* (n=15)**
SL (H=4.36)	Moulouya Basin	110.8				
	L. rifensis	0.254	130,67			
	L. maghrebensis	0.456	0.46	116.27		
	L. setivimensis	0.361	0.73	0.869	113.15	
	L. ksibi	0.079	0.494	0.361	0.322	117.9
SL/HL (H=58.48**)	Moulouya Basin	4.05				
	L. rifensis	0.850	4.04			
	L. maghrebensis	0.662	0.872	4.02		
	L. setivimensis	<0.001**	<0.001**	<0.001**	4.28	
	L. ksibi	<0.001**	<0.001**	<0.001**	0.843	4.28
SL/PrO (H=97.41**)	Moulouya Basin	12.24				
	L. rifensis	<0.001**	11.01			
	L. maghrebensis	<0.001**	0.022*	11.32		
	L. setivimensis	0.003**	<0.001**	<0.001**	12.75	
	L. ksibi	0.0223*	<0.001**	<0.001**	0.603	12.65
SL/OD (H=34.62**)	Moulouya Basin	18.22				
	L. rifensis	<0.001**	20.07			
	L. maghrebensis	<0.001**	0.83	20.5		
	L. setivimensis	0.001**	0.882	0.989	20.02	
	L. ksibi	0.028*	0.033*	0.068	0.063	19.18
SL/LB1 (H=130.5**)	Moulouya Basin	11.77				
	L. rifensis	<0.001**	18.57			
	L. maghrebensis	<0.001**	<0.001**	15.92		
	L. setivimensis	<0.001**	0.079	0.115	16.45	
	L. ksibi	<0.001**	<0.001**	0.011*	0.001**	14.75
SL/L2B (H=163.4**)	Moulouya Basin	8.84				
	L. rifensis	<0.001**	14.16			
	L. maghrebensis	<0.001**	<0.001**	12.41		
	L. setivimensis	<0.001**	0.002**	0.841	12.19	
	L. ksibi	<0.001**	<0.001**	0.024*	0.102	11.59

Appendix 1.— (*continued*)

Variables	Populations	Moulouya Basin (n=12)	*L. rifensis* (n=12)	*L. maghrebensis* (n= 27)	*L. setivimensis* (n=8)	*L. ksibi* (n=15)
SL/PrD (H=84.94**)	Moulouya Basin	1.89				
	L. rifensis	0.331	1.87			
	L. maghrebensis	<0.001**	<0.001**	1.99		
	L. setivimensis	0.038*	0.081	<0.001**	1.85	
	L. ksibi	<0.001**	<0.001**	<0.001**	<0.001**	1.98
SL/PrP (H=41.67**)	Moulouya Basin	3.81				
	L. rifensis	0.239	3.83			
	L. maghrebensis	0.031*	0.375	3.88		
	L. setivimensis	<0.001**	<0.001**	<0.001**	4.11	
	L. ksibi	<0.001**	0.009**	0.155	<0.001**	3.93
SL/PrV (H=44.95**)	Moulouya Basin	1.86				
	L. rifensis	0.002**	1.82			
	L. maghrebensis	0.015*	0.368	1.83		
	L. setivimensis	<0.001**	<0.001**	<0.001**	1.95	
	L. ksibi	0.213	0.045*	0.361	<0.001**	1.84
SL/PrA (H=51.52**)	Moulouya Basin	1.32				
	L. rifensis	<0.006**	1.31			
	L. maghrebensis	<0.006**	0.93	1.31		
	L. setivimensis	<0.001**	<0.001**	<0.001**	1.36	
	L. ksibi	<0.001**	0.305	0.204	<0.001**	1.3
SL/BD (H=20.97**)	Moulouya Basin	4.04				
	L. rifensis	0.0012**	3.92			
	L. maghrebensis	0.381	0.03*	4.01		
	L. setivimensis	0.918	0.028*	0.596	4.02	
	L. ksibi	0.057	<0.001**	0.011*	0.083	4.12
SL/BLD (H=43.37**)	Moulouya Basin	9				
	L. rifensis	0.053*	8.84			
	L. maghrebensis	0.004**	0.127	8.72		
	L. setivimensis	<0.001**	<0.001**	0.002**	8.27	
	L. ksibi	0.288	0.002**	<0.001**	<0.001**	9.11

Appendix 1.— (*continued*)

