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The discovery of *Coptodon guineensis* (Günther, 1862) (Perciformes, Cichlidae) in the Moulay Bousselham lagoon extends the species' range 1000 km northward in Morocco

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Abstract

Two specimens of *Coptodon guineensis* (Günther, 1862) were unexpectedly found in the Canal de Nador, Moulay Bousselham lagoon, almost 1000 km north of the Aabar River the northernmost river previously recorded. To confirm this identification nineteen measurements were recorded from each specimen and compared with other specimens of *C. guineensis* from Morocco and Mauritania. The COI gene was partially sequenced and compared with formerly published sequences of *Coptodon* species of the region. Both morphology and DNA revealed no differences with specimens from known populations of *C. guineensis* in Morocco.

Keywords

Barcoding, morphology, tilapia, relict population, range extension

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Introduction

During a survey of the fish species in Moulay Bousselham lagoon, Morocco (34°48′27″N, 006°18′08″W) two specimens of *Coptodon* Gervais, 1853 (Cichliformes, Cichlidae) were unexpectedly found in the Canal de Nador, an artificial spillway located south of the lagoon (Fig. 1). Only individuals of *Oreochromis* (Linnaeus, 1758) have been previously recorded for the family Cichlidae from this lagoon (Louizi et al. 2019). Species of the genera *Coptodon* and *Oreochromis* Günther, 1889 are together with species from the genera *Tilapia* Smith, 1840 and *Sarotherodon* Rüppell, 1852 are called tilapia. To date, four species of tilapia, whether indigenous or introduced, are known from Morocco. Two species belong to *Coptodon* and the other two belong to *Oreochromis*. *Oreochromis aureus* (Steindachner, 1864) and *Coptodon zillii* (Gervais, 1848) are native to Oued Drâa, south to the High Atlas Mountains (Louizi et al. 2019), and *C. guineensis* (Günther, 1862) is native further south in the Sebkha Imlili and in the Oued Chbeyka and its tributary, Oued Aabar (Qninba and Mataame 2009;

Figure 1. The Canal de Nador (34°48′27″N, 006°18′08″W), Moulay Bousselham lagoon, Morocco, where two tilapia specimens, *Coptodon guineensis*, were captured by local fishermen.

Qninba et al. 2009, 2012) (Fig. 2). *Oreochromis niloticus* has been introduced for aquaculture purposes in 2004 (MAPMDREF 2020) and then released into most of rivers and lakes in the northern part of the country (Louizi et al. 2019). A fifth species *S. galilaeus* (Linnaeus, 1758) has been reported from the Drâa river, but we agree with Louizi et al. (2019) that this is likely based on misidentifications.

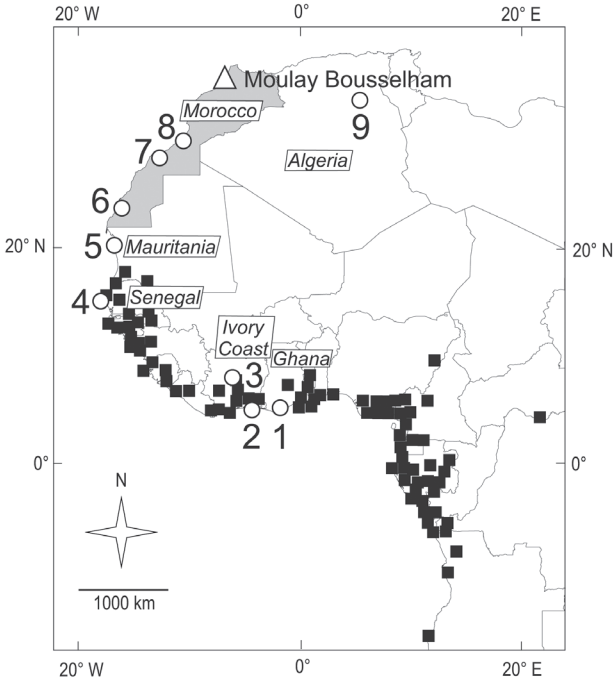

Figure 2. Map of the African continent showing the known collection sites of specimens (black squares) from Mauritania to Angola (Froese and Pauly 2021), white circles represent the populations of both *Coptodon guineensis* and *C. zillii* studied in herein: 1 = Lake Weija, Densu River, Ghana; 2 = Aby Lagoon, Bia River, Ivory Coast, 3 = Man, Sassandra River, Ivory Coast; 4 = Hann Bay, Senegal; 5 = Banc d'Arguin, Mauritania; 6 = Sebkha Imlili, Morocco; 7 = Oued Aabar, Morocco; 8 = Oued Draa, Morocco; 9 = Ouargla, Chott Oum Erraneb river basin, Algeria. The new collection site from Morocco—Canal de Nador, Moulay Bousselam Lagoon, 34°48′27″N, 006°18′08″W—is represented by a white triangle.

