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PERSPECTIVES AND NOTES

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Molecular analyses of confiscated shark fins reveal shortcomings of CITES implementations in Germany

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Abstract

A three-ton shipment of dry shark fins was examined by German customs in 2017 leading to the confiscation of 405 kg of potential CITES species. We analyzed a subsample of this material (115 specimens) using DNA sequence-based identification and compared results to morphological screening of CITES species. We found a mixture of CITES regulated (4 of 11 species) and unregulated shark species. Our results demonstrate the difficulties of identifying CITES species morphologically in large fin shipments of mixed species composition. Correct identification of CITES species based on morphology alone may be hindered by missing characters or those altered by drying. We therefore suggest random molecular screening as a uniform approach for German customs authorities to check species composition and identify CITES regulated species in transit shipments.

KEYWORDS

CITES, customs, illegal trade, IUCN Red List of Threatened Species, legislation, mislabeling, molecular identification, shark fin trade, shark finning

1 | INTRODUCTION

Overexploitation by fisheries (both direct or indirect), habitat degradation and pollution are among the main factors endangering elasmobranchs today, all of which are consequences of human activity (Dulvy et al., 2014; Fowler et al., 2005). Among sharks, 28% of the species assessed by the IUCN are globally threatened with extinction (Worm et al., 2013). Overfishing (targeted or incidental) is one of the main factors contributing to shark population reduction (Dulvy et al., 2014). Despite the trade for meat, the most significant driver of this decline is the international fin trade (Feitosa et al., 2018; Fields, Abercrombie, Eng, Feldheim, & Chapman, 2015; Wong, Shivji, & Hanner, 2009). Shark fins are the main globally traded product under the Convention on International Trade in Endangered Species of Wild Fauna and Flora—CITES (Cardeñosa et al., 2018). Trade is mainly driven by high demand for shark fin soup, a traditional Chinese dish in the Asian market (da Silva Ferrette et al., 2019). In China, the special economic area of Hong Kong is the world's largest shark fin importer of CITES-listed sharks (Cardeñosa et al., 2018; Clarke et al., 2006) and its fin market is supplied by around 130 countries (Shea & To, 2017). To reach their destination country, shark fin shipments move along international trade routes passing transit countries (Dent & Clarke, 2015).

Currently, 54 neoselachian (sharks and rays) species are CITES regulated, comprising 14 selachians and 40 batoids (CITES, 2020a). All regulated shark species are

listed in CITES Appendix 2 (CITES, 2020b- http://www.cites.org/eng/app/appendices.php), however, only a small part of traded species is regulated. Appendix 2 includes species for which trade must be controlled. CITES members agree on establishing and enforcing national laws to monitor trading of CITES regulated goods (CITES, 2020c). To transport regulated species, special import, export, and reexport permits are needed that certify usage levels will not conflict with or be detrimental to the survival of the species. However, CITES listings face implementation challenges, which can hinder protection efforts (Sims & Frost, 2019). CITES implementation requires different enforcement measures for each step of the trade chain. Fins from CITES-listed sharks can be either unreported or mislabeled to avoid detection, as has been documented for basking shark (Magnussen et al., 2007) and white shark fins (Shivji, Chapman, Pikitch, & Raymond, 2005). Therefore, even though the implementation of the CITES agreement is the first step to protect sharks from illegal trade, the effectiveness of the agreement depends on its enforcement. Signatory countries must work towards the implementation of accurate species identification methods to regulate the trade of mislabeled CITES-listed species products (Sims & Frost, 2019).

As stated in Fields et al. (2015), a great deal of responsibility lies on customs staff to enforce CITES. In order to detect illegal shipments, customs agents face the challenge of identifying over 38,700 CITES regulated species or their products (www.CITES.com). When transit countries do not detect fin shipments of regulated species, they may act as unaware partners in the business (Murdock & Rivas-Villanueva, 2019).

