**Merging two eDNA metabarcoding approaches and citizen-science based sampling to facilitate fish community monitoring along vast Sub-Saharan coastlines**

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**Running head:** eDNA assessment of aquatic systems in Mozambique

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**Competing interest:**

Kat Bruce, Cuang Tong, Vere Ross-Gillespie, Laura Balcells, Sarah Chordekar, Alex Crampton-Platt, and Judith Bakker are employed by NatureMetrics, a for profit company providing DNA-based solutions for biodiversity monitoring.

**Abstract**

The coastline of Sub-Saharan Africa hosts highly diverse fish communities of high conservation value, which are also key resources for local livelihoods. However, many costal ecosystems are threatened by overexploitation and their conservation state is frequently unknown due to limited monitoring budgets and challenges associated with their vast spatial extents. Here, we evaluated the potential of citizen science-based eDNA surveys to alleviate such chronic data deficiencies and assessed fish communities in Mozambique using two 12S metabarcoding primer sets. Samples were either collected by scientific personnel or trained local community members and results from the two metabarcoding primer sets were combined using a newly created data merging approach. Irrespective of the background of sampling personnel, a high average fish species richness was recorded (38±20 OTUs sample-1). Individual sections of the coastline largely differed in the occurrence of threatened and commercially important species, highlighting the need for regionally differentiated management strategies. A detailed comparison of the two applied primer sets revealed an important trade-off in primer choice with MiFish primers amplifying a higher number of species but Riaz primers performing better in the detection of threatened fish species. This trade-off could be partly resolved by applying our data-merging approach, which has the potential to provide a more robust baseline-data for decision-making processes. Overall, our study provides encouraging results but also highlights that eDNA-based monitoring will require further improvements of e.g., reference databases and local analytical infrastructure to facilitate routine applications in Sub-Saharan Africa.

**Introduction**

The coastline of Sub-Saharan Africa (SSA) encompasses a wide range of highly diverse natural habitats (Stuart, Adams, & Jenkins, 1990). Marine biodiversity hotspots include unique systems such as the Congo delta, the islands along the East African coast and the Mozambique Channel (Küper, et al., 2004; Griffiths, 2005). Despite the very distinct fauna and flora hosted by these ecosystems, resource use patterns are very similar across most regions in SSA. In coastal regions, such as the Mozambican coastline, artisanal fisheries are a key contribution to local livelihoods (Sowman & Cardoso, 2010), whilst offshore fish stocks are exploited by international fishing fleets (Nielsen et al., 2004; Zeeberg, Corten, & de Graaf, 2006). Monitoring of both coastal and offshore fish communities is in many regions rudimentary (Kolding, van Zwieten, Marttin, & Poulain, 2016) and in many cases even baseline community data are lacking. Effective and routine monitoring is, however, a central requirement to address the many fundamental current and future management challenges in SSA.

Across SSA, human population is projected to almost double within the next 30 years (United Nations, 2021), which is expected to substantially increase the pressure on natural resources and habitats (Ramin, 2009). The resulting increased exploitation of fish stocks may be especially problematic when fish landings are poorly monitored and fishing regulations are insufficiently enforced as is the case in many SSA coastal regions (Jacquet, Fox, Motta, Ngusaru, & Zeller, 2010). Direct anthropogenic pressures are likely to be further exacerbated by climatic changes. Temperature increases and the increased incidence of marine heat waves have already had catastrophic impacts on coral reef systems globally (Stuart-Smith, Brown, Ceccarelli, & Edgar, 2018), which provide highly valuable ecosystem services and support about a quarter of total marine biodiversity (Hughes et al., 2017). Additionally, temperature increases drive increases in the frequency of tropical storms and alterations of marine nutrient cycling with large potential consequences for natural species assemblies (Behrenfeld, 2011; Doney et al., 2012). Considering these multiple, substantial threats, frequent and accurate biodiversity monitoring is urgently required to implement adaptive management approaches and respond to early warning systems before the onset of catastrophic break-downs (Floros, Schleyer, & Maggs, 2013; Clements, Blanchard, Nash, Hindell, & Ozgul, 2017).

However, regular monitoring is a complex and challenging task (Jacquet et al., 2010). In Mozambique, the focal region of this study, fish monitoring relies on surveying methods such as electrofishing, netting, baited traps, as well as visual and acoustic surveys (Gell & Whittington, 2002; Marshall, Dudgeon, & Bennett, 2011; Samoilys et al., 2019). These methods require substantial efforts of skilled personnel, and electrofishing and netting are ecologically invasive (Hänfling et al., 2016). Furthermore, these conventional monitoring approaches are hampered by systematic sampling bias, limits of morphological identification, and elevated risks of false negative results (i.e., the failure to detect present species; Yamamoto et al., 2016; Wang et al., 2021). This leads to frequent underestimation of species distributions and diversity and a bias in knowledge towards larger, more charismatic and commercially important species. Hence, conventional methods generate without doubt very valuable ecological data, but they are likely inadequate for regular monitoring of the estimated ~1500 fish species that occur along the 2700 km coastline of Mozambique (Schneider, Buramuge, Aliasse, & Serfontein, 2005).

Molecular-based detection methods, such as environmental DNA (eDNA) metabarcoding, offer the potential for a robust, scalable, and cost-effective alternative for the monitoring of fish communities (Thomsen et al., 2011; Ruppert, Kline, & Rahman, 2019; Gilbey et al., 2021). eDNA metabarcoding refers to the amplification and sequencing of mixed-species DNA obtained from environmental samples such as water, sediment, or air (Ruppert et al., 2019; Lynggaard et al., 2022). A major advantage of eDNA-based monitoring is that field samples can be collected quickly and easily without requiring specialist survey skills. Further, eDNA preservation techniques allow for the storage of samples at room temperature for several months without performances losses (Mauvisseau, Halfmaerten, Neyrinck, Burian, & Brys, 2021).

The relatively easy collection and storage of eDNA samples makes eDNA-based approaches very attractive for citizen-science and participatory, community-led monitoring programmes (Larson et al., 2020). The involvement of local communities in decentralised monitoring networks can not only substantially reduce sampling costs but also lead to local empowerment and support conservation by increasing environmental awareness (San Llorente Capdevila et al., 2020). Hence, community-based monitoring using eDNA approaches could be a game changer for evidence-based natural resource management in Sub-Saharan Africa and enable cost-effective environmental monitoring across large spatial scales.

