Received: 20 December 2022 | Revised: 12 June 2023 | Accepted: 4 July 2023

DOI: 10.1111/conl.12971

Conservation Letters
A journal of the Society for Conservation Biology
Open Access
WILEY

LETTER

Shark-dust: Application of high-throughput DNA sequencing of processing residues for trade monitoring of threatened sharks and rays

Andhika P. Prasetyo[1,2,3] | **Joanna M. Murray**[4] | **Muh. Firdaus A. K. Kurniawan**[5] | **Naiara G. Sales**[1] | **Allan D. McDevitt**[1,6,†] | **Stefano Mariani**[7,†]

[1]School of Science, Engineering and Environment, University of Salford, Salford, UK

[2]Centre Fisheries Research, Ministry for Marine Affairs and Fisheries, Jakarta, Indonesia

[3]Research Centre for Conservation of Marine and Inland Water Resources, National Research and Innovation Agency, Bogor, Indonesia

[4]Centre for Environment, Fisheries and Aquaculture Science, Lowestoft, UK

[5]Directorate for Conservation and Marine Biodiversity, Ministry for Marine Affairs and Fisheries, Jakarta, Indonesia

[6]Marine and Freshwater Research Centre, Department of Natural Resources and the Environment, School of Science and Computing, Atlantic Technological University, Galway, Ireland

[7]School of Biological and Environmental Sciences, Liverpool John Moores University, Liverpool, UK

Correspondence
Andhika P. Prasetyo, School of Science, Engineering, and Environment, University of Salford, Salford, UK.
Email: a.p.prasetyo@edu.salford.ac.uk, andh009@brin.go.id

Stefano Mariani, School of Biological and Environmental Sciences, Liverpool John Moores University, Liverpool, UK.
Email: s.mariani@ljmu.ac.uk

Funding information
Illegal Wildlife Trade (IWT) Challenge Fund, Grant/Award Number: IWT057

Abstract

Illegal fishing, unregulated bycatch, and market demand for certain products (e.g., fins) are largely responsible for the rapid global decline of shark and ray populations. Controlling trade of endangered species remains difficult due to product variety, taxonomic ambiguity, and trade complexity. The genetic tools traditionally used to identify traded species typically target individual tissue samples, and are time-consuming and/or species-specific. Here, we performed high-throughput sequencing of trace DNA fragments retrieved from dust and scraps left behind by trade activities. We metabarcoded "shark-dust" samples from seven processing plants in the world's biggest shark landing site (Java, Indonesia), and identified 61 shark and ray taxa (representing half of all chondrichthyan orders), more than half of which could not be recovered from tissue samples collected in parallel from the same sites. Importantly, over 80% of shark-dust sequences were found to belong to CITES-listed species. We argue that this approach is likely to become a powerful and cost-effective monitoring tool wherever wildlife is traded.

KEYWORDS
DNA metabarcoding, elasmobranchs, environmental DNA, Indonesia, trade monitoring

[†]These authors contributed equally.

1 | INTRODUCTION

Continued and increasing anthropogenic stressors have devastated habitats and wildlife across the globe, including the dramatic depletion of sharks and rays (hereafter also referred to as "elasmobranchs") (Dulvy et al., 2021). Conservative life histories (Mardhiah et al., 2019) make elasmobranchs vulnerable to fisheries overexploitation, and their extirpation can destabilize functional diversity and ecosystem structure (Dulvy et al., 2021). Although some elasmobranch fisheries can be sustainably managed (Simpfendorfer & Dulvy, 2017), market demand for high-value products, such as fins, liver oil, and gill plates, typically leads to overexploitation of elasmobranch resources (Dulvy et al., 2021), which is then further fueled by illegal and unreported catches.

This combination of market demand, overexploitation, and lack of detail in catch and trade data (Cawthorn et al., 2018) requires effective mechanisms to monitor elasmobranch populations and ensure their sustainable management (Prasetyo et al., 2021). This includes improved catch reporting, special regulations for endangered species—such as the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Pavitt et al., 2021)—and a range of other transdisciplinary initiatives (Booth et al., 2019). A critical step in this context is the accurate reconstruction of the biodiversity composition of elasmobranch products at landing sites, processing plants, markets, and export hubs.

In 2023, the challenges facing trade biodiversity monitoring have more than tripled, as the number of CITES-listed species has increased from 47 to 151 (CITES, 2022a); yet, species listed in Appendix II can still be traded, by considering the viability of exploitation within the Non-Detriment Findings (NDF) framework (Smith et al., 2011). Thus, conservation managers now face a scenario where 14% of the 1120 described elasmobranch species—nearly one-third of which deemed to be under some level of conservation threat (IUCN, 2021)—can still be traded through the application of the NDF mechanism and substituted for other species (a form of species/product mislabeling). Understanding and regulating trade in these species is challenging because elasmobranch products are extremely diverse in both their usage and their value, and are processed in a myriad of different ways (Dent & Clarke, 2015). Due to their similarity in appearance and lack of distinctive features in most derivative products, shark and ray species can be deliberately or accidentally mislabeled by those involved in the trade (Figure 1). This has incentivized the use of genetic identification methods, which progressively made DNA-based inference a staple of wildlife forensics (Domingues et al., 2021). Of these, DNA barcoding (Shivji et al., 2002) and mini-barcoding (Fields et al., 2015) can robustly identify species in fresh and processed samples, while real-time qPCR (Cardeñosa et al., 2018), LAMP-based (But et al., 2020), and universal close-tube barcoding (Prasetyo et al., 2023) assays can detect target species in a matter of hours.

