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MYTH DEBUNKED: KERATINOUS PANGOLIN SCALES DO NOT CONTAIN THE ANALGESIC TRAMADOL. CONSERVATION SCIENCE AND PRACTICE

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Myth debunked: Keratinous pangolin scales do not contain the analgesic tramadol

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Abstract

Conservation plans aiming to reduce the threat of illegal wildlife trade increasingly recognize the need for multifaceted approaches that include both enhanced enforcement and demand reduction initiatives. Both are complex issues that involve understanding consumer motives. Pangolins represent some of the most heavily trafficked species, largely due to high consumer demand for their scales for use in traditional medicines. Recent media reports also suggest that demand is related to the purported presence of the analgesic tramadol in scales. We examined chemotypes of scales from 104 individual pangolins representing all extant species. None of the specimens demonstrated the presence of tramadol. Given that demand for pangolins and their parts is decimating wild populations, it is imperative that such false claims be rectified. These results could be incorporated into demand reduction campaigns in areas where this misinformation is perpetuated.

KEYWORDS

CITES, conservation, consumer demand, Manidae, *Manis*, *Phataginus*, Pholidota, *Smutsia*, wildlife trade

1 | INTRODUCTION

Consumer demand for animals, their parts, and derivatives is driving many species to the brink of extinction. This is not only true for many iconic species, such as elephants, tigers, and rhinoceros, but also threatens lesser known taxa, notably the pangolins (Bennett, 2015; Brook et al., 2014; Challender, 2011; Heinrich et al., 2017; Walston et al., 2010). Although our knowledge on the behavior and ecology of these unique species is fairly limited, they are considered to be one of the most trafficked wild mammals (Zhou, Zhou, Newman, & Macdonald, 2014).

Pangolins are nocturnal, dietary specialists that feed primarily on ants and termites (i.e., myrmecophagous; Wilson & Mittermeier, 2011). They have a conspicuous armor of epidermal keratinous scales that nearly covers the entire body (Spearman, 1967). Together they comprise eight species; four occur only in Asia, and the other four occur only in Africa (Wilson & Mittermeier, 2011). Recent analyses suggest these eight species represent three genera: *Manis* (Asian pangolins), *Smutsia* (African ground pangolins), and *Phataginus* (African tree pangolins) (du Toit et al., 2017; Gaubert et al., 2018). These taxa are the only extant members of the Family Manidae and the only extant members of the Order Pholidota (Wilson & Mittermeier, 2011). Accordingly, hunting and trade in pangolins not only threatens individual species' survival but also threatens loss of an entire order of mammals. Such dire potential consequences of the wildlife trade were recently recognized by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) when it afforded all pangolin species the highest degree of protection (Appendix I; Note: CITES currently recognizes all eight pangolin species as representing a single genus *Manis*). Thus, all international commercial trade in pangolins, including parts and derivatives, is prohibited. Despite the prohibition, illegal trade continues at an alarming rate (Heinrich et al., 2017; Nijman, Zhang, & Shepherd, 2016). For example, international trade of pangolin scales through African countries, such as Nigeria and Cameroon, has been on the rise in recent years (Ingram, Cronin, Challender, Venditti, & Gonder, 2019). In the few years following the CITES ban, there have been multiple media reports of large-scale seizures of pangolins and their parts (e.g., Anonymous, 2019; Law, 2019), highlighting the complexity involved in reducing this threat.

Recent evaluations of the illegal wildlife trade suggest that efforts to combat the threat must focus not only on increased regulation enforcement but also on initiatives to reduce demand for wildlife products (e.g., Challender & MacMillan, 2014; Cheng, Xing, & Bonebrake, 2017; Phelps, Shepherd, Reeve, Niissalo, & Webb, 2014; Veríssimo, Challender, & Nijman, 2012). Both actions were included as part of a recent Conservation Action Plan for pangolins developed by members of the International Union for Conservation of Nature (Challender, Waterman, & Bailie, 2014). Unfortunately, there is no one-size-fits-all approach to either of these actions, and each is a complex issue on its own (Challender, Harrop, & MacMillan, 2015; Olmedo, Sharif, & Milner-Gulland, 2018). That said, understanding consumer use of wildlife products, and the appropriateness of such use, can help enforcement officials and policy makers implement more targeted and effective criminal investigations and behavior change strategies (Veríssimo et al., 2012).