An important requirement for eDNA-based monitoring to live up to its potential is the robust representation of natural community composition, which strongly depends on the choice of primer sets. Primers define the phylogenetic identity of the taxa that can be detected, and they often differ in their power to discriminate among species and their sensitivity for detecting target groups (Hajibabaei, Porter, Wright, & Rudar, 2019; Schenekar, Schletterer, Lecaudey, & Weiss, 2020). Most fish eDNA metabarcoding surveys to date use primers amplifying a short fragment of the mitochondrial 12S gene, since these have generally been shown to have a higher specificity and record a greater fish diversity than primers targeting 16S rRNA or COI genes (Kelly, Port, Yamahara, & Crowder, 2014; M. Miya et al., 2015; Collins et al., 2019; Milan et al., 2020; Schenekar et al., 2020; Zhang, Zhao, & Yao, 2020; Jackman et al., 2021; Polanco et al., 2021).

Although the 12S region seems to be a clearly preferential target for metabarcoding of fishes (Zhang et al., 2020), there are different 12S primers available potentially resulting in trade-offs between primer. For example, different primer pairs may vary in (1) DNA amplification efficiency, (2) the ability to discriminate between closely-related species, and (3) the availability of reference sequences to enable confident identification to species level (Polanco et al., 2021). These performance indicators can vary even for the same primer among taxonomic groups (e.g. families), complicating the process of primer choice.

A possibility to resolve trade-offs in primer choice that is increasingly applied (Blackman et al., 2021) is the use of multiple primer pairs for analysing the same set of samples. Such multi-primer approaches can especially be advantageous in highly diverse tropical regions as primers are primarily developed and tested in temperate regions and primer choice trade-offs in tropical environments are largely underexplored (Jerde, Wilson, & Dressler, 2019). However, the data generated from multiple primer sets is not always straight forward to interpret as biodiversity patterns and the occurrence of priority conservation species might differ between datasets. The development of data merging techniques that can combine information gathered by applying two or more primer sets could therefore help to generate more complete community data supporting the management of extensive tropical coastlines.

In this study, we evaluate the potential to use eDNA metabarcoding and community-based sampling for surveying tropical and subtropical fish communities in Mozambique. Samples were collected by experienced scientific personnel as well as by trained local community members along a 600 km stretch of the Mozambican coastline. We then applied two 12S primer sets and developed a merging-algorithm to combine the resulting datasets for the analysis of fish diversity and community composition. Together with a detailed performance comparison of the two used primer sets, the “MiFish-U” (M. Miya et al., 2015) and the “Riaz” primers (Riaz et al., 2011; Kelly et al., 2014), these assessments provide an important baseline for future eDNA-based monitoring and conservation efforts in Mozambique.

**Methods:**

*Field sampling*

Water samples were collected from 47 locations (10 freshwater, 37 marine) spread throughout the southern part of Mozambique, between Ponta do Ouro and Inhambane Bay, covering 600 km of coastline, from July to September 2020 (Fig. 1; Table S1). Sample sites included marine as well as brackish and some freshwater sites (for simplicity, brackish and freshwater are subsequently referred to as freshwater sites). Samples in Inhambane Bay were collected both in July and early August as well as in September, which enabled a comparison between early and mid-late dry season. At each site, water was collected from the surface, sample volumes ranged from 800 mL to 2000 mL. Water samples were immediately filtered by pressure filtration using a 100 mL syringe. The sterile encapsulated 0.8 μm pore size PES filter (50 mm diameter) contained a 5 μm glass fibre prefilter (NatureMetrics, UK). The filter was air-dried using the syringe and the capsule subsequently filled with 1.5 mL of Longmire’s buffer for preservation (Mauvisseau et al., 2021). Disposable gloves were used for sample collection and replaced for each new sample. Sterile and disposable equipment was used at each collection site to avoid potential cross-contamination. Filters were stored in the dark at room temperature until DNA extraction.

*Sample analysis*

Samples were analysed using MiFish and Riaz primers for DNA amplification in two different metabarcoding runs. Whilst MiFish primer are specifically designed to target fish communities (Miya et al., 2015), Riaz primers target vertebrates more generally. Nonetheless, Riaz primers are 12S primers and often perform well in characterising fish communities (Zhang et al., 2020). We therefore focused in our primer comparison on their potential to characterise and monitor fish communities, which was the main goal of this study.

DNA was extracted following Spens et al., (2017), using the DNeasy Blood and Tissue kit (QIAGEN, Germany). DNA lysis was performed inside filter capsules to avoid DNA loss and contamination following a protocol scaled for larger lysate volumes. Two distinct primer sets were investigated to optimise DNA recovery of a hypervariable region of the 12S rRNA in a two-step PCR process. First, purified DNA was amplified using (i) 12S MiFish primers (M. Miya et al., 2015) amplifying a 172 bp fragment, with modification on the second base pair of the Forward primer (Cytosine replacing a Thymine) and (ii) 12S Riaz primers (Riaz et al., 2011; Kelly et al., 2014) amplifying a 106 bp fragment. Subsequently, tails were added at the 5’ end to be complementary with Illumina Nextera index primers. DNA amplifications with both primer sets were performed with 12 replicates each in a final volume of 8 μL containing 1X Phusion Green Hot Start II High Fidelity PCR Master Mix (ThermoScientific, USA), 0.4 μM of each Forward and Reverse tailed primers, 0.6 μg/μL of BSA (ThermoScientific), 3% of DMSO (ThermoScientific), 1.5 mM of MgCl2 (Invitrogen) and PCR grade water (ThermoScientific). PCR conditions were set to an initial denaturation at 98°C for 3 min followed by 45 cycles at 98°C for 20 s, 69°C for 15 s and 72°C for 15 s, and a final elongation at 72°C for 5 min. Positive control consisting of a mock community with a known composition of non-native fish species and negative control consisting of PCR grade water were analysed alongside eDNA samples. The presence of PCR products was evaluated by gel electrophoresis, and all PCR replicates for each sample were pooled and purified using MagBind TotalPure NGS (Omega Biotek, USA) magnetic beads with a 0.8:1 beads-DNA ratio to remove primer dimers.

