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 taxonomía 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*” *reini* Günther, 1874 that remains without a clear generic assignation.

In Morocco, the genera *Luciobarbus*, *Carasobarbus* and the enigmatic “*Barbus*” *reini* 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 Km² 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 characteristics 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 magniatlantis* (Pellegrin, 1919); *Luciobarbus zayanensis* Doadrio, Casal-López and Yahayoui, 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 of 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 Guefaït. 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 *h* gene.
Taxonomy of Luciobarbus populations from Moulouya Basin

We studied also Holotypes of Barbus moulouyensis Pellegrin, 1924, Barbus moulouyensis var. bouramensis Pellegrin, 1939 and the possible Holotype of Barbus moulouyensis var. grandisquamaata 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 ICTIO 290.880-290.885, 290.887-290.991); 12 specimens (3 females, 8 males and 1 undet.) from Moulouya River in Guercif, Morocco (voucher numbers: MNCN ICTIO 290.995-290.997, 290.998-291.006); 27 specimens (11 females and 16 males) from Zebzite 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 Moulouya 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 Oued Ifrane (voucher numbers: MNCN ICTIO 279.711-729, 290.731, 297.733-744) and Tizguiter (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 Ain Bouram in El ksiba- Tezghrit, Morocco (MNHN 1939-0121). Barbus moulouyensis var. grandisquamaata. 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; Röhl & 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 meridonialis 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., Guefai (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).

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

| Population assignment/ species | Locality | No. Individuals studied | Morphology/ molecular code | 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 | KX681701-81703 | 3 |
| Moulouya population           | Zobzite R. Berkine/Moulouya Basin | 27/3 | M1-M3 | KX681698-KX681700 | 4 |
| Moulouya population           | Za R. Guefai/ Moulouya Basin | 8/- | | 5 |
| *Luciobarbus rifensis*        | Laou R. Derdara/ Laou Basin | 47/4 | R1-R4 | KT003027-KT003930 | 6 |
| *Luciobarbus magrebenensis*   | 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 |
| *Luciobarbus 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 |
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).
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

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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.

| 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/                          |
| P/Ol      | 0.4139/                          | 44.44/**                            | 2.487/                           |
| ED        | 3.877/                           | 15.55/**                            | 3.664/**                         |
| P/Ol      | 16.95/**                         | 372.4/**                            | 3.905/**                         |
| B1L       | 3.171/                           | 70.95/**                            | 1.229/                           |
| B2L       | 0.0254/                          | 91.76/**                            | 2.076/                           |
| P/DD      | 0.205/                           | 75.83/**                            | 1.915/                           |
| P/PD      | 0.5848/                          | 21.88/**                            | 2.85/**                          |
| P/PV      | 0.2496/                          | 58.8/**                             | 4.23/**                          |
| P/AD      | 0.0581/                          | 58.32/**                            | 3.601/**                         |
| CPL       | 2.246/                           | 31.16/**                            | 1.445/                           |
| AFL       | 2.154/                           | 22.49/**                            | 1.77/                            |
| PVL       | 0.043/                           | 23.38/**                            | 1.371/                           |
| BD        | 3.927/                           | 33.71/**                            | 1.351/                           |
| 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/                           |
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. Triángulos: 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.

| Variables | PCI | PCII | PCIII |
|-----------|-----|------|-------|
| Eigenvalue| 0.0302 | 0.0068 | 0.0023 |
| % variance| 61.04 | 13.8 | 4.74 |

Table 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 |
|-----------|-----|------|-------|
| 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 | −0.0196 |
| DFL       | 0.1881 | 0.0720 | 0.0835 |
| DFH       | 0.1152 | 0.5012 | 0.1299 |
| CFL       | 0.0928 | 0.0598 | 0.2597 |
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 Ain 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 Bouhzz Mountain. 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. magrebensis, 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 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.

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The samples of L. ksibi and L. setivimensis had the length of the head significantly smaller than L. rifensis, L. magrebensis 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. magrebensis (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. Triángulos: *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.

| 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       | 0.0674 | 0.2385 | 0.0545 |
| BD        | 0.1277 | −0.0799| 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 those 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...
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 reophic *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; Dominguez-Dominguez 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

**DIAGNOSIS:** Diffrers 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 surpasses 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; 8.8). 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 (34.13318, -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, 290.998-290.006: 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, Guefrit (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).