The illegal trade in pangolins is largely driven by demand for their meat and scales. Pangolin meat is consumed in many countries as a delicacy, and the scales are often used in traditional medicines in Africa and Asia (Bensky & Gamble, 1993; Boakye, Pietersen, Kotzé, Dalton, & Jansen, 2014; Pantel & Chin, 2008). The greatest consumer demand for pangolin scales comes from China, as well as Vietnam and Hong Kong, where they are used in traditional Asian medicines to treat a variety of ailments (Challender, 2011; Heinrich et al., 2017), such as reducing swelling and pain (Bensky & Gamble, 1993). Although it is unclear how these keratinous structures that are of the same material as human nails (Spearman, 1967) would provide the purported medical benefits, demand and their use continues, highlighting the difficulties involved in changing behaviors with deep cultural relevance (e.g., Veríssimo et al., 2012). However, the illegal wildlife trade is also a dynamic industry; new trends, including new motivations for product use, can emerge that may not be steeped in cultural and societal history (Graham-Rowe, 2011; Milliken & Shaw, 2012; Sampson et al., 2018). Thus, efforts to reduce demand related to new trends might be more effective, underscoring the need to better understand claims behind apparent emerging wildlife trade trends.

Recently, media reports have suggested that pangolin scales contain "tramadol HCl" (Anonymous, 2010, 2015; Naveen, 2016; Victor, 2017), which is a synthetic opioid used as an analgesic. The original source of these oft-repeated claims has been difficult to pinpoint, but may be related to an article reporting results of research at Riau University in Indonesia (Anonymous, 2010). This source also claims that pangolin scales are potentially being used in the manufacture of methamphetamine because of the purported presence of tramadol HCl (Anonymous, 2010). More recent media reports have echoed these unsubstantiated claims (Anonymous, 2015; Friedman & Kriel, 2016; Naveen, 2016; Victor, 2017; but see Ewudolu, 2019), providing dangerous fuel to an already unsustainable demand for pangolin scales. Endogenous production of tramadol ($C_{16}H_{25}NO_2$) or tramadol HCl ($C_{16}H_{25}NO_2$-HCl) appeared highly questionable to us.

Tramadol HCl refers to the synthetically prepared hydrochloride salt, which facilitates absorption of the drug in the human body. Accordingly, tramadol HCl does not occur naturally. Tramadol is also not known to occur naturally. A recent study suggested that tramadol was identified in the root bark of an African plant (*Sarcocephalus latifolius*; syn. *Nauclea latifolia*) that is commonly used in African populations as a treatment for pain (Boumendjel et al., 2013). This was later confirmed to be the result of anthropogenic contamination through overuse of synthetic tramadol in humans and livestock, rather than synthesized by the plant itself (Kusari et al., 2014; Kusari, Tatsimo, Zühlke, & Spiteller, 2016). Thus, there is so far no substantiated evidence that tramadol is synthesized in biological systems. We investigated the question to address continuing inquiries from law enforcement, and found no evidence for the presence of tramadol in pangolin scales. Any demand for pangolin scales driven by this misconception is unwarranted.

2 | METHODS

In this study, we address the claim that pangolin scales contain tramadol by examining the chemical profiles of the scales representing all eight extant species (Table 1). We collected the keratin samples using standard human fingernail clippers from the scales of museum specimens (Table 1; Supporting Information Data S1). Because we are addressing whether or not tramadol might be produced or synthesized within the keratin matrix of scales, and surface contamination would confound the results, each keratin clipping was washed prior to analysis (Cappelle et al., 2014; Pinho et al., 2013). Each clipping was washed in a 2.0 mL tube with distilled water and sonicated for 10 minutes. The water was decanted, and this step was followed by a methanol wash and additional 10-minute sonication. Following the

TABLE 1 Pangolin scale sample clippings used in DART-TOFMS analysis

Species	$N_{individuals}$
Manis crassicaudata	9
Manis culionensis	4
Manis javanica	17
Manis pentadactyla	20
Phataginus tetradactyla	16
Phataginus tricuspis	18
Smutsia gigantea	10
Smutsia temminckii	10
Total	104

wash, the methanol was decanted; the sample tubes were maintained open, and the remaining methanol was left to evaporate overnight. Water and methanol washes have been commonly used to remove/dissolve surface contaminants in analyses aimed at detecting pharmaceuticals in human nails (Cappelle et al., 2014). Each scale was subsequently analyzed using direct analysis in real time (DART) and time-of-flight mass spectrometry (TOFMS).

