RESEARCH ARTICLE

Diversity and origins of giant guitarfish and wedgefish products in Singapore

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Funding information
National Research Foundation Singapore, Grant/Award Number: MSRDP-P03

Abstract
1. Giant guitarfishes (Glaucostegidae) and wedgefishes (Rhinidae) are some of the most threatened marine taxa in the world, with 15 of the 16 known species exhibiting global population declines and categorized as Critically Endangered according to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species. The recent inclusion of all species in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) necessitates more rigorous enforcement by regulatory authorities.
2. Challenges in regulating the trade of giant guitarfish and wedgefish products due to difficulties in visual identification of processed products and labelling issues impede enforcement. The aim of this study is to characterize the diversity and origins of associated traded products that were commercially available in Singapore, one of the world’s top importers and re-exporters of shark and ray products.
3. A total of 176 samples of elasmobranch products were obtained between June and December 2019 from fishery ports and various retailers in Singapore. By applying cytochrome c oxidase subunit I gene barcoding, 31 elasmobranch species were detected, with 55% of the species considered threatened (Critically Endangered, Endangered, or Vulnerable) based on the IUCN Red List and 35% of species listed in CITES Appendix II. Four species of giant guitarfishes and wedgefishes were commercially available to consumers in fresh forms of whole fish, fillet, and fin, as well as dried and cooked meats.
4. DNA barcoding has proven to be an effective tool for identifying elasmobranch products that are impossible to recognize visually and would aid enforcement of CITES trade regulations. This work underscores the urgent need to step up enforcement of marine wildlife regulations and draw public attention to the elasmobranch trade.

KEYWORDS
CITES, coastal, DNA barcoding, elasmobranch, endangered species, IUCN Red List, rays, shark fin, South-East Asia, wildlife trade
INTRODUCTION

Giant guitarfishes (family Glaucostegidae) and wedgefishes (family Rhinidae) of the cartilaginous fish order Rhinopristiformes consist of 16 valid species that are commonly known as ‘shark-like rays’ (Table 1) (Giles et al., 2016; Last, Kyne & Compagno, 2016; Simpfendorfer et al., 2019; Kyne et al., 2020). They bear the characteristic large dorsal ‘shark fin’ that gives them the appearance of a shark, but they have their gills and mouth on the underside like a ray (Md-Zain et al., 2018). Appearances aside, giant guitarfishes and wedgefishes also share similar life-history strategies with many sharks and rays. Their slow growth, late maturity, and low fecundity are characteristics of K-strategists (Pinhal et al., 2008). They are also lecithotrophic viviparous, with generally small litter sizes and low reproductive potential (Kyne et al., 2020; Mull, Yopak & Dulvy, 2020). These life-history traits tend to elevate their extinction risks (Dulvy, Sadovy & Reynolds, 2003; Dulvy et al., 2014).

Giant guitarfishes and wedgefishes are distributed in temperate to tropical neritic coastal waters throughout the ocean, although most species are concentrated within the Indo-Pacific region and some have restricted ranges (Last, Kyne & Compagno, 2016; Kyne et al., 2020). For example, the Taiwanese wedgefish (Rhynchobatus immaculatus) can only be found off the northern Taiwanese coast (Kyne & Ebert, 2019), and the false shark ray (Rhynchorhina mauritaniensis) is endemic to the coast of Mauritania (Kyne & Jabado, 2019). Giant guitarfishes and wedgefishes reside preferentially in soft bottom areas of coral reefs and seagrass meadows. Their flattened body is adapted to the benthos, and they mostly swim close to the sea floor or lie concealed within sea-bed sediments (Oh, 2016; Jabado et al., 2018a). However, these habitats face losses and fragmentation effects due to overfishing, pollution, and climate change (Wear, 2016; Unsworth et al., 2018), severely threatening giant guitarfishes and wedgefishes in their natural environment (Jabado et al., 2017; Kyne et al., 2020).

Giant guitarfish and wedgefish populations are most impacted by commercial extraction and finning (Figure 1; Davidson & Dulvy, 2017; Moore, 2017; Jabado, 2018). Demand for their characteristic dorsal and caudal fins means they are increasingly targeted by the booming international fin trade (Wainwright et al., 2018; Jabado, 2019). The demand for fins of elasmobranchs mostly originates from Chinese communities in Asia, with China’s growing middle class fuelling demand for the ultimate status symbol: shark fin soup (Fong & Anderson, 2000; Clarke, Milner-Gulland & Bjørndal, 2007; Iloulian, 2017). This dish is considered de rigueur at special occasions, like weddings (Jeffreys, 2016; Williams, 2017), and also consumed as traditional Chinese medicine, allegedly to rejuvenate the body and enhance body constitution (Cheung & Chang, 2003; Fabinyi, 2012; Ellis, 2013). The high quality and unique texture of the fins of giant guitarfishes and wedgefishes (Jabado et al., 2018b; Kyne et al., 2020) put them in a custom category known as Qun chi (Hau et al., 2018), which can fetch up to US$964 per kilogram in Hong Kong (Jabado, 2019).

