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FROM THE COVER

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Unlocking biodiversity and conservation studies in high-diversity environments using environmental DNA (eDNA): A test with Guianese freshwater fishes

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Abstract

Determining the species compositions of local assemblages is a prerequisite to understanding how anthropogenic disturbances affect biodiversity. However, biodiversity measurements often remain incomplete due to the limited efficiency of sampling methods. This is particularly true in freshwater tropical environments that host rich fish assemblages, for which assessments are uncertain and often rely on destructive methods. Developing an efficient and nondestructive method to assess biodiversity in tropical freshwaters is highly important. In this study, we tested the efficiency of environmental DNA (eDNA) metabarcoding to assess the fish diversity of 39 Guianese sites. We compared the diversity and composition of assemblages obtained using traditional and metabarcoding methods. More than 7,000 individual fish belonging to 203 Guianese fish species were collected by traditional sampling methods, and ~17 million reads were produced by metabarcoding, among which ~8 million reads were assigned to 148 fish taxonomic units, including 132 fish species. The two methods detected a similar number of species at each site, but the species identities partially matched. The assemblage compositions from the different drainage basins were better discriminated using metabarcoding, revealing that while traditional methods provide a more complete but spatially limited inventory of fish assemblages, metabarcoding provides a more partial but spatially extensive inventory. eDNA metabarcoding can therefore be used for rapid and large-scale biodiversity assessments, while at a local scale, the two approaches are complementary and enable an understanding of realistic fish biodiversity.

KEYWORDS
environmental DNA, fish assemblage, metabarcoding, reference database, tropical

1 | INTRODUCTION

Evaluating the distribution or occurrences of organisms is a crucial step in biodiversity science. Achieving these tasks can be difficult when assemblages are species-rich and/or when the organisms cannot be directly observed (Murphy & Willis, 1996). This is particularly true for fish in tropical freshwater ecosystems, where local assemblages contain dozens of species, and their observation is limited by water turbidity, depth and current velocity. Hence, fish are often sampled using nets, electricity and even toxicants (Allard et al.,

2014; Murphy & Willis, 1996; Portt, Coker, Ming, & Randall, 2006). These traditional methods are selective towards species (Gunzburger, 2007), and some of these methods, such as gill nets and toxicants, are destructive to the fauna (Dalu, Wasserman, Jordaan, Froneman, & Weyl, 2015; Snyder, 2003). Their use for scientific purposes is highly debated, and the development of alternative nondestructive methods is urgently needed to comply with ethics and laws on animal welfare and biodiversity conservation (Ellender, Becker, Weyl, & Swartz, 2012; Hickey & Closs, 2006; Thomsen & Willerslev, 2015). With advances in sequencing technologies, the use of environmental DNA (eDNA), *that is*, total DNA present in environmental samples, has drawn a large amount of attention as a method to study biodiversity in the last few years (Taberlet, Coissac, Hajibabaei, & Rieseberg, 2012; Valentini, Pompanon, & Taberlet, 2009). To date, eDNA usefulness and efficiency have been assessed in temperate freshwaters where eDNA has provided realistic pictures of fish species assemblages (Civade et al., 2016; Hänfling et al., 2016; Jerde, Mahon, Chadderton, & Lodge, 2011; Thomsen et al., 2012; Valentini et al., 2016).

The situation markedly differs in the tropics, which host higher species richness than temperate areas. For instance, French Guiana has as many fish species as Western Europe (380 species), while its surface area accounts for <1% of that of Western Europe (Le Bail et al., 2012; Melki, 2016). Moreover, our ability to make fish inventories in tropical rivers is limited because the frequently low conductivity of rainforest streams makes electrofishing ineffective, and the turbidity of large rivers limits direct underwater observations (Allard et al., 2014). These limitations led to use destructive methods (gill nets and/or rotenone) to get a picture of the fauna in both rivers and streams (Allard, Popée, Vigouroux, & Brosse, 2016; Araújo, Pinto, & Teixeira, 2009). Despite the use of such sampling methods, inventories often remain incomplete (Hercos, Sobansky, Queiroz, & Magurran, 2013; Murphy & Willis, 1996). As a consequence, gathering data on entire fish assemblages in the tropics is almost impossible without sacrificing a substantial part of the fauna and/or strongly disturbing the environment. This obviously acts as a barrier to scientific advances on ecosystem structure and functioning and the associated biodiversity conservation and management efforts. Developing fish eDNA in tropical freshwaters is therefore more than a way to reduce sampling efforts and costs (Evans, Shirey, Wieringa, Mahon, & Lamberti, 2017), as it would open avenues for tropical biodiversity research and conservation.

Nevertheless, there is no guarantee that eDNA will be as efficient in tropical rivers as it is in temperate ones. The higher temperatures of tropical waters does not affect eDNA degradation (Robson et al., 2016), but, in addition to the effect of stream characteristics (Shogren et al., 2017), the stronger solar radiation and the water acidity and turbidity might speed up eDNA degradation or impact its detection rate and thus restrict eDNA metabarcoding efficiency (Barnes et al., 2014; Matheson, Gurney, Esau, & Lehto, 2014; Pilliod, Goldberg, Arkle, & Waits, 2014). Field studies using eDNA in tropical environments are often focused on one or a few species (Bellemain et al., 2016; Lopes et al., 2017; Simpfendorfer et al., 2016), and they detect target species in fewer locations than expected. This results in uncertainties on the method efficiency in tropical rivers and/or on the spatial distribution of the species information determined by traditional sampling methods (Bellemain et al., 2016; Simpfendorfer et al., 2016). The metabarcoding approach coupled with eDNA therefore deserves to be tested on more diverse assemblages. This will require the development of a well-documented reference molecular database, which is currently lacking for most tropical freshwater species, for the target species (Ardura, Planes, & Garcia-Vazquez, 2013; Pochon, Zaiko, Hopkins, Banks, & Wood, 2015).

Here, we tested the efficiency of using eDNA metabarcoding to describe freshwater fish diversity and obtain a picture of fish assemblages in rivers and streams in French Guiana. We first developed a reference database for Guianese freshwater fish species. Then, we compared the fish species assemblages detected by metabarcoding to the known local fish fauna in these sites. We used a hierarchical framework and tested whether the metabarcoding results were consistent with the known fauna in the river drainage basin, the hydrologic unit (stream vs. river) and the local site. Finally, we measured the congruence between the diversity patterns (richness, occurrence, β-diversity) that were estimated using metabarcoding and those derived from traditional sampling methods, and we tested how these patterns fit with the theoretical knowledge on Guianese fish assemblages, considering faunistic differences among river drainages (Le Bail et al., 2012) and site position within the river continuum (Cilleros, Allard, Vigouroux, & Brosse, 2017; de Mérona, Tejerina-Garro, & Vigouroux, 2012).

2 | MATERIALS AND METHODS

2.1 | Study area and sample location

We sampled 39 watercourse sections of French Guiana for fish using both traditional methods (fish nets and toxicants) and eDNA metabarcoding (see Table 1 and Figure 1a). The sites included both small streams (less than 10 m wide and 1 m deep, Figure 1b; 31 sites) and rivers (more than 20 m wide and 1 m deep; 8 sites, Figure 1c). These sites were located within all 8 major river drainages in French Guiana, upstream from the main human settlements and away from major human disturbances (mining or deforestation). This ensured that the fish assemblages were not modified by human activities during the sampling period (2010–2015). For each river, traditional and metabarcoding sampling occurred in the same year (2015). Streams were sampled from 2010 to 2013 using traditional methods, and metabarcoding samples were performed in 2014 (Table 1). For streams, traditional sampling was not possible in 2014 because we have not been allowed to use rotenone since late 2013. Nevertheless, the local fish assemblages should not change over years because stream fish have low dispersal abilities (Cilleros, Allard, Grenouillet, & Brosse, 2016) and are strongly dependent on the physical structure of their habitat (Allard et al., 2016; Brosse, Montoya-Burgos, Grenouillet, & Surugue, 2013; Cilleros et al., 2017). Moreover, all the samples were collected during the dry season (September–November) to ensure fish assemblage differences

TABLE 1 List of studied sites with their river drainage membership and the type of watercourse (stream or river). See Figure 1 for site locations. Coordinates are given in the WGS84 coordinate reference system

