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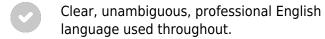
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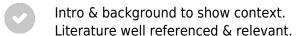
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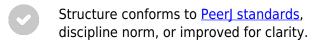
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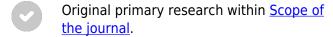




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### First DNA barcode library for the ichthyofauna of the Jos Plateau (Nigeria) with comments on potential undescribed fish species

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Located in the central region of northern Nigeria, the Jos Plateau covers approximately 9400 km<sup>2</sup> with an average altitude of 1280 m and constitutes a unique terrestrial ecoregion known as the Jos Plateau forest-grassland mosaic. The biota of the Jos Plateau include endemic elements, but very limited information is available on its ichthyofauna. This is despite the fact that the ancient plateau contributes to several large rivers spanning multiple major drainage systems including the Niger and Benue Rivers, and Lake Chad. This study provides the first species list for the fishes of the Jos Plateau based mainly on 175 DNA barcoded museum voucher specimens representing 20 species, and another three species without a DNA barcode. In total, 23 species from eight families and 17 genera were collected from the Jos Plateau including five putatively new species, four in the family Cyprinidae and one in the Clariidae. With ten species, the Cyprinidae is the most diverse fish family on the Jos Plateau, followed by Clariidae and Cichlidae, each with three species. The study also provides data on species distribution and habitat parameters including information on water chemistry that strongly suggests that selected water bodies are heavily impacted by anthropogenic activities. Urgent management steps are required to preserve the unique and diverse fish communities of the Jos Plateau and their habitats.

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#### Abstract

- Located in the central region of northern Nigeria, the Jos Plateau covers approximately 9400 km<sup>2</sup>
- with an average altitude of 1280 m and constitutes a unique terrestrial ecoregion known as the
- Jos Plateau forest-grassland mosaic. The biota of the Jos Plateau include endemic elements, but
- very limited information is available on its ichthyofauna. This is despite the fact that the ancient
- 19 plateau contributes to several large rivers spanning multiple major drainage systems including
- 20 the Niger and Benue Rivers, and Lake Chad. This study provides the first species list for the
- 21 fishes of the Jos Plateau based mainly on 175 DNA barcoded museum voucher specimens
- representing 20 species, and another three species without a DNA barcode. In total, 23 species
- 23 from eight families and 17 genera were collected from the Jos Plateau including five putatively
- 24 new species, four in the family Cyprinidae and one in the Clariidae. With ten species, the
- 25 Cyprinidae is the most diverse fish family on the Jos Plateau, followed by Clariidae and
- 26 Cichlidae, each with three species. The study also provides data on species distribution and
- 27 habitat parameters including information on water chemistry that strongly suggests that selected
- 28 water bodies are heavily impacted by anthropogenic activities. Urgent management steps are
- 29 required to preserve the unique and diverse fish communities of the Jos Plateau and their
- 30 habitats.

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#### Keywords: Checklist, COI, mitochondrial DNA, Freshwater fishes, West Africa

#### Introduction

- 34 Scientific interest in the aquatic biodiversity of the African continent dates back to the 18th
- 35 century. One important step towards an objective basis for the comparative study of its aquatic
- 36 biodiversity involved dividing the continent into more or less homogenous fish fauna regions or
- 37 ichthyological provinces (Leveque, 1997; Roberts, 1975). The Jos Plateau forest-grassland
- mosaic falls within the Nilo-Sudan ichthyological province (Stiassny et al., 2007) and is well
- 39 known as a biogeographically distinct terrestrial ecoregion (Wright & Jones, 2005). However,
- 40 the fish fauna of the Jos Plateau has received very little attention. This is surprising because the
- 41 rivers that drain from this high plateau (~ 1000 m) are unique in that they are mostly isolated
- 42 from downstream sections by waterfalls, draining geologically old granitic and basalt formations
- of different origin that span very different watersheds including the Niger River and the Chad
- and Benue Basins (Buchanan & Pugh, 1955). In fact, the only fish taxa described from the
- greater Jos Plateau are the nothobranchiid killifish *Fundulopanchax gardneri nigerianus*
- 46 (Clausen, 1963) and the labeonin cyprinid *Garra trewavasae* (Monod, 1950). Moreover,
- extremely few ichthyological records are available in museum collections as reflected in global
- databases, such as Fishbase (Froese & Pauly, 2019). However, Reid & Sydenham (1979)
- surveyed part of the Jos Plateau in their study of the fishes of the Lower Benue. Other studies on
- the fishes from the Plateau are unpublished theses (Ekeanyanwu (1980), cited in (Antony,
- 51 Eneriene, & Ufodike 1986); Oguzie (1982)). However, these studies either only refer to
- 52 commercially important fish species, some of which have been introduced (*Oreochromis*
- 53 niloticus (Linnaeus, 1758), Poecilia reticulata (Peters, 1859), Cyprinus carpio (Linnaeus,
- 54 (1758)), while there is little information on smaller fish species. Further, these studies were not



- designed to provide faunistic baseline data and lack up-to-date systematic ichthyological
- 56 expertise. As recent studies have shown; some water bodies on the Jos Plateau have been
- 57 degraded, and its undocumented fish species are potentially at risk (Ademola, 2008; Akpan &
- 58 Anadu, 1991; Anadu & Ejike, 1981).

