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HOST-PARASITE RELATIONSHIPS DURING A BIOLOGIC INVASION: 75 YEARS POSTINVASION, CANE TOADS AND SYMPATRIC AUSTRALIAN FROGS RETAIN SEPARATE LUNGWORM FAUNAS

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ABSTRACT: Invasive species may carry with them parasites from their native range, differing from parasite taxa found in the invaded range. Host switching by parasites (either from the invader to native fauna or from native fauna to the invader) may have important consequences for the viability of either type of host (e.g., their survivorship, fecundity, dispersal ability, or geographic distribution). *Rhabdias pseudosphaerocephala* (Nematoda) is a common parasite of cane toads (*Rhinella marina*) in the toad's native range (South and Central America) and also in its introduced Australian range. This lungworm can depress host viability and is capable of infecting Australian frogs in laboratory trials. Despite syntopy between toads and frogs for up to 75 yr, our analyses, based on DNA sequence data of lungworms from 80 frogs and 56 toads, collected from 2008 to 2011, did not reveal any cases of host switching in nature: toads and native frogs retain entirely different lungworm faunas. All lungworms in cane toads were the South and Central American species *Rhabdias pseudosphaerocephala*, whereas Australian frogs contained at least four taxa (mostly undescribed and currently lumped under the name *Rhabdias* cf. *hylae*). General patterns of prevalence and intensity, based on the dissection of 1,315 frogs collected between 1989 and 2011 across the toads' Australian range, show that these Australian endemic *Rhabdias* spp. are widely distributed geographically and across host taxa but are more common in some frog species (especially, large-bodied species) than they are in others.

Key words: Australian frogs, biologic invasion, *Bufo marinus*, dynamics, host-parasite, nematode, patterns of occurrence.

INTRODUCTION

Biologic invasions can influence the native biota through many ways. One of the most important mechanisms of effect may be host switching of parasites and pathogens between invasive and native species (Gozlan et al., 2005). Although introduced species often leave many of their native-range pathogens and parasites behind (Torchin et al., 2003), it is nonetheless common for an introduced species to carry new pathogens to the invaded area (Lafferty and Kuris, 2005). In some cases, transmission of those pathogens to the native biota has had devastating consequences (Daszak et al., 2000). Thus, it is important to examine the parasites of invasive species and to evaluate the likelihood and risks associated with transfer of those pathogens to the native biota (Prenter et al., 2004). The reciprocal phenomenon—transmission of parasites from the invaded range to the introduced species—is also of interest and may clarify the reasons for spatial and temporal variation in invasion success (Dunn, 2009).

The cane toad (*Rhinella marina*; formerly *Bufo marinus*) is native to South and Central America and has been introduced to more than 40 countries in misguided attempts to control insect pests (Lever, 2001). The toad has spread widely throughout tropical Australia, negatively affecting native fauna (mostly by fatal poisoning of predators that attempt to eat it [Shine, 2010] because Australian animals have not evolved with any bufonids). This invasion also may have disrupted existing host-parasite dynamics. For example, the toad may have caused a decline in the abundance of a proteocephalid tapeworm, by acting as a sink for intermediate life-history stages (Freeland, 1994). By providing additional, easily

infected hosts, toads may also have increased abundances of anuran myxosporidian parasites (Hartigan et al., 2011) and spread a previously localized pentastome more widely (Kelehear, pers. obs.). In Australia, cane toads also carry nematode lungworms that can reduce survival, growth rates, and locomotor performance of juvenile toads (Kelehear et al., 2009; Pizzatto and Shine, 2011a, c) and the growth rates of adult toads (Kelehear et al., 2011). Thus, these parasites might imperil native anurans if the lungworms are transferred to native frogs. The toad lungworm can indeed infect native frogs under laboratory conditions, and at least one native species (*Litoria splendida*) experiences high mortality rates if infected (Pizzatto and Shine, 2011b). However, most native frogs do not develop mature infections, probably because their immune systems are able to destroy the larval parasites (Pizzatto et al., 2010).