Site number	Site name	Drainage basin	Watercourse type	Latitude	Longitude	Date of traditional sampling	Date of eDNA sampling
1	Apa	Maroni	Stream	5.3432	−54.0869	2012	2014
2	Crique des Cascades	Maroni	Stream	5.3476	−54.1053	2012	2014
3	Crique Bastien	Maroni	Stream	5.27003	−54.23433	2012	2014
4	Crique Penta	Maroni	Stream	5.21758	−54.28227	2012	2014
5	Papaichton	Maroni	River	3.80456	−54.16561	2015	2015
6	Saut Sonnelle	Maroni	River	3.66042	−53.95992	2015	2015
7	Twenke	Maroni	River	3.35922	−54.05492	2015	2015
8	Pikin Tabiki	Maroni	River	3.23267	−54.08319	2015	2015
9	Apsik Icholi	Maroni	River	2.93669	−54.174	2015	2015
10	Crique Nouvelle France 6	Maroni	Stream	3.62707	−53.16569	2011	2014
11	Crique Nouvelle France 5	Maroni	Stream	3.62707	−53.16692	2011	2014
12	Crique Nouvelle France 4	Maroni	Stream	3.61284	−53.16884	2011	2014
13	Crique Nouvelle France 3	Maroni	Stream	3.59726	−53.17847	2013	2014
14	Crique Nouvelle France 2	Maroni	Stream	3.57697	−53.19268	2011	2014
15	Crique Nouvelle France 1	Maroni	Stream	3.56573	−53.1975	2011	2014
16	Crique Voltalia 4	Mana	Stream	5.37639	−53.663	2011	2014
17	Crique Voltalia 3	Mana	Stream	5.35789	−53.66336	2011	2014
18	Crique Voltalia 2	Mana	Stream	5.3427	−53.66158	2011	2014
19	Crique Petit laussat Aval	Mana	Stream	5.40887	−53.58121	2012	2014
20	Crique à l'Est	Mana	Stream	3.66264	−53.22197	2012	2014
21	Crique Organabo	Organobo	Stream	5.46971	−53.534	2012	2014
22	Crique Toussaint	Sinnamary	Stream	5.30908	−53.05873	2010	2014
23	Crique Paracou	Sinnamary	Stream	5.28692	−52.90708	2012	2014
24	Crique Eau Claire	Kourou	Stream	5.14711	−52.87149	2010	2014
25	Crique Humus	Kourou	Stream	4.91867	−52.54702	2012	2014
26	Saut Bief	Comte	River	4.55343	−52.49948	2015	2015
27	Crique Petit Approuague	Comte	Stream	4.36481	−52.32803	2011	2014
28	Crique Kapiri 6	Approuague	Stream	4.15347	−52.16586	2011	2014
29	Crique Kapiri 1	Approuague	Stream	4.13208	−52.17138	2012	2014
30	Crique Kapiri 2	Approuague	Stream	4.10373	−52.08655	2012	2014
31	Athanase	Approuague	River	4.17772	−52.35567	2015	2014
32	Machicou	Approuague	River	3.89741	−52.58253	2015	2014
33	Crique parare 2	Approuague	Stream	4.03861	−52.67697	2010	2014
34	Crique parare 5	Approuague	Stream	4.04399	−52.68746	2010	2014
35	Crique parare 8	Approuague	Stream	4.04848	−52.69214	2010	2014
36	Crique SM	Oyapock	Stream	3.8587	−51.87103	2012	2014
37	Crique Marie	Oyapock	Stream	3.87064	−51.85653	2012	2014
38	Crique Pied Saut	Oyapock	Stream	3.80605	−51.89595	2012	2014
39	Crique Minette	Oyapock	Stream	3.82033	−51.875	2012	2014

between methods were not due to seasonal changes in habitat use or migrations. For each site, we converted fish species abundance data into occurrence data to avoid potential bias due to differences in sampling techniques and potential differences in sampling efficiency among sites.

2.2 | Traditional methods

We adapted the traditional sampling method to the type of watercourse. For small streams, we conducted fish surveys under several different research projects between 2010 and 2013 (CNRS-

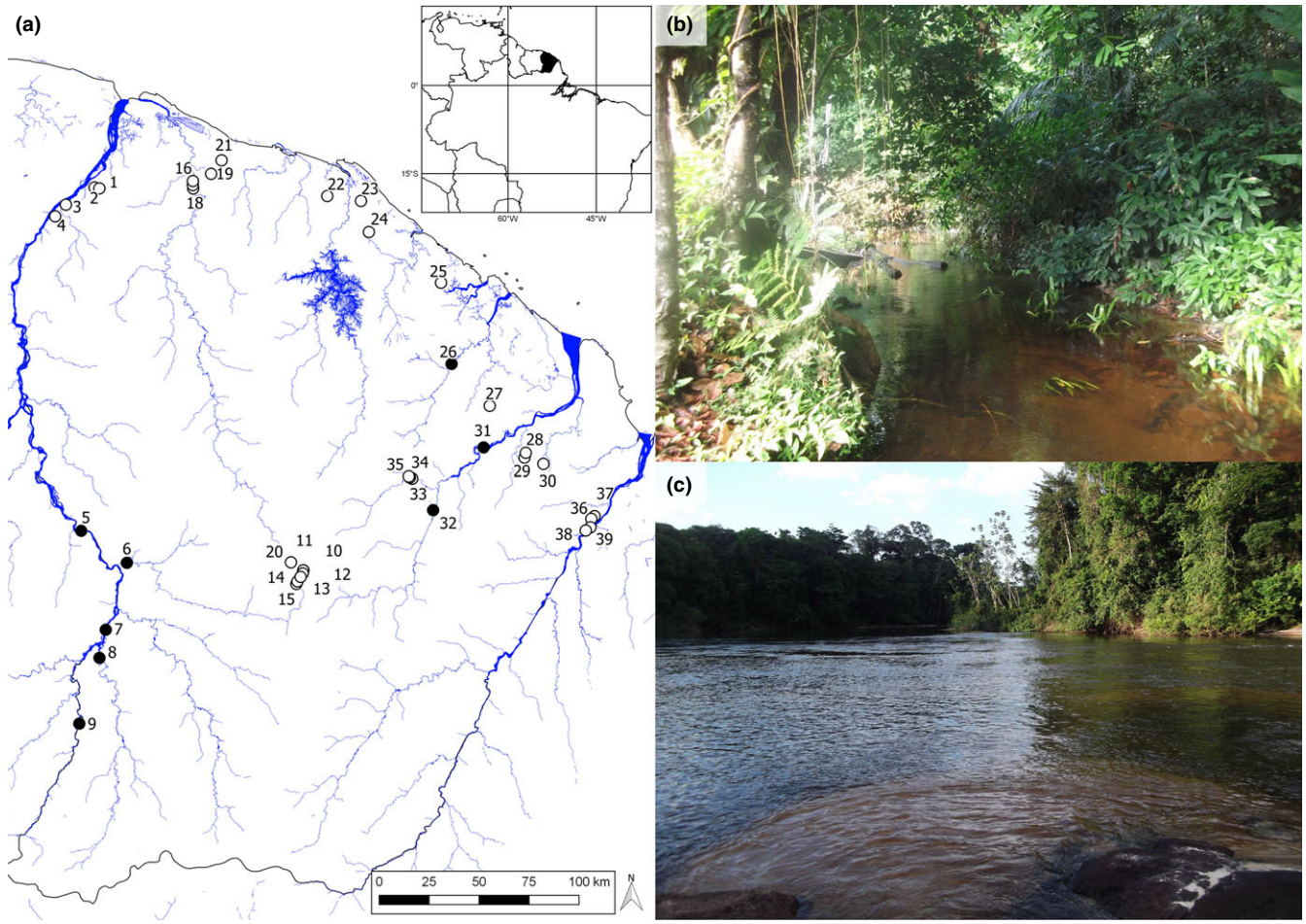

FIGURE 1 Location of the 39 studied sites (a). Stream (e.g., Apa, site 1; b) and river (e.g., Machicou, site 32; c) sites are marked with open and full circles, respectively. Numbers are as in Table 1

Nouragues, PAG-DEAL-HYDRECO, CEBA-DIADEMA projects). We standardized the sampling protocol for all sites (Allard et al., 2016). At each site, we isolated a river section located upstream of a confluence using two fine mesh (4 mm) stop nets. The length of each section was proportional to the stream width and was on average 33.67 ± 12.13 m. We collected fish after releasing a small quantity of rotenone (PREDATOX®: a 6.6% emulsifiable solution of rotenone extracted from *Derris elliptica* by Saphyr, Antibes, France) a few metres upstream of the first net. The rotenone is a nonselective piscicide traditionally used by Amazonian tribes which is also often used by scientists to sample fish in tropical streams (Allard et al., 2014; Murphy & Willis, 1996) and control fish populations (Finlayson et al., 2000). All killed fish were collected for identification, including fish recovered lying on the bottom or hidden in leaves and debris. No rotenone sample was collected after 2013 as the use of rotenone was banned by European laws 2008/296/EC and 2008/317/EC; therefore, it has been impossible to collect entire fish assemblages using this method since late 2013. There is still no alternative to rotenone, as electrofishing is not efficient due to the low conductivity of Guianese streams, and attempts to make inventories using others methods (snorkelling, hand nets, cast nets, traps) have yielded incomplete fish inventories (Allard et al., 2014). For rivers, we conducted fish surveys in November 2015. At each site, we placed 20 50-m-long gill nets with different mesh sizes (15, 20, 25, 30 and 35 mm) on the river banks and removed them after an overnight sampling to collect the fish. In addition, we collected cast-net and fine-meshed hand-net samples near the banks to complement inventories for the small species that were not captured by the gill nets. For both methods, we identified each individual to the species level.

2.3 | Reference DNA database

We sampled tissues from 503 specimens belonging to 231 species (1–3 specimens per species) and stored them in 96% ethanol. We extracted DNA from 0.05–0.25 cm^2 of tissue using the salt-extraction protocol (Aljanabi & Martinez, 1997). We performed DNA amplification in a final volume of 25 μl with 1U of GoTaq® (Promega), 5× buffer (Promega), 10 μM of dNTP, 20 μM of each primer (teleo_R 5′-CTTCCGGTACACTTACCATG-3′ and V05F_898 5′-AAACTCGTGCCAGCCACC-3′, Thomsen et al., 2016) and 1 μl of DNA template. The PCR mixture was denatured at 95°C for 10 min, followed by 35 cycles of 30 s at 95°C, 30 s at 55°C and 1 min at

72°C, which were followed by a final elongation at 72°C for 7 min; PCR was conducted in a room dedicated to DNA amplification with a negative air pressure that was physically separated from the DNA extraction rooms. A 600 bp product of the 12S rRNA gene was obtained and sequenced on the ABI3730XL Automated DNA Sanger sequencer (Genoscreen, Lille, France). For 14 species (27 individuals), no PCR product was amplified. Sequences were analysed using GENEIOUS version 9.0 (http://www.geneious.com, Kearse et al., 2012).