With a surface area of 9400 km<sup>2</sup>, the Jos Plateau is located in the central part of northern Nigeria 60 and lies at an average elevation of 1280 m above sea level, with geologically ancient granite hills 61 and rock outcrops sometimes reaching another 300 m in elevation (Lee, 1972). The area is 62 63 drained by several major rivers which are important sources of domestic water for this densely populated region. The watershed pattern on the Jos Plateau is unusual as its rivers drain into 64 three major river systems in Nigeria: rivers flowing northeast drain into the Kano River and Lake Chad (Chad drainage); east flowing rivers drain into the Gongola River, a tribituary of the Benue 66 River drainage; rivers flowing south drain as well to the Benue River; and west flowing rivers 67 drain into the Kaduna River which feeds the Niger River (Niger drainage system). Seventeen 68 69 rivers (Delimi, N'gell, Gurum, Rukuba, Jarawa, Shen-fusa, Foron, Kassa, Assop, Daffo, Gindiri, Tahoss, Korot, Magurii, Maijuju, Bokkos and Farin-ruwa) were surveyed in this study. The 70 71 Delimi River, however, is the main drainage system of the Jos Plateau and a major tributary of

the Shari River system which flows north-east before it drains into Lake Chad, covering a

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The Jos Plateau has a wet-dry climate which is largely determined by its elevation and position across the seasonal shifts of the Inter-Tropical Convergence Zone (ITCZ). Generally, the average temperatures on the Jos Plateau are lower than those in the rest of Nigeria. The dry season is dominated by the north-east trade winds while the beginning of the rainy season is marked by thunderstorms of high intensity. The plateau lies within the northern Guinea Savanna vegetation zone, an open woodland with tall grasses. Though the area has its own unique vegetation, this has been considerably altered by human activities including mining, agriculture, grazing, and the demand for timber and fire wood (Olowolafe, 2008).

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This study provides first comprehensive information on the ichthyological diversity of the Jos Plateau based on DNA barcodes and morphological identification of voucher specimens.

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#### **Materials and Methods**

distance of about 900 km.

#### 88 Fieldwork, study sites and species identification

- 89 Sampling was carried out on several field trips to the Jos Plateau during the dry season
- 90 (December 2013, January April 2014 and March 2016). Fishes were collected at 37
- 91 strategically selected sampling locations in 17 major rivers on the Jos Plateau (supplmentary
- 92 table 1 provides detailed locality information; Figures 1 & 2). Fishes were captured using
- 93 different methods (cast net, gill net, frame net, fish traps) depending on the size and habitat of the
- 94 site. Freshly caught specimens were euthanized with an overdose of anaesthetic (Benzocaine,
- 95 MS-222). Representative specimens of all taxa from each collection at each sampling site were
- 96 individually tagged, a pectoral fin clip was sampled, and whole specimens were photographed
- and fixed and eventually preserved following Neumann (2010). Tissues were placed immediately
- 98 into individually labelled tubes filled with 96% ethanol while specimens were preserved in 5%



formalin. Specimens were exported on the 17<sup>th</sup> of September, 2014 and subsequently deposited in the fish collection of SNSB-ZSM (Germany). We followed all applicable international and national guidelines of animal use and ethical standards for the collection of samples. The permit to survey in the fishes of the Jos area was obtained from the GWOM SOPP palace, Riyom, Plateau State.

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After a preliminary identification of specimens in the field to a genus level, preserved specimens were subsequently assigned to a species using standard identification keys (Paugy, Leveque & Teugels, 2003; Stiassny, Teugel, & Hopkins, 2007). In difficult cases, we consulted additional relevant literature (i.e. original descriptions) for species characteristics. If morphometric measurements were required, we measured those following protocols in the identification keys using a manual caliper. Important meristic traits were examined using a stereomicroscope (Leica MZ6) as well as with a digital X-ray using a Faxitron Ultrafocus LLC X-ray unit.

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#### Notes on collection sites (see Figure 2)

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Most of the collection sites were situated in perennial or annual headwaters of major rivers in Nigeria. On average, they originate as small streams before they flow over long stretches of fine-grained alluvial soils with few rocks. After a substantial increase in stream size, they reach the margin of the high plateau before descending, often in rapids and cascades, to the lowland plains below. On the plateau, water flow is generally moderate, whereas in rapids and waterfalls it may be torrential. Vegetation along the river banks provides partial shading and is best characterized as a savanna-forest mosaic. None of the sampled locations was pristine; all were impacted by farming, domestic waste deposition, and industrial discharges or by mining.

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#### Water chemistry (supplementary table 3)

The physico-chemical characteristics of the Assop, N'gell, Rukuba, and Tahoss rivers were 125 assessed following standard procedures. The water temperature was measured in situ using 126 mercury-in-glass bulb thermometer with calibration range ( $-10^{\circ}$  C  $- 110^{\circ}$  C). Samples for the 127 analysis of physico-chemical parameters were collected in clean 2 litre plastic bottles. However, 128 dark reagent bottles were used for Biochemical Oxygen Demand (BOD<sub>5</sub>) sample collections and 129 kept in a dark cupboard for five days for subsequent analysis. The pH, conductivity, and total 130 131 dissolved solids (TDS) of each sample were determined using a PCE-PHD1 multi-parameter meter. The analytical determinations of the physico-chemical water quality parameters were 132 carried out within the holding time of each parameter (Ademoroti, 1996; APHA, 1995; 133 Golterman, Clymo, & Ohnstadt, 1978). The BOD<sub>5</sub> was determined by iodiometric titration 134 135 (Golterman, Clymo, & Ohnstadt, 1978). Colour was investigated using a colourimeter (Jenway 6051 model) standardized with a set of potassium chloroplatinate-cobalt solutions as Pt-Co 136 137 standards, while turbidity was assessed using a turbidimeter (Ademoroti, 1996). Sulphate (SO<sub>4</sub><sup>2-</sup>) and nitrate (NO<sub>3</sub>-) were determined by spectrophotometric methods (Ademoroti, 1996). 138 Magnesium (Mg<sup>2+</sup>) was determined by the complexio-metric titration method using Na<sub>2</sub>EDTA 139 (Golterman, Clymo, & Ohnstadt, 1978). All recommended quality control (QC) and quality 140 assurance (QA) measures were taken for each determination. 141



