



Molecular phylogenetic analysis of spiny eels (Mastacembelidae: *Mastacembelus*) from the Rokel River basin and adjacent areas of Sierra Leone, West Africa

UNISA CONTEH KANU^{1,2}, E. ZHANG² & SHEN JIANZHONG¹¹Huazhong Agricultural University, Wuhan City, Hubei Province, P. R. China.²Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan City, Hubei Province, P. R. China.Corresponding author: SHEN JIANZHONG¹: [✉ jzsh@mail.hzau.edu.cn](mailto:jzsh@mail.hzau.edu.cn); Phone: +86-18627197708; [🌐 https://orcid.org/0000-0003-2068-6487](https://orcid.org/0000-0003-2068-6487)UNISA CONTEH KANU^{1,2}: [✉ kanuunisa2@gmail.com](mailto:kanuunisa2@gmail.com); [🌐 https://orcid.org/0000-0003-0282-6976](https://orcid.org/0000-0003-0282-6976)E ZHANG²: [✉ zhang@ihb.ac.cn](mailto:zhang@ihb.ac.cn); [🌐 https://orcid.org/0000-0003-2068-6487](https://orcid.org/0000-0003-2068-6487)

Abstract

Cytochrome *b* (*Cytb*) sequences from 23 specimens of *Mastacembelus* spp. from the Rokel River, Taia/Jong River basins, and Freetown Peninsula were used in this study. These sequences were used to perform a gene-based phylogenetic analysis along with closely allied congeneric species in the Upper Guinea Forest in Africa. The resulting phylogenies inferred from *Cytb* showed populations of *M. liberiensis* from the Rokel River basin clustered into an exclusive lineage being sister to the lineage formed by *M. greshoffi* from the Congo basin. The Freetown Peninsula population of *M. liberiensis* clustered with sequences previously identified as *M. taiensis* from the Taia/Jong River basin, sister to sequences of *M. taiensis* from the Rokel River basin. The paired lineages were sister to the lineage formed by sequences from samples of the Taia/Jong River basin. These findings lead to the following conclusions: (1) hidden species diversity of spiny eels exists in either the Rokel River or the Jong River basin; (2) a likely available name for this species from the Freetown Peninsula is *M. laticauda* distinct from *M. liberiensis*; (3) given the diversity within *M. liberiensis* observed in the rivers sampled in Sierra Leone, it seems likely that the remaining rivers of Liberia and Sierra Leone reported having *M. liberiensis* may also harbor undescribed diversity. It is difficult to elevate some available junior synonyms based solely on a phylogeny inferred from Cytochrome *b*. We suggest that more samples from the entire range of *M. liberiensis* are needed and an integrated approach is required.

Key words: Taxonomy, divergence, Biodiversity, Upper Guinean Forest

INTRODUCTION

The family Mastacembelidae, commonly known as spiny eels, is widespread in tropical Africa, the Middle East, South, Southeast Asia, China, and Korea (Nelson *et al.* 2016). The included species have an anguilliform body, continuous dorsal and anal fins, a moderate caudal fin attached, no pelvic fins, toothplates merged to the hypobranchial, anterior nostrils situated on distal ends of tube-like lateral extensions of the central rostral tentacle of the rostral appendage, and no interarcular cartilage (Kottelat 2006). Spiny eels occupy a vast diversity of freshwater habitats such as lakes, streams, and rivers where they subsist on zooplankton, larvae of aquatic insects, fish, and fish eggs (Nelson, *et al.* 2016). Although the generic classification of the Mastacembelidae still remains contentious (Vreven & Teugels 2005), a consensus on this issue is that three genera are presently recognized: *Macrogathus*, *Mastacembelus*, *Sinobdella* (Roberts 1986; Vreven & Teugels 2005; Kottelat 2006).

The genus *Mastacembelus* comprises species of spiny eel characterized by traits such as sharp rostral appendage with two tube-like anterior nostrils found on both sides of the central rostral tentacle, well-separated dorsal spines, several tiny isolated anal spines, and spots, bars or reticulated pattern in the body (Vreven & Teugels 1996; Vreven & Teugels 2005). Its distribution spans most of Africa's fresh and coastal waters through Syria to the Malay Archipelago and Asia/China (Brown *et al.* 2011). Sixteen and one species of *Mastacembelus* are currently recognized in Asia and the Middle East (Roberts 1986; Kottelat 2006), and 39 in Africa (Leveque 1999; Vreven & Teugels 2005; Vreven & Stiassny 2009; Brown *et al.* 2011; Steenberge *et al.* 2014). Among African species, five are present in the upper Guinean Province, as defined by Roberts (1975), namely *M. kakrimensis* Vreven & Teugels,

M. liberiensis Boulenger, and *M. nigromarginatus* Boulenger, *M. praensis* Travers, and *M. taiaensis* Travers. Only two species, *M. liberiensis* and *M. taiaensis*, have been reported in the freshwater systems of Sierra Leone (Lévêque 1999; Paugy *et al.* 1990; Payne 2018). The former is widespread in the river basins of the country, while the latter is restricted to the Taia or Jong River basin (Vreven & Teugels 2005).

