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THE DISTRIBUTION AND SYSTEMATIC STATUS OF CICHLID FISHES (TELEOSTEI, CICHLIFORMES: CICHLIDAE) FROM MOROCCO

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CICHLIDAE
MOROCCO
INTRODUCED SPECIES
MORPHOMETRY
GENETICS

ABSTRACT. – Cichlids in Morocco are relict populations of the fauna that was extant during the last glacial episodes. In recent millennia, these fishes underwent numerous bottleneck episodes that led to a significant reduction in their numbers. While the literature reports the presence of three (3) genera (*Oreochromis*, *Coptodon*, *Sarotherodon*) and four (4) species (*O. aureus*, *C. guineensis, C. zillii*, *S. galilaeus*) in Morocco, an intensive search for these fishes throughout this country and a thorough genetic and morphometric study in fact revealed the presence of only two (2) genera (*Oreochromis*, *Coptodon*) and four (4) species: three (3) native (*O. aureus, C. guineensis, C. zillii*) and one (1) introduced (*O. niloticus*). *Sarotherodon galilaeus* was not found, even in the watershed from which it was originally reported. Species encountered were identified morphologically and their identification was confirmed genetically (ND2/COI). For *O. niloticus*, we found two haplotypes with a difference of 7.5 % between Oued Sebou and Oued Bouregreg watersheds. For *C. guineensis* sampled in Oued Aabar and Sebkha Imlili, a taxonomic incongruence occurred on the basis of significant differences between seventeen (17) of the thirty-seven (37) morphometric characters studied (including dentition).

INTRODUCTION

The family Cichlidae Heckel, 1840 is a monophyletic group of fishes (Stiassny 1981, Zihler 1982) presenting the most remarkable biological diversity, endemicity, and evolution among vertebrates (Salzburger & Meyer 2004), with 1712 valid species and 250 genera (Froese & Pauly 2019). Cichlids are known for their mechanisms of diversification, differentiation and explosive speciation ending in rapid adaptive radiation in terms of numbers, varieties, shapes, colorations and behaviors (Trewavas 1983). They have become one of the best models to study biological diversity and species flocks (Barluenga & Meyer 2010, Pariselle *et al*. 2011). These fish are the object of socioeconomic interest at the international level, and tilapias (*Coptodon* Gervais, 1848; *Oreochromis* Günther, 1889; *Sarotherodon* Rüppel, 1852; *Tilapia* Smith, 1840 and *Pelmatolapia* Thys van den Audenaerde, 1969) are the most commonly introduced fishes as the result of the global aquaculture trade. Their natural geographical distribution extends throughout most of Africa, Madagascar, the Middle East (Israel, Lebanon, and Syria), Asia (Iran, South of India, and Sri Lanka), the Caribbean (Cuba and Haiti), and the Americas (from southern Texas, USA, through northern Argentina). In addition, fossils were found in Europe (Italy) (Nelson 2016).

In Morocco, cichlids are at the northwestern limit of their African range and, according to the literature, only three genera and four species are reported there: *Coptodon*, with *C. zillii* (Gervais, 1848) and *C. guineensis* (Günther, 1862); *Oreochromis*, with *O. aureus* (Steindachner, 1864); and *Sarotherodon*, with *S. galilaeus* (Linnaeus, 1758) (see Qninba & Mataame 2009, Qninba *et al*. 2009, 2012, Clavero *et al*. 2014, 2017). However, morphological similarities among species make identification of cichlids difficult, whether they are closely related [e.g., *C. zillii* and *C. guineensis* in Qninba *et al*. (2009)] or broadly distributed because of the occurrence of species complexes [e.g., *C. guineensis* in Kidé *et al*. (2016)]. Nowadays molecular techniques approves the accuracy of identification.

The aim of this survey was to verify and update the list of Cichlidae species currently present in the Moroccan watersheds, to update and delimit their range, and to determine and clarify their taxonomic status using both morphometric and molecular analyses.

MATERIELS AND METHODS

Fish sampling: Fish were captured from the major river basins of Morocco from 2010 to 2018 (Fig. 1) by either gillnets (length: 20 m, height: 2 m; extended mesh size: 40 and 80 mm) or electrofishing using a portable Samus 725G Fish Shocker Stunner (adjusted according to the physicochemical descriptors of the water) connected to a 12V battery. Each fish was photographed. A piece of the pectoral fin was collected and stored in 95° ethanol as a source of DNA for molecular study.

For fish identification, we adopted the nomenclature and criteria used by Teugels & Thys van den Audenaerde (2003). Fish names comply with FishBase (Froese & Pauly 2019).

DNA isolation and PCR amplification: DNA isolation was carried out according to the protocol of Aljanabi & Martinez (1997). Approximately 50 µg of pectoral fin fragment was sheared into small pieces before being digested at 55 °C overnight with 20 µl of proteinase K (20 mg/ml) in 180 µl of extraction buffer solution (1M Tris, 0.5M Na Cl_2, 1 % SDS). The extracted DNA was suspended in 150 µl sterile double distilled water and stored at –20 °C until amplified by PCR.

A 900 bp fragment of the ND2 gene was amplified for each sample by PCR using the two primers: ND2F 5'-CAT ACC CCA AAC ATG TTG GT-3' (forward) and ND2R 5'-GGA GAT TTT CAC TCC CGC TTA-3' (reverse) (Agnèse *et al*. 2018). The partial mitochondrial Cytochrome Oxidase (COI) gene was also amplified for *O. niloticus* using FishF1/F2 and FishR1 universal primers (Ward *et al*. 2005). Each amplification was performed in a volume of 50 µl containing 0.25 mM of MgCl 2, 0.2 mM of each dNTP, 1 mM of each primer, 5 µl buffer (10×) and 10 units of Taq polymerase. The replication cycle was as follows: 94 °C (3 min), 48 °C (30 s), 72 °C (5 min) for 30 cycles with a final step at 72 °C for 10 min.

Phylogenetic analyses and genetic distance calculation: The sequence alignments obtained for each data set were performed using Clustal W multiple alignments (Thompson *et al*. 1994) incorporated in MEGA V.6 (Molecular Evolutionary Genetics Analysis) (Kumar *et al*. 2004). The uncorrected P-distances between the sequences of the various cichlid species reported in this and other studies from which sequences were imported from GenBank were calculated using the MEGA V.6 software. The best adapted DNA evolution model was determined using the Akaike Information Criterion in jModelTest 2.1.10 (Darriba *et al*. 2012). The aligned sequences were analyzed using Maximum Likelihood with PAUP 4b10 (Swofford 2002). Supports for inferred clades were obtained by nonparametric boot-

