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Cast netting new species: Integrative taxonomy of *Distichodus notospilus* (Characiformes: Distichodontidae) discovers new species and overlooked areas of endemism in Central Africa

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Abstract

Distichodus notospilus was described from the Ogooué River and is considered to occur throughout the Lower Guinea ichthyofaunal province and the western tributaries of the middle and lower Congo River. Recent expeditions in Equatorial Guinea collected *D. notospilus* specimens in the Mbini River drainage and the Mbia River; a small coastal river that is located between the Ntem and Mbini river drainages. Detailed morphological analyses and multilocus molecular analyses confirm that these two populations are distinct from one another. Topotypic populations of *D. notospilus* were included in the analyses and demonstrated that populations in the Mbini and Mbia rivers are distinct and these two new species are described herein. *Distichodus microps* **sp. nov.** is endemic to the Mbia River drainage and is distinguished from *D. notospilus* in having more scales along the lateral line (41, rarely 40 versus 37–39, rarely 40), a nearly inferior mouth versus subterminal in *D. notospilus*, a curved posterolateral margin of the opercle versus straight in *D. notospilus*, a smaller eye (56.7–80.4 versus 70.1–104.3 % of snout length), and a less prominent elongated spot at the base of the caudal fin. *Distichodus mbiniensis* **sp. nov.** is endemic to the upper Mbini River drainage and distinguished from *D. notospilus* in having more scales along the lateral line (41–42, rarely 40 versus 37–39, rarely 40), a much less prominent elongated dark spot at the base of the caudal fin, and a shorter dorsal fin (21.4–27.2 versus 22.7–34.2% standard length). *Distichodus microps* is distinguished from *D. mbiniensis* in having a shallower body (usually six scales from lateral line to the pelvic fin versus seven), fewer anal-fin rays (usually 12 total rays versus 13 or 14), a more inferior mouth, a deeper and longer caudal peduncle, a smaller eye, and differences in several features associated with the head. In addition to the two new species described this study also revealed potential undescribed diversity in the *D. notospilus* species complex in the Ntem River and Dja River (Congo R. basin) in Cameroon. The biogeography of these fishes in the rivers of Lower Guinea suggests that the Mbini River and smaller coastal rivers are overlooked areas of endemism. Studies of other reported widespread species will likely reveal additional diversity and further elucidate the processes promoting and maintaining freshwater diversity in Central Africa.

Key words: Freshwater biodiversity, barcoding, morphometrics, biogeography, IUCN Red List, allometric correction

Introduction

The Lower Guinea ichthyofaunal province in Central Africa is comprised of the coastal rivers emptying into the Atlantic Ocean between the mouths of the Niger and Congo Rivers. This diverse province contains over 575 species of fishes with many being endemic to the area (Stiassny *et al.* 2007), though it is suggested that forested rivers and streams in the region have many more species than currently recognized (Brummett & Teugels, 2002). The fishes and other freshwater organisms in these rivers, and more broadly globally, face a number of threats to their long-term survival (Dudgeon *et al.* 2006; Darwall *et al.* 2018; Fouchy *et al.* 2019). Foremost among these is habitat destruction and a lack of reliable baseline information on the biodiversity in these systems. Though some rivers and areas within the Lower Guinea province have been recently surveyed (Cutler *et al.* 2019), much more work is needed to better understand the diversity and the biogeographical processes that facilitated the endemism in the region.

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Members of the genus *Distichodus* occur throughout sub-Saharan Africa but have high levels of diversity in the Lower Guinea and Congo River ichthyofaunal provinces. There are currently 26 valid species of *Distichodus* with several species recently described or elevated (Mamonekene & Vreven 2008; Moelants *et al.* 2014; Moelants *et al.* 2018; Abwe *et al.* 2019; Fricke *et al.* 2020). *Distichodus notospilus* Günther, 1867 was described from the Ogooué River in Gabon based on specimens collected by R.B.N. Walker (Walker 1865). This species is reported to be widespread throughout the Lower Guinea province and also occurs in the western tributaries of the lower and middle Congo River (Vari 2007).

A recent expedition to Equatorial Guinea collected putative specimens of *Distichodus notospilus* in two different river systems. Observations in the field suggested that these two populations were morphologically distinct from one another. This study set out to determine if these populations were distinct through an integrative approach. In addition to examining the two populations collected in Equatorial Guinea, this study includes topotypic material from the Ogooué River, comparative material from the Dja River, and published molecular data. Our study determined that these two populations collected in Equatorial Guinea were indeed morphologically and genetically distinct from one another and also distinct from the *D. notospilus* populations in the Ogooué River. These two new species are described herein. We also discuss the biogeography for the group in Lower Guinea, the additional diversity discovered, and future work needed.

Materials and methods

Fishes were collected across Equatorial Guinea over several weeks in June and July of 2017 (Fig. 1). *Distichodus* specimens were collected at several localities with a cast net, seine, or electrofisher. Representative specimens were photographed and tissue samples and fin clips were collected and preserved in 95% ethanol. Voucher specimens and other material were then fixed in 10% formalin and subsequently stored in 75% ethanol. All specimens were collected under the supervision and with permission of the República de Guinea Ecuatorial Instituto Nacional de Desarrollo Forestal Y Gestión del Sistema Nacional de Áreas Protegidas (INDEFOR).

Two mitochondrial markers (CO1 and CYTB) and one nuclear marker (MYH6) were examined for specimens collected in Equatorial Guinea and from borrowed comparative material following published methods (Arroyave & Stiassny, 2011; Arroyave *et al.* 2020).Published sequences from *D. notospilus*, *D. maculatus* Boulenger, *D. kolleri* Holly, and *D. affinis* Günther, were also included in the analyses (Calcagnotto *et al.* 2005; Arroyave & Stiassny, 2011; Sonet *et al.* 2019; Arroyave *et al.* 2020). PartitionFinder2 (Lanfear *et al.* 2017) was used to determine the best model of evolution for each codon position in the datasets. Phylogenetic analyses were conducted with MrBayes version 3.2 (Ronquist *et al*. 2012) on the CIPRES Science Portal (Miller *et al.* 2010). Posterior probabilities were assessed with five million generations, sampling trees every 100 generations. The first 25% of trees were discarded as burn-in. The CO1 sequences and metadata associated with the specimens collected in Equatorial Guinea are available in the Barcode of Life Database (BOLDsystems.org). All specimens included in the molecular analyses and associated GenBank accession numbers are provided in table 1. Institutional abbreviations follow Sabaj (2016).

Morphological measurements and counts were taken with a digital caliper and modified from Mamonekene & Vreven (2008). We added several measurements including base length and length/height of various fins (Table 3). Dorsal-fin terminus to adipose fin is measured from the terminus of the base of the dorsal fin to the origin of the adipose fin, and head length was measured from the snout to the posterior margin of the subopercle. This resulted in morphological analyses including 24 measurements, six counts, and three relative measurements. A principal components analysis of log-transformed measurements using the covariance matrix, descriptive statistics, and Mann-Whitney U tests on relative measurements (*i.e.,* measurements as a percentage of head or standard length) were completed in MYSTAT (SYSTAT Software Inc.).

Shape variation within principal components and relative measurements correlated with size were assessed through reduced-major axis (RMA) regression lines in the SMATR package (Warton *et al.* 2006). This analysis allows one to test if the allometric trajectories (*i.e.,* slope) for each variable (*e.g.,* PC1 and individual relative measurements) are the same for each population/species and further tests for significant differences among the elevations (*i.e.,* y-intercept) of the different variables from different populations/species. This method was effective in elucidating morphological differences, usually obscured by size differences or discarded, among cryptic species in *Leporinus* spp. (Sidlauskas *et al.* 2011), *Enteromius* spp. (Schmidt *et al.* 2019), and *Chiloglanis* spp. (Schmidt &

TABLE 1. Specimens, metadata, and associated GenBank accession numbers included in the molecular analyses. Complete locality data can be found in additional materials examined. Bolded accession numbers are sequences produced during this study.

Species	Locality	Drainage	Country	Voucher #	Catalog/Field#	CO1 Acc#	CYTB Acc#	MYH6 Acc#
Distichodus mbiniensis **n. sp.**	Rio Ntoro at Aconibe—Acurenam Rd.	Mbini River (Rio Wele)	Equatorial Guinea	EqGui2017_127	USNM 451317	**MN968316**	**MN918621**	**MN918648**
Distichodus mbiniensis **n. sp.**	Rio Ntoro at Aconibe—Acurenam Rd.	Mbini River (Rio Wele)	Equatorial Guinea	EqGui2017_128	MRAC 2020,005, P,0001	**MN968317**	**MN918622**	**MN918642**
Distichodus mbiniensis **n. sp.**	Rio Ntoro at Aconibe—Acurenam Rd.	Mbini River (Rio Wele)	Equatorial Guinea	EqGui2017_129	USNM 451318	**MN968315**	**MN918623**	**MN918640**
Distichodus mbiniensis **n. sp.**	Rio Ntoro at Aconibe—Acurenam Rd.	Mbini River (Rio Wele)	Equatorial Guinea	EqGui2017_130	USNM 451319		**MN918624**	
Distichodus mbiniensis **n. sp.**	Rio Ntoro at Aconibe—Acurenam Rd.	Mbini River (Rio Wele)	Equatorial Guinea	EqGui2017_135	MRAC 2020,005, P,0002		**MN918625**	
Distichodus mbiniensis **n. sp.**	Rio Abia at Evinayong—Aconibe Rd	Mbini River (Rio Wele)	Equatorial Guinea	EqGui2017_147	USNM 451321	**MN968313**	**MN918626**	**MN918641**
Distichodus mbiniensis **n. sp.**	Rio Abia at Evinayong—Aconibe Rd	Mbini River (Rio Wele)	Equatorial Guinea	EqGui2017_149	USNM 451322	**MN968318**	**MN918627**	
Distichodus mbiniensis **n. sp.**	Rio Nuik near Alam	Mbini River (Rio Wele)	Equatorial Guinea	EqGui2017_191	USNM 451324	**MN968312**	**MN918628**	**MN918643**
Distichodus microps **n. sp.**	Rio Mbia at Menzong	Mbia River	Equatorial Guinea	EqGui2017_274	USNM 451457	**MN968314**	**MN918629**	**MN918637**
Distichodus microps **n. sp.**	Rio Mbia at Menzong	Mbia River	Equatorial Guinea	EqGui2017_282	MRAC 2020,005, P,0004	**MN968311**	**MN918630**	**MN918644**
Distichodus aff. *notospilus*	Dja River at Somalomo ferry crossing	Dja River	Cameroon	JPF 2049	CUMV 97190	**MN935441**	**MN918631**	**MN918638**
Distichodus notospilus	Left of Ogooué River at Doumé village	Ogooué	Gabon	BLS14-021	OS 19453		**MN918632**	**MN918645**
Distichodus notospilus	LEBAMBA B	Ogooué-Ngounié-Louetsi	Gabon	GAB17-1446	OS 20757		**MN918633**	
Distichodus notospilus	SINDARA C	Ogooué-Ngounié	Gabon	GAB17-1460	OS 21266		**MN918635**	