The species identity and distribution of *M. liberiensis* remain to be resolved. There are two species, *M. laticauda* Ahl, and *M. reticulatus* Boulenger, currently considered junior synonyms of *M. liberiensis* (Vreven & Teugels 2005). Currently, *M. liberiensis* is known widely in Sierra Leone's coastal river basins and the West African countries of Senegal, Gambia, Guinea, Liberia, and Ivory Coast (Vreven & Teugels 2005). Unlike all other currently recognized congeneric species of the upper Guinean Province, this species shows a marked intraspecific variation in the counts of preopercular spines, dorsal-anal-fin soft rays, and predorsal, abdominal, caudal, and total vertebrae, which are used to distinguish among species of *Mastacembelus* (Vreven & Teugels, 2005). This suggests that the species, as currently recognized, has the potential to include multiple species.

The type locality of *M. liberiensis* is in Solymah drainage (Mano River basin) which runs along the border between Sierra Leone and Liberia. Current taxonomic revisions of freshwater fishes from most river basins in the upper Guinea Province, particularly Sierra Leone, were scarce and largely morphology-based (Leveque 1992; Payne *et al.* 2010; Payne 2018). The present data on morphological characters are insufficient for detailed species identification of these populations, and it seems likely that hidden species diversity remains to be described in the freshwater river systems of Sierra Leone (Paugy *et al.* 1990). This is the first molecular analysis conducted among species of spiny eels from the Rokel River and adjacent river basins in Sierra Leone.

Field surveys of fishes, conducted by the first author over 2018-2021 to the Rokel River, Pampana/Jong River and Freetown Peninsula, yielded many specimens of *Mastacembelus*. The mitochondrial *Cytb* gene sequences were amplified and then utilized for phylogenetic analysis published sequences of many congeneric species from Africa and Asia. Based on molecular phylogenetic analysis the present study aims to scrutinize the species identity of spiny eels from the Rokel River and its nearby river basins and provide some notes on the taxonomic biogeographic implications of our findings. This study will help narrow the existing gaps in the taxonomic understanding of spiny eels within the Upper Guinean Forests.

MATERIALS AND METHOD

Fish sampling

Samples used for this study were caught during field surveys conducted from December 2018 to March 2019 and July 2021 to the Rokel River, Taia/Jong River basins, and Freetown Peninsular basins in Sierra Leone (Fig. 1). 39 specimens of spiny eels were collected with local traps and seine nets of one finger-lap mesh size (5-10 mm). Specimens were frozen with benzocaine intermediately after capture. All caught specimens after removing the right pectoral-fin clips, all caught specimens were initially stored in 10% formalin preservative and then transferred to 70% ethanol for permanent preservation as well. These fin clips were preserved in 95% ethyl alcohol for molecular analysis. Species identification following Vreven & Teugels (2005). All voucher specimens are preserved in the Ichthyological collection at the Institute of Hydrobiology, Chinese Academy of Sciences (IHB), Wuhan City, Hubei Province, and P.R. China.

DNA and species delimitation analysis

A partial fragment of cytochrome b was amplified for 23 samples of spiny eels. Total genomic DNA was extracted from pelvic or pectoral fin clips and preserved in ethanol utilizing TIANamp Genomic DNA Kit (Tiangen Biotech, Beijing) with the recommended protocol. This gene was determined by primer: MNCN- Fish Pro R (5'AGTTTAATTTAGAATYTTTRGCTTTGG'3) (Zardoya & Rüber 2001). Amplification reaction volume involved 25 µl of 0.5 µl of each primer, 12.5 µl Master mix Taq (Beijing TsingKe Biotech Co., Ltd.), 1 µl template DNA and 10.5 µl double distilled water (dd H₂O). The thermocycling conditions were as follows: initial denaturation for 3 min at 94°C, denaturation 45 s at 94°C, annealing for 40 s at 53°C and extension for 1:30 min at 72°C. After 35 cycles, the final extension was done for 5 min at 72°C, and the PCR products were stored at 4°C. Sequencing was conducted by the Tianyihuiyuan Biotechnology Company using the same PCR primers for amplification.