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#### Laboratory protocols

- For DNA extraction, we subsampled the ethanol-preserved fin clips and transferred them to 96-
- well plates. These plates were sent to the Canadian Center for DNA Barcoding (CCDB, Guelph,
- 146 Canada) for DNA extraction, PCR amplification, and Sanger sequencing (all protocols are
- avilable online: ccdb.ca/resources/). PCR amplification of all samples employed the fish primer
- 148 cocktail C FishF1t1/C FishR1t1 (Ivanova et al., 2007) for the CO1 (mitochondrial cytochrome
- 149 c oxidase subunit 1) barcode fragment. The same primer cocktail was used for subsequent
- bidirectional Sanger sequencing reactions. The resulting sequence data and trace files were
- uploaded to BOLD (Ratnasingham & Hebert, 2007) as well as all corresponding metadata
- voucher information (i.e. locality data, altitude, taxonomic classification, habitat images, primer
- information etc.) and are publicly accessible in the "NGAJO" dataset in BOLD. DNA barcodes
- were also subsequently uploaded to GenBank (accession numbers available after manuscript
- 155 review).

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#### Molecular data anaylsis

- We used the "Sequence Analysis" toolbox on BOLD and restricted analysis to sequences >500
- bp. MUSCLE (Edgar, 2004) was employed to align the sequences and sequence divergences,
- 159 (i.e. mean and maximum intraspecific variation as well as the minimum genetic distance to the
- nearest-neighbour species) were calculated using the 'Barcode Gap Analysis' tool on BOLD
- employing the Kimura-2-Parameter (K2P) distance metric (Puillandre et al., 2012). As well a
- neighbour-joining (NJ) tree was constructed to graphically represent the BIN clusters. Within
- BOLD, closely similar COI barcode sequences are assigned a globally unique identifier, termed
- a "Barcode Index Number" or BIN (Ratnasingham & Hebert, 2013). The "BIN Discordance"
- report on BOLD was used to reveal cases where BINs were shared between species clusters or
- restricted to certain species clusters (BIN Discordance and BIN Sharing).
- In addition, we created additional CO1 DNA barcode sequence datasets for all genera for which
- putatively undescribed species (see below) appeared to be present on the Jos Plateau region. This
- was done by screening the BOLD database for all available CO1 sequences for each genus (with
- a minimum length of 500bp) using the "Record Search" tool. Subsequently, we constructed a NJ
- tree for each genus as indicated above.

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#### Results

- Nearly 800 specimens were collected during the survey and, based on morphological keys, they
- were assigned to 23 species (8 families, 17 genera), some of which appeared to represent
- undescribed species (see below for further information). Potentially new species fell into four
- genera Clarias (Scopoli, 1777), Enteromius (Cope, 1867), Labeo (Cuvier, 1816) and
- 178 *Labeobarbus* (Rüppell, 1835). Further, two species were observed to have different phenotypes
- 179 (morphs) Coptodon zillii (Gervais, 1848) and Enteromius perince (Rüppell, 1835). Poecilia
- 180 reticulata (Peters, 1859) was the only non-native species found on the Jos Plateau. The order and
- family with the highest species diversity was the Cypriniformes (family Cyprinidae) with 10 of
- 23 species so it represented nearly half of all recorded species. It was followed by the
- Siluriformes (Mochokidae, Clariidae) with five species and by Cichliformes (Cichlidae) with



- three species. The Osteoglossiformes (Mormyridae) and Cyprinodontiformes (Nothobranchiidae,
- Poecilidae) were each represented by two species while there was a single species of
- 186 Characiformes (Alestidae). Representative specimens of all species and morphs found on the Jos
- Plateau are depicted in Supplementary Figures 1-4. The distribution of the species according to
- sampling sites are noted in supplementary table 2.

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#### DNA barcode library

- 191 In total, 176 CO1 DNA barcode sequences were generated with lengths >635 bp (1 shorter
- barcode (444 bp) was excluded from further sequence analysis). These sequences represented 20
- of the 23 fish species collected on the Jos Plateau. Three species lacked barcode coverage
- because of suboptimal tissue preservation and low sample size: *Chiloglanis* cf. *benuensis* (Daget
- 8 Stauch 1963), Clarias cf. gariepinus (Burchell, 1822) and Marcusenius mento (Boulenger,
- 1890). Only a single barcode was obtained for five species (Heterobranchus longifillis, Labeo sp.
- 197 Assop, *Labeobarbus bynni*, *Synodontis violaceus*, *Poecilia reticulata*) whereas the other species
- all had two or more sequences. Overall, CO1 amplification was successful for 92% of the 190
- submitted specimens. The sequence divergence values for the CO1 barcode region
- 200 (supplementary table 4) show that most species of the Jos Plateau possessed very low
- 201 intraspecific genetic distance values.