The two newly discovered tilapia specimens from the Canal de Nador were initially identified in the field as *Coptodon guineensis*. However, due to their poor condition after capture (Fig. 3), their original color pattern could not be precisely determined, hence, hampering their correct identification. As these two specimens were found almost 1000 km north of the northernmost known populations of this species in Oued Aabar (Fig. 2), we were motivated to carry out more in-depth analyses to unambiguously identify them. Louizi et al. (2019) were able to discriminate between Moroccan populations (Oued Aabar vs. Sebkha Imlili) of *C. guineensis* based on morphological data. Kidé et al. (2016) highlighted differences between several populations of *C. guineensis* from Mauritania to Gabon by comparing partial cytochrome oxidase I (COI) gene sequences. We used both techniques to determine the identity of our two specimens from the Canal de Nador. We discuss the hypotheses that could explain the presence of these fish in the Canal de Nador.

Methods

Sampling. The two tilapia specimens (Fig. 3) were captured by local fishermen in the Canal de Nador (34° 48′27″N, 006°18′08″W), Moulay Bousselham lagoon, Morocco (Figs. 1, 2) in November 2018 using a gill net (mesh size of 17 and 40 mm) and kept frozen. For morphological analyses, the specimens were thawed in the laboratory and photographed. In addition, clips of the pectoral fin were taken and stored in tubes with absolute ethanol for molecular analysis. Additional specimens of *C. guineensis* came from previous studies (Kidé et al. 2016; Louizi et al. 2019) and were collected using fishing rod and gill nets (mesh size of 17 and 40 mm) from Sebkha Imlili (20 specimens) and Oued Aabar (20 specimens) in Morocco and Banc d'Arguin (20 specimens) in Mauritania.

Morphometric study. A total of 19 measurements commonly used for cichlids (Barel et al. 1977; Snoeks 1988, 2004; Teugels and Thys van den Audenaerde 2003) were taken on each specimen using calipers: standard length (SL), head length (HL), eye diameter (ED), interorbital width (IoW), snout length (SnL), pre-orbital bone length (PoL), pre-dorsal distance (PrD), pre-pectoral distance (PrP), pre-ventral distance (PrV), pre-anal distance (PrA), caudal peduncle width (APL), dorsal fin length (DFL), pectoral fin length (PFL), ventral fin length (VFL), anal fin length (AFL), 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). Additional measurements of 40 specimens of *C. guineensis* from Morocco (20 from the Oued Aabar and 20 from the Sebkha Imlili) (Louizi et al. 2019. 2020) and 20 specimens of *C. guineensis* from Mauritania (Banc d'Arguin National Park) (Kidé et al. 2016) were included in the analysis.

As there are significant size differences between the specimens of the different groups (Table 1), the measurements obtained were logarithmically transformed and additionally double-centered according to Lewi (2005) to remove the size effect and a principal component analysis (PCA) was performed on these log-transformed double-centered measurements. Statistical analyses were executed using the Statistica v. 6 program (Stat Soft 2001).

Genetic study. DNA was extracted from the clips of the pectoral fin preserved in absolute ethanol using a rapid salt-extraction protocol (Aljanabi and Martinez 1997). Extracted DNA was then suspended in sterile double distilled water and stored at –20 °C until PCR amplification.

The universal molecular barcode mitochondrial gene COI already used to differentiate between *Coptodon* species and populations (Kidé et al. 2016) was amplified from the two specimens from Canal de Nador, two

Table 1. Measurements (in mm) taken on 62 specimens of *Coptodon guineensis* from four locations, Sebkha Imlili, Oued Aabar, Canal de Nador in the Moulay Bousselham Lagoon, Morocco, and Banc d'Arguin National Park, Mauritania. Standard length (SL), head length (HL), eye diameter (ED), interorbital width (IoW), snout length (SnL), pre-orbital bone length (PoL), pre-dorsal distance (PrD), pre-pectoral distance (PrP), pre-ventral distance (PrV), pre-anal distance (PrA), caudal peduncle width (APL), dorsal fin length (DFL), pectoral fin length (PFL), ventral fin length (VFL), anal fin length (AFL), 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).

