zoosystema

2021 • 43 • 27

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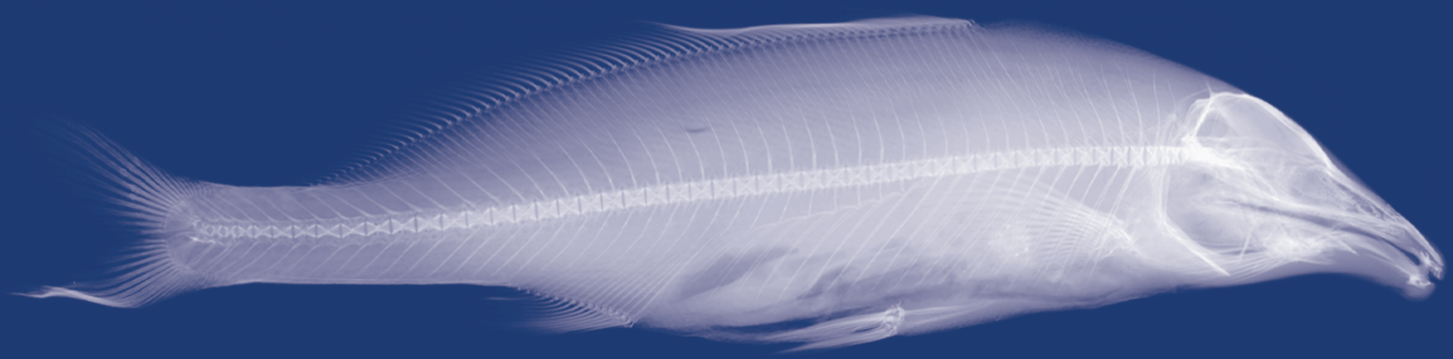

art. 43 (27) — Published on 20 October 2021
www.zoosystema.com

PUBLICATIONS SCIENTIFIQUES

MUSÉUM NAL HIST NATURELLE

Couverture / *Cover* :
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CP 41 – 57 rue Cuvier F-75231 Paris cedex 05 (France)
Tél. : 33 (0)1 40 79 48 05 / Fax : 33 (0)1 40 79 38 40
diff.pub@mnhn.fr / https://sciencepress.mnhn.fr

ISSN (imprimé / *print*) : 1280-9551/ ISSN (électronique / *electronic*) : 1638-9387

From fin rays to DNA: supplementary morphological and molecular data to identify *Mormyrus subundulatus* Roberts, 1989 (Pisces: Mormyridae) from the Bandama River in Côte d'Ivoire

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Submitted on 5 January 2021 | Accepted on 26 March 2021 | Published on 20 October 2021

urn:lsid:zoobank.org:pub:C79AC41A-0192-4951-B7F3-F0E625F716CB

Prié V., Adam B., Melki F. 2021. — From fin rays to DNA: supplementary morphological and molecular data to identify *Mormyrus subundulatus* Roberts, 1989 (Pisces: Mormyridae) from the Bandama River in Côte d'Ivoire. *Zoosystema* 43 (27): 649-658. https://doi.org/10.5252/zoosystema2021v43a27. http://zoosystema.com/43/27

ABSTRACT
The genus *Mormyrus* Linnaeus, 1758 has been well studied in western Africa where five species are currently recognized. *Mormyrus subundulatus* Roberts, 1989 was described in 1989, and, although morphologically and genetically close to *Mormyrus rume* Valenciennes, 1847, is still considered by the most recent studies as a distinct species. It is endemic to the Côte d'Ivoire and Ghana River systems. It was historically recorded from only three locations but is today very likely extirpated from one of them and threatened in the remaining two. It is categorized as "Vulnerable B1ab(iii)" by the IUCN Red List (Dankwa 2020). Although a few papers describe precisely the morphology and electric signal of the species, they are based on a very limited number of samples. In this paper, we present new data on the morphological characteristics of the species (extending the dorsal fin rays count range, which is the main morphometric criterium to distinguish *M. subundulatus* and *M. rume*) based on Museum samples and samples collected recently a few kilometers away from the type locality. Determination of the newly collected material was confirmed by the analysis of a fragment of the Cytochrome b gene, and we produced the first sequence fragment of the cytochrome oxydase subunit 1 (COI) for this species.

KEY WORDS
Conservation,
Ivory Coast,
Barcoding,
Dorsal Fin Ray Count.

RÉSUMÉ
Des rayons de nageoire à l'ADN : données morphologiques et moléculaires supplémentaires pour identifier Mormyrus subundulatus *Roberts, 1989 (Pisces : Mormyridae) de la rivière Bandama en Côte d'Ivoire.*
Le genre *Mormyrus* Linnaeus, 1758 a été bien étudié en Afrique de l'Ouest où cinq espèces sont actuellement reconnues. *Mormyrus subundulatus* Roberts, 1989 a été décrit tardivement en 1989, et, bien que morphologiquement et génétiquement proche de *Mormyrus rume* Valenciennes, 1847, est toujours considéré comme une espèce valide par les études récentes. Endémique des hydrosystèmes de Côte d'Ivoire et du Ghana, il est historiquement connu de seulement trois localités, mais a vraisemblablement disparu de l'une d'elles, et est menacé sur les deux autres. Sur la Liste rouge de l'UICN, il est catégorisé comme « Vulnérable B1ab(iii) ». Même si quelques publications décrivent précisément sa morphologie et son signal électrique, elles ne sont basées que sur un nombre limité d'échantillons. Dans cet article, nous présentons de nouvelles données sur la morphologie de l'espèce (en élargissant l'amplitude de variation du nombre de rayons de la nageoire dorsale, le principal critère pour séparer de *M. subundulatus* et de *M. rume*) basées sur des échantillons de collection et sur des échantillons collectés récemment à quelques kilomètres de la localité type. L'identification de ces échantillons est confirmée par l'analyse d'un fragment du gène mitochondrial Cytochrome b et nous avons généré les premières séquences pour le fragment du gène codant pour la première sous-unité de la cytochrome oxydase (COI) pour cette espèce.

MOTS CLÉS
Côte d'Ivoire,
conservation,
barcoding,
nombre de rayons
de la nageoire dorsale.

INTRODUCTION

Among the more than 200 known mormyrid fish species of Africa, the genus *Mormyrus* Linnaeus, 1758 comprises 22 species (according to Froese & Pauly 2018), five of which occur in western Africa.