All these methods require the collection and analysis of individual specimens, which is a significant limitation when large volumes of samples, across many locations, must be inspected in a limited timeframe (Prasetyo et al., 2021). Recent advances in next-generation sequencing have shaped the transformation of general DNA barcoding (Hebert et al., 2003) into a technique that allows the simultaneous identification of multiple taxa from an inordinate mixture, known as DNA metabarcoding (hereafter referred to as just "metabarcoding") (Riaz et al., 2011). Metabarcoding has been broadly applied to analyzing environmental DNA (eDNA) samples—trace DNA fragments left behind by organisms in water, soil, and air, an approach that effectively complements, and in some cases surpasses, traditional monitoring (Aglieri et al., 2021; Boussarie et al., 2018). Such developments are unlocking novel applications in trade monitoring, allowing bulk mixtures to be analyzed, and tackling the limitations of existing tools.

Here, we propose a novel metabarcoding application, by targeting seven key shark and ray trading hubs in the island of Java, Indonesia, the top elasmobranch-landing country in the world (FAO, 2022). The seven sites were selected by considering their importance in trade flows (role and volume, Prasetyo et al., 2021) as well as accessibility. We used high-throughput metabarcoding to screen the byproducts of processing plant activities (which we term "shark-dust") and compare them with single-specimen barcoding. This unconventional application is poised to minimize labor requirements, enhance the detection of species that are not visible at the time of inspection, and be implemented globally.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Dust collections and shark/ray tissue samples (Figure 1) were collected from January to February 2020. Here, we targeted seven locations across cities on Java Island, Indonesia (Figure S1), the main export hub for various commodities, including elasmobranch products. We collected two sets of samples: first, using gloves, we gathered 28 mixtures (number of samples per location varies due to accessibility) of residual material from floors where shark products were processed, sorted, and stored for later

FIGURE 1 The diversity of elasmobranch products on sale can lead to misidentification and mislabeling. Our samples included, among others: (a) shark-dust from a pile of small dried fins, (b) tissue sample from a finless juvenile scalloped hammerhead shark whose cephalofoil (the distinctive "face" in this Family, also known as "blade") had been cut, (c) trunk (headless-finless shark body), (d) ray's wing, (e) piles of dried shark skin, (f) diced meat from shark heads, (g) shredded fin, (h) shark cartilage, (i) mobulid gill rack, and (j) lower lobes of shark caudal fins.

shipping, henceforth referred to as "dust" samples (Table S1); then, we selected 183 tissue samples from individual specimens (Table S2). Replicated samples (4 ± 3 samples) were collected in seven locations representative of Indonesia's processing, export, and regulatory activity. About 10 grams (about two tablespoons) of dust were scooped and stored at room temperature in sterilized 5 mL Click-Seal flat bottom tubes without a preservative. From the same location, about 2 × 1 × 0.5 cm of tissue was collected from individual specimens, trying to cover the broadest possible spectrum of morphological diversity observed at the sites, including both fresh and processed products. The tissue was then stored in 2.0 mL screw-cap microcentrifuge tubes, submerged in 90% ethanol, and stored at 4°C. The sampling tools were either changed or sterilized each time between samples.

2.2 | Laboratory and bioinformatic procedures

DNA was extracted from all samples (dust and tissue samples) following the Mu-DNA protocol for tissue samples (Sellers et al., 2018). All DNA extractions were diluted to 10–15 ng/μL prior to DNA amplification. The Elas02 primer pair (Elas02-F, 5′-GTTGGTHAATCTCGTGCCAGC-3′; Elas02-R, 5′-CATAGTAGGGTATCTAATCCTAGTTTG-3′) was used to target an ~180 bp amplicon from a variable region of the 12S rRNA mitochondrial gene (Miya et al., 2015; Taberlet et al., 2018). Given that dust was sampled from the floor, an elasmobranch-specific 12S marker was selected to avoid nontarget amplification, as the use of a COI-based marker would likely lead to the vast majority of reads coming

from other organisms (Collins et al., 2019). Samples were amplified in triplicate to minimize amplification stochasticity, and replicates were later pooled into a single representative sample. Meanwhile, the sequencing of individual tissue samples followed a massively parallel framework hereafter termed "high-throughput barcoding". The Leray-XT primer pair targeting an ~313 bp amplicon from a region of the COI mitochondrial gene (Wangensteen et al., 2018) was used for DNA amplification from tissue samples.

Adapters were ligated to PCR products using the KAPA HyperPrep Kit PCR-Free, and then quantified by qPCR using the NEBNext® Library Quant Kit for Illumina sequencing. The dust-generated library was diluted to 9 pM, and sequenced on an Illumina MiSeq run using a 2×150 bp v2 kit; the tissue sample libraries were diluted to 18 pM and sequenced on an Illumina MiSeq run using a 2×300 bp v3 kit. PhiX spike was at 1% for both runs. Bioinformatic analysis was based on OBITools, a python-based pipeline specifically designed for analyzing massively parallel sequencing data in a DNA metabarcoding context (Boyer et al., 2016), with taxonomic assignment conducted against a custom reference database (Table S3). Details on laboratory, bioinformatic, and statistical procedures can be found in the Supplementary Materials, and the scripts and dataset associated with the study are provided in a dedicated GitHub repository (https://github.com/andhikaprima/sharkdust) which has been archived (https://doi.org/10.5281/zenodo.7997300).

3 | RESULTS AND DISCUSSION

3.1 | Dust metabarcoding analysis

We obtained 5,580,616 reads from 28 discrete dust samples. We refined the final dataset (by removing contaminants and non-elasmobranch reads) to 4,640,239 (83.15% of initial reads) elasmobranch-only reads, partitioned into 61 molecular operational taxonomic units (MOTUs) (Figures S1, S2, S5, and Table S4) belonging to seven different orders: requiem sharks (Carcharhiniformes), mackerel sharks (Lamniformes), dogfish sharks (Squaliformes), cow sharks (Hexanchiformes), carpet sharks (Orectolobiformes), stingrays (Myliobatiformes), and shark-like rays (Rhinopristiformes). Taxonomic assignment successfully identified 54 of the 61 MOTUs to species level, with five assigned to genus level and two only attributable to family level.