2.4 | eDNA metabarcoding sampling and analysis

We collected eDNA samples in November 2014 for streams and in October 2015 for rivers, based on the protocol of Valentini et al. (2016) for running waters. For each sample, we used a filtration kit made of a sterile filtration capsule (Envirocheck HV 1 μm; Pall Corporation, Ann Arbor, MI, USA), a peristaltic pump (Vampir Sampler; Bürkle GmbH, Bad Bellingen, Germany) and sterile, single-use tubing. All the materials were handled with sterile gloves. The peristaltic pump, although not in contact with the water, was sterilized using sodium hypochlorite between each site to avoid contamination. At each site, we placed the input part of the tubing in a high-flow part of the watercourse located in the middle of the stream or river channel. Sampling was achieved in rapid hydromorphological units to ensure an optimal homogenization of the water throughout the water column. The operator always remained downstream from the filtration area and stayed on the bank (for small streams) or on emerging rocks (for larger streams and rivers). Water was pumped ~20 cm below the surface, and each filtration lasted 30 min at 1.67 L/min. Each sample therefore results from the filtration of ~50 L of water. For sites located along the same river course, we sampled from downstream to upstream to avoid contamination by eDNA transported by the boat (for rivers) or our clothes. For the same reason, we took eDNA samples just upstream from the nets. At the end of the filtration, we emptied the filtration capsule of water, filled it with 150 mL of preservation buffer (Tris–HCl 0.1 M, EDTA 0.1 M, NaCl 0.01 M and N-lauroyl sarcosine 1%, pH 7.5–8) and stored it in the dark in individual sterile plastic bags. Samples were then stored at room temperature before DNA extraction. Preliminary tests demonstrated that the preservation buffer was suitable for room temperature storage up to a month. The extraction was conducted at the end of the field session, 1–3 weeks after sampling. For the procedure, filtration capsules were left at 56°C for 2 hr, agitated manually for 5 min and then emptied into three 50 ml tubes. In total, ~120 ml was divided among three tubes that were centrifuged for 15 min at 15,000 *g*. The supernatant was removed with a sterile pipette, leaving 15 ml of liquid at the bottom of the tube. Subsequently, 33 ml of ethanol and 1.5 ml of 3 M sodium acetate were added to each 50 ml tube. The three tubes were centrifuged at 15,000 *g* for 15 min at 6°C, and the supernatant was discarded. After this step, 360 μl of ATL Buffer of the DNeasy Blood & Tissue Extraction Kit (Qiagen) was added to the first tube, the tube was vortexed, and the supernatant was transferred to the second tube (Tréguier et al., 2014). This operation was repeated for all tubes. The supernatant of the third tube was finally transferred to a 2-ml tube, and the DNA extraction was performed following the manufacturer's instructions. Two negative extraction controls were also performed. They were amplified and sequenced in the same way as and in parallel to the samples to monitor possible contaminants. After the DNA extraction, the samples were tested for inhibition by qPCR following the protocol in Biggs et al. (2015). If the sample was considered inhibited, it was diluted 5-fold before the amplification.

We performed DNA amplifications in a final volume of 25 μl including 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA), 10 mM of Tris–HCl, 50 mM of KCl, 2.5 mM of MgCl2, 0.2 mM of each dNTP, 0.2 μM of "teleo" primers (teleo_R 5′- CTTCCGGTACACTTACCATG-3′ and teleo_F 5′-ACACCGCCCGTCACTCT-3′, Valentini et al., 2016) and 3 μl of DNA template. We also added 4 μM of human blocking primer for the "teleo" primers and 0.2 μg/μl of bovine serum albumin (BSA, Roche Diagnostic, Basel, Switzerland) to the mixture. The "teleo" primers were 5′-labelled with an eight-nucleotide tag unique to each PCR replicate (with at least three differences between any pair of tags), allowing each sequence to be assigned to the corresponding sample PCR replicate during the sequence analysis and therefore to check for low variance between PCR replicates. The forward and reverse primer tags were identical for each PCR replicate. PCR was conducted for 12 replicates with the same protocol described in the "Reference DNA database" section, increasing the number of PCR cycles to 50.

We also amplified two negative extraction controls and three PCR controls and sequenced them in parallel with the 39 samples. We pooled the purified PCR products in equal volumes to achieve an expected sequencing depth of 400,000 reads per sample. Library preparation and sequencing were performed at Fasteris facilities (Geneva, Switzerland). Four libraries were prepared using the Metafast protocol (https://www.fasteris.com/dna/?q=content/metafast-protocol-amplicon-metagenomic-analysis). For the stream samples, we carried out the paired-end sequencing (2 × 125 bp) in an Illumina MiSeq sequencer (Illumina, San Diego, CA, USA) using the Paired-end MiSeq Reagent Kit V2 (Illumina, San Diego, CA, USA), following the manufacturer's instructions. For the river samples, we ran the libraries on an Illumina HiSeq 2500 (2 × 125 bp; Illumina, San Diego, CA, USA) using the HiSeq SBS Kit v4 (Illumina, San Diego, CA, USA) following the manufacturer's instructions. We analysed the sequence reads using the programs in the OBITOOLS package (http://metabarcoding.org/obitools; Boyer et al., 2016) following the protocol described in Valentini et al. (2016). We performed the taxonomic assignment of molecular operational taxonomic units (MOTUs) using the ecotag program with the local reference database built for this study and the GenBank nucleotide database. The program works in two steps: first, it searches for the reference sequence that has the highest similarity to the query sequence. This similarity value is then used as a threshold to search for sequences in the reference database with a similarity to the initial reference sequence that is equal to or lower than the threshold. The query sequence is then assigned to the most recent common ancestor of first and second matched sequences. MOTUs with a similarity to a sequence in the reference

database (local or GenBank) lower than 98% were discarded. To take tag jumping (Schnell, Bohmann, & Gilbert, 2015) into account, we discarded all sequences with a frequency of occurrence below 0.0003 per taxon per sample and per sequencing run. These thresholds were empirically determined to clear all reads from the extraction and PCR negative controls included in our global data production procedure (De Barba et al., 2014).

2.5 | Statistical analyses

First, we compared the species detected by eDNA metabarcoding to the lists of known species at three different spatial scales: the river drainage scale, the watercourse type scale and the site scale. At the drainage scale, we used an updated version of the Le Bail et al. (2012) and Melki (2016) checklists to verify if the species detected using eDNA metabarcoding belonged to the species assemblage known to inhabit the considered river drainage. At the watercourse type scale, we merged all available species ecological data reported by Keith, Le Bail, and Planquette (2000), Le Bail, Keith, and Planquette (2000) and Planquette, Keith, and Le Bail (1996) to determine if each species inhabits only streams, only rivers or both streams and rivers. At the site scale, we compared the list of species, genera and families obtained by metabarcoding to the list of species, genera and families obtained by traditional sampling. Then, for each site, we calculated the percentage of species detected by each method as the number of species detected by a method divided by the total number of species detected. In addition to the observed measures of species richness, we computed estimates of richness derived from traditional and eDNA metabarcoding methods using the bias-corrected form of the Chao2 richness estimator (Chao, 1987; Chiu, Wang, Walther, & Chao, 2014). Ninety-five percentage confidence intervals were calculated to test for differences in the estimated richness between the sampling methods, with nonoverlapping confidence intervals reflecting differences in estimated richness. Richness estimates were calculated for separate streams and rivers types and all sites pooled together.

Afterwards, we tested the congruence of the diversity patterns at each site given by the two methods. We calculated the species richness at each site for both methods and tested the correlation between them using Pearson's r correlation coefficient. We calculated the relative occurrence of species as the percentage of sites where a species was detected, and we tested whether the occurrence patterns were correlated using Kendall's τ rank correlation coefficient.

Finally, we used the β-diversity between sites to characterize spatial patterns in fish assemblage diversity. We calculated β-diversity using the turnover component of the Jaccard's dissimilarity index (Baselga, 2012) and used nonmetric multidimensional scaling (NMDS) to visualize β-diversity patterns in two-dimensional plots. To test the congruence of the ordinations produced by the two methods, we used Procrustes analyses with 999 permutations. We measured the goodness-of-fit between ordinations using the m^2 statistic, which varies from 0 (perfect congruence) to 1 (no congruence). We ran this procedure after assigning metabarcoding reads to species. We also tested the congruence between methods using the MOTUs without the species assignation matrices to determine if raw metabarcoding data can be used to describe spatial patterns in fish assemblages. We used nonparametric multivariate analysis to test if fish assemblages had differing compositions and variabilities (a) between streams and rivers and (b) between river drainages. In this last analysis, in addition to the complete data set, we also used a reduced data set comprising only the streams. The difference in the species composition of river fish fauna between river drainages (excluding streams) was not tested because the number of river sites ($n = 8$) was not sufficient to provide a meaningful representation of each river drainage.

We tested composition differences between groups with a permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001), and we tested differences in the intragroup assemblage variation with an analysis of homogeneity of multivariate dispersion (PERMADISP, Anderson, Ellingsen, & McArdle, 2006). The significance was assessed with 999 permutations.

All statistical analyses were performed using R.3.3.2 (R Core Team, 2015) and the "VEGAN" package version 2.4-1 (Oksanen et al., 2016), the "WIQID" package version 0.1.3 (Meredith, 2017), the "BETAPART" package version 1.3 (Baselga, Orme, Villeger, Bortoli, & Leprieur, 2013) and the "DUNN.TEST" package version 1.3.4 (Dinno, 2017). The significance threshold was fixed at $p < 0.05$.

3 | RESULTS

With traditional methods, we collected 7,029 individuals belonging to 203 species (Table 2) and 12 orders. At almost all sites, cryptic fish were collected, including highly cryptic Siluriformes species and litter bank fishes such as small killifishes. Several species of Gymnotiformes that inhabit small Guianese streams, although known to be resistant to rotenone, were also caught.

From the 615 specimens (belonging to 218 species) sequenced to build the reference database, 68 specimens did not provide information for the 12S metabarcoding fragment and were excluded from the reference database (thus resulting in 17 removed species without a sequence). In total, 193 unique sequences were obtained and assigned a species using the 12S metabarcoding fragment in accordance with Valentini et al. (2016) (see Materials and Methods). When a sequence matched several reference taxa, it was assigned to the lowest taxonomic level (genera, family or order) that grouped all the matched reference taxa (Table 2). A total of 181 unique sequences were identified to the species level, eight were identified to the genus level (*Astyanax*, *Cyphocharax*, *Bryconops*, *Gymnotus*, *Hemigrammus*, *Leporinus*, *Moenkhausia*, *Pimelodella*), three were identified to the family level (Characidae, Hypopomidae, Prochilodontidae) and one was identified to the order level (Characiformes). Those 10 sequences that were assigned to a higher level than species represent 28 potential species (Table 2).