The two short-snouted species, *Mormyrus hasselquistii* Valenciennes, 1847 and *M. macrophtalmus* Günther, 1866, have relatively wide distributions in Africa. *M. hasselquistii* is known from Senegal to Ethiopia, in the Sahelo-Sudanese basins, rivers Géba, Bandama, Comoé, Mono and lagoons Ebrié and Aguien. *Mormyrus macrophthalmus* is patchily distributed from Guinea, in the Niger and Volta basins, to Cameroon, in the Cross and Sanaga Rivers.

Among the three long-snout species, *Mormyrus rume* Valenciennes, 1847 has the widest distribution, being found from Senegal westward to Ghana eastward, through Gambia, Niger and Côte d'Ivoire (Lévêque & Bigorne 1985; Paugy *et al.* 2003; Stiassny *et al.* 2008). It is presently known only from the great Sahelo-Sudanese basins and from certain coastal basins, e.g. Cavally, Bandama, Sassandra, Mono, Ouémé, Ogun and Culufi basins (Stiassny *et al.* 2008). *Mormyrus tapirus* Pappenheim, 1905 has a disjunct distribution in coastal rivers systems of Guinea, Sierra Leone, Liberia, and Cameroun. There are no records from the region between Cameroon and Upper Guinea. *Mormyrus goheeni* Fowler, 1919 is considered a synonym of *M. tapirus* (Lalèyè *et al.* 2010).

Mormyrus subundultaus Roberts, 1989 from the Bandama River, in the Lamto Nature Reserve was described in relation to the sympatric *M. rume*. Its description is based on anatomical features, mainly the higher number of dorsal fin rays count (DFRC), higher range of both anal fin rays and scales in lateral series. Subsequent studies from Crawford & Hopkins (1989) confirmed the distinction of *M. rume* and *M. subundulatus* by analyzing and describing *M. subundulatus* specific electric discharge. *M. subundulatus* is currently known only from three locations according to Entsua-Mensah & Lalèyè (2010): two in the Bandama River in Côte d'Ivoire and one in the Tano River in Ghana (restricted to two locations only according to Dankwa 2020).

While the available literature precisely describes the morphology (Lévêque & Bigorne 1985; Kramer 2013) and electric signal (Crawford & Hopkins 1989; Kramer 2013) of the species, these studies are based on a very limited number of samples and only one DNA fragment sequence is available (Lavoué *et al.* 2000). We here report on the observation of five specimens collected close to the type locality in the Bandama River, present the results of DNA analyzes for the genes COI and Cyt b and discuss the morphological traits of *M. subundulatus*. Although still relying on a small number of specimens, this additional data is valuable as the species is threatened in and around its type locality, as well as in its entire range, in particular, by pollution, placer mining and hydroelectric power plants.

MATERIAL AND METHODS

Specimens examined

The type material of *M. subundulatus* is held at the Muséum national d'Histoire naturelle in Paris (MNHN). The type series consists in one holotype (MNHN-IC-1987-1610) and a batch of 16 paratypes (MNHN-IC-1987-1611, nine adults and seven juveniles) preserved in 90° ethanol, all coming from the type locality, the Lamto Nature Reserve in the Bandama River (Côte d'Ivoire). The original publication also mentions as paratype a specimen from the Tano River in Ghana, held in the Stanford University collection in the California Academy of Sciences (CAS – SU 63507). This specimen was pictured and radiograph for dorsal fin rays count (Fig. 1A).

An additional specimen (MNHN-IC-1963-0241) collected in 1961 in Beoumi, upper Bandama was examined in