For morphological identification of CITES-listed shark fins, there are a number of fin identification guides (e.g., Abercrombie, 2019; Abercrombie & Hernandez, 2017; Jabado, 2019; Marshall & Barone, 2016) as well as the landmark-based software iSharkFin (FAO & University of Vigo, 2014). In Germany, only CITES regulated species can be confiscated under the German federal law on nature protection (Bundesnaturschutzgesetz [BNatSchG]; article 51 paragraph 2 sentence 1). Generally, this law states that only specimens under appendices A, B or C of the regulation number 338/97 of the European Community can be confiscated. The aforementioned appendices correspond to appendices I, II, and III of CITES, respectively. This legislation allows customs authorities dealing with species trade to seek help from external experts listed in the webpage of the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU—https://www.bfn.de/themen/cites/recherche-Sachverstaendige.html).

Here, we examine a particular case of an in-transit country seizure in Germany in a likely attempt to bypass CITES regulations. A total of 3,049 kg of unprocessed dried shark fins, exported from Mexico in transit to Hong Kong, were stopped by German customs authorities on December 28, 2017 at Frankfurt Airport. The shipment was accompanied by a CITES document stating that it only comprises fins from the non CITES-listed Sandbar shark (*Carcharhinus plumbeus*). German customs contacted coauthor J. P. as an external consultant for fin identification. Despite J. P.'s profession as customs officer, he is an authorized CITES expert for sharks, rays, and skates at the BMU. A subsample of eight fins was provided to J. P. for a preliminary screening of CITES regulated species. J. P. identified five species in these eight fins, three of which are CITES-listed. The identification was performed using free available fin identification keys (Abercrombie, Chapman, Gulak, & Carlson, 2013; Abercrombie & Hernandez, 2017; Marshall & Barone, 2016) and fins were further compared to results obtained using iSharkFin software (FAO & University of Vigo, 2014).

Morphological identification was subsequently provided to customs authorities along with fin identification keys for further screening for CITES species in the shipment. German customs officers then identified that 405 kg originated from CITES species and confiscated this material. The remaining 2,644 kg were cleared. A random subsample of 50 kg of seized fins (approximately 500 fin specimens) were provided to the Bavarian State Collection of Zoology (ZSM) for further analyses with the intention of depositing reference material for future seizures and education of customs staff. In this study, we report on the species composition of a subsample of the shipment and identify the presence of CITES-listed species using mitochondrial DNA sequence analysis. DNA-based identifications are compared to findings from morphological identification keys. Issues of a European transit country to enforce CITES regulations on shark fin shipments are discussed in light of our results.

2 | MATERIALS AND METHODS

2.1 | Available shipment information and sample collection

From the 50 kg of unprocessed dried fins provided to the ZSM (Figure 1), we randomly selected 115 specimens. Each fin was photographed from both sides, labeled and tissue sampled (Appendix 2). In addition, we included the eight fins initially analyzed by J. P. We extracted approximately 250 mg of tissue from the internal part of each fin through either drilling or cutting small fragments from the base below the skin. For subsequent DNA extraction, 20–25 mg of tissue was used, while the

remaining tissue was stored in 96% EtOH at −20°C in the tissue collection of the ZSM. An overview of the samples is given in Table S1 including information on the eight fins initially analyzed by J. P.

2.2 | DNA extraction and molecular species identification

Genomic DNA was extracted from fin tissue using the Macherey & Nagel NucleoSpin Tissue kit (MACHEREY-NAGEL GmbH & Co. KG, Germany). DNA extracts were stored at −20°C in elution buffer until further processing. Fins were identified by amplifying the mitochondrial NADH dehydrogenase subunit 2 gene (NADH2). Polymerase chain reaction (PCR) amplification and sequencing specifications can be found in Appendix 1 in the Supporting Information.

Forward and reverse sequences were edited and aligned using Geneious 7.1.9 (http://www.geneious.com, Kearse et al., 2012). Consensus sequence alignments were done using the Geneious Alignment algorithm and each DNA sequence was translated to amino acids to check for stop codons. Fin species identification was performed in two steps: first, we used the Basic Local Alignment Search Tool (BLAST) (Altschul, Gish, Miller, Myers, & Lipman, 1990) to compare sequences generated in this study to the public database, GenBank—NCBI (National Center for Biotechnology Information - http://www.ncbi.nlm.nih.gov/genbank/) for molecular species identification. We considered a species ID reliable for similarity >98%.

