



TAXONOMY OF RHEOPHILIC *LUCIOBARBUS* HECKEL, 1842 (ACTINOPTERYGII, CYPRINIDAE) FROM MOROCCO WITH THE DESCRIPTION OF TWO NEW SPECIES

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ABSTRACT

The genus *Luciobarbus* Heckel, 1843 is characterized by medium to large fish species occurring in large rivers and downstream zones with slow-moving waters. Remarkably also rheophilic *Luciobarbus* occur in Morocco, which are of small size and exhibit distinct morphological traits as well as different habitat requirements. These rheophilic species have traditionally been assigned to *Luciobarbus nasus* (Günther, 1874) and *L. magniatlantis* (Pellegrin, 1919), although some authors consider *L. magniatlantis* as a junior synonym of *L. nasus*. This lack of consensus on the taxonomy of rheophilic barbs is constrained by limited population studies that do not encompass their entire distribution range. Using molecular, morphometric, and osteological data we studied populations of rheophilic barbs of three drainage basins in which they are currently present: Tensift, Moulouya and Oum er Rbia. Our results clearly identified the rheophilic barbs of each basin as different species. The species *Luciobarbus magniatlantis* is a well-recognized species endemic to Tensift Basin. In contrast, the investigated populations from the Moulouya and Oum er Rbia basins could not be assigned to any previously described species. Consequently, we describe two new *Luciobarbus* species in the Moulouya and Oum er Rbia basins.

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

RESUMEN

Taxonomía de las especies reófilas del género *Luciobarbus* Heckel, 1842 (Actinopterygii, Cyprinidae) de Marruecos con la descripción de dos nuevas especies

El género *Luciobarbus* Heckel, 1843 se caracteriza por presentar especies de medio y gran tamaño que viven en las zonas bajas de grandes ríos con aguas lentas. Singularmente, en Marruecos también existen especies reófilas del género *Luciobarbus* de pequeño tamaño y que presentan diferentes caracteres morfológicos y distintos requerimientos de hábitat. Estas especies reófilas han sido tradicionalmente asignadas a *Luciobarbus nasus* (Günther, 1874) y *L. magniatlantis* (Pellegrin, 1919) aunque algunos autores consideran a *L. magniatlantis* como un sinónimo de *L. nasus*. Esta falta de consenso en la taxonomía de los barbos reófilos está limitada por los escasos estudios poblacionales realizados que no abarcan la totalidad del área de distribución de estos barbos. Nosotros, usando datos moleculares, morfométricos y osteológicos, estudiamos las poblaciones de los barbos reófilos de tres cuencas hidrográficas, en las cuales están actualmente presentes: Tensift, Moulouya y Oum er Rbia. Nuestros resultados claramente identifican a los barbos reófilos de cada una de estas cuencas como diferentes especies. La especie *L. magniatlantis* es una especie bien definida y endémica de la cuenca del Tensift. En contraste, las poblaciones analizadas de las cuencas del Moulouya y Oum er Rbia no pudieron ser asignadas a ninguna especie previamente descrita. Consecuentemente, nosotros describimos dos nuevas especies de *Luciobarbus* en las cuencas del Moulouya y del Oum er Rbia.

Palabras clave: Norte de África; *Luciobarbus*; Sistemática; ADN mitocondrial; morfología.

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Introduction

The genus *Luciobarbus* Heckel, 1843 comprises more than 30 species of medium to large size widely distributed along rivers draining into the Persian Gulf and the Mediterranean, Caspian, and Black seas (Doadrio, 1994; Kottelat & Freyhof, 2007). With approximately 20 species, North Africa and the Iberian Peninsula show the highest diversity in *Luciobarbus* populations, with the genus being one of the main component of the freshwater fish fauna of these regions (Doadrio, 1994; Machordom *et al.*, 1995). Phylogenetic studies using morphological characters, have provided evidence for *Luciobarbus* monophyly (Doadrio, 1990), but monophyly has not been unambiguously retrieved by molecular markers, due to the position of the genus *Capoeta* within *Luciobarbus* (Levin *et al.*, 2012; Yang *et al.*, 2015). The genera *Capoeta* and *Luciobarbus* comprise limnetic species, most of which migrate upstream to spawning areas, characterized by the presence of gravel and clear waters (Banarescu, 1999; Doadrio *et al.*, 2011). Although they can survive in a variety of habitats, outside of the reproductive period they are most abundant in downstream areas. Remarkably, in the freshwaters of Morocco rheophilic *Luciobarbus* species can also be found, which have habitat requirements and morphological traits that differ from other *Luciobarbus* species (Doadrio, 1990).

The rheophilic species of *Luciobarbus* are sympatric with limnetic *Luciobarbus* and *Carasobarbus* species in rivers of the Atlas Mountains in Morocco (Doadrio, 1994), but their habitat is restricted to riffle areas, while other Atlas Mountain *Luciobarbus* are more widespread, with preference for slower waters (Doadrio 1990). The rheophilic *Luciobarbus* in Morocco have been assigned, so far, to two species: *Luciobarbus nasus* (Günther, 1874) and *Luciobarbus magniatlantis* (Pellegrin, 1919), which are morphologically characterized, with respect to other *Luciobarbus* species, by size rarely reaching 20 cm standard length; thick barbels placed at the extreme anterior of the snout; and males with equal-sized nuptial tubercles uniformly distributed over the body (Almaça, 1970; Doadrio, 1990). This difference in morphology placed *L. nasus* and *L. magniatlantis* in an unresolved taxonomic position, and they could not be assigned to *Luciobarbus* when the genus was formally defined on the basis of osteological traits (Doadrio, 1990). Subsequently, molecular studies of mitochondrial genes have unambiguously

placed the rheophilic *L. nasus* in the genus *Luciobarbus* together with other North African species (Machordom & Doadrio, 2001a, 2001b).

Within rheophilic *Luciobarbus* from Morocco, the taxonomic position of *L. magniatlantis* remains unclear. This species has been traditionally considered a junior synonym of *L. nasus* (Estève, 1947; Lévêque & Daget, 1984). However, several authors reported morphological traits that unequivocally separate the rheophilic *Luciobarbus* into two morphological types and consider *L. magniatlantis* as a separate species (Pellegrin, 1939; Almaça, 1970, 1971; Doadrio, 1990).

So far there were no available studies on the population structure of the rheophilic barbs of the four Moroccan basins in which they occur: Moulouya Basin, on the Mediterranean slope, and Oum er Rbia, Tensift, and Kasab basins, on the Atlantic slope (Almaça, 1970; Doadrio, 1994; Borkenhagen & Krupp, 2013). This lack of population studies compounds confusion about the type locality of *Luciobarbus nasus* and has hindered the taxonomic work. While the type locality, Tensift Basin, of *L. magniatlantis* is not in question (Pellegrin, 1919), the type locality of *L. nasus* is unclear, because the two syntype specimens were preserved together with sea fishes from Mogador (Essaouira) on the seacoast of Morocco (Günther, 1874). It is probable that the two syntypes of *Barbus nasus* came from material collected in the Kasab River near Mogador by K. v. Fritsch and J. Rein in 1872 (Borkenhagen & Krupp, 2013).

We review the taxonomy of rheophilic *Luciobarbus* from Morocco, investigating morphology and genetic traits of specimens from Moulouya, Tensift, and Oum er Rbia basins, where they have been previously reported (Almaça, 1970). The recognition of two new distinct taxonomic entities, lead us to the description of two new species of *Luciobarbus* for the freshwaters of Morocco.

Material and Methods

Our study of *Luciobarbus* populations was based on 15 specimens (14 males, one female) from Tensift Basin, 33 specimens (21 males, 12 females) from Oum er Rbia Basin, and 12 specimens (12 males) from Moulouya Basin (Fig. 1; Table 1). Material from Kasab Basin could not be collected despite consecutive efforts over the course of several years. The Kasab River has been intensively modified in recent years by anthropogenic pressure as a consequence of the touristic development of Essaouira (Mogador).

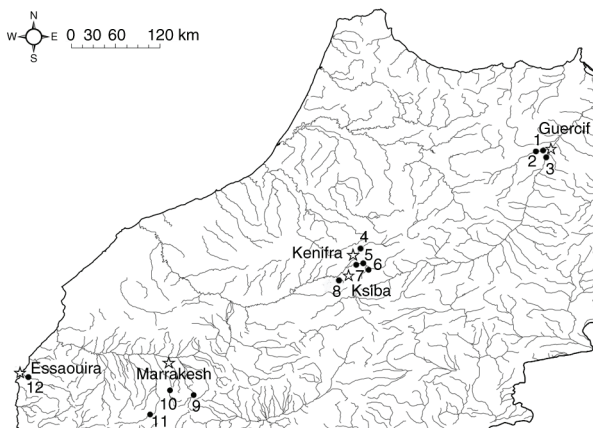


Fig. 1.— Sampling localities of *Luciobarbus* populations in Morocco. Melloul River, Guercif (1, 2); Moulouya R., Ghafoula (3); Oum er Rbia R., El Borj (4); Chbouka R., El Herri (5); Douna R., El Khemis (6); Serrou R., El Herri (7); Arba R., Ksiba (8); Ourika R., (9); Reraia R., Moulay Brahim (10); N'Fiss R., Kasbah Goundafi, and Imaounane (11); Kasab R., Essaouira (Former Mogador) (12).