### TABLE 1 Sixteen valid species of giant guitarfishes and wedgefishes

| Species | Common name |
|---------|-------------|
| **Glaucostegidae (giant guitarfishes)** | |
| *Glaucostegus cemiculus* (Geoffroy Saint-Hilaire, 1817) | Blackchin guitarfish |
| *Glaucostegus granulatus* (Cuvier, 1829) | Granulated guitarfish |
| *Glaucostegus halavi* (Forsskål, 1775) | Halavi guitarfish |
| *Glaucostegus obtusus* (Müller & Henle, 1841) | Widenose guitarfish |
| *Glaucostegus thouin* (Anonymous [Lacepède], 1798) | Clubnose guitarfish |
| *Glaucostegus typus* (Anonymous [Bennett], 1830) | Giant guitarfish |
| **Rhinidae (wedgefishes)** | |
| *Rhina ancylostoma* Bloch & Schneider, 1801 | Bowmouth guitarfish |
| *Rhynchobatus australiae* Whitley, 1939 | Bottlenose wedgefish |
| *Rhynchobatus djiddensis* Forsskål, 1775 | Whitespotted wedgefish |
| *Rhynchobatus cooki* Last, Kyne & Compagno, 2016 | Clown wedgefish |
| *Rhynchobatus immaculatus* Last, Ho & Chen, 2013 | Taiwanese wedgefish |
| *Rhynchobatus laevis* (Bloch & Schneider, 1801) | Smoothnose wedgefish |
| *Rhynchobatus lueberti* Ehrenbaum, 1915 | African wedgefish |
| *Rhynchobatus palpebratus* Compagno & Last, 2008 | Eyebrow wedgefish |
| *Rhynchobatus springeri* Compagno & Last, 2010 | Broadnose wedgefish |
| *Rhynchorhina mauritaniensis* Séret & Naylor, 2016 | False shark ray |

Note: All have decreasing population trends and, with the exception of *R. palpebratus* (Near Threatened), are Critically Endangered under the International Union for Conservation of Nature Red List of Threatened Species. All species are in Convention on International Trade in Endangered Species of Wild Fauna and Flora Appendix II.
Apart from their fins, giant guitarfish and wedgefish meats in fresh and salted forms are consumed by communities along the coasts and in many developing countries. When sold fresh, these elasmobranchs can fetch a relatively high price of US$4 per kilogram (Choy CPP & Choo MY, 2020, unpublished data), and a whole wedgefish over 2 m in total length can be sold at prices as high as US$680 (Jabado, 2018) owing to its reputation as good quality meat (Moore, 2017). Together, artisanal and commercial fisheries put extreme pressure on harvested populations (Last, White & Pogonoski, 2008; Dulvy et al., 2014; Jabado et al., 2018; D’Alberto et al., 2019), with declining catch rates in trawl surveys and reductions in landings reported at fishing ports across the Indo-West Pacific and Indian Ocean despite a substantial increase in fishing effort (Jabado et al., 2018b). Indeed, the most recent global assessment based on the International Union for Conservation of Nature (IUCN) Red List Categories and Criteria (IUCN, 2012) indicates that all species except the eyebrow wedgefish, *Rhynchobatus palpebratus*, have undergone >80% population decline over the last 30–45 years and are classified as Critically Endangered (Kyne et al., 2020).

In August 2019, at the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) 18th meeting of the Conference of the Parties, all six species of Glaucostegidae were added to CITES Appendix II (CITES, 2019a). *Glaucostegus cemiculus* and *Glaucostegus granulatus* were included due to severe declines to 30% of their historical abundances and as trade regulation is necessary to halt further declines (Jabado, 2018). The remaining four species in the Glaucostegidae were also added to Appendix II to facilitate enforcement because of difficulties in distinguishing the congeneric species (CITES, 2019a). Similar to Rhinidae, *Rhynchobatus australiae* and *Rhynchobatus djiddensis* were included on the basis of rapid population declines in recent years of ≥80% (Jabado et al., 2018b) and that trade regulation is necessary to halt the reduction of wild populations (Fields et al., 2017). Because of considerable overlap between intra- and interspecific variabilities in dorsal coloration and morphology, as well as the high likelihood of the existence of cryptic species within the family Rhinidae (Giles et al., 2016; Moore, 2017; Jabado, 2018), all 10 Rhinidae species were added to Appendix II (CITES, 2019b).

Singapore ranks amongst the world's top importers and re-exporters of elasmobranch fins and meats (Boon, 2017; Tan, 2017). Under its Endangered Species (Import and Export) Act (Cap 92A, 2008 Rev Ed), Singapore’s National Parks Board (NParks) has the power to implement and enforce trade regulations under CITES. CITES-listed sharks have been seized at customs checkpoints over the last decade by regulatory authorities, but information on these seizures is not publicly accessible (Boon, 2017). Owing to the listing of giant guitarfishes and wedgefishes in CITES Appendix II, the aforementioned legislation was amended on 26 November 2019 in order to properly control and facilitate the trade of these taxa. Despite a stringent import and export permitting process, the sale of these endangered species forms a hidden part of the elasmobranch trade. Fresh specimens that are relatively whole can be easily identified by enforcement officers, who have been trained accordingly since the listing came into effect (Wong A, 2020, personal communications;
Figure 1). However, fillets and fins in dried and cooked forms and when packaged with other meats are generally impossible to visually identify to species (Moore, 2017).