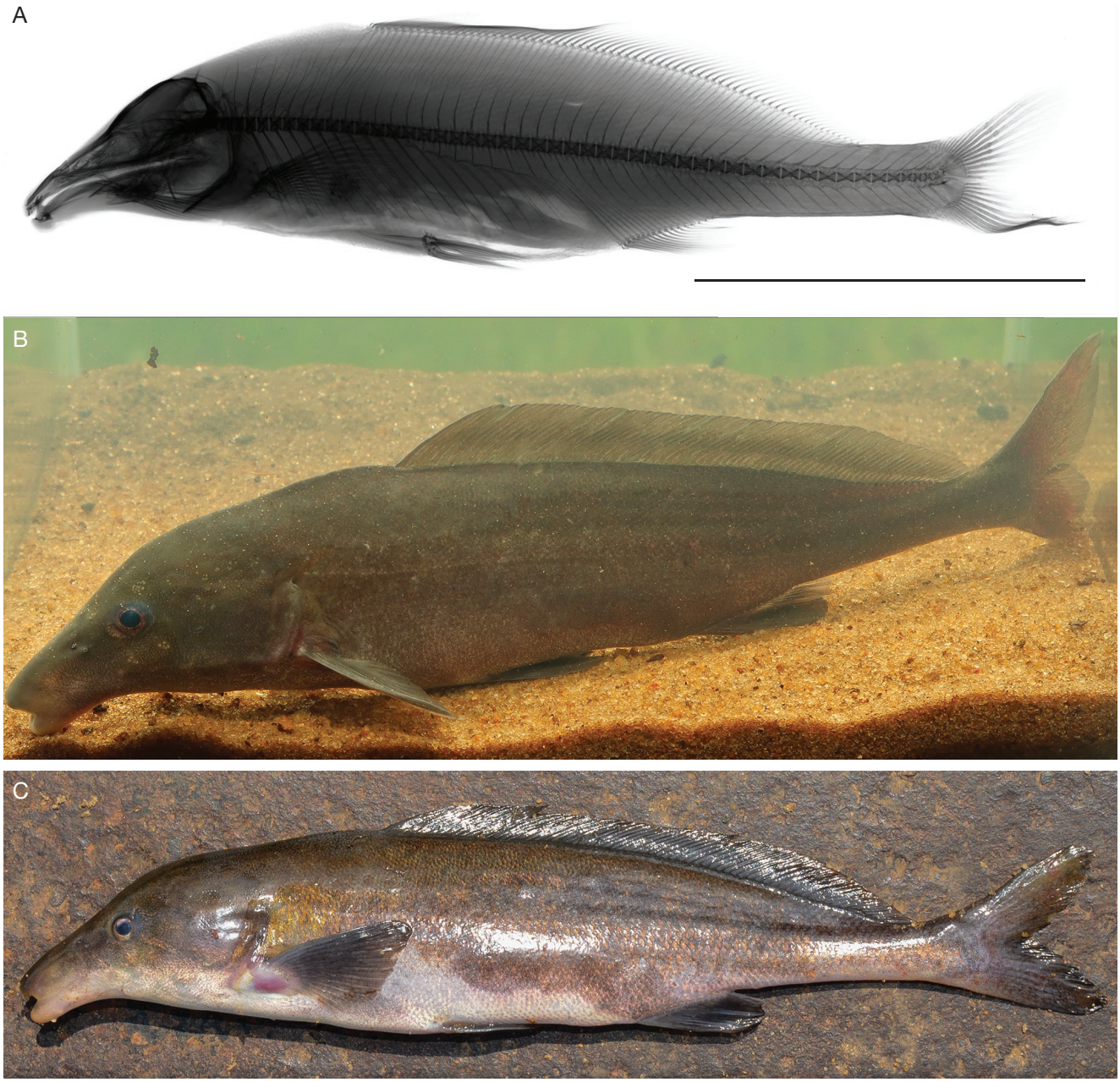

Fig. 1. — **A**, radiography of the paratype SU 63507 *Mormyrus subundulatus* Roberts, 1989 from the Tano River (© California Academy of Sciences, Dept. of Ichthyology); **B**, specimen number MNHN-IC-2018-0558 caught in the Bandama River near the type locality; **C**, specimen number MNHN-IC-2018-0559. Scale bar: A, 10 cm.

the MNHN collections. Four specimens are preserved in the Institut royal des Sciences naturelles de Belgique in Bruxelles: two specimens were collected by G. Teugels on the upper Bandama River near Marabadiassa in 1985 (88-055-P-0027 and 88-055-P-0028), and another two specimens by the same author, further upstream where the Bandama River crosses the road between Niakaramandougou and Tortiva (87-018-P-0040 and 87-018-P-0041).

These museum specimens represent the only distribution data available for the species, except for a doubtful record from Lévêque & Bigorne (1985) in the Sassandra River, west of the Bandama (see Roberts 1989 and Discussion).

Three specimens were sampled alive by us in the Bandama River for morphometric and genetic analyses (Fig.1B, C): MNHN-IC-2018-0559 was collected on the 7th of December 2017, 6°6'14"N, 4°56'52"W; MNHN-IC-2018-0558 (female with eggs) was collected on the 9th of December 2017, 6°6'13"N, 4°57'10"W; MNHN-IC-2018-0560 (tissue sample only) was collected on the 11th of December 2017, 6°7'35"N, 4°57'18"E.

Two additional specimens caught by local fishermen were photographed in the field.

For all the specimens examined, a precise DFRC was performed on photographs (available upon request).

TABLE 1. — Detailed references of the sequences and specimens studied for molecular analyses.

GenBank	Species	Reference	Gene	Location	Voucher nb	Haplotype
KT192785	*M. caballus bumbanus* Boulenger, 1909	Decru *et al.* 2011	COI	Democratic Republic of the Congo: Ituri River	MRAC:A9_29_DNA_3224	Mc_coi_1
KT193005	*M. caballus bumbanus* Boulenger, 1909	Decru *et al.* 2011	COI	Democratic Republic of the Congo: Ituri River	MRAC:A9_29_DNA_3935	Mc_coi_2
KT193007	*M. caballus bumbanus* Boulenger, 1909	Decru *et al.* 2011	COI	Democratic Republic of the Congo: Ituri River	MRAC:A9_29_DNA_3943	Mc_coi_3
HM882746	*M. hasselquistii* Valenciennes, 1847	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 59	Mh_coi_1
HM882747	*M. hasselquistii* Valenciennes, 1847	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 60	Mh_coi_1
HM882748	*M. hasselquistii* Valenciennes, 1847	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 61	Mh_coi_1
HM882749	*M. hasselquistii* Valenciennes, 1847	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 63	Mh_coi_1
HM882750	*M. hasselquistii* Valenciennes, 1847	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 64	Mh_coi_1
HM882751	*M. hasselquistii* Valenciennes, 1847	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 65	Mh_coi_1
HM882752	*M. hasselquistii* Valenciennes, 1847	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 66	Mh_coi_1
HM882753	*M. hasselquistii* Valenciennes, 1847	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 67	Mh_coi_1
HM882754	*M. hasselquistii* Valenciennes, 1847	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 68	Mh_coi_1
HM882755	*M. hasselquistii* Valenciennes, 1847	Nwani *et al.* 2011	COI	Nigeria: Enugu, South-East, Ebonyi, Abakaliki	BNF 69	Mh_coi_1
HM882756	*M. hasselquistii* Valenciennes, 1847	Nwani *et al.* 2011	COI	Nigeria: South-East, Ebonyi, Abakaliki	BNF 70	Mh_coi_1
JF510513	*M. hasselquistii* Valenciennes, 1847	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 62	Mh_coi_1
HM882759	*M. macrophthalmus* Günther, 1866	Nwani *et al.* 2011	COI	Nigeria: Anambra, South-East, Otuocha	BNF 73	Mm_coi_1
AP011577	*M. rume* Valenciennes, 1847	Lavoué *et al.* 2012	COI	Ouémé R., Bénin	??	Mr_coi_1
MH792419	*M. subundulatus* Roberts, 1989	This study	COI	Bandama River, Côte d'Ivoire	MNHN-IC-2018-0558	Ms_coi_1
MH792420	*M. subundulatus* Roberts, 1989	This study	COI	Bandama River, Côte d'Ivoire	MNHN-IC-2018-0559	Ms_coi_1
MH792421	*M. subundulatus* Roberts, 1989	This study	COI	Bandama River, Côte d'Ivoire	MNHN-IC-2018-0560	Ms_coi_1
HM882737	*M. tapirus* Pappenheim, 1905	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 50	Mt_coi_1
HM882738	*M. tapirus* Pappenheim, 1905	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 51	Mt_coi_1
HM882739	*M. tapirus* Pappenheim, 1905	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 52	Mt_coi_1
HM882740	*M. tapirus* Pappenheim, 1905	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 53	Mt_coi_1
HM882741	*M. tapirus* Pappenheim, 1905	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 54	Mt_coi_1
HM882742	*M. tapirus* Pappenheim, 1905	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 55	Mt_coi_1
HM882743	*M. tapirus* Pappenheim, 1905	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 56	Mt_coi_1
HM882744	*M. tapirus* Pappenheim, 1905	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 57	Mt_coi_1
HM882745	*M. tapirus* Pappenheim, 1905	Nwani *et al.* 2011	COI	Nigeria: Anambra, South-East, Otuocha	BNF 58	Mt_coi_2
HM882760	*M. tapirus* Pappenheim, 1905	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 74	Mt_coi_1
HM882761	*M. tapirus* Pappenheim, 1905	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 75	Mt_coi_1
HM882762	*M. tapirus* Pappenheim, 1905	Nwani *et al.* 2011	COI	Nigeria: Abai, South-East, Ebonyi, Afikpo	BNF 76	Mt_coi_1
HM882735	*Marcusenius senegalensis* (Steindachner, 1870)	Nwani *et al.* 2011	COI	Nigeria: Anambra, South-East, Otuocha	BNF 48	Extra group
KT962115	*M. kannume* Forsskål, 1775	Direct submission	Cytb	Ethiopia	C395 Baro R.	Mk_cytb_1