We investigated patterns of native lungworm occurrence in Australian frogs and compared the genetic identity of the parasites infecting frogs vs. toads. Our aims were to investigate 1) whether host switching of lungworms has occurred between cane toads and native frogs (as indicated by overlap in the parasite taxa found in the two host types), and 2) how host traits (e.g., body size, sex, species, geographic distributions, phylogenetic relationships) affect the patterns of occurrence of lungworms in Australian frogs.

MATERIALS AND METHODS

Lungworms of the genus *Rhabdias* have a direct life cycle: the infective larvae (L3) enter the host via cutaneous penetration and migrate through the body to reach the lungs (Baker, 1979), where they feed on blood and develop, over 5–28 days, into protandrous, hermaphroditic adults (Kelehear et al., 2012). Eggs laid in the lungs pass up the trachea and into the intestine, where they hatch into first-stage larvae (Baker, 1979). Larvae exit the toad with feces (on which they feed) and molt to become free-living, adult males and females that mate in the soil over the ensuing 24–48 hr. The L3 larvae develop inside the fertilized, free-living, adult female and consume her organs, before breaking her cuticle and entering the soil (4–10 days postdefecation) to search for a toad to infect.

We dissected cane toads and native frog species from throughout Australian areas invaded (Queensland [Qld] and Northern Territory [NT]), or soon to be invaded (Western Australia [WA]), by cane toads, to sample their lung nematodes. We unambiguously identified the parasites by DNA analyses of fresh lungworms collected from live toads and some live frogs, but mostly from road-killed frogs collected during the wet season. The timing of our samples was constrained by seasonality because frogs are inactive (and thus, largely inaccessible) during the dry season in the wet-dry Australian tropics. Live anurans were euthanized by pithing (when processed in the field and in the laboratory before 2010) or an overdose injection of sodium pentobarbital (50% lethabarb diluted in saline, 1 mL/kg) and then dissected. All procedures were approved by the University of Sydney Animal Ethics Committee (approvals L04/4-2008/2/4788 and L04/5-2010/2/5334).

Queensland frog and toad samples that provided DNA for parasite identification were gathered in 2008, and the NT and WA samples were gathered more recently (2008–2011). Our DNA samples included those used by Dubey and Shine (2008), which comprised lungworms from anurans collected in Qld and the NT ($n=34$ toads and $n=37$ frogs), as well as specimens from the cane toad *Rhinella marina* ($n=7$) and a related toad species *Rhinella schneideri* ($n=3$) from the toads' native range (in Brazil). In addition to this previous sample, we collected and analyzed DNA from both toads and frogs from the NT (one location), as well as the toads' recently colonized range in WA (one location). We also analyzed lungworms from an isolated (stowaway-founded) population of cane toads in Sydney, New South Wales, Australia. In total, we examined 12 new samples from toads and 33 from frogs (including three species not previously sampled), to provide a broader geographic and taxonomic sample than was available for the Dubey and Shine (2008) analysis.

To describe patterns of prevalence and intensity of lungworms in native frogs, we also included data from 1) an earlier survey in Qld (1989–1992; Barton, 1999), and 2) preserved specimens collected in WA from 1996 to 2007 and held in the Western Australian Museum. This latter sample was collected before cane

toads arrived in WA. Preservation methods did not allow us to conduct genetic analyses on the nematodes collected from either of those surveys. For each dissected frog, we counted the number of mature *Rhabdias* in each lung (intensity) and recorded host sex and snout-urostyle length (SUL), when possible (some road-killed frogs were too damaged to be measured). Our sampling methods were opportunistic and variable, mostly conducted during the wet season; and our reliance on road-killed samples and small sample sizes for some species may have biased patterns of occurrence (Wilson et al., 2002).

