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Molecular tracing of confiscated pangolin scales for conservation and illegal trade monitoring in Southeast Asia

Huarong Zhang [a,*], Mark P. Miller [b], Feng Yang [a], Hon Ki Chan [a], Philippe Gaubert [c], Gary Ades [a], Gunter A. Fischer [a]

[a] Kadoorie Farm and Botanic Garden, Lam Kam Road, Tai Po, N.T., Hong Kong Special Administrative Region

[b] U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, 3200 SW Jefferson Way, Corvallis, OR, 97331, USA

[c] Institut des Sciences de l'Evolution de Montpellier (ISEM) – UM2-CNRS-IRD-CIRAD-EPHE, Université de Montpellier, Place Eugène Bataillon, Montpellier Cedex 05, France

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ABSTRACT

Despite being protected by both international and national regulations, pangolins are threatened by illegal trade. Here we report mitochondrial DNA identification and haplotype richness estimation, using 239 pangolin scale samples from two confiscations in Hong Kong. We found a total of 13 genetically distinct cytochrome c oxidase I (COI) haplotypes in two confiscations (13 and ten haplotypes respectively, with ten shared haplotypes between confiscations). These haplotypes clustered in two distinct clades with one clade representing the Sunda pangolin (*Manis javanica*). The other clade did not match with any known Asian pangolin sequences, and likely represented a cryptic pangolin lineage in Asia. By fitting sample coverage and rarefaction/regression models to our sample data, we predicted that the total number of COI haplotypes in two confiscations were 14.86 and 11.06 respectively, suggesting that our sampling caught the majority of haplotypes and that we had adequately characterized each confiscation. We detected substantial sequence divergence among the seized scales, likely evidencing that the Sunda pangolins were harvested over wide geographical areas across Southeast Asia. Our study illustrates the value of applying DNA forensics for illegal wildlife trade monitoring.

1. Introduction

Illegal trade of wildlife is a major threat to biodiversity (Corlett, 2007; Nijman, 2009; Rosen and Smith, 2010). Overexploitation driven by wildlife trade has pushed many species to the edge of extinction, as in the well-known cases of tigers, rhinos and elephants. South-East Asia is among the world's "wildlife trade hotspots", where the trade is usually in high volume (Li et al., 2000; Nijman, 2009; Phelps and Webb, 2015). It is estimated that illegal wildlife trade is worth US$2.5 billion a year in East Asia and the Pacific (UNODC, 2013). Monitoring and regulating wildlife trade has become a critical conservation priority (Wasser et al., 2008; Oldfield, 2013; Welton et al., 2013; Chan et al., 2015; Challender et al., 2015).

Pangolins (family Manidae) are heavily poached for their meat and scales, to supply the illegal food and traditional medicine trade (Challender, 2011; Challender and Hywood, 2012; Zhou et al., 2014). There are eight species of living pangolins (Gaubert, 2011), and four pangolin species in the genus *Manis* are found in Asia (Fig. 1; Appendix 1 in

* Corresponding author.
E-mail address: hzhang@kfbg.org (H. Zhang).

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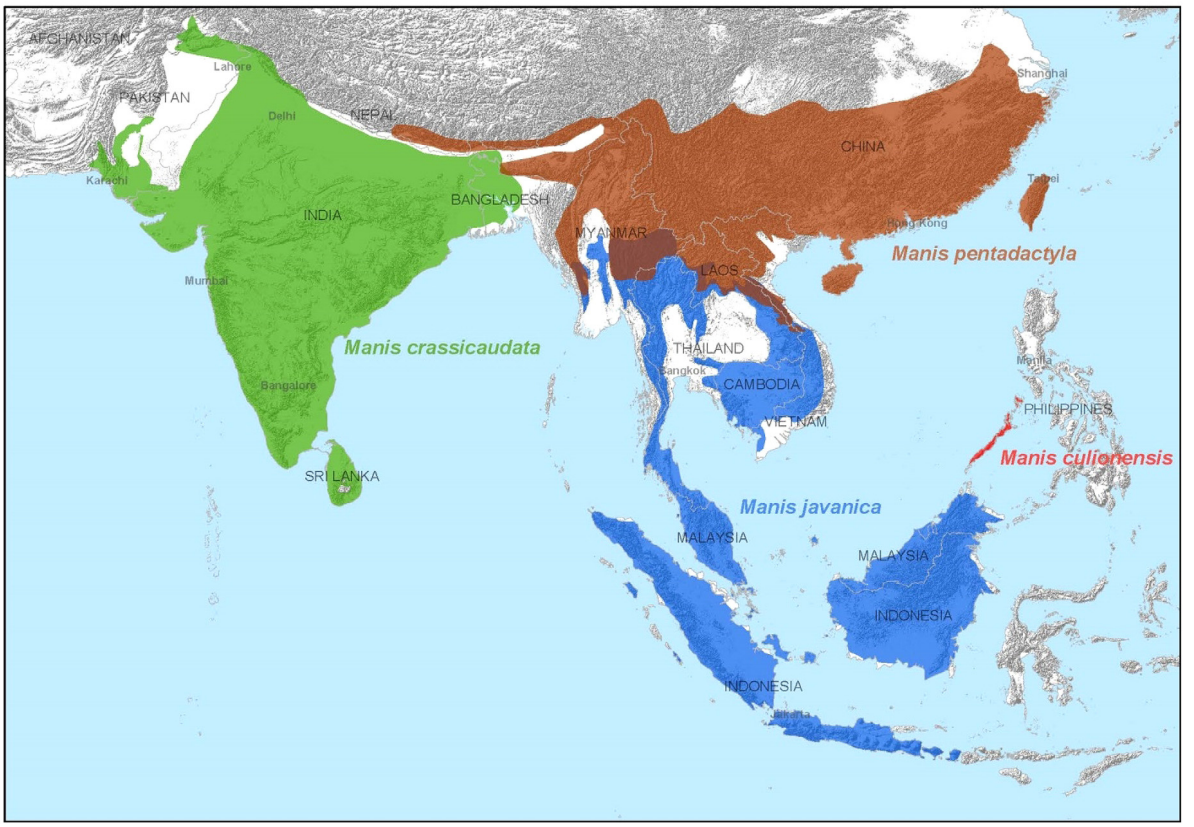