Table 1. — Continuation.

GenBank	Species	Reference	Gene	Location	Voucher nb	Haplotype
AF201600	*M. ovis* Boulenger, 1898	Sullivan *et al.* 2000	Cytb	Central African Republic, Sangha Rvier	AMNH 228161	Mo_cytb_1
AF095291	*M. rume* Valenciennes, 1847	Lavoué *et al.* 2000	Cytb	Niger River, Mali	MNHN-IC-1999-275	Mr_cytb_1
AF201601	*M. rume* Valenciennes, 1847	Sullivan *et al.* 2000	Cytb	Mali, Niger R.	MNHN-IC-1999-613	Mr_cytb_2
AP011577	*M. rume* Valenciennes, 1847	Lavoué *et al.* 2012	Cytb	Ouémé R., Bénin		Mr_cytb_3
AF095292	*M. subundulatus* Roberts, 1989	Lavoué *et al.* 2000	Cytb	Bandama River, Côte d'Ivoire	MNHN « not registered »	Ms_cytb_1
MH792422	*M. subundulatus* Roberts, 1989	This study	Cytb	Bandama River, Côte d'Ivoire	MNHN-IC-2018-0558	Ms_cytb_1
MH792423	*M. subundulatus* Roberts, 1989	This study	Cytb	Bandama River, Côte d'Ivoire	MNHN-IC-2018-0559	Ms_cytb_1
AF201592	*Marcusenius senegalensis* (Steindachner, 1870)	Sulivan *et al.* 2000	Cytb	Niger River, Mali	MNHN-IC-1999-612	Extra-group

All the available sequences of *Mormyrus* spp. for the mitochondrial genes cytochrome oxydase subunit 1 (COI) and cytochrome b (Cytb) were mined in GenBank (Table 1).

DNA extraction, amplification, and sequencing

A small flesh sample was collected in the field and placed directly into 90% ethanol. The whole genomic DNA was extracted from the tissue samples using the DNeasy blood and tissue kit (Qiagen) according to the manufacturer's protocol.

A fragment of 710 bp of the mitochondrial cytochrome c oxidase subunit I (COI) gene and the complete cytochrome b (1100 bp) gene were amplified by Polymerase Chain Reaction (PCR) using the primers LCO1490 and HCO2198 (Folmer *et al.* 1994) for COI and L14724 (GAC TTG AAA AAC CAC CGT TG) and H15915 (CTC CGA TCT CCG GAT TAC AAG AC) (Schmidt & Gold 1993; Brito *et al.* 1997) for Cyt b. Amplifications were performed in 25 µl total volume including 0.5 µl of gDNA, 1 × GoTaq Green reaction buffer (Promega), 200 µM of dNTPs (Promega), 0.5 µM of both primers and 0.1 U of GoTaq DNA polymerase (Promega). PCR conditions were: 4 min at 94 °C followed by 30 cycles of 45 s at 94 °C, 45 s at annealing temperature (45 °C for COI and 48 °C for Cyt b) and 45 s at 72 °C, and then a final extension of 10 min at 72 °C. PCR products were visualized on 1% agarose gel and then purified and sequenced with each universal primer on an automated ABI3730XL Genetic Analyzer. The new sequences obtained in this study were submitted to GenBank (Table 1).

Analyses

Chromatograms were checked by eye and the sequences (COI and Cyt b) were aligned with ClustalW, using Bioedit v. 5.0.9. (Hall 1999) and then adjusted manually. COI and Cyt b mtDNA sequences of *Marcusenius senegalensis* (Steindachner, 1870) were included as outgroups (HM882735, Nwani *et al.* 2011 for COI and AF201592, Sullivan *et al.* 2000 for Cytb).

Nucleotide substitution models were selected for each gene separately using the program jModeltest 0.1.1 (Posada 2008), based on the AIC criterion. Saturation was tested for the three codon positions of the COI gene, and the third position was proved to be largely homoplasic (results not shown): we thus defined three partitions (one for each codon position) for the COI gene in all subsequent Bayesian analyses (BI). BI analyses have been performed running two parallel analyses in MrBayes V 3.1.2 (Huelsenbeck & Ronquist 2001), consisting each of two Markov chains of 10 000 000 generations each, sampled every 1 000 Generations. Convergence of the analysis was checked using Tracer V 1.4.1 (Rambaut & Drummond 2007); all ESS values were >200 (default burnin). Both chained had converged after 2 665 000 trees for COI and 730 000 trees for Cyt b. A consensus tree was then calculated after omitting the first 10% trees as burn-in.

Abbreviations

DFRC	Dorsal fin ray count;
Cytb	fragment of the Cytochrome b gene;
COI	fragment of the Cytochrome Oxydase subunit 1 gene.

Institutions

CAS	California Academy of Science, San Francisco;
MNHN	Muséum national d'Histoire naturelle, Paris;
MRAC	Royal Museum for Central Africa in Belgium, Tervuren.

RESULTS

DNA analyses

Cytb sequences were successfully amplified for the three specimens sampled. They all shared the same haplotype as the sequence produced by Lavoué *et al.* (2000), supporting the correct identification of the specimens collected (Fig. 2A).