DNA extraction and amplification

We isolated total cellular DNA from intact *Rhabdias*. Then, tissues were placed in 200 μL of 5% Chelex (BioRad, Hercules, California, USA) containing 0.2 mg/mL of proteinase K (Fermentas, Glen Burnie, Maryland, USA), incubated overnight at 56 C, boiled at 100 C for 10 min, and finally centrifuged at 13,200 × G for 10 min. Lastly, the supernatant containing purified DNA was collected and stored at −20 C. Double-stranded DNA amplifications of *cyt-b* were performed with the primer pairs Rh_cytbf/Rh_cytbr (Dubey and Shine, 2008). Amplification conditions included a hot-start denaturation of 95 C for 3 min, followed by 35 cycles of 95 C for 45 sec, 50 C annealing temperature for 45 sec, 72 C for 90 sec, and a final extension of 72 C for 7 min (Dubey and Shine, 2008).

The sequences were aligned by eye, using the software BioEdit 7.1.3. Tests were conducted on the total fragment (553 base pairs); all codon positions were used. The tree was rooted using a sequence of *Heligmosomoides polygyrus* (Order, Rhabditida; family, Heligmosomatidae; AJ608862). For maximum likelihood (ML) analyses, we used jModelTest 0.1.1 (Guindon and Gascuel, 2003; Posada, 2008) to select the model of DNA substitution. The HKY+I+G model best fitted the data set using a Bayesian information criterion (BIC; Schwarz, 1978). The ML heuristic searches and bootstrap analyses (1,000 replicates) were performed using phyml (Guindon and Gascuel, 2003).

Analyses

We assessed normality and variance homogeneity before conducting statistical analyses. Data on the intensity of parasitism (number of parasites per infected host; Margolis et al., 1982) and abundance per frog (including numbers in uninfected as well as infected individuals; Margolis et al., 1982) were highly skewed ($k=0.25$, SE$=0.02$) because most individuals were uninfected, across all species. Thus, we used generalized linear models (glm) with a negative binomial distribution to analyze patterns involving numbers of parasites (e.g., Wilson and Grenfell, 1997). These analyses were run in the software program R (R Foundation for Statistical Computing, Wien, Austria), using the package pscl. The main effects for the glm were estimated by Wald tests using the package aod. We used logistic regressions to examine predictors (sex, SUL, species, and geographic location) of infection status (infected vs. uninfected), and paired *t*-tests to compare parasite intensity in left vs. right lungs. We used analysis of variance to compare differences in the mean intensity and prevalence per species between habitat-use categories and family categories, and linear regressions to analyze relationships between prevalence and mean intensity, mean SUL and mean intensity, and mean SUL and prevalence, per species. Data on SUL, mean intensity, and mean prevalence were log-transformed for all analyses.

RESULTS

Genetic identification of the parasites

The 136 samples of lungworms showed 35 different haplotypes of 553 base pairs (GenBank accessions JQ316473–JQ316482 and EU836833–EU836862, from this study and Dubey and Shine, 2008, respectively; Fig. 1). Phylogenetic analysis of sequence data revealed only a single lungworm taxon in Australian populations of cane toads (Fig. 1). The parasite is *Rhabdias pseudosphaerocephala*, virtually identical, at the loci we studied, to the parasite found in the native range (Brazilian) of cane toads (Fig. 1). The other Brazilian toad species, *Rhinella schneideri*, was also infected with *Rhabdias pseudosphaerocephala* and another closely related congeneric taxon (Fig. 1).