Purified amplicons were indexed in a second PCR following the Illumina 16S Metagenomics Sequencing Preparation protocol in a final volume of 20 μL containing 1X Phusion Green Hot Start II High-Fidelity PCR Master Mix (ThermoScientific), 2 μL of Nextera XT i7 Index Primer (Illumina, USA), 2 μL of Nextera XT i5 Index Primer (Illumina), 4 μL of PCR grade water (ThermoScientific), and 2 μL of pooled and purified first-round PCR product. After the second amplification, PCR products were again purified (1:1 beads:DNA ratio), quantified using a Qubit dsDNA HS Assay Kit, sized using a TapeStation D1000 ScreenTape System (Agilent, USA) and normalised to 4 nM. Libraries were pooled in equimolar concentrations and sequenced on a MiSeq platform (Illumina) with a V2 2 × 250 bp kit. The final library was loaded at 12 pM with a 10% PhiX control spike.

*Bioinformatic and statistical analyses*

Samples were demultiplexed based on the combination of the i5 and i7 index tags. Paired-end reads for each sample were merged using USEARCH (Edgar, 2010) requiring a minimum of 80% agreement in the overlap. Forward and reverse primers were trimmed from the merged sequences using Cutadapt (Martin, 2011) and length-filtered to retain sequences of the expected size for each primer set. Sequences were quality filtered using USEARCH to retain only those with an expected error rate per base of 0.01 or below and dereplicated by sample, retaining singletons. Unique reads from all samples were denoised in a single analysis using UNOISE (Edgar, 2016), requiring retained ZOTUs (zero-radius Operational Taxonomic Units) to have a minimum abundance of 8 in at least one sample. ZOTUs were then clustered at 99% similarity with USEARCH to form OTUs (Operational Taxonomic Units) A taxon-by-sample table was generated by mapping all dereplicated reads for each sample to the OTU representative sequences using USEARCH with an identity threshold of 97%.

OTUs were identified via BLAST searches of the representative sequences against the nucleotide database of NCBI and a local curated database of 12S fish sequences, requiring an e-score of 1e-20 and a hit length of at least 90% of the query sequence. Species-, genus-, and higher-level assignments required at least one hit of ≥99%, ≥95%, and ≥92% similarity respectively. Where there were multiple hits meeting these criteria, public GBIF records for Mozambique were used to resolve the conflicts where possible. Low abundance detections were omitted using a filter threshold of 0.02%, or 10 reads, whichever was the greater threshold for the sample. Common contaminant species (human and domestic animals) and unidentified OTUs were also excluded.

Sequencing depth per sample of datasets generated by the application of MiFish and Riaz primers were 62139 ± 15015 and 57228 ± 20088, respectively (Fig. S1). The primer comparison was implemented on all samples that contained at least 8000 reads of fish DNA per dataset (*n* = 44). 8000 reads per sample was chosen as threshold for this part of the analysis as it provides a reasonable compromise between (a) reliable richness assessments in rarefied samples and (b) avoiding the exclusion of too many samples because of high threshold values. In order to assess differences in alpha diversity (richness and evenness per sample) between primer-sets, we performed paired t-tests after data were log-transformed (if necessary) to achieve statistical requirements such as variance homogeneity and normality. We also used a paired t-test (or Mann-Whitney u-test in case of non-parametric data) to test for differences in taxonomic coverage, which was defined as the fraction of genera that were recorded by one primer-set and were also present in the second primer set. Further, differences between the alpha diversity of habitat types (freshwater, southern marine habitats, and central marine habitats) were tested using ANOVA after performing a log-transformation to achieve variance homogeneity. Additionally, we tested for systematic differences in primer performance along diversity gradients using a model 2 major-axis regression using the lmodel2 package in R (Legendre, 2018). A model 2 regression was also used to test for common patterns in beta-diversity (calculated as Bray-Curtis dissimilarity) recorded with the two primer approaches by pairwise comparing similarity values. Assessments of threatened species, which were also used for primer comparisons were based on the IUCN’s red-list (IUCN, 2021).

Finally, we developed an approach to create a joined data-set based on the data provided by the two individual primer sets. The main function of the algorithm is to capitalise on the availability of two different datasets to detect false negative results that would emerge if only one primer pair were applied. This is accomplished by comparing records of occurrence and the taxonomic assignment of the OTUs from both data-sets (See Box 1 for method explanation). Under certain circumstances, it is not possible to determine whether entries in two datasets represent two different or the same species (e.g., if one assay reports a species and the other assay reports a taxon of the same genus without species affiliation). Under such circumstances, we chose conservative procedures (Box 1) to avoid the artificial inflation of taxon richness as a result of data merging procedures. Nevertheless, errors in taxonomic assignment can negatively affect the merging process. A detailed discussion of potential error sources and an in-depth description of the merging algorithm are provided in the Supplementary Information, Section 1. An annotated R-Script to implement data merging procedures, which can in principle be applied to merge any metabarcoding dataset, is included as an annex. All data processing and statistical analyses were performed in R version 4.1.0 [(R Core Team Foundation)](https://www.zotero.org/google-docs/?NBLlMg).

**Results**

Both Riaz and MiFish primers recovered generally a high fish diversity per sample (Fig. 2). Yet, the identity of fish species and the overall performance differed between assays. Whist the MiFish primers detected a higher number of fish OTUs across all samples (317 vs 263 OTUs), the Riaz dataset encompassed a higher number of fish orders and threatened species (Fig. 2, S2, S3, S4). Riaz primers by design also facilitate the detection of other vertebrate species (53 OTUs, 23% of all reads, 98% of non-fish taxa belonging to birds), which can be valuable auxiliary data for fisheries management. Though, a high contribution of bird eDNA to total vertebrate eDNA decreases the number of fish target reads and resulted here in the exclusion of five samples (<3000 reads of fish sequences per sample, decreasing the reliability of biodiversity comparisons).