Indeed, mislabelling of products is a well-known issue in the elasmobranch trade globally (Almerón-Souza et al., 2018; Wainwright et al., 2018; Pazartzi et al., 2019), with many instances where products are vaguely labelled in order to meet loose labelling regulations (Donlan & Luque, 2013; Ho et al., 2020). Inappropriate labelling impacts the sustainability of elasmobranch populations, as sales of at-risk species are obscured even if they are protected under existing trade regulations. Consequently, efforts to mitigate the decline of particular regulated species could be compromised, as overall demand continues to drive populations away from recovery. Furthermore, the known presence of harmful chemicals in certain products makes mislabelling a serious food safety problem. For example, mercury and arsenic have been detected in elasmobranch products on many occasions above permissible levels (Clarke, Milner-Gulland & Bjørndal, 2007; Whitcraft, O’Malley & Hilton, 2014; Barcia et al., 2020). Clearly, product labelling is an issue that requires the immediate attention of regulatory authorities in order to safeguard consumer safety.

While the Harmonized System Code developed by the World Customs Organization has included commodities such as shark fin as well as shark and ray meat (Boon, 2017), there were no species-specific commodity codes for giant guitarfishes and wedgefishes prior to their recent listing in CITES Appendix II. This has resulted in inadequate traceability of the threatened species moving through the supply chain and severe limitations in our understanding of the scale and dynamics of the trade (see Gerson et al., 2008). The paucity of academic scrutiny on the giant guitarfish and wedgefish trade is evident in Singapore, where a female specimen of the clown wedgefish (*Rhynchobatus cooki*) was found at Jurong Fishery Port in May 2019. This was the first record of the species since 1996 (Clark-Shen et al., 2019), and only 12 *R. cooki* specimens had been collected at fishing ports in Singapore and Jakarta prior to 1996 (Last, Kyne & Compagno, 2016). A recent study broadly targeting traded fins, meats, and mobulid gill plates identified a further three wedgefish species: bowmouth guitarfish (*Rhina ancylostoma*; n = 7), bottlenose wedgefish (*R. australis*; n = 18), and whitespotted wedgefish (*R. djiddensis*; n = 1) (Wainwright et al., 2018). A giant guitarfish was also detected, but its species identity could not be confirmed. Lack of precise species information is a major hurdle when planning for conservation and population recovery.

This paper aims to shed light on the giant guitarfish and wedgefish trade in Singapore by sampling products readily available to consumers in Singapore just before and as the CITES listing of these species came into effect and by performing DNA barcoding to identify the products to species. The filleted, dried, cooked, and other processed products are impossible to identify by visual observation alone, as the processing methods generally remove any resemblance to a single species. Here, detection of one giant guitarfish species and three wedgefish species in poorly and erroneously labelled products highlights potential challenges in the enforcement of marine wildlife trade regulations and draws further attention on the elasmobranch trade.

2 | METHODS

2.1 | Specimen collection

Potential sources of elasmobranch products were compiled from an earlier study by Wainwright et al. (2018) and from a broader search of vendors in Singapore to encompass suspected giant guitarfish and wedgefish species (Supporting Information Data S1). The two government-run facilities for fishing vessels to land their catches, Jurong Fishery Port and Senoko Fishery Port, were also included in the compilation (Figure 2). Products sold commercially to the general public and deemed probable to be elasmobranchs were purchased from each targeted retailer once between June and December 2019.
in a snapshot survey. A total of 176 samples were collected (Supporting Information Data S1), ranging from whole fish to fillets, fins, and gill rakers, in fresh, dried, and cooked forms (Figure 1).

2.2 DNA barcoding

DNA was extracted from approximately 25 mg of tissue from each sample. For products potentially containing multiple individuals (e.g. multiple meat pieces), clearly separated tissues were selected at random (Fields et al., 2017). Tissue was cut out from the centre of each sample using sterile razor blades, scissors, and tweezers that had been autoclaved and not been in contact with any other material to prevent contamination. Digestion was performed overnight at 55 °C in 100 μl of hexadecyltrimethylammonium bromide buffer and 40 μl of proteinase K (20 mg ml⁻¹), followed by standard phenol:chloroform:isoamyl alcohol (25:24:1) extraction and ethanol precipitation.

Polymerase chain reaction (PCR) was performed to amplify a 313 bp fragment of the cytochrome c oxidase subunit I (COI) gene using the mCiOlinF (5’-GGW ACW TGA ACW GTW TAY CCY CC-3’) and LoboR1 (5’-TAA ACY TCW GGR TGW CCR AAR AAY CA-3’) primers (Leray et al., 2013; Lobo et al., 2013). The thermal cycling profile was 94 °C for 10 s; five cycles of 94 °C for 30 s, 48 °C for 120 s, 72 °C for 60 s; five cycles of 94 °C for 30 s, 54 °C for 120 s, 72 °C for 60 s; and 72 °C for 5 min. Each reaction was performed in a total volume of 25 μl containing 1.0 μl of magnesium chloride (2.5 mM), 2.0 μl of each primer at 5 μM, 1 μl bovine serum albumin (20 mg ml⁻¹), 2 μl of DNA template, 12.5 μl of GoTaq DNA polymerase, and 4.5 μl of water. Amplification success was assessed using 1% gel electrophoresis. PCR products were purified using Sera-Mag™ Magnetic SpeedBeads™ and sequenced on an Applied Biosystems 3730XL DNA Analyser. To identify any contamination issues, negative controls with no sample DNA were tested for every round of PCR.

Raw sequence data were assembled with Geneious Prime 2020.1.1 (https://www.geneious.com). The COI sequence data produced here have been deposited in GenBank (for samples with validated species identifications; accession numbers MT933185-MT933190) and at Zenodo (https://doi.org/10.5281/zenodo.3894031).