Australian frogs contained lungworm parasites from four main lineages (Fig. 1). One lineage was found only in two Qld frog specimens (both *Cyclorana alboguttata*), and a second lineage was found in two *Litoria fallax* and two *Opisthodon ornatus* from Qld. The remaining two lineages were represented by

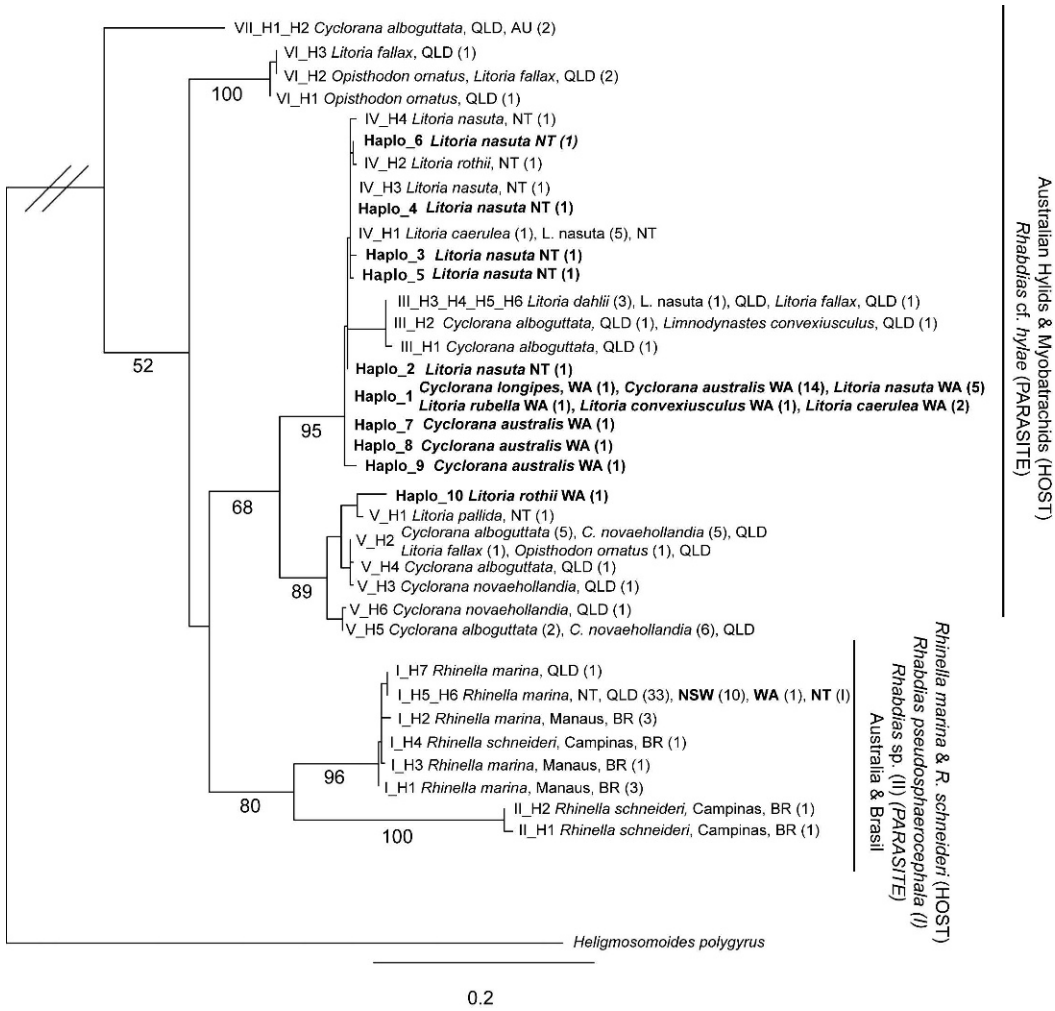

FIGURE 1. Phylogenetic relationships of nematode lungworms (*Rhabdias* spp.) from toads in Brazil (native range) and Australia (invaded range), and from Australian native frogs, using a maximum likelihood procedure and based on cytochrome b sequence data; bootstrap values are shown at tree nodes. Samples not included in Dubey and Shine (2008) are shown in bold, and sample sizes appear in parentheses after each haplotype.

larger numbers of samples and were widely distributed both taxonomically (in both hylids and myobatrachids) and geographically (from Qld across to WA). More-extensive sampling might reveal wider taxonomic and geographic distributions in the other two (small) lineages as well.