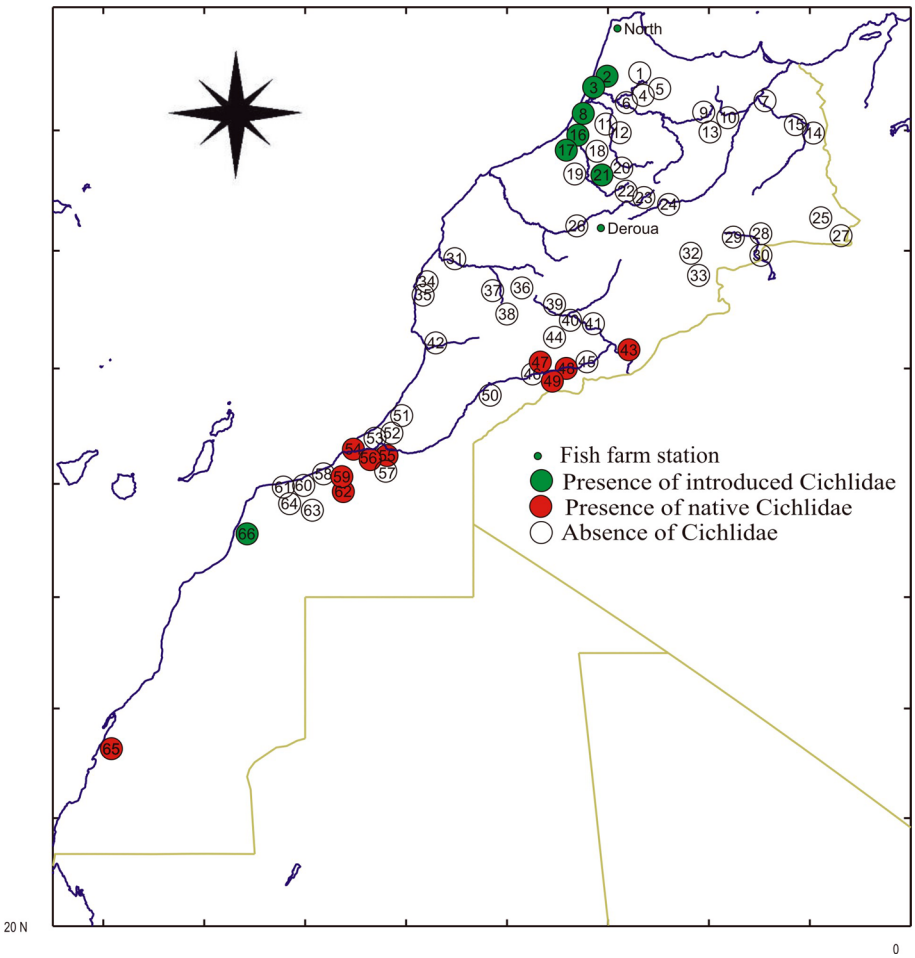

Fig. 1. – Sampled localities in Morocco. Numbers corresponds to localities listed in Table I.

strap (Felsenstein 1985) with 2000 replicates. For ND2 and COI sequences, the SYM + G model was chosen as the most suitable evolutionary model. For ND2 sequences, additional sequences obtained from GenBank were included in the analysis: *C. zillii* (Egypt, #AB195555), *C. guineensis* (Senegal, #MG755417)), and *O. aureus* (Israel, #DQ465029). The sequence of *Tylochromis leonensis* Stiassny, 1989 (#AF317274) was added as an outgroup to root the tree following Pouyaud & Agnèse (1995). COI haplotypes of *O. niloticus* available in GenBank (220 sequences) were included in the analysis.

Breeding experiment: In cichlid fishes, body coloration is an important systematic character, especially during the breeding period. However, because coloration can also be influenced by the environment, its stability was tested in the *C. guineensis* population of hole 121 from the Sebkha Imlili (with typical dark color for both males and females) via a breeding experiment under controlled conditions. For this experiment, an initial breeding couple was selected based upon the appearance of the female's soft dilated abdomen and her protruding genital papilla and, for the male, the pinkish coloring of its genital papilla. This pair was transferred to an aquarium (50 × 40 × 60 cm) filled with water (salinity 35 ppm) and maintained at a constant temperature (27 °C). Spawning occurred within one week of paring and F1 individuals were isolated in other aquaria to prevent cannibalism.

Morphometrics and statistical analyses: For each individual, 22 metric measurements (expressed in mm) were recorded using a dial caliper (Mitutoyo): total length (TL), standard length (SL), head length (HL), eye diameter (ED), snout length (SnL), preorbital bone length (PoL), interorbital width (IoW), predorsal distance (PrD), pre-pectoral distance (PrP), pre-ventral distance (PrV), pre-anal distance (PrA), caudal peduncle length (CPL), caudal peduncle width (APL), dorsal fin length (DFL), pectoral fin length (PFL), ventral fin length (VFL), anal fin length (AFL), caudal fin length (CFL), body depth (BD), caudal peduncle length (CPD), length of the longest dorsal fin spine (LDFS), and length of the third spine in the anal fin (L3SAF). Following the methods of Bitja Nyom *et al*. (2012), seven meristic characters were also studied: dorsal fin rays (NDR), dorsal fin spines (NDS), anal fin rays (NAR), anal fin spines (NAS), scales along the upper lateral line (LATUP), scales along the lower lateral line (LATLOW), and total number of gill rakers on the first ceratobranchial arch (GRTOTAL).

Since descriptors of the dentition are among the criteria used in cichlid fish systematics alongside classical metric and meristic measurements, we added four metric dental characters to the analysis: lower pharyngeal length (PhJL), lower pharyngeal width (PhJW), dentigerous area length (DeAL), and dentigerous area width (DeAW)] as well as four meristic dental characters: number of external teeth counted on upper jaw (UPPER), number of external teeth counted on the lower jaw (LOWER), number of rows of internal teeth on the upper jaw (ROWSUP), and number of rows of internal teeth on the lower jaw (ROWLOW).

Metric data were standardized by first being log transformed, after which the logarithmic data were centered-reduced in rows with a second centering in columns [i.e., the additive double centering on the logarithms (Rasch 1963 *in* Lewi 1995)]. Meristic data (or counts) were analyzed directly without any conversion and separately from the metric data. The non-parametric Mann-Whitney U test was performed to identify characters that differed significantly ($P \leq 0.05$) between populations compared by pair. These analyses were performed using Statistica software (Stat Soft, version 6).

RESULTS

A total of 271 cichlid individuals of four species (*C. zillii, C. guineensis, O. aureus, O. niloticus*) were collected in 17 of the 66 sampled localities. Seven of these localities were inhabited only by the introduced species, *O. niloticus* (Fig. 1, Table I).

Checklist of Cichlidae species present in Morocco

Coptodon zillii characteristics: a well-marked black spot (tilapiine spot) between last spine and fourth soft ray of dorsal fin; body brown or olive with iridescent reflections; 28 to 31 scales in lateral line; 7 to 10 transverse black bands; dorsal, anal, and caudal fins brownish and spotted with yellow; one black spot on operculum; dorsal fin often bordered with a yellow band; 14 to 16 spiny rays and 10 to 14 soft rays in dorsal fin; 3 spiny rays and 8 to 10 soft rays in anal fin; 8 to 11 branchiospines on lower part of first branchial arch; maximum TL observed 115 mm.

Coptodon guineensis characteristics: a well-marked tilapiine spot between the last spine and the third soft ray of the dorsal fin; a silvery color turning whitish on belly and green yellow on back and top of head; 27 to 33 scales in lateral line; 6 to 8 vertical bands dark, not very marked; both caudal fins colored with a greyish upper part and a yellowish lower part; greyish anal fin with a darker lower edge; white, sometimes reddish, coloration under mouth and continuous on part of abdomen; 14 to 16 spiny rays and 12 to 13 soft rays in dorsal fin; 3 spiny rays and 8 to 10 soft rays in anal fin; 8 to 10 branchiospines on the lower part of the first branchial arch; maximum TL observed 190 mm. Specimens from Sebkha Imlili (Fig. 1, #65) showed some unique features (an overall black coloration, especially during the breeding period, and a proportionally larger head) compared to those from Oued Aabar (Fig. 1, #62) and from other localities in Africa.