	SL	HL	ED	IoW	SnL	PoL	PrD	PrP	PrV	PrA	CpL	DFL	PFL	VFL	AFL	BD	CPD	LDFS	L3SAF
Sebkha Imlili	90	32	7	9	13	12	45	30	36	67	12	47	7	3	15	36	13	5	6
	71	26	7	8	10	10	31	28	36	59	10	41	5	3	11	29	10	4	4
	71	25	8	9	8	8	27	28	31	52	12	39	5	2	11	34	10	2	1
	90	27	8	10	11	12	40	34	40	73	15	47	6	3	15	37	13	6	4
	80	27	7	9	9	9	31	29	32	55	12	41	4	3	13	32	11	3	1
	70	28	9	7	9	9	33	29	32	54	9	36	5	2	10	30	10	4	3
	90	30	9	10	12	11	40	31	36	55	13	42	4	3	14	32	12	3	3
	81	32	10	11	11	12	38	31	37	67	16	42	7	5	14	35	14	6	5
	70	25	7	9	8	8	41	29	32	54	8	43	5	3	13	35	11	2	3
	80	30	9	9	10	11	37	29	35	61	9	45	5	3	14	35	10	6	4
	80	25	8	8	8	8	30	27	31	56	11	41	5	3	13	29	12	2	3
	71	28	9	9	11	11	34	24	36	59	13	37	5	4	14	30	12	2	3
	80	26	8	7	8	10	39	29	32	49	10	38	5	3	11	28	11	5	3
	80	27	7	9	9	10	33	28	34	59	14	43	5	2	11	32	11	2	3
	71	27	6	8	9	10	31	30	33	55	11	37	5	2	8	25	10	2	3
	70	26	7	7	10	9	32	27	31	56	11	38	4	3	11	28	10	4	3
	80	30	10	9	10	10	39	29	33	62	10	40	4	3	14	33	10	4	3
	61	23	8	9	9	10	31	26	27	50	9	34	4	3	10	26	9	4	4
	90	32	7	9	13	12	45	30	36	67	12	47	7	3	15	36	13	5	6
	90	27	8	10	11	12	40	34	40	73	15	47	6	3	15	37	13	6	4
	80	27	7	9	9	9	31	29	32	55	12	41	4	3	13	32	11	3	1
Oued Aabar	140	47	9	18	18	16	51	49	61	105	17	79	7	4	28	63	22	4	5
	141	48	12	16	17	16	56	48	60	99	18	76	9	7	26	63	23	8	8
	131	44	9	17	17	18	55	43	54	98	15	76	9	5	23	55	20	6	8
	151	48	9	17	18	15	57	53	65	109	17	86	9	7	30	67	27	8	8
	151	52	13	22	22	20	67	57	70	112	22	88	12	9	30	72	30	8	11
	161	51	11	19	20	18	61	53	67	116	17	91	11	7	31	67	26	6	8
	131	41	10	16	16	14	53	49	56	94	14	73	8	5	27	59	21	6	7
	140	47	9	18	18	16	51	49	61	105	17	79	7	4	28	63	22	4	5
	141	48	12	16	17	16	56	48	60	99	18	76	9	7	26	63	23	8	8
	131	44	9	17	17	18	55	43	54	98	15	76	9	5	23	55	20	6	8
	81	23	7	10	8	10	30	26	35	62	10	47	5	4	15	34	12	3	5
	81	26	7	10	8	10	29	32	37	64	11	46	5	3	16	35	12	4	5
	80	23	6	9	9	8	25	26	33	57	11	44	5	4	15	30	13	3	5
	90	30	9	12	9	11	33	33	40	65	12	51	4	5	17	36	15	3	5
	81	28	9	10	9	10	31	32	36	61	10	46	5	5	15	35	14	3	6
	80	24	7	10	7	11	27	30	36	60	13	45	6	5	17	32	11	3	6
	80	25	6	10	7	10	30	31	36	60	13	46	5	5	16	33	14	3	5
	80	24	8	13	10	11	26	27	33	55	12	43	4	5	14	30	12	4	6
	70	25	9	10	8	8	25	27	34	55	10	40	4	5	16	27	13	3	5
	81	23	7	10	9	10	30	26	35	62	10	47	5	4	15	34	12	3	5
Moulay Bousselham lagoon, Canal de Nador																			
H433	141	50	9	20	21	20	60	51	60	110	20	90	10	9	21	51	21	5	6
H434	151	50	10	20	21	21	61	51	60	111	21	81	10	8	21	60	30	5	5

Table 1. Continued.