Patterns of parasite occurrence

We found *Rhabdias* spp. in the lungs of 298 of the 1,315 native frogs (22.7%) we dissected, belonging to 17 of the 19 species analyzed. Because we cannot distinguish the native *Rhabdias* species by morphology alone, we combine the taxa for analysis of general patterns of occurrence. There was no consistent trend for greater parasite intensity in the right vs. left lung of a host (paired $t=0.01$, df=1,182, $P=0.76$), so we looked only at total numbers of worms and at whether or not an individual host was infected.

Treating each frog as a separate sampling unit (i.e., disregarding its species), the probability of a frog containing lungworms was

TABLE 1. Broad habitat use, mean snout-urostyle length (SUL) of specimens examined, sample sizes (n total=1,315), prevalence, and mean intensity of lungworms (*Rhabdias* spp.) in Australian frogs.

Species	Habitat	Mean SUL (mm)	n total	Prevalence	Mean intensity
Cyclorana alboguttata	Terrestrial	62.4	20	55.0	2.7
Cyclorana australis	Terrestrial	67.1	151	28.5	4.1
Cyclorana longipes	Terrestrial	38.3	51	3.9	1.5
Cyclorana novaehollandiae	Terrestrial	71.3	24	37.5	11.4
Cyclorana vagitus	Terrestrial	46.0	12	8.3	1.0
Limnodynastes convexiusculus	Terrestrial	37.3	49	6.1	2.7
Limnodynastes tasmaniensis	Terrestrial	26.8	5	0	
Litoria caerulea	Arboreal	63.2	105	8.5	3.2
Litoria coplandi	Rock-dwelling	31.5	30	6.7	1.0
Litoria dahlii	Aquatic	44.2	107	5.6	2.3
Litoria fallax	Arboreal	24.8	8	37.5	1.7
Litoria inermis	Terrestrial	28.8	197	53.8	2.3
Litoria nasuta	Terrestrial	36.1	150	33.3	2.6
Litoria pallida	Terrestrial	26.4	32	15.6	2.2
Litoria rothii	Arboreal	42.5	92	4.3	3.0
Litoria rubella	Arboreal	30.4	70	5.7	1.0
Litoria serrata/genimaculata	Arboreal	42.2	54	0	
Litoria wotjulumensis	Terrestrial	40.5	31	46.2	4.4
Opisthodon ornatus	Terrestrial	30.3	127	18.9	3.1

dependent on sex and body size. Females were more likely to be infected than were males (91 of 251 females infected [36%] vs. 87 of 308 males [28%]; $\chi^2=4.07$, df=1, $P=0.04$) but, if infected, had similar intensities of parasitism ($\chi^2=0.74$, df=1, $P=0.39$). Larger frogs were also more frequently infected ($\chi^2=6.19$, df=1, $P=0.013$), and larger, infected hosts had more worms ($\chi^2=23.8$, df=1, $P<0.001$). If we include uninfected, as well as infected, hosts to analyze parasite abundance, larger frogs tended to have a greater abundance of parasites ($\chi^2=3.7$, df=1, $P=0.053$). However, none of these sex or body size effects were significant within any given species, so are likely associated with species differences in parasite prevalence as well as other attributes. Thus, the following analyses were applied separately within each species.

The prevalence of lungworms differed significantly among the 19 anuran species: from zero in *Litoria serrata/genimaculata* (we were unable to reliably distinguish between these two closely related taxa) to >45% of specimens in *Litoria inermis*, *Litoria wotjulumensis*, and *Cyclorana alboguttata*: see Table 1 (logistic regression of presence/absence of worms: $\chi^2=262.62$, df=17, $P<0.001$). Overall, frogs from Qld were more likely to be infected (163 of 449 infected, 36%) than were those from WA (100 of 578, 17%) or the NT (31 of 281, 11%; $\chi^2=77.14$, df=2, $P<0.001$). The likelihood of widely distributed species (with distributions spanning the three states: *Cyclorana australis*, *Limnodynastes convexiusculus*, *Litoria caerulea*, and *Litoria rothii*) being infected also tended to differ among states ($\chi^2=11.97$, df=6, $P=0.06$). For example, 31% of *Litoria caerulea* in Qld were infected (16 frogs analyzed), compared with 4% of WA conspecifics (70 frogs analyzed), and 5% infected (19 frogs analyzed) in the NT. However, prevalence and intensity were dependent on sample size and may also vary through time. Our samples from different states and species were collected at different times and differ in number, possibly increasing error.