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TABLE 1. (Continued)

Species	Locality	Drainage	Country	Voucher #	Catalog/Field#	CO1 Acc#	CYTB Acc#	MYH6 Acc#
Distichodus notospilus	NDOUBI Y	Ogooué-Ngounié-Louetsi	Gabon	GAB17-259	OS 21554	**MN935443**	**MN918636**	**MN918647**
Distichodus notospilus	FOUGAMOU L	Ogooué-Ngounié	Gabon	GAB17-1700	OS 20917	**MN935442**	**MN918634**	**MN918639**
Previously published data								
Distichodus affinis	Lulua River, Dijiba		DRC		AMNH 252633	KF541762	KF541891	KF542213
Distichodus affinis	Liau Stream		DRC		AMNH 247062	KF541761	KF541890	KF542236
Distichodus kolleri	Djerem River near Mbakaou Village		Cameroon		CU 93515	KF541802	KF541912	
Distichodus kolleri	Monatele, sanaga river		Cameroon		AMNH 236538	KF541801	KF541911	KF542248
Distichodus kolleri	Ebebda		Cameroon		AMNH 249814			JF801092
Distichodus maculatus	Basse-Kotto | Oubangui River shoreline at uncompleted bridge at Mobaye		Central African Republic		CU 91523	KF541805	KF541896	KF542276
Distichodus maculatus	Kigoma | Malagarasi River at Lower Igamba Falls (Kasagwe)		Tanzania		CU 95265	KF541769	KF541892	KF542277
Distichodus aff. *notospilus*	Region of Campo, Bitande River near village Afan-Esokie (Georeferenced)	Ntem River (lower)	Cameroon		AMNH 249523	KF541772	KF541898	KF542298
Distichodus aff. *notospilus*	Stream near Metondo Village, Kribia-Ebolowa Road (Georeferenced)	Ntem River (middle)	Cameroon		AMNH 249537	KF541773	KF541897	KF542299
Distichodus notospilus	Ntem river, at auberge d'ayengbe near village of doan (Georeferenced)	Ntem River (upper)	Gabon		AMNH 231537		AY791395	
Distichodus notospilus	riv. Nyanga à Nyanga, juste au pont	Nyanga	Republic of the Congo	A7-31-589		MK074209		

......*continued on the next page*

TABLE 1. (Continued)

Species	Locality	Drainage	Country	Voucher #	Catalog/Field#	CO1 Acc#	CYTB Acc#	MYH6 Acc#
Distichodus notospilus	riv. Nyanga à Nyanga, juste au pont	Nyanga	Republic of the Congo	A7-31-590		MK074210		
Distichodus notospilus	riv. Nyanga à Nyanga, juste en amont du pont	Nyanga	Republic of the Congo	A7-31-609		MK074214		
Distichodus notospilus	riv. Nyanga à Nyanga, juste en amont du pont	Nyanga	Republic of the Congo	A7-31-610		MK074204		
Distichodus notospilus	riv. Ngongo à Ngongo, juste en aval du pont	Ngongo_Ogowe	Republic of the Congo	A7-31-644		MK074208		
Distichodus notospilus	riv. Ngongo à Ngongo, juste en aval du pont	Ngongo_Ogowe	Republic of the Congo	A7-31-645		MK074207		
Distichodus notospilus	riv. Ngongo à Ngongo, juste en aval du pont	Ngongo_Ogowe	Republic of the Congo	A7-31-657		MK074206		
Distichodus notospilus	riv. Ngongo à Ngongo, juste en aval du pont	Ngongo_Ogowe	Republic of the Congo	A7-31-658		MK074211		
Distichodus notospilus	riv. Niari (Kouilou) à Pont Niari, juste en aval du pont	Kouilou-Niari	Republic of the Congo	A7-31-696		MK074213		
Distichodus notospilus	riv. Niari (Kouilou) à Pont Niari, juste en aval du pont	Kouilou-Niari	Republic of the Congo	A7-31-697		MK074212		
Distichodus notospilus	riv. Niari (Kouilou) à Pont Niari, juste en aval du pont	Kouilou-Niari	Republic of the Congo	A7-31-731		MK074203		
Distichodus notospilus	riv. Niari (Kouilou) à Pont Niari, juste en aval du pont	Kouilou-Niari	Republic of the Congo	A7-31-732		MK074205		

Barrientos 2019).

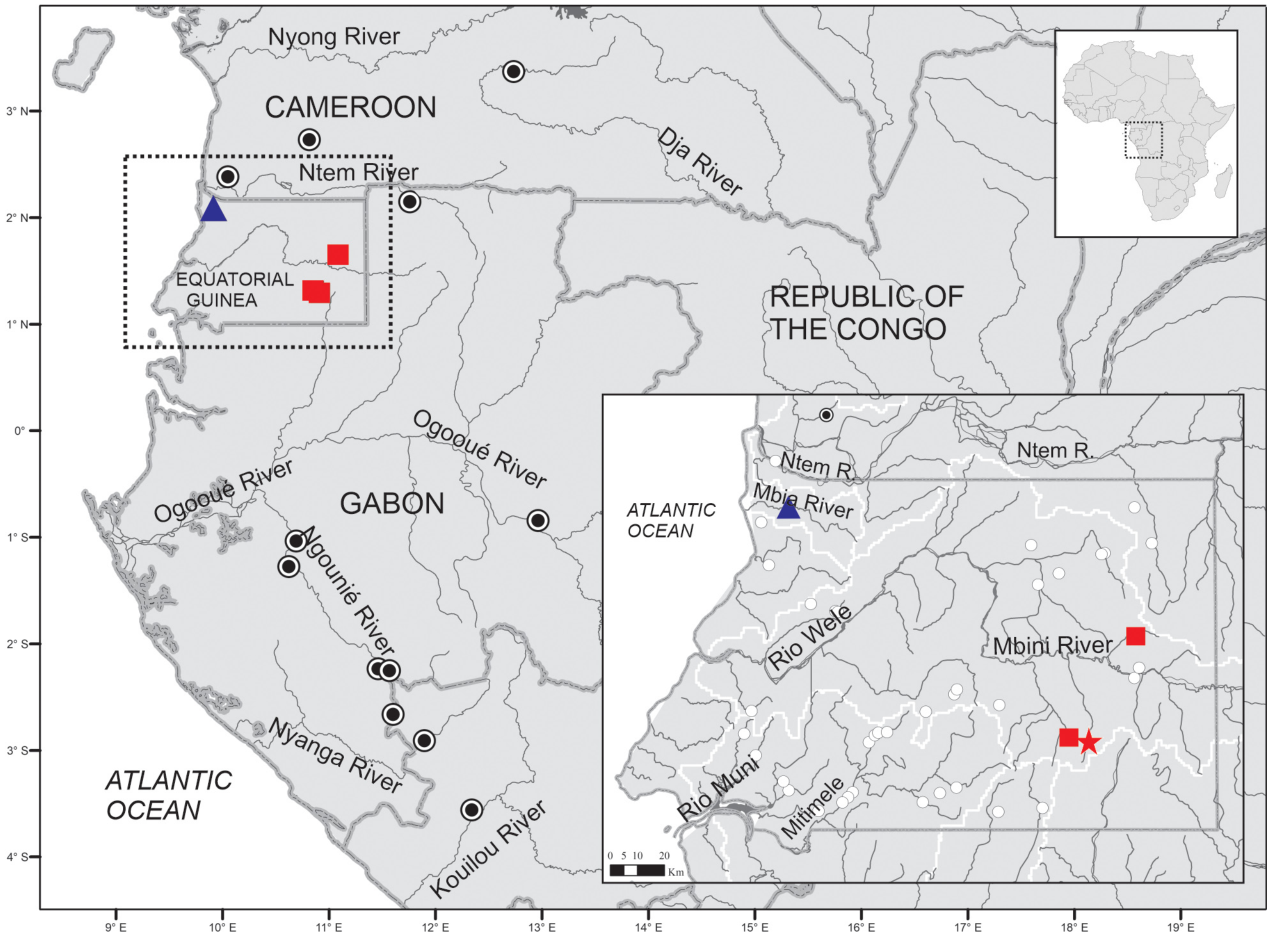

FIGURE 1. Localities of *Distichodus notospilus* populations included in the analysis. Holotype locality for *Distichodus microps* **sp. nov.** (triangle) and *Distichodus mbiniensis* **sp. nov.** (star), and localities of paratypes of *D. mbiniensis* (square). Localities of specimens included in the molecular analyses (closed circle) and all localities sampled during the 2017 expeditions (open circle). White line denote boundaries between drainages.

Results

The phylogeny inferred from 996 base pairs (bp) of CYTB shows four distinct clades within what is currently recognized as *D. notospilus* (Fig. 2A). The relationships among these four clades are unresolved but those specimens collected in the Mbini River, Dja River, and the Mbia and lower/middle Ntem River drainages are each distinct from topotypic material collected in the Ogooué River drainage. The uncorrected p-distances among populations in the Ogooué River drainage and those in the Mbia and Mbini rivers are 3.6% and 3.9% respectively, and the uncorrected p-distance between the Mbia and Mbini river populations is 4.6% (Table 2). The phylogeny inferred from 627 bp of CO1 also showed unresolved relationships but clear divergence among the four clades within the *D. notospilus* complex (Fig. 3). The uncorrected p-distance among populations in Ogooué River drainage and those in the Mbia and Mbini rivers are 2.4% and 3.2% respectively, and the uncorrected p-distance between the Mbia and Mbini river populations is 3.7% (Table 2). The phylogeny inferred from 792 bp of MYH6 is less resolved (not shown) with uncorrected p-distance among populations in Ogooué River drainage and those in the Mbia and Mbini rivers are 0.5% and 0.8% respectively, and the uncorrected p-distance between the Mbia and Mbini river populations is 0.4% (not shown). A phylogeny inferred from a combined dataset of CYTB, CO1, and MYH6 showed a similar topology, though it does provide moderate evidence of a relationship among populations in the Ogooué River, Ntem River, and Mbia Rivers (Fig. 2B). The models of evolution implemented for each codon position were for CYTB (K80+I, F81+1, and HKY+G), CO1 (K80+I, F81, and GTR), and MYH6 (F81, F81, and K80).