The average OTU richness per rarefied sample was very similar between the two primer sets (t-test, *p* = 0.16) and a pairwise comparison of OTU richness demonstrated their consistent coupling (slope of model 2 regression was not significantly different from 1; confidence interval 0.66-1.13). However, MiFish primers had a significantly better taxonomic coverage at the genus level (i.e., MiFish primers had a lower probability to miss genera that were detect by Riaz primers than vice-versa; Mann-Whitney u-test, *p* < 0.01; Fig. 2). The rate of successful taxonomic assignment was generally low and only 28% of MiFish and 37% of Riaz OTUs could be identified to the species level. These values were in the range of a GenBank-based in-silico analysis, which revealed that 12S sequences were available for 37% of fish species listed for Mozambique (see Annex 2 for species-by-species results). Assignment success increased at lower taxonomic resolution, but even at the family level more than 10% of MiFish OTUs remained unassigned.

Patterns in community composition showed a clear distinction among fish communities from Inhambane Bay, the southern Mozambican coastline, and freshwater habitats (Fig. 3). This differentiation was consistent for the datasets generated with Riaz and MiFish primers, and a pairwise comparison of beta-diversity showed a good accordance among the two assays (model 2 regression, confidence interval of slope: 0.95 - 1.02; Fig. 3C). Nonetheless, less than 50% of all fish genera per sample were identified by both assays individually, indicating that detected taxa differed substantially between assays.

In order to mediate trade-offs between different primer sets and minimise the rate of false negatives, we developed a data-set merging algorithm (Box 1) and applied this procedure for further biodiversity analyses. The resulting fish OTU richness was 38 (±20) taxa per sample and no significant differences were found between samples collected by either local community members or research personnel (t-test, *p* = 0.29). Moreover, we recorded 29 different threatened fish species with an average of two threatened species per sample. The highest number of threatened species was found at the end points of bays and in estuaries characterised by both marine and freshwater influences (Fig. 1, S5, S6).

OTU richness did not differ among target-ecosystems (ANOVA, *p* = 0.32) but an assessment of the most abundant OTUs, as well as of species with high commercial and conservation value revealed large regional differences (Fig. 4). As expected, species occurrence and abundance differed substantially between the southernmost Mozambican coastline and Inhambane Bay. However, we also found large differences in the threatened species composition recorded in Inhambane Bay in July-August compared to September, highlighting the importance of an appropriate temporal resolution to support conservation planning in the region.

**Discussion**

Recurrent monitoring of the vast seascapes of SSA will be crucial for their protection and to respond to the growing anthropogenic pressures these habitats are expected to face in upcoming decades (Ramin, 2009; Ibe & Amikuzuno, 2019). Here, we have tested the potential of eDNA-based techniques, using two different primer sets, to survey coastal fish communities in Mozambique. eDNA-based monitoring, which was partly implemented by local communities was able to reveal a high species richness per sample, reflecting its sensitivity and potential to complement conventional surveying methods. However, several improvements are still required to aid routine applications. First, reference databases for Mozambique show large gaps and even some of the most abundant species failed to match known reference sequences. Further, we found substantial species-recovery trade-offs between the different primer sets. However, these trade-offs can be overcome by using multiple primers combined with data-merging approaches such as the one developed here. Hence, our results demonstrate that once methodological hurdles are overcome, eDNA-based monitoring techniques can be powerful tools to address the diverse conservation challenges encountered in coastal habitats of SSA.

*Primer selection*

In this study, we used two 12S primer sets, which have previously been shown to outperform assays targeting other gene regions for fish community assessments (Kelly et al., 2014; M. Miya et al., 2015; Collins et al., 2019; Milan et al., 2020; Schenekar et al., 2020; Zhang et al., 2020; Polanco et al., 2021). Although the two primer sets reached similar results in some regards (e.g., alpha diversity, community characterization), we found also substantial trade-offs in primer performance. Similar trade-offs among metabarcoding primer sets have already been encountered in previous studies evaluating a wider primer range (Polanco et al., 2021). For example, “teleo” primers targeting 12S mtDNA (Valentini et al., 2016) have been shown to recover higher species richness compared to MiFish primers, despite the higher power of the latter to discriminate among taxa at species, genus and family levels (Polanco et al., 2021). In our application in Mozambique, MiFish primers detected a substantially higher number of fish OTUs across all samples, whilst Riaz primers facilitated the detection of a 50% higher number of threatened fish species. Further, Riaz primers are designed more generally to target vertebrate taxa, and have the advantage of providing data on water birds and other taxa of potential interest (in Mozambique, this may help to detect e.g., Dugongs and other critically endangered marine vertebrates). However, this lower specificity means abundant non-fish eDNA may overlay the molecular signal of target fish species (Zhang et al., 2020). In our study, this resulted in seven samples with substantially fish sequence reads, which translates into a lower detection power and a higher risk of false negative results for our primary target group. Hence, the optimal choice of the primers for fish community characterisation will depend on specific conservation and research targets and will need to be determined on a case-by-case basis.

A possibility to capitalise on the strengths of individual primer sets is to apply multiple primer sets and merge the resulting datasets following, e.g., the procedure developed in our study (Box 1). However, the procedure is not trivial, as uncertainties and errors during taxonomic assignment can potentially result in both under and overestimation of species richness during the data-merging process. One potential challenge is that cross-contamination or tag jumps during MiSeq sequencing runs (Schnell, Bohmann, & Gilbert, 2015; Bohmann et al., 2021) can potentially be amplified by data merging and lead to an inflation of the species richness per sample. On the other hand, the power of combining datasets to detect false negative results in one assay, a major strength of the merging procedure, is lower when reference databases are incomplete. For example, merging data entries of the two assays that have both been assigned to the same species, is much more robust than merging entries that only were assigned to family-level. However, in spite of these methodological challenges, data merging approaches have the power to resolve primer-related trade-offs, provide more robust estimates of local biodiversity and enhance community data quality to support a more robust conservation planning.