In a next step, we downloaded all available NADH2 sequences from corresponding BLAST results best hits and an out-group sequence (*Hypanus longus*) from GenBank. Sequences are published in Naylor et al. (2012a). All sequences were aligned with our consensus sequences. Thereafter, we checked the placement of our sequences and the species divergence patterns using a clustering approach with a neighbor-joining analysis conducted in Mega 7.0.26 (Kumar, Stecher, & Tamura, 2016), using the K2P distance model. We used 1,000 bootstrap replicates for evaluating statistical node support.

For morphological identification, we tested four morphological fin guides (Abercrombie, 2019; Abercrombie & Hernandez, 2017; Jabado, 2019; Marshall & Barone, 2016) on images of all 115 fins analyzed above. Used keys are intended to identify shark fins of CITES listed species only and do not provide species level identification for nonregulated species. Morphological results were then compared with results from molecular analysis.

3 | RESULTS

3.1 | Species identification

We successfully extracted DNA from 110 of 115 randomly selected fins. Six of the eight fins initially identified by J. P. were also sequenced. The NADH2 gene was effectively amplified for all 116 samples and 109 edited consensus sequences were 1,044 bp in length (348 amino acids). The exceptions were seven consensus sequences identified as *Galeocerdo cuvier*, which were 1,041 bp (347 amino acids), as documented before (Naylor et al., 2012b). Amino acid sequences showed no stop codons within the analyzed sequences (Figure 1).

In total, we identified 11 shark species representing two families, Carcharhinidae (8 species) and Sphyrnidae (3 species) and 3 genera, *Carcharhinus*, *Galeocerdo*, and *Sphyrna*. *Carcharhinus brevipinna* was the most abundant species (34%), followed by *Carcharhinus falciformis* (18%) and *C. plumbeus* (17%) (Table 1). Four of the species identified are CITES regulated and listed in Appendix 2: *Sphyrna lewini*, *C. falciformis*, *Sphyrna zygaena*, and *Sphyrna mokarran*. Then, 6 of 11 identified species (54.5%) are assessed under the threatened categories of the IUCN, two of which are listed as endangered and four as vulnerable. If we also include those species categorized as "Near Threatened," 90.9% are rated under concern by the IUCN Red List of Threatened Species (Figure 3).

Results from BLAST analyses showed that all fins matched with the query cover and percent identity of >98% with reference sequences from Naylor et al. (2012a, 2012b) (Table S1). The neighbor-joining tree analysis placed all sequences derived from fins to the corresponding sequences

FIGURE 1 Representative image of confiscated shark fins provided to the Bavarian State Collection of Zoology for further scientific analyses

TABLE 1 Shark species identification based on molecular analyses. One hundred and ten specimens of seized dried shark fins were analyzed using the mitochondrial NADH2 gene

Packaging label ID	Molecular species ID	Frequency	Family	IUCN category	CITES listing
Carcharhinus plumbeus	*Carcharhinus brevipinna*	37	Carcharhinidae	NEAR THREATENED	Not listed
	Carcharhinus falciformis	20	Carcharhinidae	VULNERABLE	Listed in Appendix 2
	Carcharhinus plumbeus	19	Carcharhinidae	VULNERABLE	Not listed
	Carcharhinus perezii	8	Carcharhinidae	NEAR THREATENED	Not listed
	Carcharhinus signatus	7	Carcharhinidae	VULNERABLE	Not listed
	Galeocerdo cuvier	7	Carcharhinidae	NEAR THREATENED	Not listed
	Sphyrna lewini	7	Sphyrnidae	CRITICALLY ENDANGERED	Listed in Appendix 2
	Carcharhinus limbatus	2	Carcharhinidae	NEAR THREATENED	Not listed
	Sphyrna zygaena	1	Sphyrnidae	VULNERABLE	Listed in Appendix 2
	Sphyrna mokarran	1	Sphyrnidae	CRITICALLY ENDANGERED	Listed in Appendix 2
	Carcharhinus altimus	1	Carcharhinidae	DATA DEFICIENT	Not listed

Abbreviation: NADH2, NADH dehydrogenase subunit 2 gene.