TABLE 2. Average uncorrected p-distances of CYTB and CO1 among populations/species of *Distichodus* spp. in lower Guinea and the Congo River basin.

CYTB between groups									
D. maculatus									
D. kolleri	0.0944								
D. affinis	0.1155	0.0713							
D. notospilus (upper Ntem R.)	0.1037	0.0639	0.0811						
D. notospilus (Ogooué R.)	0.1046	0.0662	0.0844	0.0032					
D. aff. *notospilus* (Dja R.)	0.1064	0.0668	0.0969	0.0272	0.0295				
Distichodus mbiniensis **sp. nov.**	0.1098	0.0693	0.0808	0.0368	0.0393	0.0387			
D. aff. *notospilus* (middle Ntem R.)	0.0929	0.0587	0.0768	0.0171	0.0195	0.0321	0.0397		
D. aff. *notospilus* (lower Ntem R.)	0.0949	0.0633	0.0808	0.0302	0.0321	0.0392	0.0437	0.0171	
Distichodus microps **sp. nov.**	0.1024	0.0713	0.0868	0.0342	0.0361	0.0392	0.0457	0.0351	0.0211

CO1 between groups											
D. maculatus											
D. kolleri	0.1018										
D. affinis	0.0938	0.0633									
D. notospilus (Ngongo R.)	0.0909	0.0581	0.0585								
D. notospilus (Nyanga R.)	0.0917	0.0589	0.0577	0.0014							
D. notospilus (Niari R.)	0.0925	0.0629	0.0605	0.0088	0.0096						
D. notospilus (Ogooué R.)	0.0921	0.0561	0.0585	0.0020	0.0028	0.0100					
D. aff. *notospilus* (Dja R.)	0.0970	0.0593	0.0585	0.0276	0.0284	0.0325	0.0288				
Distichodus mbiniensis **sp. nov.**	0.1056	0.0570	0.0693	0.0308	0.0316	0.0357	0.0321	0.0353			
D. aff. *notospilus* (middle Ntem R.)	0.0954	0.0593	0.0569	0.0052	0.0060	0.0100	0.0064	0.0288	0.0321		
D. aff. *notospilus* (lower Ntem R.)	0.0954	0.0529	0.0553	0.0228	0.0236	0.0276	0.0240	0.0256	0.0369	0.0240	
Distichodus microps **sp. nov.**	0.0954	0.0529	0.0553	0.0228	0.0236	0.0276	0.0240	0.0256	0.0369	0.0240	0.000

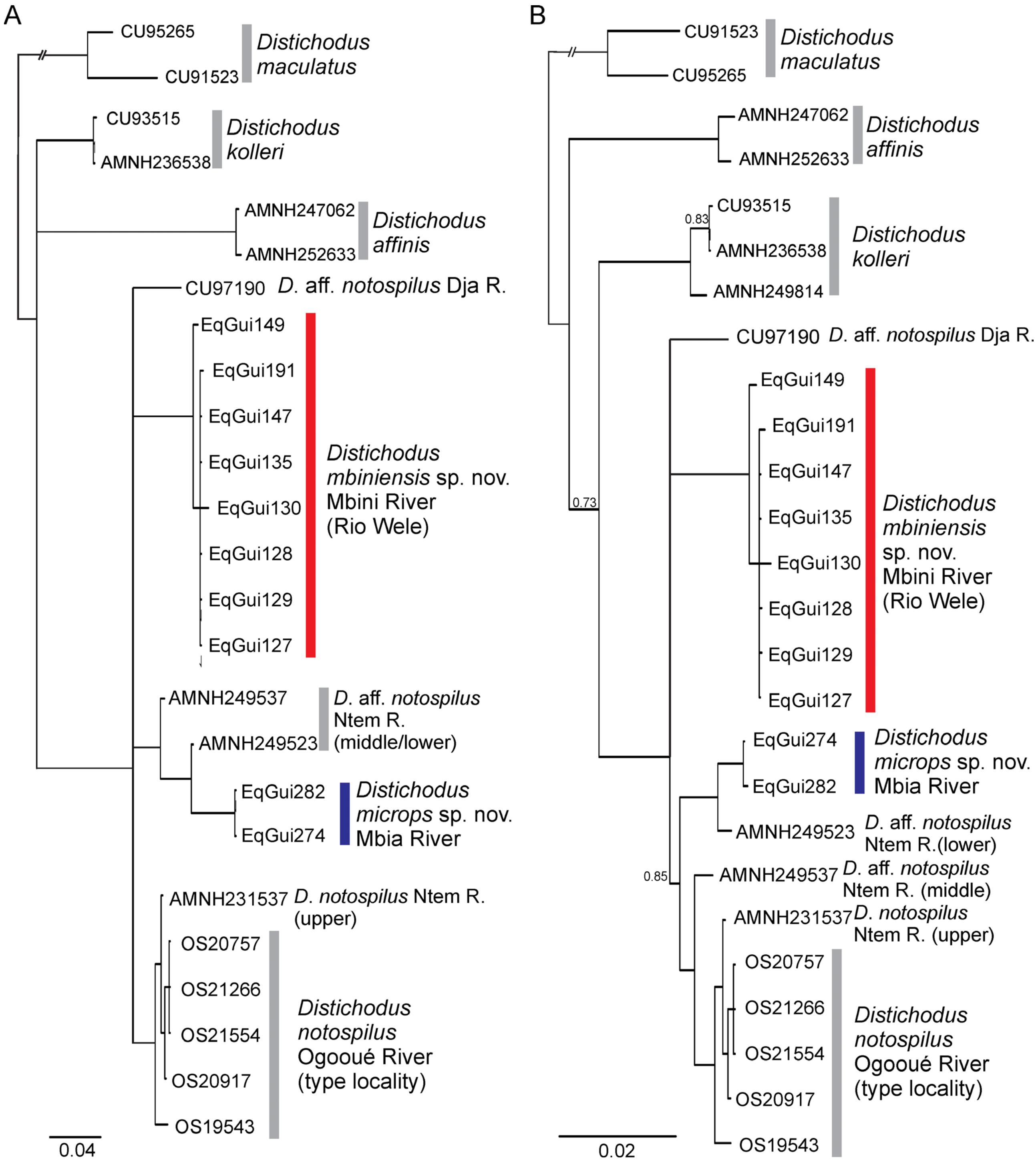

FIGURE 2. Phylogeny of *Distichodus* spp. inferred from 996 bp of cytochrome b (A). Phylogeny inferred from concatenated dataset of 996 bp CYTB, 729 bp MYH6, and 627 bp of CO1(B). Posterior probabilities from Bayesian inference above major branches; above 0.95 not shown and branches with support values <70 were collapsed. Metadata and accession numbers for specimens included in the analyses shown in table 1 and average uncorrected p-distances among these different taxa shown in table 3.

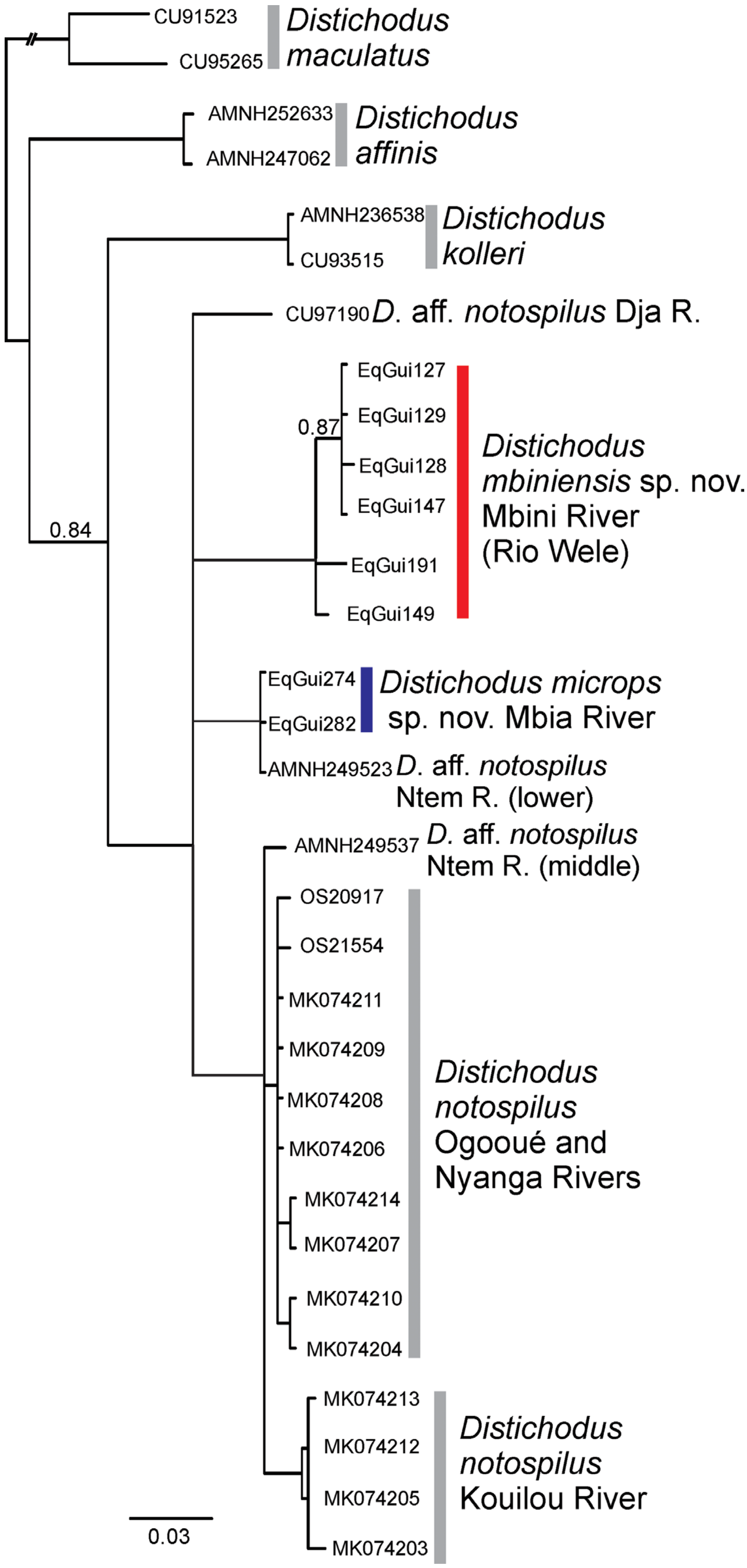

FIGURE 3. Phylogeny of *Distichodus* spp. inferred from 627 bp of CO1. Posterior probabilities from Bayesian inference above major branches; above 0.95 not shown and branches with support values <70 were collapsed. Metadata and accession numbers for specimens included in the analyses shown in table 1 and average uncorrected p-distances among these different taxa shown in table 3.