	SL	HL	ED	IoW	SnL	PoL	PrD	PrP	PrV	PrA	CpL	DFL	PFL	VFL	AFL	BD	CPD	LDFS	L3SAF
Mauritania, Banc d'Arguin National Park																			
	260	70	17	25	32	20	69	66	83	166	21	130	63	66	31	90	44	22	20
	230	63	14	22	28	17	64	66	80	155	20	121	67	46	30	83	38	19	18
	248	65	14	22	27	18	60	62	80	164	21	130	62	57	32	89	38	20	21
	221	60	12	18	25	15	55	57	72	152	19	112	59	57	32	80	35	26	19
	253	70	13	26	35	20	72	74	82	169	22	130	69	65	43	94	42	21	21
	251	71	17	26	34	21	65	67	86	170	24	131	65	70	38	91	42	28	24
	267	75	19	28	37	25	77	75	90	182	28	143	85	54	44	93	44	31	22
	223	67	14	26	32	22	55	67	82	158	25	131	66	63	38	89	45	29	21
	230	65	15	25	31	20	63	69	82	163	22	135	59	82	34	87	42	30	23
	240	70	16	26	34	21	68	72	87	162	23	135	78	65	42	94	44	27	21
	329	82	15	43	28	20	85	75	105	200	32	171	83	87	48	101	41	33	22
	328	79	16	40	22	21	85	74	91	201	28	162	91	83	45	102	49	46	27
	236	84	15	40	30	24	74	77	95	200	30	158	99	97	43	110	49	41	25
	319	93	18	46	31	25	95	85	117	210	35	173	100	90	52	122	55	45	30
	225	74	11	47	26	21	88	70	90	188	32	150	86	74	42	99	44	32	25
	282	87	17	41	35	28	93	87	111	210	35	161	110	94	49	120	46	40	31
	255	74	15	37	28	23	81	68	91	186	32	149	87	85	41	92	42	40	28
	241	72	15	31	26	21	72	70	90	185	29	140	81	78	37	101	43	31	22
	281	81	17	41	29	26	83	80	101	201	41	159	85	96	43	111	50	47	32
	226	77	12	38	26	23	75	73	97	190	30	152	85	81	44	104	45	31	23

specimens from Oued Aabar, and two from Sebkha Imlili using the primers FishF1 and FishF2, used together as forward primers and FishR1 as reverse primer (Ward et al. 2005). Each amplification was performed following the standard PCR protocol for Taq DNA polymerase with the standard Taq buffer (New England Biolabs) in a volume of 50 µl containing 5 µl buffer (10× Standard Taq Reaction Buffer), 1 µl of 10 mM dNTPs, 1 µl of 10 µM forward primers, 1 µl of 10 µM reverse primer, 0.50 µl (2.5 units) of Taq polymerase, 1 µl of genomic DNA (0.1–0.5 ng), and 40.5 µl of nuclease-free water. The conditions of PCR reaction were as follows: 94 °C (3 min), 30 cycles of 94 °C (30 s), 56 °C (30 s), and 72 °C (30 s), with a final step at 72 °C for 10 min.

Additional COI sequences of *C. guineensis* from Kidé et al. (2016) and of *C. zillii* from Geiger et al. (2014) were downloaded from GenBank and included in the analysis. These represented eight different haplotypes. For *C. guineensis* these include two from Banc d'Arguin Mauritania (#KJ938198, #KJ938202); one from Baie de Hann Senegal (#KJ938159); one from Aby Lagoon, Bia River, Ivory Coast (#KJ938236); and one from Lake Weija, Densu River, Ghana (#KJ938155). For *C. zillii*, these include one from Ouargla, Chott Oum Erraneb basin, Algeria (#KJ938220); one from Man, Sassandra River, Ivory Coast (#KJ938177); and one from Oued Drâa, Morocco (#KJ553249). All COI sequences were aligned using Muscle as implemented in MEGA v. 10.2.0 (Kumar et al. 2018). Kimura two-parameter (K2P) distance (Kimura 1980) and average uncorrected *p* distance (Srivathsan and Meier 2012) were also computed with MEGA v. 10.2.0 (Kumar et al. 2018). The neighbor-joining tree (NJ) (Saitou and Nei 1987) representing the genetic relationships between all these haplotypes was established based on the K2P distances as implemented in MEGA v. 10.2.0 (Kumar et al. 2018). Support values for branches were estimate using the non-parametric bootstrap analysis (Felsenstein 1985) with 1000 replicates.

Results

Family Cichlidae Heckel, 1840
Genus *Coptodon* Gervais, 1853

***Coptodon guineensis* (Günther, 1862)**
Figures 1–3A, B

New record. MOROCCO • Kenitra Province; Moulay Bousselham, Canal de Nador; 34°48′27″N, 006°18′08″W; 2 m elev.; 25.XI.2018; local fishermen leg.; collected using gill nets; 2 spec. (sex unknown), preserved in alcohol (voucher numbers H433 and H434; GenBank OK104167, OK104168).

The specimens were first identified in the field by two of us (AB and AP). They were deposited in the collection of the Mohammed V University, Faculty of Sciences, Rabat Morocco.

Identification. Based on the description and the key given by Teugels and Thys van den Audenaerde (2003), the two individuals were identified as *C. guineensis* by the length of the head (between 29.7 and 34.1% of the standard length), the dorsal fin with 14–16 spines and 12 or 13 soft rays, a black spot on the gills, and six black vertical bars only faintly visible on flanks. These vertical bands distinguish *C. guineensis* from *C. zillii* (Teugels et al. 2003). Due to their poor condition, morphometric and genetic comparisons with other tilapia specimens were necessary for unambiguous species identification.