Oreochromis aureus characteristics: a dark green to dark blue opercular spot; overall coloration of flanks and fins light silvery gray; 30 to 33 scales on lateral line; dorsal, anal, and caudal fins with pink-red border on their upper edge; white maculae between rays of dorsal and caudal fins; caudal fin truncated; 15 to 16 spiny rays and

Table I. – Sampling localities and identification of Cichlidae species present in Morocco according to the present study: Numbers in parentheses correspond to the numbers of each locality in Fig. 1. *O = Oreochromis; C = Coptodon*; O = Oued and G = Guelta.

Locality	Longitude	Latitude	Species
(1) O. Zendoula	34.916	–5.53811	–
(2) O. Drader	34.861792	–6.258908	*O. niloticus*
(3) Canal Nador	34.817175	–6.295419	*O. niloticus*
(4) O. Elbiad	34.72819445	–5.54856	–
(5) Elborj	34.6843889	–5.216750	–
(6) O. Ardat	34.4907778	–5.8303	–
(7) O. Za	34.41080555	–2.87475	–
(8) O. Sebou	34.26335	–6.678334	*O. niloticus*
(9) O. Lahdar	34.2424167	–4.064972	–
(10) O. Melloullou	34.18102778	–3.53323	–
(11) Dam Lake Ganzra	34.070722	–5.938361	–
(12) O. Beht	34.0707222	–5.936138	–
(13) O. Saghor	34.0344525	–3.929328	–
(14) Ain Beni Mathar	34.000083	–2.066264	–
(15) O. Charef	33.9973056	–2.085361	–
(16) Dam Lake Smba	33.9697778	–6.730083	*O. niloticus*
(17) O. Korifla	33.768111	–6.7325	*O. niloticus*
(18) O. Grou	33.591111	–6.43044	–
(19) O. Elmachraa	33.53277774	–6.62767	–
(20) O. Boulhmayel	33.330446	–6.004194	–
(21) O. Bouregreg	33.3147778	–6.081916	*O. niloticus*
(22) Lahri	32.8591111	–5.624694	–
(23) O. Serrou	32.807777	–5.57166	–
(24) O. Moulouya	32.6987222	–5.197555	–
(25) Dfilia	32.55144166	–1.891416	–
(26) O. Zaidouh	32.265	–6.907972	–
(27) O. Tisserfine	32.1673	–1.362	–
(28) O. Bouanane	32.0778	–3.09403	–
(29) Boudnib	31.949	–3.6077	–
(30) O. Guir	31.87	–3	–
(31) O. Tensift Bis	31.853194	–9.173583	–
(32) O. Zouala	31.79219448	–4.245291	–
(33) O. Ziz	31.5263056	–4.18612	–
(34) O. Ksob	31.4640833	–9.757027	–
(35) O. Ksob Bis	31.456639	–9.752	–
(36) O. near Asni	31.277492	–7.961844	–
(37) O. Tensift	31.22319444	–8.111138	–
(38) O. near Tinmel	30.990081	–8.203961	–
(39) Lake Ouarzazate	30.968461	–6.723544	–
(40) Guelta of O. Draa	30.892669	–6.675942	–
(41) O. Tansikht	30.6887	–6.2074	–
(42) O. Massa	30.526	–9.648222	–
(43) O. Draa	30.1867333	–5.579816	*O. aureus/C. zillii*
(44) O. Ouhmidi	30.4682334	–6.9767	–
(45) O. Dades	30.023217	–6.486175	–
(46) Amtoudi	29.8524725	–7.256778	–
(47) O. El Maleh	29.880003	–7.256472	*C. zillii*

12 to 15 soft rays in dorsal fin; 3 spiny rays and 9 to 11 soft rays in anal fin; 18 to 26 branchiospines on lower part of the first branchial arch; maximum TL observed 210 mm.

Oreochromis niloticus characteristics: a dark grayish body compressed laterally; back olive-green color; 6 to 9 transverse bands not very visible on flanks; 32 to 33 scales on lateral line; regular black vertical bands on caudal fin; 16 to 18 spiny rays and 12 to 13 soft rays in dorsal fin; 3 spiny rays and 9 to 10 soft rays in anal fin; 19 to 25 branchiospines on lower part of first branchial arch; maximum TL 330 mm. It should be noted that this is the first record of this species in Moroccan natural habitats.

Geographical distribution of Cichlidae species in Morocco (Fig. 1)

Coptodon zillii: the distribution of this species was found to be limited to the Draa River basin (Oued Draa: bridge between Zagora and Mhamid lghizlane (43), Oued El Maleh at waterfalls (47), Oued El Maleh near Mrimima, (48) Oued Tissint (49), mouth of Oued Draa (54), Guelta Zerga (55) and in the Guelta Kehla (56)).

Coptodon guineensis: individuals of this species were found in the Oued Chbeyka (59) and its tributary, Oued Aabar (61), and in the Sebkha Imlili (65), where it was first identified [although reported at that time as *C. zillii* (Qninba *et al.* 2009)] (see discussion).

Oreochromis aureus: this species was found in sympatry with *C. zillii*, i.e., its distribution was limited to the Draa watershed [Oued Draa bridge between Zagora and Mhamid lghizlane (43), Oued El Maleh near Mrimima (48), Oued Tissint (49), mouth of Oued Draa (54), Guelta Zerga (55) and Guelta Kehla (56)].

Oreochromis niloticus: this introduced species, previously known only from aquaculture facilities near the cities of Beni Mellal and Tanger

Table I. – Continued.

Locality	Longitude	Latitude	Species
(48) Mrimima	29.823234	–6.9767	*C. zillii*
(49) O. Tissint	29.76	–7.16	*C. zillii*
(50) O. near Akka	29.434542	–8.268083	–
(51) O. Assaka bis	29.118558	–10.37124	–
(52) O. Assaka	28.87	–10.78	–
(53) O. Izahar	28.87	–10.78000	–
(54) Mouth of O. Draa	28.575983	–11.07035	*O. aureus/C. zillii*
(55) G. Zerga	28.497416	–10.88561	*O. aureus*
(56) G. Kehla	28.45	–10.86	*O. aureus*
(57) O. Win Madkour	28.389603	–10.83909	–
(58) O. Ouma Fatma bis	28.164986	–11.76591	–
(59) O. Chebika	28.102886	–11.41852	*C. guineensis*
(60) Lagoon Khnifis	28.05	–12.25	–
(61) Lagoon Khnifis bis	27.963969	–12.31810	–
(62) O. Aabar	27.9360832	–11.42336	*C. guineensis*
(63) Khawi Nam	27.68	–12.2	–
(64) O. Ouma Fatma	27.679078	–12.21835	–
(65) Sebkha Imlili	23.27272222	–15.92147	*C. guineensis*
(66) Foum el Oued	27.208454	13.352880	*O. niloticus*

(Fig. 1), was found to be largely distributed in the Northern part of Morocco: the Bouregreg watershed [Dam lake Sidi Mohammed Ben Abdellah (16), Oued Bouregreg (21) and Oued Korifla (17)], the Sebou watershed [Oued Sebou (8)], and in the Merja Zerga [Nador Canal (2) and Oued Drader (3)].