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- A NJ tree (Figure 3) showed that barcodes reliably discriminated most species as all conspecific
- specimens were recovered as monophyletic lineages assigned to a single BIN (19 BINs in total).
- 205 Oreochromis niloticus and Sarotherodon galilaeus shared a BIN, but formed two different
- 206 haplotype clusters with a low sequence divergence (see Figure 3). These results are similar to
- 207 those in an earlier barcoding study on the freshwater fishes of south-eastern Nigeria (Nwani et
- 208 al., 2011) which found very low genetic divergence between O. niloticus and Sarotherodon
- 209 galilaeus boulengeri. The low inter-specific variation between these two species may reflect
- 210 introgressive hybridisation as the species hybridize in captivity (Otubusin, 1988) and are
- commonly farmed in aquaculture facilities throughout Nigeria (Ayinla, 2007). In any case, the
- 212 reason for the BIN sharing between the two species is worth further investigation.

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#### Notes on new candidate species from the Jos Plateau

- Based on the COI results and morphological data, this study suggests the presence of at least five
- 216 candidate species (following the terminology regarding potentially undescribed species of
- 217 (Padial *et al.*, 2010; Vieites *et al.*, 2009); four Cyprinidae, and one Clariidae.
- Among the potentially new cyprinid species, two belong to the genus *Enteromius* (Cope, 1867).
- 219 This genus was recently revalidated by (Yang et al., 2015) for the small diploid African barbs
- 220 formerly placed in the genus *Barbus* (Daudin, 1805), but is known to be polyphyletic (Yang et
- 221 al., 2015). Although a comprehensive taxonomic re-evaluation of the diploid African barbs is
- 222 needed, this interim generic assignment is currently generally accepted (Englmaier, Tesfaye, &
- Bogutskaya 2020; Hayes & Armbruster, 2017; Martin & Chakona, 2019; Skelton, 2016).



- The first species, herein referred to as *Enteromius* sp. Silver (see supplementary Figure 2 C, was
- 225 widespread on the Jos Plateau, being present at most collection sites. It is phenotypically similar
- 226 to Enteromius callipterus (Boulenger 1907), but differs by its possession of a faint greyish
- longitudinal band (vs. no lateral colour pattern for *E. callipterus*). In addition, the dorsal spot in
- 228 the dorsal fin is less pronounced, i.e. irregular and not deep black but rather greyish with
- 229 translucent orange (base colour of the dorsal fin), as compared with E. callipterus (Leveque,
- 230 2003). In a NJ tree based on all available CO1 sequences of *Enteromius* on BOLD (see
- supplementary Figure 5) *Enteromius* sp. Silver was a distinct mitochondrial lineage not
- clustering with *E. callipterus*.
- 233 The second candidate species, referred herein as *Enteromius* sp. Gold (see supplementary Figure
- 234 2 D), was collected from four rivers (N'gell, Delimi, Shen Fusa, Rukuba). It is a comparatively
- small species easily recognized by its yellowish to golden body coloration and small irregular
- blackish spots forming a more or less confluent longitudinal band on the sides which terminates
- before the base of the caudal fin in a slightly larger blackish spot. Morphologically, *Enteromius*
- sp. Gold could not be assigned to any *Enteromius* species known from West Africa or Lower
- Guinea based on standard keys (Leveque, 2003; De Weirdt et al., 2007). Interestingly, a NJ tree
- based on all CO1 sequences for *Enteromius* on BOLD (see supplementary Figure 5) revealed
- 241 that Enteromius sp. Gold was a sister taxon to Enteromius cf. macrotaenia (Worthington 1933), a
- species known from south-eastern Africa. The sister group of this clade included *Enteromius*
- 243 lukusiensis (David & Poll, 1937), Enteromius greenwoodi (Poll, 1967), and the goldie barb
- 244 Enteromius pallidus (Smith, 1841), which is endemic to the eastern Cape Fold Ecoregion of
- South Africa (Martin & Chakona, 2019). These results suggest the closest relatives of
- 246 Enteromius sp. Gold are found in the southern parts of Africa, raising interesting questions on its
- biogeographic history (i.e., when and how it colonized the Jos Plateau area).
- The third potentially new cyprinid species, referred herein as *Labeo* sp. Assop, was only
- collected from the Assop River (see supplementary Figure 2 G). It shows affinities with *Labeo*
- 250 parvus (Boulenger 1902) which is widespread on the Jos Plateau. A NJ tree including all
- available CO1 sequences for *Labeo* on BOLD (see supplementary Figure 6) revealed that *Labeo*
- sp. Assop was a sister taxon to a clade including *Labeo lukululae* (Boulenger, 1902) *Labeo*
- 253 quadribarbis (Poll & Gosse, 1963), and Labeo sp. aff. rectipinnis (Tshibwabwa, 1997).
- The fourth potentially new cyprinid species, referred herein as *Labeobarbus* sp. Assop, is the
- second candidate species collected only from the Assop River (see supplementary Figure 3 A).
- 256 It could not be assigned to any known *Labeobarbus* species found in western Africa based on
- morphological data and following the key in Leveque, (2003). The collected specimens were
- recovered as sister group to *Labeobarbus brevispinis* (Holly, 1927) in a NJ tree based on all
- 259 available CO1 sequences (see supplementary Figure 6).
- The last candidate species, referred herein to as *Clarias* sp. White dots (see supplementary
- 262 Figure 4D), was found in four rivers (N'gell, Gurum, Kassa, Rukuba). It could not
- unambiguously assigned to any described *Clarias* species known from Western Africa based in
- standard morphological keys (Teugels, 2003; Teugels et al., 2007). Nevertheless, it is similar to
- 265 Clarias agboyienesis (Sydenham 1980), a species known from West African coastal basins
- 266 including the lower course of the Niger. The latter species is, however, characterized by a very