Variables	Populations	Moulouya Basin (n=12)	*L. rifensis* (n=12)	*L. maghrebensis* (n= 27)	*L. setivimensis* (n=8)	*L. ksibi* (n=15)
SL/CPL (H=77.5**)	Moulouya Basin	2.77				
	L. rifensis	0.117	2.75			
	L. maghrebensis	<0.001**	0.017*	2.7		
	L. setivimensis	<0.001**	<0.001**	<0.001**	2.57	
	L. ksibi	<0.001**	<0.001**	0.002**	0.013*	2.63
SL/APL (H=55.87**)	Moulouya Basin	5.28				
	L. rifensis	0.022*	5.41			
	L. maghrebensis	<0.001**	0.02*	5.54		
	L. setivimensis	<0.001**	<0.001**	<0.001**	4.72	
	L. ksibi	0.045*	0.839	0.135	<0.001**	5.43
SL/PVL (H=52.61**)	Moulouya Basin	3.58				
	L. rifensis	<0.001**	3.47			
	L. maghrebensis	<0.001**	0.337	3.45		
	L. setivimensis	0.88	0.017*	0.003**	3.57	
	L. ksibi	<0.001**	<0.001**	<0.001**	0.001**	3.79
SL/DFL (11.41*)	Moulouya Basin	7.25				
	L. rifensis	0.211	7.36			
	L. maghrebensis	0.256	0.06	7.01		
	L. setivimensis	0.388	0.172	0.864	7.2	
	L. ksibi	0.008**	0.231	0.02*	0.006**	7.47
SL/DFH (139.2**)	Moulouya Basin l	5.18				
	L. rifensis	<0.001**	6.1			
	L. maghrebensis	<0.001**	0.778	6.11		
	L. setivimensis	<0.001**	<0.001**	<0.001**	4.7	
	L. ksibi	0.452	<0.001**	<0.001**	<0.001**	5.22
SL/PFL (H=67**)	Melloulou	5.33				
	L. rifensis	<0.001**	5.77			
	L. maghrebensis	0.422	<0.001**	5.36		
	L. setivimensis	<0.001**	<0.001**	<0.001**	4.6	
	L. ksibi	0.961	<0.001**	0.43	<0.001**	5.29

Appendix 1.— (*continued*)

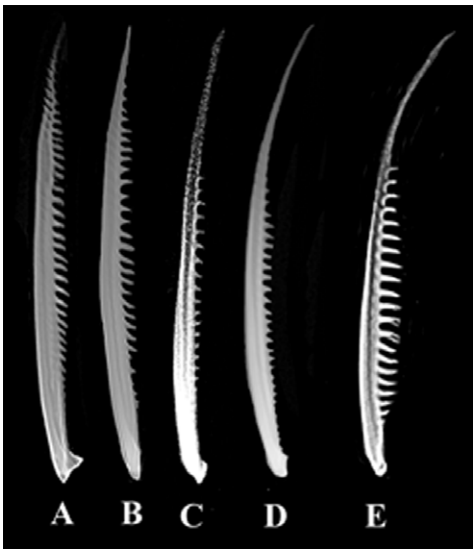
Variables	Populations	Moulouya Basin (n=12)	*L. rifensis* (n=12)	*L. maghrebensis* (n= 27)	*L. setivimensis* (n=8)	*L. ksibi* (n=15)
SL/VFL (H=104.7**)	Moulouya Basin	5.85				
	L. rifensis	<0.001**	6.77			
	L. maghrebensis	0.003**	<0.001**	6.08		
	L. setivimensis	<0.001**	<0.001**	<0.001**	5.42	
	L. ksibi	0.009**	<0.001**	0.785	<0.001**	6.05
SL/AFL (H=29.11**)	Moulouya Basin	12.44				
	L. rifensis	0.557	12.59			
	L. maghrebensis	0.539	0.317	12.34		
	L. setivimensis	0.001**	0.011*	0.005**	13.28	
	L. ksibi	<0.001**	<0.001**	<0.001**	0.674	13.45
SL/AFH (H=113.5**)	Moulouya Basin	5.37				
	L. rifensis	<0.001**	6.18			
	L. maghrebensis	<0.001**	<0.001**	5.58		
	L. setivimensis	<0.001**	<0.001**	<0.001**	4.87	
	L. ksibi	0.063	<0.001**	0.201	<0.001**	5.49
SL/CFL (H=47.84**)	Melloulou	4.34				
	L. rifensis	<0.001**	4.66			
	L. maghrebensis	0.016**	<0.001**	4.23		
	L. setivimensis	0.064	<0.001**	0.886	4.21	
	L. ksibi	<0.001**	0.227	<0.001**	<0.001**	4.53
CPL/BLD (H=27.1**)	Moulouya Basin	3.25				
	L. rifensis	0.497	3.22			
	L. maghrebensis	0.534	1	3.24		
	L. setivimensis	0.404	0.882	1	3.21	
	L. ksibi	<0.001**	<0.001**	<0.001**	<0.001**	3.47
APL/BLD (H=65.05**)	Moulouya Basin	1.71				
	L. rifensis	<0.001**	1.64			
	L. maghrebensis	<0.001**	0.001**	1.58		
	L. setivimensis	0.012*	<0.001**	<0.001**	1.75	
	L. ksibi	0.162	0.097	<0.001**	0.004**	1.68