*Ecological considerations and reference databases*

Costal habitats contributing to the Mozambique’s exclusive economic zone sustain approximately half of the country’s population living in coastal areas (Hoguane, Jose, Francisco, & Simbine, 2018). In our study, the application of eDNA-metabarcoding facilitated the detection of a large range of economically important as well as threatened fish taxa. However, there were also several gaps in our taxonomic assessment. A major shortcoming of currently available fish metabarcoding primers is their poor coverage of elasmobranch species (Asbury et al., 2021). Whilst MiFish primers rarely detect elasmobranch taxa (Masaki Miya, Gotoh, & Sado, 2020), Riaz primers revealed the presence of the critically endangered bowmouth guitarfish *Rhina ancylostoma,* as well as the threatened cownose ray *Rhinoptera jayakari,* and whiptail stingray *Himantura* spp. in Inhambane Bay. However, these species only represent a fraction of the elasmobranch communities present in the target ecosystems (Schneider et al., 2005; Ebert, Dando, & Fowler, 2021). Hence, a refinement of currently available metabarcoding primers targeting ray and shark species is urgently required to complement eDNA-based assessments of threatened marine fish communities (Asbury et al., 2021).

Moreover, we found large temporal turn-over of the threatened fish species recorded with both primer sets in Inhambane Bay. These differences might result from changes in habitat use as the sampling period between July and September marks the transition from the rainy to the dry season. However, there might also be other factors contributing to the observed differences. First, threatened species are often rare and difficult to detect. The resulting low eDNA concentrations shed by these rare species increases the stochasticity of their detection (Currier, Morris, Wilson, & Freeland, 2018) and make sample comparisons based on rare species less comparable, especially field replication is low (Mauvisseau et al., 2019). Second, sampling conducted by local community members was not repeated at the same locations in both time periods. In July/August more sampling points from the outer Bay were recorded, whereas in September sampling focused more on the endpoints of the lagoons (Fig. S5). Hence, spatial differences might have been a confounding factor of temporal effects on community composition recorded in our study. Nonetheless, large spatio-temporal differences in the occurrence of threatened species are an important finding for the implementation of temporary fishing restrictions and other habitat management strategies.

A critical measure that will increase the ecological insight generated through eDNA applications in Mozambique and in many Sub-Saharan countries is the improvement of available reference databases. In our study, species level assignment was merely 26.5%, a very low rate compared to what can be achieved with fish reads in well-studied temperate regions (for example, up to >90% in Collins et al., 2019). Low species-level assignment can partly be attributed to targeting the 12S region instead of COI, which is the most common marker used for fish phylogeny and taxonomic classification (Zhang et al., 2020). However, the use of COI as target region results in the amplification of many non-target taxa such as invertebrate, bacterial, and human DNA (Günther, Knebelsberger, Neumann, Laakmann, & Martínez Arbizu, 2018; Collins et al., 2019), which can substantially decrease the detection power for fish as a target taxa of eDNA primers sets. Another reason for the low species assignment rates is that Mozambique is expected to still host a relatively high number of undescribed fish species (Gell & Whittington, 2002). In this context, eDNA applications coupled with more complete reference databases might aid in the detection of previously undescribed species, eventually facilitating their description (Zinger et al., 2020).

*Possible future improvements*

The regular and reliable monitoring of coastal fish communities in SSA will require a cost-effective, scalable, highly sensitive, and robust surveying method. Our results demonstrate that the eDNA-based approach used in this study can fulfil these requirements if it is appropriately adjusted to management needs. Sensitivity of assessments could for example be further increased if the highly heterogeneously distribution of eDNA in natural habitats (Troth, Sweet, Nightingale, & Burian, 2021) is accounted for by an increase in the number of field replicates (Capo, Spong, Königsson, & Byström, 2019; Mauvisseau et al., 2019). Implementation of field replication would also enable the application of occupancy models and other post-processing tools to increase the robustness of data interpretation and decision-making processes (Burian et al., 2021). Another exciting possibility is to combine metabarcoding with the quantification of eDNA in a sample (Shelton et al., 2019; Everts et al., 2021). Such quantification using for example qPCR and droplet digital PCR (ddPCR), can be used to infer fish biomass (Brys et al., 2020; Mauvisseau et al., 2021), which would provide very valuable additional information for the sustainable management of fish stocks.

Finally, current monitoring strategies in most Sub-Saharan countries rely strongly on centralised implementation structures, which are linked to substantial travel and field sampling costs. Our results and earlier studies have shown that eDNA-based tools provide an excellent basis for decentralised community-based monitoring and citizen science initiatives (Biggs et al., 2015; Aylagas et al., 2020; Howell, LaRue, & Flanagan, 2021). Such initiatives would be very well suited to complemented centralised survey efforts and thereby help to reduce associated costs for flights, per diems, boat time, fuel, etc. The freed-up resources could then be used to extent survey efforts and the coverage of remote and currently often marginalised regions. eDNA preservation techniques can play an important role in supporting such efforts as they facilitate the storage of samples at ambient temperatures without eDNA degradation for several months (Mauvisseau et al., 2021). Additionally, a stronger integration of local communities into environmental monitoring often leads to increased agency and environmental awareness (Trimble & Berkes, 2013; Wiber, Charles, Kearney, & Berkes, 2009). Sub-Saharan Africa, costal resources are often primarily managed and administrated by local communities and such ‘side-effects’ of community-based monitoring can be equally important for conservation as the availability of reliable monitoring data. Consequently, eDNA-based methods can provide powerful tools that help to attain a sustainable use of costal ecosystems. Critical further steps to attain this goal will be the development of local analytical infrastructure, the complementation of local genetic reference databases, and the establishment of suitable organisational structures to facilitate routine monitoring.

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**Data availability statement**

Data and R-script are provided in supplementary information.

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**Figure legends**

**Fig. 1**: Location of sampling sites along the Southern coastline of Mozambique (*n* = 47). The number of recorded OTUs is reflected by the size of the dots and the number of threatened species that have been recorded is indicated by colour intensity. A focus area of our study was Inhambane bay, which is highlighted by a black rectangular in **A** and in greater detail in **B**.

**Fig. 2**: Comparison of richness and success of species assignment in datasets generated with MiFish and Riaz primers. Displayed are the number of bony fish OTUs, threatened species (including fish and other vertebrates) and fish taxa at the order levels (**B**) and the assignment success of the two primer sets at the family, genus, and species level (**B**). In **C**, the OTU richness per sample and in **D**, the comparative taxonomic coverage at the genus level for the two primer sets is presented. The comparative taxonomic coverage is thereby defined as the proportion of genus that were recorded by both primers at a specific site. In **E**, the OTU richness per sample is compared between the two primer sets with the red line indicating the 1:1 ratio and the black line representing the model 2 regression slope. The confidence interval of the regression slopes included 1 (0.74-1.24) and is indicated as a grey band. **C**-**E** are all based on samples that are amplified by both primer sets and on rarefied data to account for a potentially confounding effect of differential sequencing depth.