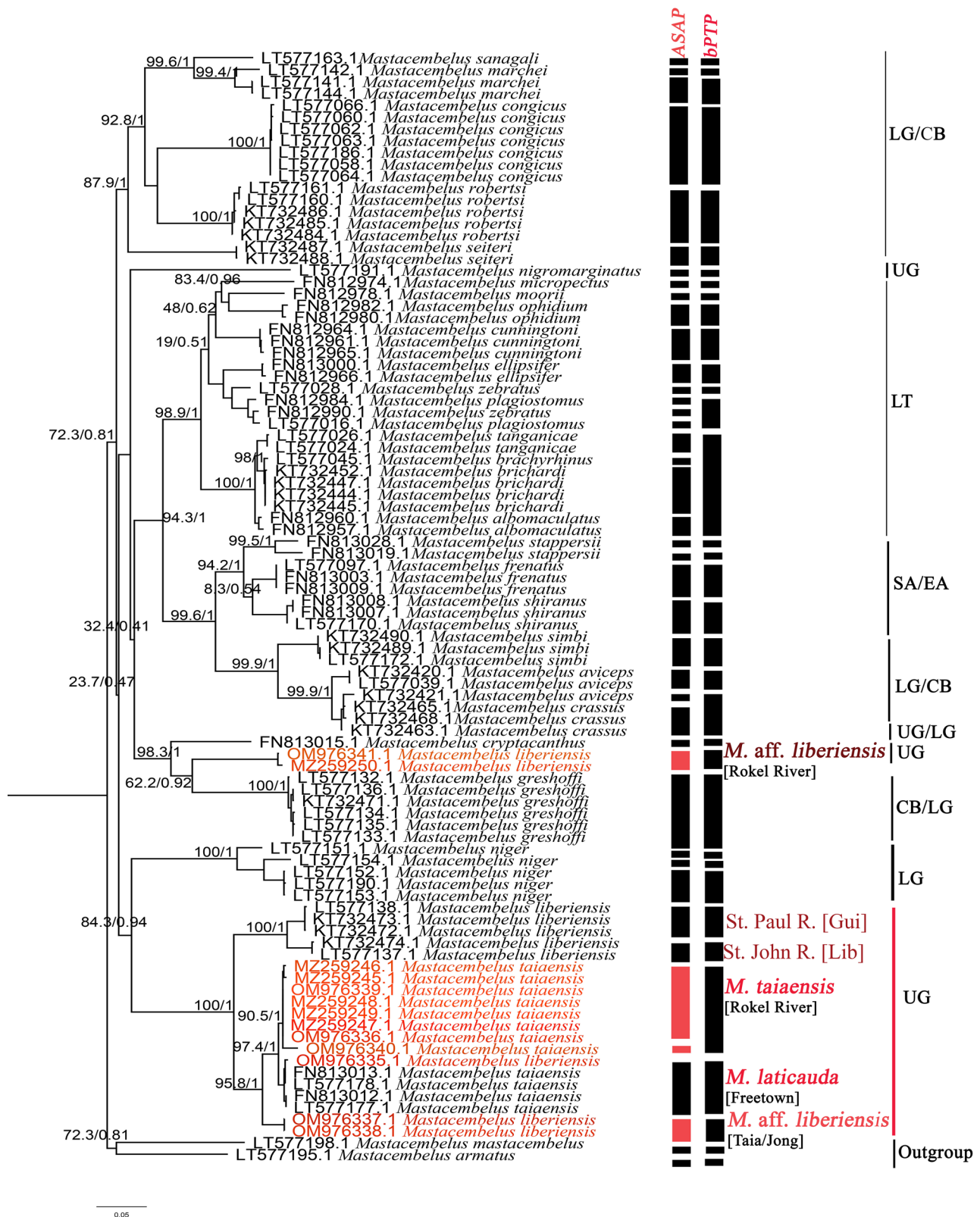


FIGURE 2. Phylogeny of species of *Mastacembelus* inferred from partial cytochrome b. Branch support for each node is shown from Bayesian Inference (BI)/maximum likelihood (ML), illustrating the *M. liberiensis* (1 & 2): LT (Lake Tanganyika); LCR/CB (Lower Congo Region/Congo Basin); UG (Upper Guinean Province); LG (Lower Guinean Province) taxa and Asia populations.

Thirteen *Cytb* gene sequences of spiny eels were amplified successfully. These sequences were used for phylogenetic analysis together with 94 sequences retrieved from GenBank for congeneric species of Asia (2) and Africa (79), including two closely allied species: *M. mastacembelus* Banks & Solander, and *M. armatus* Lacépède, which served as outgroup (Table 1). The sequences were aligned using Mega v7 (Kumar *et al.* 2016) and ends trimmed, for a total alignment length of 1090 bp. The genetic distance based on the Kimura 2-parameter model (Kimura 1980) were calculated with Mega v7 (Kumar *et al.* 2016). The substitution saturation for these genes was estimated to quantify if they could be used for phylogenetic analysis with DAMBE (Xia 2013). Haplotypes for each gene were detected by DnaSP v5 (Librado & Rozas 2009).

TABLE 1. Reference information of sequences produced in this study or downloaded from GenBank.

Species	Sampling location/Country	GenBank Acc#	Sources
<i>M. tanganyicae</i>	Lake Tanganyika	LT577024.1, 26	Day <i>et al.</i> 2017
<i>M. marcheii</i>	LCR/DRC	LT577141-42, 44.1	Day <i>et al.</i> 2017
<i>M. albomaculatus</i>	Lake Tanganyika	FN812953, 59	Brown <i>et al.</i> 2010
<i>M. brachyrhinus</i>	LCR/DRC	LT577040.1	Day <i>et al.</i> 2017
<i>M. zebratus</i>	Lake Tanganyika	FN812990.1	Brown <i>et al.</i> 2010
<i>M. aviceps</i>	LCR/DRC	KT732421.1	Alter <i>et al.</i> 2015
<i>M. ophidium</i>	Lake Tanganyika Lake Tanganyika	FN812982.1 LT577014.1	Brown <i>et al.</i> 2010 Day <i>et al.</i> 2017
<i>M. paucispinis</i>	LCR/DRC LCR	KT732481.1 LT577159.1	Alter <i>et al.</i> 2015 Day <i>et al.</i> 2017
<i>M. stappersii</i>	Lake Tanganyika	FN813019.1	Brown <i>et al.</i> 2010
<i>M. plagiostomus</i>	Lake Tanganyika	LT577016, 24.1	Day <i>et al.</i> 2017
<i>M. ellipsifer</i>	Lake Tanganyika Lake Tanganyika	FN812971.1 FN812968.1	Brown <i>et al.</i> 2010 Brown <i>et al.</i> 2010
<i>M. aff. micropectus</i>	Lake Tanganyika	FN812995.1	Brown <i>et al.</i> 2010
<i>M. simbi</i>	LCR/DRC	KT732489-93	Alter <i>et al.</i> 2015
<i>M. brichardi</i>	LCR/DRC LCR/DRC	KT732449, 454.1 KT732454.1, 451	Alter <i>et al.</i> 2015 Alter <i>et al.</i> 2015
<i>M. frenatus</i>	SA, EA SA, EA	LT577106.1 LT577104.1	Day <i>et al.</i> 2017 Day <i>et al.</i> 2017
<i>M. shiranus</i>	SA, EA	FN813007-08.1	Brown <i>et al.</i> 2010
<i>M. cunningtoni</i>	Lake Tanganyika	FN812964-65.1	Brown <i>et al.</i> 2010
<i>M. moorii</i>	Lake Tanganyika	FN812975, 78.1	Brown <i>et al.</i> 2010
<i>M. congicus</i>	Lake Tanganyika Lake Tanganyika	LT577058.1, 63 LT577063.1	Day <i>et al.</i> 2017 Day <i>et al.</i> 2017
<i>M. greshoffi</i>	LCR/DRC LCR/DRC LCR/DRC LCR/DRC LCR/DRC	LT577132.1 LT577136.1 KT732471.1 LT577133.1 LT577134, 35	Day <i>et al.</i> 2017 Day <i>et al.</i> 2017 Alter <i>et al.</i> 2016 Day <i>et al.</i> 2017 Day <i>et al.</i> 2017
<i>M. cryptacanthus</i>	St. Paul river, Liberia	FN813015.1	Brown <i>et al.</i> 2010
<i>M. niger</i>	Nigeria, LCR Nigeria, LCR	LT577152.1 LT577156.1	Day <i>et al.</i> 2017 Day <i>et al.</i> 2017