Systematic status of Cichlidae species present in Morocco

Genetic identification

The ND2 sequences obtained were compared with ND2 sequences from GenBank using BLAST search. High identities of 99-100 % occurred between sequences from this study and sequences of *C. zillii* from Egypt (#AB195555), *C. guineensis* from Senegal (#MG755417), and *O. aureus* from Israel (#DQ465029), confirming the presence of these three species in our samples. It should be noted that sequences of *C. guineensis* from Oued Aabar and Sebkha Imlili were represented by three different haplotypes, one from Oued Aabar (*C. guineensis* Morocco A) and two from Sebkha Imlili (*C. guineensis* Morocco I-a, *C. guineensis* Morocco I-b), the latter being characterized by a pair of synonymous substitutions at location 768 (G-A nucleotides).

COI sequences from *O. niloticus* from Morocco revealed the existence of two haplotypes with 7.5 % difference: haplotype A in the Oued Sebou (Fig. 1, #8) watershed and Merja Zerga (Fig. 1, #2-3), and haplotype B in the Oued Bouregreg (Fig 1, #21) watershed and Oued Korifla (Fig. 1, #17), both being highly identical (99-100 %) to homologuous sequences in GenBank, with neither morphological nor genetic inconsistency being detected.

Phylogenetic analyses of sampled species

The Maximum likelihood phylogenetic tree based on ND2 sequences (Fig. 2) confirmed the BLAST results, with bootstrap values ranging from 53 % to 100 % (Fig. 2). The nodes of the species *O. aureus* (Morocco, current study, #MK955803), *O. aureus* (Israel), *C. zillii* (Morocco, current study, #MK955802) and *C. zillii* (Egypt) were very reliable and strongly supported with values ranging from 94 to 100 %. Those of *C. guineensis* Morocco I-a (current study, #MG75500) and I-b (current study, #MG755474)/*C. guineensis* Morocco A (current study, #MK955801) (bootstrap = 54 %) and the *C. guineensis* Morocco I a-b/*C. guineensis* Morocco A/*C. guineensis* Senegal (bootstrap = 53 %) were poorly supported. Calculated pairwise distances are in Table II, the analysis revealed no significant differences between *C. guineensis* from Oued Aabar and *C. guineensis* from Sebkha Imlili (0.2 %).

The distance values of 13.3-14.9 % indicated a

Fig. 2. – Maximum-Likelihood phylogenetic tree for Cichlidae fish. *O = Oreochromis; C = Coptodon*. GenBank accession numbers: *C. zillii* #AB195555 (Egypt); *C. guineensis* #MG755417 (Senegal); *O. aureus* #DQ465029 (Israel) and *Tylochromis leonensis* #AF317274 (Lake Tanganyika). Number are bootstrap support values in %.

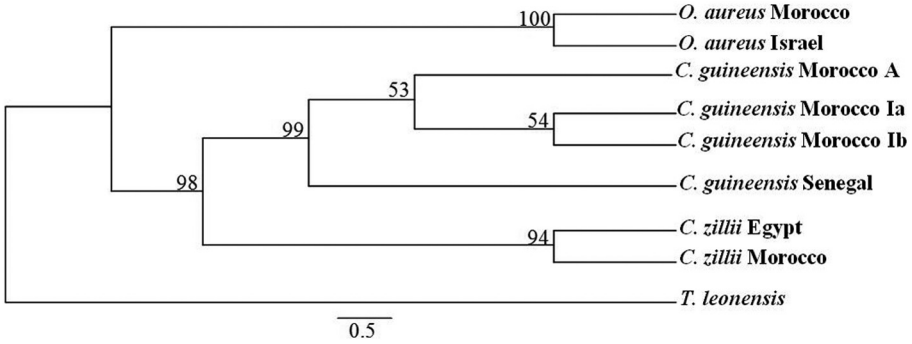

Table II. – Pairwise distances between partial ND2 sequences from populations of the three native cichlid species in Morocco *Oreochromis aureus*, *Coptodon zillii* and *C. guineensis*.

	1	2	3	4	5
1. O. aureus Morocco	–	0.149	0.147	0.148	0.133
2. C. guineensis Morocco A	0.149	–	–	0.002	0.102
3. C. guineensis Morocco I a	0.147	0.002	–	0.002	0.104
4. C. guineensis Morocco I b	0.148	0.002	0.002	–	0.104
5. C. zillii Morocco	0.133	0.102	0.104	0.104	–

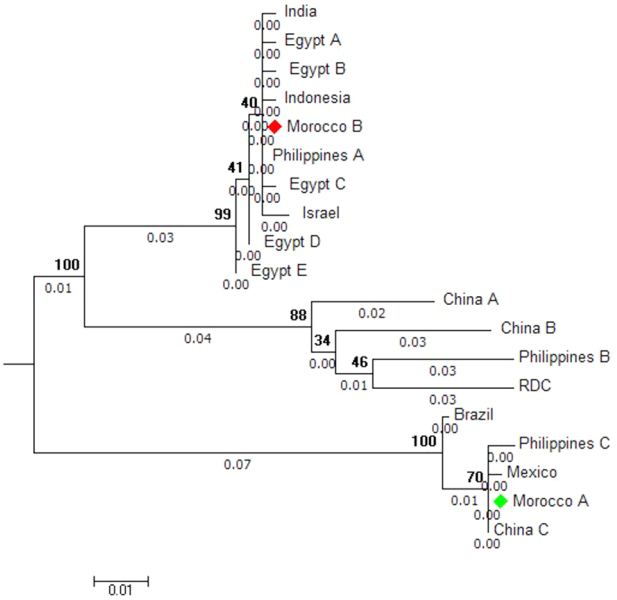

Fig. 3. – Maximum Likelihood phylogenetic tree for *Oreochromis niloticus* COI haplotypes found in Morocco (current study, red and green diamonds) and the World (retrieved from GenBank). GenBank accession numbers: India (#JX260932); Egypt A (#KJ443696); Egypt B (#KJ443702); Indonesia (#KP856791); Philippines A (#KU565843); Egypt C (#KJ443698); Israel (#FJ348103); Egypt D (#KJ443697); Egypt E (#KJ443695); China A (#DQ856613); China B (#DQ426668); Philippines B (#HQ654742); RDC (#KT193494); Brazil (#KM897268); Philippines C (#HQ654744); Mexico (#EU751881) and China B (#DQ856612).

high level of genetic divergence between *O. aureus* and *Coptodon* species for the ND2 gene.

The 220 COI sequences available for *O. niloticus* in GenBank could be grouped into 17 haplotypes (Fig. 3). The two haplotypes found in Morocco matched haplotypes of individuals from aquaculture strains: haplotype A from Oued Sebou watershed (Oued Sebou and Merja Zerga), (current study, #MK955804) exists also in China (#DQ856612), the Philippines (#KU565830), Nigeria (#HM882785) and Egypt (#MG428623); haplotype B from Oued Bouregreg watershed (Oued Bouregreg and Korifla) (current study, #MK955805) was reported from the Philippines (#KU565843; #KC789549), China (#DQ426666), Thailand (#JQ742041), and Indonesia (#HM345941). Alignments and distances calculated confirmed this finding (haplotype A/#DQ856612 (China) = 0 % difference; haplotype B/#KU565843 (Philippines) = 0% difference) (Fig. 3). Haplotype A probably originated from West Africa because of the presence of *O. aureus* mitochondrial DNA introgressed into the *O. niloticus* genome (Rognon & Guyomard 2003). Haplotype B may have originated from Egypt [B/#KJ443695/#KJ443697 = 0 % difference] and was scattered in the Philippines and other Asian countries in the 1970s [haplotype B/#KU565843/#KC789549 (from the Philippines) = 0 % difference] (Ordoñez *et al.* 2016).