Nearly, 84% of the filtered reads belonged to 32 CITES-listed taxa, including high-profile pelagic bycatch species, such as hammerhead sharks (*Sphyrna* spp.), silky shark (*Carcharhinus falciformis*), and spot-tail shark (*Carcharhinus sorrah*) (Figure 2a). The scalloped hammerhead shark (*S. lewini*) could be found almost everywhere and was most prevalent in the processing plants in Indramayu (IDM2 and IMD3), Banyuwangi (BYW7), and Surabaya (SBY6). Spot-tail shark, recently added to the CITES list, showed the highest read abundance in the Indramayu processing plants (Figure 2b). Among non-CITES-listed species, tiger shark (*Galeocerdo cuvier*) was the predominant species across sampling locations, followed by zebra shark (*Stegostoma fasciatum*), the Australian weasel shark (*Hemigaleus australiensis*), whitespotted whipray (*Himantura gerrardi*), and spotless smooth-hound (*Mustelus griseus*) (Figure 2c). These five species contributed about 70% of the non-CITES-listed read count overall, but their relative proportions varied greatly among locations.

The prevalence and abundance of reads from CITES-listed species detected in dust samples show that these animals continue to be major trade commodities and that monitoring efforts need to be intensified. Such species of conservation concern—primarily pelagic taxa—are found in abundance in processing plants (IDM2, IDM3, CLP4, and BYW7) and exporter warehouses in the main export hub cities (i.e., Jakarta and Surabaya—JKT1 and SBY6). These results corroborate earlier indications that CITES-listed species, such as thresher sharks, hammerhead sharks, silky sharks, wedgefishes, and guitarfishes, are still being traded in major Indonesian markets (Fahmi et al., 2021) and may still be exported through NDF mechanisms (CITES, 2022b). In Hong Kong, which is the main destination market, fin products of CITES-listed species are modeled to be ~10% of the overall traded volume (Fields et al., 2017). Based on our results from the world's largest exporter—and the recent expansion of CITES listings—these figures are likely an underestimation. Dust samples also contained the DNA of several key reef-associated sharks, such as blacktip reef shark (*C. melanopterus*), whitetip reef shark (*Triaenodon obesus*), and sand tiger shark (*Carcharias taurus*). These species play an important part in the equilibria of coral reef ecosystems, which is particularly concerning for Indonesia, where reef sharks have been driven to near functional extinction (MacNeil et al., 2020). Several mesopredatory ray species were also detected, including Hortle's whipray (*Himantura hortlei*), mangrove whipray (*Himantura granulata*), pale-edged stingray (*Dasyatis zugei*), and bluespotted stingray (*Neotrygon kuhlii*). These species, albeit not controlled under CITES, significantly contribute to trophic interactions in key coastal ecosystems (Flowers et al., 2021); in fact, 90% of non-CITES-listed species detected from dust samples are currently designated as threatened species under the IUCN (International Union for Conservation of Nature) Red List (IUCN, 2021). Therefore, beyond trade enforcement aspects, obtaining information on these taxa is critical for monitoring the impact of exploitation on population dynamics and ecosystem health.