Distribution. Africa: coastal fresh, brackish, and marine

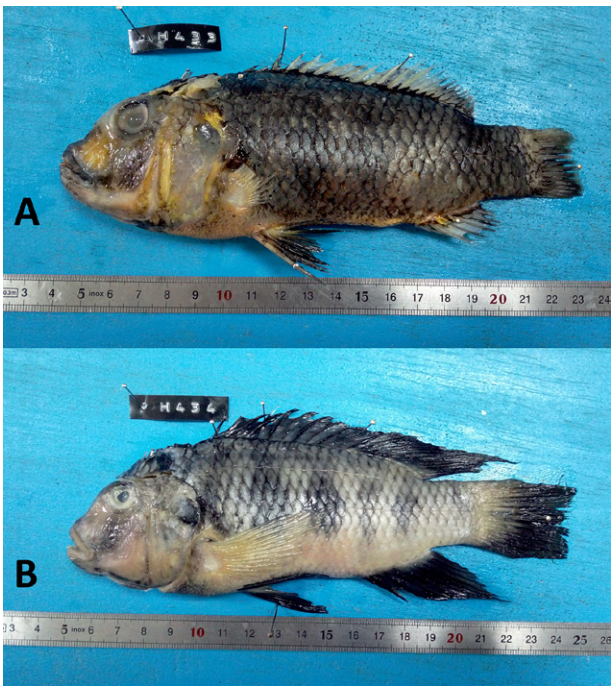

Figure 3. *Coptodon guineensis* from the Canal de Nador (34°48′27″N, 006°18′08″W), Moulay Bousselham lagoon, Morocco. Specimens are deposited in the collection of the Faculté des Sciences in Rabat (University Mohammed V). **A.** Voucher number H433. **B.** Voucher number H434.

waters from Oued Chbeyka and its tributary Oued Aabar (Morocco) to the mouth of the Cuanza River (Angola) (Qninba and Mataame 2009; Qninba et al. 2012; Stiassny et al. 2008)

Morphometric analysis. A PCA (Fig. 4) was carried out on the 19 log transformed and centered metrics measured from the 62 specimens originating from the Sebkha Imlili (20 specimens), the Oued Aabar (20 specimens), Banc d'Arguin National Park (20 specimens), and the Canal de Nador (2 specimens) (Table 1). After double centering, the first component PC1 (68.2 %) was not correlated with size as shown in the table of loadings (Table 2). PC1 was defined mainly by a combination of multiple characters including ED, HL, PoL, PrV, PrP, PrD, VFL, PFL, and LDFS (Table 2). PC2 (8.18 %) was defined mainly by BD, CPD, and DFL (Table 2).

The PCA allowed distinction between, on one hand, the populations of *C. guineensis* from Morocco (Sebkha Imlili and Oued Aabar) and the two specimens from the Canal de Nador, all located in the positive side of the first axis and, on the other hand, the population from Mauritania (Banc d'Arguin National Park) all located on the negative side. The second axis discriminated between the two populations in Morocco. The specimens from Oued Aabar were all in the positive side, while the specimens from Imlili were all except one in the negative side. The two specimens from the Canal de Nador were located in the positive side of the first axis and on either side of second axis close to the Imlili specimens. These results indicate that the specimens collected in the Canal Nador were more similar to the previously recorded Moroccan

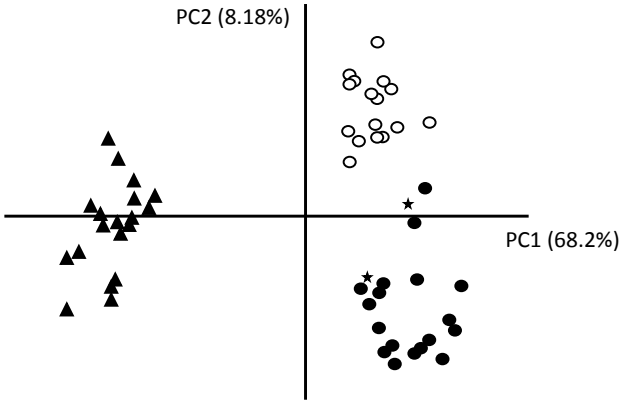

Figure 4. Plot of the second and third principal components taken from a principal component analysis of 19 metric variables on three populations of *Coptodon guineensis* (Mauritania = black triangle; Sebkha Imlili = black circle; Oued Aabar = open circle) and the two specimens from the Canal de Nador (= black star).

specimens of *C. guineensis* from Sebkha Imlili than to specimens from the Banc d'Arguin National Park in Mauritania.