**Fig. 3**: Comparison among results generated with Riaz and MiFish primer sets to capture patterns in fish community composition. Displayed are results of a non-metric dimension scaling (NMDS) of datasets based on MiFish (**A**) and Riaz primers (**B**) reflecting similarity in community composition within specific environments. The relationship of community similarities obtained by the two primer sets is depicted in **C**, with the red line representing the prediction of a model 2 regression. In **D**, the number of genera in perciform and non-perciform fish are presented for both primer sets in a Venn-diagram. We chose these presentation as perciforms were better covered by MiFish and non-perciform were better covered by Riaz primers, highlighting primer-specific differences in group resolution.

**Fig. 4**: Heat map displaying differences in species occurrence in non-marine sites, in Inhambane bay, and in the most Southern part of the Mozambican coast (between the South African border and Maputo). Displayed are the 10 most abundant species (blue), species of high commercial interest (green), and threatened species (red). Abundant OTUs that were not assigned to species level are displayed with the lowest taxonomic resolution available.

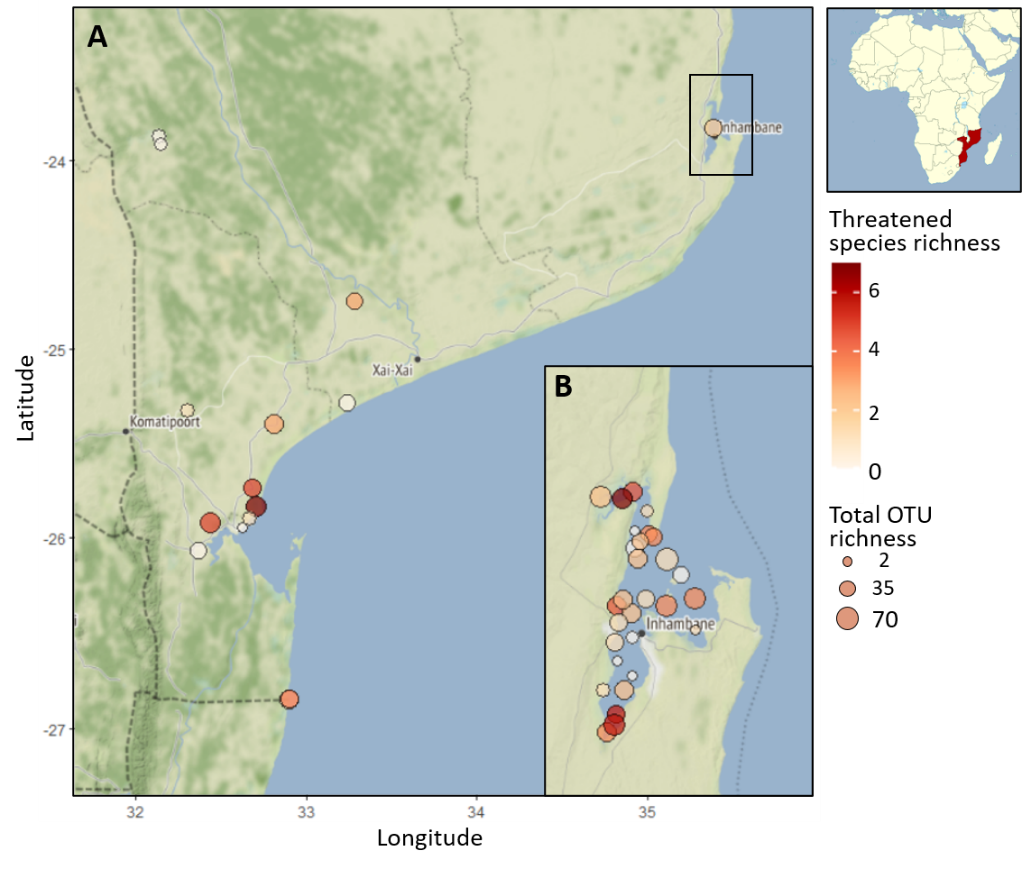


Fig. 1

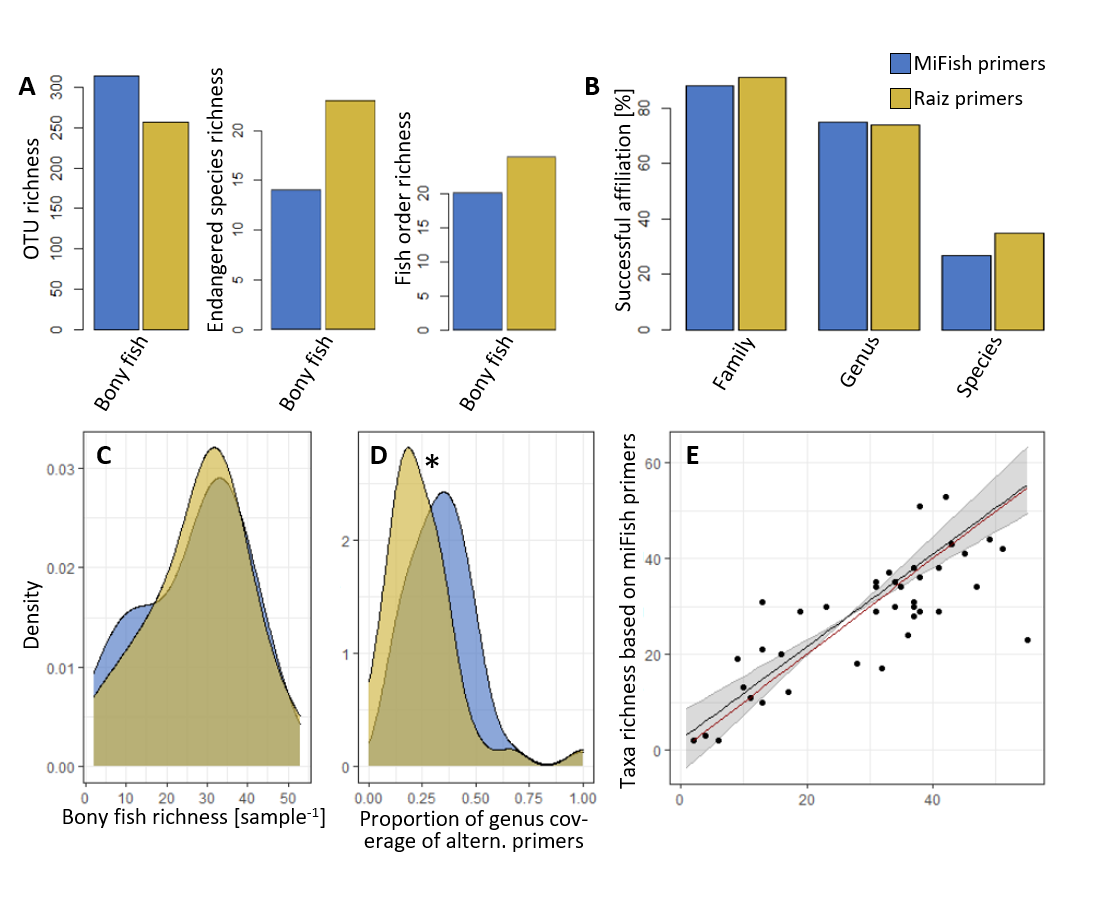


Fig . 2

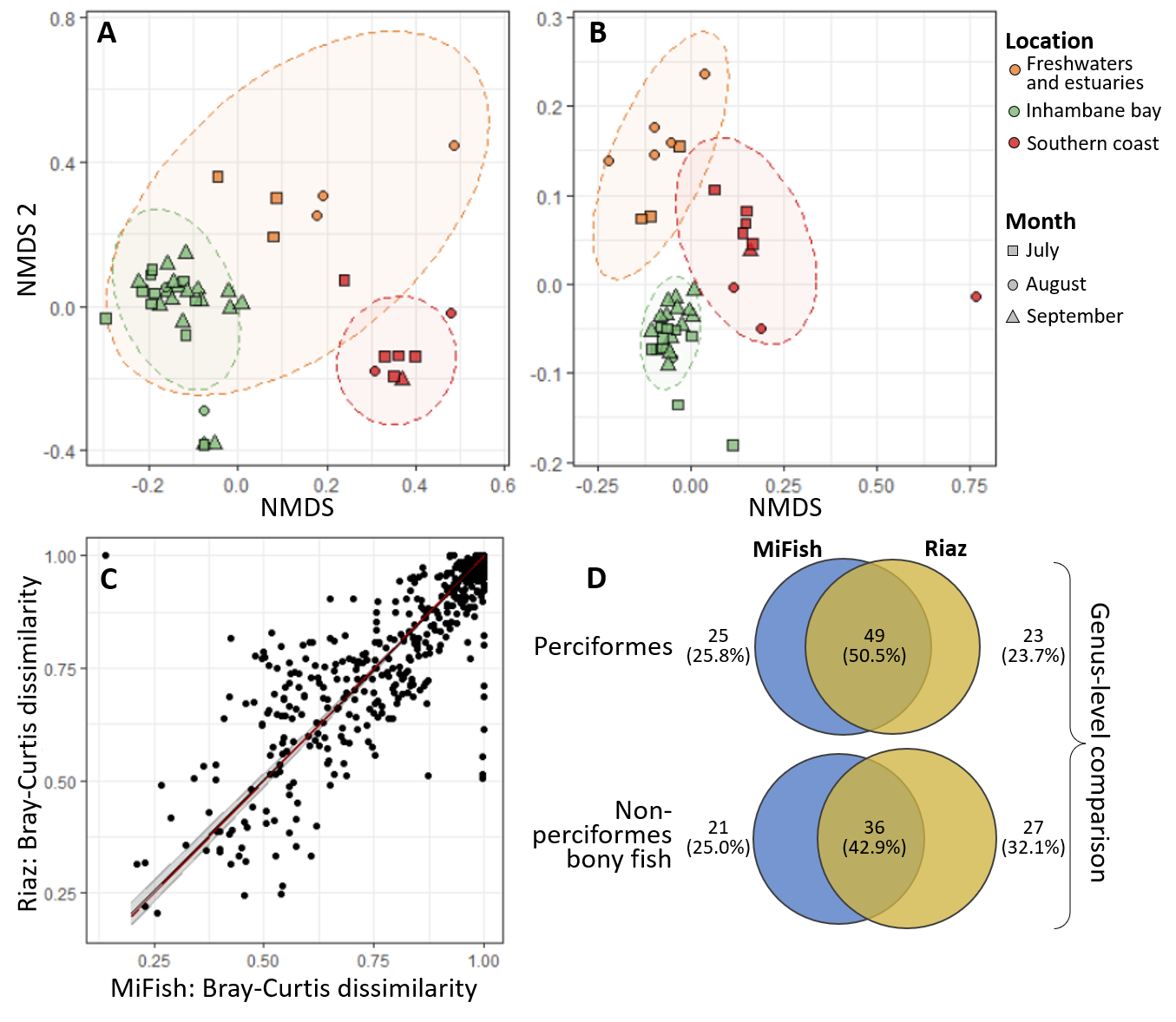


Fig. 3

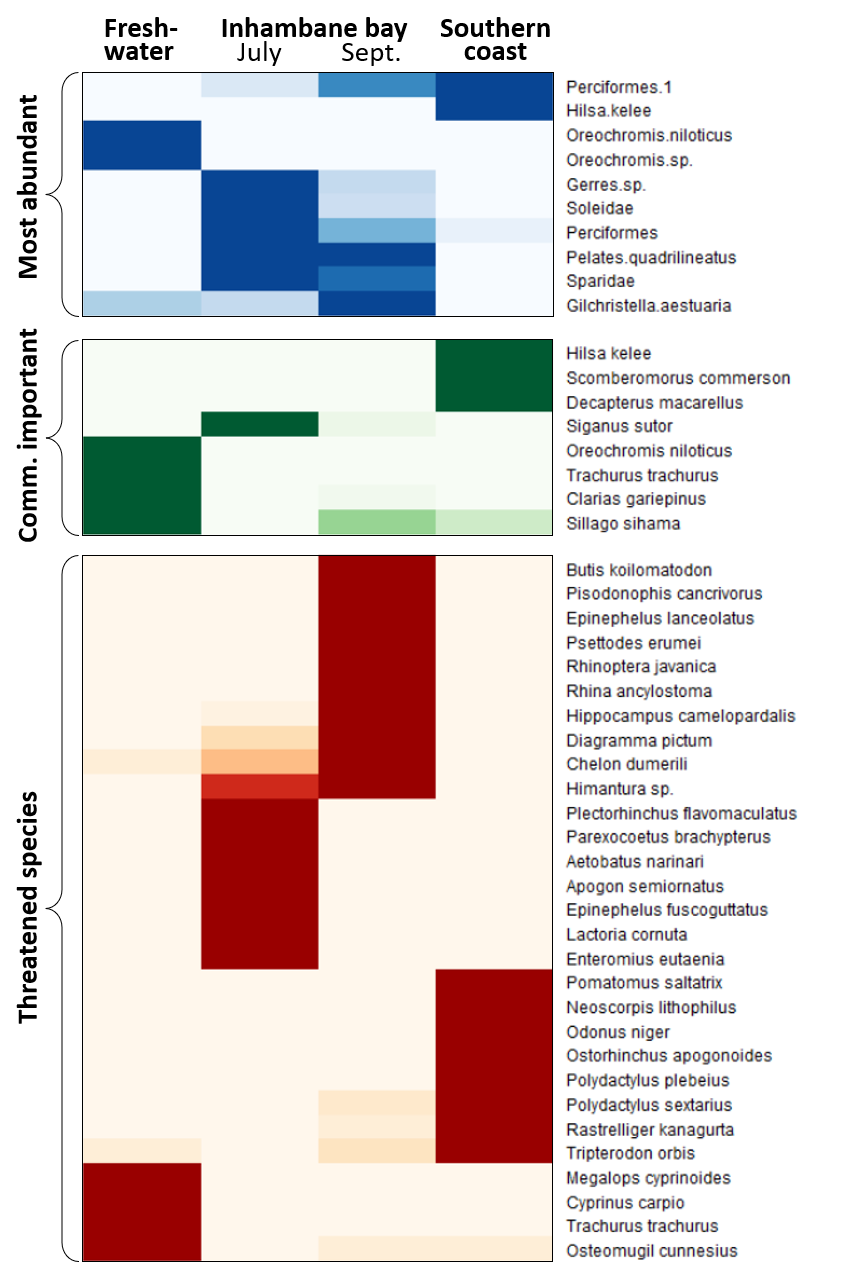
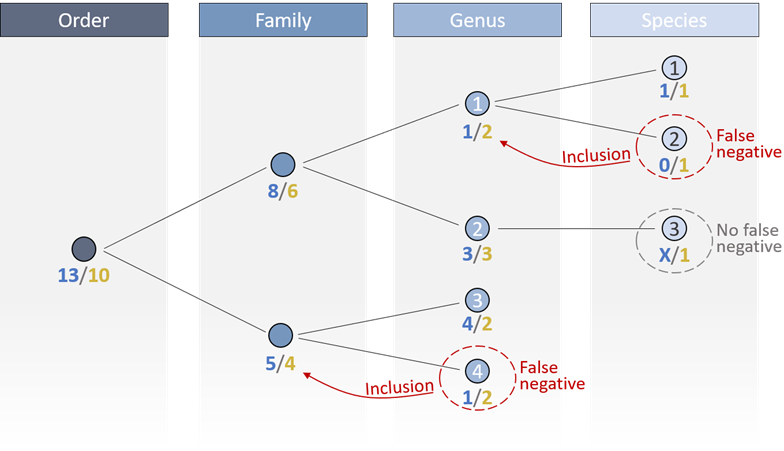


Fig. 4