Appendix 1.— (*continued*)

Variables	Populations	Moulouya Basin (n=12)	*L. rifensis* (n=12)	*L. maghrebensis* (n= 27)	*L. setivimensis* (n=8)	*L. ksibi* (n=15)
LB1/OD (H=73.81**)	Moulouya Basin	1.6				
	L. rifensis	<0.001**	1.12			
	L. maghrebensis	<0.001**	<0.001**	1.3		
	L. setivimensis	<0.001**	0.087	0.148	1.23	
	L. ksibi	<0.001**	<0.001**	0.977	0.092	1.32
LB2/OD (H=94.86**)	Moulouya Basin	2.08				
	L. rifensis	<0.001**	1.46			
	L. maghrebensis	<0.001**	<0.001**	1.68		
	L. setivimensis	<0.001**	0.022*	0.668	1.65	
	L. ksibi	<0.001**	0.003**	0.91	0.96	1.67
PrVD/PrDD (H=72.93**)	Moulouya Basin	1.02				
	L. rifensis	0.262	1.03			
	L. maghrebensis	<0.001**	0.007**	1.06		
	L. setivimensis	<0.001**	<0.001**	<0.001**	0.95	
	L. ksibi	<0.001**	<0.001**	0.218	<0.001**	1.08
HL/PrO (H=75.35**)	Moulouya Basin	3.02				
	L. rifensis	<0.001**	2.72			
	L. maghrebensis	<0.001**	0.021*	2.82		
	L. setivimensis	0.51	<0.001**	0.001**	2.98	
	L. ksibi	0.163	<0.001**	0.02*	0.638	2.95
PrDL/CPL (H=87.22**)	Moulouya Basin	1.47				
	L. rifensis	0.541	1.46			
	L. maghrebensis	<0.001**	<0.001**	1.4		
	L. setivimensis	<0.001**	<0.001**	0.548	1.39	
	L. ksibi	<0.001**	<0.001**	<0.001**	0.003**	1.33
PrAL/APL (H=56.12**)	Moulouya Basin	3.99				
	L. rifensis	0.012*	4.13			
	L. maghrebensis	<0.001**	0.075	4.24		
	L. setivimensis	<0.001**	<0.001**	<0.001**	3.46	
	L. ksibi	<0.013**	0.789	0.3	<0.001**	4.17

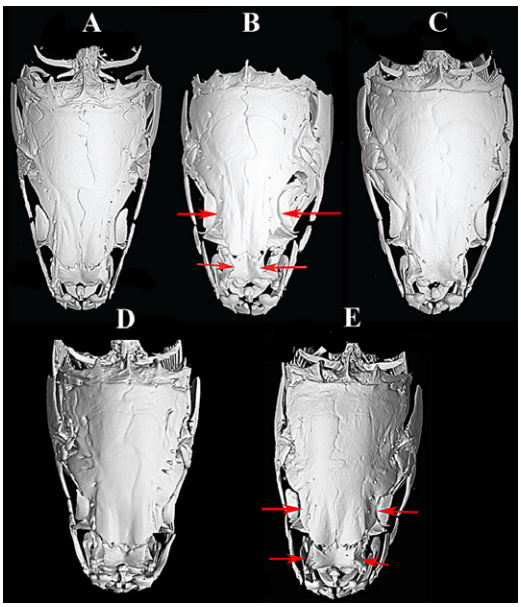
Appendix 1.— (*continued*)

Variables	**Populations**	**Moulouya Basin (n=12)**	***L. rifensis* (n=12)**	***L. maghrebensis* (n= 27)**	***L. setivimensis* (n=8)**	***L. ksibi* (n=15)**
LL (H=78.72**)	Moulouya Basin	43				
	L. rifensis	<0.001**	44			
	L. maghrebensis	<0.001**	0.043	44		
	L. setivimensis	<0.001**	0.108	0.009**	44	
	L. ksibi	<0.001**	<0.001**	<0.001**	0.16	45
RSA (H=179.6**)	Moulouya Basin	8				
	L. rifensis	<0.001**	8.5			
	L. maghrebensis	<0.001**	<0.001**	8		
	L. setivimensis	0.64	<0.001**	<0.001**	8	
	L. ksibi	<0.001**	<0.001**	<0.001**	<0.001**	9
RSB (H=114.2**)	Moulouya Basin	5				
	L. rifensis	<0.001**	4.5			
	L. maghrebensis	<0.001**	<0.001**	5.5		
	L. setivimensis	<0.001**	<0.001**	<0.001**	6	
	L. ksibi	<0.001**	<0.001**	<0.001**	0.028*	6