TABLE 3. Component loadings from principal components analysis of 24 log-transformed measurements from 51 specimens. Result of Mann-Whitney U test of relative measurements from specimens > 80 mm SL. Values bolded are significant after Bonferoni correction (p = 0.0166); *Distichodus microps* **sp. nov.** (n=11), *Distichodus mbiniensis* **sp. nov.** (n=9), and topotypic *Distichodus notospilus* (n=16).

	Morphometrics PC component loadings			Mann-Whitney U test (p-value)		
	PC1 (90.5%)	PC2 (3.4%)	PC3 (1.2%)	*D. microps* to *D. notospilus*	*D. mbiniensis* to *D. notospilus*	*D. microps* to *D. mbiniensis*
Standard Length	0.101	0.003	-0.001			
Predorsal length	0.098	0.007	0.002	0.007	0.027	0.552
Head length	0.079	0.007	0.012	**0.000**	0.084	0.456
Eye diameter	0.058	0.016	0.021	**0.000**	**0.003**	0.603
Snout length	0.088	0.088	0.001	0.753	0.718	0.552
Interorbital distance	0.092	0.015	0.002	**0.000**	**0.000**	0.095
Internasal distance	0.111	-0.002	0.003	0.827	0.207	0.295
Prepectoral length	0.08	0.007	0.010	0.017	0.020	0.824
Prepelvic length	0.098	0.005	0.001	0.064	0.169	0.941
Preanal length	0.102	0.004	-0.001	0.716	0.095	0.230
Dorsal-fin terminus to adipose fin	0.107	0.008	0.001	0.305	0.215	0.080
Body depth	0.114	0.015	0.009	**0.000**	0.452	**0.000**
Pectoral-fin length	0.098	-0.005	0.005	0.422	0.207	0.456
Pectoral-fin base length	0.095	-0.006	-0.015	0.039	0.637	0.112
Pelvic-fin length	0.085	0.013	0.002	0.020	**0.014**	0.412
Pelvic-fin base length	0.117	0.008	-0.028	0.904	0.251	0.261
Dorsal-fin height	0.07	0.02	0.008	**0.000**	**0.000**	0.824
Dorsal-fin base length	0.108	0.004	-0.001	0.716	0.452	0.370
Adipose-fin height	0.102	-0.037	0.016	0.054	**0.004**	0.152
Adipose-fin base length	0.116	-0.072	0.001	**0.001**	**0.001**	1.000
Anal-fin length	0.077	0.008	-0.008	0.342	**0.005**	**0.002**
Anal-fin base length	0.102	0.003	0.009	0.865	0.121	0.131
Caudal peduncle depth	0.096	0.011	-0.011	0.162	**0.000**	**0.000**
Caudal peduncle length	0.095	-0.005	-0.023	**0.001**	0.388	**0.000**
Eye diameter as % of snout length				**0.000**	0.084	0.261
Eye diameter as % of head length				**0.002**	**0.001**	0.552
Snout length as % of head length				**0.006**	0.452	0.261

The principal components analysis of 24 measurements from 51 specimens shows morphological variation among the populations from the Mbini River, Mbia River, and topotypic material from the Ogooué River in Gabon. A plot of PC2 to PC3 shows clear separation among these three populations (Fig. 4A). Snout length and measurements associated with the adipose fin contribute the most variation to PC2; and eye diameter, caudal peduncle length, and pelvic-fin length contribute the most variation to PC3 (Table 3). Principal component 1 was strongly correlated to size (Pearson Correlation 0.995) and the RMA regression showed the slopes were not different among the groups (p = 0.419), and there were no differences in the elevations among the groups (p = 0.82603). Principal components 2 and 3 were not correlated to size (Pearson correlation 0.03 and -0.012 respectively). RMA regressions were informative in looking at differences in several relative measurements among the different populations. Eye diameter as a percentage of snout length is negatively correlated to standard length (-0.54); the allometric trajectories (*i.e.*, the slope) for each group are not different (p = 0.7613) and the elevations are different (p = 0.0002) with the population from the Mbia River being significantly different (p < 0.01) from the populations in the Mbini and Ogooué rivers (Fig. 4B). To reduce the effects of allometry in the Mann-Whitney U tests only specimens larger than 80 mm SL were included in the analyses, and there were several measurements that were significantly different among the different populations (Table 3)

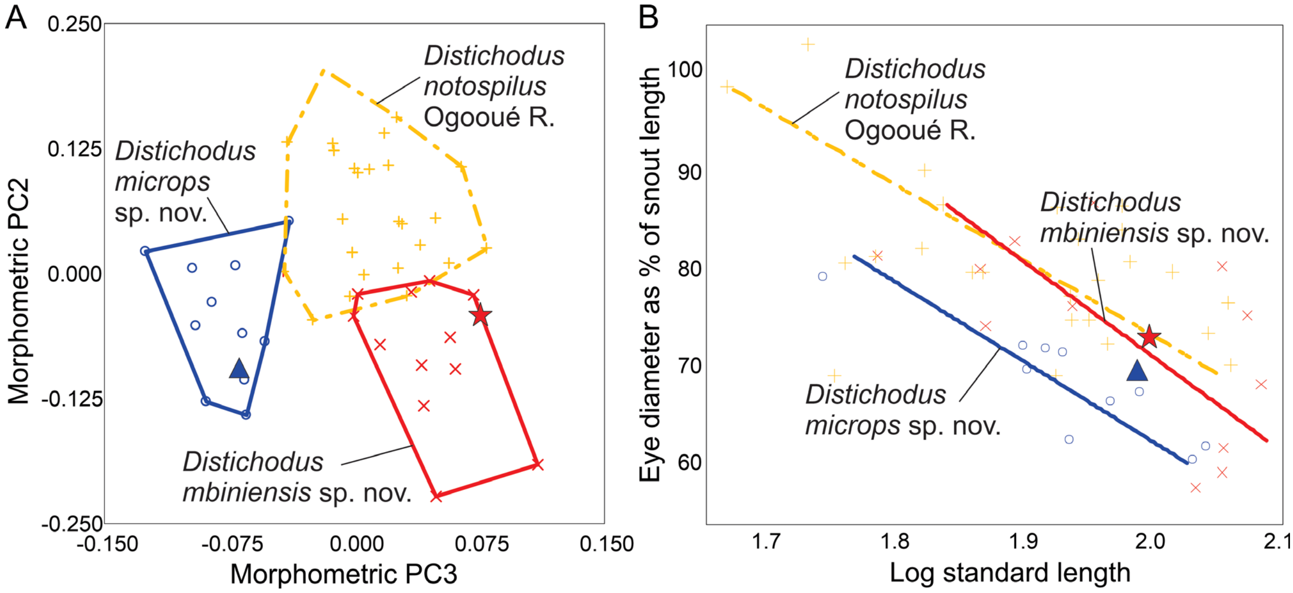

FIGURE 4. Plot of PC2 and PC3 from principal component analysis of 24 log-transformed measurements from 51 specimens (A). Reduced-major axis regression of eye diameter as a percentage of snout length on log-transformed standard length (B). Trendlines are shown for each species; slopes for each species are not different (p-value = 0.7613), and the elevation among species are significantly different (p-value = 0.0002) with *Distichodus* microps being significantly different (p-value < 0.01) from *D. mbiniensis* and topotypic *D. notospilus*. The holotype of *Distichodus microps* and *D. mbiniensis* are denoted by a filled triangle and star respectively. Component loadings and results of Mann-Whitney U tests shown in table 3.

***Distichodus microps* sp. nov.**

urn:lsid:zoobank.org:act:C350FA00-3277-4202-9998-46AF78E9E681

Figures 2, 3, 4, 5, 8, 9; Table 4

Holotype. USNM 451325, ALC, 98.0 mm SL, Equatorial Guinea, Litoral, Rio Mbia at Menzong, 32 m elev, 2.07529° N, 9.92378° E, 2017 Equatorial Guinea expedition team, 30 June 2017.

Paratypes. MRAC 2020,005,P,0004, 1 ALC, 86.5 mm SL, voucher EqGui2017_0282, collection information the same as the holotype.—MRAC 2020,005,P,0005-0008, 4 ALC, 80.1–98.1mm SL, collection information the same as the holotype.—USNM 451457, ALC, 81.6 mm SL, voucher EqGui2017_0274, collection information the same as the holotype.—USNM 451458, 5 ALC, 55.5–110.5 mm SL, collection information the same as the holotype.

