An introduced pentastomid parasite (*Raillietiella frenata*) infects native cane toads (*Rhinella marina*) in Panama

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SUMMARY

The pentastomid parasite, *Raillietiella frenata*, is native to Asia where it infects the Asian House gecko, *Hemidactylus frenatus*. This gecko has been widely introduced and recently *R. frenata* was found in introduced populations of cane toads (*Rhinella marina*) in Australia, indicating a host-switch from introduced geckos to toads. Here we report non-native adult *R. frenata* infecting the lungs of native cane toads in Panama. Eight of 64 toads were infected (median = 2·5, range = 1–80 pentastomids/toad) and pentastomid prevalence was positively associated with the number of buildings at a site, though further sampling is needed to confirm this pattern. We postulate that this pattern is likely due to a host shift of this parasite from an urban-associated introduced gecko. This is the first record of this parasite infecting cane toads in their native range, and the first instance of this parasite occurring in Central America.

Key words: Amphibian, Asian House gecko, *Bufo marinus*, *Hemidactylus frenatus*, Invasive species, lung parasite, Panama Canal, urban–rural gradient.

INTRODUCTION

Biological invasions are increasing with the expansion of global travel and trade causing billions of dollars in economic damage annually (Meyerson and Mooney, 2007). Invasive species are also a major threat to biodiversity (Sandlund *et al*. 2001) and introduced parasites are a serious concern in this respect (Daszak *et al*. 2000; Sandlund *et al*. 2001). Introduced parasites can 'spillover' to infect native hosts which were previously naïve to the parasite (Daszak *et al*. 2000). This can be particularly severe if transmission of introduced parasites is amplified by high densities of their invasive hosts (Torchin *et al*. 2002) and parasites 'spillover' to less common native species. Major travel and trade hubs (such as ports) serve as focal points for the establishment of invaders (Hulme, 2009). For example, the red imported fire ant (*Solenopsis invicta*) was introduced to the USA through the port of Mobile Bay and has spread focally from there (Tschinkel, 2006), and the distribution of the introduced rough-tailed gecko (*Cyrtopodion scabrum*) in Texas, USA hugs commercial shipping docks (Dixon, 2013). Similarly, ports may also provide entry points for parasites and disease vectors (Tatem *et al*. 2006).

Recently, an introduced parasite (the pentastomid *Raillietiella frenata* = *frenatus*) was discovered infecting the lungs of invasive cane toads (*Rhinella marina*, previously *Bufo marinus*) in the tropical port city of Darwin, Australia (Kelehear *et al*. 2011, 2013). The prevalence of *R. frenata* declined with distance from the Port of Darwin, suggesting that the parasite was probably introduced to the port (Kelehear *et al*. 2013). Importantly, this parasite originally infected only the introduced Asian House gecko (*Hemidactylus frenatus*) in Darwin and a few other isolated towns (Barton, 2007; Kelehear *et al*. 2013). The distribution of this gecko is strongly linked to the presence of buildings (Newbery and Jones, 2007; McKay *et al*. 2009; Hoskin, 2011; Yang *et al*. 2012); therefore, its parasites were initially restricted to urban areas (Kelehear *et al*. 2013). However, *R. frenata* switched hosts to infect cane toads after toads first colonized Darwin in 2005 (Kelehear *et al*. 2013). Since cane toads are ubiquitous through the Australian tropics and occur in both rural and urban habitats (Lever, 2001), this host shift enabled the pentastomid to colonize Australia more widely than was originally possible in its urban gecko host (Kelehear *et al*. 2013).