Genetic analysis. From the studied specimens, partial (621 bp) COI sequences were obtained and deposited in GenBank: two identical sequences from the two specimens from Oued Aabar (Morocco 1, accession numbers OK104169, OK104170), two identical sequences from the two specimens from Sebkha Imlili (Morocco 2, accession numbers OK104165, OK104166), and two identical sequences from the two specimens from Canal de Nador (accession numbers OK104167, OK104168). The comparison of these COI sequences with those of *C.*

Table 2. First three PC loadings of the PCA on the additive double-centered on log-transformed measurements of three populations of *C. guineensis* (Mauritania; Sebkha Imlili; Oued Aabar) and the two specimens from the Canal de Nador.

Variable	PC 1	PC 2	PC 3
Log SL	−0.878060	0.075685	0.027072
Log HL	0.908295	−0.202055	0.004327
Log ED	0.936153	−0.123728	0.068985
Log IoW	0.658995	0.146337	0.097304
Log SnL	0.764197	−0.087833	−0.201843
Log PoL	0.968923	−0.042735	0.139390
Log PrD	0.884691	−0.217707	0.166215
Log PrP	0.933329	0.026535	0.098467
Log PrV	0.896882	0.183051	0.249081
Log PrA	−0.758485	0.128217	0.394123
Log APL	0.903341	−0.149429	0.137928
Log DFL	−0.779715	0.475936	0.103919
Log PFL	−0.954577	−0.127130	−0.178902
Log VFL	−0.960010	−0.001138	−0.170267
Log AFL	0.774308	0.454044	0.061019
Log BD	0.530024	0.564616	−0.219165
Log CPD	0.503390	0.525071	−0.550751
Log LDFS	−0.846377	−0.281482	−0.042345
Log L3SAF	−0.622978	0.467010	0.514575

guineensis already present in GenBank (Table 3) were done using BLAST search. The identical sequences obtained from the two tilapias from the Canal de Nador were 100% identical to three sequences from GenBank of *C. guineensis* from Mauritania (KJ938195, KJ938202) and Senegal (KJ938159). The unique haplotype obtained from the two specimens of *C. guineensis* from Sebkha Imlili was 0.16% (K2P or average uncorrected *p*-distance) divergent from the unique haplotype observed in the two specimens of *C. guineensis* from Oued Aabar. Haplotypes of *C. guineensis* present in Ivory Coast and Ghana were more differentiated with divergences ranges from 6.8 to 11.3% (Table 4). The divergence was even greater (9.4–11.3%) with the haplotypes of *C. zillii*.

A NJ tree of all the different haplotypes of *C. guineensis* and *C. zillii*, including the sequences of the two specimens from the Canal de Nador (Table 3), is presented in Figure 5.

Haplotypes were clustered in three different groups all supported by high bootstrap values (100%). One group was composed of the three haplotypes of *C. zillii* from Ivory Coast, Algeria, and Morocco. Another group was composed of the haplotypes of *C. guineensis* from Ghana and Ivory Coast. The third group was composed of haplotype of *C. guineensis* from Senegal, the two haplotypes from Mauritania (1 and 2 both from the Banc d'Arguin population), and the two haplotypes from Morocco: Morocco 1 (Oued Aabar) and Morocco 2 (Imlili and Canal de Nador) (Fig. 5).

Discussion

Species generally known as tilapias are native to continental Africa and the Middle East (Trewavas 1983), although they are now widely introduced around the world (Prabu et al. 2019). Three native species of tilapias are found in Morocco and represent relict populations of a tropical fauna from the Miocene (Lévêque 1990). *Oreochromis aureus* occurs naturally in most of West Africa, including the Senegal, Niger, and Chad basins, the Nile, and the Jordan River (Trewavas 1983). In Morocco, this species occurs in the watershed of the Drâa (Qninba and Mataame 2009; Louizi et al. 2019). *Coptodon zillii* is present in West and Central Africa including the Congo basin, Lake Turkana, and the Nile (Teugels and Thys van den Audenaerde 1991). In Morocco, it occurs in the Oued Drâa basin (Qninba and Mataame 2009; Louizi et al. 2019). Other relict populations of *C. zillii* are found in gueltas in Algeria and Tunisia (Lévêque 1990). *Coptodon guineensis* occurs in brackish or even marine waters from Mauritania to Angola and is present with two distinct populations in Morocco in the Sebkha Imlili (Qninba et al. 2009) and in Oued Chbeyka and its tributary Oued Aabar (Qninba et al. 2012).

In addition to these native populations, introduced populations of tilapias are also present in Morocco. *Oreochromis niloticus*, originally introduced in 2004 for aquaculture from Egypt (MAPMDREF 2020), is now present in the Bouregreg and Sebou basins and in the Moulay Bousselham area (Canal de Nador), as well as in other watershed in northern Morocco (Louizi et al. 2019).

Table 3. Sequences used in this study: GenBank accession number, location, haplotype code. Sequences in bold are those produced in this study.