For the eDNA metabarcoding analysis, a total of 17,775,665 reads were obtained, among which 2,015,596 received a taxa assignment when the GenBank database was used as a reference (11%, see

TABLE 2 Species occurrences, expressed as the percentage of sites where each species was found using eDNA metabarcoding and traditional methods

Species	Taxonomic detection level	Occurrence with eDNA metabarcoding	Occurrence with traditional methods
Acestrorhynchus falcatus (Bloch, 1794)	Species	46.15	56.41
Acestrorhynchus microlepis (Müller & Troschel, 1844)	No sequence	–	20.51
Acnodon oligacanthus (Müller & Troschel, 1844)	Species	2.56	2.56
Aequidens tetramerus (Heckel, 1840)	Species	25.64	10.25
Ageneiosus inermis (Linnaeus, 1766)	Species	2.56	15.38
Ageneiosus ucayalenis (Castelnau, 1855)	Species	0	5.13
Anableps anableps (Linnaeus, 1758)	Species	0	0
Anablepsoides gaucheri (Keith, Nandrin & Le Bail, 2006)	Species	0	0
Anablepsoides holmiae (Eigenmann, 1909)	Species	0	2.56
Anablepsoides igneus (Huber, 1991)	Species	28.21	25.64
Anablepsoides lungi (Berkenkamp, 1984)	Species	0	10.26
Anchovia surinamenis (Bleeker, 1865)	Species	0	2.56
Ancistrus aff. hoplogenys (Günther, 1864)	No sequence	–	10.26
Ancistrus aff. temminckii (Valenciennes, 1840)	Species	33.33	2.56
Ancistrus cf. leucostictus (Günther, 1864)	Species	28.21	23.08
Anostomus brevior (Géry, 1963)	Species	5.13	5.13
Aphyocharacidium melandetum (Eigenmann, 1912)	No sequence	–	2.56
Apistogramma gossei (Kullander, 1982)	Species	12.82	5.13
Apteronotus albifrons (Linnaeus, 1766)	No sequence	–	2.56
Astyanax bimaculatus (Linnaeus, 1758)	Species	0	7.69
Astyanax validus (Géry, Planquette & Le Bail, 1991)	Species	10.26	10.26
Auchenipterus dentatus (Valenciennes, 1840)	No sequence	–	2.56
Auchenipterus nuchalis (Spix & Agassiz, 1829)	Species	0	7.69
Batrochoglanis raninus (Valenciennes, 1840)	Species	35.90	43.59
Bivibranchia bimaculata (Vari, 1985)	Species	2.56	10.26
Boulengerella cuvieri (Spix & Agassiz, 1829)	Species	0	0
Brachyhypopomus beebei (Schultz, 1944)	Species	2.56	5.13
Brachyplatystoma rousseauxii (Castelnau, 1855)	Species	0	0
Brachyplatystoma vaillantii (Valenciennes, 1840)	Species	0	2.56
Brycon falcatus (Müller & Troschel, 1844)	Species	2.56	7.69
Brycon pesu (Müller & Troschel, 1845)	Species	2.56	7.69
Bryconamericus aff. hyphesson	Species	0	2.56
Bryconamericus guyanensis (Zarske, Le Bail & Géry, 2010)	Species	43.59	41.03
Bryconops aff. caudomaculatus (Günther, 1864)	Genus	Bryconops	10.26
Bryconops affinis (Günther, 1864)	Genus	Bryconops	46.15
Bryconops caudomaculatus (Günther, 1864)	Genus	Bryconops	17.95
Bryconops melanurus (Bloch, 1794)	Genus	Bryconops	20.51
Caenotropus maculosus (Eigenmann, 1912)	Species	2.56	7.69
Callichthys callichthys (Linnaeus, 1758)	Species	25.64	2.56
Cetopsidium orientale (Vari, Ferraris & Keith, 2003)	Species	15.38	12.82
Chalceus macrolepidotus (Cuvier, 1818)	Species	2.56	7.69
Characidium zebra (Eigenmann, 1909)	Species	28.21	48.72
Charax aff. pauciradiatus	Species	0	0
Charax niger (Lucena, 1989)	Species	0	0

(Continues)

TABLE 2 (Continued)

Species	Taxonomic detection level	Occurrence with eDNA metabarcoding	Occurrence with traditional methods
Charax gibbosus (Linnaeus, 1758)	Species	2.56	10.26
Chasmocranus brevior (Eigenmann, 1912)	Species	10.26	7.69
Chasmocranus longior (Eigenmann, 1912)	Species	41.03	17.95
Chilodus zunevei (Puyo, 1946)	Species	5.13	5.13
Cichla ocellaris (Bloch & Schneider, 1801)	Species	5.13	5.13
Cichlasoma bimaculatum (Linnaeus, 1758)	Species	5.13	0
Cleithracara maronii (Steindachner, 1881)	Species	23.08	12.82
Copella arnoldi (Regan, 1912)	Species	2.56	0
Copella carsevennensis (Regan, 1912)	No sequence	–	48.72
Corydoras aeneus (Gill, 1858)	Species	7.69	0
Corydoras aff. guianensis	Species	5.13	5.13
Corydoras amapaensis(Nijssen, 1972)	Species	0	7.69
Corydoras oiapoquensis (Nijssen & Isbrücker, 1983)	Species	0	0
Corydoras geoffroy (Lacepède, 1803)	Species	12.82	10.26
Corydoras solox (Nijssen & Isbrücker, 1983)	Species	5.13	0
Corydoras spilurus (Norman, 1926)	Species	0	2.56
Creagrutus melanzonus (Eigenmann, 1909)	Species	5.13	0
Creagrutus planquettei (Géry & Renno, 1989)	Species	2.56	2.56
Crenicichla albopunctata (Pellegrin, 1904)	Species	12.82	15.38
Crenicichla johanna (Heckel, 1840)	Species	0	5.13
Crenicichla multispinosa (Pellegrin, 1903)	Species	5.13	2.56
Crenicichla saxatilis (Linnaeus, 1758)	Species	15.38	28.21
Cteniloricaria platystoma (Günther, 1868)	Species	2.56	0
Curculionichthys sp	Species	7.69	2.56
Curimata cyprinoides (Linnaeus, 1766)	Species	2.56	7.69
Curimatopsis crypticus (Vari, 1982)	Species	2.56	2.56
Cynodon meionactis (Géry, Le Bail & Keith, 1999)	Species	2.56	7.69
Cynopotamus essequibensis (Eigenmann, 1912)	No sequence	–	10.26
Cyphocharax aff. spilurus	No sequence	–	2.56
Cyphocharax gouldingi (Vari, 1992)	Species	0	0
Cyphocharax helleri (Steindachner, 1910)	Species	20.51	12.82
Cyphocharax spilurus (Günther, 1864)	Species	10.26	15.38
Doras carinatus (Linnaeus, 1776)	Species	5.13	7.69
Doras micropoeus (Eigenmann, 1912)	Species	2.56	7.69
Eigenmannia virescens (Valenciennes, 1836)	Species	41.03	35.90
Electrophorus electricus (Linnaeus, 1766)	Species	23.08	5.13
Eleotris pisonis (Gmelin, 1789)	Species	2.56	2.56
Erythrinus erythrinus (Bloch & Schneider, 1801)	Species	25.64	20.51
Farlowella reticulata (Boeseman, 1971)	Species	15.38	12.82
Farlowella rugosa (Boeseman, 1971)	No sequence	–	2.56
Gasteropelecus sternicla (Linnaeus, 1758)	No sequence	–	15.38
Geophagus camopienis (Pellegrin, 1903)	Species	0	2.56
Geophagus harreri (Gosse, 1976)	Species	10.26	7.69
Geophagus surinamensis (Bloch, 1791)	Species	7.69	10.26
Glanidium leopardum (Hoedeman, 1961)	Species	12.82	7.69

(Continues)

TABLE 2 (Continued)

Species	Taxonomic detection level	Occurrence with eDNA metabarcoding	Occurrence with traditional methods
Guianacara geayi (Pellegrin, 1902)	Species	2.56	10.26
Guianacara owroewefi (Kullander & Nijssen, 1989)	Species	15.38	7.69
Guyanancistrus brevispinis (Heitmans, Nijssen & Isbrücker, 1983)	Species	10.26	5.13
Guyanancistrus longispinis (Heitmans, Nijssen & Isbrücker, 1983)	Species	0	0
Gymnotus carapo (Linnaeus, 1758)	Genus	Gymnotus	69.23
Gymnotus coropinae (Hoedeman, 1962)	Genus	Gymnotus	46.15
Harttia guianensis (Rapp Py-Daniel & Oliveira, 2001)	No sequence	–	12.82
Hartiella longicauda (Covain & Fish-Muller, 2012)	Species	0	0
Harttiella lucifer (Covain & Fisch-Muller, 2012)	No sequence	–	5.13
Helogenes marmoratus (Günther, 1863)	No sequence	–	61.54
Hemiancistrus medians (Kner, 1854)	No sequence	–	2.56
Hemibrycon surinamensis (Géry, 1962)	Species	15.38	15.38
Hemigrammus boesemani (Géry, 1959)	Species	0	5.13
Hemigrammus guyanensis (Géry, 1959)	Genus	Hemigrammus	2.56
Hemigrammus ocellifer (Steindachner, 1882)	Genus	Hemigrammus	23.08
Hemigrammus ora (Zarske, Le Bail & Géry, 2006)	Species	0	2.56
Hemigrammus rodwayi (Durbin, 1909)	Species	0	17.95
Hemigrammus unilineatus Gill, 1858	Species	5.13	35.90
Hemiodus aff. unimaculatus	Species	2.56	17.95
Hemiodus huraulti (Géry, 1964)	Order	Characiformes	5.13
Hemiodus quadrimaculatus (Pellegrin, 1909)	Species	2.56	7.69
Hemiodus unimaculatus (Bloch, 1794)	Species	2.56	7.69
Heptapterus bleekeri (Boeseman, 1953)	Species	2.56	0
Hoplerythrinus unitaeniatus (Spix & Agassiz, 1829)	Species	30.77	5.13
Hoplias aimara (Valenciennes, 1847)	Species	46.15	25.64
Hoplias malabaricus (Bloch, 1794)	Species	69.23	17.95
Hyphessobrycon borealis (Zarske, Le Bail & Géry, 2006)	No sequence	–	33.33
Hyphessobrycon copelandi (Durbin, 1908)	Species	0	2.56
Hyphessobrycon roseus (Géry, 1960)	Species	0	5.13
Hyphessobrycon simulatus (Géry, 1960)	Species	0	7.69
Hyphessobrycon takasei (Géry, 1964)	Species	0	0
Hypomasticus despaxi (Puyo, 1943)	Species	10.26	7.69
Hypopomus artedi (Kaup, 1856)	Species	46.15	23.08
Hypopygus lepturus (Hoedeman, 1962)	Family	Hypopomidae	5.13
Hypostomus gymnorhynchus (Norman, 1926)	Species	10.26	12.82
Hypostomus plecostomus (Linnaeus, 1758)	No sequence	–	2.56
Imparfinis pijpersi (Hoedeman, 1961)	Species	2.56	0
Ituglanis amazonicus (Steindachner, 1882)	Species	35.90	10.26
Ituglanis nebulosus (de Pinna & Keith, 2003)	Species	25.64	17.95
Japigny kirschbaum (Meunier, Jégu & Keith, 2011)	Species	12.82	7.69
Jupiaba abramoides (Eigenmann, 1909)	No sequence	–	38.46
Jupiaba keithi (Géry, Planquette & Le Bail, 1996)	Species	7.69	7.69
Jupiaba maroniensis (Géry, Planquette & Le Bail, 1996)	Species	0	2.56
Jupiaba meunieri (Géry, Planquette & Le Bail, 1996)	No sequence	–	2.56
Krobia aff. guianensis sp1	Species	33.33	10.26