Analysis of the Moroccan C. guineensis *populations*

Due to weak genetic and morphological differences observed between *C. guineensis* populations from Oued Aabar and Sebkha Imlili (see above), we undertook a morphometric analysis to estimate their divergence. Two F1 individuals from the breeding experiment were also included in this study.

Analysis of metric characters

The SL range of *C. guineensis* individuals in this study was between 61-90 mm and 70-90 mm for fish from the Sebkha Imlili hole 35 and hole 121, respectively; and 70-90 mm ("small") and 131-161 mm ("large") for fish from Oued Aabar. The two F1 individuals measured 50 and 50.4 mm SL.

The scatter plot relative to the set of morphometric variables (Fig. 4A) allowed the differentiation of two distinct groups. The first group comprised specimens from the two holes of Sebkha Imlili (1 and 2 in Fig. 4) as well as the two F1 individuals, while the second group included individuals from Oued Aabar (3 and 4 in Fig. 4). In the latter, two subgroups were apparent, with one including large individuals (3) and the other one small individuals (4). However it should be noted that one large specimen was located near the small individuals' cluster. Axis 1 was defined by a combination of characters including DFL, PhJL, PhJW and AFL. Axis 2 was defined by CFL, LDFS and APL.

Several significant characters were revealed among the four populations studied (Supplementary data 1). Among these characters, the best scores were obtained for DFL (hole 35/hole 121), PhJL (hole 35/Oued Aabar "large individuals"), PrD (hole 35/Oued Aabar "small individuals"), PoL (hole 35/F1), ED (hole 121/"large individuals"), DFL (hole 121/"small individuals"), PoL (hole 121/F1), SnL (Oued Aabar "large individuals"/Oued Aabar "small individuals"), SnL (Oued Aabar "large individuals"/F1) and ED (Oued Aabar "small individuals"/F1).

Only two characters (L3SAF and DFL) differed significantly between the two populations (hole 35 and hole 121)

of the Sebkha Imlili, whereas 12 variables were different between hole 35 and OA "large individuals", 18 variables between hole 35 and OA "small individuals", 4 variables between hole 35 and F1, 14 variables between hole 121 and OA "large individuals", 15 variables between hole 121 and OA "small individuals", 6 between hole 121 and F1, 16 variables between OA "large individuals" and OA "small individuals", 10 variables between OA "large individuals" and F1 and 9 variables between OA "small individuals" and F1 (Supplementary data1).

Analysis of meristic characters

The PCA of raw meristic data (Fig. 4 b) separated the populations of Oued Aabar and that of Sebkha Imlili into four major groups, with the clouds of points representing Sebkha Imlili (1, 2), F1 (5), Oued Aabar "large" (3) and "small individuals" (4). The upper boundary of the cloud formed by the populations of hole 35 and hole 121 of Sebkha Imlili was slightly intertwined with the scatterplot of Oued Aabar "large individuals" specimens. Factor 1 was structured mainly by UPPER, ROWLOW, ROWSUP, and LATUP. Factor 2 was supported by both GRTOTAL and LATLOW.

Significant differences among groups occurred for all characters, except for NAR and NAS (Supplementary data 2). There were significant differences for 5 meristic characters between Sebkha Imlili hole 35/Oued Aabar "Large individual", for 5 characters between Sebkha Imlili hole 121/Oued Aabar "Large individual", for 5 characters between Sebkha Imlili hole 35/F1, for 5 characters between Sebkha Imlili hole 121/F1, for 6 characters between Sebkha Imlili hole 121/Oued Aabar "Small individuals", for 4 characters between Sebkha Imlili hole 35/Oued Aabar "Small individuals", for 8 characters between Oued Aabar" Large individual"/Oued Aabar "Small individuals", for 5 characters between Oued Aabar "Large individuals"/F1, and for 2 characters between Oued Aabar "Small individuals"/F1. In contrast, only two descriptors (DFL, L3SAF) allowed the separation of the two populations of Sebkha Imlili (hole 35/hole 121).

The best value of the variables was obtained for GRTOTAL (Supplementary data 2). This character varied greatly and depended on the size of the fish, with the smallest individuals typically having the greatest number of gill rakers and vice versa for the largest individuals, who showed the lowest number of gill rakers. This was particularly clear in the individuals of the Oued Aabar population.

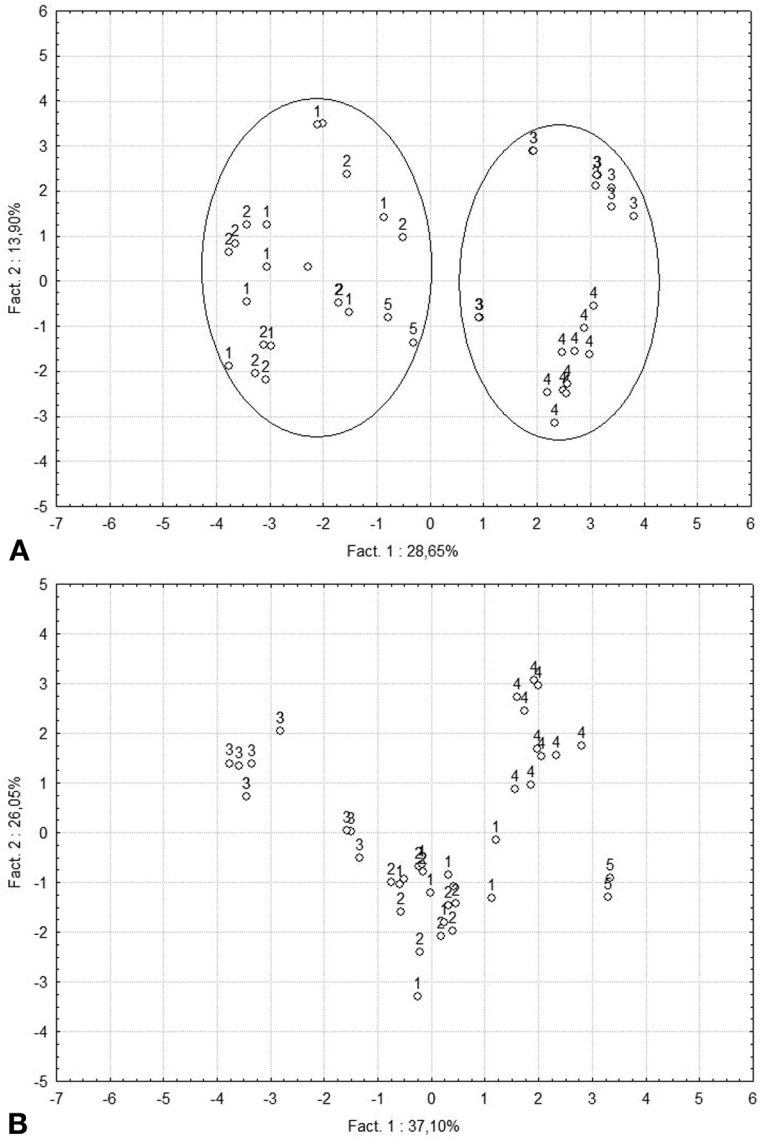

Fig. 4. – Scatter plots of the principal component analysis of metric (**A**) and of meristic (**B**) on the axes 1 and 2 (Moroccan populations of *C. guineensis*). 1 = Sebkha Imlili (hole 35), 2 = Sebkha Imlili (hole 121), 3 = Oued Aabar (Large individuals), 4 = Oued Aabar (Small individuals), 5 = F1.