Pentastomids are haematophagous endoparasites that infect the respiratory system of reptiles, and to a lesser extent, anurans, birds and mammals (including humans; Paré, 2008; Kelehear *et al*. 2011). They can cause pulmonary haemorrhage and potentially lethal secondary bacterial infections in their hosts (Jacobson, 2007). *Raillietiella frenata* primarily infects small insectivorous lizards (Ali *et al*. 1981) and occasionally anurans, of which the cane toad is the only known suitable definitive host (Kelehear *et al*. 2013). *Raillietiella frenata* has a complex life cycle and uses coprophagic insect intermediate hosts (such as cockroaches) and insectivorous lizard or toad definitive hosts (Ali and Riley, 1983;

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Kelehear *et al*. 2013). This parasite is widely distributed throughout its native range in Asia (Ali *et al*. 1981), has recently become established in Australia (Kelehear *et al*. 2011; 2013), and also occurs in the USA (Hawaii and Texas) and Brazil (Pence and Selcer, 1988; Goldberg and Bursey, 2000; Anjos *et al*. 2008). It is likely spreading to new ranges via either a cockroach intermediate host, or a gecko definitive host, both of which are common invaders (Kraus, 2009; Rabitsch, 2010).

We discovered lung pentastomids of the genus *Raillietiella* in toads in the Republic of Panama near the Panama Canal, a hub for international shipping. *Raillietiella* is the largest pentastomid genus and contains ~43 species (Poore, 2012), some of which may have been described as new species in error as the morphological characteristics used for species identification change ontogenetically (Kelehear *et al*. 2011). Therefore, morphology alone is unreliable for distinguishing species of this genus and molecular confirmation is required. Herein we (i) document the presence of pentastomids infecting cane toads in Panama; (ii) confirm their identity using molecular techniques; (iii) begin to examine whether the distribution of this parasite follows an urban–rural gradient and consider potential pathways by which it was introduced to Panama.

MATERIALS AND METHODS

We collected 64 cane toads from eight sites across three provinces in central Panama (Fig. 1) over the period 22 August 2013 – 14 September 2013 (Table 1). We dissected toads, examined their lungs and preserved parasites in 70% ethanol.

We prepared two pentastomid DNA extractions with a QIAamp DNA Micro Kit (Qiagen), using 100 μL of Buffer AE in the final elution. One extraction included a single individual and the other included three pentastomids. We initially tried to amplify the COX1 region using the primer pair LCO1490–HCO2198 (Folmer *et al*. 1994) but were only able to obtain cane toad COX1 with these primers. Our subsequent attempts using the HCO2198 reverse primer paired with the forward primer crustF2 (Costa *et al*. 2007) were successful at obtaining pentastomid sequence. Each polymerase chain reaction had a total volume of 25 μL and contained 2·5 μL of 10 × PCR buffer, 2 mM of $MgCl_2$, 50 μmol each dNTP, 0·2 μmol of each primer, 2% DMSO, 1·25 U AmpliTaq Gold polymerase and 2 μL of DNA extract. We used a PCR thermal regime of 94 °C for 12 min; 5 cycles of 94 °C for 45 s, 50 °C for 45 s and 72 °C for 60 s; 35 cycles of 94 °C for 45 s, 55 °C for 45 s and 72 °C for 1 min; followed by 72 °C for 7 min. We gel-purified PCR products using GELase enzyme (Epicentre) and sequenced them in both directions on an ABI 3100 × 1 automated sequencer. We edited and aligned sequences using Sequencher 5.1 (GeneCodes Corp.) and identified the species using BLAST (NCBI).

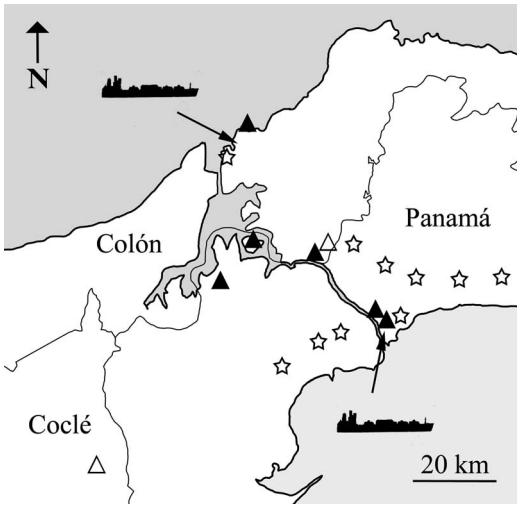

Fig. 1. Map of central Panama showing collection sites (triangles: closed triangles indicate presence of *R. frenata*; open triangles indicate absence of *R. frenata*), major cities with populations >35 000 (stars), and the two shipping ports (ships with arrows) at either end of the Panama Canal.