(Continues)

TABLE 2 (Continued)

Species	Taxonomic detection level	Occurrence with eDNA metabarcoding	Occurrence with traditional methods
Krobia aff. guianensis sp2	Species	7.69	17.95
Krobia itanyi (Puyo, 1943)	Species	33.33	15.38
Laimosemion aff. geayi (Mol *et al.*, 2012)	Species	2.56	0
Laimosemion agilae (Hoedeman, 1954)	Species	2.56	30.77
Laimosemion cf. geayi	Species	0	2.56
Laimosemion cladophorus (Huber, 1991)	Species	0	2.56
Laimosemion geayi (Vaillant, 1899)	No sequence	–	17.95
Laimosemion xiphidius (Huber, 1979)	No sequence	–	23.08
Leporinus acutidens (Valenciennes, 1837)	Genus	Leporinus	5.13
Leporinus fasciatus (Bloch, 1794)	Species	7.69	10.26
Leporinus friderici (Bloch, 1794)	Species	12.82	12.82
Leporinus gossei (Géry, Planquette & Le Bail, 1991)	Genus	Leporinus	2.56
Leporinus granti (Eigenmann, 1912)	Genus	Leporinus	12.82
Leporinus lebaili (Géry & Planquette, 1983)	Species	10.26	10.26
Leporinus maculatus (Müller & Isbrücker, 1993)	Genus	Leporinus	2.56
Leporinus melanostictus (Norman, 1926)	Genus	Leporinus	2.56
Leporinus nijsseni (Garavello, 1990)	Genus	Leporinus	2.56
Lithoxus boujardi (Muller & Isbrücker, 1993)	Species	2.56	2.56
Lithoxus planquettei (Boeseman, 1982)	Species	10.26	28.21
Lithoxus stocki (Nijssen & Isbrücker, 1990)	Species	0	5.13
Loricaria aff. parnahybae	Species	0	0
Loricaria cataphracta (Linnaeus, 1758)	Species	0	7.69
Lycengraulis batesii (Günther, 1868)	Species	0	5.13
Mastiglanis cf. asopos (Bockmann, 1994)	Species	2.56	2.56
Megalechis thoracata (Valenciennes, 1840)	No sequence	–	7.69
Melanocharacidium blennioides (Eigenmann, 1909)	Species	2.56	5.13
Melanocharacidium dispilomma (Buckup, 1993)	Species	2.56	5.13
Metaloricaria paucidens (Isbrücker, 1975)	Species	2.56	5.13
Metynnis lippincottianus (Cope, 1870)	No sequence	–	2.56
Microcharacidium eleotrioides (Géry, 1960)	Species	35.90	15.38
Micropoecila bifurca (Eigenmann, 1909)	Species	0	0
Moenkhausia aff. grandisquamis	Genus	Moenkhausia	7.69
Moenkhausia aff. intermedia	Species	5.13	2.56
Moenkhausia chrysargyrea (Günther, 1864)	Genus	Moenkhausia	38.46
Moenkhausia collettii (Steindachner, 1882)	Species	5.13	28.21
Moenkhausia georgiae (Géry, 1965)	Species	12.82	7.69
Moenkhausia grandisquamis (Müller & Troschel, 1845)	Genus	Moenkhausia	12.82
Moenkhausia hemigrammoides (Géry, 1965)	Species	0	10.26
Moenkhausia moisae (Géry, Planquette & Le Bail, 1995)	Genus	Moenkhausia	23.08
Moenkhausia oligolepis (Günther, 1864)	Species	25.64	41.03
Moenkhausia surinamensis (Géry, 1965)	Genus	Moenkhausia	15.38
Myloplus rhomboidalis (Cuvier, 1818)	Species	2.56	7.69
Myloplus rubripinnis (Müller & Troschel, 1844)	No sequence	–	12.82
Myloplus ternetzi (Norman, 1929)	Species	30.77	20.51
Nannacara aureocephalus (Allgayer, 1983)	Species	35.90	38.46

(Continues)

TABLE 2 (Continued)

Species	Taxonomic detection level	Occurrence with eDNA metabarcoding	Occurrence with traditional methods
Nannostomus beckfordi (Günther, 1872)	Species	2.56	5.13
Nannostomus bifasciatus (Hoedeman, 1954)	Species	12.82	15.38
Ochmacanthus cf. alternus (Myers, 1927)	No sequence	–	2.56
Ochmacanthus reinhardtii (Steindachner, 1882)	Species	0	2.56
Otocinclus mariae (Fowler, 1940)	Species	5.13	2.56
Pachypops fourcroi (Lacepède, 1802)	Species	2.56	7.69
Parodon guyanensis (Géry, 1959)	Species	2.56	5.13
Phenacogaster wayampi (Le Bail & Lucena, 2010)	Species	0	0
Phenacogaster wayana (Le Bail & Lucena, 2010)	Species	0	10.26
Phenacorhamdia tenuis (Mees, 1986)	Species	7.69	7.69
Piabucus dentatus(Koelreuter, 1763)	Species	0	5.13
Pimelabditus moli (Parisi & Lundberg, 2009)	Species	2.56	2.56
Pimelodella cristata (Müller & Troschel, 1849)	Genus	Pimelodella	33.33
Pimelodella geryi (Hoedman, 1961)	Genus	Pimelodella	10.26
*Pimelodella procera (*Mees, 1983)	Species	7.69	10.26
Pimelodus blochii (Valenciennes, 1840)	No sequence	–	2.56
Pimelodus ornatus (Kner, 1858)	Species	2.56	7.69
Plagioscion auratus (Castelnau, 1855)	Species	10.26	7.69
Plagioscion squamosissimus (Heckel, 1840)	Species	0	0
Platydoras costatus (Linnaeus, 1758)	Species	2.56	2.56
Polycentrus schomburgkii (Müller & Troschel, 1849)	Species	15.38	5.13
Poptella brevispina (Reis, 1989)	Species	10.26	43.59
Potamorrhaphis guianensis (Jardine, 1843)	Species	0	5.13
Potamotrygon orbignyi (Castelnau, 1855)	Species	7.69	5.13
Pristella maxillaris (Ulrey, 1894)	Species	0	5.13
Pristobrycon striolatus (Steindachner, 1908)	Species	2.56	2.56
Prochilodus rubrotaeniatus (Jardine, 1841)	Species	2.56	7.69
Pseudancistrus barbatus (Valenciennes, 1840)	Species	7.69	7.69
Pseudoplatystoma fasciatum (Linnaeus, 1766)	Species	7.69	0
Pterengraulis atherinoides (Linnaeus, 1766)	Species	2.56	0
Pyrrhulina filamentosa (Valenciennes, 1847)	Species	56.41	56.41
Retroculus septentrionalis (Gosse, 1971)	Species	0	0
Rhamdia quelen (Quoy & Gaimard, 1824)	Species	2.56	28.21
Rhamphichthys rostratus (Linnaeus, 1766)	Species	0	5.13
Rineloricaria nsp1 aff. stewarti	Species	23.08	15.38
Rineloricaria platyura (Müller & Troschel, 1849)	Species	2.56	0
Roeboexodon geryi (Myers, 1960)	Species	2.56	10.26
Satanoperca rhynchitis (Kullander, 2012)	Species	7.69	5.13
Schizodon fasciatus (Spix & Agassiz, 1829)	Order	Characiformes	10.26
Sciades couma (Valenciennes, 1840)	Species	0	0
Semaprochilodus varii (Castro, 1988)	Family	Prochilodontidae	5.13
Serrapinnus gracilis (Géry, 1690)	Family	Characidae	5.13
Serrasalmus eigenmanni (Norman, 1929)	Species	2.56	12.82
Serrasalmus rhombeus (Linnaeus, 1766)	No sequence	–	12.82
Steindachnerina varii (Géry, Planquette & Le Bail, 1991)	Species	12.82	0

(Continues)

TABLE 2 (Continued)