DART uses an ambient (open air) ion source for sample analysis. Advantages of the DART-TOFMS technique include the following: solid samples can be analyzed without sample preparation; it produces high-resolution mass spectrometric data; and it is sensitive, thus allowing small samples to be analyzed for tramadol with low detection thresholds (100 parts per billion).

For analysis, each keratin clipping was placed in the heated gas stream of helium. Described compounds were then ionized by protonation and eventually detected in the TOFMS. Polyethylene glycol (PEG) was used as a mass calibration standard (Ultra Scientific, North Kingstown, RI, USA), which was run between every fifth sample. The tramadol reference sample was obtained by dissolving a tramadol tablet in methanol. A capillary tube was dipped in the solution and then held in the heated gas stream of helium. The DART source parameters and mass spectrometer settings follow Price, McClure, Jacobs and Espinoza, (2018). Each pangolin scale spectrum was probed for tramadol and tramadol HCl within a 15 mmu window and a threshold of 5% of the base peak using Mass Mountaineer software (RBC Software, Peabody, Massachusetts).

3 | RESULTS

Our mass spectra sample represents a single spectrum from one scale of 104 individual pangolins (Table 1; Supporting Information Data S1 and S2). We compared the mass spectrum of each specimen to the reference tramadol ($C_{16}H_{25}NO_2$). As expected, the protonated ion with highest intensity in tramadol has an *m/z* 264.206 (Figure 1; Supporting Information Data S2). Our probe of tramadol and tramadol HCl indicates that none of the scales representing all eight pangolin species contained tramadol, tramadol HCl, or its corresponding isotopes within the 15 mmu window and threshold of 5% of the base peak. Figure 1 provides a representative spectrum for each pangolin species compared to the chemical profile of tramadol. All raw spectra are provided in Supporting Information Data S2.

4 | DISCUSSION

The results of our study do not corroborate recent reports that pangolin scales contain the analgesic tramadol (Anonymous, 2010, 2015; Friedman & Kriel, 2016; Naveen, 2016). Our analysis of 104 pangolin scale spectra found no evidence of tramadol based on our detection threshold. The claim that pangolin scales contain tramadol has been repeated without scientific evidence, and here, we demonstrate evidence to the contrary. Some reports further suggest that demand for pangolin scales is driven in part by the purported presence of tramadol (Friedman & Kriel, 2016; Naveen, 2016). In fact, some media articles refer to an Interpol report, suggesting the presence of tramadol HCl in pangolin scales is driving demand in the United States (Friedman & Kriel, 2016; Naveen, 2016). We were unable to confirm the existence of this Interpol report (see also Ewudolu, 2019). To the extent that such false claims might augment illegal trade in pangolin scales, it is important that they be corrected, and that they are not perpetuated, as there remains no evidence that tramadol, or tramadol HCl, occurs naturally in pangolins or any other biological systems. The use of pangolin scales and other animal products in traditional medicines is common in many cultures (Bensky & Gamble, 1993; Boakye et al., 2014; Pantel & Chin, 2008), but it is unsustainable on a global commercial scale. Despite attempts to curtail this trade through increased protections, large-scale illegal trade in pangolins continues through multiple trade routes worldwide (Heinrich et al., 2017). We hope that our results will contribute to discussions that seek to reduce demand for pangolin scales by informing decisions about financial resource allocation, public education efforts, enforcement priorities, and management plans that accurately address present and future threats to pangolin populations. Accurate knowledge of pangolin biology is a crucial first step in this process as policy makers and enforcement officials attempt to implement effective behavior change strategies that seek to reduce pressure on fragile pangolin populations.