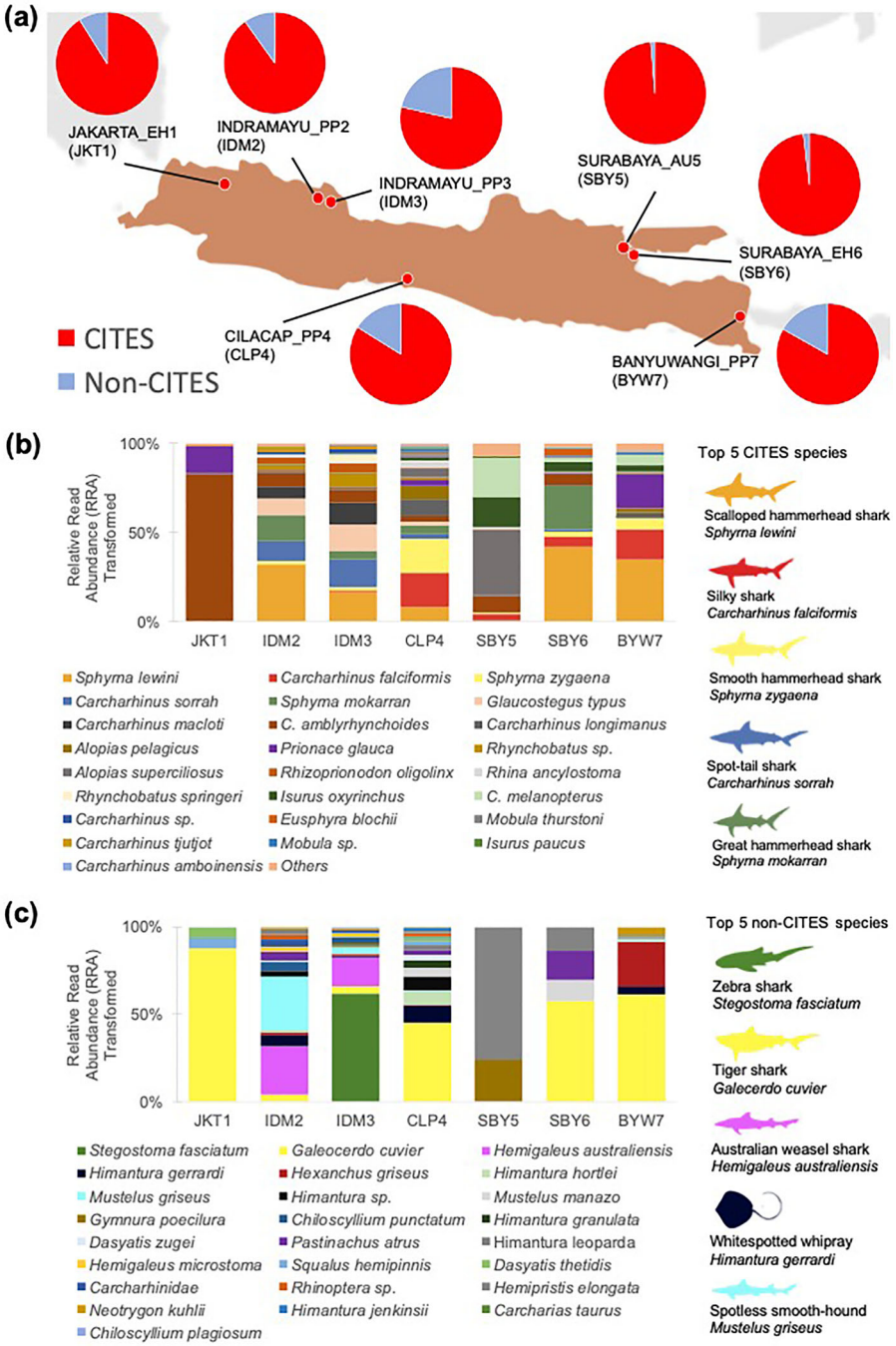

FIGURE 2 The species composition of CITES and non-CITES-listed species (square-rooted and normalized read abundance) across sampled locations (a), composition of CITES-listed species (b), and composition of non-CITES-listed species (c). The top 5 species are visualized with silhouettes and the same color in the bar chart after data transformation and normalization.

3.2 | Comparison of species detections from dust and tissue samples

Tissue-based barcoding successfully identified 175 out of 183 samples associated with the locations where dust samples were taken. Specimens were partitioned into 36 taxa, nearly all of which were also detected in the dust samples (Figure 3a). Overall, we were able to identify more than 64 taxa across methods; however, the dust samples detected 17 more genera than tissue samples, and uniquely identified 10 CITES-listed species (Figures 3b and 4a, Table S5). When sequencing reads from the dust samples were transformed into presence and absence data, species compositions between dust and tissue samples were shown to be significantly different (PERMANOVA: $F = 3.49$, $p = 0.001$; Figure 3c and Table S6). Tissue samples showed a greater

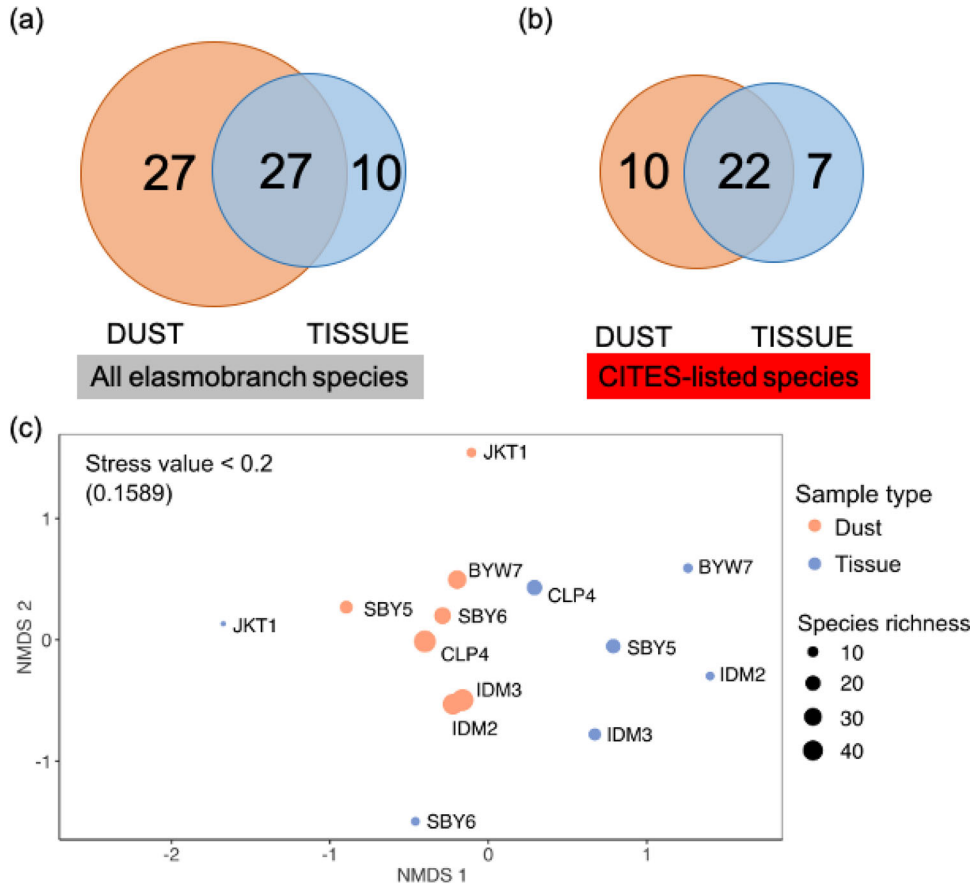

FIGURE 3 Comparison between species recovery from dust and tissue samples; Venn diagrams of all elasmobranch species (a), CITES-listed species only (b), and non-metric multidimensional scaling (nMDS) based on Jaccard similarity index between two sample types in different locations (c). Samples have been pooled into the seven locations. Nb. Only species-level taxa are considered except for *Mobula* sp. and *Rhynchobatus* sp. as these taxa, detected by dust metabarcoding, could only be confidently assigned to genus using the 12S marker. The stress value measures how well the original distance matrix between samples is reduced in two dimensions. Values below 0.2 are generally considered a good representation. Moreover, the size of the circles on the NMDS plot is proportional to species richness (number of species being detected in a particular location and sample type).

separation among locations, due to the high-grading bias introduced by the single-specimen approach to sampling (which may also select for more "notable" samples). Dust samples showed a consistently greater species richness across locations, detecting an average of 31.57 (± 16.34) taxa per collection, with tissue samples averaging 11.14 (± 6.01), as is also shown by the taxon accumulation curve (Figure 4b).