DISCUSSION

Checklist of Cichlidae species present in Morocco

The diversity of Cichlidae in Morocco is low, probably because the only three native species encountered (*C. guineensis*, *C. zillii*, *O. aureus*) occur at the northernmost limit of their range, which is situated at the level of the Draa Basin (about 30° N). Cichlidae in Morocco have been considered to be tropical relics of the fauna extant during the last glacial episodes when the Sahara was a sub-

tropical humid region. These fishes probably underwent numerous bottleneck episodes and are now represented by diminutive isolated populations in small gueltas where abiotic conditions are suboptimal.

The species *O. niloticus* is widely introduced across the world for aquaculture purposes (Deines *et al.* 2016). It was imported into Morocco into fishery facilities in Tanger and Beni Mellal, probably during the late 1990s. While it is not possible to track the origin of the *O. niloticus* reported herein, it most likely was accidentally introduced in rivers and lakes from the Beni Mellal aquaculture facility, since it is reported herein in several surrounding river basins for the first time. This species now seems to be well adapted to the environment, and large populations occur in various watersheds north of the High Atlas mountain range. While *O. niloticus* has been targeted by local fishermen (pers com) for about the past four years for commercial purposes, its impact on the native fish fauna should be monitored because tilapia are known to cause "damage to native fish species and biodiversity" (Canonico *et al.* 2005). Future monitoring of the southern watershed in the region of Lâayoune [Foum el Oued (66)], where we recently found individuals of *O. niloticus* that we presumed had escaped from an upstream aquaculture facility, will allow us to determine how much of a threat *O. niloticus* is to the native fauna in Morocco.

Species with questionable status in Morocco

Sarotherodon galilaeus

Although individuals of *S. galilaeus* were reported from gueltas of Oued Aguemamou (Draa basin) by Vienille in 1939 and by de Lépiney, Rungs et Sauvage in 1941, and more recently from the central Oued Draa (Qninba *et al.* 2012), none were found in our current survey. The fact that we sampled at different times of the year and in different localities along the Draa basin may indicate that this species does not occur throughout this basin and/or not throughout the year. However, because individuals of *O. aureus* occur broadly in this basin and because this species is also known to have a negative ecological impact (de Moor & Britton 1988), it may have outcompeted *S. galilaeus*. One other possibility, however, is that these fish were misidentified in the earlier studies, since individuals of *Oreochromis* and *Sarotherodon* are very closely related. Natural fertile hybrids between the two genera are not uncommon (Bakhoum *et al.* 2009) and fish of both species are morphologically very similar and are distinguished mainly by their reproductive behavior (Clavero *et al.* 2017).

Coptodon guineensis

The aggregation of small (70-90 mm SL) and large (131-161 mm SL) individuals from Oued Aabar (Fig. 4A) demonstrated that, while these fish differ significantly in characters related to their size (DB, HL, CPD, and GRTOTAL), they are otherwise morphologically similar. Furthermore, the PCA also showed that the two F1 individuals resulting from the breeding of a couple from hole 121 of Sebkha Imlili were clustered with the specimens of the two holes (#35 and #121) despite their difference in size (F1's were only 50 mm *vs* 61-90 mm SL for the latter), further supporting morphological similarity between the F1's and fish from holes #35 and #121 of Sebkha Imlili.

In contrast, when individuals of similar size from Sebkha Imlili and Oued Aabar (61-90 mm and 70-90 mm SL, respectively) were compared, different groups within these two populations appeared based on 19 of the 26 variables analyzed, with the main discriminating characters being ED, HL, PrD, DFL, CPD, PhJL, PhJW, and AFL (Supplementary data 1). Among these discriminating characters, the pharyngeal jaw variables (PhJL, PhJW) are not surprising, since this apparatus is highly variable, is rapidly adaptive in the Cichlidae, and is considered one of the key evolutionary innovations that has contributed to the remarkable diversity of cichlid fishes (Gunter *et al.* 2013). Furthermore, it was also not unexpected for the type and shape of teeth to be discriminating characters, since fish in the two analyzed populations lived in very different environments and thus had adapted to different diets. In the same vein, F1 individuals obtained via the breeding experiment in the present study were fed exclusively with pellets, while in Sebkha Imlili fish feed on a variety of organisms, including crustaceans and gastropods, and by grazing and swallowing sand (pers obs). However, while differences in the pharyngeal apparatus (PhJL, PhJW) of the fish in these two populations occur and may be due to this major difference in diet, fish displayed the same shape and type of teeth. It is also probable that characters such as the number of teeth, both on the upper (UPPER) and lower jaws (LOWER), as well as the number of rows of internal teeth (ROWSUP and ROWLOW) and the size of the lower pharyngeal bone (PhJL and PhJW) are only dependent on fish size and age.

Regarding coloration, which is recognized to be an essential character in cichlid systematics and evolutionary processes (Maan & Sefc 2013), *C. guineensis* males and females from Oued Aabar showed a strong dimorphism, as is commonly observed in other natural populations of this species (pers obs). However, males and females from Sebkha Imlili were difficult to distinguish from one another, even more so during the breeding season when individuals of both sexes become almost entirely black. The F1 fish born and breed in captivity displayed the same uniform color pattern as those from Sebkha Imlili, indicating that this exceptional uniformity in coloration may be due to underlying genetic divergence of *C. guineensis* in the Sebkha.

CONCLUSION

In conclusion, we identified three native species (*Oreochromis aureus*, *Coptodon zillii*, and *C. guineensis*) as well as the introduced species, *O. niloticus*, during this large survey of cichlids in natural basins in Morocco. Although reported in past surveys, no *S. galilaeus* was found in the present study, and we suspect that the species was either outcompeted by *O. aureus* or, more probably, originally misidentified because of its morphological similarity to *O. aureus*.

The geographic distribution of *O. aureus*, *C. zillii*, and *C. guineensis* was updated and its Northern limit defined as being the Draa basin. A significant morphometric variability was observed between the only two known populations of *C. guineensis* in Morocco (Oued Aabar and Sebkha Imlili) and the analysis of metric and meristic data led to classifying this species into two main phenotypes corresponding to the two populations. However, because these phenotypes showed only 0.2 % divergence in their ND2 gene, we recognize that further studies are deemed necessary to clarify the taxonomic status of cichlids from Sebkha Imlili, and we continue to identify these fish as *C. guineensis* until further data is available.

The introduced species, *O. niloticus*, was reported herein for the first time from the wild in Morocco. This species is invasive, has the potential to outcompete native cichlid species, and harms ecosystems in general. Hence, populations of *O. niloticus* encountered in the present study should be monitored in order to minimize further spreading in the Draa basin and southern Oueds, where native species are found.

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REFERENCES

Agnèse JF, Louizi H, Gilles A, Berrada Rkhami O, Benhoussa A, Qninba A, Pariselle A 2018. A euryhaline fish, lost in the desert: The unexpected metapopulation structure of *Coptodon guineensis* (Günther, 1862) in the Sebkha of Imlili. *C R Biol* 341: 75-84.

Aljanabi SM, Martinez I 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Res* 25: 4692-4693.

Bakhoum SA, Sayed-Ahmed MA, Ragheb EA 2009. Genetic evidence for natural hybridization between Nile Tilapia (*Oreochromis niloticus* Linnaeus, 1757) and Blue Tilapia (*Oreochromis aureus* Steindachner, 1864) in Lake Edku, Egypt. *Global Vet* 3: 91-97.