Fig. 1.— Localidades de las poblaciones de *Luciobarbus* de Marruecos muestreadas. Río Melloul, Guercif (1, 2); Río Moulouya, Ghafoula (3); Río Oum er Rbia, El Borj (4); Río Chbouka, El Herri (5); Río Douna, El Khemis (6); Río Serrou, El Herri (7); Río Arba, Ksiba (8); Río Ourika, (9); Río Reraia, Moulay Brahim (10); Río N'Fiss, Kasbah Goundafi and Imaounane (11); Río Kasab, Essaouira (antiguo Mogador) (12).

Three cyprinid species were collected in the Kasab River during the past two decades: *Luciobarbus ksibi* (Boulenger, 1905), "*Labeobarbus*" *reinii* (Günther, 1874), and *Carasobarbus fritschii* (Günther, 1874). However, the endemic "*L.* *reinii*" and *L. nasus* from the Kasab River have not been found in the past ten years, and it is likely that the species have disappeared or at least they are extremely rare. Therefore, our study of rheophilic *Luciobarbus* from the Kasab River was limited to the museum specimens of the two syntypes of *Barbus nasus*.

The material studied comprised the following specimens and locations: *Tensift Basin*: 3 specimens from the Reraia River, Moulay Brahim (31.2862, -7.9597), Morocco (voucher numbers: MNCN 280454-280456); 2 specimens from the N'Fiss River, Imaounane (30.9899, -8.2011), Morocco (voucher numbers: MNCN 208172-280173); 7 specimens from the Ourika River, Morocco (voucher numbers: MNHN-IC-1919-0431-0437); Lectotype and Paralectotypes of *Barbus magniatlantis* Pellegrin, 1919; 3 specimens from N'Fiss River, Kasbah Goundafi (30.9899, -8.2011), Morocco (voucher numbers: MNHN-IC-1919-0438-040). *Oum er Rbia Basin*: 1 specimen from the Douna River, El Khemis (32.7508, -5.5416) Morocco, (voucher number: MNCN 55094); 2 specimens from the Serrou River, El Herri (32.8276, -5.6152), Morocco (voucher numbers: MNCN 208115-280116); 14 specimens from Arba River, Ksiba (32.5668, -6.0174), Morocco (voucher numbers: MNCN: 54987-55000); 9 specimens from the Oum er Rbia River, El Borj (33.0157, -5.6295),

Morocco (voucher numbers: MNCN 208168-208169; MNCN 71972-71978); 7 specimens from the Chbouka River, El Herri (32.8595, -5.6213), Morocco (voucher numbers: MNCN 279704-279707; MNCN 280083; MNCN 279695-279696). *Moulouya Basin*: 3 specimens from the Melloulou River, Guercif (34.2180, -3.3467 and 34.215, -3.3756), Morocco (voucher numbers: MNCN 286595-286596; MNCN-290831); 9 specimens from the Moulouya River, Ghafoula (34.145, -3.388), Morocco (voucher numbers: MNCN 290832-290840). *Kasab Basin*: 2 syntype specimens of *Barbus nasus* Günther, 1874 from the Kasab River preserved at the Natural History Museum (BMNH 1874.1.30.25-26).

All sampling sites (Fig. 1) presented similar riverine morphology, with clear water, rapid current and gravel bottom.

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 for 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 assess sexual dimorphism and 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).

Table 1.— Sampling localities for *Luciobarbus* from Moroccan and GenBank Accession numbers.Tabla 1.— Localidades de muestreo para *Luciobarbus* de Marruecos y números de acceso de GenBank.

Population assignment/species	Locality	No. Individuals studied Morphology/molecular	Number in Phylogenetic tree	GenBank Accession Numbers	Number in map
Moulouya population	Melloulou R. Guercif/ Moulouya Basin	3/1	M3	KU257527	1, 2
Moulouya population	Moulouya R. Ghafoula/ Moulouya Basin	9/3	M1, M2, M4	KU257525, KU257526, KU257528	3
Oum er Rbia population	Oum er Rbia R. El Borj/Oum er Rbia Basin	9/3	R1, R2, R3	AY004744, KU257531, KU257532	4
Oum er Rbia population	Chbouka R. El Herri/Oum er Rbia Basin	7/-			5
Oum er Rbia population	Douna R. El Khemis/Oum er Rbia Basin	1/-			6
Oum er Rbia population	Serrou R. El Herri/Oum er Rbia Basin	2/1	R4	KU257533	7
Oum er Rbia population	Arba R. Ksiba/Oum er Rbia Basin	14/-			8
Tensift population. Lectotype and Paralectotypes of <i>Barbus magniatlantis</i>	Ourika R./Tensift Basin	7/-		xxx	9
Tensift population	Reraia R. Moulay Brahim/ Tensift Basin	3/2	T2, T3	KU257536, KU257537	10
Tensift population. Paralectotypes of <i>Barbus magniatlantis</i>	N'Fiss R. Kasbah Goudafi/ Tensift Basin	3/-		xxx	11
Tensift population	N'Fiss R. Imaounane/Tensift Basin	2/2	T1, T4	KU257534, KU257535	11
Kasab population. Syntypes of <i>Barbus nasus</i>	Kasab R. Essaouira/Kasab Basin	2/-			12
<i>L. ksibi</i>	Kasab R. /Kasab Basin.	-/2	1, 2	KU257523, KU257524	12
<i>L. ksibi</i>	Reraia R./Tensift basin	-/2	3, 4	KU257538, KU257539	10
<i>L. ksibi</i>	Chbouka R./Oum er Rbia basin	-/2	5, 6	KU257529, KU257530	5
<i>L. bocagei</i>	Duratón R./Duero Basin	-/1			
<i>L. comizo</i>	Tajo R./Tajo Basin	-/1			
<i>L. graellsii</i>	Irati R./Ebro Basin	-/1			
<i>L. guiraonis</i>	Turia R./Turia Basin	-/1			
<i>L. microcephalus</i>	Zujar R./Guadiana Basin	/1			
<i>L. sclateri</i>	Segura R./Segura Basin	/1			
<i>L. setivimensis</i>	Soumman R./Soumman Basin	/1			

For molecular analyses, we obtained specimens of rheophilic *Luciobarbus* from Oum er Rbia, Tensift, and Moulouya basins along with the limnophilic *L. ksibi* from Oum er Rbia, Tensift and Kasab basins, (Table 1). Also, the Iberian species of *Luciobarbus* and *L. setivimensis* (Valenciennes, 1842) from Algeria were added. *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 fin-clip tissue using the commercial kit Biosprint15 for tissue and blood (Qiagen).

For each specimen, the complete region (1140 bp) of the mitochondrial cytochrome b (cytb) was amplified. Primers and protocols used for PCR for cytb 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. Sequences were deposited in the GenBank database under accession numbers KU257523-KU257539.

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. Bayesian inference 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 a majority-rule consensus tree was estimated after discarding the first 10% of generations. The robustness of the clades was assessed using Bayesian posterior probabilities. The average uncorrected p-distances among *Luciobarbus* populations were calculated for the cytb gene using MEGA package v. 6.0 (Tamura *et al.*, 2013).

Results and Discussion

COMPARISON OF MORPHOLOGY AMONG POPULATIONS

Due to the sexual dimorphism of *Luciobarbus* (Doadrio, 1990), and because few female specimens were available, we removed females from the subsequent morphological analyses. Non-parametric Kruskal–Wallis and Mann–Whitney *post hoc* comparison analyses were used to assess differences in size and body shape among populations (Appendix 1). No differences in SL were found among *Luciobarbus* populations. However we found significant differences in morphometric measures, with the exception of pre-ventral and preanal distances, anal and dorsal fin lengths, pre and post-orbital distances, and caudal peduncle length (Appendix 1).

The population from Moulouya basin exhibited an elongated body shape with the smallest body depth of any studied population. The dorsal fin was placed slightly more anterior, and the predorsal distance was shorter, than in Tensift and Oum er Rbia populations. All fins in the Moulouya population were larger than the ones present in the remaining populations, with the height of the anal fin significantly bigger than in other populations, in some specimens reaching, or nearly reaching, the anterior rays of the caudal fin when folded. The population of Oum er Rbia Basin possessed the longest anal caudal peduncle. This population had a narrow skull, characterized by the lowest inter-orbital width. The population of Tensift Basin had the smallest head, with the head and prepectoral distances shorter than in Moulouya and Oum er Rbia populations. The deepest caudal peduncle was also found in the Tensift population. The fins in the Tensift population were significantly smaller than in Moulouya and Oum er Rbia populations. Some specimens of the Tensift population, belonging to paralectotypes of *Barbus magniatlantis*, presented three pairs of barbels, a characteristic that was not observed in any other populations. An overview of the general morphology of the analysed populations is illustrated in Figs. 2 and 3.