Dust metabarcoding has much greater power to unveil a comprehensive portrayal of shark and ray species being traded, for a considerably lower sampling effort (N_{dust} = 28 vs. N_{tissue} = 175) and less disruption of the processing and trading operations in the visited hubs (Figure 4c). Dust samples revealed some cryptic and rare species, such as winghead shark (*Eusphyra blochii*), pigeye shark (*C. amboinensis*), sand tiger shark (*Carcharias taurus*), smooth hammerhead (*S. zygaena*), knifetooth sawfish (*Anoxypristis cuspidata*), manta, and devil rays (*Mobula* spp.). The latter three are hardly ever seen at landing places, given their fully protected status under Indonesia's regulations (Ministerial Decree No. 61 in 2018 concerning the utilization of protected and/or CITES-listed species, Prasetyo et al., 2021). These findings mirror the performance of eDNA studies on elasmobranchs from natural environments, which consistently reveal important "dark diversity" that is missed by pre-existing biomonitoring tools (Boussarie et al., 2018). In this sense, the "shark-dust" metabarcoding approach can boost and streamline all the biodiversity, fishery, and trade control operations that have up to this point been carried out via earlier-generation DNA monitoring tools.

There were 40 CITES-listed taxa identified in total, with 22 taxa—including thresher sharks (*Alopias* spp.), mako sharks (*Isurus* spp.), and two hammerhead species—that are commonly found at landing sites (*S. lewini* and *S. mokkaran*) identified using both dust and tissue samples.

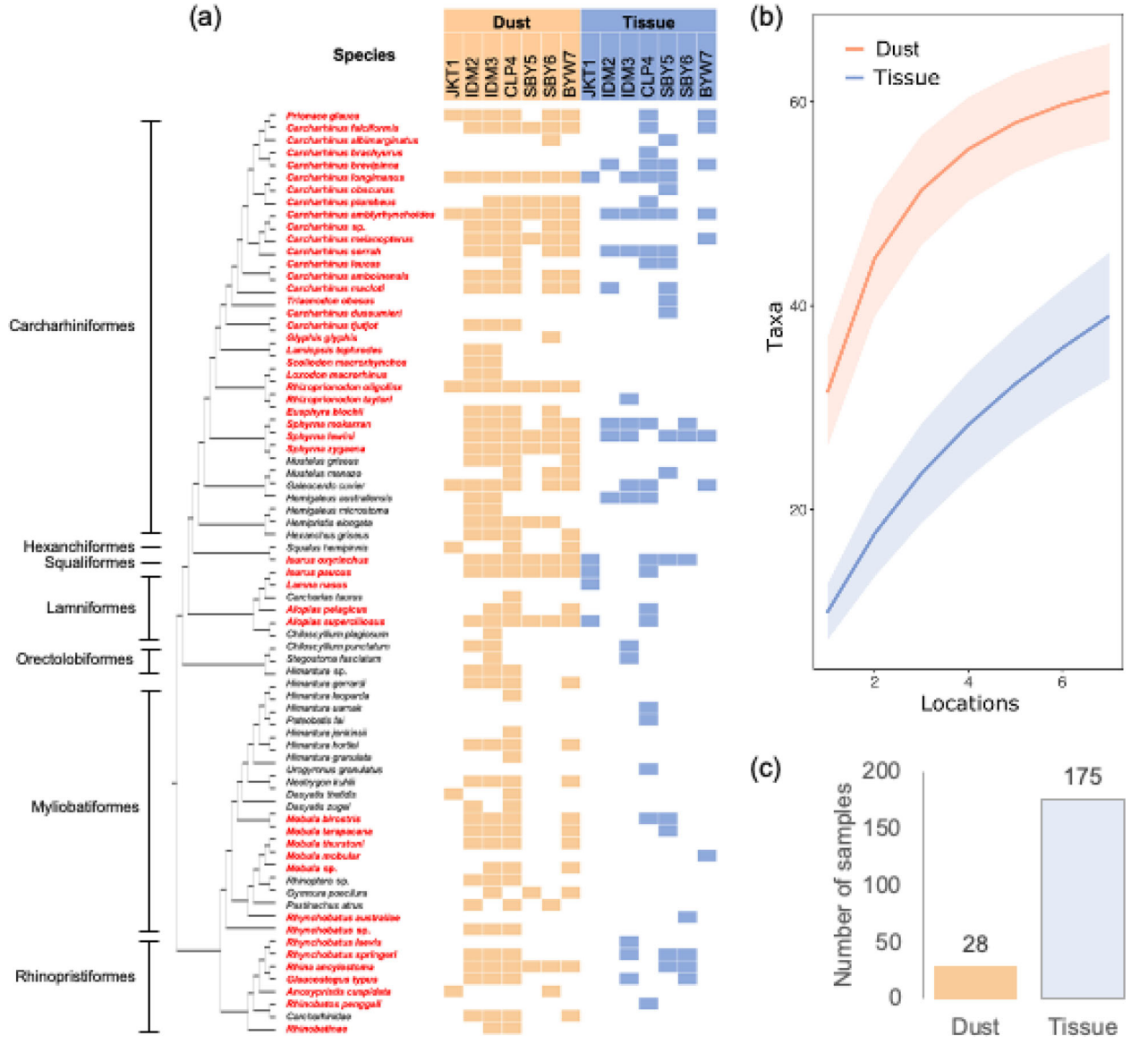

FIGURE 4 The cladogram (a) was generated using FigTree 1.4.4 using NADH2 region sequences (Naylor et al., 2012) from the NCBI database. Colors represent sample type, such as dust samples (ORANGE) and tissue samples (BLUE) for results from each sampling location, with CITES-listed species written in RED. Species accumulation curves (b) emphasize the differences in taxon detection rate between methods; differences in sampling effort (c) are also visualized.