Anuran species differed in infection intensity as well as prevalence (Table 1).

Mean intensity per host species ranged from 1.0 (in *Cyclorana vagitus*, *Litoria coplandi*, and *Litoria rubella*) to 11.4 (in *Cyclorana novaehollandiae*; $\chi^2=65.8$, df=14, $P<0.001$). Species with a high prevalence also had a high mean intensity of parasitism ($r=0.49$, $n=17$, $P=0.048$). Species with larger mean body sizes (in our samples) also had higher mean intensities ($r=0.57$, $n=17$, $P=0.02$), but did not have higher prevalence ($r=0.10$, $n=17$, $P=0.71$). Prevalence did not differ between terrestrial (22.6±5.4%) and arboreal taxa (including rock-dwellers: 20.7±8.5%; $F_{1,16}=0.02$, $P=0.89$). Mean intensity also did not differ between these two habitat-defined groups (terrestrial=3.2±0.8 worms, arboreal=2.3±0.7 worms; $F_{1,16}=1.21$, $P=0.289$). Prevalence was slightly higher in frogs of the family Hylidae than it was in those of the family Myobatrachidae (23.3±4.9% vs. 12.5±6.4%), but that difference was not statistically significant ($F_{1,16}=0.25$, $P=0.63$). Mean intensity of infection also did not differ between the families (Hylidae, 3.0±0.7; Myobatrachidae, 2.9±0.2; $F_{1,16}=0.18$, $P=0.68$).

DISCUSSION

Patterns of occurrence of lungworms in Australian frogs

Broadly, intensity of lungworm infection tended to be greater in large-bodied anurans than in smaller taxa, independent of habitat use (Table 1). The green tree frog (*Litoria caerulea*) had the fourth highest intensity of lungworm parasitism and is the third-largest species. This taxon is common around human habitations (the same sites that attract toads: Zug and Zug, 1979) and is often active on the ground and in low branches (Pizzatto, pers. obs.). It also has high prevalence of trophically transmitted spirurid nematodes encysted in the stomach (Kelehear and Jones, 2010), suggesting that toads and green tree frogs forage on similar prey and, therefore, overlap in microhabitat use.

The intensity of lungworm infections increased with host body size, possibly because rates of exposure to infective stages vary with host size (affected by the history of exposure [age], host surface area, and host behavior), or larger frogs may have invested more in growth and less in immune defense (Wilson et al., 2002).

In our study, parasite prevalence was greater in female frogs than in conspecific males, but parasite infection intensity was not related to host sex (even after correction for body size). The role of host sex in the dynamics of *Rhabdias* lungworms in frogs seems variable among species and is difficult to interpret. In experimental studies, patterns of intensity, prevalence, abundance, and size of *Rhabdias ranae* differed between males and females of *Lithobates sylvatica* (previously *Rana sylvaticus*) and *Lithobates pipiens* (previously *Rana pipiens*) but in a complex way that depended on differential rates of development through metamorphosis, breeding status, and age (Dare and Forbes, 2008, 2009; Dare et al., 2008).

Host switching of lungworms between cane toads and native frogs

Despite decades of sympatry, the lungworm faunas of cane toads are entirely separate taxonomically from those of Australian frogs (Fig. 1); we found no evidence of host switching of lungworms between frogs and toads.