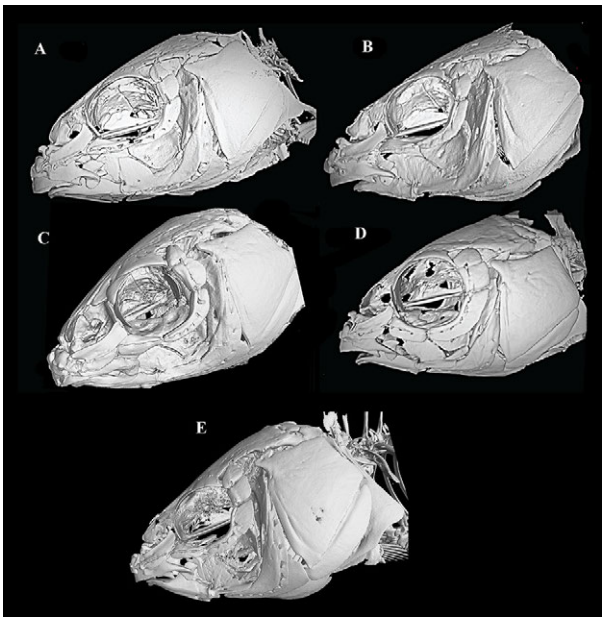
Appendix 2.1.— Last unbranched dorsal-fin ray in adult specimens (SL>120 mm) of the studied populations. A: *Luciobarbus rifensis* (Laou River). B*: Luciobarbus maghrebensis* (Ifrane River). C: *Luciobarbus setivimensis* (Soummam River). D: *Luciobarbus ksibi* (Kasab River). E: Moulouya population (Moulouya River, El Ksabi).

Apéndice 2.1.— Último radio no ramificado de la aleta dorsal en ejemplares adultos (SL>120 mm) de las poblaciones estudiadas. A: *Luciobarbus rifensis* (Laou River). B*: Luciobarbus maghrebensis* (Ifrane River). C: *Luciobarbus setivimensis* (Soummam River). D: *Luciobarbus ksibi* (Kasab River). E: Población del Moulouya (Moulouya River, El Ksabi).



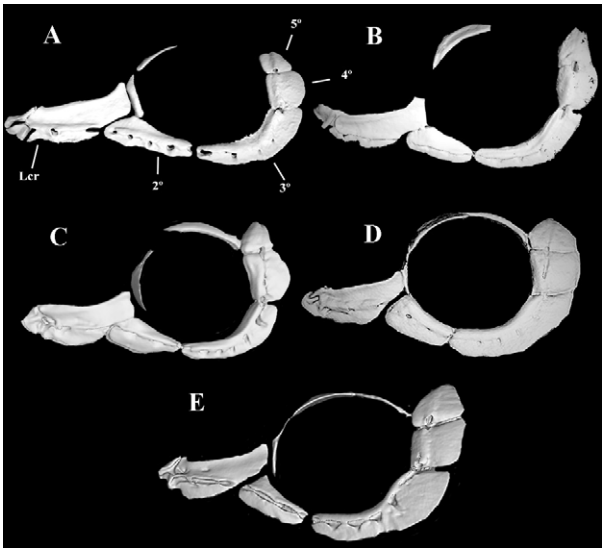
Appendix 2.2.— Dorsal view of the skull of the populations under study. Arrows indicate width of the ethmoid bone and interorbital distance. A: *Luciobarbus rifensis*. B: *Luciobarbus maghrebensis*. C: *Luciobarbus setivimensis*. D: *Luciobarbus ksibi*. E: Moulouya population.

Apéndice 2.2.— Cráneo de las diferentes poblaciones estudiadas. Entre flechas se señala la diferente anchura del etmoides y del espacio interorbitario. A: *Luciobarbus rifensis*. B: *Luciobarbus maghrebensis*. C: *Luciobarbus setivimensis*. D: *Luciobarbus ksibi*. E: Población del Moulouya.