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.

|                        | *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               | 0.8            |
| Moulouya Basin         | 5.6               | 5.7           | 6.8        | 9.6               | 8.8               | 0.1            |

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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                |
| PDD                    | 63.2                        | 58.6            | 95.6–34.7       | 14.1               |
| PPD                    | 33.1                        | 29.1            | 47.5–16.4       | 7.5                |
| PVD                    | 65.8                        | 59.8            | 104.2–33.8      | 15.7               |
| PVD                    | 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                |

Fig. 8.— Holotype of *Luciobarbus yahyaouii* from the Moulouya River, Moulouya 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.
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. setivimenis 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. magrebensis and L. rifensis, with preorbital
length 6-9% of SL. The iris, as in other Moroccan spe-
cies of Luciobarbus, is yellowish pigmented at the supe-
rior 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 lon-
ger 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. magrebensis 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 dor-
sal 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
Yahyaouii, 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 ubiqui-
tous generally inhabiting rivers with sandy and gravel
substrates and in downstream muddy substrates.

Luciobarbus yahyaouii is the most common fish spe-
cies in the Moulouya Basin where are also present
other species as the trout (Salmo sp.) in upstream
currents, the North African shad (Alosa algeriensis
Regan, 1916) and the sea lamprey (Petromyzon mari-
lius Linnaeus, 1758) in the lower courses of rivers.

Another two cyprinid species are known in Moulouya
Basin; the scarce and rare species Luciobarbus guer-
cifensis that inhabits riffle areas; and Carasobarbus
cf. fritschii that shows habitat requirements similar
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 com-
mon species in Moulouya Basin but the populations are
decailing 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 down-
stream where there are both a dam and a reservoir.

Therefore our suggestion would be to include this spe-
cies 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 yahy-
aouii 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    | 11.08                  | 0.254, 13.067      | 0.456, 0.46            | 0.361, 0.73          | 0.079, 0.494    |
|                    | L. rifensis       |                        |                    |                        |                      |                 |
|                    | L. maghrebensis   |                        |                    |                        |                      |                 |
|                    | L. setivimensis   |                        |                    |                        |                      |                 |
|                    | L. ksibi          |                        |                    |                        |                      |                 |
| SL/HL (H=58.48**)  | Moulouya Basin    | 4.05                   | 0.850, 4.04        | 0.662, 0.872           | <0.001**, 4.02       |                 |
|                    | L. rifensis       |                        |                    |                        |                      |                 |
|                    | L. maghrebensis   |                        |                    |                        |                      |                 |
|                    | L. setivimensis   |                        |                    |                        | <0.001**, 0.843       | 4.28            |
|                    | L. ksibi          |                        |                    |                        | <0.001**, 0.001**     | 4.28            |
| SL/PrO (H=97.41**) | Moulouya Basin    | 12.24                  | <0.001**, 11.01    | <0.001**, 0.022*       | <0.001**, 11.32     |                 |
|                    | L. rifensis       |                        |                    |                        |                      |                 |
|                    | L. maghrebensis   |                        |                    |                        |                      |                 |
|                    | L. setivimensis   |                        |                    |                        | <0.001**, 12.75       |                 |
|                    | L. ksibi          |                        |                    |                        | <0.001**, 0.603       | 12.65           |
| SL/OD (H=34.62**)  | Moulouya Basin    | 18.22                  | <0.001**, 20.07    | <0.001**, 0.83         | 0.001**, 20.5       |                 |
|                    | L. rifensis       |                        |                    |                        |                      |                 |
|                    | L. maghrebensis   |                        |                    |                        |                      |                 |
|                    | L. setivimensis   |                        |                    |                        | <0.001**, 20.02       |                 |
|                    | L. ksibi          |                        |                    |                        | 0.063, 0.068         | 19.18           |
| SL/LB1 (H=130.5**) | Moulouya Basin    | 11.77                  | <0.001**, 18.57    | <0.001**, 0.079        | <0.001**, 15.92      |                 |
|                    | L. rifensis       |                        |                    |                        |                      |                 |
|                    | L. maghrebensis   |                        |                    |                        | <0.001**, 16.45       |                 |
|                    | L. setivimensis   |                        |                    |                        | 0.011*, 0.001**       |                 |
|                    | L. ksibi          |                        |                    |                        | 14.75                |                 |
| SL/L2B (H=163.4**) | Moulouya Basin    | 8.84                   | <0.001**, 14.16    | <0.001**, 0.079        | 0.002**, 12.41       |                 |
|                    | L. rifensis       |                        |                    |                        | 12.19                |                 |
|                    | L. maghrebensis   |                        |                    |                        | 0.011**              |                 |
|                    | L. setivimensis   |                        |                    |                        | 0.024*, 0.102         | 11.59           |
|                    | L. ksibi          |                        |                    |                        | 11.59                |                 |
### 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 | 1.87 | 1.99 | 1.85 | 1.98 |
|---------------------|-----------------|------|------|------|------|------|
| *L. rifensis*       | 0.331           |      |      |      |      |      |
| *L. maghrebensis*   | <0.001**        |      |      |      |      |      |
| *L. setivimensis*   | 0.030*          | 0.081| <0.001** |      |      |      |
| *L. ksibi*          | <0.001**        | <0.001** | <0.001** |      | <0.001** |      |