Diagnosis. *Distichodus microps* is readily distinguished from the larger-bodied, higher-scale count *Distichodus* spp. (group B in Arroyave *et al.* 2020: *D. antonii* Schilthuis, *D. atroventralis* Boulenger, *D. fasciolatus* Boulenger, *D. lusosso* Schilthuis, *D. mossambicus* Peters, *D. sexfasciatus* Boulenger, *D. langi* Nichols & Griscom, *D. rostratus* Günther, *D. engycephalus* Günther, *D. kasaiensis* Vreven, Moelants, & Snoeks, *D. ingae* Moelants & Snoeks, *D. polli* Abwe, Snoeks, Manda, & Vreven, *D. petersii* Pfeffer, *D. nefasch* Bonnaterre, *D. brevipinnis* Günther, and *D. schenga* Peters) in having fewer lateral line scales (40–41 versus >60) and in achieving a smaller maximum standard length (<15 cm versus >30 cm). This new species is also distinguished from the smaller-bodied, lower-scale count *Distichodus* spp. (group A in Arroyave *et al.* 2020) in the following ways. *Distichodus microps* is distinguished from *D. decemmaculatus* Pellegrin, and *D. teugelsi* Mamonekene & Vreven, in having two rows of teeth on the lower jaw versus one. *Distichodus microps* can be readily distinguished from D. *noboli* Boulenger, *D. hypostomatus* Pellegrin, and *D. maculatus* in having fewer scales along the lateral line (40–41 versus 45, 53–60, and 75 respectively), and distinguished from *D. rufigiensis* Norman, in not having prominent vertical bars along the sides. *Distichodus microps* is distinguished from *D. kolleri* and *D. altus* Boulenger, in having fewer dorsal-fin rays (15–17 total rays versus 21–26 and 17–18 respectively), and further distinguished from *D. altus* and *D. affinis* Günther, in having fewer total anal-fin rays (12–13 versus 21–22). *Distichodus microps* is distinguished from *D. notospilus* in having more scales along the lateral line (41, rarely 40 versus 37–39, rarely 40; Tables 4 & 5), a nearly inferior mouth versus subterminal in *D. notospilus*, a curved posterolateral margin of the opercle versus straight in *D. notospilus*, a smaller eye (56.7–80.4 versus 70.1–104.3 % of snout length; Figs. 5, 6, & 8), and a less prominent elongated spot at the base of the caudal fin. *Distichodus microps* is distinguished from *D. mbiniensis* in having a shallower body (usually six scales from LL to the pelvic fin versus seven; 34.1–38.7 versus 33.6–42.4% SL), fewer anal-fin rays (usually 12 total rays versus 13 or 14), a longer anal fin (15.0–17.8 versus 13.4–17.3% SL), a more inferior mouth versus the subterminal mouth in *D. mbiniensis*, a deeper (12.4–14.2 versus 11.8–13.0% SL) and a longer caudal peduncle (11.9–14.8 versus 9.7–12.6% SL), a smaller eye (56.7–80.4 versus 58.6–88.0% of snout length; Figs. 4B, 5, 7, & 8), a more narrow and elongate subopercle (Fig. 8), and a deeper infraorbital 1 with a more rectangular anterolateral margin versus a more shallow infraorbital 1 with a curved anterolateral margin in *D. mbiniensis* (Fig. 8).

TABLE 4. Morphometric measurements and meristics of *Distichodus microps* **sp. nov.** (n=12; holotype and 11 paratypes). Standard length expressed in mm. All other measurements expressed in percent SL unless noted. Meristic data from holotype are identified by an "*".

MORPHOMETRICS	Holotype	Range	Mean±%SD
Standard Length (mm)	98.0	55.5–110.5	
Predorsal length	51.7	49.5–55.4	52.4±1.6
Head length	23.0	21.2–25.2	23.7±1.1
Eye diameter	5.7	5.2–7.4	6.2±0.6
Snout length	8.1	8.1–11.0	9.0±0.8
Interorbital distance	9.3	8.9–9.9	9.4±0.3
Internasal distance	5.3	4.9–5.8	5.3±0.3
Prepectoral length	23.5	22.3–27.2	24.9±1.6
Prepelvic length	52.0	51.7–56.1	53.8±1.3
Preanal length	77.7	77.5–82.5	79.4±1.7
Dorsal-fin terminus to adipose fin	19.1	19.1–24.2	21.0±1.3
Body depth	36.5	34.1–38.7	36.1±1.5
Pectoral-fin length	19.1	17.8–19.5	18.8±.5
Pectoral-fin base length	5.8	4.4–5.8	5.3±0.5
Pelvic-fin length	19.4	17.4–20.2	19.2±0.8
Pelvic-fin base length	5.2	4.3–5.8	5.0±0.5
Dorsal-fin height	23.5	20.2–26.8	23.0±2.1
Dorsal-fin base length	19.8	15.6–19.8	18.1±1.3
Adipose-fin height	8.1	5.9–8.2	7.1±0.7
Adipose-fin base length	3.3	2.2–3.6	2.9±0.5
Anal-fin length	17.4	15–17.8	16.5±0.9
Anal-fin base length	13.8	10.3–13.9	12.3±1.2
Caudal peduncle depth	13.9	12.4–14.2	13.6±0.4
Caudal peduncle length	12.2	11.9–14.8	13.2±1.0
Eye diameter as % of snout length	70.9	56.7–80.4	68.5±6.5
Eye diameter as % of head length	24.9	23.9–29.3	26±1.9
Snout length as % of head length	35.1	35.1–43.9	38.2±2.5
MERISTICS			
Scales in lateral line	40 (2), 41* (10)		
Scales LL to dorsal	6.5 (2), 7.5* (10)		
Scales LL to pelvic	6* (12)		
Scales around caudal peduncle	16* (12)		
Dorsal-fin count	iii-12 (1), iii-13 (9), iii-14* (1)		
Anal-fin count	iii-9* (10), iii-10 (2)		

TABLE 5. Morphometric measurements and meristics of topotypic *Distichodus notospilus* (n=26). Standard length expressed in mm. All other measurements expressed in percent SL unless noted.

MORPHOMETRICS	Range	Mean±%SD
Standard Length (mm)	46.8–115.6	
Predorsal length	51.7–55.5	53.9±1.0
Head length	21.3–28.4	25.8±1.4
Eye diameter	5.8–9.6	7.4±0.7
Snout length	7.7–10.3	9.0±0.6
Interorbital distance	9.0–10.9	10.1±.4
Internasal distance	4.7–5.9	5.3±0.3
Prepectoral length	24.7–29.9	27.0±1.2
Prepelvic length	53.0–56.5	54.3±0.8
Preanal length	75.2–82.3	79.3±1.7
Dorsal-fin terminus to adipose fin	19.9–22.9	21.4±0.8
Body depth	34.8–44.3	39.6±2.2
Pectoral-fin length	16.9–20.2	18.8±0.9
Pectoral-fin base length	3.8–5.7	4.9±0.4
Pelvic-fin length	17.6–22.4	20.4±1.1
Pelvic-fin base length	3.8–5.8	4.8±0.5
Dorsal-fin height	22.7–34.2	27.1±2.3
Dorsal-fin base length	16.4–20.3	18.2±1.0
Adipose-fin height	5.0–8.3	6.8±0.8
Adipose-fin base length	1.8–3.1	2.4±0.3
Anal-fin length	12.9–19.4	16.5±1.4
Anal-fin base length	11.2–14.8	12.5±0.8
Caudal peduncle depth	12.3–14.1	13.4±0.4
Caudal peduncle length	10.2–14.4	12.0±0.9
Eye diameter as % of Snout length	70.1–104.3	82.1±8.2
Eye diameter as % of Head length	26.5–33.8	28.6±1.7
Snout length as % of Head length	30.3–40.3	35.0±2.4
MERISTICS		
Scales in lateral line	37 (2), 38 (9), 39 (14), 40 (1)	
Scales LL to dorsal	6.5 (13), 7.5 (11)	
Scales LL to pelvic	6 (7), 7 (19)	
Scales around caudal peduncle	16 (26)	
Dorsal-fin count	ii-14 (3), ii-15 (1), iii-13 (11), iii-14 (11)	
Anal-fin count	iii-10 (12), iii-11 (14)	

Description. Morphometrics and meristics for holotype and paratypes of *Distichodus microps* are summarized in table 4. Holotype shown in figure 5 and the live color of a paratype is shown in figure 9. Maximum observed size: 110.5 mm SL. Body laterally compressed and moderately elongate. Dorsal profile with convex curve from snout to anterior margins of head; weakly convex to dorsal-fin origin. Profile from origin of dorsal fin to adipose fin straight to slightly convex; concave from adipose to caudal fin. Ventral profile broadly convex from tip of snout to the terminus of the anal fin; slightly concave in area from anal-fin terminus to caudal fin. Body covered in relatively large ctenoid scales; extending over proximal half of adipose and caudal fins; extending beyond proximal half in lobes of caudal fin.

Mouth inferior to nearly subterminal; two rows of bicuspid teeth in upper and lower jaws. Dorsal fin with straight to slightly concave distal margin; its origin anterior to vertical of pelvic-fin origin. Adipose fin origin nearly two thirds the distance from dorsal-fin terminus to caudal fin; anterior to vertical of anal-fin terminus and extends beyond vertical of anal-fin terminus. Caudal fin forked; tips of upper and lower lobe slightly rounded with moderate point. Anal fin with slightly concave margin; base about five-sixths the length of longest ray; not reaching caudal fin when flexed to body. Pelvic-fin origins at mid-length of snout to caudal fin distance; nearly as long as head length. Pectoral-fin origin posterior to vertical of posterior edge of subopercle; horizontal of pectoral-fin origin near or slightly inferior to mouth; nearly as long as pelvic fin.

FIGURE 5. *Distichodus microps*, a new species, holotype, USNM 451325, ALC, 98.0 mm SL, Equatorial Guinea, Litoral, Rio Mbia at Menzong, 32 m elev, 2.07529° N, 9.92378° E; Photograph by S. Raredon.

FIGURE 6. Topotypic *Distichodus notospilus*, OS 21266, Gabon, Province de la Ngounié, SINDARA C—Below the rapids of Sindara on the left bank, in a small inundation zone with a sandy bottom that is exposed in the dry season, 1.03492° S, 10.69116° E; Photograph by S. Raredon.

Coloration. Live coloration: specimens silver with some red and gold flecks along head and sides anteriorly and becoming more diffuse posteriorly. Pectoral and anal fins red. Dorsal fin cream at based with black band beginning about one-third the length of the third ray stretching nearly to the distal tip and roughly to the base of the fourth ray through the base of the tenth ray (Fig. 9). Diffuse ovoid black spot at base of caudal fin. Typical coloration of preserved specimens shown in figure 5. Specimens light grey to silver along sides. Scales darker gray on upper quarter of sides. Pores along the lateral line with melanophores forming a diffuse stripe. Faint black ovoid spot at base of caudal fin. Fins clear with black spot in dorsal fin as previously described.