Barluenga M, Meyer A 2010. Phylogeography, colonization and population history of the Midas cichlid species complex (*Amphilophus* spp.) in the Nicaraguan crater lakes. *BMC Evol Biol* 10: 326.

Bitja Nyom AR, Gilles A, Pariselle A, Snoeks J, Bilong Bilong CF 2012. Divergences morphologiques allopatriques et sympatriques de *Tilapia kottae* (Perciformes, Cichlidae) endémique des lacs Barombi Kotto et Mboandong et affinités avec des tilapias de la Ligne Volcanique du Cameroun. *Cybium* 36: 335-348.

Canonico GC, Arthington A, McCrary JK, Thieme ML 2005. The effects of introduced tilapias on native biodiversity. *Aquat Conserv Mar Freshw Ecosyst* 15: 463-483.

Clavero M, Esquivias J, Qninba A, Riesco M, Calzada J, Ribeiro F, Fernández N, Delibes M 2014. Fish invading deserts: non-native species in arid Moroccan rivers. *Aquat Conserv: Mar Freshw Ecosyst*

Clavero M, Qninba A, Riesco M, Esquivias J, Calzada J, Delibes M 2017. Moroccan desert rivers: fish on the arid extreme of Mediterranean streams. *Fish Medit* 3: 1-21.

Darriba D, Taboada GL, Doallo R, Posada D 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 9: 772.

Deines AM, Wittmann ME, Deines JM, Lodge DM 2016. Tradeoffs among ecosystem services associated with global *Tilapia* introductions. *Rev Fish Sci Aquacult* 24: 178-191.

de Moor IJ, Bruton MN 1988. Atlas of alien and translocated indigenous aquatic animals in southern Africa. A report of the Committee for Nature Conservation Research National Programme for Ecosystem Research. *South Afr Sci Prog Rep* 144: 1-310.

Felsenstein J 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.

Froese R, Pauly D (eds) 2000. FishBase 2000: Concepts, Design and Data Sources. Los Baños: ICLARM, 344.

Froese R, Pauly D (eds) 2019. FishBase (version Feb 2018). *In* Roskov Y, Ower G, Orrell T, Nicolson D, Bailly N, Kirk PM, Bourgoin T, DeWalt RE, Decock W, Nieukerken E, Zarucchi J, Penev L, eds Species 2000 & ITIS Catalogue of Life, 26th February 2019. Digital resource at www.catalogueoflife.org/col. Species 2000: Naturalis, Leiden, the Netherlands.

Gunter HM, Fan S, Xiong F, Franchini P, Fruciano C, Meyer A 2013. Shaping development through mechanical strain: the transcriptional basis of diet-induced phenotypic plasticity in cichlid fish. *Mol Ecol* 22: 4516-531.

Kidé NG, Dunz A, Agnèse JF, Dilyte J, Pariselle A, Carneiro C, Correia E, Brito JC, Yarba LO, KoneY, Durand JD 2016. Cichlids of the Banc d'Arguin National Park, Mauritania: insight into the diversity of the genus *Coptodon*. *J Fish Biol* 88: 1369-1393.

Kumar S, Tamura K, Nei M 2004. MEGA3: Integrated software for molecular evolutionary genetic analysis and sequence alignment. *Brief Bioinform* 5: 150-163.

Lewi PJ 1995. Spectral mapping of drug-test specificities. *In* van de Waterbeemd-Weinheim H ed, Chemo Metric Methods in Molecular Design, Vol II. VCH, Chap 4.5: 219-253.

Maan ME, Sefc KM 2013. Colour variation in cichlid fish: developmental mechanisms, selective pressures and evolutionary consequences. *Semin Cell Dev Biol* 24: 516- 528.

Nelson JS 2016. Fishes of the World. Fourth edit. John Wiley & Sons, Hoboken, New Jersey.

Ordoñez JFF, Ventolero MFH, Santos MD 2016. Maternal mismatches in farmed tilapia strains (*Oreochromis* spp.) in the Philippines as revealed by mitochondrial COI gene. *Mit DNA Part A* 28: 526-535.

Pariselle A, Boeger WA, Snoeks J, Billon Bilong CF, Morand S, Vanhove MPM 2011. The monogenean parasite fauna of cichlids: a potential tool for host biogeography. *Int J Evol Biol* 2011: 15.

Pouyaud L, Agnèse JF 1995. Phylogenetic relationships between 21 species of three tilapine genera *Coptodon*, *Sarotherodon* and *Oreochromis* using allozyme data. *J Fish Biol* 47: 26-38.

Qninba A, Mataame A 2009. Mise au point sur la répartition au Maroc des Cichlidés (Pisces, Perciformes) basée sur les échantillons conservés dans les collections du Muséum national d'Histoire naturelle de l'Institut Scientifique (Rabat, Maroc). *Bull Inst Sci* 31: 57-61.

Qninba A, Ibn Tattou M, Radi M, El Idrissi Essougrati A, Bensouiba H, Ben Moussa S, Ougga T, Bouzrou J, Azaguagh I, Bensbai J, Khayya ML 2009. Sebkhet Imlily, une zone humide originale dans le Sud marocain. *Bull Inst Sci* 31: 51-55.

Qninba A, El Agbani M, Radi M, Pariselle A 2012. Sur la présence de *Tilapia guineensis* (Teleostei, Cichlidae) dans les gueltas d'un affluent de l'Oued Chbeyka, l'Oued Aabar (Province de Tan Tan, Sud Ouest du Maroc). *Bull Inst Sci* 34: 125-126.

Rognon X, Guyomard R 2003. Large extent of mitochondrial DNA transfer from *Oreochromis aureus* to *O. niloticus* in West Africa. *Mol Ecol* 12: 435-445.

Salzburger W, Meyer A 2004. The species flocks of East African cichlid fishes: recent advances in molecular phylogenetics and population genetics. *Naturwissenschaften* 91: 277-290.

Stiassny MLJ 1981. The phyletic status of the family Cichlidae (Pisces: Perciformes): a comparative anatomical investigation. *Neth J Zool* 31: 275-314.

Swofford DL 2002. PAUP*: Phylogenetic Analysis using Parsimony (and other Methods), Version 4. Sinauer Associates; Sunderland.

Teugels GG, Thys van den Audenaerde DFE 2003. Cichlidae: *In* Paugy D, Lévêque C, Teugels GG eds, Poissons d'Eaux douces et saumâtres d'Afrique de l'Ouest. IRD Éditions Paris, MNHN Paris, MRAC Tervuren, Collection Faune et Flore tropicales 40, tome 2: 520-600.

Thompson JD, Higgins DG, Gibson TJ 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22: 4673-4680.

Trewavas E 1983. Tilapiine fishes of the genera *Sarotherodon, Oreochromis* and *Danakilia*. British Museum of Natural History, London, Publication 878.

Ward RD, Zemlak TS, Ines BH, Last PR, Heber PDN 2005. DNA barcoding Australia's fish species. *Phil Trans R Soc, Lond* Ser B 360: 1847-1857.

Zihler F 1982. Gross morphology and configuration of digestive tracts of Cichlidae (Teleostei, Perciformes): phylogenetic and functional significance. *Neth J Zool* 32: 544-571.

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SUPPLEMENTARY DATA

1: Results of the Mann-Whitney U test ($p > 0.05$) on metrics. In bold: significant different measurements.