| SU/PrP (H=41.67**) | Moulouya Basin | 3.81 | 3.83 | 3.88 | 4.11 | 3.93 |
|---------------------|-----------------|------|------|------|------|------|
| *L. rifensis*       | 0.239           |      |      |      |      |      |
| *L. maghrebensis*   | 0.031*          | 0.375|      |      |      |      |
| *L. setivimensis*   | <0.001**        | <0.001** | <0.001** |      |      |      |
| *L. ksibi*          | <0.001**        | 0.009** | 0.155 | <0.001** |      |      |

| SL/PrV (H=44.95**) | Moulouya Basin | 1.86 | 1.82 | 1.83 | 1.95 | 1.84 |
|---------------------|-----------------|------|------|------|------|------|
| *L. rifensis*       | 0.002**         |      |      |      |      |      |
| *L. maghrebensis*   | 0.015*          | 0.368|      |      |      |      |
| *L. setivimensis*   | <0.001**        | <0.001** | <0.001** |      |      |      |
| *L. ksibi*          | 0.213           | 0.045* | 0.361 | <0.001** |      |      |

| SL/PrA (H=51.52**) | Moulouya Basin | 1.32 | 1.31 | 1.31 | 1.36 | 1.3 |
|---------------------|-----------------|------|------|------|------|------|
| *L. rifensis*       | <0.006**        |      |      |      |      |      |
| *L. maghrebensis*   | <0.006**        | 0.93 | 1.31 |      |      |      |
| *L. setivimensis*   | <0.001**        | <0.001** | <0.001** |      |      |      |
| *L. ksibi*          | <0.001**        | 0.305 | 0.204 | <0.001** |      |      |

| SL/BD (H=20.97**) | Moulouya Basin | 4.04 | 3.92 | 4.01 | 4.02 | 4.12 |
|-------------------|-----------------|------|------|------|------|------|
| *L. rifensis*     | 0.0012**        |      |      |      |      |      |
| *L. maghrebensis* | 0.381           | 0.03* |      |      |      |      |
| *L. setivimensis* | 0.918           | 0.029* | 0.596 |      |      |      |
| *L. ksibi*        | 0.057           | <0.001** | 0.011* | 0.083 |      |      |