light coloration with yellowish brown flanks and a light grey belly whereas the body ground 267 coloration of *Clarias sp.* White dots is dark with larger specimens having small whitish to 268 yellowish spots on the flanks and on unpaired fins. Interestingly, the sequenced specimens of 269 Clarias sp. White dots clustered with sequences of Clarias gabonensis (Günther, 1867) in the NJ 270 tree based on all CO1 sequences for *Clarias* on BOLD (see supplementary Figure 7). The 271 coloration of C. gabonensis is either uniform brownish yellow or marbled with small yellowish 272 spots (Hanssens, 2007; Teugels et al., 2007) which shows some correspondence to the coloration 273 pattern of *Clarias* sp. White dots, but it has a distinctively shorter head. Furthermore, C. 274 gabonensis occurs in the Congo basin and different drainage systems of Lower Guinea, e.g. the 275 Ogowe, Noya, Kouilou and Chilango rivers (Hanssens, 2007; Teugels et al., 2007), and it was 276 only recently recorded from Nigeria (Nwani et al., 2011). However, the taxonomic assignment of 277 the Nigerian specimens of *Clarias gabonensis* might be incorrect. Itshould be carefully validated 278 as the identification of *Clarias* specimens to a species is often challenging. Indeed, C. 279 gabonensis was revealed to be polyphyletic in our NJ tree (see supplementary Figure 7), 280 indicating probable misidentifications or cryptic species. Our sequences of *Clarias* sp. White 281 dots group with some Nigerian C. gabonensis in one of the larger clades identified as C. 282 gabonensis. Taken together it would be premature to assign our specimens of Clarias sp. White 283 dots to Clarias gabonensis until more thorough morphological and genetic investigations are 284 285 made. A thorough taxonomic investigation and the formal description of these unconfirmed candidate 286 species is in progress and will be presented in a follow up study. 287 In addition to these five putatively new species, we observed substantial phenotypic variation in 288 two species. The first, Coptodon zillii, is widespread on the Jos Plateau and occurs in two color 289 morphs. Both morphs occurred sympatrically at one single location (Delimi River). Although 290 differences between these morphs is modest, the adults of the two morphs have different 291 292 colouration. The flanks of the first morph (see supplementary Figure 1) are yellowish whereas those of the second morph are whithish. Furthermore, the first morph has a pale green upper lip 293 and and a whitish lower lip while the second morph has greenish to brownish upper lips and 294 blueish lower lips. Members of the two morphs formed two distinct mitochondrial lineages with 295 low genetic divergence (see Figure 3). 296 Specimens of *Enteromius perince* (Rüppell, 1835) also showed clear intraspecific variation. 297 Most specimens showed the colouration typical of this species (i.e., silver body ground 298

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#### Discussion

By providing the first DNA barcode library for the ichthyofauna of the Jos Plateau, this study represents important progress in the taxonomic study of Nigerian fishes as few earlier investigations have employed DNA barcoding (Falade, Opene, & Benson, 2016; Iyiola *et al.*, 2018; Nneji *et al.*, 2020; Nwakanma, Ude & Unachukwu, 2015; Nwani *et al.*, 2011; Sogbesan *et al.*, 2017; Ude *et al.*, 2020). Aside from providing barcode records for known species, the results

coloration with three spots aligned along the mid-lateral line, but a few specimens had additional

spots. These individuals resemble the so-called "lepidus form" (Leveque, 2003), but no CO1

barcodes were recovered from them so their divergence from the nominate form is uncertain.



- 309 suggest the presence of at least five species new to science. As well, the new DNA barcode
- 310 records constitute an important contribution to the growing reference library for Nigerian
- freshwater fishes, especially because most of the species are shared with the Niger, Benue, and
- Chad River drainages, the major river systems in Nigeria.
- 313 Most rivers on the Jos Plateau flow into the lower Benue River drainage and hence a part of the
- wider Nilo-Sudan Ichthylogical Province (Roberts, 1975). Among the areas sampled by Reid &
- Sydenham, (1979) in their study of fishes from the lower Benue river basin, two were on the Jos
- Plateau. They reported eight species (Enteromius nigeriensis, Enteromius ablabes (as "Barbus
- spurellii), Labeobarbus\_cf. wurtzi (as "Barbus cf. holasi"), Garra waterloti or G. ornata, Garra
- 318 trewavasae or G. waterloti, Fundulopanchax gardneri, E. ablabes, and a species provisionally
- identified as G. waterloti). However, they could not identify the original collection site for G.
- 320 trewavaseae described by (Monod, 1950) as 'a small tributary of the Gongola River, South West
- 321 of the Bargesh district'.
- Just two of the species (G. trewavasae, F. gardneri) reported by Reid & Sydenham (1979) were
- encountered during our study, perhaps because we did not resample their sites (see Figure 1).
- Therefore, the species count for the Jos Plateau area based on this study might be expanded to
- include four more species (E. nigeriensis, E. ablabes, Labeobarbus cf. wurtzi, G. waterloti).
- 326 Alternatively, some species reported earlier may reflect misidentifications. This might be the
- case for specimens assigned to "Barbus spurelli", a species described by Boulenger, (1913) from
- Ghana that was later synonymized with *Barbus ablabes* (now *E. ablabes*) by Leveque, (1983) as
- he was unable to find any differences between these two taxa apart from differences in the
- coloration; these differences are the presence of a longitudinal stripe on the middle of the sides in
- E. ablabes, which should be absent in "Barbus spurelli". Enteromius sp. Silver has only a very
- faint longitudinal stripe and matches with some aspects of the description of "Barbus spurelli"; it
- has, e. g., dark-edged scales which are darker at the base, indicating that our specimens of E. sp.
- 334 Silver might be conspecific with the specimens identified as "Barbus spurelli" by Reid &
- Sydenham (1979). In fact, E. sp. Silver is readily distinguished from E. ablabes by its possession
- of both longer barbels and more than 3.5 scales between the origin of the dorsal fin and the
- lateral line (Paugy, Leveque & Teugels, 2003) However, without careful re-examination of fish
- specimens collected by Reid & Sydenham, (1979) this remains speculative. It is clear that the
- sampling locations visited by Reid & Sydenham, (1979) should be resampled to help resolve the
- 340 identity of the species at these two localities
- The overall number of fish species (23) recorded from the Jos Plateau is low in comparison with
- other river basins in Nigeria such as the Imo (142 species) and Ogun (119 species). As well,
- more than half (12) of the fish species on the Jos Plateau are found in the Benue, Chad, and
- Niger drainages (F. gardneri, M. hasselquisti, B. nurse, E. perince, L. parvus, R. nigeriensis, R.
- 345 senegalensis, H. longifillis, S. violaceous, O. niloticus, S. galilaeus, T. zillii). The only described
- 346 fish species endemic to the area is G. trewavasae and the subspecies Fundulopanchax gardneri
- 347 nigerianus rendering the regional endemism low, and M. mento is found in both the Upper Niger
- and Lower Benue basin but not in the Chad basin. However, if the potentially new species
- detected in this study are considered, the level of endemicity would reach  $\sim 30 \%$  (7 out 23
- recorded species), a result emphasizing the need for further taxonomic and biogeographic
- 351 research.