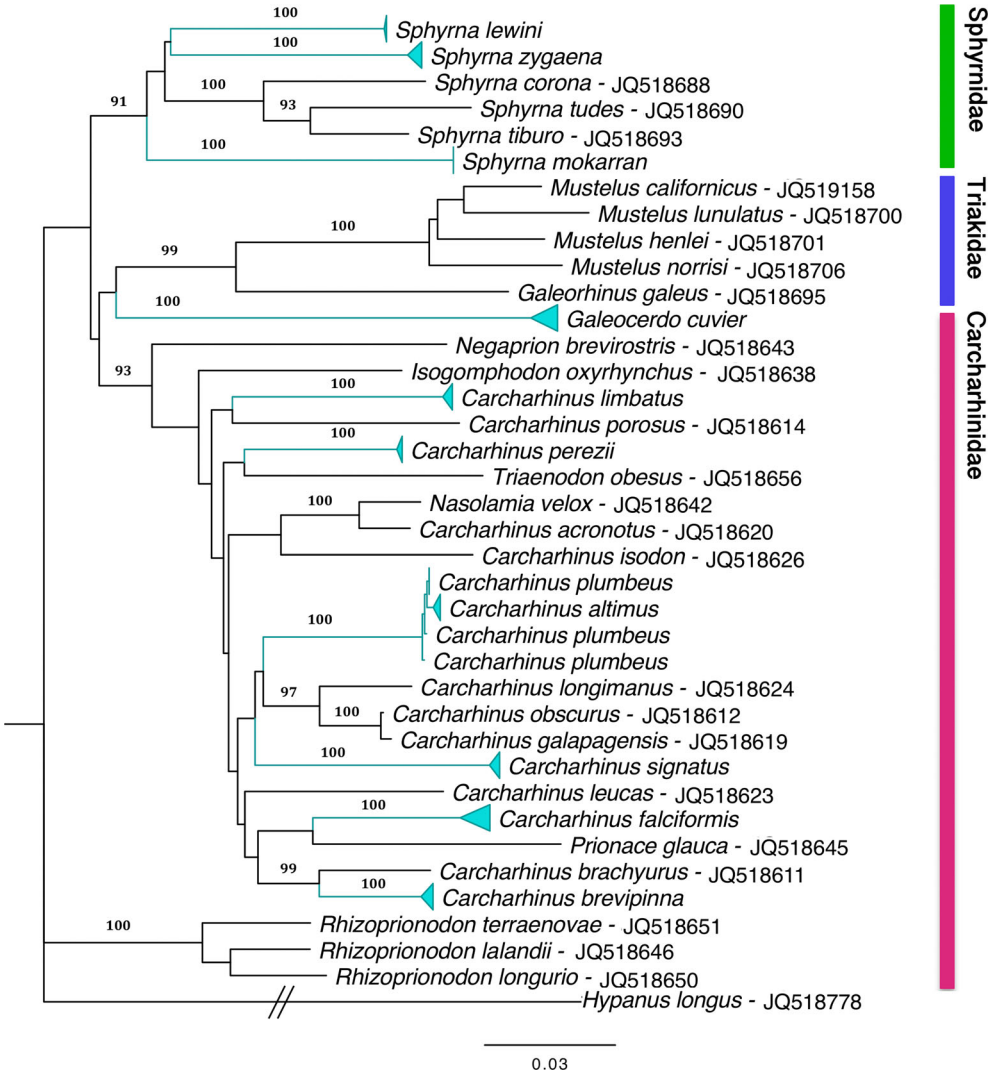

FIGURE 2 Neighbor-Joining tree computed from mitochondrial NADH dehydrogenase subunit 2 gene (NADH2) sequences ($N = 110$) from confiscated shark fins and reference sequences based on Naylor et al. (2012a, 2012b). *Hypanus longus* was defined as outgroup taxon. Only bootstrap values >90 are shown. Collapsed turquoise clades comprise sequences obtained in this study with matching reference sequences (Table S1)