The number of scales along the lateral line (median=45), superior transverse line (median=7.5) and inferior transverse line (median=5.5) was significantly lower in the Moulouya population than in the Tensift and Oum er Rbia (Appendix 1). Scale number along the lateral line (median=51) and superior transverse line (median=9.5) were significantly higher in the Oum er Rbia population than in Tensift and Moulouya populations.

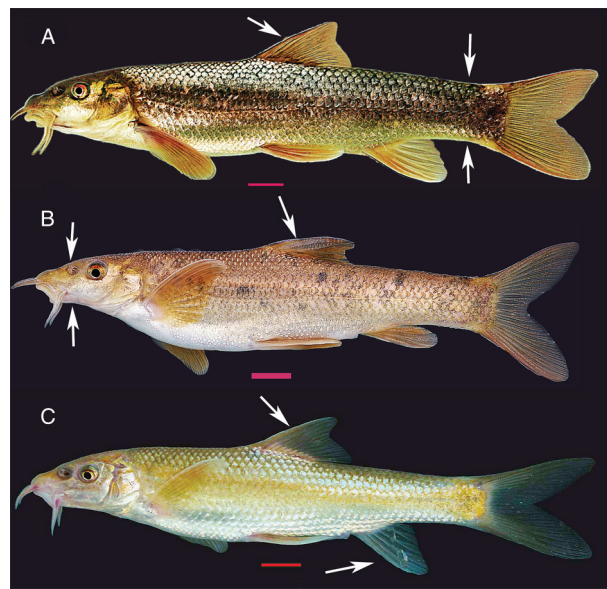


Fig. 2.— Morphology of the analysed populations. Arrows show principal traits of each population. A: Tensift Basin population; B: Oum er Rbia Basin; C: Moulouya Basin. Scale: 10 mm.

Fig. 2.— Morfología de las poblaciones analizadas. Las flechas muestran los principales rasgos de cada población. A: población del Tensift; B: población del Oum er Rbia; C: población del Moulouya. Escala: 10 mm.



Fig. 3.— Syntypes of *Barbus nasus* showing the characteristic deep caudal peduncle. Figure from the Natural History Museum (London).

Fig. 3.— Sintipos de *Barbus nasus* mostrando su característico y profundo pedúnculo caudal. Figura del Museo de Historia Natural de Londres.

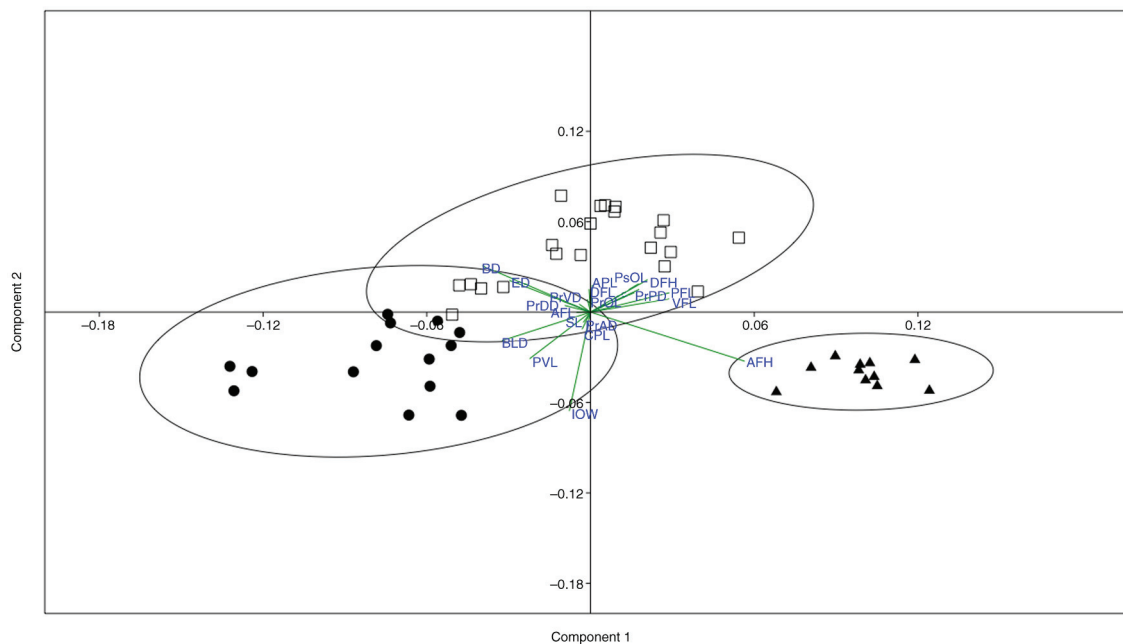


Fig. 4.— Variables that most contributed to the PCA analysis. Dots: Tensift population. Squares: Oum er Rbia population. Triangle: Moulouya population. Acronyms are defined in Materials and Methods.

Fig. 4.— Variables que más contribuyeron al análisis de PCA. Puntos: población del Tensift. Cuadrados: población del Oum er Rbia. Triángulo: población del Moulouya. Los acrónimos están definidos en la sección de Material y Métodos.

The principal component analysis (PCA) divided the specimens into three groups, corresponding to the populations of the three investigated basins (Fig. 4). The eigenvalues of the three first principal components, with the Burnaby-corrected matrix, explained most of the variance (Table 2). The highest eigenvector values (anal fin height and inter-orbital width) were in agreement with results of Kruskal–Wallis and Mann–Whitney analyses (Table 2).

OSTEOLOGICAL FEATURES (APPENDIX 2)

As shown by morphometric analyses, the skull of the Oum er Rbia population was narrower than the one present in Tensift and Moulouya populations. In particular, the ethmoid bone was longer and narrower (Appendix 2-1). In contrast, the kinethmoid bone of the Oum er Rbia population was shorter and more robust than in other populations (Appendix 2-2). The posterior branch of the lachrymal bone was elongated in the Oum er Rbia population and shorter in the Moulouya population (Appendix 2-3). The dentary bone was shorter in the Tensift population than in other populations (Appendix 2-4). The anterior process of the maxilla of the Tensift population was lesser developed than in Oum Er Rbia and Moulouya populations (Appendix 2-5). The number of pharyngeal teeth in Tensift, and Oum er Rbia populations was usually 4.3.2. As in other *Luciobarbus* species, the juveniles possessed five teeth in the external row. In the Tensift population, the fifth tooth was occasionally retained in the adults. In the Moulouya population, we found

pharyngeal teeth in a 4.2.1 or 4.3.1 configuration (Appendix 2-6). The Oum er Rbia population had a thick inner branch of the pharyngeal bone, with reduced pharyngeal lamina. In the Tensift population, the superior branch of the pharyngeal bone was strongly flexed (Appendix 2-7). In Oum er Rbia populations, the last single ray of the dorsal fin was strongly ossified with a maximum width of 12–14.5% (median=13.8) of its height (Fig. 5). In Tensift (6.7–9.5%, median=8.3) and Moulouya (6.2–9.2%, median=7.4) populations, the last single ray of the dorsal fin showed a lower level of ossification (Fig. 5). The Tensift and Moulouya populations showed weaker denticulations in the last single ray of the dorsal fin, but higher denticulate density (Moulouya 3.1–2.4 teeth/mm, median=2.6 teeth/mm; Tensift 2.2–1.8 teeth/mm, median=2.1 teeth/mm) (Fig. 5). The population from Oum er Rbia had scarce and strong denticulations on the last single ray of the dorsal fin at a density of 1.1–1.4 teeth/mm, median=1.2 teeth/mm (Fig. 5).

MOLECULAR DATA

The phylogenetic analysis using BI, with *Aulopyge huegelli* and *Barbus meridionalis* as outgroup taxa, revealed two main clades corresponding primarily to the Iberian and African species (Fig. 6). The Iberian group clustered with *Luciobarbus setivimensis* of Algeria, as was previously reported (Machordom & Doadrio, 2001b). Unexpectedly, the populations of rheophilic *Luciobarbus* were not monophyletic, and the Moulouya Basin population was clustered with

Table 2.— Eigenvalues and eigenvectors for the first three principal components (PC1-PC3) of 21 morphometric variables for all rheophilic *Luciobarbus* populations. Acronyms are defined in the Material and Methods section. Variables with the highest eigenvalues for each PC are in bold.

Tabla 2.— Eigenvalores y eigenvectores para los tres primeros componentes principales (CP1-CP3) de 21 variables morfométricas para todas las poblaciones de barbos reófilos. 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	0.0050	0.0019	0.0009
% variance	46.48	18.05	8.85
Eigenvectors			
SL	-0.2605	-0.0678	-0.1058
PrDD	-0.0926	0.0432	-0.1735
PrPD	0.1747	0.1477	0.0542
PrVD	-0.0406	0.0515	0.0110
PrAD	-0.0046	-0.0412	0.0021
PVL	-0.2190	-0.3034	-0.3745
CPL	-0.0301	-0.1112	-0.3078
APL	0.0062	0.1765	-0.3697
BD	-0.3888	0.302	0.1404
BLD	-0.3187	-0.1862	0.0311
HL	0.1575	0.1267	-0.1107
PrOL	-0.0114	0.0839	0.4119
ED	-0.2916	0.2065	0.1496
PsOL	0.1731	0.1844	-0.0512
PFL	0.2832	0.1257	0.1731
VFL	0.2832	0.0867	0.2835
DFL	-0.0053	0.1496	0.1400
DFH	0.2053	0.2113	-0.0097
AFL	-0.0504	-0.0029	-0.1324
AFH	0.5536	-0.3194	-0.1403
IOW	-0.0760	-0.6432	0.4349

the Iberian group, while the populations from Oum er Rbia and Tensift basins constituted a monophyletic group with *Luciobarbus ksibi* from the same basins and from its type locality (Kasab River). The phylogenetical position of the Moulouya population within the Iberian group was not resolved.