.....Continued on the next page

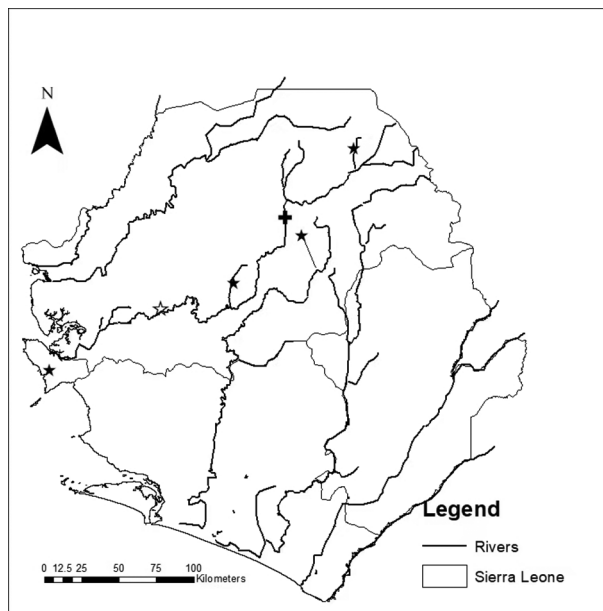
TABLE 1. (Continued)

Species	Sampling location/Country	GenBank Acc#	Sources
<i>M. taiaensis</i>	Jong/Taia River basin	LT577177.1	Day <i>et al.</i> 2017
	Sierra Leone	FN813012.1	Brown <i>et al.</i> 2010
	Jong/Taia River basin	LT577178.1	Day <i>et al.</i> 2017
	Jong/Taia River basin	FN813013.1	Brown <i>et al.</i> 2010
<i>M. liberiensis</i>	Liberia (St. John River)	KT732474.1	Alter <i>et al.</i> 2015
	Liberia (St. John River)	LT577137.1	Day <i>et al.</i> 2017
	Guinea (St. Paul drainage)	LT577138.1	Day <i>et al.</i> 2017
	Guinea (St. Paul drainage)	KT732473.1	Alter <i>et al.</i> 2015
		KT732472.1	
<i>M. crassus</i>	LCR/DRC	KT732467, 69	Alter <i>et al.</i> 2015
<i>M. traversi</i>	LCR/DRC	LT577179.1	Day <i>et al.</i> 2017
<i>M. robertsi</i>	LCR/DRC	LT577160, 61.1	Day <i>et al.</i> 2017
<i>M. sanagali</i>	LCR/DRC	LT577163.1	Day <i>et al.</i> 2017
<i>M. aff. liberiensis</i>	Rokel River, S/Leone,	OM976341	This study
	Rokel River, S/Leone	MZ595250	This study
<i>M. taiaensis</i>	Rokel River, S/Leone,	MZ595245	This study
	Rokel River, S/Leone,	MZ595246	This study
	Rokel River, S/Leone,	MZ595247	This study
	Rokel River, S/Leone,	MZ595248	This study
	Rokel River, S/Leone	MZ595249	This study
	Rokel River, S/Leone	OM976336.1	This study
	Rokel River, S/Leone	OM976339.1	This study
	Rokel River, S/Leone	OM976340.1	This study
<i>M. laticauda</i>	Freetown Peninsula, SL	OM976335.1	This study
<i>M. aff. liberiensis</i>	Taia/Jong, S/Leone	OM976337.1	This study
	Taia/Jong, S/Leone	OM976338.1	This study
<i>M. nigromarginatus</i>	West Africa	LT577191.1	Day <i>et al.</i> 2017
<i>M. seiteri</i>	Cameroon	KT732487-88	Alter <i>et al.</i> 2015
<i>M. mastacembelus</i>	Asia	LT577198.1	Day <i>et al.</i> , 2017
<i>M. armatus</i>		LT577197.1	Day <i>et al.</i> , 2017