COI sequences were successfully amplified for the three specimens sampled, resulting in a single haplotype. This haplotype

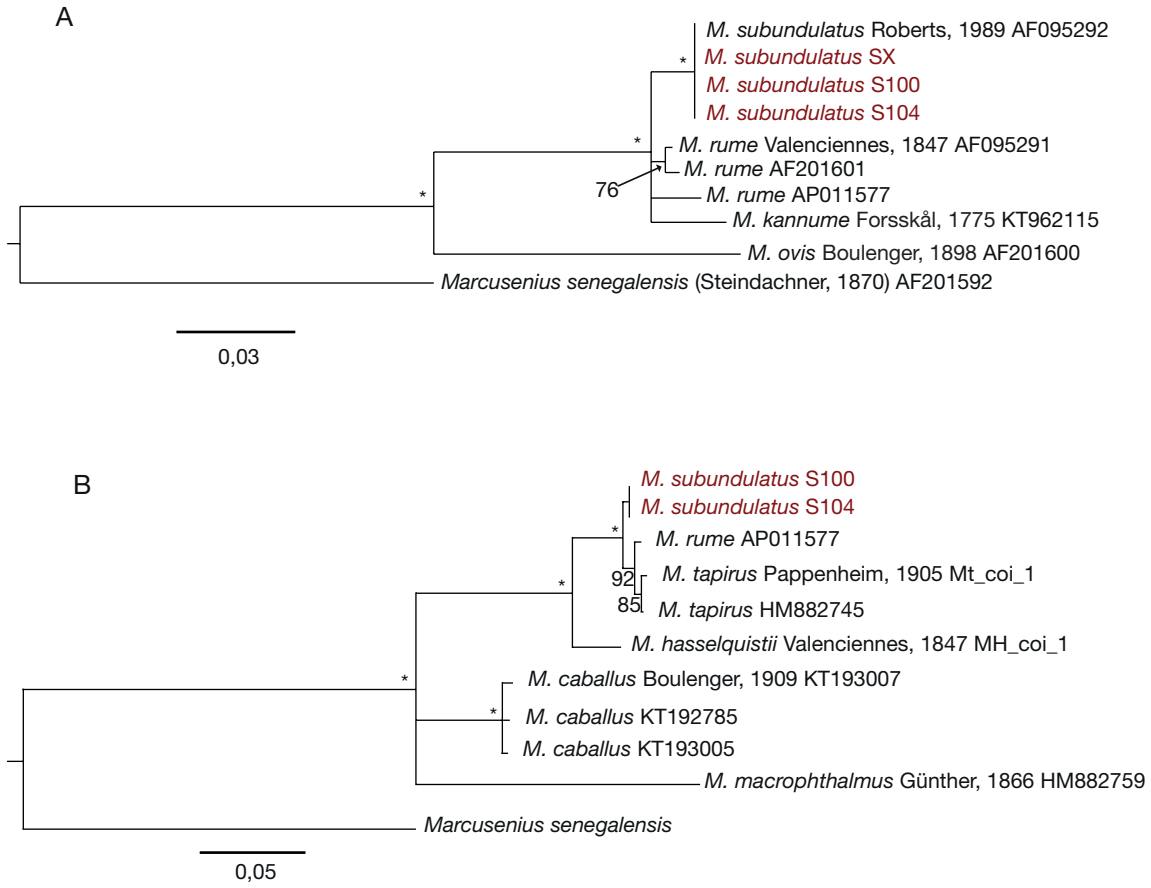

Fig. 2. — Bayesian phylogenetic analyses base on Cyt b gene fragment (**A**) and COI gene fragment (**B**). Numbers on branch node indicate posterior probability values, only node with posterior probability above 85 are represented. Nodes with probability > 98 are identified with *. Numbers on specimens indicate the GenBank accession number of the sequence or, when many specimens shared the same haplotype, the haplotype names in Table 1.

was significantly divergent from *M. rume* and *M. rume* was found closer to *M. tapirus* than to *M. subundulatus* (Fig. 2B). Average COI distance between *M. subundulatus* and *M. tapirus* was 0.77%, versus 0.6% between *M. subundulatus* and *M. rume*. COI distance between *M. rume* and *M. tapirus* is only 0.46%.

Dorsal fin rays count

The number of dorsal fin rays is the most reliable morphological character to distinguish *M. subundulatus* from *M. rume*. Dorsal fin rays were counted for the 18 specimens of the type series held in MNHN, the paratype held in CAS, the four specimens held in MRAC and the five specimens observed by us (Fig. 3). DFRC ranged for *M. subundulatus* from 57 (specimens 88-055-P-0027 and 88-055-P-0028 collected upstream the Bandama River) to 73 (specimen MNHN-IC-2018-0558 collected by us downstream the Bandama River).

Other morphological characters

According to the original description, anal fin ray count should be between16 and 18. Our specimens had respectively 18 (MNHN-IC-2018-0558) and 15 (MNHN-IC-2018-0559) anal fin rays. Interestingly, the paratype SU 63507 also has 15 anal fin rays (Fig. 1A). Depth of caudal peduncle was about 17 times in standard length for both specimens observed, in accordance with the original description. The color of the live specimens observed in the Bandama was almost uniformly black (specimen MNHN-IC-2018-0558, fig. 1B) and seem to turn paler on the belly and throat after death (specimen MNHN-IC-2018-0559, fig. 1 C).

DISCUSSION

DNA analyses

The correct identification of the sampled specimens was supported by the Cyt b gene fragment amplified, as those specimens had the same haplotype as the one produced by Lavoué *et al.* (2000). We here provide the first COI reference sequence for *M. subundulatus* for barcoding analyses.

The COI marker confirms that *M. subundulatus* is genetically divergent from *M. rume*, as identified by Lavoué *et al.* (2012). The genetic distances are rather low, but the fact that both species live in the same river stretches support their distinction as different species.