2.3 Data analysis

Sequences were searched against GenBank (https://www.ncbi.nlm.nih.gov) with BLASTn using a 98% sequence similarity as a minimum threshold following Wainwright et al. (2018) (see also Hebert et al., 2003; Hebert, Ratnasingham & de Waard, 2003; Armani et al., 2016). The best match sequence identity was regarded as a positive identification. Species accumulation plots were performed in R 4.0 (R Core Team, 2013) with the package vegan (Oksanen et al., 2019). The ‘collector’ and ‘rarefaction’ methods were used to describe the actual sampling sequence and determine if species richness had saturated with samples collected, respectively.

Sample data and species identification for each sample were consolidated together with global conservation and trade regulation statuses obtained from the IUCN Red List of Threatened Species (IUCN, 2020; Kyne et al., 2020) and CITES (2020) respectively (Supporting Information 1).

3 RESULTS

3.1 Species identification and diversity

All 176 samples collected were successfully sequenced for the COI genetic marker, and all sequences were matched to GenBank sequences at >99.3% sequence similarity (Supporting Information Data S1). Among all samples, 99 were sharks (Selachimorpha) and 77 were rays (Batoidea). Thirty of the ray samples were identified as one giant guitarfish and three wedgefish species (Table 2). Of these samples, 47% were fresh whole fish, fillet, or fin (n = 14), 30% were dried (n = 9), and 23% were cooked (n = 7). From the fresh samples, eight were from fishery ports and six from easily accessible supermarkets and food centres. Only two wedgefish samples of R. australiae were available as fresh fins, and no giant guitarfish or wedgefish species were sold as shark fin.

The silky shark (Carcharhinus falciformis; n = 33), devil fish (Mobula mobular; n = 23), and bottlenose wedgefish (R. australiae; n = 22) were the most commonly sampled elasmobranch species. Recent taxonomic revisions have synonymized the spinetail mobula (Mobula japonica) as a junior synonym of the devil fish (M. mobular) (Bustamante et al., 2016; White et al., 2018), so two samples that had identical sequence similarity with M. mobular and M. japonica in GenBank were considered to be positive matches for M. mobular. Relatedly, the Alfred manta (Mobula alfredi) and giant manta (Mobula birostris) were recently recognized as distinct species (Kashiwagi et al., 2011). Previous M. alfredi and M. birostris barcodes in GenBank may not have been distinguished according to the current taxonomy and thus were considered as a single species (M. alfredi/birostris).

Species accumulation curves showed the number of species approaching an asymptote, and thus close to the expected species richness (Mao, Colwell & Chang, 2005) for giant guitarfish or wedgefish species (S = 4) but not for sharks (Selachimorpha; S = 21) and other rays (Batoidea, excluding Rhinopristiformes; S = 6) (overall S = 31; Figure 3). The actual sampling process (‘collector’ method) showed that the rate of increase in number of species slowed considerably by about 100 samples amassed (Figure 3), from 29 of 42 collection events (Figure 2).

3.2 Species statuses, availability, and origins

Overall, collections here comprised six species which are Critically Endangered (n = 33), three species listed as Endangered (n = 36), and eight species listed as Vulnerable (n = 62) under the IUCN Red List of
Threatened Species (IUCN, 2020), including 11 species listed in CITES Appendix II ($n = 106$) (Table 2).

Critically Endangered giant guitarfishes and wedgefishes were predominantly found at Jurong Fishery Port (fresh whole fish; $n = 6$) and Senoko Fishery Port (fresh fins; $n = 2$), as well as a supermarket (fresh fillet; $n = 1$) and food centre (cooked meat; $n = 7$) in the vicinity of the former (Figure 2). There were 20 samples of Endangered and Vulnerable *Mobula* (*M. mobular*, *M. tarapacana*, and *M. alfredi/birostris*) in a single location in central Singapore (Chinatown).

### Table 2

Frequencies and tissue types of 176 elasmobranchs identified in this study, specifying their common names, conservation status according to the International Union for Conservation of Nature (IUCN) Red List of Threatened Species, and Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II