Genetic distances within Moulouya, Oum er Rbia and Tensift populations of rheophilic barbels ranged from 0.1 to 0.3%. The Moulouya population showed genetic distances from Tensift and Oum er Rbia populations ranging from 8.1 to 8.4%. The genetic distances between Oum er Rbia and Tensift populations was 1.1% (Table 3). Genetic distances between limnophilic *L. ksibi* and rheophilic barbels of the same basins (Oum er Rbia and Tensift) were from 5.6 to 5.9%. The genetic distances of Moulouya population with respect to other *Luciobarbus* species were similar or higher

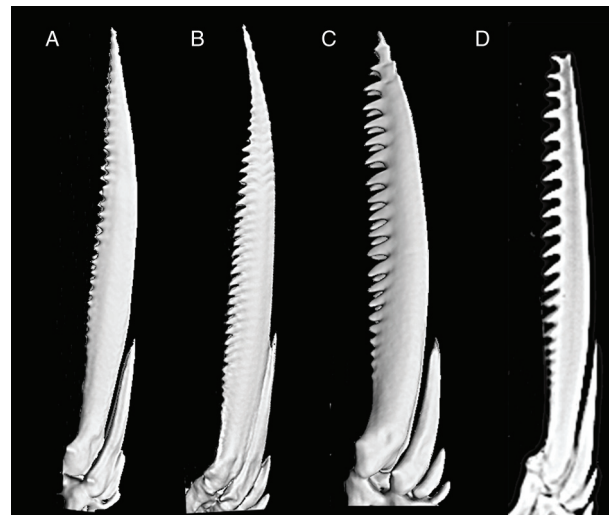


Fig. 5.— Denticulation of the last single ray of the dorsal fin in specimens of the populations from Tensift (A, SL 135.8 mm), Moulouya (B, SL 139 mm), Oum er Rbia (C, SL 126.5 mm) and syntype of *Barbus nasus* (D, SL 108.7 mm).

Fig. 5.— Último radio sencillo denticulado de la aleta dorsal en individuos de las poblaciones del Tensift (A, SL 135,8 mm), Moulouya (B, SL 139 mm), Oum er Rbia (C, SL 126,5 mm) and sintipo de *Barbus nasus* (D, SL 108,7 mm).

than the ones reported to sister species of cyprinid fishes (Doadrio & Carmona, 2003, 2006; Doadrio & Madeira, 2004; Robalo *et al.*, 2005; Doadrio & Elvira, 2007; Domínguez-Domínguez *et al.*, 2007, 2009). In contrast, genetic distances between Tensift and Oum er Rbia population were lower than the ones found in sister species of *Luciobarbus* (Casal-López *et al.*, 2015).

TAXONOMIC REMARKS

The three studied populations from the Oum er Rbia, Moulouya, and Tensift basins possessed molecular, osteological, and morphometric traits that identified them as different species. The assignment of Oum er Rbia, Moulouya, and Tensift populations to the rheophilic species *L. nasus* was not possible, as the combination of diagnostic traits of *L. nasus* was not shared by the studied populations. The combination of the diagnostic traits of all populations and the syntypes of *Barbus nasus* are showed in Table 4. The population morphologically most similar to *L. nasus* was that of the Oum er Rbia Basin. Differences between the Oum er Rbia population and *L. nasus* were found, primarily in the morphology of the last single ray of the dorsal fin, number of scales in the lateral line, morphology of the pharyngeal teeth, kinethmoid bone shape and caudal peduncle depth. The maximum width of the last single dorsal fin ray of the syntype of *Barbus nasus* was 11.5% of its height, and does not reach the robustness of that of the Oum er Rbia population (12-14.5%, median=13.8) (Fig. 5). The number of scales in the lateral line was 46-44 in *Barbus nasus*

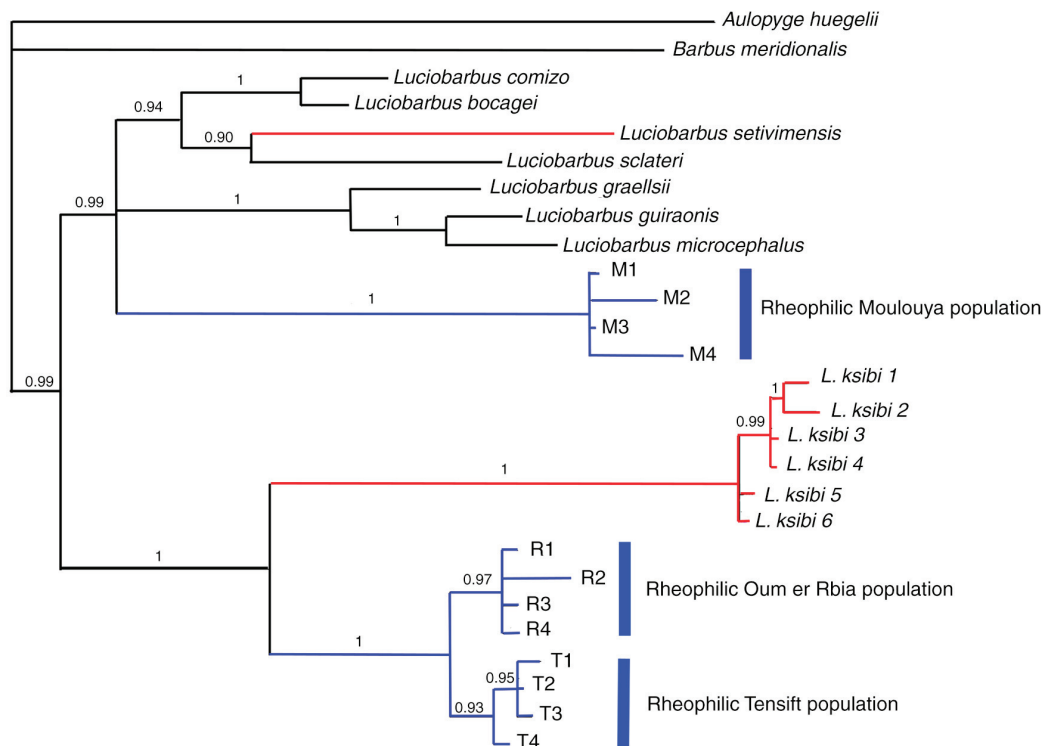


Fig. 6.— Phylogenetic tree rendered by Bayesian Inference of the mitochondrial cytochrome *b* gene. Numbers on branches indicate posterior probability values. Identification of localities is defined in Table 1. Red branches show limnophilic North African species. Blue branches represent North African rheophilic populations.

Fig. 6.— Árbol filogenético del gen mitocondrial 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. Las ramas marcadas en rojo representan las especies limnófilas del Norte de África. Las ramas marcadas en azul señalan las poblaciones reófilas del Norte de África.

syntypes and 49-53 scales ($\bar{x}=51$, median=51) in Oum er Rbia population. Pharyngeal teeth and kinethmoid bone were more robusts in Oum er Rbia than in *L. nasus* (Appendix 2-2, 2-7). Anal peduncle length was less of two times the BLD while in Oum er Rbia population was always more of two times.

In the absence of molecular data and a greater number of specimens for morphological studies, *L. nasus*

must be considered an endemic species of the Kasab Basin and *L. magniatlantis* an endemic species of the Tensift Basin.

The populations of Moulouya and Oum er Rbia basins exhibit multiple diagnostic traits differing from those of *L. nasus* and *L. magniatlantis* (Table 4).

DESCRIPTION OF LUCIOBARBUS POPULATIONS

The high degree of morphological and genetic differentiation of *Lucio barb* populations endemic to the Moulouya and Oum er Rbia basins justifies the consideration of these population as distinct species. No available names for these populations exist, and therefore these are described as new species in the present study.

***Lucio barb* guercifensis sp. nov.** Doadrio, Perea & Yahyaoui

<http://lsid:zoobank.org:act:6DF6332F-F73B-437F-88E1-2F4AC7F15F4F>

HOLOTYPE: Fig. 7, Table 5. MNCN 290835 male, 139.4 mm (SL); Moulouya River, Moulouya Basin, Ghafoula, Mediterranean slope in Morocco (34.14534, -3.38847) (Fig. 1); 22/6/2015. 378 ATSL, Collected by (Coll.) Doadrio, I; Yahyaoui, A and Perea, S.

Table 3.— Genetic distances for the complete mitochondrial cytb gene. Uncorrected-p genetic distances between species are below the diagonal. Uncorrected-p genetic distances within species are shown in the diagonal.