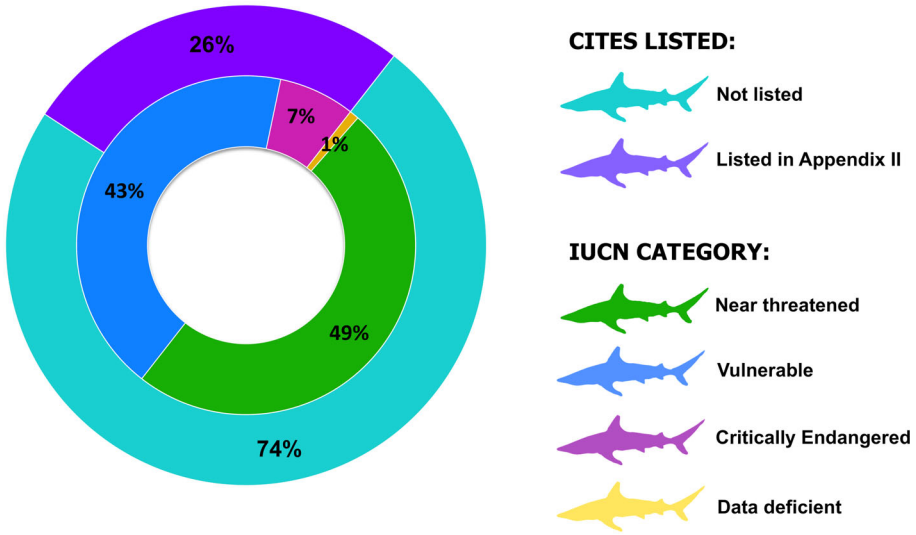

FIGURE 3 Proportion of species listed under the Appendix 2 of CITES (outer circle) and their corresponding IUCN status (inner circle) [Correction added on 22nd March, after first online publication: Figure 3 was revised.]

found through the BLAST search with high bootstrap support (= 100) (Figure 2). The only exceptions are sequences of *C. plumbeus* and *Carcharhinus altimus*. Even though specimens from both species were differentiated by our BLAST searches, *C. altimus* clustered within sequences from *C. plumbeus*.

Sequences of *G. cuvier* match two GenBank reference sequences that represent distinct *G. cuvier* NADH2 haplotype clusters. One of the clusters is confined to the Pacific and Indian Oceans (two specimens) and the other to the Atlantic Ocean (five specimens) (Naylor et al., 2012b).

The morphological identification approach yielded less accurate results in comparison to the molecular identification. While all nonlisted species were classified as such, six fin specimens were classified as non-CITES listed even though molecular results identified these fins as CITES listed species (Table S1).

4 | DISCUSSION

This assessment is the first genetic identification of confiscated unprocessed dried shark fins in Germany. Our results contradict the original CITES documentation of the shipment stating to contain fins from the non CITES-listed Sandbar shark (*C. plumbeus*). Only 17% of our samples were actually *C. plumbeus* (Table 1, Figure 3). The rest were a mixture of CITES and non-CITES listed species, suggesting an intention to bypass CITES regulations. In total, we identified 11 shark species, of which four are CITES regulated.

The genus *Carcharhinus* was the most abundant (63.6%) (Table 1), including the CITES-listed species *C. falciformis*. In recent years, silky shark populations have declined and are now listed as vulnerable to extinction by the International Union for Conservation of Nature—IUCN (Grant et al., 2019). Silky sharks are one of the most heavily exploited (both as target and nontarget species) and frequently traded tropical shark species worldwide (Fields et al., 2017; Grant et al., 2019).

Likewise, tiger shark (*G. cuvier*) catch rates have declined by approximately 65% since 1986 (Baum et al., 2003). The tiger shark haplotypes identified in this study allow us to draw conclusions on the origin of fins. The only origin information provided on the shipment label stated "Mexico." No detailed information was provided with respect to where the sharks were actually fished. As *G. cuvier* sequences in this study match haplotypes reported from both Pacific and Atlantic clusters (Naylor et al., 2012b), multiple geographic origins of fins are likely.

S. lewini, *S. mokarran*, and *S. zygaena* are part of a large coastal shark species complex that is considered overfished. Both *S. lewini* and *S. mokarran* are listed as critically endangered in the most recent IUCN (2019) assessment. All three species of this group were also documented in our study (Table 1). Baum and Blanchard (2010) reported a decline of 76% in their abundance between 1992 and 2005. They are caught both incidentally and intentionally in large quantities by multispecies fisheries worldwide and are among the most valuable and popular fins traded in Hong Kong (Abercrombie, Clarke, & Shivji, 2005; Gallagher & Klimley, 2018).