| Species                          | Common name                     | IUCN Red List/CITES Appendix II | $n$ | Tissue type                           |
|----------------------------------|---------------------------------|---------------------------------|-----|--------------------------------------|
| **Giant guitarfish**             | *Glaucostegus typus*            | CR/yes                          | 2   | Whole fish; dried meat               |
| **Wedgefishes**                  |                                  |                                 |     |                                      |
| *Rhina ancylostoma*              | Bowmouth guitarfish             | CR/yes                          | 5   | Dried meat                           |
| *Rhynchobatus australiae*        | Bottlenose wedgefish            | CR/yes                          | 22  | Whole fish; fresh fillet; fin; cooked, dried meat |
| *Rhynchobatus springeri*         | Broadnose wedgefish             | CR/yes                          | 1   | Cooked meat                          |
| **Rays (excluding Rhinopristiformes)** |                                  |                                 |     |                                      |
| *Mobula mobular*                 | Devil fish                      | EN/yes                          | 23  | Dried gill rakers                    |
| *Mobula tarapacana*              | Chilean devil ray               | EN/yes                          | 8   | Dried gill rakers                    |
| *Mobula alfredi/birostris*       | Alfred/Giant manta              | VU/yes                          | 4   | Dried gill rakers                    |
| *Maculabatis gerrardi*           | Sharpnose stingray              | VU/no                           | 7   | Fresh fillet; dried meat             |
| *Maculabatis pastinacoides*      | Round whip ray                  | VU/no                           | 2   | Dried meat                           |
| *Pastinachus sephen*             | Cowtail stingray                | NT/no                           | 3   | Dried meat                           |
| **Sharks**                       |                                  |                                 |     |                                      |
| *Sphyrna lewini*                 | Scalloped hammerhead            | CR/yes                          | 2   | Frozen cooked fin; dried meat        |
| *Sphyra mokarran*                | Great hammerhead                | CR/yes                          | 1   | Dried meat                           |
| *Alopias pelagicus*              | Pelagic thresher                | EN/yes                          | 5   | Dried meat                           |
| *Carcharhinus falciformis*       | Silky shark                     | VU/yes                          | 33  | Dried fin; dried meat                |
| *Carcharhinus plumbeus*          | Sandbar shark                   | VU/no                           | 1   | Dried fin                            |
| *Hemigales microstoma*           | Sicklefin weasel shark          | VU/no                           | 9   | Dried meat                           |
| *Hemipristis elongata*           | Snaggletooth shark              | VU/no                           | 4   | Dried meat                           |
| *Nebrius ferrugineus*            | Tawny nurse shark               | VU/no                           | 2   | Dried meat                           |
| *Carcharhinus amblyrhynchoïdes*  | Graceful shark                  | NT/no                           | 5   | Dried fin; dried meat                |
| *Carcharhinus brevipinna*        | Spinner shark                   | NT/no                           | 1   | Dried meat                           |
| *Carcharhinus leucas*            | Bull shark                      | NT/no                           | 9   | Dried meat                           |
| *Carcharhinus limbatus*          | Blacktip shark                  | NT/no                           | 4   | Dried meat                           |
| *Carcharhinus sealei*            | Blackspot shark                 | NT/no                           | 2   | Dried meat                           |
| *Carcharhinus sorrah*            | Spot-tail shark                 | NT/no                           | 11  | Dried fin; dried meat                |
| *Chiloscyllium hasselti*         | Hasselt's bamboo shark          | NT/no                           | 1   | Cooked meat                          |
| *Chiloscyllium punctatum*        | Brownbanded bamboo shark        | NT/no                           | 1   | Dried meat                           |
| *Galeocerdo cuvier*              | Tiger shark                     | NT/no                           | 4   | Dried meat                           |
| *Mustelus lenticulatus*          | Spotted estuary smooth-hound    | LC/no                           | 1   | Dried fin                            |
| *Rhizoprionodon acutus*          | Milk shark                      | LC/no                           | 1   | Frozen cooked fin                    |
| *Rhizoprionodon oligolinx*       | Grey sharpnose shark            | LC/no                           | 1   | Frozen cooked fin                    |
| *Carcharhinus amboinensis*       | Pigeye shark                    | DD/no                           | 1   | Dried fin                            |

Abbreviation: CR, Critically Endangered; DD, Data Deficient; EN, Endangered; LC, Least Concern; NT, Near Threatened; VU, Vulnerable.
The countries listed on packaging labels or obtained from vendors, where available ($n = 124$), were used to determine the locations where products were packed or extracted (Supporting Information Data S1). A total of 99 samples originated from Malaysia, all in dried form. Five whole bottlenose wedgefish ($R. australis$) and a whole giant guitarfish ($Glaucostegus typus$) were claimed to originate from Indonesia and landed at Jurong Fishery Port. Two fresh samples of the bottlenose wedgefish ($R. australis$) were claimed to be caught off Pedra Branca, an outlying island 45 km east of mainland Singapore, landed at Senoko Fishery Port. Five samples from China ($n = 2$) and Spain ($n = 3$) were shark fins (all Carcharhinidae) in dried and frozen forms. Two frozen instant shark fin products originated from the Philippines, including one positively identified as the Critically Endangered scalloped hammerhead ($Sphyrna lewini$; Rigby et al., 2019).

About 15% of all sampled products ($n = 26$) were labelled as ‘dried fish’ or ‘salted fish’ (Supporting Information Data S1), including those identified as snaggertooth shark ($Hemipristis elongata$), sicklefin weasel shark ($Hemigaleus microstoma$), tawny nurse shark ($Nebrius ferrugineus$), blackspot shark ($Carcharhinus sealei$), spot-tail shark ($Carcharhinus sorrah$), and tiger shark ($Galeocerdo cuvier$). Samples of three bowmouth guitarfishes ($Rhina ancylostoma$) and a bottlenose wedgefish ($R. australis$) were indicated as ‘ikan kurau’. Fresh fillets identified here as $R. australis$ were sold as ‘shovelnose rays’.

4 | DISCUSSION

4.1 Giant guitarfish and wedgefish identification

This study draws conservation focus on the retail availability of giant guitarfishes and wedgefishes to evaluate their recent listing on the CITES Appendix II. It also assesses in greater detail the efficacy of using COI barcoding for trade surveillance, building on recent work performed on elasmobranch products in Singapore (Wainwright et al., 2018), and discusses the possibility of utilizing this technique for practical, regular enforcement of the Endangered Species (Import and Export) Act (Cap 92A, 2008 Rev Ed). To better understand the trade in giant guitarfishes and wedgefishes specifically, a broad range of shark and ray products in Singapore were targeted to capture suspected giant guitarfish and wedgefish samples and to more generally examine the robustness of the approach for recognizing shark and ray species sold in Singapore.