Tabla 3.— 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.

	Moulouya	Oum er Rbia	Tensift	<i>L. ksibi</i>
Moulouya	0.2			
Oum er Rbia	8.4	0.3		
Tensift	8.1	1.1	0.1	
<i>L. ksibi</i>	9.1	5.9	5.7	0.5

Table 4.— Diagnostic morphological characters of the four populations studied.

Tabla 4.— Caracteres morfológicos diagnósticos de las cuatro poblaciones estudiadas.

	Moulouya population	Oum er Rbia population	Tensift <i>L. magniatlantis</i>	Kasab <i>L. nasus</i>
Width of the last single ray of the dorsal fin	<10% Height	>10% Height	<10% Height	>10% Height
Denticulations of the last single ray of the dorsal fin	Profusely serrated 24-26 denticulations in adults	Sparsely serrated 11-13 denticulations in adults	Profusely serrated 18-22 denticulations in adults	Sparsely serrated 20-21<25 denticulations in adults
No. scales in lateral line	45-48≤48	49-53>48	51-47≤48	44-46≤48
No. scales above lateral line	6.5-8.5	8.5-9.5	8.5	9.5
Inner row of pharyngeal teeth	1	2	2	2
Pharyngeal Teeth	Robust	Robust	Robust	Weak
Lachrymal manubrium	Wide	Narrow	Narrow	Narrow
Ethmoid bone	Wide	Narrow	Wide	Narrow
Kinethmoid bone	Narrow	Wide	Narrow	Narrow
Caudal peduncle	Low and elongated. ACL is ≥2.1 the BLD	Low and elongated. ACL is ≥2.1 the BLD	Short and high. ACL is <2 the BLD	Short and high. ACL is <2 the BLD

PARATYPES: Table 5. MNCN 286595-96: 2 specimens from Melloulou River, Moulouya Basin, Guercif, Mediterranean slope in Morocco (34.218035, -3.346732); 9/4/2007. Coll. Doadrio, I; Doadrio, I jr. and Perea, S. MNCN 290831: 1 specimen from Melloulou River, Moulouya Basin, Guercif, Mediterranean slope in Morocco (34.21526, -3.375668). 2/5/2015. Coll. Doadrio, I; Garzón, P; Perea, S and Yahyaoui, A. MNCN 290832: 1 specimen from Moulouya River, Moulouya Basin, Ghafoula, Mediterranean slope in Morocco (34.14534, -3.38847); 2/5/2015. Coll. Doadrio, I; Perea, S; Garzón, P and Yahyaoui, A. MNCN 290833-290834, 290836-290840: 7 specimens from Moulouya River, Moulouya Basin, Ghafoula, Mediterranean slope in Morocco (34.14534, -3.38847); 22/6/2015. Coll. Doadrio, I; Perea, S and Yahyaoui, A.

The holotype and a series of paratypes (12 specimens) have been deposited at the Fish Collection of the Museo Nacional de Ciencias Naturales, Madrid, Spain.

DIAGNOSIS: Differs from other known *Luciobarbus* species by the following combination of characters: 45-48 scales along the lateral line (\bar{x} =45.6, median=45), 6.5-8.5 (\bar{x} =7.4, median=7.5) above lateral line, and 5.5 below lateral line. The last single ray of the dorsal fin is strongly ossified with the maximum width 6.2-9.2% (median=7.4) of its length. The last single dorsal fin ray is densely denticulated along its length,



Fig. 7.— Holotype of *Luciobarbus guercifensis* from the Moulouya River, Ghafoula, Morocco. MNCN 290835. SL=139.4 mm.

Fig. 7.— Holotipo de *Luciobarbus guercifensis* del Río Moulouya, Ghafoula, Marruecos. MNCN 290835. SL=139,4 mm.

and, in adults specimens, the number of denticulations exceeds 25 (Fig. 6). The ethmoid bone is wider than its length. Most specimens possess a single pharyngeal tooth in the inner row. The number of vertebrae is 39-41 (\bar{x} =39.7, n=11). The interorbital distance is 1.7 to 2.2 times (median=2) the eye diameter. The caudal peduncle is longer than the one seen in *L. nasus* and *L. magniatlantis*, with depth 2.1-2.6 times the length of the anal peduncle. The posterior segment (manubrium) of the lachrymal bone is short and high. Differences in diagnostic characters among analysed *Luciobarbus* populations are presented in Table 4.

DESCRIPTION: D III-V 8, A III 5, P I 15-16, V I 6, C 18; LL 45-48 (\bar{x} =45.6, median=45), RSA 6.5-8.5 (\bar{x} =7.4, median=7.5), RSB 5.5. Pharyngeal teeth in adults 4.3.1 or 4.2.1. GR 13-15 (\bar{x} =14.1, median=14), VE 39-41 (\bar{x} =39.7, median=40). Rarely reaches 200 mm SL. The body is elongated, relative to maximum body depth compared to other *Luciobarbus* species. Maximum body depth is 16-19% of SL. Head length (\bar{x} =28.5 mm) is 25-27% of SL and greater than body depth (\bar{x} =20.1 mm). The skull is wide, with the ethmoid bone width greater than its length. The interorbital distance is 1.7 to 2.2 times eye diameter. Infraorbital bones are narrow and the lachrymal bone is shorter than in *Oum er Rbia* population as consequence of a shorter manubrium. The height of the manubrium is 20-23% the lachrymal length. Thick lips and barbels display granular appearance. The first pair of barbels is short and located at the anterior extreme of the mouth, reaching the insertion of the second pair of barbels. The second pair of barbels is thick and reaches the posterior edge of the eye. The anterior barbel is 23.2-29.7%, and the second 34.6-38.6%, of HL. The snout is prominent, with preorbital length 10-12.6% of SL. The preorbital length (\bar{x} =13.6 mm) is larger

Table 5.— Morphometric and meristic measurement of the holotype and paratypes of *Luciobarbus guercifensis*. Acronyms are defined in the Material and Methods section.

Tabla 5.— Medidas morfométricas y merísticas del holotipo y paratipos de *Luciobarbus guercifensis*. Los acrónimos están definidos en la sección de Material y Métodos.

Morphometric variable	Holotype	Paratypes n=11		
	MNCN 290835 Measurements (mm)	Range	Mean	Standard Deviation
SL	139.4	75.7-149.4	109.4	23.5
PrDD	66.7	37.1-70.3	52.5	10.4
PrPD	35.2	20.1-38.2	28.3	5.7
PrVD	61.2	35.4-69.8	50.5	10.8
PrAD	97.7	49.7-107.8	75.9	18.4
PVL	30.4	15.4-34.3	24.3	5.6
CPL	58.1	30.9-60.6	44.6	9.5
APL	32.8	19.1-34.8	26.3	4.9
BD	26	12.4-28.1	19.5	4.9
BLD	14.9	7.8-16.2	11.5	2.7
HL	35	20.5-37.8	27.8	5.6
PrOL	17.3	8.1-18.8	12.6	3.2
ED	5	3-5.2	4	0.7
PsOL	15.4	8.7-16	12.1	2.3
IOW	10.5	5.1-11.3	7.9	1.9
B1L	9.7	4.7-11	7.4	2
B2L	12.8	7.1-13.7	10.2	2.1
PFL	30	16.8-33.3	24.1	4.9
VFL	27.3	16.8-31	22.7	4.5
DFL	17.1	9.2-17.7	13.4	2.8
DFH	27.2	15.7-28.5	22	4
AFL	11	5.8-12.1	8.6	2.0
AFH	26.4	16.3-28.5	22.1	3.9
CFL	32.5	16.5-34.1	26.1	5.3
LL	45	45-48	45.7	0.9
RSA	7.5	5.5-8.5	7.4	0.5
RSB	5.5	5.5	5.5	-

than the postorbital length (\bar{x} =12 mm). The dorsal fin is posterior on the body but slightly more anterior than in other rheophilic barbs, with the predorsal distance being 46.2-50% of SL. The profile of the dorsal fin is concave, with the last single ray ossified with more than 25 denticulations (Fig. 5). In males, the anal fin is longer than the one seen in *L. nasus* and *L. magniatlantis* with its height 76-92% of APL. The caudal peduncle is less deep than in *L. magniatlantis* and *L. nasus*, with a height 9.9-10.9% of SL. The length of the anal caudal peduncle is 2.1 to 2.6 times its height. In males, the pectoral and ventral fins are long with pectoral fins usually reaching the origin of the ventral fins. Males exhibited numerous nuptial tubercles of equal size distributed over the body and fins. The caudal fin

length is 16.6-27.3% of SL. Morphometric and meristic measurements for the holotype and paratypes of *Luciobarbus guercifensis* are presented in Table 5. The colouration of *L. guercifensis* is silver-yellowish with a paler ventral area (Fig. 2).

DISTRIBUTION: This species is endemic to Moulouya basin, inhabiting Moulouya and Melloul rivers in riffle areas near to the Guercif village (Fig. 1).

ETYMOLOGY: The species name 'guercifensis' was selected, as because the species is mainly distributed around the Guercif village in Morocco.

COMMON NAME: We propose the English common name 'Guercif barbel' for this new species.