Lungworms occur in native frogs in all geographic areas that we sampled in Australia (albeit, with spatial variation in prevalence and intensity). Our genetic analyses revealed at least four lineages distinctive enough to warrant species-level recognition, but allocating names to these taxa is problematic. Currently, there are only two recognized species of *Rhabdias* known to occur in Australian frogs: *Rhabdias australensis* (described from *Rana daemeli*) and *Rhabdias hylae* from several frog species (Barton, 1994; Kuzmin, 2012). Lungworms of the frog species used in this study were previously

identified as *Rhabdias* cf. *hylae* (Dubey and Shine, 2008), already suspected to be a species complex (Barton, 1998). The task of linking the genetically distinctive taxa in Figure 1 to existing names is further complicated by the apparent lack of host-specificity and previous misidentification of cryptic species (at least within the *Rhabdias hylae* complex). Thus, knowing which anuran was the host of some previously described nematode taxon does not help to link it to the taxa revealed by our genetic analyses. Clarifying the taxonomy of Australian *Rhabdias* will require a more-extensive sampling of both hosts and parasites and detailed morphologic analyses combined with genetic analyses.

Our extensive sampling across the toads' Australian range revealed that only one lungworm species infects the cane toad (the American taxon *R. pseudosphaerocephala*), supporting the findings of Dubey and Shine (2008). These lungworms are widely distributed in Australian populations of the cane toad (Phillips et al., 2010). The South and Central American *Rhabdias pseudosphaerocephala* were probably brought to Australia with the original toads, which were collected from a translocated Hawaiian population in 1935 (Lever, 2001). These toads were kept in a large communal outdoor enclosure, where adults were mixed with their progeny (Mungomery, 1936; Easteal, 1981), creating ideal conditions for metamorphs to become infected. Also, some of the original adult toads (as well as thousands of metamorphs) may have been released or escaped (Mungomery, 1936). Apparently inconsistent with this scenario, lungworms are not reported in Hawaiian cane toad populations (Marr et al., 2010). Either the lungworms have disappeared from Hawaii within the past several decades or were missed in the surveys (only 48 toads were sampled from three separate locations in a single field trip: Barton and Riley, 2004). It is difficult to imagine any alternative route these nematodes could have taken to Australia because they are only otherwise recorded from *Rhinella marina* (Kuzmin et al., 2007) and *R. schneideri* (Dubey and Shine, 2008), both from South and Central America.

The host range of a parasite is determined by two factors: the encounter filter and the compatibility filter, and for a parasite to occur in a specific host (or for the occurrence of host switching), both filters must be open (Combes, 2001). Throughout their invasion history, cane toads have acquired several parasites from Australian frogs. For example, myxosporidium parasites probably spread to toads, increasing parasite abundance through a spillback mechanism (Hartigan et al., 2011). Although certainty of identification is reduced by the lack of genetic studies (e.g., Criscione et al., 2005), cane toads apparently also acquired several other protozoans of Australian frogs (as well as carrying a range of American taxa: Delvinquier and Freeland, 1988) and a proteocephalid tapeworm of the python *Antaresia maculosa* (Freeland, 1994). Because cane toads are not eaten by most snake predators, the tapeworm uptake prevents the parasite from completing its complex life cycle, probably altering host-parasite dynamics (Freeland, 2005).

Whether or not cane toads are suitable hosts for Australian *Rhabdias* is uncertain, but the prevalence and intensity of infection by native lungworms is much lower in native frogs (even when pooling all native lungworm species together: Table 1), than it is for *R. pseudosphaerocephala* in well-established populations of *Rhinella marina* in eastern Australia (82.2% prevalence and 16.1 mean intensity, $n=580$ toads from one population: Barton, 1998) or northern Australia (Pizzatto and Kelehear, pers. obs.). Thus, the low abundance of native lungworms would partially close the encounter filter, reducing the likelihood of host switching from frogs to toads.