352 353 354 355 356 357 358 359 360 361 362 363 364 365	The closest relative of <i>Enteromius</i> sp. Gold seems to be found in Southern Africa which underscores the important gap in knowledge of the biogeography of the small African <i>Enteromius</i> . This lack in knowledge is largely due to the limited understanding of phylogenetic interrelationships within the group despite past studies (Howes, 1991; Rainboth, 1991). However, the recent study of Yang <i>et al.</i> , (2015) has shed more light on the biogeographical distribution of the cyprinine fishes. Their study suggests that the distribution of the cyprinids could not have been influenced by Gondwanaland separation as the dispersal events of the cyprinids are much more recent than the separation between 50-80 mya. Their findings suggest several independent dispersal events for cyprinids. Among the Smiliogastrini, two hypothetical dispersal events were identified; those involving the small-sized African barbs and allies and another involving the small-sized Asian <i>Puntius</i> and allies. However, there is inadequate information to explain the factors that might have shaped the odd distribution of the newly found <i>Enteromius</i> sp. Gold between western and southern Africa, if the preliminary DNA barcode based association is maintained.
366 367 368 369 370 371 372 373	The Jos Plateau is dotted with waterfalls along its main rivers (Farin Ruwa, Kwall, Assop, Kura) at the southern, western, and eastern margins of the Jos Plateau respectively. Waterfalls are ordinarily viewed as a dispersal barrier to fishes (Torrente-Vilara <i>et al.</i> , 2011), but the impact of those on the Jos Plateau appear to be limited since upstream and downstream populations of fishes did not show sequence divergence at CO1. Furthermore, not all rivers that originate on the Jos plateau feature a waterfall because the north-eastern part of the Plateau slopes gradually towards the lowlands (Payne & Firefinch, 1998). <i>G. trewavasae</i> has apparently not dispersed beyond the Jos Plateau area because of the lack of comparatively cold streams in the lowlands.
374 375 376 377 378 379 380 381 382 383 384	The sampling program for this study occurred during the dry season when most water bodies were nearly dry, being fragmented into remnant pools along the river course. Only a few were flowing. This situation suggests that rivers on the Jos Plateau are already heavily impacted by climate change that has reduced rainfall in the area by more than 5% (Fasona & Omojola, 2005). On the positive side, most water quality parameters were within ranges tolerated by fishes except the high turbidity and colour of many water bodies, especially on the N'gell River (supplementary table 3). The fact that all assessed water bodies were turbid underscores widespread anthropogenic impacts across the plateau, indicating the need for urgent management steps to safeguard fish communities from intensified impacts in the future. Some of the key activities leading to this high turbidity were domestic washing, small-scale mining, making building blocks, and the release of industrial waste (Figure 2).

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#### Conclusion

In conclusion, this study provides a valuable COI barcode dataset which will serve as reference for further studies on the ichthylogical diverstiy of western Africa. It indicates the need for further taxonomic studies on Nigerian fishes with an obvious need to evaluate the taxonomic status of the five potentially new species found on the Jos Plateau.

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#### **Author contributions**

408	M.O.P. and U.K.S designed the study while M.O.P organized and conducted the sampling.
409	Specimen identification was done by F.D.B.S, U.K.S and M.O.P. Subsequent analysis and
410	corresponding figures and tables were prepared by F.D.B.S. and M.O.P. F.D.B.S. and M.O.P
411	wrote togehter the first draft of the manuscript and U.K.S. and P.D.N.H. provided edits and
412	comments. All authors read and approved the final manuscript.