MODELTEST v2.1.7 (Darriba *et al.* 2020) was utilized to ascertain the best model of molecular evolution for each dataset and selected under the Akaike Information Criterion. RAxML v8.2 (Stamatakis 2014) was used for Maximum Likelihood (ML) analysis, with model substitution rates from Modeltest applied. MRBAYES 3.1.1 (Lakner *et al.* 2008) was used for Bayesian Inference (BI) analysis with the best-fit model TIM2+I+G for each partition selected by Partitionfinder2 in PhyloSuite v1.1.16 (Zhang *et al.* 2020). Four independent MCMC chains were constructed with 10,000,000 replicates, sampling one tree per 1000 replications for each run. The distribution of log-likelihood scores was assessed to ascertain both stationary for each search and the requisite for extra runs to reach overlap in log-likelihoods. The initial trees with non-stationary log-likelihood (burn-in) were discarded. The outstanding trees resulting in confluence log-likelihood scores from both independent searches were concerted. A 50% majority-rule consensus tree was used in constructing the trees. The average standard deviation of split frequencies and the potential scale reduction factor was evaluated. The support values are Posterior probabilities and Bootstrap support. The BI/ML trees gave similar topology, statistical node support of both approaches are visualized on the BI tree.

The ASAP analysis using the online server (<https://bioinfo.mnhn.fr/abi/public/asap>) was divided to identify the group into hypothetical species based on the genetic distance, which can be observed whenever the divergence among populations that belonged to the same species is smaller than the divergence among populations from different species. The coalescent clustering-based method (bPTP) was performed using the online server (<http://species.h-it>

s.org/) and the Bayesian Inference trees from MrBayes 3.2 (Lakner *et al.* 2008). We ran bPTP analyses for 500,000 MCMC generations with a thinning of 500 and a burn-in of 0.1. Convergence of the MCMC chain was assessed as recommended by Zhang *et al.* (2020). Outgroups were pruned before conducting bPTP analyses to avoid bias that may arise if some of the outgroup taxa were too distantly related to the ingroup taxa. The phylogenetic trees were visualized and edited in FigTree v.1.4.3 (<http://tree.bio.ed.ac.uk/>), Adobe Illustrator CS6, and Adobe Photoshop CS6 (Adobe Systems Inc., San Jose, California, USA).



Results

The *Cytb* gene dataset included 94 sequences (1090 bp), 13 of which were amplified in this study and 81 available in GenBank of the same gene from West and West-central African congeners were subjected to phylogenetic analyses, with two Asian species, namely *M. armatus*, and *M. mastacembelus*, used as outgroup. The mean nucleotide composition of the species under study was A=26.6%, T=28.3%, C=31.9%, and G=13.2%; the base composition was A-T rich (54.9%). BI and ML approach yielded similar tree topology with most branches receiving strong support. Both ASAP and bPTP analyses delimited four putative species for spiny eels from the Freetown Peninsula, the Taia/Jong and the Rokel River basins (Fig. 2). The genetic distance values of four putative species of spiny eels from our target area with more closely related congeneric species ranged from 3.9% to 19.2% (Table 2).

Sequences from the sample here identified as *M. liberiensis* from the Freetown Peninsular united with GenBank-retrieved sequences (LT577177, FN813012, LT577178, and FN813013) from samples identified as *M. taitaensis* from the Taia River basin (type locality of *M. taitaensis*) into a highly-supported (99.4% BS and 1.0 PP) lineage. This lineage was sister to the lineage constituted by samples recognized as *M. taitaensis* from the Rokel River basin. These lineages were sister to the lineage formed by samples here identified as *M. liberiensis* from the Pampana/Jong River basin. The sister group of these three lineages was the one made up of two lineages formed by sequences from samples of *M. liberiensis* from St. John River in Liberia (KT732474 and LT577137) and St. Paul R. in Southeastern Guinea (KT732473, LT577138 and KT732472), respectively. Sequences from samples here identified *M. liberiensis* from the Rokel River basin formed a lineage sister to the lineage which included sequences from samples (LT577132-35 and KT732471) of *M. greshoffi* from the Congo River basin and river basins of the Lower Guinean ecoregion. The paired lineages were well supported with 98.2% BS and 1.0 PP to be sister to the lineage including the sequence from the sample of *M. cryptacanthus* (FN813015), an upper and lower Guinean ecoregion species (Fig. 2).

TABLE 2. Average pairwise K2P genetic distances (%) between mastacembelids based on Cytb gene. The following denotes, **Lib**: Liberia; **Gui**: Guinea; **T/J**: Taia/Jong River basin; **RR**: Rokel River basin; **FP**: Freetown Peninsula