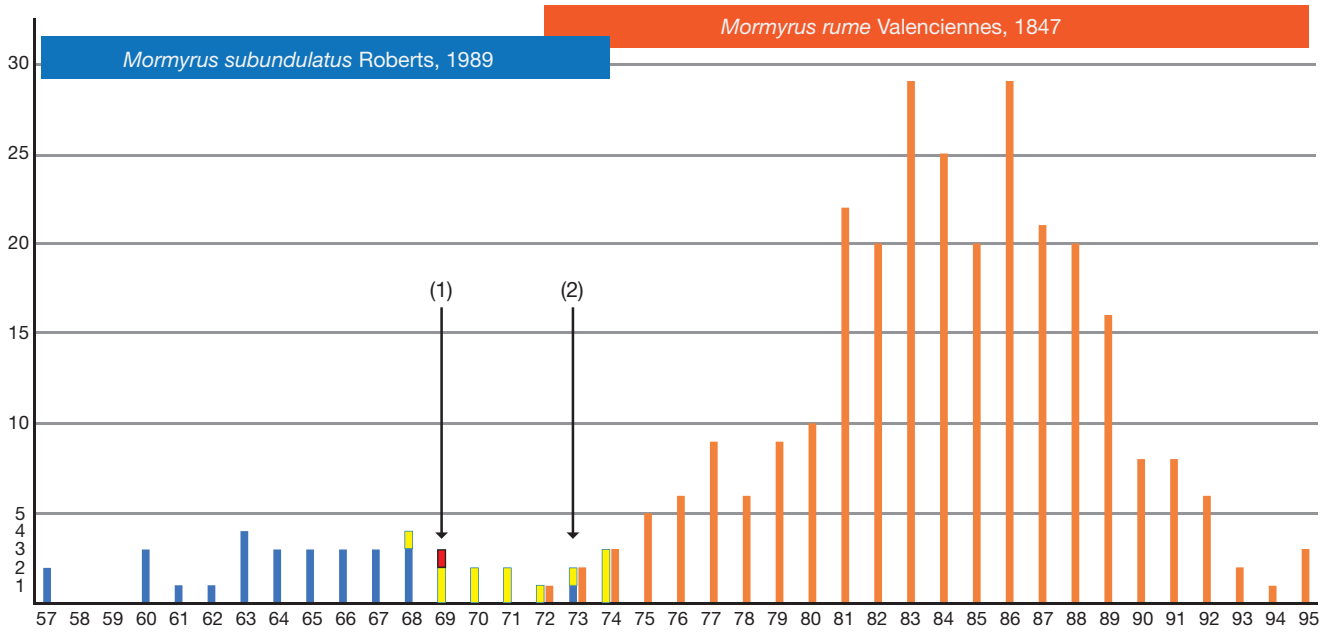

FIG. 3. — Distribution of the dorsal fin rays counts for the specimens of *M. subundulatus* Roberts, 1989 examined by us (**blue bars**) and for the specimens of *M. rume* Valenciennes, 1847 (data from Lévêque and Bigorne, 1985; **orange bars**). **Yellow bars**: specimens presumably from the Sassandra River population. (**1**) position of paratype CAS-SU63507 from the Tano River in Ghana (red bar); (**2**) position of specimen MNHN-IC-2018-0558, for which genetic data confirms the identification as *M. subundulatus*.

Regarding *M. rume*, the single specimen sampled in Benin by Lavoué *et al.* (2012) has a Cyt b sequence fragment which does not cluster with the other two specimens (sampled in Niger and Mali), suggesting it might belong to a distinct species.

Dorsal fin rays count and field identification

Intermediate between the two morphologically similar species *M. rume* and *M. tapirus*, *M. subundulatus* was described by Roberts (1989) based on morphological characters. *Mormyrus subundulatus* differs from *M. tapirus* in having a "larger eye, more slender caudal peduncle, more anal fin rays (21-29) and fewer circumpeduncular scales (14-19)". However, the author notices that *M. tapirus* "agrees with *M. subundulatus* in DFRC (60-74) and number of scales in lateral series (83-98)". According to Roberts (1989), *M. subundulatus* superficially resembles *M. rume* but differs in the DFRC which ranges from 60 to 71, while *M. rume*'s counts range from 72 to 95. This gap between DFRC allows the author to identify both species in a batch of specimens collected in the Sassandra River by Lévêque & Bigorne (1985). However, the DFRC in the supposed *M. subundulatus* of the Sassandra River would range from 68 (one specimen) to 74 (three specimens) (data from Lévêque & Bigorne 1985: 327).

We here report on a larger range of DFRC than in the original description, with barcoded specimens having 73 dorsal fin rays. These data agree with the fin ray count observed in the Sassandra-provided the specimens in question are really *M. subundulatus* and really come from the Sassandra. Indeed, the population of *M. subundulatus* purportedly identified in the Sassandra River by Roberts (1989) is enigmatic from two points of views: first because it is unclear which of the material deposit in the MNHN by Lévêque & Bigorne (1985) comes from the Sassandra, as locality labels indicate both the Sassandra and Bandama River, and second because DFRC is slightly different from that of the Bandama population. The fact that fin ray count is significantly different in this batch supports the hypothesis that it comes from a different basin, as genetic isolation and genetic drift or selection could have produced morphological divergences.

As a conclusion, reliable identification of both species in the field based only on DFRC is difficult because they both overlap, at least between 72 and 74 dorsal fin rays. Only 39 specimens were examined for *M. subundulatus*. It is therefore questionable whether our data reflect the actual variability of this character. The fin ray count range could exceed the upper value of 74 given here. However, on average, *Mormyrus subundulatus* has fewer fin rays than *M. rume*: 15-18 vs 16-21 for the anal fin, 60-74 vs 72-95 for the dorsal fin (Fig. 3).

According to Roberts (1989), local fishermen in the Tano River in Ghana were able to tell the two species apart because *M. rume* does not give perceptible electric shocks when handled, while *M. subundulatus* does produce a slight electric shock. During our field trip, local fishermen warned us about the fact that *Mormyrus* species could give a perceptible electric shock but did not specify if this could be a differentiating feature between *M. subundulatus* and *M. rume*. Such an electric shock was experienced by one of us (BA) while handling the fish MNHN-IC-2018-0558 (Fig. 1B) from a tank to another. This characteristic is highly subjective and may not be considered diagnostic for field identification. Indeed, apart from this unique experience, the specimens we caught and handled in the Bandama River have not given any other electric shocks,