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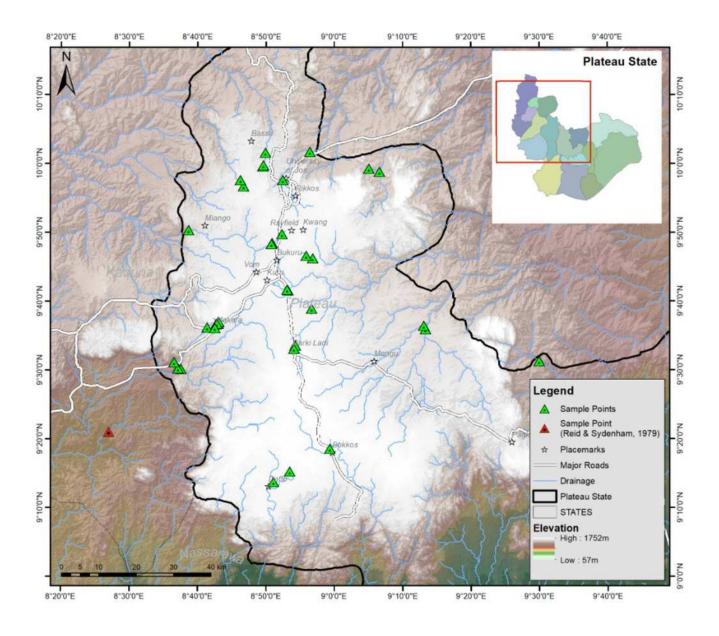


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### Figure 1

Map of the Jos Plateau area showing the sampling locations (green) examined in this study and the sampling point (red) examined by Reid & Sydenham (1979).



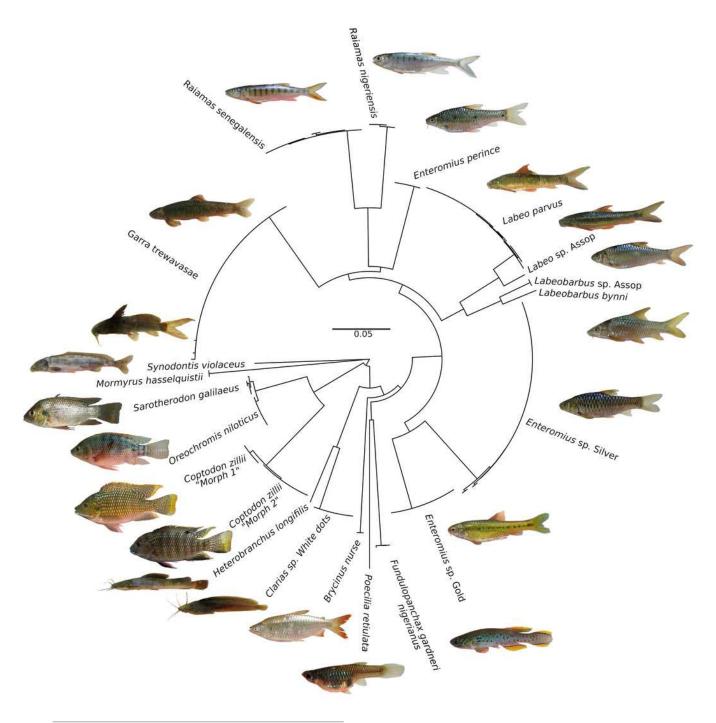
### Figure 2

Overview of sampling sites. A) Magurji River B) Dilimi River (highly polluted C) Assop River D) Gindiri River (seasonal) E) Shen Fusa River (seasonal) F) Rukuba River (seasonal) G) Artisanal mining at Ngell River H) Cattle trough at the Assop River



### Figure 3

Neighbour-Joining tree based on 176 CO1 barcode sequences of fish species occurring in the Jos Plateau, created in BOLD using "Taxon ID tree" tool. The circularized NJ tree was created using FigTree v1.4.4 ( http://tree.bio.ed.ac.uk/software/figtre/)



Order	Family	Genus			CO1 barcode	Distribution	
G: 11:0	C' 11'1	G 1	1111 O. F. 1 T.	(G : 1040)	available:	11 12 10 20 21	
Cichliformes	Cichlidae	Coptodon	zillii (Morph I)	(Gervais, 1848)	Yes	11, 13, 18, 28, 31.	
Cichliformes	Cichlidae	Coptodon	zillii (Morph II)	(Gervais, 1848)	Yes	1, 4, 10, 15, 17, 22, 27 28, 36.	
Cichliformes	Cichlidae	Oreochromis	niloticus	(Linnaeus, 1758)	Yes	2, 13, 14, 23, 28, 36.	
Cichliformes	Cichlidae	Sarotherodon	galilaeus	(Linnaeus, 1758)	Yes	14, 19, 24.	
Cypriniformes	Cyprinidae	Enteromius	perince	(Rüppell, 1835)	Yes	1, 5, 11, 12, 13, 15,16, 18, 22, 29, 31, 33.	
Cypriniformes	Cyprinidae	Enteromius	sp. Silver	Unconfirmed candidate species[PH1]	Yes	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 22, 23, 24, 27, 29, 31, 32, 33.	
Cypriniformes	Cyprinidae	Enteromius	sp. Gold	Unconfirmed candidate species	Yes	1, 5, 14, 15, 18, 19, 22, 29, 36.	
Cypriniformes	Cyprinidae	Garra	trewavasae	(Monod, 1950)	Yes	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 14, 15, 16, 22.	
Cypriniformes	Cyprinidae	Labeobarbus	bynni	(Fabricius, 1775)	Yes	2	
Cypriniformes	Cyprinidae	Labeobarbus	sp. Assop	Unconfirmed candidate species	Yes	4	
Cypriniformes	Cyprinidae	Labeo	parvus	Unconfirmed candidate species	Yes	1, 2, 15, 16, 19, 23, 29, 31.	
Cypriniformes	Cyprinidae	Labeo	sp. Assop	Unconfirmed candidate species	Yes	4	
Cypriniformes	Cyprinidae	Raiamas	senegalensis	(Steindachner, 1870) Ye		2, 17, 23/2, 4, 14, 18, 24, 25.	
Cypriniformes	Cyprinidae	Raiamas	nigeriensis	((Daget, 1959)	(Daget, 1959) Yes 19, 24 and 2[PH2		
Cyprinodontiformes	Nothobranchidae[PH3]	Fundulopanchax	gardneri	(Boulenger, 1911)	Yes	4, 5, 11.	
Cyprinodontiformes	Poecilidae[PH4]	Poecilia	reticulata	(Peters, 1859)	Yes	29	
Characiformes	Alestidae	Brycinus	Nurse	(Rüppell, 1832)	Yes	2	
Osteoglossifromes	Mormyridae	Mormyrus	hasselquistii	(Valenciennes, 1847)	Yes	es 2	
Osteoglossifromes	Mormyridae	Mracusenius[PH5]	mento (Boulenger, 1890) No		No	2	
Siluriformes	Mochokidae	Chiloglanis	cf. benuensis	(Daget & Stauch, 1963)	No	2	
Siluriformes	Mochokidae	Synodontis	violaceus	(Pellegrin, 1919)	Yes	2	
Siluriformes	Clariidae	Clarias	cf. gariepinus	(Burchell, 1822)	No	14	
Siluriformes	Clariidae	Clarias	sp. White dots	lots <b>Potential new species</b> Yes 13, 17, 20, 23.		13, 17, 20, 23.	
Siluriformes	Clariidae	Heterobranchus	longifilis	(Valenciennes, 1840)	Yes	4	