V: Variable, L: Locality 1/2: (Sebkha Imlili hole 35/hole 121); 1/3: (Sebkha Imlili hole 35/Oued Aabar "Large individuals"); 2/3: (Sebkha Imlili hole 121/Oued Aabar "Large individuals"); 1/5: (Sebkha Imlili hole 35/F1); 2/5: (Sebkha Imlili hole 121/F1); 2/4: (Sebkha Imlili hole 121/Oued Aabar "Small individuals"); 1/4: (Sebkha Imlili hole 35/Oued Aabar "Small individuals"); 3/4: (Oued Aabar "Large individuals"/"Small individuals"); 3/5: (Oued Aabar "Large individuals"/F1); 4/5: (Oued Aabar "Small individuals"/F1).

V/L	1/2	1/3	2/3	1/5	2/5	2/4	1/4	3/4	3/5	4/5
SL	1.0294	0.3526	0.1230	0.6060	0.4848	**0.0003**	**0.0185**	**0.0089**	0.7575	0.1212
TL	0.7393	0.0542	**0.0232**	0.6060	0.6060	**0.0020**	**0.0038**	**0.0354**	0.0606	**0.0303**
HL	0.3526	0.0892	0.1051	0.3636	0.4848	**0.0185**	**0.0007**	**0.0288**	0.9090	0.2727
ED	0.7393	**0.0003**	**0.00001**	0.1212	0.9090	**0.0028**	0.2474	**0,0007**	**0.0303**	0.1212
IoW	0.8534	**0.0028**	**0.0010**	0.9090	0.4848	**0.0038**	**0.0089**	0.4812	**0.0303**	**0.0303**
SnL	0.6842	0.1903	0.2474	0.1212	0.1818	**0.00007**	**0.0007**	**0.00001**	0.2727	**0.0303**
PoL	0.7959	0.0752	0.0524	**0.0303**	**0.0303**	**0.0354**	**0.0432**	0.9705	**0.0303**	**0.0303**
PrD	0.8534	0.0524	**0.0007**	0.2727	0.1212	**0.0002**	**0.00001**	**0.0015**	0.9090	**0.0303**
PrP	0.1051	0.0752	**0.0354**	0.3636	0.3636	0.8534	0.9117	0.2798	1.0909	0.4848
PrV	0.4358	0.0752	0.8534	0.2727	0.1212	**0.0020**	**0.0068**	**0.0003**	0.0606	0.4848
PrA	0.1614	0.9117	0.2798	0.9090	0.7575	0.3149	**0.0185**	**0.0003**	0.9090	0.1212
CPL	0.0630	**0.0232**	**0.0020**	0.3636	0.1212	0.0752	0.4385	**0.0056**	0.3636	1.0909
APL	0.1051	**0.0354**	**0.0051**	0.3636	0.9090	0.0752	0.8534	0.2175	**0.0303**	0.1212
DFL	**0.0288**	**0.00004**	**0.00004**	0.2727	0.9090	**0.00001**	**0.00001**	**0.0432**	**0.0303**	**0.0303**
PFL	0.0630	0.1431	0.2474	0.6060	0.0606	0.1903	**0.0146**	0.1654	0.2727	0.0606
VFL	0.4358	**0.0068**	0.0892	**0.0303**	**0.0303**	**0.0015**	**0.0001**	**0.0232**	**0.0303**	**0.0303**
AFL	0.3526	**0.0001**	**0.00004**	0.0606	**0.0303**	**0.00004**	**0.0001**	**0.0089**	0.9090	0.7575
CFL	0.1903	0.8534	0.1230	0.0606	0.2727	0,1903	**0.0020**	0.6842	**0.0303**	0.6060
BD	0.1903	**0.00007**	**0.0004**	0.3636	0.1212	0.4358	0.0603	**0.0015**	**0.0303**	0.1212
CPD	0.6842	**0.0004**	**0.00001**	0.0606	**0.0303**	0.0524	**0.0288**	0.0892	0.9090	0.3636
LDFS	0.1903	0.5787	0.3526	1.0909	0.4848	**0.0068**	0.0892	**0.0015**	0.4848	0.1818
L3SAF	**0.0232**	0.0752	**0.00004**	0.1212	**0.0303**	**0.00001**	**0.0051**	**0.0432**	**0.0303**	**0.0303**
PhJL	0.2798	**0.00001**	**0.00002**	**0.0303**	**0.0303**	**0.0007**	**0.00007**	0.6030	**0.0303**	**0.0303**
PhJW	0.5787	**0.0003**	**0.0051**	**0.0303**	0.0606	**0.0288**	**0.0114**	0.1903	0.6060	0.2727
DeAL	0.3142	**0.0004**	0.0524	0.7575	0.7575	0.9117	0.2175	**0.0146**	0.3636	0.7575
DeAW	0.5787	0.6842	0.8534	0.2727	0.3636	0.6842	0.5787	1.0294	0.3636	0.4848

SUPPLEMENTARY DATA

2: Results of the Mann-Whitney U test ($p > 0.05$) on meristic. In bold: significant different measurements.

V: Variable, L: Locality. 1/2: (Sebkha Imlili hole 35/hole 121); 1/3: (Sebkha Imlili hole 35/Oued Aabar "Large individuals"); 2/3: (Sebkha Imlili hole 121/Oued Aabar "Large individuals"); 1/5: (Sebkha Imlili hole 35F1); 2/5: (Sebkha Imlili hole 121/F1); 2/4: (Sebkha Imlili hole 121/Oued Aabar "Small individuals"); 1/4: (Sebkha of Imlili hole 35/Oued Aabar "Small individuals"); 3/4: (Oued Aabar "Large individuals"/"Small individuals"); 3/5: (Oued Aabar "Large individuals"/F1); 4/5: (Oued Aabar "Small individuals"/F1)

V/L	1/2	1/3	2/3	1/5	2/5	2/4	1/4	3/4	3/5	4/5
NDR	0.9705	0.8534	0.7393	0.6060	0.4848	**0.0354**	0.0630	**0.0232**	0.6060	0.1212
NDS	0.2474	0.0752	0.3930	0.4848	0.1818	**0.0028**	0.0752	**0.002**	0.1212	0.9090
NAR	–	–	–	–	–	–	–	–	–	–
NAS	0.0892	0.8534	0.0752	0.9090	0.1212	0.630	0.6842	0.5288	1.0909	0.6060
LATUP	**0.0089**	0.1230	0.4812	**0.0303**	**0.0303**	**0.00001**	**0.00001**	**0.00001**	**0.0303**	0.4848
LATLOW	0.6842	1.0294	0.3930	**0.0303**	0.0606	**0.00002**	**0.00001**	**0.00001**	**0.0303**	0.1212
GRTOTAL	0.2474	**0.00013**	**0.0185**	**0.0303**	**0.0303**	**0.00001**	**0.00001**	**0.00002**	0.0606	0.1818
UPPER	0.8534	**0.00001**	**0.00001**	**0.0303**	**0.0303**	0.5288	0.3930	**0.00001**	**0.0303**	**0.0363**
LOWER	0.1654	**0.00001**	**0.00001**	**0.0303**	**0.0303**	**0.0068**	**0.0015**	0.0752	**0.0303**	**0.0363**
ROWSUP	1.0294	**0.0004**	**0.00048**	0.3636	0.3636	0.7393	0.7393	**0.0010**	**0.0303**	0.2727
ROWLOW	–	**0.0068**	**0.0068**	–	–	–	–	**0.0068**	0.1818	–