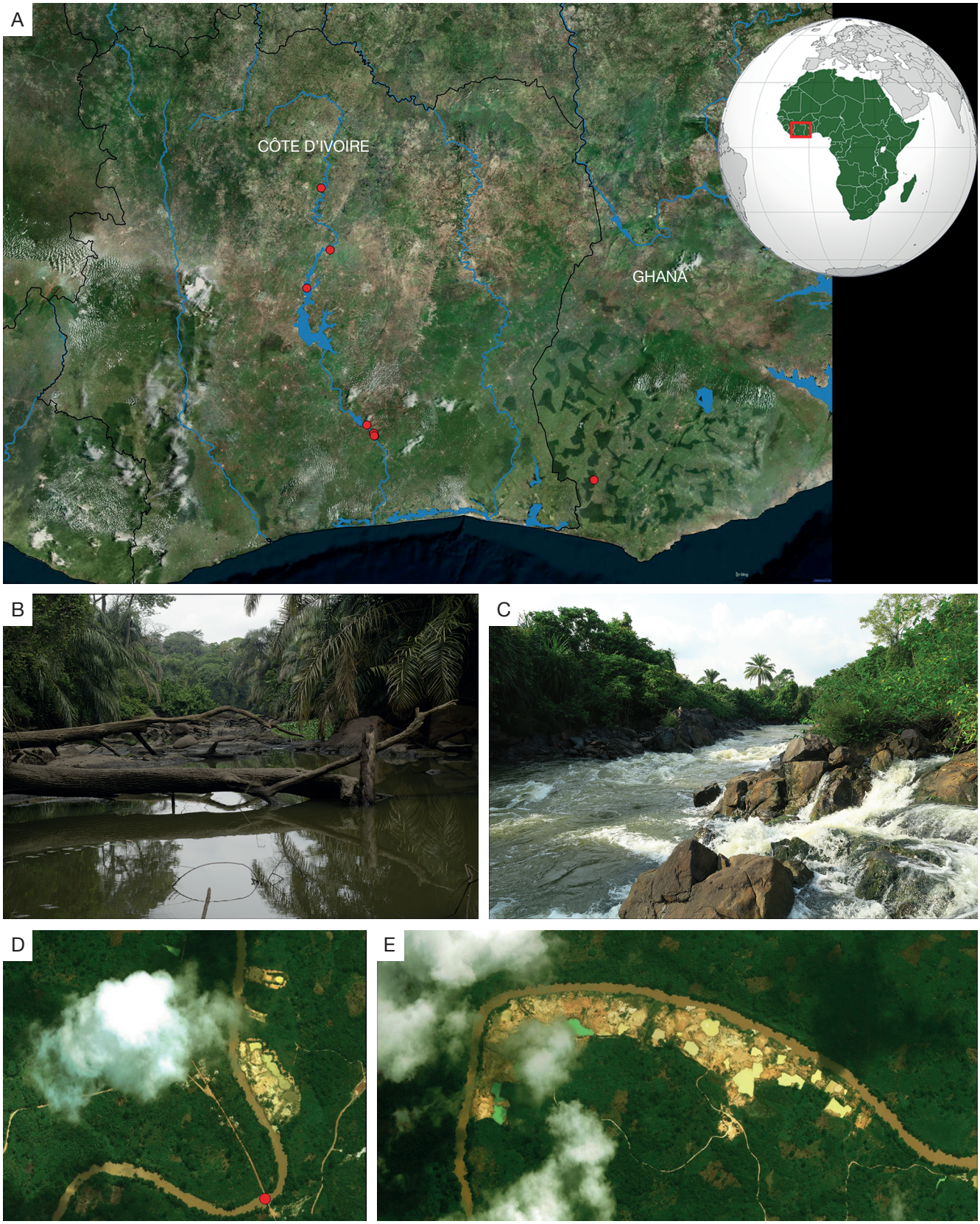

FIG. 4. — **A**, Distribution of *Mormyrus subundulatus* Roberts, 1989 according to available data; **B**, the Bandama River in the type locality is impacted by the Taabo dam just upstream; **C**, preserved stream habitat downstream the type locality where *M. subundulatus* still lives. This part of the River will be lost after the impoundment of another big dam, planned for the next few years; **D**, aerial view (GoogleEarth) of the Tano River in the type locality (**red dot**); **E**, upstream, showing the important buildup of soil and mud due to mining activities. The Tano River does not seem to host suitable habitat for *M. subundulatus* anymore, at least around the historical locality.

although annoyed repeatedly by all of us, for the sake of repeating the experience. Both *M. rume* and *M. subundulatus* may produce electric shocks according to Hopkins (pers. comm.), although the current may be stronger in *M. subundulatus* than *M. rume*. The comparatively thicker caudal peduncle in *M. subundulatus* might support this. But the strength of the electric shock is also likely dependent on specimen size.

Actual distribution and conservation status

The distribution of *Mormyrus subundulatus* is currently known only from museum-preserved specimens. Its natural distribution seems to be restricted to the Tano River in Ghana, the Bandama River and possibly the Sassandra River in Côte d'Ivoire. However, reliable data is very scarce (Fig. 4A) and the species is likely to also live in other rivers between the Tano and the Sassandra Rivers such as the Bia or Comoe Rivers basins.

In the type locality, the Taabo dam has changed the flow regime from fast-flowing waters and rapids to muddy substrate with an important drawdown (Fig. 4B). The site is no longer suitable for *M. subundulatus*. We have caught specimens downstream of the type locality in fast-flowing river stretches (Fig. 4C). These are the southernmost known localities for the species in the Bandama River. But this area is also threatened by another dam under construction in 2020 and we can assume that all the river downstream Beoumi will soon be threatened by river management and will no longer host the species. Probably, the only remaining preserved habitats in the Bandama are located upstream the village of Beoumi. However, the river is quite narrow upstream, which makes it more vulnerable to human impact in this very populated area, pollution due to intensive agriculture, draughts and climate change effects.

From an aerial view, the Tano River in Ghana seems to be mostly slow flowing, with very few stream habitats (Fig. 4D, E). While the riversides are still forested, vast areas of mining have probably led to a significant buildup of soil and mud in the river and increased turbidity, leaving very few suitable habitats for sustainable populations of *M. subundulatus*.

Studies on the Sassandra River to confirm the occurrence of a population of *M. subundulatus* are pending. Aerial photos suggest it hosts similar habitats to those of the Bandama River, apparently well preserved, apart from the few hundreds of kilometers affected by the huge Irebouo dam. If a population of the species exists in the Sassandra River, the extent of occurrence (EOO of the IUCN) of *M. subundulatus* would be of about 170 000 km². If absent from this basin, the EOO would only be about 46 000 km². Considering the possible extirpation of the downstream populations in the Bandama and Tano Rivers, the EOO of the remaining healthy population of the species in the upstream Bandama River will soon be restricted to about 3000 km².