HABITAT AND BIOLOGY: The species inhabits large rivers, mainly in mid-stream, usually in riffle areas. No information exists on the spawning period or reproductive behaviour.

CONSERVATION: Currently, *Luciobarbus guercifensis* is a rare species that has been found in few places, localized in riffle areas. These riffle areas are becoming scarce in Moulouya River as a consequence of increasing crop irrigation at its headwaters. The water taken for irrigation, as well as the impact of fertilizers and pesticides on water quality in the lower courses has likely been the primary cause of the recent decline of this population. No quantification of the decline in numbers is available. We suggest that this species should be included in the IUCN category of Endangered.

GENETICS: Uncorrected-*p* distance of mitochondrial cytb gene between *Luciobarbus guercifensis* and the other analysed species are presented in Table 3. *L. guercifensis* shows 63 diagnostic positions in the cytb gene with respect to other rheophilic barbels.

***Luciobarbus zayanensis* sp. nov.** Doadrio, Casal-López & Yahyaoui

<http://lsid:zoobank.org:act:7A79DAD4-B60A-43F9-8BF6-617B34670E64>

HOLOTYPE: Fig. 8, Table 6. MNCN 279706, male, 113 mm (SL); Chbouka River, Oum er Rbia Basin, El Herri (Elhri), Atlantic slope in Morocco (32.859510, -5.621355) (Fig. 1); 27/3/2013. 872 ATSL. Coll. Doadrio, I; Yahyaoui, A; Garzón, P and Perea, S.

PARATYPES: Table 6. MNCN 55094 one specimen from the Douna River, Oum er Rbia Basin, El Khemis, Atlantic slope in Morocco (32.750866, -5.541695); 7/5/1988. Coll. Doadrio, I; Merino, M; Cubo J and González, JL. MNCN 208115-208116: 2 specimens from Serrou River, Oum er Rbia Basin, El Herri, Atlantic slope in Morocco (32.827621, -5.615255). 27/4/2000. Coll. Doadrio, I; Garzón, P; Doadrio, A and Doadrio, I Jr. MNCN 54987-55000: 14 specimens from the Arba River, Oum er Rbia Basin, Ksiba, Atlantic slope in Morocco (32.566810, -6.017450). 8/5/1988. Coll. Doadrio, I; Merino, M; Cubo J and González, J.L. MNCN 208168-208169,



Fig. 8.— Holotype of *Luciobarbus zayanensis* from the Chbouka River, Oum er Rbia Basin, El Herri, Morocco. MNCN 279706. SL=113 mm.

Fig. 8.— Holotipo de *Luciobarbus zayanensis* del Río Chbouka, Cuenca del Oum er Rbia, El Herri, Marruecos. MNCN 279706. SL=113 mm.

Table 6.— Morphometric and meristic measurement of the holotype and paratypes of *Luciobarbus zayanensis*. Acronyms are defined in the Material and Methods section.

Tabla 6.— Medidas morfométricas y merísticas del holotipo y paratipos de *Luciobarbus zayanensis*. Los acrónimos están definidos en la sección de Material y Métodos.

Morphometric measurement	Holotype	Paratypes n=32		
	MNCN 279706 Measurements (mm)	Range	Mean	Standard Deviation
SL	113	64.5-178.0	118.2	25.1
PrDD	56.7	33.3-92.5	59.5	12.7
PrPD	30	17.9-41.5	29.7	5.3
PrVD	54.7	33.3-79.4	55.1	10.5
PrAD	78.4	44.6-121.6	81.7	17.2
PVL	26.4	13.6-42.3	27.1	6.6
CPL	43.7	25.4-76.3	48.5	11.3
APL	28.3	16.0-45.2	29.8	6.3
BD	20.8	12.0-43.4	25.7	7.0
BLD	12.3	6.1-20.6	13.3	3.0
HL	28.9	17.4-43.4	29.6	5.8
PrOL	14.9	7.8-20.5	13.8	2.8
ED	4.6	3.5-6.3	4.7	0.6
PsOL	12	8.8-17.3	12.8	1.9
IOW	7.1	4.1-12.5	7.8	1.8
B1L	8.1	4.8-8.0	6.5	1.1
B2L	10.5	6.2-11.8	9.4	1.6
PFL	23.4	15.1-33.4	24.1	4.1
VFL	22.1	14.0-30.6	22.1	3.5
DFL	15.6	8.0-23.0	14.7	3.1
DFH	25	14.7-32.7	22.8	4.1
AFL	8.3	5.3-14.1	9.2	2.1
AFH	21	11.3-29.2	20.2	4.2
CFL	27.1	15.1-37.5	25.5	4.9
LL	51	49.0-53.0	51.1	1.2
RSA	9.5	8.5-9.5	9.3	0.4
RSB	5.5	5.5-7.5	6.6	0.6

MNCN 71972-71978: 9 specimens from the Oum er Rbia River, El Borj, Atlantic slope in Morocco Morocco (33.015769, -5.629534). 18/4/1991. Coll. Doadrio, I; Perdices, A and Cubo, J. MNCN 279704-279705, MNCN 279707, MNCN 280083, MNCN 279695-279696: 6 specimens from the Chbouka River, Oum er Rbia Basin, El Herri, Atlantic slope in Morocco (32.859510, -5.62135527/3/2013. 27/3/2013. Coll. Doadrio, I; Yahyaoui, A; Garzón, P; and Perea, S.

Holotype and a series of paratypes (33 specimens) have been deposited at the Fish Collection of the Museo Nacional de Ciencias Naturales, Madrid, Spain.

DIAGNOSIS: Differs from other known species of *Luciobarbus* by the following combination of characters: 49-53 scales along the lateral line (\bar{x} =51, median=51); 8.5-9.5 scales (\bar{x} =9.3, median=9.5) above lateral line and 5.5-7.5 (\bar{x} =6.5, median=6.5) below lateral line. The last single ray of the dorsal fin is strongly ossified with a maximum width of 12-14.5% (median=13.9%) of its length. The last single dorsal fin ray is densely denticulate along its length and, in adults, the number of denticulations is smaller than 23 (Fig. 5). The skull is narrow, with the width of the ethmoid bone smaller than its length. The inter-orbital distance is 1.2 to 1.9 times (median=1.6) eye diameter. The pharyngeal teeth formula in adults is 4.3.2. The number of vertebrae is 41-43 (\bar{x} =42, n=10). Large deep caudal peduncle 2.1-2.6% of APL. Lachrymal bone with narrow posterior segment (manubrium). Differences in diagnostic characters among analysed *Luciobarbus* populations are presented in Table 4.

DESCRIPTION: D III-V 8, A III 5, P I 15-16, V I 6, C 18; LL 49-53 (\bar{x} =51, median=51), RSA 8.5-9.5 (\bar{x} =9.3, median=9.5), RSB 5.5-7.5 (\bar{x} =6.5, median=6.5). Pharyngeal teeth in adults 4.3.2. GR 13-16 (\bar{x} =14.5, median=14.5), VE 41-43 (\bar{x} =42, n=10). Similarly to other rheophilic *Luciobarbus*, it is a small-sized species, rarely reaching 200 mm SL. Females are larger (\bar{x} =128.6 mm) than males (\bar{x} =112.9 mm). The body is elongated with maximum body depth 18-24% of SL in males and 21-24% in females. The head is typically sharp and narrow. The inter-orbital distance is 1.2 to 1.9 times the eye diameter in males and 1.4 to 1.9 times in females. The skull is narrow, with the ethmoid bone longer than wide. The frontal bones are narrower relative to other rheophilic barbs, notably in the anterior part. Infraorbital bones are narrow, and the lachrymal bone is longer than in other rheophilic barbs and possesses a long manubrium. The height of the manubrium is 10-13% the lachrymal bone length. The kinethmoid bone is more robust than the one found in other rheophilic barbs. Thick lips and barbels with granular appearance. The first pair of barbels is short and positioned at the anterior extreme of the mouth, reaching the insertion of the second pair of barbels. The second pair of barbels is thick and reaches the posterior edge of the eye. The anterior barbel length is 20.1-29.9%, and the second 29.7-39.5%

of HL. The snout is prominent, with preorbital length 10.9-12.1% of SL in females and 10.6-13.2 in males. The preorbital length (\bar{x} =14.8 mm in females and \bar{x} =13.5 mm in males) is greater than the postorbital length (\bar{x} =13.5 mm in females and \bar{x} =12.3 mm in males). The pectoral and ventral fins are larger in males than in females. The pectoral fin length is 76-83% (\bar{x} =80%) of HL in females and 72-90% (\bar{x} =83%) of HL in males. The ventral fin length is 65.5-74% (\bar{x} =70%) of HL in females and 65.5-85% (\bar{x} =78%) of HL in males. The dorsal fin is located posteriorly on the body, with the predorsal distance being 48.9-52.5% of SL in females and 48.5-52.5% in males. The profile of the dorsal fin is concave, with the last single ray of the dorsal fin ossified with few and strong denticulations (Fig. 5). The caudal peduncle is elongated, and the least body depth is 11-11.9% SL in females and 9.5-11.8% SL in males. The length of the anal caudal peduncle is 2.1 to 2.3 times the height of the anal peduncle in females and 2.1-2.6 times in males. Males exhibited numerous equal-sized nuptial tubercles distributed over the body and fins. The caudal fin length is 20-23% of SL in females and 18.4-24.5% of SL in males. Morphometric and meristic measurements for the holotype and paratypes of *Luciobarbus zayanensis* are represented in Table 6. The coloration of *L. zayanensis* is brownish with a paler ventral area and some black spots along the body (Fig. 2).