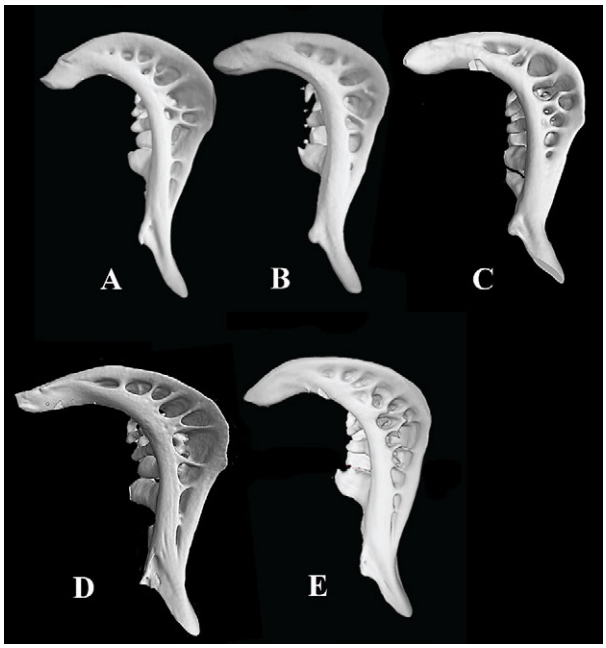
Appendix 2.3.— Lateral view of the skull of the studied populations. A: *Luciobarbus rifensis*. B: *Luciobarbus maghrebensis.* C: *Luciobarbus setivimensis*. D: *Luciobarbus ksibi*. E: Moulouya Population.

Apéndice 2.3.— Cráneo de las diferentes poblaciones estudiadas. Entre flechas se muestra la longitud del opercular. A: *Luciobarbus rifensis*. B: *Luciobarbus maghrebensis*. C: *Luciobarbus setivimensis*. D: *Luciobarbus ksibi*. E: Población del Moulouya.



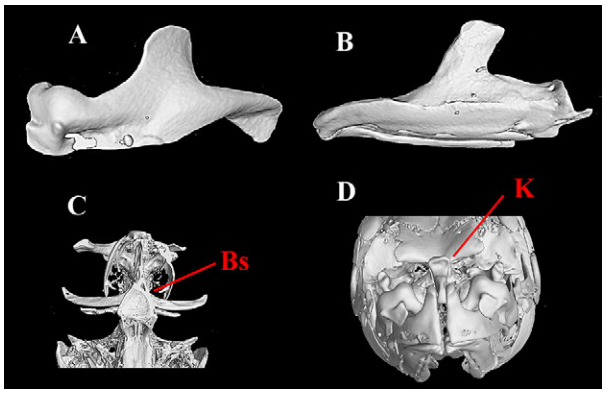
Appendix 2.4.— Infraorbital bones of the studied populations. A: *Luciobarbus rifensis*. B: *Luciobarbus maghrebensis*. C: *Luciobarbus setivimensis*. D: *Luciobarbus ksibi*. E: Moulouya Population. Lcr = Lacrymal. 2°-5°: Infraorbitals.

Apéndice 2.4.— Huesos infraorbitarios de las diferentes poblaciones estudiadas. A: *Luciobarbus rifensis*. B: *Luciobarbus maghrebensis*. C: *Luciobarbus setivimensis*. D: *Luciobarbus ksibi*. E: Población del Moulouya. Lcr: Lacrimal. 2°-5° Infraorbitales.



Appendix 2.5.— Pharyngeal teeth of the studied populations. The arrows show the width of the pharyngeal bone. A: *Luciobarbus rifensis*. B: *Luciobarbus maghrebensis*. C: *Luciobarbus setivimensis*. D: Moulouya Population. E: *Luciobarbus ksibi*.

Apéndice 2.5.— Dientes faríngeos de las diferentes poblaciones estudiadas. Entre flechas la anchura del hueso faríngeo. A: *Luciobarbus rifensis*. B: *Luciobarbus maghrebensis*. C: *Luciobarbus setivimensis*. E: Población del Moulouya. D: *Luciobarbus ksibi*.



Appendix 2.6.— Morphology of different osteological structures and bones of an individual of Moulouya Population. A: Maxillar. B: Dentary. C: Basioccipital. D. Frontal view of the skull. Bc Basioccipital Plate. K Kinethmoids

Apéndice 2.6.— Morfología de diferentes huesos y estructuras óseas de un ejemplar la población del Moulouya. A: Maxilar. B: Dentario. C: Basioccipital. D. Vista frontal del cráneo. Bs: Placa Basioccipital. K: Kinetmoides.