In the light of these alarming declines, implementation of CITES regulation in transit countries is essential. In Germany, confiscated CITES material either remains in custody until the sender can deliver the missing documents or it becomes property of the German government. Mislabeling can be fined with a maximum of

50,000 € according to article 69 of the BNatSchG. Penalty amount depends on the protection status of the confiscated material and counteracting legislation can only be filed once, that is, a three-ton shipment is a single violation. Since all shark species regulated under CITES agreements are listed in Appendix 2, relatively low penalties will result. Put into context of the large shipment size (>3 tons), mislabeling is not of great economic risk to the sender as the value of the total shipment likely exceeds 1 million € (Wu, 2016). Assuming the maximum possible penalty, the cleared part of the shipment (2,644 kg) is still of very high value.

Despite central Europe playing no active role in the consumption of shark fin products, its logistics network and transportation routes are used in the global shark fin trade, facilitating the shipping of shark fins from harvesting countries to the Asian markets (Hobbs, Potts, Walsh, Usher, & Griffiths, 2019). According to TRAFFIC (2020) (https://www.traffic.org/what-we-do/species/sharks-and-rays/), traceability is crucial for the effective conservation of shark species listed under Appendix 2. In that sense, the use of molecular approaches could provide key information on the shark species being traded and on their place of origin (coastal and pelagic shark species were reported in this study). EU Transit countries' collecting such detailed information about shark fin shipments is crucial for accurate tracking of the species trade. Understanding trade dynamics is fundamental to better monitor the fin trade and create better action plans and conservation efforts (Shea & To, 2017).

Initial morphological identification led to the confiscation of 405 kg of fins, based on the premise that they were CITES regulated species. However, molecular diagnostic techniques later indicated the presence of CITES and non-CITES regulated species mixed together in the confiscated material (Table S1). Our results further suggest that CITES regulated species might have been present in the shipment part that was not confiscated and was forwarded to the recipient in Hong Kong. This demonstrates how morphological identification keys are useful as a first step during the process of screening for CITES-listed species; but also highlights the difficulties of the identification process based on morphological characteristics alone. Dried shark fins could be missing representative diagnostic characteristics, making morphological identification a challenging task that can give unreliable results even for experts (da Silva Ferrette et al., 2019). Fin desiccation often results in color changes and drying may morph and curl the tissue, as was evidenced in this shipment with several fins in poor morphological condition. Furthermore, morphological identification by in-transit countries customs staff is largely impeded by the large size of such shipments, which often contain several thousand fins from different ontogenetic stages.

Molecular identification is independent of the morphological condition of the specimens and outperformed morphological identification in this study (Table S1). Based on the fin identification keys, it was only possible to identify CITES-listed species-to-species level. Therefore, we suggest random molecular screening of subsamples as a uniform approach for species identification. It can also provide information on nonregulated species allowing for data collection on such, which may be valuable for future evaluations. Molecular methods can also be applied to shipments containing processed specimens and can ultimately prevent camouflage of CITES-listed species as legally traded goods (Dudgeon et al., 2012; Magnussen et al., 2007; Sims & Frost, 2019). A tissue sampling protocol is provided in Appendix 2. Tissue sampling does not require any special training of customs staff and DNA sequence-based identification can be outsourced.

Current German CITES implementation appears ineffective when handling shark fin shipments, since they can contain a mixture of CITES regulated and unregulated material. We suggest a uniform German procedure using molecular identification for detecting violations of species conservation laws. This approach has proven to be a superior monitoring and controlling tool for shark products across borders and a better safeguard against mislabeling of illegal products (Sims & Frost, 2019). We further suggest that revealed mislabeling should result in the complete destruction of the shipment, representing a huge economic risk to the sender, as practiced in other EU countries.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Nicolas Straube, **Friederike Kremer-Obrock**, and **Jürgen Pollerspöck**: Designed the study. **Melany Villate-Moreno**: Carried out lab work and analyzed the data. **Melany Villate-Moreno** and **Nicolas Straube**: Wrote the manuscript. All authors drafted and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

All DNA sequence data are available in Genbank, please see Table S1 for corresponding Genbank numbers.

ETHICS STATEMENT

No ethics review for animal handling was necessary for the work described in this article.

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SUPPORTING INFORMATION

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

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