Species	Taxonomic detection level	Occurrence with eDNA metabarcoding	Occurrence with traditional methods
Sternopygus macrurus (Bloch & Schneider, 1801)	Species	48.72	46.15
Synbranchus marmoratus (Bloch, 1795)	Species	61.54	7.69
Tatia brunnea (Mees, 1974)	Species	15.38	2.56
Tatia intermedia (Steindachner, 1877)	Species	10.26	7.69
Tetragonopterus chalceus (Spix & Agassiz, 1829)	Species	2.56	12.82
Tetragonopterus rarus (Zarske, Géry & Isbrücker, 2004)	Species	10.26	2.56
Thayeria ifati (Géry, 1959)	Species	2.56	10.26
Tometes trilobatus (Valenciennes, 1850)	Species	0	0
Trachelyopterus galeatus (Linnaeus, 1766)	Species	2.56	5.13
Triportheus brachipomus (Valenciennes, 1850)	No sequence	–	12.82

Note. For each species, the taxonomic unit at which the 12S rRNA metabarcoding fragment was identified is indicated. "–" indicates species absent from the reference database. A nil occurrence for both methods indicates a species was included in the reference database but not detected in the field

Supporting information: Table S1). Most of the assigned taxa corresponded to fish species present in French Guiana or neighbouring Amazonian regions (1,050,515 reads), but reads were also assigned to other diverse taxa (Anura, Mammalia, Aves, Insecta and Reptilia; Supporting information: Table S2). Among the reads assigned to fish taxa, 904,782 (86%) were assigned to 28 taxonomic units occurring in French Guiana (24 species and 4 genera; Supporting information: Table S2). The remaining 14% of the reads were assigned to species closely related to Guianese species (all belonged to genera present in French Guiana). At the site scale, the number of reads assigned to GenBank data ranged from 0 to 296,917 (median: 19,715 reads).

Using our custom reference database, 8,109,492 reads (46%) were given an assignment, with the number of assigned reads from each site ranging from 18,895 to 870,235 (median: 112,997 reads, Supporting information: Table S1). The reads assigned to the reference database were distributed among 148 taxonomic units: 132 species (7,521,532 reads), nine genera (550,264 reads), two subfamilies (638 reads), four families (29,503 reads) and one order (495 reads). When comparing the complete lists of the species detected by the two methods (i.e., all individuals and assigned reads identified at the genus level or higher were removed from the analysis), we found that 119 species were detected by the two methods, with 13 only detected by metabarcoding and 84 only detected by traditional methods (Table 2). Within these 84 species, 53 were not classified at the species level in our database, due to missing sequences (27), or were assigned a higher taxonomic level (26). The 31 other species, while classified by our reference database, were not detected with eDNA. Globally, even if the number of species caught with traditional sampling was higher (Table 3), the Chao2 richness estimates did not differ between the two methods.

At the river drainage scale, five species (i.e., 3.79% of the 132 species detected by metabarcoding) were detected outside their known Guianese river drainage distribution. These detections represented 12 of a total of 680 fish occurrences (i.e., 1.76% of the fish occurrences). Moreover, three of the five species were detected no more than twice in river drainages beyond their known distribution: *Corydoras aeneus* was detected at one site in the Oyapock River drainage, *Hemiodus quadrimaculatus* was detected once in the Maroni River drainage, *Ancistrus aff. temminckii* was detected once in the Kourou River drainage and once in the Sinnamary River drainage and *Krobia itanyi* was detected in two sites in the Approuague River drainage. The remaining species, *Krobia aff. guianensis sp1*, was detected six times in the Approuague River drainage.

TABLE 3 Overall species richness of the stream and river sites. Global indicates the total species richness for streams and rivers combined. Species richness represents the number of observed species using the eDNA metabarcoding or traditional sampling methods, and Chao II represents the bias-corrected Chao II estimates, with the 95% confidence interval in parentheses

	eDNA		Traditional	
	Species richness	Chao II	Species richness	Chao II
Global	132	207.23 (166.86–294.38)	203	230.56 (216.10–260.99)
Streams	86	104.38 (92.24–140.16)	133	155.21 (142.41–185.43)
Rivers	87	151.97 (119.19–218.12)	110	145.99 (126.83–186.98)

With regard to the watercourse type, 27 species (20.45% of the 132 species) detected in the rivers have not been caught in rivers before and are only known to live in streams. Although more species were collected in rivers with traditional methods than the eDNA metabarcoding method (Table 3), the Chao2 estimate of species richness for all rivers did not greatly differ between the metabarcoding and traditional methods (Table 3). For streams, only three detected species (2.27% of the 132 species) have not been caught in streams before (*Crenicichla multispinosa*, *Mastiglanis cf. asopos* and *Plagioscion squamosissimus*). Both the observed and estimated stream species richness values were higher based on traditional sampling methods than the metabarcoding method (Table 3).

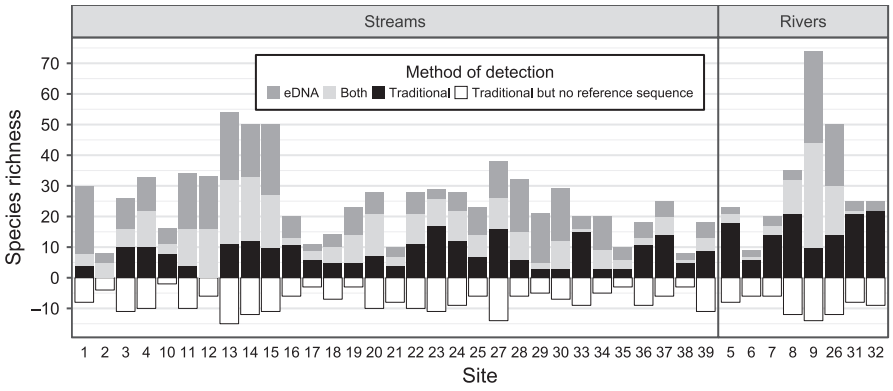

FIGURE 2 Species richness per site detected with traditional and eDNA metabarcoding methods. The species caught only with traditional methods are indicated with black (species received a reference database assignment) or white (species was absent from the reference database), those detected only with metabarcoding are indicated with dark grey, and those detected by both metabarcoding and traditional methods are indicated with light grey

At the site scale, the number of species detected was lower for metabarcoding than traditional methods in 29 of the 39 sampled sites, and the number of species common to both methods was low (mean ± *SD*: 20.74 ± 10.90 percentage of species in common). When we excluded the species that were not classified by the reference database, eDNA metabarcoding gave a lower number of species than traditional methods at 20 sites (Figure 2). For three sites, the methods produced equal species richness estimates, and a higher species richness was detected by metabarcoding in 16 sites.

The total percentage of species detected by each method (shared by the two methods, only metabarcoding vs. shared and only traditional) did not differ (Wilcoxon rank-sum test: $z = -1.03$, $p = 0.30$, Figure 3). This trend was verified for streams ($z = 0.61$, $p = 0.54$, Figure 3), but in the rivers, the traditional methods detected a greater percentage of species than the metabarcoding method ($z = -2.68$, $p = 0.0073$, Figure 3).

Considering higher scales of taxonomic resolution, the results were similar at the genus taxonomic level (Supporting information: Figure S1A, S2A and S3A). The congruence in the detected genera was low between the two methods (28.35 ± 14.26 percentage genera in common), and a higher percentage of genera was detected by traditional methods when all the sites grouped together (global) or only rivers were considered (Wilcoxon test: global: $z = -3.20$, $p = 0.0013$; streams: $z = -1.84$, $p = 0.066$; rivers: $z = -2.97$, $p = 0.0030$. The percentage of genera detected by both methods (shared genera) was similar to that obtained at the species taxonomic level (Dunn's test for stochastic dominance: $z = 0.87$, $p = 0.19$; Figure 4a,c). At the family level, the congruence between the two methods increased (45.63 ± 18.48 percentage families in common; Supporting information: Figures S1B, S2B and S3B), and the traditional methods still detected more families when all the sites were grouped together or only rivers were considered (Wilcoxon test: global: $z = -4.56$, $p < 0.001$; streams: $z = -0.26$, $p = 0.79$; rivers: $z = -2.36$, $p = 0.018$). The percentage of families detected by the two methods was higher than the percentage of species (Dunn's test: $z = 4.34$, $p < 0.001$; Figure 4b,c) and genera (Dunn's test: $z = 3.47$, $p < 0.001$; Figure 4c) detected by both methods.

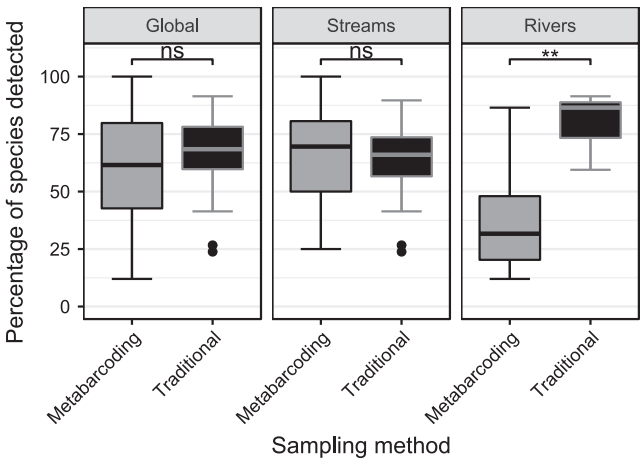

FIGURE 3 Percentage of species detected at each site with metabarcoding (dark grey) or traditional methods (black). The differences between the eDNA and traditional methods were tested using the Wilcoxon rank-sum test, ns: $p > 0.05$; $^{**}p < 0.01$

Considering the assemblage descriptors, the species richness values estimated by the two methods were significantly correlated ($r = 0.64$, $p < 0.001$, Figure 5a,b). Species occurrences were also correlated, with the most widespread species detected in a greater number of sites with metabarcoding ($\tau = 0.39$, $p < 0.001$, Figure 5c). The site ordination based on β-diversity showed a significant but weak concordance between the two methods (Procrustes analysis: $m^2 = 0.57$, $p = 0.001$). When analysing rivers and streams separately, the concordances between the two methods were also significant but weak (rivers: $m^2 = 0.42$, $p = 0.029$; streams: $m^2 = 0.70$, $p = 0.001$). When using complete MOTU data, the concordance between the traditional and metabarcoding methods was still significant and a little higher than that based on species-level data (Procrustes analysis: $m^2 = 0.44$, $p = 0.001$).