To examine whether parasite prevalence ([# of toads infected/# of toads examined] × 100) was associated with urban development, we counted the number of buildings within a 650 m radius of each site using aerial images from Google Earth (version 7.1.2.2041; images taken: 07 December 2012 – 09 July 2013; see Kelehear *et al*. (2013) for full methods). We performed a weighted (by sample size per site to account for uneven sampling) one-way ANOVA with number of buildings within a 650 m radius of the site as the independent variable, and pentastomid prevalence (% infected) as the dependent variable. All analyses were performed in JMP Pro 9.0.

RESULTS

All pentastomids matched the appearance of *R. frenata* morphologically (Ali *et al*. 1985; Kelehear *et al*. 2011). This identification was confirmed by our molecular analysis: we obtained a single 657 bp sequence from both DNA extractions that was identical to a *R. frenata* specimen (GenBank Accession No. JF975594) sequenced from cane toad hosts in Australia (Kelehear *et al*. 2011).

Eight (4 male, 4 female) of the 64 dissected toads (25 male, 39 female) were infected with *R. frenata* (Table 1). Mean infection intensity (# parasites per infected toad) was 15·13 ± 9·8 S.E. (median = 2·5, range = 1–80; Table 1). Pentastomid prevalence was positively associated with the number of

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Table 1. Collection site details (locations and the number of buildings within a 650 m radius) and the prevalence and intensity of pentastomids (*R. frenata*) infecting cane toads (*R. marina*) at each site surveyed in Panama

Collection site	Latitude, longitude	# buildings	# toads collected	Pentastome prevalence (%)	Mean ± 1 S.E. pentastome intensity	Maximum pentastome intensity
Albrook	8°58′47″N, 79°33′40″W	267	1	100	28 ± 0	28
Clayton	8°59′59″N, 79°34′47″W	165	2	50	3 ± 0	3
Gamboa	9°7′7″N, 79° 42′8″W	148	5	40	1·5 ± 0·5	2
Lagarterita	9°4′46″N, 79° 54′48″W	58	4	50	42 ± 38	80
San Antonio	9°8′3″N, 79° 41′38″W	22	2	0	–	–
Barro Colorado Island	9°9′48″N, 79° 50′16″W	13	19	5·3	1 ± 0	1
Galeta	9°24′12″N, 79°51′46″W	8	7	14·3	2 ± 0	2
Chiguirí Arriba	8°40′28″N, 80°11′16″W	1	24	0	–	–

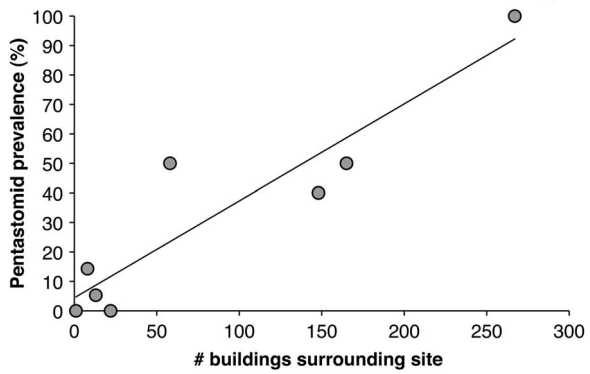

Fig. 2. Relationship between the level of urban development at a collection site (# buildings within a 650 m radius of each site) and the prevalence of an introduced pentastomid parasite (*R. frenata*) infecting native cane toads (*R. marina*) in Panama.

buildings surrounding a site ($R^2 = 0{\cdot}80$, $F_{1,6} = 23{\cdot}71$, $P = 0{\cdot}0028$; Fig. 2). At one of our sites (Albrook), only one toad was sampled so we repeated the analysis excluding this site and the association remained significant ($R^2 = 0{\cdot}72$, $F_{1,5} = 12{\cdot}74$, $P = 0{\cdot}016$).