No.	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	<i>M. liberiensis</i> Lib														
2	<i>M. liberiensis</i> Gui	0.039													
3	<i>M. tataensis</i> RR	0.115	0.111												
4	<i>M. laticauda</i> FP	0.100	0.097	0.027											
5	<i>M. aff. liberiensis</i> T/J 1	0.103	0.095	0.052	0.040										
6	<i>M. aff. liberiensis</i> RR 2	0.192	0.191	0.192	0.178	0.183									
7	<i>M. niger</i>	0.198	0.192	0.185	0.172	0.176	0.169								
8	<i>M. roberisi</i>	0.180	0.175	0.198	0.186	0.184	0.168	0.169							
9	<i>M. cryptacanthus</i>	0.182	0.189	0.185	0.173	0.171	0.131	0.162	0.144						
10	<i>M. greshoffi</i>	0.203	0.194	0.204	0.193	0.196	0.131	0.182	0.170	0.130					
11	<i>M. brachyrhinus</i>	0.182	0.176	0.197	0.180	0.180	0.162	0.186	0.143	0.148	0.165				
12	<i>M. brichardi</i>	0.183	0.177	0.195	0.179	0.176	0.160	0.182	0.146	0.145	0.165	0.007			
13	<i>M. tanganicae</i>	0.182	0.178	0.194	0.178	0.177	0.161	0.176	0.153	0.144	0.172	0.023	0.019		
14	<i>M. albomaculatus</i>	0.188	0.182	0.198	0.182	0.183	0.158	0.174	0.153	0.150	0.168	0.022	0.018	0.018	
15	<i>M. crassus</i>	0.190	0.179	0.188	0.169	0.167	0.195	0.203	0.205	0.184	0.205	0.156	0.160	0.169	0.168

Discussion

The present study reveals unrecognized diversity of spiny eels from the Rokel River basin and adjacent rivers in Sierra Leone. All analyzed specimens under the name of *M. liberiensis* were clustered within three lineages that were delimited as three putative species, and these putative species are distantly allied to *M. liberiensis* from the St. John River basin in Liberia and its adjacent river basin population from the St. Paul River basin southeastern Guinea (Fig. 2). Samples here identified as *M. liberiensis* from the Rokel River basin possibly represent an undescribed species, as it is phylogenetically more closely related to *M. greshoffi* from the Lower Congo River basin (type locality) and to *M. cryptacanthus*, a widespread species from West Africa, than to congeneric species within the two adjacent/neighbouring river basins. This species is deeply diverged from these two populations by a genetic divergence of 13.1% and 13.2%, respectively. Another possible undescribed species is represented by specimens, under the name of *M. liberiensis*, collected from the Pampana/Jong River basins. These taxa are sister to *M. taiaensis* from the Rokel River basin and *M. laticauda*, which may be the appropriate name for these taxa (see elaboration below), from the Freetown Peninsula and the Jong River basin as well. It had a 3.9% and 5.2% genetic distance, respectively.

The type locality of *M. laticauda* is the Freetown Peninsula of Sierra Leone (Ahl 1937). The species has been synonymized with *M. liberiensis* based on Vreven & Teugels (2005). In our analysis (Fig. 2), the samples from the Freetown Peninsular clustered with the published sequences (FN813012, FN813013, LT577177 and LT577178). These sequences were previously identified as *M. taiaensis* from the Jong River basin (Day *et al.* 2017). In our analysis, these taxa are sister to *M. liberiensis* in the Rokel River basin. Both species delimitation analyses showed that the two lineages represent two putative species (Fig. 2) and their interspecific genetic divergence was 2.7%. The Freetown peninsular specimens and voucher specimens of GenBank-retrieved sequences from the Jong River basin likely represent a single species, given an intraspecific genetic divergence of 0.2%.

Mastacembelus taiaensis, initially described from the Jong River basin of Sierra Leone, is characterized by a filamentary tail of the caudal fin (Travers 1992). This character is shared with the Rokel River basin specimens but not with the specimens collected by us from the Freetown Peninsula. This finding leads us to conclude that voucher specimens of sequences (FN813012, FN813013, LT577177 and LT577178) under the name of *M. taiaensis* from the Jong River basin were probably misidentified. These voucher specimens represent the same species as the Freetown Peninsula specimens. We suggest that *M. laticauda* is likely the available scientific name for this species, additional samples from its known distribution range and the use of both molecular and morphological methods needs known distribution range and molecular and morphological methods to be completed before it could be elevated. In addition to *M. taiaensis*, there are another two species occurring in the Jong River basin: *M. laticauda* and an unnamed species.

More specimens of *M. liberiensis* from their entire range are needed to get a full picture of the distribution of this species. Still based on the study, many *Mastacembelus spp.* are restricted to individual basins. This suggests that populations of the Rokel and Taia/Jong rivers currently considered *M. liberiensis* are likely distinct from *M. liberiensis* from the Mano River. Two species likely occur in the Rokel River basin (*e.g.*, an unnamed species and *M. taiaensis*) and three species in the Taia/Jong River basin (*e.g.*, another unnamed species, *M. laticauda*, and *M. taiaensis*). The discovery of at least two species within the *M. liberiensis* complex in the Rokel River and the Jong/Taia River basin suggests that some of the main coastal river basins of the upper Guinean Province have the potential to harbor multiple members of a species complex each river. Recent studies in other groups have also shown that multiple species previously thought taxon can co-occur in these Upper Guinean Rivers (Schmidt & Pezold 2011; Schmidt *et al.* 2016; 2017; Kanu *et al.* 2022). However, in the current taxonomy of African *Mastacembelus* species (Fricke *et al.* 2021), this genus is represented in each of these rivers by a single species—*M. liberiensis*. The present study accentuates a need to recheck the current species identification of spiny eels from coastal rivers of West Africa, particularly for *M. liberiensis*.