The NMDS ordination of all sites showed a distinction between the river and the stream sites for both methods (Figure 6a,b). Intragroup assemblage variability (i.e., within the stream or river group) did not differ between methods (PERMADISP: eDNA metabarcoding: $F_{1,37} = 2.08$, $p = 0.16$; traditional: $F_{1,37} = 2.29$, $p = 0.14$). However, species composition differed between the rivers and streams, and the difference was greater when traditional methods were used (PERMANOVA: eDNA metabarcoding: $F_{1,37} = 7.07$, $p = 0.001$; traditional: $F_{1,37} = 12.12$, $p = 0.001$).

When testing the effect of river drainage membership, the assemblages assessed by eDNA differed among river drainages (PERMANOVA: $F_{7,31} = 3.06$, $p = 0.001$), but their variability within each river drainage did not differ (PERMADISP: $F_{7,31} = 1.75$, $p = 0.13$).

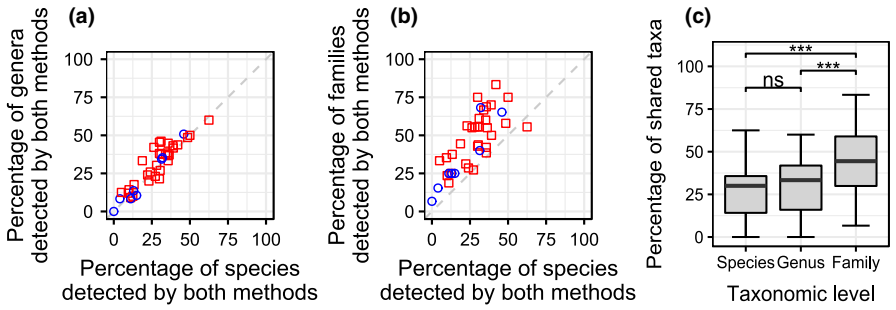

FIGURE 4 Percentage of genera (a) and families (b) detected by either the eDNA or traditional methods compared to the percentage of species detected by both methods. The 1:1 line is represented by the dashed line on all plots, and the sites are classified according to the watercourse type (□ for streams and ○ for rivers). (c) Percentage of taxa detected by both metabarcoding and traditional methods according to the taxonomic level (species, genus and family). Differences between taxonomic levels were tested using Dunn's test for stochastic dominance, ns: $p > 0.05$; ***$p < 0.001$

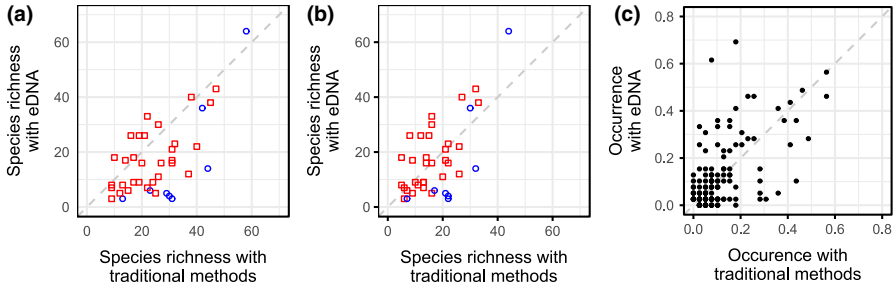

FIGURE 5 Relationship between species richness (a, b) and species occurrences (c) obtained with metabarcoding and traditional methods for (a) all data and (b) after species not in the reference database were removed. Species occurrences are expressed as the percentage of sites where a species was detected. The 1:1 line is represented by the dashed line on all plots. For (a) and (b), sites are classified according to the watercourse type (□ for streams and ○ for rivers)

When traditional methods were used, both assemblage variability (PERMADISP: $F_{7,31} = 8.95$, $p = 0.001$) and species composition differed among river drainages. However, those differences were weaker than those obtained with metabarcoding (PERMANOVA: $F_{7,31} = 1.56$, $p = 0.011$). Nevertheless, metabarcoding provided a better discrimination of the stream fauna between river drainages than the traditional methods (Figure 6c,d).

For streams, the assemblages detected using metabarcoding did not vary significantly within each river drainage (Figure 6e, PERMADISP: $F_{7,23} = 1.64$, $p = 0.20$), but their composition differed among river drainages (PERMANOVA: $F_{7,23} = 3.85$, $p = 0.001$). Based on traditional methods, the variability of fish assemblages within river drainages and the assemblage compositions differed significantly among river drainages (Figure 6f, PERMADISP: $F_{7,23} = 7.11$, $p = 0.001$; PERMANOVA: $F_{7,23} = 1.64$, $p = 0.004$).

4 | DISCUSSION

Despite imperfect local species detection, the fish assemblages derived from the metabarcoding samples were consistent with the fauna known to occur at greater spatial scales as only five of the 132 species detected using metabarcoding were outside of their spatial distribution range. Three of these species probably represent actual occurrences in the considered river drainage, although they have never been detected using traditional methods. The three species are indeed known to be in adjacent river drainages (*Krobia aff. guianensis sp1* or *Satanoperca rhynchitis*; Le Bail et al., 2000, 2012) or to have a large distribution in the Neotropics encapsulating French Guiana. This is the case for *Corydoras aeneus* (Froese & Pauly, 2015), a species whose presence in the Oyapock River drainage is therefore probable. The two remaining species were probably erroneously assigned to closely related species due to the incompleteness of our reference database. For instance, *Ancistrus aff. temminckii* was detected outside of its known range in areas colonized by the closely related species *Ancistrus aff. hoplogenys*. *A. aff. hoplogenys* was not in our reference database, so sequences of *A. aff. hoplogenys* were probably wrongly assigned to *A. aff. temminckii*, the most similar species in the reference database. Likewise, *Hemiodus quadrimaculatus* was detected in the Maroni River drainage, instead of *Hemiodus hurault*i, a closely related species, that was not in the reference database. Within river drainages, we adequately differentiated between small-stream fauna from large rivers using metabarcoding. Only three of the 86 species detected by metabarcoding in small streams were only detected in rivers using the traditional methods, but two of those (*Crenicichla multispinosa* and *Mastiglanis cf. asopos*) are known to occur in small streams (Keith et al., 2000; Planquette et al., 1996), although they were not found

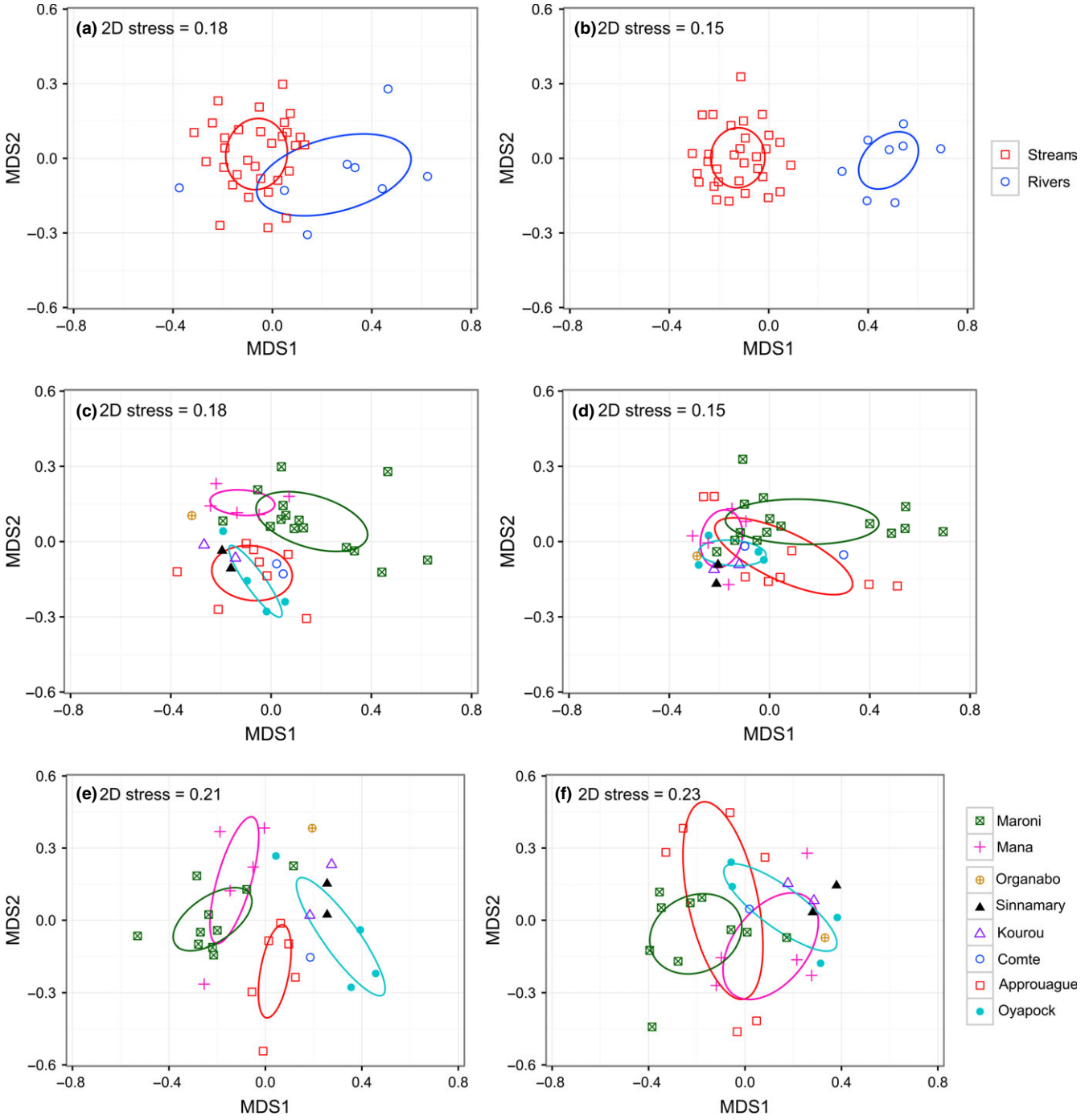

FIGURE 6 Nonmetric multidimensional scaling (NMDS) ordination of (a, c) the entire data set for metabarcoding, (b, d) the entire data set for traditional methods, (e) only stream assemblages detected with metabarcoding and (f) only stream assemblages detected with traditional methods. For (a) and (b), sites are classified according to the watercourse type (□ for streams and ○ for rivers), and for c, d, e and f, sites are classified according to the river drainage (see legend for river drainage classification). Ellipses represent the standard deviation of each group

using traditional methods in our study. Only one occurrence of the species *Plagioscion squamosissimus* in a small stream was unexpected.