GenBank number	Location	Haplotype code	Species
KJ938198	Banc d'Arguin	Mauritania 1	*C. guineensis*
KJ938202	Banc d'Arguin	Mauritania 2	*C. guineensis*
OK104165 / OK104166	Sebkha Imlili	Morocco 2	*C. guineensis*
OK104167 / OK104168	Moulay Bousselham lagoon	Canal de Nador	*C. guineensis*
OK1041659 / OK104170	Oued Aabar	Morocco 1	*C. guineensis*
KJ938236	Aby Lagoon	Ivory Coast	*C. guineensis*
KJ938155	Lake Weija	Ghana	*C. guineensis*
KJ938159	Baie de Hann	Senegal	*C. guineensis*
KJ938177	Man, Sassandra River	Ivory Coast	*C. zillii*
KJ553249	Morocco, Oued Draa	Morocco	*C. zillii*
KJ938220	Ouargla, Chott Oum Erraneb basin	Algeria	*C. zillii*

Table 4. Genetic distances between the nine haplotypes observed. Lower left, Kimura 2-parameter distance (Kimura 1980); upper right, Uncorrected *p*-distances. Cg = *Coptodon guineensis*; Cz = *C. zillii*.

	1	2	3	4	5	6	7	8	9
1-Cg-Mauritania 1		0.0032	0.0036	0.0016	0.0660	0.0611	0.0966	0.0998	0.1030
2-Cg-Morocco 1	0.0032		0.0032	0.0016	0.0660	0.0611	0.0966	0.0998	0.1030
3-Cg-Senegal	0.0032	0.0032		0.0016	0.0660	0.0611	0.0966	0.0998	0.1030
4-Cg-Mauritania 2, Morocco 2, Canal de Nador	0.0016	0.0016	0.0016		0.0644	0.0595	0.0950	0.0982	0.1014
5-Cg-Ghana	0.0702	0.0702	0.0702	0.0683		0.0064	0.0885	0.0885	0.0917
6-Cg-Ivory Coast	0.0647	0.0647	0.0647	0.0629	0.0065		0.0869	0.0869	0.0901
7-Cz-Ivory Coast	0.1065	0.1055	0.1055	0.1026	0.0961	0.0942		0.0128	0.0161
8-Cz- Algeria	0.1094	0.1094	0.1094	0.1075	0.0961	0.0942	0.0131		0.0032
9-Cz-Morocco	0.1132	0.1132	0.1132	0.1113	0.0998	0.0979	0.0163	0.0032	

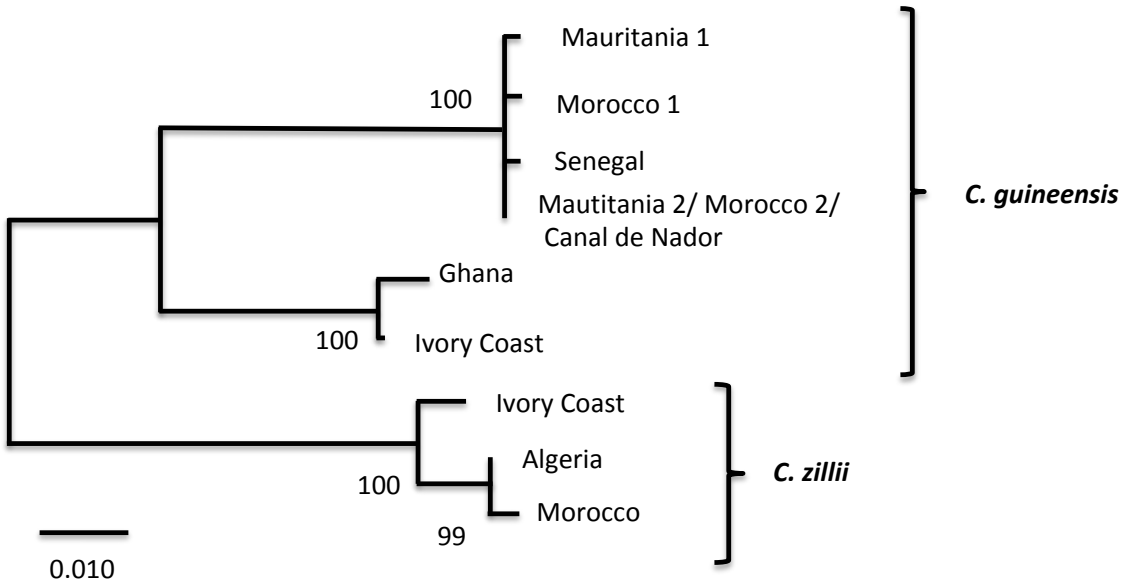

Figure 5. Neighbor-joining tree (Saitou and Nei 1989) based on distances computed using the Kimura 2-parameter method (Kimura 1980; Table 4) representing the genetic relationships between all the different haplotypes of *Coptodon guineensis* and *C. zillii* (Table 3), including the sequences of the two specimens from the Canal de Nador. Percentage of replicates tree in which the associated haplotypes clustered together in the bootstrap test (1000 replicates) are shown next to the nodes.