Every giant guitarfish and wedgefish sample sequenced here has an unambiguous, high-similarity or perfect match to a single species in GenBank. All samples of $R. australis$ and $R. ancylostoma$ match unambiguously to GenBank records of conspecifics, including sequences from Wainwright et al. (2018). Two additional species, the giant guitarfish ($G. typus$) and broadnose wedgefish ($Rhynchobatus springeri$) that were not identified previously
(Wainwright et al., 2018), have been detected here. However, Wainwright et al. (2018) found a single whitespotted wedgefish (R. djiddensis) as dried shark meat that is not detected here. It should be noted that there is taxonomic confusion between R. australiae and R. djiddensis (Last et al., 2016; Jabado, 2018), and it is possible that they may ultimately belong to the same species—more taxonomic work is needed on this species complex—although Wainwright et al. (2018) recorded both species. Nevertheless, these detections mirror other studies that identified giant guitarfishes and wedgefishes based on unambiguous, high-percentage or perfect matches with published sequences on GenBank (e.g. Bineesh et al., 2014; Muttaqin et al., 2019; Wannell et al., 2020). We also note that all the species in Glaucostegidae (giant guitarfishes) and Rhinidae (wedgefishes) have been included in CITES Appendix II in part due to these taxonomic issues (CITES, 2019a; CITES, 2019b), so uncertainties over particular species are not expected to impact CITES implementation and enforcement significantly.

Genetic techniques are commonly used in the field of wildlife forensics to identify particular species or populations that are illegally poached and sold (Caniglia et al., 2010; Dalton & Kotze, 2011; Sanches et al., 2011; Domingues, de Amorim & Hildsdorf, 2013; Hobbs et al., 2019). The ubiquitous application of COI gene sequencing has made it the de facto gene marker in DNA barcoding work (Smith, Poyarkov & Hebert, 2008; Pentinsaari et al., 2016; Ip et al., 2019), especially for its efficiency in identifying animal products (Yang et al., 2018). The widespread availability of COI sequences across many taxa also means that matching new sequences to archived data in genetic databases (e.g. GenBank and BOLD) are very likely to yield informative matches (Meusnier et al., 2008; Appleyard et al., 2018; Wannell et al., 2020). Though DNA degradation during processing of food products may limit recovery of full-length barcodes, sequencing shorter fragments, or mini-barcoding (Shokralla et al., 2015), has been shown to be effective in identifying species in various types of preserved and processed meats (Hellberg, Hernandez & Hernandez, 2017; Ho et al., 2020).

Indeed, samples in the present study have undergone various processes, such as drying, freezing, and even cooking, but the 313 bp fragment of the COI gene could be sequenced effectively with minimal problems. Furthermore, identifiable features have been removed from many fresh samples after being portioned for fillets and steak cuts. Visual identification of species from these products is therefore highly challenging (Thommasen et al., 1989; Hau et al., 2018; but see Jabado, 2019). By using COI gene sequencing to provide positive identification, this study adds to the growing body of work showing that DNA barcoding can facilitate the investigation of species among landed elasmobranchs and animal parts in fishing ports (Chuang et al., 2016). The technique can also help detect seafood fraud and illegal products in locally consumed shark and fish products, even in cooked dishes such as shark fin and meat soups (Almerón-Souza et al., 2018; Wainwright et al., 2018; Pazartzii et al., 2019).

### 4.2 Supply, distribution, and labelling of elasmobranch products

All the products examined here have been acquired with ease at government-run fishing ports, major supermarket outlets, and even family-owned mini-marts and food centres. Samples obtained from fishery ports have been purchased directly from fish merchants operating at the fishing ports. These include six whole Critically Endangered giant guitarfishes and wedgefishes at the Jurong Fishery Port, as well as cooked meat purchased from a food centre in its vicinity, suggesting that proximity to the fishing port facilitates the availability of these threatened species to consumers.

In order to enforce regulations associated with CITES Appendix II-listed species, knowledge on the origins of wildlife products is paramount. Under Singapore’s Sale of Food Act (Cap. 283, Food Regulations, 2005 Rev Ed), the country from which each product originates needs to be labelled on the packaging. However, only 70% of all the products obtained in this study could be traced to at least the country where they are packaged. Even fewer (65%) contain information about their countries of origin. We furthermore note that the frozen shark fin of a grey sharptail shark (Rhizoprionodon oligolinx) labelled as originating from Spain is likely to have been caught in its native range of the Indo-West Pacific rather than in Spanish waters. These issues clearly highlight that traceability in the elasmobranch trade is a key challenge.

In particular, landings at the fishing ports are extremely difficult to track. Any attempt by enforcement officers to determine the country of origin for landings would be based solely on the fish merchant’s word or records in the logbook of the vessel, which may not contain precise information about fishes caught (see Fisheries Act, Cap. 111, Fisheries (Fishing Harbour) Rules, 1996 Rev Ed). There is generally no reliable means of gathering evidence to determine that a catch has been fished outside of Singapore’s waters, thereby limiting the enforcement of Singapore’s Endangered Species (Import and Export) Act (Cap 92A, 2008 Rev Ed). Nevertheless, with just two pectoral fins of the bottlenose wedgefish (R. australiae) clearly claimed to have originated from Singapore, it appears that almost all of the elasmobranch products sold here were imported. We note that NParks, working with the Singapore Food Agency, has stepped up surveillance measures at the fishing ports and also informed merchants and traders on the regulations and penalties for non-compliance since the new CITES listing came into effect (Thiagarajan, 2020).