Turning the focus from species distribution to fish assemblages revealed that eDNA metabarcoding and traditional methods provided different patterns. This contrasts with results in temperate areas, where eDNA metabarcoding provided an exhaustive representation of the fish assemblages (Civade et al., 2016; Valentini et al., 2016). The discrepancies between the local fish assemblage results of eDNA metabarcoding and traditional methods in Guianese stream and river sites might be partially explained by the incompleteness of the fish inventories created using traditional methods. Indeed, gill nets are known to be species selective and investigate the fish in a limited range of habitats (Murphy & Willis, 1996; Mojica, Lobón-Cerviá, & Castellanos, 2014). Similarly, rotenone samples investigate fauna from restricted reaches within streams that do not encompass all available habitats (Allard et al., 2016). Thus, some species inhabiting particular

habitats probably remain undetected by traditional methods. In contrast, eDNA metabarcoding provides a way to detect fish independent of their habitat use (Olds et al., 2016), and it integrates fish data over larger scales (from a few 100 m to several kilometres) than those of the local habitats sampled using traditional methods (Civade et al., 2016; Deiner & Altermatt, 2014; Deiner, Fronhofer, Mächler, Walser, & Altermatt, 2016; Fukumoto, Ushimaru, & Minamoto, 2015). This explains why the river eDNA samples detected both stream and river fish fauna, making eDNA metabarcoding less efficient than traditional methods at discriminating fauna from large rivers from those of nearby streams. In contrast, the ability of eDNA metabarcoding to detect distant fauna makes it an efficient tool to measure diversity at regional scales (e.g., over a drainage basin scale, Deiner et al., 2016) and therefore makes metabarcoding an efficient method to assess regional biodiversity. The integrative characteristic of eDNA metabarcoding across a large spatial scale (Civade et al., 2016; Deiner & Altermatt, 2014; Deiner et al., 2016) also explains why eDNA metabarcoding was more efficient than traditional methods at distinguishing between small-stream fauna from distinct river drainages. Although the species detection using eDNA metabarcoding remains incomplete, data were not influenced by the physical characteristics of the stream. In contrast, deep pools or burden areas, such as fallen submerged trees, cannot be sampled by rotenone (no access to the fish lying above branches or at the bottom), although these areas are known to be inhabited by a rich fish fauna (Wright & Flecker, 2004). Using traditional methods, the same habitat types are therefore sampled at all investigated sites (Allard et al., 2016), which probably hides interdrainage discrepancies and therefore causes the underestimation of faunistic distinctiveness between river drainages.

Our eDNA inventories are nevertheless incomplete, as a substantial part of the fauna captured using traditional methods was not detected using metabarcoding (Table 3). This might be due to imperfect detection (Mojica et al., 2014; Willoughby, Wijayawardena, Sundaram, Swihart, & DeWoody, 2016) or the erroneous attribution of reads to species. The incompleteness of the reference database (~25% of the species caught are not in the reference database, representing 24.31 ± 7.23% of the species at each site) might, for instance, explain the grouping of some reads in higher taxonomic units (genera or families). In other words, slight differences between reference sequences of the same rank, especially the genus rank, can result in the assignment of reads to one unique unit (Ardura et al., 2013; Pochon et al., 2015). That was probably the case for some genera with closely related species from a morphological point of view and probably also from a molecular point of view (Brown, Chain, Crease, MacIsaac, & Cristescu, 2015; Flynn, Brown, Chain, MacIsaac, & Cristescu, 2015), such as species in the *Bryconops*, *Leporinus* or *Pimelodella* genera. Those genera were indeed represented by a high number of reads in our results, but species discrimination was not possible, and those genera were excluded from our analyses. Enhancing the relevance of eDNA samples requires more molecular data on species to be gathered. This is a crucial step in the development of a precise method to inventory species-rich ecosystems based on eDNA (Valentini et al., 2009). Public repositories, at the moment, lack information on the species occurring in these ecosystems. For instance, using GenBank to classify our eDNA sequences yielded few Guianese fish taxa assignments, which underlines the need to develop reference data for most species. Here, we expanded the reference databases of Neotropical fauna using the 12S rRNA molecular marker for 114 new species. Although these species account for only 5% of the 4035 Neotropical freshwater fish species reported by Lévêque, Oberdorff, Paugy, Stiassny, and Tedesco (2007), they nevertheless account for a wide range of genera (18.6% of the 705 Neotropical freshwater fish genera) and families (60.8% of the 74 Neotropical freshwater fish families). As the species considered in this study represent most of the major fish orders in the Neotropics, the reference database can be used in future metabarcoding fish inventory work throughout the Neotropics using a family-level taxonomic resolution. Metabarcoding fish inventories at a finer taxonomic resolution (genus or species) over larger spatial areas (Guiana shield, Amazon River drainage or the entire Neotropical area) will nevertheless require additions to the reference database. We therefore appeal to forthcoming studies to complement our reference data with more species.

Another potential pitfall lies in the limitation of using a single marker for species assignments. Although the "teleo" primers were designed to amplify Teleostei DNA, they may also amplify nontarget taxa without the occurrence of mismatches in the primers (Valentini et al., 2016). In addition, low divergences between closely related species for the considered marker can prevent species discrimination within the same genus (as experienced here for the *Bryconops* or *Leporinus* genera). One way to overcome this limitation is to use several markers (Marcelino & Verbruggen, 2016; Miya et al., 2015), which would help to complement the species list and to confirm species occurrences (Olds et al., 2016). Metagenomic methods, although still expensive and time consuming, are known to efficiently discriminate species and therefore also represent an alternative to the use of multiple markers (Gómez-Rodríguez, Crampton-Platt, Timmermans, Baselga, & Vogler, 2015; Srivathsan, Sha, Vogler, & Meier, 2015). In addition, targeting particular species, e.g., rare species or species caught with traditional methods but not detected with metabarcoding, with species-specific approaches (barcoding approaches including qPCR and ddPCR) might also enhance the efficiency of eDNA methods in tropical freshwater ecosystems (Evans et al., 2017; Schmelzle & Kinziger, 2016; Simmons, Tucker, Chadderton, Jerde, & Mahon, 2015). These tools might allow the determination of whether the nondetection of a species is due to its absence in the considered ecosystem or due to its low abundance, which might reduce the quantity of eDNA present in samples and thus affect molecular and bioinformatic analyses (due to no amplification or a read number below the analysis threshold).

5 | CONCLUSION

Despite pitfalls and limitations, eDNA metabarcoding is a promising approach for the assessment of fish biodiversity in tropical areas.

Given the rarity of erroneous species detection, the significant correlations between fish diversity and occurrences for both traditional methods and eDNA metabarcoding, and the higher capacity of metabarcoding than traditional methods to discriminate between river drainages, it appears that metabarcoding can be used as a rough but rapid biodiversity assessment method in the Neotropics. eDNA metabarcoding should therefore be used as a complementary tool to traditional methods, pending future developments that make this methodology more exhaustive. Turning eDNA metabarcoding into a more exhaustive inventory tool will need to expand reference databases and optimize field and laboratory protocols (Rees, Gough, Middleditch, Patmore, & Maddison, 2015; Roussel, Paillisson, Tréguier, & Petit, 2015). Such developments are crucial because destructive inventory tools (e.g., rotenone, gill nets) are now banned from most countries for both ethical and legal reasons. For instance, in Europe, the use of rotenone has been regulated since 2008 (European laws 2008/296/CE and 2008/317/CE). Although a few exceptional authorizations have been obtained to conduct scientific studies, its use has now completely been banned. Developing a new, nondestructive sampling method would unlock the current situation, where scientists and environmental managers can no longer achieve complete species inventories. The implementation of eDNA-based methods would therefore allow the collection of information on fish assemblages in tropical freshwaters to continue, which is of particular importance given the current increase in anthropogenic disturbances and associated declines in the aquatic biodiversity of Neotropical ecosystems (Allard et al., 2016; Hammond, Gond, de Thoisy, Forget, & DeDijn, 2007; Winemiller et al., 2016).

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CONFLICTS OF INTEREST

P.T. is the inventor of a patent on "teleo" primers and on the use of amplified fragments to identify amphibian and fish species from environmental samples. This patent only restricts commercial applications and has no impact on the use of this method by academic researchers. A.V. and T.D. are research scientists in a private company that specializes on the use of eDNA for species detection.

DATA ACCESSIBILITY

The reference database sequences, all Illumina raw sequence data, the raw metabarcoding data (MOTUs data), analysis codes, the sampling results from the traditional sampling methods and the sequences assignation results are available on Dryad https://doi.org/10.5061/dryad.dc25730.

AUTHOR CONTRIBUTIONS

K. C. and S. B. designed the study and discussed the results; K. C., G. G., P.T. and S.B. conducted the metabarcoding sampling; K.C., L.A., G.G., R.V. and S.B. conducted the traditional sampling; A.V., R.E. and A.I. conducted the laboratory work; A.V. and T.D. ran the bioinformatic analysis; K.C. analysed the data; and K.C. led the writing, with contributions from all authors.

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SUPPORTING INFORMATION

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