The present study unearths some critical perceptions about the taxonomy and distribution of *M. liberiensis* in the upper Guinean Province. In the Cytb gene-based trees (Fig. 2), St. John River basin (Liberia) and St. Paul River basin (southeastern Guinea) samples of *M. liberiensis* clustered together, but formed two exclusive lineages, which were delimited as two putative species. A 3.9% genetic distance was detected between both populations, greater than the 2% threshold value (Table 2). This genetic variation corresponds to the morphological variation observed in previous studies (Vreven & Teugels 2005). Another junior synonym of *M. liberiensis* is *M. reticulatus* Boulenger. This species type locality was noted as Sierra Leone. Additional surveys in the rivers of Sierra Leone and more

detailed morphological analyses of these populations and type material are needed before determining which of the taxa revealed in the study, if any, represent *M. reticulatus*.

Identifying species is essential for species-based conservation, and accurate species recognition is needed for effective conservation management (Lalèyè *et al.* 2021). The ongoing development in the regions means that some of these undescribed species may become threatened or even extinct before they are formally recognized. This is especially true for the Rokel River basin. Using integrative methods can be an asset for traditional taxonomy in increasing the pace of species detection and description. This study discovered several distinct populations of spiny eel in several rivers in Sierra Leone providing the basis for future targeted morphological analyses.

Acknowledgment

The authors are indebted to Kanu A. R. and Brima S. U. (deceased) for their kind support in sampling throughout our stay in Sierra Leone. We also thankfully appreciate Mrs. Ellie, E. and Mrs. Jalloh, K. (the Ministry of Fishery and Marine Resources) for their help to facilitate sampling and export permits. Our thanks go to An, C.T., Chen, X., Melaku, Y.A., Guo, D.M.; Cao, L., Deng, S.Q., Shao, W.H., and Nguyen, D.T. (IHB) for their help with molecular technique experiment, and Liu, Y. (IHB) for making figures using Adobe. This research is supported by a grant from the Sino-Africa Joint Research Centre (Nos. SAJC201320 and SAJC201612).

References

- Ahl, E. (1937) Ueber eine kleine Fischeammlung aus Sierra Leone. *Mitteilungen aus dem Zoologischen Museum in Berlin*, 22, 239–243.
<https://doi.org/10.1002/mmzn.19370220208>
- Alter, S.E., Brown, B. & Stiassny, M.L. (2015) Molecular phylogenetics reveals convergent evolution in lower Congo River spiny eels. *BMC Evolutionary Biology*, 15 (1), 1–12.
<https://doi.org/10.1186/s12862-015-0507-x>
- Beshera, K.A., Harris, P.M. & Mayden, R.L. (2016) Novel evolutionary lineages in *Labeobarbus* (Cypriniformes; Cyprinidae) based on phylogenetic analyses of mtDNA sequences. *Zootaxa*, 4093 (3), 363–381
<https://doi.org/10.11646/zootaxa.4093.3.4>
- Brown, K.J., Rüber, L., Bills, R. & Day, J.J. (2010) Mastacembelid eels support lake tanganyika as an evolutionary hotspot of diversification. *BMC Evolutionary Biology*, 10, 188.
<https://doi.org/10.1186/1471-2148-10-188>
- Brown, K.J., Britz, R., Bills, R., Rüber, L. & Day, J.J. (2011) Pectoral fin loss in the Mastacembelidae: A new species from Lake Tanganyika. *Journal of Zoology*, 284 (4), 286–293.
<https://doi.org/10.1111/j.1469-7998.2011.00804.x>
- Day, J.J., Fages, A., Brown, K.J., Vreven, E.J., Stiassny, M.L., Bills, R., Friel, J.P. & Rüber, L. (2017) Multiple independent colonizations into the Congo Basin during the continental radiation of African Mastacembelus spiny eels. *Journal of Biogeography*, 44 (10), 2308–2318.
<https://doi.org/10.1111/jbi.13037>
- Darriba, D., Posada, D., Kozlov, A.M., Stamatakis, A., Morel, B. & Flouri, T. (2020) ModelTest-NG: A New and Scalable Tool for the Selection of DNA and Protein Evolutionary Models. *Molecular Biology and Evolution*, 37 (1), 291–294. <https://doi.org/10.1093/molbev/msz189>
- Fricke, R., Eschmeyer, W., Van Der laan, R. & Utrecht, H. (2021) *Catalog of Fishes : Genera , Species , References. September.*
- Ivanova, N.V., Zemlak T.S., Hanner, R.H. & Hebert, P.D.N. (2007) Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*. *Molecular Ecology Notes*, 7 (4), 544–548.
<https://doi.org/10.1098/rstb.2005.1716>
- Kanu, U.C., Liang, C., Nwafor, C.C., Shen, J. & Zhang, E. (2022) Phylogenetic Analyses of Cyprinid Species from the Rokel River Basin of Sierra Leone, West Africa: Taxonomic, Biogeographic, and Conservation Implications. *Diversity*, 14 (4), 299.
<https://doi.org/10.3390/d14040299>
- Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16 (2), 111–120.
<https://doi.org/10.1007/BF01731581>
- Kottelat, M. (2006) The Fishes of the Inland Waters of Southeast Asia: A Catalogue and Core Bibliography of the Fishes Known to Occur in Freshwaters, Mangroves and Estuaries Maurice. *Raffles Bulletin of Zoology*, 54 (2).