Etymology. The specific epithet is a combination of the Greek "micro" and "ops" and refers to the small eye relative to the snout and head length.

Distribution. *Distichodus microps* is only known from the type locality in the Mbia River drainage in Equatorial Guinea. The type specimens were collected by cast net in ~2 m deep flowing water along the bank downstream from the older bridge crossing on the road from Bata to Rio Campo. Roman collected at two localities on the Mbia River in the 1960s but did not record any *Distichodus* species (Roman 1971). Additional surveys are needed to determine the distribution of this species within the Mbia River drainage. Published sequences from populations in the lower Ntem River suggest that *D. microps* species may also be present in the Lower Ntem and potentially in other coastal rivers in southern Cameroon (Fig. 2; see discussion for further comments).

Conservation status. *Distichodus microps* is presently only known to occur in the Mbia River drainage in Equatorial Guinea. The extant of occurrence (EOO) is 3,467 km^2 and this species occurs in less than five threat-based locations. The ongoing threats to the habitats preferred by *D. microps* include habitat destruction and sedimentation resulting from road construction and logging activities in the region. Due to the restricted EOO and limited number of locations available to this species, this species is assessed in the IUCN Red List as Endangered (Schmidt *et al.* 2020[a]).

Distichodus mbiniensis sp. nov.

urn:lsid:zoobank.org:act:8B5B9875-DB26-4417-B719-0869D8CFA19E
Figures 2, 3, 4, 7, 8, 9; Table 6

Holotype. USNM 451317, ALC, 99.9 mm SL, voucher EqGui2017_0127, Equatorial Guinea, Wele-Nzas, Rio Ntoro at Aconibe—Acurenam Rd., 668 m elev, 1.29091° N, 10.9174° E, 2017 Equatorial Guinea expedition team, 23 June 2017.

Paratypes. MRAC 2020,005,P,0001, 1 ALC, 114.1 mm SL, voucher EqGui2017_0128, collection information the same as the holotype.—MRAC 2020,005,P,0002, 1 ALC, 74.4 mm SL, voucher EqGui2017_0135, collection information the same as the holotype.—MRAC 2020,005,P,0003, 1 ALC, 113.9 mm SL, collection information the same as the holotype.—USNM 451319, ALC, 61.3 mm SL, voucher EqGui2017_0130, collection information the same as the holotype.—USNM 451318, ALC, 122.1 mm SL, voucher EqGui2017_0129, collection information the same as the holotype.—USNM 451320, 3 ALC, 78.4–119.0 mm SL, collection information the same as the holotype.—USNM 451324, ALC, 73.7 mm SL, voucher EqGui2017_0191, Equatorial Guinea, Wele-Nzas, Rio Nuik near Alam, 660 m elev, 1.64875° N, 11.08593° E, 2017 Equatorial Guinea expedition team, 27 June 2017.—USNM 451321, ALC, 108.6 mm SL, voucher EqGui2017_0147, Equatorial Guinea, Wele-Nzas, Rio Abia at Evinayong—Aconibe Rd, 664 m elev, 1.31069° N, 10.86025° E, 2017 Equatorial Guinea expedition team, 23 June 2017.—USNM 451322, ALC, 113.8 mm SL, voucher EqGui2017_0149, collection information the same as USNM 451321.—USNM 451323, ALC, 90.3 mm SL, collection information the same as USNM 451321.

Diagnosis. *Distichodus mbiniensis* is readily distinguished from the larger-bodied, higher-scale count *Distichodus* spp. (group B in Arroyave *et al.* 2020; *D. antonii*, *D. atroventralis*, *D. fasciolatus*, *D. lusosso*, *D. mossambicus*, *D. sexfasciatus*, *D. langi*, *D. rostratus*, *D. engycephalus*, *D. kasaiensis*, *D. ingae*, *D. polli*, *D. petersii*, *D. nefasch*, *D. brevipinnis*, and *D. schenga*) in having fewer lateral line scales (40–42 versus >60) and in achieving a smaller maximum standard length (<15 cm versus >30 cm). This new species is also distinguished from the smaller-bodied, lower-scale count *Distichodus* spp. (group A in Arroyave *et al.* 2020) in the following ways. *Distichodus mbiniensis* is distinguished from *D. decemmaculatus* and *D. teugelsi* in having two rows of teeth on the lower jaw versus one. *Distichodus mbiniensis* can be readily distinguished from *D. noboli, D. hypostomatus*, and *D. maculatus* in having fewer scales along the lateral line (40–42 versus 45, 53–60, and 75 respectively), and distinguished from *D. rufigiensis* in not having prominent vertical bars along the sides. *Distichodus mbiniensis* is distinguished from *D. kolleri* in having fewer dorsal-fin rays (16–18 total rays versus 21–26), and distinguished from *D. altus* and *D. affinis* in having fewer total anal-fin rays (13–14 versus 21–22). *Distichodus mbiniensis* is distinguished from *D. notospilus* in having more scales along the lateral line (41–42, rarely 40 versus 37–39, rarely 40; Tables 5 & 6), a much less prominent elongated dark spot at the base of the caudal fin, a shorter dorsal fin (21.4–27.2 versus 22.7–34.2% SL), and shallower caudal peduncle (11.8–13.0 versus 12.3–14.1% SL). *Distichodus mbiniensis* is distinguished from *D. microps* in having a deeper body (usually seven scales from LL to the pelvic fin versus six), a subterminal mouth versus the nearly inferior mouth of *D. microps*, a shallower (11.8–13.0 versus 12.4–14.2% SL) and shorter caudal peduncle (9.7–12.6 versus 11.9–14.8% SL), a larger eye (58.6–88.0 versus 56.7–80.4% of snout length, Figs. 4B, 5, 7, & 8), a wider subopercle (Fig. 8), and a shallower infraorbital 1 with a more curved anterolateral margin versus a deeper infraorbital 1 with a more rectangular anterolateral margin in *D. microps* (Fig. 8).

TABLE 6. Morphometric measurements and meristics of *Distichodus mbiniensis* **sp. nov.** (n=13; holotype and 12 paratypes). Standard length expressed in mm. All other measurements expressed in percent SL unless noted. Meristic data from holotype are identified by an "*".

MORPHOMETRICS	Holotype	Range	Mean±%SD
Standard Length (mm)	99.9	61.3–122.1	
Predorsal length	52.1	49.5–54.4	52.6±1.5
Head length	23.9	21.8–28.9	25±2.1
Eye diameter	6.3	5.7–8.5	6.7±0.9
Snout length	8.5	7.1–10.7	9.0±1.2
Interorbital distance	9.6	8.7–9.6	9.1±0.3
Internasal distance	5.4	4.8–6.2	5.6±0.6
Prepectoral length	26.1	22.7–29.0	26.0±2.1
Prepelvic length	53.0	50.9–56.8	54.0±1.9
Preanal length	78.7	76.6–80.1	78.4±1.2
Dorsal-fin terminus to adipose fin	21.9	18.6–23.2	21.4±1.3
Body depth	38.7	33.6–42.4	38.2±2.5
Pectoral-fin length	18.6	18.0–21.9	19.9±1.4
Pectoral-fin base length	4.5	4.3–5.7	5.0±0.4
Pelvic-fin length	17.8	16.3–20.6	18.8±1.5
Pelvic-fin base length	4.2	3.9–5.5	4.6±0.5
Dorsal-fin height	21.6	21.4–27.2	23.6±1.9
Dorsal-fin base length	18.3	16.5–19.6	18.5±0.9
Adipose-fin height	7.7	6.7–9.5	7.8±0.8
Adipose-fin base length	2.8	2.8–4.2	3.2±0.5
Anal-fin length	13.4	13.4–17.3	14.8±1.2
Anal-fin base length	14.2	11.7–14.8	13.3±1.0
Caudal peduncle depth	12.2	11.8–13.0	12.4±0.4
Caudal peduncle length	11.5	9.7–12.6	11.6±0.8
Eye diameter as % of snout length	74.1	58.6–88.0	74.7±9.4
Eye diameter as % of head length	26.4	24.1–29.6	26.6±1.8
Snout length as % of head length	35.6	32–41.1	36.0±2.9

MERISTICS	
Scales in lateral line	40 (2), 41 (6), 42* (5)
Scales LL to dorsal	7.5* (13)
Scales LL to pelvic	6 (1), 7* (12)
Scales around caudal peduncle	16* (13)
Dorsal-fin count	ii-14 (1), iii-13 (1), iii-14* (10), iii-15 (1)
Anal-fin count	iii-10* (10), iii-11 (3)

Description. Morphometrics and meristics for the holotype and paratypes of *Distichodus mbiniensis* are summarized in table 6. Holotype shown in figure 7 and the live color of the holotype and a paratype are shown in figure 9. Maximum sized observed: 122.1 mm SL. Body laterally compressed and moderately elongate. Dorsal profile with a convex curve from tip of snout to the vertical of anterior margin of eye, weakly concave from the vertical of the eye to posterior margin of head, and convex to origin of dorsal fin. Profile weakly convex from origin of dorsal to adipose fin origin; weakly concave from adipose fin origin to caudal fin. Ventral profile broadly convex from snout to origin of anal fin; weakly concave from anal-fin terminus to caudal fin. Body covered in relatively large ctenoid scales; extending over the proximal half of adipose fin and caudal fin.

Mouth subterminal; two rows of bicuspid teeth in the upper and lower jaws. Dorsal fin with convex margin; its origin just anterior to the vertical of the origin of the pelvic fins. Adipose fin two thirds the distance from dorsal-fin terminus to caudal fin origin; base entirely anterior to the vertical of anal-fin terminus; distal tip extending beyond vertical of anal-fin terminus. Caudal fin forked; upper and lower lobes slightly rounded with moderate point. Anal fin with concave margin; base nearly equal to longest ray. Pelvic-fin origin at mid-length of snout to caudal fin distance; nearly as long as head length. Pectoral-fin origin just posterior to vertical of posterior margin of subopercle; horizontal just inferior to inferior margin of subopercle; slightly longer than pelvic fin.