The opposite scenario (transfer of parasites from toads to frogs) seems more feasible, and there is some evidence that,

in this case, the encounter filter may be open: 1) cane toads have a broad geographic distribution in Australia, and their frequent proximity to native frogs (around water bodies and in shelter sites) suggests substantial opportunities for transmission; and 2) laboratory studies confirm that the toads' lungworms are capable of infecting native frogs (Pizzatto et al., 2010; Pizzatto and Shine, 2011a, b). However, behavior is an important parameter that can open and close the encounter filter, and, at present, we know very little about the behavior of these anurans and their parasites under natural conditions. A lack of host switching can also reflect an inability for infective larvae to survive and reproduce inside the "wrong" host (closed compatibility filter). In support of this interpretation, in the laboratory, *R. pseudosphaerocephala* rarely established in the lungs of Australian frogs; instead, larvae migrated to inappropriate parts of the frog's body or were attacked by the host's immune system (Pizzatto et al., 2010). Presumably, similar problems could arise if native lungworms attempt to infect cane toads. Australia lacks native bufonids, so the phylogenetic distance between toads and Australian frogs may have created much greater physiologic barriers to host switching than would occur if invader and natives were closely related (Poulin, 2007).

Dubey and Shine (2008) noted that a lack of records of toad nematodes (*Rhabdias pseudosphaerocephala*) in native frogs was not conclusive evidence of a lack of host switching; the same pattern could occur if infected frogs all died quickly, so infections would not be detected in surveys. Fortunately, recent laboratory studies show that native frogs infected by *Rhabdias pseudosphaerocephala* generally show few ill effects (Pizzatto and Shine, 2011a). Nonetheless, the rapid mortality of one species (*Litoria splendida*) means that generalizations may prove difficult (Pizzatto and Shine, 2011b). *Rhabdias* spp. are often mentioned as an important cause of morbidity and mortality in captive frogs, but very little is known about their host-parasite dynamics and pathogenicity in wild populations. Experiments using *Rhabdias elegans* in the hosts *Eleutherodactylus coqui* suggested very mild pathogenicity (a reduction in burst performance, but no overt effects on survivorship, growth, or endurance) under optimal laboratory conditions (Marr et al., 2010). However, stressful conditions may compromise the immunocompetence of animals and are likely to affect parasite life histories and pathogenicity, altering host-parasite interactions (Christin et al., 2003). For example, dose-dependent exposure to agricultural pesticides compromised the immune system of *Lithobates pipiens*, resulting in higher migration and establishment and earlier maturation (without loss of fecundity) of their lungworm *Rhabdias ranae* (Gendron et al., 2003), generating higher parasite prevalence and abundance (Christin et al., 2003; Gendron et al., 2003).

The transmission of novel pathogens to previously unexposed, native fauna can be devastating, as shown by the effects of European colonization on many native people (O'Fallon and Fehren-Schmitz, 2011), trichomonosis in British finches (Passeriformes) (Robinson et al., 2010), and distemper in African wild dogs (*Lycaon pictus*) (Woodroffe, 2006). Cane toads may well have disrupted native host-parasite systems in some cases (e.g., snake tapeworms: Freeland, 1994) and, in other cases, increased the abundance of native parasites in native hosts (e.g., myxosporidium: Hartigan et al., 2011). However, toad invasion did not cause the replacement of native lungworms or the acquisition of the exotic nematode by native anurans. This lack of host switching suggests that we might be able to use the negative effects of *Rhabdias pseudosphaerocephala* (Kelehear et al., 2011) as a component of cane toad control. However, even if practical ways to increase nematode abundance could be developed,

caution is needed in any such attempt. The duration of sympatry between toads and native frogs in Australia is still less than a century, and host switching may occur over a longer period. Also, even closely related species of native frogs may differ greatly in their susceptibility to infection (Pizzatto and Shine, 2011b; see above), so that at least some native anurans in yet-to-be-colonized areas may prove vulnerable to this aspect of cane toad invasion.

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