Our morphological and mtDNA study of the two tilapia specimens captured in the Canal de Nador near the Moulay Bousselham Lagoon confirmed our initial field identifications. The PCA analysis of morphometric data shows that the two specimens group together with individuals of *C. guineensis* from the Sebkha Imlili. Congruently, the analysis of the partial COI sequences also show that the two individuals from the Canal de Nador were conspecific to *C. guineensis*, as the unique sequence obtained was identical to sequences of this species from Mauritania (KJ938195, KJ938202) and Senegal (KJ938159). Hence our finding expands the known range of *C. guineensis* by about 1000 km north of the previously known northmost record at Oued Chbeyka, Oued Aabar basin (Fig. 2).

It now remains to be determined how this population of *C. guineensis* became established in the Canal de Nador, so far from the nearest population. There are two possible origins: (i) either the fish were transported by humans, or (ii) they arrived there on their own. The presence of an introduced population of *O. niloticus* in the Moulay Bousselham area at the level of the Canal de Nador suggests that *C. guineensis* could have been co-introduced. However, *Coptodon guineensis* is not a species used in aquaculture and has never been officially introduced in Morocco, whereas *O. niloticus* has been (MAPMDREF 2020). Nevertheless, juveniles of *C. guineensis* could have been accidentally introduced to an aquaculture station together with *O. niloticus* and then released into the natural environment with *O. niloticus* or even unintentionally escaped from a pond. The strain of *O. niloticus* introduced in Morocco came from Egypt (MAPMDREF 2020), where *C. guineensis* is not present. It is unlikely that *C. guineensis* would have been introduced with these fish, but it cannot be ruled out that some unrecorded aquaculture farms exist or some aquaculture trials in Morocco have taken place in the area where *C. guinenesis* is present (Oued Aabar basin) and that mixtures of both fry occurred on this occasion and then were dumped in the area drained by the Canal de Nador. This would account for the observation that the fish of the Canal de Nador are genetically very close to the other fish of Morocco.

Nevertheless, this scenario seems rather unlikely, and the most probable hypothesis is that the two individuals found in the Canal de Nador are part of a previously overlooked native population. The aptitude of this species to live in the marine environment in particular in Senegal and Mauritania (Kidé et al 2016) is well known. It is, therefore, not improbable that this species was able to colonize the Moulay Bousselham lagoon via the sea. However, one can wonder whether this colonization is ancient (Holocene) or contemporary due to climate change, which could have allowed this species to move further north. Shifts in species' distributions are occurring globally in response to climate change. Champion et al. (2021) estimated poleward rates of climate-driven range shifts in core oceanographic habitats over 21 years for four coastal-pelagic fishes from Australia between 148.7 and 278.6 km per decade. Rindsjdorp et al. (2009) reported that Lusitanian species (sprat anchovy and horse mackerel) have increased at the northern limit of their distribution areas in recent decades, while boreal species decreased at the southern limit of their distribution range (cod and plaice) but increased at the northern limit (cod). They considered that climate change would act directly or indirectly on the recruitment success. Perry et al. (2005) reported boundary shifts northward with warming for half of the marine species with northerly or southerly range margins in the North Sea.

To be able to determine the events that led to the presence of this species in the Canal de Nador, it will be necessary to study genetic markers that evolve faster than mitochondrial DNA such as microsatellites. Additionally, it will be necessary to determine the exact part of the lagoon where this population thrives. Most likely it is not the Canal de Nador because the species is very rare in catches there, although fishing is as intensive in the Canal as in the lagoon. The Moulay Bousselham lagoon is actually strongly shaped by human. The connections of the lagoon and the ocean are periodically reopened by digging. The Canal de Nador is artificial and the Drader River that flows into the northwestern part of the lagoon is strongly transformed for agriculture purposes (Carruesco 1989). There are many schorres around the lagoon. Schorres are flat natural areas with low vegetation located near the seaside inundated by salt water only during high tides. These areas, as well as the Drader River, will need to be surveyed next. Within the last decade, species detection from environmental DNA (eDNA) (i.e., extra-organismal DNA released by organisms into their environment) has shown great potential for routine species surveys (Rees et al. 2014; Goldberg et al. 2016; Deiner et al. 2017; Langlois et al. 2020). This approach has been used in many studies as promising and a complementary or alternative method for monitoring fish in lakes (Civade et al 2016). Thus, eDNA is a potential method to detect the presence, but not reveal the actual abundance, of this cichlid in Moulay Bousselham lagoon and in other suspected areas.

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