FIGURE 7. *Distichodus mbiniensis*, a new species, holotype, USNM 451317, ALC, 99.9 mm SL, voucher EqGui2017_0127, Equatorial Guinea, Wele-Nzas, Rio Ntoro at Aconibe—Acurenam Rd., 668 m elev, 1.29091° N, 10.9174° E; Photograph by S. Raredon.

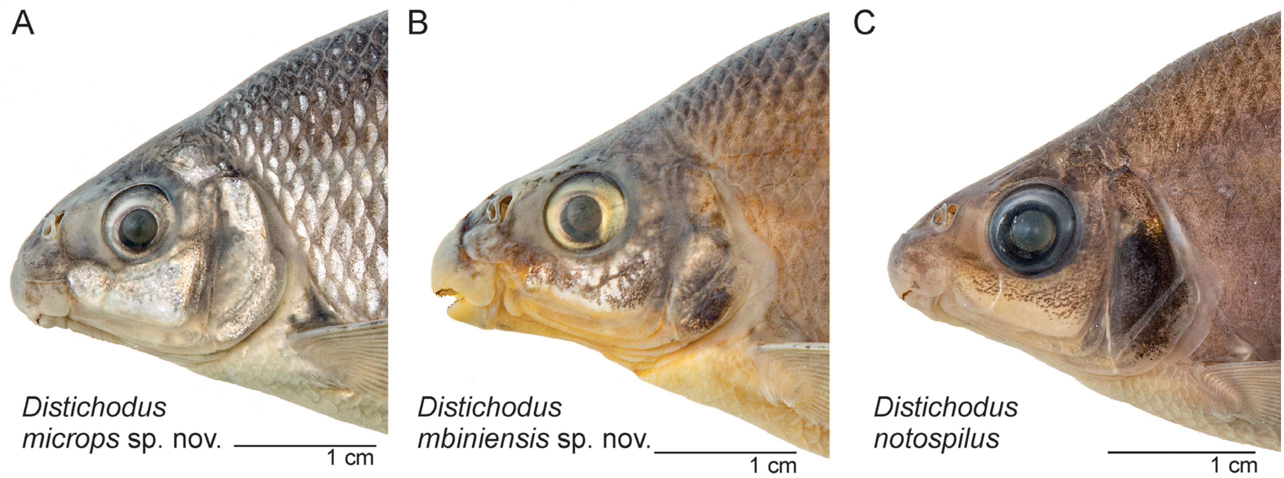

FIGURE 8. Lateral views of head region in *Distichodus microps* **sp. nov.** (A), *Distichodus mbiniensis* **sp. nov.** (B), and topotypic *Distichodus notospilus (C)*. Same specimens and locality information as previous figures. Photographs by S. Raredon.

Coloration. Live coloration: in both large and small specimens the ground color is bronze with some yellow and gold along the sides of the head. Some dark melanophores on subopercle in larger specimens which appears to be absent in smaller specimens. Anal and pelvic fins bright red in smaller specimens; a darker brick-red in larger individuals (Fig. 9). Pectoral fins, adipose fin, and caudal fin clear to yellow. The dorsal fin with prominent black spot originating at the base of the third or fourth ray and extending anteriorly at an angle to the first ray. In small specimens this black marking extends to the distal tip of the dorsal fin but only extends distally about two-thirds in larger individuals (Figs. 7 & 9). The last third of the dorsal fin is clear to yellow in smaller specimens and a darker red to yellow in larger specimens. Typical coloration after preservation is shown in figure 7. Specimens are medium to dark brown along the back and upper quarter of sides, lighter below. Scales along sides have darker spot on anterior and poster margin providing an effect of light horizontal stripes. Pigmentation on opercle and subopercle, and the spot a base of caudal fin more distinct than in live specimens. Fins beige with the dorsal fin with distinct black marking as described previously.

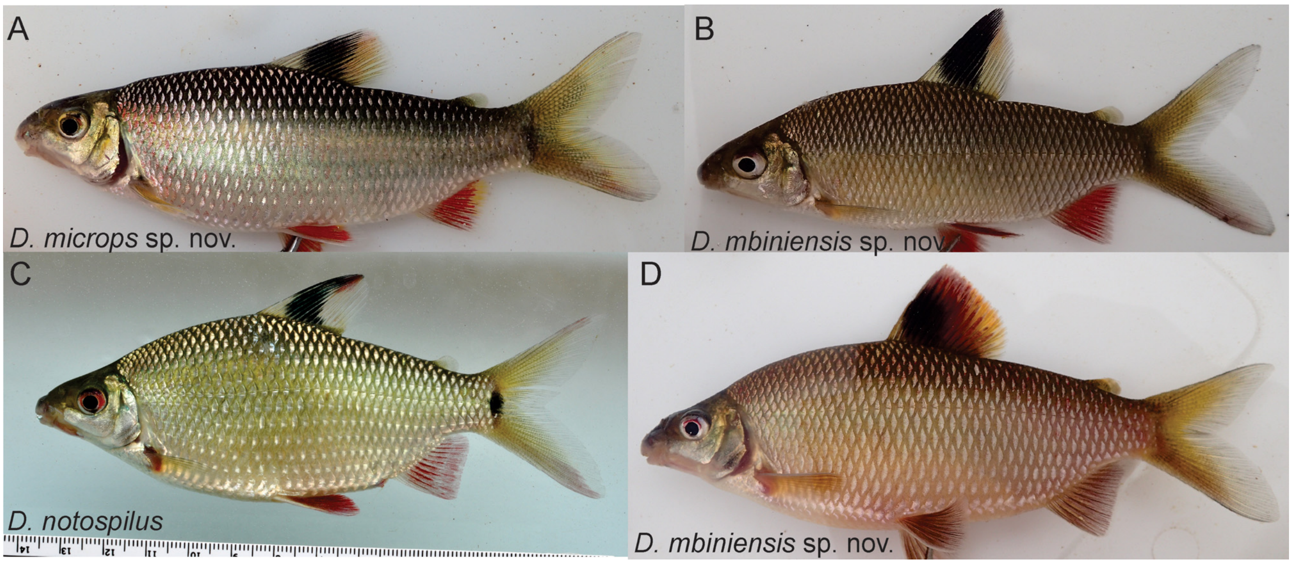

FIGURE 9. Live coloration of *Distichodus microps*, USNM 451457, ALC 81.6 mm SL, voucher EqGui2017_0274, Equatorial Guinea, Litoral, Rio Mbia at Menzong, 32 m elev, 2.07529° N, 9.92378° E photograph by R.C. Schmidt (A); *Distichodus mbiniensis*, USNM 451319, ALC 61.3 mm SL, voucher EqGui2017_0130, Equatorial Guinea, Wele-Nzas, Rio Ntoro at Aconibe—Acurenam Rd., 668 m, 1.29091° N, 10.9174° E, photograph by R. C. Schmidt (B); Topotypic *Distichodus notospilus*, OS 19544, ALC, 104.1 mm SL, Gabon, Province de l'Ogooué-Lolo, Right bank of Ogooué River at Doumé village, 0.84245° S, 12.96249° E, Photograph by B. Sidlauskas, included with permission (C); Holotype of *Distichodus mbiniensis*, USNM 451317, ALC, 99.9 mm SL, voucher EqGui2017_0127, Equatorial Guinea, Wele-Nzas, Rio Ntoro at Aconibe—Acurenam Rd., 668 m elev, 1.29091° N, 10.9174° E; Photograph by R. C. Schmidt (D).

Etymology. The specific epithet refers to the Mbini River which is the Ndowe name for the Rio Wele. The Ndowe are Bantu-speaking people that live along the coastal region in Equatorial Guinea.

Distribution. This species is only known from the upper Mbini River drainage (Rio Wele) in Equatorial Guinea but may also occur within this drainage in Gabon (Fig. 2). Research expeditions in the Woleu R. (Mbini River drainage) in Gabon discovered a new species of *Synodontis*, but no specimens of *Distichodus* were collected at that time (Friel & Sullivan 2008). *Distichodus mbiniensis* was collected in relatively deep (~2 meters) pools with some flow. At the type locality they were collected in the outflow of a black-water reservoir impounded by the recent road construction. Additional sampling in the region is needed to determine how widespread this species is within the region. The expedition in 2017 was one of the first to collect fishes in the tributaries of the Mbini River in Equatorial Guinea.

Conservation Status. *Distichodus mbiniensis* is only known to occur within the upper reaches of the Mbini River drainage in Equatorial Guinea but may also occur in this drainage in Gabon. The extant of occurrence (EOO) for this species is estimated at 19,085 km^2, determined in the IUCN Freshwater Mapping Application, and the species likely occurs in less than ten threat-based locations. These locations are the mainstrem of the Mbini River and the major tributaries that flow into the Mbini River. The habitats of *D. mbiniensis* are under threat due to pollution and sedimentation caused by ongoing road construction and logging activities in the region. Due to the restricted EOO, fewer than ten threat-based locations, and ongoing threats in the region that are negatively affecting the quality and extant of habitat for *D. mbiniensis*, this species is assessed as Vulnerable in the IUCN Red List (Schmidt *et al.* 2020[b])

Discussion

New species of *Distichodus* from Lower Guinea

Described from the Ogooué River in Gabon, *Distichodus notospilus* was thought to occur throughout the Lower Guinea ichthyoregion, but this study reveals two new species in Equatorial Guinea distinct from *D. notospilus* and what may be undescribed diversity in the Ntem and Dja Rivers of southern Cameroon. The two new species described herein are genetically distinct from topotypic material and can be readily distinguished from *D. notospilus* through a combination of morphological and meristic characters (Figs. 2, 3, & 4). *Distichodus microps* is distinguished from *D. notospilus* in having more scales along the lateral line, a nearly inferior mouth versus subterminal in *D. notospilus*, a curved posterolateral margin of the opercle versus straight in *D. notospilus*, a smaller eye, and a less prominent elongated spot at the base of the caudal fin. *Distichodus mbiniensis* is distinguished from *D. notospilus* in having more scales along the lateral line, a much less prominent elongated dark spot at the base of the caudal fin, a shorter dorsal fin, and a shallower caudal peduncle. *Distichodus microps* is distinguished from *D. mbiniensis* in having a shallower body, fewer anal-fin rays, a longer anal fin, a more inferior mouth versus the subterminal mouth in *D. mbiniensis*, a deeper and a longer caudal peduncle, and differences in several features associated with the head (*e.g.,* eye size, shape of infraorbital 1, and shape of subopercle). These features associated with the head were also informative in comparing several species in the southern Congo (Abwe *et al.* 2019). Utilizing the RMA analysis allows us to better understand how some of these characters (*e.g.,* eye diameter as % of snout length) are informative even if the ranges overlap (Fig. 4B).