**Box 1**: The rapid development of eDNA-based technologies has led to the availability of multiple primer sets for individual taxonomic groups. Different primer sets are associated with specific advantages leading at times to substantial trade-offs in primer choice [(Alberdi et al., 2019)](https://www.zotero.org/google-docs/?TJkolm). Although a combination of primers is often recommended to increase species coverage [(Blackman et al., 2021)](https://www.zotero.org/google-docs/?TcrVbj), the merging of resulting datasets is not trivial and standard procedures have not yet been developed. Caveats include systematic under- or overestimation of species richness, the distortion of relative abundance measures, and increased risk of contamination and sequencing errors (see SI for detailed discussion). These can be mitigated but not ultimately resolved, especially when reference databases are incomplete, and hence merging procedures need to be implemented with caution.

Nonetheless, the application of multiple assays and subsequent combination with data merging procedures can be an effective solution to resolve trade-offs in primer choice and improve the robustness of eDNA-based monitoring. The simplest data merging approach, which helps to decrease the risk of false negative results, is to include species that occur in either one of the datasets into a presence-absence matrix. However, such basic approaches are linked to potential inflation of species richness and result into the loss of relative abundance data. We developed here a more refined data-merging approach is based on the three simple procedures (Box Fig. 1). These rules are implemented separately for each sample and have the advantage to reduce the risk of false negative detections, result in more robust species richness estimates and allow to maintain relative abundance data as basis for more detailed community assessments.



**Box Fig. 1**: Schematic overview of the merging procedure developed in this study. Circles represent taxa, numbers below indicate the OTU counts for a taxon using MiFish (blue) and Riaz (yellow) primers. An example is provided where not all taxa were identified to species level. As first step, the dataset with a higher OTU count at the order level is used as the primary dataset and the respective OTUs are included in the merged dataset. Then, the secondary data set, which has a lower taxa count at the order level, is used to detect false negatives in the primary dataset. False negatives are identified as taxa that are present in both reference taxonomies but for the sample of interest only found in the secondary dataset (e.g. species 2, which is added to the merged dataset to prevent a false negative). An X signifies that a species is missing in a reference taxonomy and hence in the example above no statement can be made about whether or not species 3 is a false negative. Finally, an additional control mechanism is implemented to detect false negatives based on the number of OTUs assigned to a taxon. E.g., see genus 4: even though none of the OTUs are identified to species level, the secondary dataset contains more OTUs of that genus indicating again a false negative in the primary dataset. Further methodological details and an r-script for are provided in the SI.