DISTRIBUTION: This species is endemic to Oum er Rbia basin, inhabiting riffle areas with clear waters in the Atlas Mountains (Fig. 1).

ETYMOLOGY: The species name 'zayanensis' has been selected because it is mainly distributed around Middle Atlas region, in the area inhabited by the Zayanes shepherds.

COMMON NAME: We suggest the English common name 'Zayan barbel' for this new species.

HABITAT AND BIOLOGY: The species inhabits riffle areas in headwaters of large rivers. Spawning takes place from late April to early May.

CONSERVATION: The habitat of *L. zayanensis* is being transformed by dam and reservoir constructions and by increased pollution linked to agriculture and urban development for tourism. The population of *L. zayanensis* is in decline, therefore we suggest that this species should be included in the IUCN category of Vulnerable.

GENETICS: Uncorrected-*p* distances of mitochondrial gene cytb between *L. zayanensis* and the other analysed species are presented in Table 3. *Luciobarbus zayanensis* shows 5 diagnostic positions in the cytb gene.

Acknowledgments

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

Apéndice 1.— Test de Kruskal–Wallis y comparaciones *post hoc* no paramétricas de Mann–Whitney para todas la poblaciones. Los valores del test de Kruskal–Wallis (H) aparecen debajo de las variables. Los valores del test de Mann–Whitney están debajo de la diagonal. La mediana es la diagonal de cada variable. Diferencias significativas $p < 0.05$ (*); $p < 0.01$ (**). Los acrónimos están definidos en la sección de Material y Métodos.

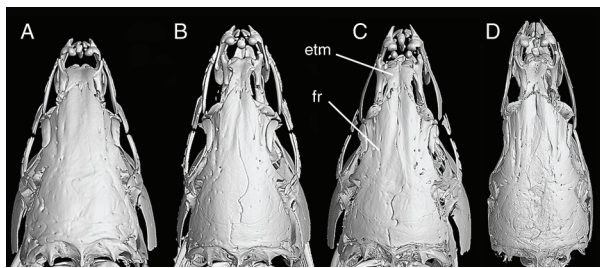
Variables	Populations	Tensift (n=14)	Oum er Rbia (n=21)	Moulouya (n=12)
SL (H=0.502)	Tensift	108.6		
	Oum er Rbia	0.449	112.9	
	Moulouya	0.763	0.968	109.2
PrDD (H=14.63**)	Tensift	52.9/0.49		
	Oum er Rbia	0.88	55.8/0.5	
	Moulouya	<0.0001**	<0.0001**	53.3/0.48
PrPD (H=16.54**)	Tensift	25.2/0.24		
	Oum er Rbia	0.005**	29.4/0.26	
	Moulouya	<0.0001**	0.052	28.7/0.26
PrVD (H=4.197)	Tensift	50.2/0.46		
	Oum er Rbia	0.96	54.7/0.47	
	Moulouya	0.095	0.057	52/0.46
PrAD (H=1.556)	Tensift	73.4/0.68		
	Oum er Rbia	0.827	78.4/0.68	
	Moulouya	0.529	0.177	77.2/0.7
PVL (H=13.73**)	Tensift	24.9/0.24		
	Oum er Rbia	0.002**	24.9/0.22	
	Moulouya	0.0005**	0.8427	25/0.22
CPL (H=1.89)	Tensift	42.7/0.41		
	Oum er Rbia	0.372	44.2/0.4	
	Moulouya	0.978	0.177	44.3/0.41
APL (H=17.8**)	Tensift	25.3/0.24		
	Oum er Rbia	<0.0001**	28.3/0.25	
	Moulouya	0.019	0.06	26/0.24
BD (H=24.63**)	Tensift	22.4/0.21		
	Oum er Rbia	0.259	23.4/0.2	
	Moulouya	<0.0001**	<0.0001**	19.7/0.18
BLD (H=34.48**)	Tensift	12.8/0.12		
	Oum er Rbia	<0.0001**	12.5/0.11	
	Moulouya	<0.0001**	<0.0001**	11.7/0.11
HL (H=29.07**)	Tensift	25.1/0.24		
	Oum er Rbia	<0.0001**	28.9/0.25	
	Moulouya	<0.0001**	0.234	28.5/0.25
PrOL (H=2.33)	Tensift	12.7/0.12		
	Oum er Rbia	0.578	13.5/0.12	
	Moulouya	0.106	0.365	13.6/0.11
PsOL (H=22.8**)	Tensift	11.1/0.1		
	Oum er Rbia	<0.0001**	12.3/0.11	
	Moulouya	0.0002**	8427	12/0.11
ED (H=30.41**)	Tensift	4.7/0.04		
	Oum er Rbia	0.002	4.6/0.04	
	Moulouya	<0.0001**	<0.0001**	4.2/0.04

Appendix 1.— (Continued)

Variables	Populations	Tensift (n=14)	Oum er Rbia (n=21)	Moulouya (n=12)
PFL (H=28.05**)	Tensift	20.9/0.2		
	Oum er Rbia	0.0001**	24.2/0.21	
	Moulouya	<0.0001**	0.0009**	24.5/0.22
VFL (H=24.74**)	Tensift	19.4/0.18		
	Oum er Rbia	0.0009**	22.2/0.2	
	Moulouya	<0.0001**	0.001**	23.5/0.2
DFL (H=3.7)	Tensift	13.8/0.12		
	Oum er Rbia	0.699	14.8/0.13	
	Moulouya	0.132	0.074	13.6/0.12
DFH (H=26.43**)	Tensift	19.6/0.18		
	Oum er Rbia	<0.0001**	23.1/0.2	
	Moulouya	<0.0001**	0.7812	21.9/0.2
AFL (H=1.27)	Tensift	8.3/0.08		
	Oum er Rbia	0.88	9.1/0.08	
	Moulouya	0.218	0.451	8.9/0.08
AFH (H=25.74**)	Tensift	17.6/0.16		
	Oum er Rbia	0.1623	19.9/0.17	
	Moulouya	<0.0001**	<0.0001**	22.4/0.2
IOW (H=32.72**)	Tensift	7.9/0.07		
	Oum er Rbia	<0.0001**	7.1/0.06	
	Moulouya	0.132	<0.0001**	8.1/0.07
LL (H=37.66**)	Tensift	49		
	Oum er Rbia	<0.0001**	51	
	Moulouya	<0.0001**	<0.0001**	45
RSA (H=40.5**)	Tensift	8.5		
	Oum er Rbia	<0.0001**	9.5	
	Moulouya	<0.0001**	<0.0001**	7.5
RSB (H=21.65**)	Tensift	6.5		
	Oum er Rbia	0.03*	6.5	
	Moulouya	0.002	<0.0001**	5.5

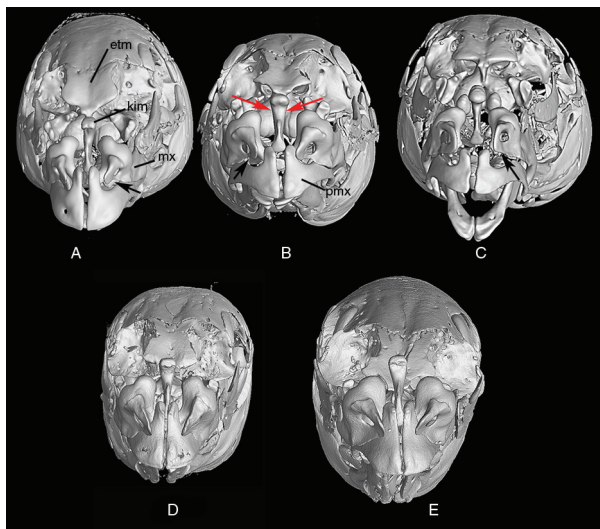
Appendix 2.— Osteological features.

Apéndice 2.— Características osteológicas.



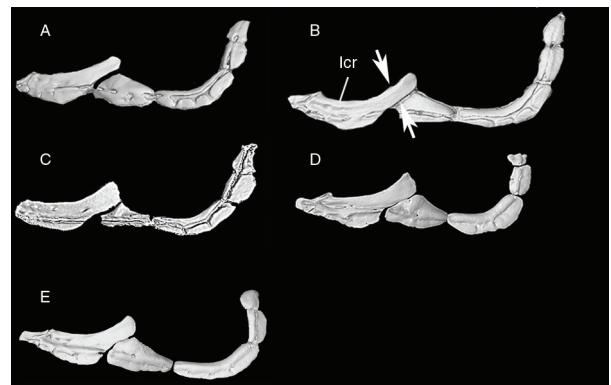
Appendix 2-1.— Dorsal view of the skull of the populations under study. A: Tensift population (N'Fiss River), SL 135.8 mm. B: Oum er Rbia population (Chbouka river), SL 126.5 mm. C: Moulouya population (Moulouya River), SL 139 mm. D: Syntype of *Barbus nasus*, SL 108.7 mm. Etm: ethmoid bone; fr: frontal.