This study also suggests that additional undescribed diversity exists in the Ntem River, the Dja River, and potentially in other coastal rivers in southern Cameroon. Based on one specimen from the Dja River, these populations are genetically distinct from topotypic *D. notospilus* with nearly 3% uncorrected p-distance in CYTB and 2.9% divergence in CO1 (table 2). Morphological analyses of this population are needed before determining if specific recognition is warranted. The populations in the lower and middle Ntem River are also genetically distinct from *D. notospilus* from the Ogooué River (Figs. 2 & 3), but the relationships resolved from different markers are discordant (further discussed in biogeography section). Morphological analyses of these populations and additional populations from other small coastal drainages in western Cameroon are needed to determine if they represent undescribed species or if they are divergent populations of *D. microps*.

Biogeography of the *Distichodus notospilus* complex in Lower Guinea

The discovery of distinct species of *Distichodus* in the Mbini River and the coastal Mbia River provides an opportunity to reexamine the proposed freshwater biogeography for the region. Frequent past connections among the rivers in Lower Guinea are suggested (Lévêque 1997) and evidence of past headwater capture events promoting geodispersal *(i.e.,* faunal exchange) seems apparent when viewing maps of the rivers in the region. The Dja River originally drained into the Nyong River in the Lower Guinea province but seems to have been captured by the Sangha River and now is part of the Congo River basin (Roberts 1975). This headwater capture event would have allowed for geodispersal between these two drainages as evidenced by the presence of *Hepsetus lineatus* Decru, Vreven, & Snoeks 2013 and several other Lower Guinean species in the Dja River as detailed by Decru *et al.,* (2015). Several shared species also suggest recent geodispersal events between the Congo and Ogooué River drainages (Roberts 1975) and the Ogooué and Ntem River drainages (Thys van den Audenaerde 1966). More recent studies working with high resolution Digital Elevation Maps (DEMs) of the region suggest recent and older (< 5 MYA), pre-Oligocene, headwater capture events between the Ogooué R. drainage (Ivindo River) and upper Ntem River (Eisenburg 2012; Markwick 2019). River capture events among the upper Ntem and upper Mbini river drainages are also suggested since several tributaries of the Ntem arise on the plateau nearly exclusively drained by the Mbini River drainage (Eisenburg 2012).

The genetic divergences observed between *D. mbiniensis* in the upper Mbini River drainage and *D. notospilus* in the Ogooué and Ntem rivers suggest that no recent geodispersal events have occurred among these river drainages (*i.e.,* Mbini—Ntem & Mbini—Ogooué) or, if occurred, have not included *Distichodus* spp. If headwater capture occurs in small, first-order streams, where *Distichodus* spp. may not be present, they would not be able to participate in geodispersal events as hypothesized for *Hydrocynus* spp. (Goodier *et al* 2011). There are many species reported to co-occur in the upper Ntem, Ogooué, and Mbini rivers (Stiassny *et al*. 2007), but based on the results of this study these populations should be examined in detail. *Synodontis woleuensis* Friel & Sullivan 2008 was described from the upper Mbini River drainage in Gabon and is also known to occur in the Kyé River (upper Ntem River drainage), but only one specimen of several reported from the Kyé River was available for study and a thorough comparison between the two populations was not possible (Friel & Sullivan 2008). *Enteromius martorelli* Román 1971 is another species reported to occur in these three rivers drainages, but recent molecular studies of these populations suggest genetic divergences that may warrant specific recognition (Schmidt unpublished). This study also suggests recent geodispersal between the Ogooué and upper Ntem River as evidenced by little genetic divergences observed between those two populations of *D. notospilus* (Figs. 2, 3, & Table 2); which corresponds to previous biogeographical hypotheses (Thys van den Audenaerde 1966). There is also very little divergence among *D. notospilus* populations in the Ogooué, Ngounié, Nyanga, and Kouilou rivers suggesting ongoing or recent geodispersal among these drainages.

The presence of the new species, *Distichodus microps*, in the short coastal Mbia River provides further evidence that endemic diversity in these coastal rivers is often overlooked. These smaller coastal river drainages can remain isolated from neighboring larger basins (Schmidt *et al.* 2016; 2017), and the effects of sea level changes on paleo drainages can also promote isolation and diversity (Chakona *et al.* 2013). Studies have looked at the evolution of the Ntem River drainage and the unique inland delta in particular, but possible headwater capture events between the Mbia and Ntem rivers have not been elucidated. The proximity of headwaters of the Mbia River and southern tributaries of the Ntem River could allow for geodispersal events between the two. Interestingly, the phylogenies produced from different markers from *Distichodus* specimens in the lower and middle Ntem River are discordant (Figs. 2 & 3). *Distichodus microps* in the Mbia River and the specimens from the lower Ntem River are 2.1% divergent in CYTB but identical in their CO1 sequences. The *Distichodus* specimen from the middle Ntem is more closely aligned with topotypic *D. notospilus* in the CO1 analysis, but sister to the specimen from the lower Ntem R. and *D. microps* in the CYTB analysis (Figs. 2 & 3). The published sequences from the Ntem River populations are from previous studies (Arroyave *et al.* 2020; Calcagnotto *et al.* 2005) and these tissues/sequences may need to be verified and additional markers are needed since CO1 was not published from the upper Ntem R. population. Additional specimens from the Ntem River drainage and the smaller coastal rivers in Cameroon are needed to further understand the relationships observed herein. Interestingly, the broad inland delta in the middle reaches of the Ntem River is suggested to have been a closed basin during past uplifting events and climatic fluctuations which could have effectively isolated populations of fishes in the upper and lower reaches of the system (Runge *et al.* 2006).

This study, looking at just one group of fishes in the Mbia, Ntem, Mbini, and Ogooué rivers, suggests that the biogeography of fishes in the rivers in Lower Guinea is more complicated than previously thought. There is evidence for recent or ongoing connections between the Ogooué and upper Ntem, but the Mbini, Mbia, and lower Ntem rivers have distinct taxa suggesting that these systems, or at least these taxa, were not involved in recent geodispersal events. The new species described herein and other recent discoveries in the region (Mipounga *et al.* 2019; Schmidt & Barrientos 2019) suggest that the diversity within African rainforest rivers, especially those in Lower Guinea, is drastically underestimated (Brummett & Teugels 2002). Additional collections in the small coastal streams in Lower Guinea and within the Mbini River drainage will likely yield more new species and will allow us to better understand the biogeography and evolutionary history of these diverse fishes.

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Additional material examined

Distichodus notospilus: BMNH 2013.4.17.2-4, Syntypes, 3 ALC, 65.0–78.3 mm SL, Gabon, Ogooué River, R.B.N. Walker.—CUMV 98377, 5 ALC, Cameroon, East Cameroon, Haut-Nyong Co., Dja River at Somalomo ferry crossing, 3.37154° N, 12.73367° E, Armbruster, Friel, et al, 9 Dec 2011.—OS 19529, 2 ALC, 92.6–115.6 mm SL, Gabon, Province de l'Ogooué-Lolo, Left bank of Ogooué River at Doumé village, 0.84245° S, 12.96249° E, Cutler, Joe; Mve Beh, Jean Hervé, 15 Sept 2014.—OS 19543, ALC, 84.4 mm SL, Gabon, Province de l'Ogooué-Lolo, Left bank of Ogooué River at Doumé village, 0.84245° S, 12.96249° E, Cutler, Joe; Mve Beh, Jean Hervé, 15 Sept 2014.—OS 19544, ALC, 104.1 mm SL, Gabon, Province de l'Ogooué-Lolo, Right bank of Ogooué River at Doumé village, 0.84245° S, 12.96249° E, Cutler, 17 Sept 2014.—OS 20634, ALC, 111.0 mm SL, Gabon, Province de la Ngounié, FOUGAMOU N—Both banks of the Ngounié near the mouth of Bitoukou creek where it empties into the main channel, 1.22903° S, 10.59027° E, Cutler, Joe; Yedi, Marie-Louise; Nzigou, Franck; Tsinga Keyi, 12 Sept 2017.—OS 20757, 2 ALC, 56.7–57.8 mm SL, Gabon, Province de la Ngounié, LEBAMBA B—Louetsi River approximately 200m downstream, 2.23563° S, 11.46126° E, Mve Beh, Jean Hervé; Nzengue, Edouard, 7 Sept 2017.—OS 20917, ALC, 86.7 mm SL, Gabon, Province de la Ngounié, FOUGAMOU L—Right bank Ngounié mainstem upstream from Fougamou town, 1.27503° S, 10.62342° E, Cutler, Joe; Yedi, Marie-Louise; Nzigou, Franck; Tsinga Keyi, 11 Sept 2017.—OS 20927, 9 ALC, 54.1–95.1 mm SL, Gabon, Province de la Ngounié, SINDARA J—Confluence of the Louga River with the Ngounié, -1.10782° S, 10.70504° E, Mve Beh, Jean Hervé; Nzengue, Edouard; Mipounga, Hans, 11 Sept, 2017.—OS 20984, 2 ALC, 84.7–115.1 mm SL, Gabon, Province de la Ngounié, FOUGAMOU L—Right bank Ngounié mainstem upstream from Fougamou town, 1.27503° S, 10.62342° E, Cutler, Joe; Yedi, Marie-Louise; Nzigou, Franck; Tsinga Keyi, 11 Sept 2017.—OS 21139, 3 ALC, 46.8–86.9 mm SL, Gabon, Province de la Ngounié, LEBAMBA A—Louetsi River right bank approximately 50m downstream from the dam at Lebamba, 2.23362° S, 11.46178° E, Nzigou, Franck; Nzengue, Edouard, 7 Sept 2017.—OS 21266, 4 ALC, 91.1–96.4 mm SL, Gabon, Province de la Ngounié, SINDARA C—Below the rapids of Sindara on the left bank, in a small inundation zone with a sandy bottom that is exposed in the dry season, 1.03492° S, 10.69116° E, Mve Beh, Jean Hervé; Nzengue, Edouard; Mipounga, Hans, 10 Sept 2017.

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