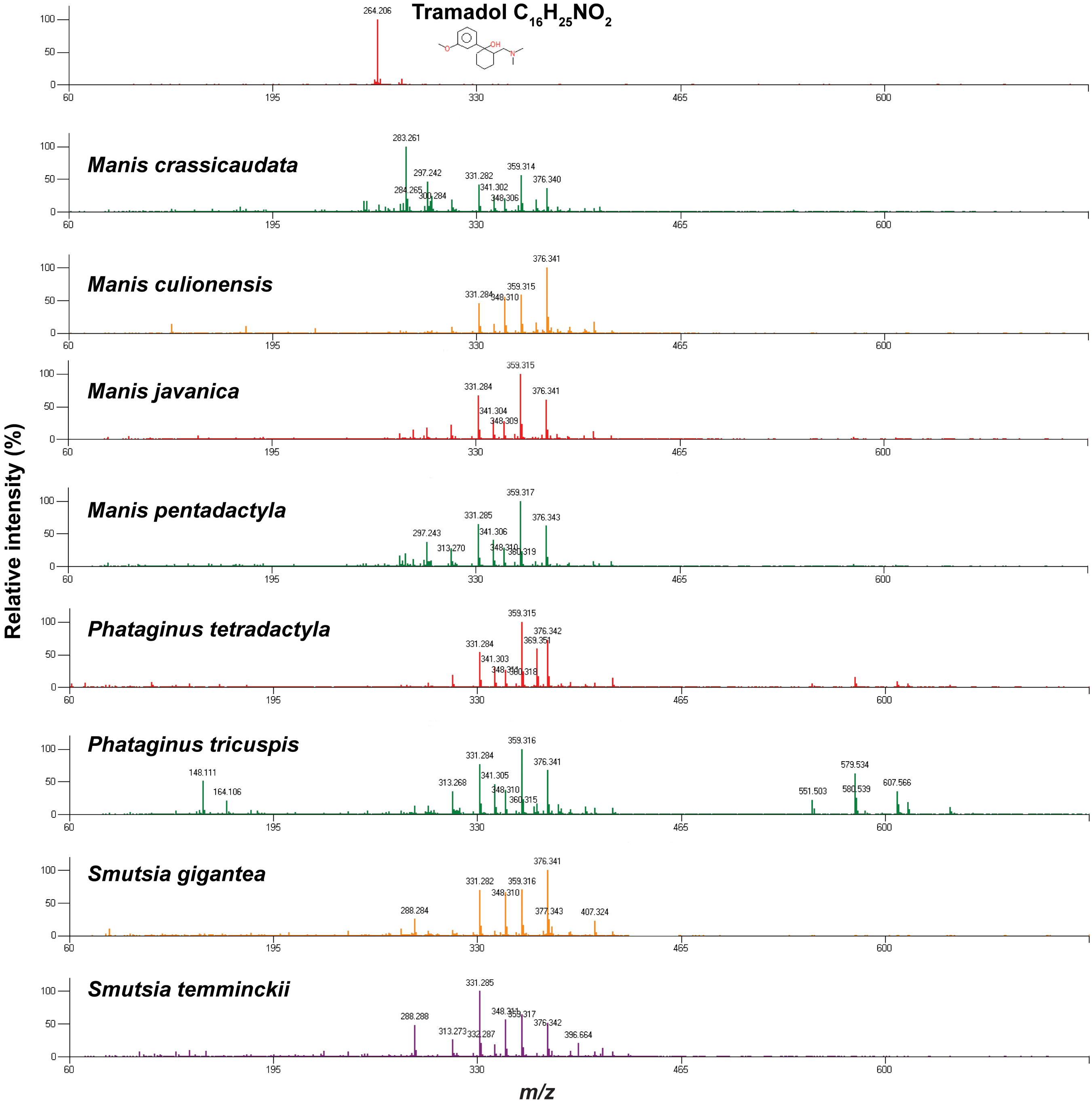

FIGURE 1 Full spectra of individual keratin scales representing each pangolin species compared to the spectrum of tramadol obtained using DART-TOFMS. *m/z* refers to the mass-to-charge ratio. Samples represented for each species are as follows: *Manis crassicaudata* (FMNH 91353); *Manis culionensis* (FMNH 62921); *Manis javanica* (AMNH M-32637); *Manis pentadactyla* (AMNH M-26637); *Phataginus tetradactyla* (FMNH 62209); *Phataginus tricuspis* (AMNH M-53875); *Smutsia gigantea* (AMNH M-53846); *Smutsia temminckii* (AMNH M-83772). See Supporting Information Data S2 for spectra representing all 104 scale samples

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authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

The project was conceived by all authors. Sample collection was conducted by R.L.J. E.O.E. and P.J.M. analyzed and interpreted data. R.L.J. drafted the manuscript, and all authors contributed to editing/revising its contents.

ETHICS STATEMENT

This study did not require ethics approval.

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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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