One of the tissue samples belonged to a species that is not distributed in Indonesian waters: porbeagle shark (*Lamna nasus*); but this was a single sample obtained from the exporter's reference collection that was used for education purposes.

3.3 | A cutting-edge tool for trade monitoring

Our findings showed that trade monitoring using dust metabarcoding expands the reach of traditional barcoding methods. However, seven MOTUs could not be identified to species level from dust samples (Table S7), including two families and five genera with species listed in CITES appendices, namely, wedgefishes (*Rhynchobatus* sp.), devil rays (*Mobula* sp.), requiem sharks (*Carcharhinus* sp.), and guitarfishes (Rhinobatinae). We had anticipated this issue by developing an additional 12S reference database for our analyses, but recent studies (Mariani et al., 2021; Miya et al., 2020) had already shown that the size (170–180 bp) and resolution of the 12S Elas02 fragment will not allow discrimination between some closely related species, as shown for *Rhynchobatus, Mobula*, Rhinobatinae, and also for some species in the polyphyletic genus *Carcharhinus* (Sorenson et al., 2014). Yet, despite these limitations, the marker used remains the most effective metabarcoding tool for elasmobranch identification while also avoiding

nontarget amplification (Collins et al., 2019), and this could be further strengthened through the ongoing expansion of 12S and mitogenomic reference libraries (Collins et al., 2021) and the development of further taxon-specific assays, which may in the future accurately distinguish between the most closely related species.

Another advantage of bulk metabarcoding of processing byproducts includes the ability to detect trace DNA in situations where the original tissue source is no longer available, either due to the complexity of trading operations or as a result of deliberate concealment (Challender et al., 2015). This may also allow for coarse estimation of relative volumes traded (Ershova et al., 2021; Shelton et al., 2023), which would be impossible through the pain-staking tissue sampling from individual specimens. Finally, dust metabarcoding is also more cost-effective: the collection of several dry processing residues is easier than collecting and preserving hundreds of individual tissue samples. Along with the significant reduction in both time and costs of processing these dust samples in the lab right through to high-throughput sequencing, this much-reduced sample size is then sufficient to garner higher species richness estimates than the individual-based tissue analysis (Figure 4c). Technically, the collection of dust residues, compared to tissue sampling, is open to environmental contamination, whereby DNA traces can be detected from species that had passed through the sampled establishment days, weeks, and potentially months earlier. Still, this "contamination" is an inherent feature of the approach, which purposely seeks to investigate the biodiversity extracted, processed, and traded through a given hub. Certainly, a formal framework will be required and agreed by key stakeholders (traders, exporters, and inspectors) on how to operationally implement shark-dust; one possible step would be to ask exporters to use brand-new/cleaned containers for each batch of exports. Another useful approach would be to identify robust, conservative "thresholds for detection" parameters in the bioinformatic workflow.

Recent developments in fast and portable technologies open up new opportunities to run metabarcoding in the field. Our existing approach relies on laboratory equipment, which may be prohibitive in some contexts, especially in developing countries. Optimization of third-generation sequencing technologies (Johri et al., 2019; van der Reis et al., 2023) will most likely advance in situ bulk metabarcoding techniques, enabling a wide range of applications in wildlife forensics and fisheries management and benefiting the global conservation community.

The CITES Secretariat promotes capacity development and the transmission of information and skills between countries in order to "efficiently, reliably, and cost-effectively identify shark items in commerce" (CoP18 Doc. 21.2), including genetic procedures. With a current list of 151 species (CITES, 2022a), which now include over 50 species of requiem sharks (*Carcharhinus* spp.), over 50 species between wedgefishes and guitarfishes, as well as thresher sharks, hammerhead sharks, manta rays, and freshwater stingrays, the difficulties that countries face in complying with CITES regulations have never been greater. Decades of overexploitation have devastated elasmobranch populations; but the use of trade bans will only be successful in tandem with the implementation of reliable and cost-effective monitoring tools. Indonesia, the global epicenter of shark and ray trade, was the best place to assess the effectiveness of shark-dust metabarcoding to improve trade monitoring. With this novel approach proving successful, the Ministry for Marine Affairs and Fisheries immediately invested in certification schemes and biotechnological facilities, with the view of adopting these DNA-based monitoring tools to improve sustainability in the sector. The present approach based on the residues of shark and ray processing activities should effectively assist conservation strategies, working toward the sustainability of elasmobranch populations across the world, and inspiring the design of similar methods to combat a wealth of other illegal wildlife trading activities.

ACKNOWLEDGMENTS

This study was funded by the Illegal Wildlife Trade (IWT) Challenge Fund number IWT057 through collaboration between the University of Salford (UoS), the Ministry for Marine Affairs and Fisheries (MMAF)—Indonesia, the Centre for Environment, Fisheries and Aquaculture Science (Cefas), the Rekam Nusantara Foundation—Indonesia, and Liverpool Jhon Mores University (LJMU). We also thank the editor and two anonymous reviewers for providing constructive criticism.

DATA AVAILABILITY STATEMENT

The scripts and dataset associated with the study are provided in a dedicated GitHub repository (https://github.com/andhikaprima/sharkdust) which has been archived (https://doi.org/10.5281/zenodo.7997300).