Mislabelling and non-specific labelling of products are prominent features of the shark and ray trade (Bornatowski, Braga & Vitule, 2010; Boon, 2017). Indeed, many shark species have been detected here in products labelled simply as ‘dried fish’ or ‘salted fish’. Though it is true that elasmobranchs are technically fishes, the vague wording of the labels would likely lead to the interpretation that the products contain non-threatened bony fishes. Importantly, the ‘ikan kurau’ labelled on three bowmouth guitarfishes (Rhina ancylostoma) and a bottlenose wedgefish (R. australiae) is the Malay name for the Indian threadfin (Leptomelanosoma indicum), a bony fish. The use of a non-English name on the packaging is permitted under...
Singapore’s Sale of Food Act (Cap. 283, Food Regulations, 2005 Rev Ed) as long as it is a common name for the precise species in the local context (i.e. L. indicum), but in these cases the elasmobranch products are clearly labelled erroneously. Wainwright et al. (2018), and the present study also found Endangered (H. elongata), Vulnerable (H. microstoma, N. ferrugineus, and C. falciformis), and Near Threatened (C. sealei, C. sorrah, Carcharinus amblyrhynchos, Carcharinus brevipinna, and G. cuvier) shark species in ‘dried fish’, ‘salted fish’, or ‘ikan kurau’. In addition, ‘shovel-nosed ray’ refers to other species of guitarfishes belonging in the genus Aptocyotrema but has been applied on fresh fillets identified here as R. australiae. As unremarkable as it may seem, labelling is an important issue that has direct impacts on consumers’ health and their ethical and religious beliefs, especially in the context of Singapore’s multicultural and multi-religious society (Ho et al., 2020).

4.3 | Recommendations to strengthen trade regulations

The lack of clear identifiable features in filleted and processed products of giant guitarfish and wedgefish species adds to the enforcement burden on regulatory authorities and officers and may affect how effectively international trade in these CITES Appendix II-listed species can be controlled (Boon, 2017; CITES, 2020). More detailed field-based identification tools would help bridge the gap in enforcement by enabling enforcement officers to identify products based on species’ external morphology and verify if the appropriate commodity codes and trade permits have been applied (Ramasvel & Vannuccini, 2015). For example, including information for identifying fins (e.g. Jabado, 2019) and fresh samples from meat cuts—taking into account the common processing techniques applied on elasmobranch products—would help enforcement officers overcome the problem of lack of identifiable features. These materials ought to be based on industry and scientific knowledge, such as morphological features used by shark fish traders to categorize fins of giant guitarfishes and wedgefishes (Mundy-Taylor & Crook, 2013). Enforcement officers also need to be trained to use these materials proficiently and be able to apply alternative methods of species identification, such as DNA barcoding.

By law, all imported shipments entering Singapore need to be checked for contraband, but with the large volume of shipments and limited time for inspection, ensuring controlled entry of CITES-regulated species can be challenging. NParks adopts a risk-based approach to identify particular import shipments for further inspections, based on a set of risk profiles and risk indicators as well as information shared by regulatory authorities in other countries (NParks, 2019). Shark fin shipments are also checked by random sampling for DNA sequencing to verify the authenticity of the products that have been declared (Boon, 2017). To increase the throughputs and accuracy of these processes, robust sampling and test kits that can handle numerous samples should be utilized as soon as they are available and effective for customs surveillance of trade in endangered species. With rapid, affordable, and high-capacity DNA sequencing tools such as MinION (Oxford Nanopore Technologies; Johri et al., 2019; Chang et al., 2020a; Chang et al., 2020b), large volumes (up to 1,000) of mixed-species products can be analysed in one flow cell at the low cost of <US$10 per sample (Ho et al., 2020). Loop-mediated isothermal amplification, which requires minimal laboratory set-up (e.g. pipettes and water bath), has also been used to amplify the DNA of Atlantic cod (Gadus morhua) and to identify mislabelled products in a UK supermarket (Saull et al., 2016). Development of test kits utilizing these new technologies will facilitate their deployment at import checkpoints, retail markets, and fishery ports. By utilizing a toolbox of specimen identification protocols that includes DNA barcoding for processing large volumes of elasmobranch imports, there will be minimal room for CITES-listed giant guitarfishes and wedgefishes to enter the country without trade permits, as another product, or as a mixed-species product. Relatedly, information on traded giant guitarfishes and wedgefishes now needs to be reported with the recently established (November 2019) species-specific Harmonized System commodity codes. Verification performed using the aforementioned identification toolbox would improve traceability and trade monitoring of the supply chain. Data on customs seizures of CITES-listed goods, if made more widely available, would also lead to more effective regulation and enforcement efforts (Boon, 2017).