Despite this alarming suggestion, we must acknowledge that African rivers are poorly studied and the possibility remains that unnoticed populations are still to be discovered. On the other hand, the overwhelming demographic growth of African countries provides reason for concern about the future of natural habitats, freshwaters foremost among them. The conservation status of *M. subundulatus* is probably a function of both our unawareness of this species' extant distribution and the accelerating degradation of its habitat.

Acknowledgements

Olivier Pauwels (IRSNB), Jonathan Pfliger and Patrick Pruvost (MNHN), Jon Fong and Dave Catania (CAS) gave us access to the specimens held in their respective museum collections. We thank the *Direction des Services vétérinaires de Côte d'Ivoire* for allowing us to export the biological material, *autorisation d'exportation* number 275. The sequencing of the tissue samples has been outsourced to Lise-Marie Pigneur, Université de Lièges. We are grateful to Carl D. Hopkins and John P. Sullivan for their constructive review of a previous version of the manuscript.

REFERENCES

Brito R. M., Briolay J., Galtier N., Bouvet Y. & Coelho M. M. 1997. — Phylogenetic relationships within the genus *Leuciscus* (Pisces, Cyprinidae) in Portuguese fresh waters, based on mitochondrial DNA cytochrome b sequences. *Molecular Phylogenetics and Evolution* 8: 435-442.

Crawford J. D. & Hopkins C. D. 1989. — Detection of a previously unrecognized mormyrid fish (*Mormyrus subundulatus*) by electric discharge characters. *Cybium* 13: 319-326.

Dankwa H. 2020. — *Mormyrus subundulatus*. *The IUCN Red List of Threatened Species* 2020: e.T182111A135009211. https://doi.org/10.2305/IUCN.UK.2020-3.RLTS.T182111A135009211.en

Decru E., Moelants T., De Gelas K., Vreven E., Verheyen E. & Snoeks J. 2016. — Taxonomic challenges in freshwater fishes: a mismatch between morphology and DNA barcoding in fish of the north-eastern part of the Congo basin. *Molecular Ecology Resourources* 16 (1): 342-352. https://doi/10.1111/1755-0998.12445

Entusa-Mensah M. & Laleye P. 2010. — *Mormyrus subundulatus*. The IUCN Red List of Threatened Species 2010: e.T182111A7802871. https://doi.org/10.2305/IUCN.UK.2010-3.RLTS.T182111A7802871.en

Folmer O., Black M., Wr H., Lutz R. & Vrijenhoek R. 1994. — DNA primers for amplification of mitochondrial Cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular marine biology and biotechnology* 3: 294-9.

Froese R. & Pauly D. 2018. — FishBase. World Wide Web electronic publication. www.fishbase.org, consulted in January 2021.

Hall T. A. 1999. — BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95-98.

Huelsenbeck J. P. & Ronquist F. 2001. — MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755. https://doi/10.1093/bioinformatics/17.8.754

Kramer B. 2013. — A morphological study on species of African *Mormyrus* (Teleostei: Mormyridae) and their electric organ discharges. *African Journal of Aquatic Science* 38 (1): 1-19.

Lalèyè P., Moelants T. & Olaosebikan B. D. 2010. — *Mormyrus tapirus*. The IUCN Red List of Threatened Species 2010: e.T181605A7688024. https://doi.org/10.2305/IUCN.UK.2010-3.RLTS.T181605A7688024.en

Lavoué S., Bigoprne R., Lecointre G. & Agnèse J-F. 2000. — Phylogenetic relationships of mormyrid electric fishes (Mormyridae; Teleostei) inferred from cytochrome b sequences. *Molecular Phylogenetics and Evolution* 14: 1-10.

LAVOUÉ S., MIYA M., ARNEGARD M. E., SULLIVAN J. P., HOPKINS C. D. & NISHIDA M. 2012. — Comparable Ages for the Independent Origins of Electrogenesis in African and South American Weakly Electric Fishes. *PLoS ONE* 7 (5): e36287. https://doi/10.1371/journal.pone.0036287

LÉVÊQUE C. & BIGORNE R. 1985. — Répartition et variabilité des caractères méristiques et métriques des espèces du genre *Mormyrus* (Pisces – Mormyridae) en Afrique de l'Ouest. *Cybium* 9: 325-340.

NWANI C. D., BECKER S., BRAID H. E., UDE E. F., OKOGWU O. I. & HANNER R. 2011. — DNA barcoding discriminates freshwater fishes from southeastern Nigeria and provides river system-level phylogeographic resolution within some species. *Mitochondrial DNA* 22(S1): 43-51. https://doi/10.3109/19401736.2010.536537

PAUGY D., LÉVÈQUE C. & TEUGELS G. G. 2003. — *Poissons d'eaux douces et saumâtres de l'Afrique de l'Ouest*. Tome 1. Collection Faune et Flore Tropicales, Institut de Recherche pour le Développement, Paris, France, Muséum national d'Histoire naturelle, Paris, France, Musée royal de l'Afrique centrale, Trevuren, Belgique, 457 p.

POSADA D. 2008. — jModelTest: Phylogenetic Model Averaging. *Molecular biology and evolution* 25: 1253-1256. https://doi/10.1093/molbev/msn083

RAMBAUT A. & DRUMMOND A. 2007. — Tracer v1.4 (Internet) http://tree.bio.ed.ac.uk/software/tracer/

ROBERTS T. R. 1989. — *Mormyrus subundulatus*, a new species of mormyrid fish with a tubular snout from West Africa. *Cybium* 13: 51-54.

SCHMIDT T. R. & GOLD J. R. 1993. — Complete sequence of the mitochondrial cytochrome b gene in the cherryfin shiner, *Lythrurus roseipinnis* (Teleostei: Cyprinidae). *Copeia* 3: 880-883.

SULLIVAN J. P., LAVOUÉ S. & HOPKINS C. D. 2000. — Molecular systematics of the African electric fishes (Mormyroidea: teleostei) and a model for the evolution of their electric organs. *Journal of Experimental Biology* 203: 665-683

STIASSNY M. L. J., TEUGELS G. G. & HOPKINS C. D. 2008. — *Poissons d'eau douces et saumâtres de basse Guinée, Ouest de l'Afrique centrale*. Volumes 1 and 2. IRD Éditions / MNHN – Collection Faune et Flore tropicales, 1408 p.

Submitted on 5 January 2021;
accepted on 26 March 2021;
published on 20 October 2021.