- Kottelat, M. (2010) *Ichthyological Exploration of Freshwaters*. *Ichthyological Exploration of Freshwaters*, 24 (2).
- Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, 33 (7), 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Lakner, C., Van Der Mark, P., Huelsenbeck, J.P., Larget, B. & Ronquist, F. (2008) Efficiency of markov chain monte carlo tree proposals in bayesian phylogenetics. *Systematic Biology*, 57 (1), 86–103. <https://doi.org/10.1080/10635150801886156>
- Lalèyè, P., Olaosebikan, D.B., Dankwa, H., Schmidt, R.C., Diallo, I. & Darwall, W.R. (2021) The status and distribution of freshwater fishes in western Africa. Identification and Validation of Western African Freshwater Key Biodiversity Areas, 22.
- Leveque, C. (1999) *Fishes of African continental waters: diversity, ecology, human activities*. IRD Editions, Paris (France), 521 pp. [<https://www.researchgate.net/publication/282169802%0ARichesse>]
- Librado, P. & Rozas, J. (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25 (11), 1451–1452. <https://doi.org/10.1093/bioinformatics/btp187>
- Nelson, J.S., Grande, T.C. & Wilson, M.V.H. (2016) *Fishes of the World 5th edition*. John Wiley & Sons, Hoboken, New Jersey, 707 pp. <https://doi.org/10.1002/9781119174844>
- Paugy, D., Leveque, C., Teugels, G.G., Bigorne, R. & Romand, R. (1990) Freshwater Fishes of Sierra Leone and Liberia Annotated Checklist and Distribution. *Revue d'Hydrobiologie Tropicale*, 23 (1), 329–350.
- Paugy, D., Lévêque, C. & Teugels, G.G. (2003) *The Fresh and Brackish Water Fishes of West Africa, vols 1 & 2*. Muséum national d'Histoire naturelle, Paris, 1272 p. [Faune et Flore tropicales; 40]
- Payne, I.A. (2018) The ecology, distribution and diversity of fish species in Sierra Leone rivers and response to human impacts. *Environmental Biology of Fishes*, 101 (5), 843–864. <https://doi.org/10.1007/s10641-018-0742-2>
- Payne, A., Ndomahina, T.E. & Wakeford, R.C. (2010) Fish distribution and zonation along a tropical African river, the Rokel/Seli River, Sierra Leone, West Africa. *Smithiana Bulletin* 12, 25–36.
- Roberts, R. (1986) Systematic Thailand, Review of the Mastacembelidae or Spiny Eels of Burma and with Description of Two New Species of Macrogynathus. *Japanese Journal of Ichthyology*, 33 (2), 95–109. <https://doi.org/10.1007/BF02905838>
- Roberts, T.R. (1975) Geographical distribution of African freshwater fishes. *Zoological Journal of the Linnean Society*, 57 (4), 249–319. <https://doi.org/10.1111/j.1096-3642.1975.tb01893.x>
- Stamatakis, A. (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30 (9), 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Schmidt, R.C. & Pezold, F. (2011) Morphometric and molecular variation in mountain catfishes (Amphiliidae: Amphilius) in Guinea, West Africa. *Journal of Natural History*, 45 (9-10), 521–552. <https://doi.org/10.1080/00222933.2010.534560>
- Schmidt, R.C., Bart Jr, H.L. & Pezold, F. (2016) High levels of endemism in suckermouth catfishes (Mochokidae: Chiloglanis) from the Upper Guinean forests of West Africa. *Molecular Phylogenetics and evolution*, 100, 199–205. <https://doi.org/10.1016/j.ympev.2016.04.018>
- Schmidt, R.C., Bart Jr, H.L., Pezold, F. & Friel, J.P. (2017b) A biodiversity hotspot heats up: Nine new species of suckermouthcatfishes (Mochokidae: Chiloglanis) from Upper Guinean forest streams in West Africa. *Copeia*, 105 (2), 301–338. <https://doi.org/10.1643/CI-16-474>
- Travers, R.A. (1992) *Caecomastacembelus tafiaensis* and *Aethiomastacembelus praensis*, two new species of mastacembelid spiny-eels from West Africa. 2 (4), 331–340.
- Van Steenberge, M., Vreven, E.J. & Snoeks, J. (2014) The fishes of the Upper Luapula area (Congo basin): a fauna of mixed origin. *Ichthyological Exploration of Freshwaters*, 24 (4), 289–384.
- Vreven, E.J. & Teugels, G.G. (2005) Redescription of *Mastacembelus liberiensis* Boulenger, 1898 and description of a new West African spiny-eel (Synbranchiformes: Mastacembelidae) from the Konkoure River basin, Guinea. *Journal of Fish Biology*, 67 (2), 332–369. <https://doi.org/10.1111/j.0022-1112.2005.00729.x>
- Vreven, E.J. & Teugels, G.G. (1996) Description of a new Mastacembelid species (Synbranchiformes; Mastacembelidae) from the Zaire River Basin in Africa *Copeia*, 130–139. <https://doi.org/10.2307/1446948>
- Vreven, E.J. & Stiassny, M.L.J. (2009) *Mastacembelus simbi*, a new dwarf spiny eel (Synbranchiformes: Mastacembelidae) from the lower Congo River. *Ichthyological Exploration of Freshwaters*, 20 (3), 213–222.
- Xia, X. (2013) DAMBE5: A comprehensive software package for data analysis in molecular biology and evolution. *Molecular Biology and Evolution*, 30 (7), 1720–1728. <https://doi.org/10.1093/molbev/mst064>
- Zhang, D., Gao, F., Jakovlić, I., Zou, H., Zhang, J., Li, W.X. & Wang, G.T. (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources*, 20 (1), 348–355. <https://doi.org/10.1111/1755-0998.13096>