Given that 53% of the samples obtained here have been packaged in Singapore, raising packaging and labelling standards in Singapore would aid enforcement of CITES trade regulations. Currently, Singapore’s Sale of Food Act (Cap. 283, Food Regulations, 2005 Rev Ed) requires sellers to indicate ‘the common name, or a description’, and labels only need to be ‘sufficient to indicate the true nature of the food’. These minimal requirements add another layer of uncertainty on the identity of potential CITES-listed products and limit their traceability. The ambiguity in labelling also allows sellers to adopt vague or ‘creative labelling’ for their products (Ho et al., 2020), especially in the interest of selling protected species. The lack of transparency in labelling of seafood products has come under scrutiny after a recent study on seafood labelling detected considerable amounts of pig DNA in certain seafood products sold in Singapore (Ho et al., 2020). The results of the study were quickly picked up by the local news media (Lim, 2019; Liu, 2019) and has spurred discussions for Singapore to adopt similar labelling regulations as those in the EU. The EU mandates that both the commercial and scientific names of the product be printed on the label, and that products can only be sold under approved commercial names published by EU member countries (Regulation (EU) No. 1379/2013). Food labels also need to contain allergen information, the origin of primary ingredients if it differs from the food label, with a set minimum font to ensure legibility. Furthermore, fish, molluscan, crustacean, and algal products must bear information on whether they were extracted from the wild—with associated catch area and type of fishing gear used—or farmed.

More generally, authorities should consider implementing labelling requirements that reflect transparency in the supply chains and sustainability of harvested products. Well-established eco-labelling
standards backed by sustainable fishery certification programmes (e.g. Marine Stewardship Council) could form the basis for labelling reforms. In particular, seafood products with certified labelling by the Marine Stewardship Council, though costly (Asche & Bronnmann, 2017; Wang & Chang, 2017), have been found to be nearly free (<1%) of mislabelling (Barendse et al., 2019). Accurate labelling would place consumers in a better position to make informed purchasing decisions in the interest of compliance and sustainability. Public trust in food safety remains high in Singapore (Singapore Food Agency, 2019), but unless authorities take steps to improve packaging and labelling standards, this trust may erode with food scares, especially in the digital era of misinformation (Vaqué, 2018).

Apart from the application of advanced scientific techniques and knowledge for CITES enforcement, outreach and engagement by relevant authorities and non-governmental organizations are needed to educate the public on better buying choices and product labelling (Cardeñosa et al., 2018). Consumers ought to be persuaded to consistently opt for products with labels that account for sustainability and traceability (Ward & Phillips, 2009). There already exists among general consumers a preference for traceability information, which they perceive to be a good indication of quality assurance (Pazartzi et al., 2019). However, consumer preferences may not translate into a willingness to pay the price premium associated with these products or to accept the cost burden of accurate labelling that sellers might pass on to consumers. Studies specifically targeting Singapore consumers on attitudes towards labelling and traceability are urgently needed, as our current understanding of their consumption patterns and willingness to pay for shark and ray products is mostly derived from research performed elsewhere (Alfnes, Chen & Rickertsen, 2018; but see Supartini, Oishi & Yagi, 2018). The varying availabilities (Table 2) and prices fetched by different giant guitarfish and wedgefish products (Jabado, 2018) also indicate that the demand may be driven by distinct uses and market segments. Future studies need to examine consumption at the product and species levels and would therefore benefit from the enhanced DNA sequencing capabilities described herein. Information gained from such targeted local research would aid relevant agencies in changing consumption behaviour and guide reforms in the seafood industry.

4.4 | Conclusions

The lack of identifiable features in elasmobranch products has challenged enforcement agencies in ensuring that CITES-listed species are imported with appropriate permits. Limited regulations in labelling further add burden on enforcement, especially with the large volume of imports entering Singapore and the landings at fishing ports. There have also been hardly any studies characterizing shark and ray products that are being traded in Singapore (but see Wainwright et al., 2018). Results here show that DNA barcoding can unambiguously identify giant guitarfish and wedgefish species in unknown elasmobranch samples. Though this study gives a snapshot of the products that were available to consumers just before and as the CITES listing of these species came into effect, continued sampling of the same vendors repeated over a longer period of time would provide insight into the impacts of enhanced trade regulations concerning giant guitarfishes and wedgefishes.

More broadly, DNA barcoding has immense potential to be an effective trade surveillance tool at customs checkpoints, retail markets, or fishery ports, especially with the adoption of new DNA amplification, sequencing, and detection technologies. In line with these scientific developments, the Centre for Wildlife Forensics was established by NParks in August 2020 to facilitate investigations relating to the illegal wildlife trade (NParks, 2020). We suggest that active surveillance and consumer attitudes are also key to curbing illegal imports, and the results and recommendations presented here and elsewhere (e.g. Wainwright et al., 2018) could form a baseline for these efforts. Clearly, Singapore is becoming better positioned to enhance enforcement capacity and increase public awareness to ensure that marine products are accurately labelled and traceable for safe and sustainable consumption.

ACKNOWLEDGEMENTS

We thank members of the Reef Ecology Lab, National University of Singapore, for assistance and support, and are grateful for comments by Anna Wong and Renhui Xie of the National Parks Board. This study was funded by the National Research Foundation, Prime Minister’s Office, Singapore, under its Marine Science R&D Programme (MSRDP-P03). MR was supported by the Shark Conservation Fund through the Wildlife Conservation Society.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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

**How to cite this article:** Choo MY, Choy CPP, Ip YCA, Rao M, Huang D. Diversity and origins of giant guitarfish and wedgefish products in Singapore. *Aquatic Conserv: Mar Freshw Ecosyst*. 2021:1–14. https://doi.org/10.1002/aqc.3553