DISCUSSION

We discovered non-native pentastomids infecting cane toads in their native range in Panama. Overall, eight of 64 toads were infected with *R. frenata* and infection intensities ranged from 1 to 80 pentastomids. These parasites are genetically identical to *R. frenata* infecting invasive cane toads and Asian House geckos in Australia (Kelehear *et al*. 2011). This is the first report of *R. frenata* occurring in Central America and the first instance of it infecting cane toads in their native range.

The two most likely pathways by which this parasite could have been introduced to Panama are: (1) spread from adjacent infected areas, or (2) introduction to Panama via cargo (likely from Asia). *Raillietiella frenata* has been recorded in introduced *Hemidactylus turcicus* in Texas, USA (Pence and Selcer, 1988) and *Hemidactylus mabouia* in Brazil (Anjos *et al*. 2007, 2008; Almeida *et al*. 2008), however neither of these gecko species are established in Panama (R. Ibáñez, personal communication). If the parasite spread into Panama from either Texas or Brazil, we would expect infected toads across their entire native range (southern Texas to Brazil; Lever, 2001). Numerous surveys of the cane toad's parasite fauna throughout its native range have never reported *R. frenata* (Espinoza-Jiménez *et al*. 2007; Espinola-Novelo and Guillen-Hernandez, 2008; C. Bursey and J. Santos, personal communication 2013), suggesting either a recent introduction to Panama and/or a recent host switch to cane toads. The Panama Canal is a hub for global shipping, receiving over 12 500 ships annually, including many from Asia (Ruiz *et al*. 2009; Kaluza *et al*. 2010). It is possible that this parasite was introduced through the ports in Panama, similar to the introduction of *R. frenata* to the port city of Darwin in Australia (Kelehear *et al*. 2013). Two non-native geckos (*Lepidodactylus lugubris* and *H. frenatus*) that are established in Panama (Savage, 2002; Kraus, 2009) host *R. frenata* in ranges outside of the Americas (see Kelehear *et al*. 2013) and may have brought this parasite with them when they were introduced.

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In our surveys, *R. frenata* occurred at all sites in the vicinity of the Panama Canal but was not found outside the Panama Canal Watershed, suggesting that the Panama Canal may have been the point of introduction. Further, we found that the prevalence of *R. frenata* was positively associated with the number of houses in the proximity of our collection sites. This result suggests that urban-associated geckos may be shedding pentastomid eggs that are being transferred to cane toads (via an unknown insect intermediate host). However, since our sampling was uneven with low sample sizes at some sites (due to scarcity of toads) further research is needed to substantiate this pattern of urban association, which has also been observed in cane toad infections in Australia (Kelehear *et al*. 2013).

While some pentastomids can kill or debilitate their hosts (Jacobson, 2007) and some species cause zoonotic disease in humans (Riley, 1986; Drabick, 1987), little is known about the pathological effects of raillietiellids on their hosts. Kelehear *et al*. (2012) found no relationship between body condition of invasive cane toads and infection with *R. frenata* in Australia, implying negligible effects; however, the impact of this introduced parasite in Panama is yet to be determined. Considering that cane toads are competent definitive hosts of *R. frenata*, are infected at a modest prevalence with intensities as high as 80 parasites, and have a broad distribution over diverse habitats extending from Texas, USA to Brazil, they have the potential to spread this parasite more effectively than the presumed original gecko host (Kelehear *et al*. 2013). Since *R. frenata* infects at least six families of anurans and lizards globally (Kelehear *et al*. 2013), this parasite may spill-over from cane toads to infect additional native hosts such as other anurans and small carnivorous lizards throughout the Neotropics. Comparing the spread of this parasite within the cane toad's introduced range in Australia and its native range in Panama offers a unique natural experiment which may offer insight to disease transmission in native *vs* introduced host–parasite systems.

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