Apéndice 2-1.— Vista dorsal del cráneo de las poblaciones estudiadas. A: población del Tensift (Río N'Fiss), SL 135,8 mm. B: población del Oum er Rbia (Río Chbouka), SL 126,5 mm. C: población del Moulouya (Moulouya River), SL 139 mm. D: Sintipo de *Barbus nasus*, SL 108,7 mm. Etm: ethmoid; fr: frontal.



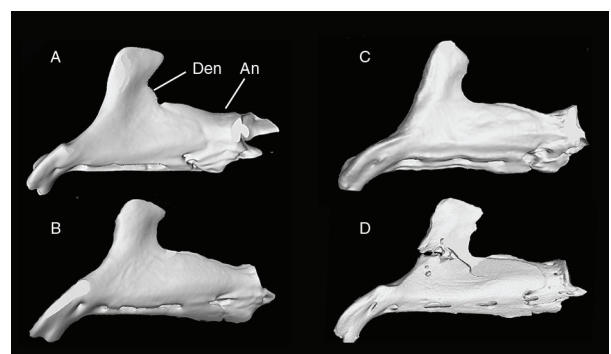
Appendix 2-2.— Frontal view of the skull of the populations under study. A: Tensift population (N'Fiss River), SL 135.8 mm. B: Oum er Rbia population (Chbouka river) showed a short and wide kinethmoid bone, SL 126.5 mm. C: Moulouya population (Moulouya River), SL 139 mm. D: Syntype of *Barbus nasus*, SL 108.7 mm. E: Syntype of *Barbus nasus*, SL 81.5 mm. Etm: Ethmoid bone; Kim: Kinethmoid bone; Pmx: Premaxilla. Arrows show the width of kinethmoid bone.

Apéndice 2-2.— Vista frontal del cráneo de las poblaciones estudiadas. A: población del Tensift (Río N'Fiss), SL 135,8 mm. B: la población del Oum er Rbia (Río Chbouka) mostró un hueso kinetmoides corto y ancho, SL 126,5 mm. C: población del Moulouya (Río Moulouya), SL 139 mm. D: Sintipo de *Barbus nasus*, SL 108,7 mm. E: Sintipo de *Barbus nasus*, SL 81,5 mm. Etm: Etmoides; Kim: Kinetmoides; Pmx: Premaxilar. Las flechas muestran la anchura del kinetmoides.



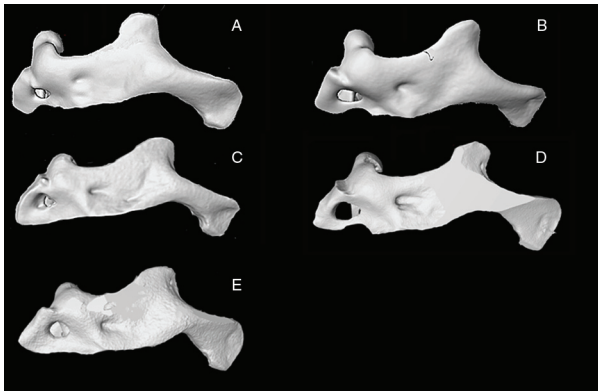
Appendix 2-3.— Infraorbital bones of the studied populations. A: Tensift population (N'Fiss River), SL 135.8 mm. B: Oum er Rbia population (Chbouka river), SL 126.5 mm. C: Moulouya population (Moulouya River), SL 139 mm. D: Syntype of *Barbus nasus*, SL 108.7 mm. E: Syntype of *Barbus nasus*, SL 81.5 mm. Lcr: Lacrymal. Arrows show the width of lacrymal manubrium.

Apéndice 2-3.— Huesos infraorbitarios de las poblaciones estudiadas. A: población del Tensift (Río N'Fiss), SL 135,8 mm. B: población del Oum er Rbia (Río Chbouka), SL 126,5 mm. C: población del Moulouya (Río Moulouya), SL 139 mm. D: Sintipo de *Barbus nasus*, SL 108,7 mm. E: Sintipo de *Barbus nasus*, SL 81,5 mm. Lcr: Lacrymal. Las flechas muestran la anchura del manubrio del lacrymal.



Appendix 2-4.— Dentary bone of the studied populations. A: Tensift population (N'Fiss River), SL 135.8 mm. B: Oum er Rbia population (Chbouka river), SL 126.5 mm. C: Moulouya population (Moulouya River), SL 139 mm. D: Syntype of *Barbus nasus*, SL 108.7 mm. Den: Dentary; An: Anguloarticular.

Apéndice 2-4.— Hueso dentario de las poblaciones estudiadas. A: población del Tensift (N'Fiss River), SL 135,8 mm. B: población del Oum er Rbia (Río Chbouka), SL 126,5 mm. C: población del Moulouya (Río Moulouya), SL 139 mm. D: Sintipo de *Barbus nasus*, SL 108,7 mm. Den: Dentario; An: Anguloarticular.



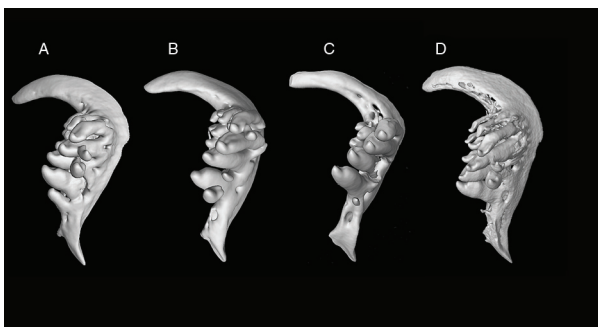
Appendix 2-5.— Maxilla of the studied populations. A: Tensift population (N'Fiss River) showed a short anterior process, SL 135.8 mm. B: Oum er Rbia population (Chbouka River), SL 126.5 mm. C: Moulouya population (Moulouya River), SL 139 mm. D: Syntype of *Barbus nasus*, SL 108.7 mm. E: Syntype of *Barbus nasus*, SL 81.5 mm.

Apéndice 2-5.— Maxilar de las poblaciones estudiadas. A: la población del Tensift (Río N'Fiss) mostró un proceso anterior corto, SL 135,8 mm. B: población del Oum er Rbia (Río Chbouka river), SL 126,5 mm. C: población del Moulouya (Río Moulouya), SL 139 mm. D: Sintipo de *Barbus nasus*, SL 108,7 mm. E: Sintipo de *Barbus nasus*, SL 81,5 mm.



Appendix 2-7.— External view of the pharyngeal teeth of the populations studied. Width of the inner branch between arrows. A: Tensift population (N'Fiss River) showed a flexed superior branch, SL 135.8 mm. B: Oum er Rbia population (Chbouka river) showed a strong inner branch, SL 126.5 mm. C: Moulouya population (Moulouya River) showed a thin pharyngeal bone. SL 139 mm. D: Syntype of *Barbus nasus*, SL 108.7 mm. E: Syntype of *Barbus nasus*, SL 81.5 mm. ib: Inner Branch. obr: Over branch.

Apéndice 2-7.— Vista externa de los dientes faríngeos de las poblaciones estudiadas. Anchura de la rama interna entre flechas. A: población del Tensift (Río N'Fiss) mostró una rama superior flexionada, SL 135,8 mm. B: población del Oum er Rbia (Río Chbouka) mostró una rama interna robusta, SL 126,5 mm. C: población del Moulouya (Río Moulouya) mostró un hueso faríngeo delgado, SL 139 mm. D: Sintipo de *Barbus nasus*, SL 108,7 mm. E: Sintipo de *Barbus nasus*, SL 81,5 mm. ib: Rama interna. obr: Rama superior.



Appendix 2-6.— Pharyngeal teeth of the populations studied. A: Tensift population (N'Fiss River) showed 5 teeth in the external row, 3 in the middle, and 2 in the inner row, SL 135.8. B: Oum er Rbia population (Chbouka river) showed 4 teeth in the external row, 3 in the middle, and 2 in the inner row, SL 126.5 mm. C: Moulouya population (Moulouya River) showed 4 teeth in the external row, 2 in the middle, and one in the inner row, SL 139 mm. D: Syntype of *Barbus nasus* showed 4.3.2 pharyngeal teeth, SL 81.5 mm.

Apéndice 2-6.— Dientes faríngeos de las poblaciones estudiadas. A: población del Tensift (Río N'Fiss) mostró 5 dientes en la fila externa, 3 en la del medio y 2 en la fila interna, SL 135,8 mm. B: población del Oum er Rbia (Río Chbouka) mostró 4 dientes en la fila externa, 3 en la del medio y 2 en la fila interna, SL 126,5 mm. C: población del Moulouya (Río Moulouya) mostró 4 dientes en la fila externa, 2 en la del medio y una en la fila interna, SL 139 mm. D: Sintipo de *Barbus nasus* mostró 4.3.2 dientes faríngeos, SL 81,5 mm.