Fig. 1. Geographic distribution of the four Asian pangolins: the Chinese pangolin (*Manis pentadactyla*), the Sunda pangolin (*M. javanica*), the Palawan pangolin (*M. culionensis*), and the Indian pangolin (*M. crassicaudata*).

supplementary material): the Chinese pangolin (*Manis pentadactyla*), the Sunda pangolin (*M. javanica*), the Palawan pangolin (*M. culionensis*), and the Indian pangolin (*M. crassicaudata*). The remaining four pangolin species occur in Africa: the tree pangolin (*Phataginus tricuspis*), the long-tailed pangolin (*Uromanis tetradactyla*), the Cape or ground pangolin (*Smutsia temminckii*) and the giant pangolin (*Smutsia gigantea*). Pangolins are nocturnal animals that prey on ants and termites. They are "EDGE" (Evolutionarily Distinct and Globally Endangered; Isaac et al., 2007) mammals (constituting the Order Pholidota) that possess scale-covered bodies (Gaubert, 2011). Pangolins play an important role in controlling ant and termite populations in natural ecosystems (Swart et al., 1999; Wu et al., 2004). With their slow growth and low reproductive rates, pangolin populations are highly vulnerable to hunting. Many populations may now be locally extinct and recovery of the small number of individuals remaining in some severely impacted areas is likely to be slow (Wu et al., 2002; Newton et al., 2008).

Due to rapid declines in their populations, all pangolin species are listed as 'threatened' in the International Union for Conservation of Nature (IUCN) Red List (IUCN, 2015). They are also included in CITES Appendix II (CITES, 2013). Although pangolins are also protected by national and regional laws in their range states, they are still subject to illegal trade. Consumption of pangolins is driven by the folklore belief of their health benefits. However, pangolin scales consist of keratin, which has no proven pharmacological effects (Leader et al., 2008).

In the past decade, tons of pangolin scales and meat have been seized worldwide every year (TRAFFIC, 2013) and these seizure records are likely to represent only a small fraction of the massive illegal trade these species have been subject to (Pantel and Anak, 2010; Challender et al., 2015). In 2013, the illegal pangolin trade in Asia-Pacific was estimated to be valued at between US$100–150 million (UNODC, 2013). As the trade in pangolins raises serious legal and conservation concerns, accurate species identification is critical for trade regulation and law enforcement. However, it is difficult to confirm species identity from isolated scales (Challender, 2011).

Wildlife crime investigation has benefited from advances in DNA techniques which are increasingly used to assist cases (Ogden et al., 2009). Species or even populations can be distinguished by nucleotide substitutions in a selected fragment of DNA sequence such as the cytochrome c oxidase subunit 1 (COI) gene, the standard barcoding fragment for species identification (Hebert et al., 2003). Previous studies have shown application of DNA forensics in species identification from rhino horns (Hsieh et al., 2003), ivories (Ishida et al., 2013), whale and dolphin products (Baker et al., 1996), shark fins (Clarke et al., 2006), bushmeat including pangolins (Gaubert et al., 2015), and seized pangolin scales (Hsieh et al., 2011).