Order	Family	Species	Number of barcode sequences	Bacrcode Index Number (BIN)	Mean intraspecific distance	Maximum intraspecific distance	Nearest Neighbour	Nearest Species	Average genetic distance to nearest neighbour species
Characiformes	Alestidae	Brycinus nurse	2	BOLD:AAI8453	0	0	NGAJO135-14	Labeobarbus sp. Assop	21.95
Cichliformes	Cichlidae	Coptodon zillii	14	BOLD:AAB9042	0.21	0.46	NGAJO007-14	Oreochromis niloticus	12.3
Cichliformes	Cichlidae	Oreochromis niloticus	8	BOLD:AAA6537	0	0	NGAJO053-14	Sarotherodon galilaeus	0.46
Cichliformes	Cichlidae	Sarotherodon galilaeus	5	BOLD:AAA6537	0.15	0.31	NGAJO007-14	Oreochromis niloticus	0.46
Cypriniformes	Cyprinidae	Enteromius perince	5	BOLD:ACR9287	0	0	NGAJO008-14	Enteromius sp. Gold	12.38
Cypriniformes	Cyprinidae	Enteromius sp. Gold	13	BOLD:ACR9412	0	0	NGAJO027-14	Enteromius perince	12.38
Cypriniformes	Cyprinidae	Enteromius sp. Silver	38	BOLD:ACR9413	0.05	0.46	NGAJO008-14	Enteromius sp. Gold	13.07
Cypriniformes	Cyprinidae	Garra trewavasae	31	BOLD:ACR9351	0.04	0.46	NGAJO182-14	Labeo parvus	15.81
Cypriniformes	Cyprinidae	Labeo parvus	21	BOLD:AAA4266	0.12	0.31	NGAJO095-14	Labeo sp. Assop	4.14
Cypriniformes	Cyprinidae	Labeo sp. Assop	1	BOLD:ACR9419	N/A	0	NGAJO067-14	Labeo parvus	4.14
Cypriniformes	Cyprinidae	Labeobarbus bynni occidentalis[PH1]	1	BOLD:ACR9402	N/A	0	NGAJO135-14	Labeobarbus sp. Assop	6
Cypriniformes	Cyprinidae	Labeobarbus sp. Assop	2	BOLD:ACR9057	0	0	NGAJO104-14	Labeobarbus bynni occidentalis	6
Cypriniformes	Cyprinidae	Raiamas nigeriensis	4	BOLD:ACR9172	0.08	0.15	NGAJO048-14	Raiamas senegalensis	14.08
Cypriniformes	Cyprinidae	Raiamas senegalensis	13	BOLD:ACR9171	0.13	0.31	NGAJO032-14	Raiamas nigeriensis	14.08
Cyprinodontiformes	Nothobranchiidae	Fundulopanchax gardneri	3	BOLD:ACR9440	0.1	0.15	NGAJO008-14	Enteromius sp. Gold	22.56
Cyprinodontiformes	Poeciliidae	Poecilia reticulata	1	BOLD:ACE3484	N/A	0	NGAJO162-14	Enteromius perince	22.96
Osteoglossiformes	Mormyridae	Mormyrus hasselquistii	2	BOLD:AAL5815	0	0	NGAJO129-14	Synodontis violaceus	22.68
Siluriformes	Clariidae	Clarias sp. White dots	8	BOLD:AAJ1105	0	0	NGAJO088-14	Heterobranchus longifilis	10.87
Siluriformes	Clariidae	Heterobranchus longifilis	1	BOLD:AAM0293	N/A	0	NGAJO045-14	Clarias sp. White dots	10.87
Siluriformes	Mochokidae	Synodontis violaceus	1	BOLD:ACH7500	N/A	0	NGAJO011-14	Clarias sp. White dots	19.96