ORCID

Andhika P. Prasetyo https://orcid.org/0000-0002-7412-8432
Stefano Mariani https://orcid.org/0000-0002-5329-0553

REFERENCES

Aglieri, G., Baillie, C., Mariani, S., Cattano, C., Calò, A., Turco, G., Spatafora, D., Di Franco, A., Di Lorenzo, M., Guidetti, P., & Milazzo, M. (2021). Environmental DNA effectively captures functional diversity of coastal fish communities. *Molecular Ecology*, *30*, 3127–3139.

Booth, H., Squires, D., & Milner-Gulland, E. J. (2019). The mitigation hierarchy for sharks: A risk-based framework for reconciling

trade-offs between shark conservation and fisheries objectives. *Fish and Fisheries*, *21*, 269–289.

Boussarie, G., Bakker, J., Wangensteen, O. S., Mariani, S., Bonnin, L., Juhel, J.-B., Kiszka, J. J., Kulbicki, M., Manel, S., Robbins, W. D., Vigliola, L., & Mouillot, D. (2018). Environmental DNA illuminates the dark diversity of sharks. *Science Advances*, *4*, eaap9661.

Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., & Coissac, E. (2016). obitools: A unix-inspired software package for DNA metabarcoding. *Molecular Ecology Resources*, *16*, 176–182.

But, G. W.-C., Wu, H.-Y., Shao, K.-T., & Shaw, P.-C. (2020). Rapid detection of CITES-listed shark fin species by loop-mediated isothermal amplification assay with potential for field use. *Scientific Reports*, *10*, 4455.

Cardeñosa, D., Quinlan, J., Shea, K. H., & Chapman, D. D. (2018). Multiplex real-time PCR assay to detect illegal trade of CITES-listed shark species. *Scientific Reports*, *8*, 16313.

Cawthorn, D.-M., Baillie, C., & Mariani, S. (2018). Generic names and mislabeling conceal high species diversity in global fisheries markets. *Conservation Letters*, *11*, e12573.

Challender, D. W. S., Harrop, S. R., & Macmillan, D. C. (2015). Towards informed and multi-faceted wildlife trade interventions. *Global Ecology and Conservation*, *3*, 129–148.

CITES. (2022a). 19.218 - 19.225 Sharks and rays (*Elasmobranchii* spp.). *Decisions of the Conference of the Parties to CITES in Effect After the 19th Meeting*. Panama: CITES.

CITES. (2022b). What's an NDF and why is it important? *News*. CITES.

Collins, R. A., Bakker, J., Wangensteen, O. S., Soto, A. Z., Corrigan, L., Sims, D. W., Genner, M. J., & Mariani, S. (2019). Non-specific amplification compromises environmental DNA metabarcoding with COI. *Methods in Ecology and Evolution*, *10*, 1985–2001.

Collins, R. A., Trauzzi, G., Maltby, K. M., Gibson, T. I., Ratcliffe, F. C., Hallam, J., Rainbird, S., Maclaine, J., Henderson, P. A., Sims, D. W., Mariani, S., & Genner, M. J. (2021). Meta-Fish-Lib: A generalised, dynamic DNA reference library pipeline for metabarcoding of fishes. *Journal of Fish Biology*, *99*, 1446–1454.

Dent, F., & Clarke, S. (2015). *State of the global market for shark products*. Food and Agriculture Organization of the United Nations.

Domingues, R. R., Bunholi, I. V., Pinhal, D., Antunes, A., & Mendonça, F. F. (2021). From molecule to conservation: DNA-based methods to overcome frontiers in the shark and ray fin trade. *Conservation Genetics Resources*, *13*, 231–247.

Dulvy, N. K., Pacoureau, N., Rigby, C. L., Pollom, R. A., Jabado, R. W., Ebert, D. A., Finucci, B., Pollock, C. M., Cheok, J., Derrick, D. H., Herman, K. B., Sherman, C. S., Vanderwright, W. J., Lawson, J. M., Walls, R. H. L., Carlson, J. K., Charvet, P., Bineesh, K. K., Fernando, D., … Simpfendorfer, C. A. (2021). Overfishing drives over one-third of all sharks and rays toward a global extinction crisis. *Current Biology*, *31*, 4773–4787.e4778.

Ershova, E. A., Wangensteen, O. S., Descoteaux, R., Barth-Jensen, C., & Præbel, K. (2021). Metabarcoding as a quantitative tool for estimating biodiversity and relative biomass of marine zooplankton. *ICES Journal of Marine Science*, *78*, 3342–3355.

Fahmi, O. S., Koeshendrajana, S., Wiadnyana, N. N., & Nugroho, D. (2021). Summary. In S. O. Fahmi, S. Koeshendrajana, N. N. Wiadnyana, & D. Nugroho (Eds.), *3rd Shark and Ray Symposium in Indonesia*. Jakarta: Ministry for Marine Affairs and Fisheries and WWF, 12–14.

FAO. (2022). Fishery and aquaculture statistics. *Global capture production 1950–2020 (FishStatJ)*. FAO Fisheries and Aquaculture Division [online].

Fields, A. T., Abercrombie, D. L., Eng, R., Feldheim, K., & Chapman, D. D. (2015). A novel mini-DNA barcoding assay to identify processed fins from internationally protected shark species. *PLoS ONE*, *10*, e0114844.

Fields, A. T., Fischer, G. A., Shea, S. K. H., Zhang, H., Abercrombie, D. L., Feldheim, K. A., Babcock, E. A., & Chapman, D. D. (2017). Species composition of the international shark fin trade assessed through a retail-market survey in Hong Kong. *Conservation Biology*, *32*, 376–389.

Flowers, K. I., Heithaus, M. R., & Papastamatiou, Y. P. (2021). Buried in the sand: Uncovering the ecological roles and importance of rays. *Fish and Fisheries*, *22*, 105–127.

Hebert, P. D. N., Cywinska, A., Ball, S. L., & Dewaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London Series B: Biological Sciences*, *270*, 313–321.

IUCN. (2021). *The IUCN Red List of Elasmobranch Species Version*. IUCN.