| SL/BLD (H=43.37**) | Moulouya Basin | 9 | 8.84 | 8.72 | 8.27 | 9.11 |
|---------------------|-----------------|---|-----|-----|-----|-----|
| *L. rifensis*       | 0.053*          |      |      |      |      |      |
| *L. maghrebensis*   | 0.004**         | 0.127|      |      |      |      |
| *L. setivimensis*   | <0.001**        | <0.001** | 0.002** |      |      |      |
| *L. ksibi*          | 0.288           | 0.002** | <0.001** | <0.001** |      |      |
### 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    | Moulouya Basin | 2.77                   | 0.117                | <0.001**               | <0.001**               | 2.7              |
|           | *L. maghrebensis* |                      | 0.017*              | 2.7                    |                       |                  |
|           | *L. setivimensis* |                      | <0.001**            | <0.001**               | <0.001**               | 2.57             |
|           | *L. ksibi* |                      | <0.001**            | <0.001**               | 0.002**                | 0.013*           |
| SL/APL    | Moulouya Basin | 5.28                   | 0.022*              | <0.001**               | <0.001**               | 5.14             |
|           | *L. maghrebensis* |                      | 0.02*              | 5.54                   |                       |                  |
|           | *L. setivimensis* |                      | <0.001**            | <0.001**               | <0.001**               | 4.72             |
|           | *L. ksibi* |                      | 0.045*              | 0.839                  | 0.135                 | <0.001**         |
| SL/PVL    | Moulouya Basin | 3.58                   | <0.001**            | 3.47                   |                       |                  |
|           | *L. maghrebensis* |                      | 0.337              | 3.45                   |                       |                  |
|           | *L. setivimensis* |                      | 0.88                | 0.017*                 | 0.003**               | 3.57             |
|           | *L. ksibi* |                      | <0.001**            | <0.001**               | <0.001**               | 0.001**          |
| SL/DFL    | Moulouya Basin | 7.25                   | 0.211               | <0.001**               | 6.1                    |                  |
|           | *L. maghrebensis* |                      | 0.256              | 7.01                   |                       |                  |
|           | *L. setivimensis* |                      | 0.388              | 0.172                  | 0.864                 | 7.2              |
|           | *L. ksibi* |                      | 0.008**            | 0.231                  | 0.02*                 | 0.006**          |
| SL/DFH    | Moulouya Basin | 5.18                   | <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**         |
| SU/PFL    | Melloulou | 5.33                   | <0.001**            | 5.77                   |                       |                  |
|           | *L. rifensis* |                      | <0.001**            | 5.36                   |                       |                  |
|           | *L. maghrebensis* |                      | 0.422              | <0.001**               |                       |                  |
|           | *L. setivimensis* |                      | <0.001**            | <0.001**               | <0.001**               | 4.6              |
|           | *L. ksibi* |                      | 0.961              | <0.001**               | 0.43                  | <0.001**         |
| Variables | Populations     | Moulouya Basin (n=12) | L. rifensis (n=12) | L. maghrebensis (n=27) | L. setivimensis (n=8) | L. ksibi (n=15) |
|-----------|-----------------|-----------------------|--------------------|------------------------|-----------------------|-----------------|
| SL/VFL    | Moulouya Basin  | 5.85                  | 6.77               |                        |                       |                 |
|           | L. rifensis     | <0.001**              |                    |                        |                       |                 |
|           | 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            |
| SU/AFL    | 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           |
| SU/AFH    | Moulouya Basin  | 5.37                  |                    |                        |                       |                 |
|           | L. rifensis     | <0.001**              |                    |                        |                       |                 |
|           | 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**              |                 |
| SU/CFL    | Melloulou       | 4.34                  |                    |                        |                       |                 |
|           | L. rifensis     | <0.001**              |                    |                        |                       |                 |
|           | 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**              |                 |
| CPL/BLD   | 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**              |                 |
| APL/BLD   | 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            |
### Taxonomy of Luciobarbus populations from Moulouya Basin

#### 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                   | <0.001**           | 1.12                   |                       |                 |
|               | L. rifensis        |                       | <0.001**           |                       |                       |                 |
|               | 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                  | <0.001**           | 1.46                   |                       |                 |
|               | L. rifensis        |                       | <0.001**           |                       |                       |                 |
|               | 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            |
| PVD/PDD (H=72.95**) | Moulouya Basin   | 1.02                  | 0.262              | 1.03                   |                       |                 |
|               | L. rifensis        |                       | 0.262              |                       |                       |                 |
|               | 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                  | <0.001**           | 2.72                   |                       |                 |
|               | L. rifensis        |                       | <0.001**           |                       |                       |                 |
|               | 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                  | 0.541              | 1.46                   |                       |                 |
|               | L. rifensis        |                       | 0.541              |                       |                       |                 |
|               | 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                  | 0.012*             | 4.13                   |                       |                 |
|               | L. rifensis        |                       | 0.012*             |                       |                       |                 |
|               | 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        | Moulouya Basin            | 43                    | <0.001**           | 44                     |                       |                 |
|           | 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       | Moulouya Basin            | 8                     | <0.001**           | 8.5                    |                       |                 |
|           | 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       | Moulouya Basin            | 5                     | <0.001**           | 4.5                    |                       |                 |
|           | 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 longitud del opercular. 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 = Lacrymal. 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. Bs Basioccipital Plate. K Kinetmoides

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.