Johri, S., Solanki, J., Cantu, V. A., Fellows, S. R., Edwards, R. A., Moreno, I., Vyas, A., & Dinsdale, E. A. (2019). 'Genome skimming' with the MinION hand-held sequencer identifies CITES-listed shark species in India's exports market. *Scientific Reports*, *9*, 4476.

Macneil, M. A., Chapman, D. D., Heupel, M., Simpfendorfer, C. A., Heithaus, M., Meekan, M., Harvey, E., Goetze, J., Kiszka, J., Bond, M. E., Currey-Randall, L. M., Speed, C. W., Sherman, C. S., Rees, M. J., Udyawer, V., Flowers, K. I., Clementi, G., Valentin-Albanese, J., Gorham, T., … Cinner, J. E. (2020). Global status and conservation potential of reef sharks. *Nature*, *583*, 801–806.

Mardhiah, U., Booth, H., Simeon, B. M., Muttaqin, E., Ichsan, M., Fahmi, D., Prasetyo, A. P., & Yulianto, I. (2019). Quantifying vulnerability of sharks and rays species in Indonesia: Is biological knowledge sufficient enough for the assessment? *IOP Conference Series: Earth and Environmental Science*, *278*, 012043.

Mariani, S., Fernandez, C., Baillie, C., Magalon, H., & Jaquemet, S. (2021). Shark and ray diversity, abundance and temporal variation around an Indian Ocean Island, inferred by eDNA metabarcoding. *Conservation Science and Practice*, *3*, e407.

Miya, M., Gotoh, R. O., & Sado, T. (2020). MiFish metabarcoding: A high-throughput approach for simultaneous detection of multiple fish species from environmental DNA and other samples. *Fisheries Science*, *86*, 939–970.

Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., Minamoto, T., Yamamoto, S., Yamanaka, H., Araki, H., Kondoh, M., & Iwasaki, W. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. *Royal Society Open Science*, *2*, 150088.

Naylor, G. J. P., Caira, J. N., Jensen, K., Rosana, K. A. M., White, W. T., & Last, P. R. (2012). A DNA sequence–based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. *Bulletin of the American Museum of Natural History*, *2012*, 1–262.

Pavitt, A., Malsch, K., & King, E. (2021). *CITES and the sea: Trade in commercially exploited CITES-listed marine species*. FAO.

Prasetyo, A. P., Cusa, M., Murray, J. M., Agung, F., Muttaqin, E., Mariani, S., & Mcdevitt, A. D. (2023). Universal closed-tube barcoding for monitoring the shark and ray trade in megadiverse conservation hotspots. *iScience*, *26*(7), 107065.

Prasetyo, A. P., Mcdevitt, A. D., Murray, J. M., Barry, J., Agung, F., Muttaqin, E., & Mariani, S. (2021). Shark and ray trade in and out of Indonesia: Addressing knowledge gaps on the path to sustainability. *Marine Policy*, *133*, 104714.

Riaz, T., Shehzad, W., Viari, A., Pompanon, F., Taberlet, P., & Coissac, E. (2011). ecoPrimers: Inference of new DNA barcode markers from whole genome sequence analysis. *Nucleic Acids Research*, *39*, e145.

Sellers, G. S., Di Muri, C., Gómez, A., & Hänfling, B. N. (2018). Mu-DNA: A modular universal DNA extraction method adaptable for a wide range of sample types. *Metabarcoding and Metagenomics*, *2*, e24556.

Shelton, A. O., Gold, Z. J., Jensen, A. J., D′Agnese, E., Andruszkiewicz Allan, E., Van Cise, A., Gallego, R., Ramón-Laca, A., Garber-Yonts, M., Parsons, K., & Kelly, R. P. (2023). Toward quantitative metabarcoding. *Ecology*, *104*, e3906.

Shivji, M., Clarke, S., Pank, M., Natanson, L., Kohler, N., & Stanhope, M. (2002). Genetic identification of pelagic shark body parts for conservation and trade monitoring. *Conservation Biology*, *16*, 1036–1047.

Simpfendorfer, C. A., & Dulvy, N. K. (2017). Bright spots of sustainable shark fishing. *Current Biology*, *27*, R97–R98.

Smith, M. J., Benítez-Díaz, H., Clemente-Muñoz, M. Á., Donaldson, J., Hutton, J. M., Noel Mcgough, H., Medellin, R. A., Morgan, D. H. W., O'criodain, C., Oldfield, T. E. E., Schippmann, U., & Williams, R. J. (2011). Assessing the impacts of international trade on CITES-listed species: Current practices and opportunities for scientific research. *Biological Conservation*, *144*, 82–91.

Sorenson, L., Santini, F., & Alfaro, M. E. (2014). The effect of habitat on modern shark diversification. *Journal of Evolutionary Biology*, *27*, 1536–1548.

Taberlet, P., Bonin, A., Zinger, L., & Coissac, E. (2018). *Environmental DNA: For biodiversity research and monitoring*. Oxford University Press.

Van Der Reis, A. L., Beckley, L. E., Olivar, M. P., & Jeffs, A. G. (2023). Nanopore short-read sequencing: A quick, cost-effective and accurate method for DNA metabarcoding. *Environmental DNA*, *5*, 282–296.

Wangensteen, O. S., Palacín, C., Guardiola, M., & Turon, X. (2018). DNA metabarcoding of littoral hard-bottom communities: High diversity and database gaps revealed by two molecular markers. *PeerJ*, *6*, e4705.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Prasetyo, A. P., Murray, J. M., Kurniawan, M. F. A. K., Sales, N. G., McDevitt, A. D., & Mariani, S. (2023). Shark-dust: Application of high-throughput DNA sequencing of processing residues for trade monitoring of threatened sharks and rays. *Conservation Letters*, e12971. https://doi.org/10.1111/conl.12971