



Acquired and introduced macroparasites of the invasive Cuban treefrog, *Osteopilus septentrionalis*



Nicole Ortega ^{a,*}, Wayne Price ^b, Todd Campbell ^b, Jason Rohr ^a

^a Department of Integrative Biology, University of South Florida, 4202 E. Fowler Ave – SCA 110, Tampa, FL 33620-5150, United States

^b Department of Biology, 401 W. Kennedy Blvd., University of Tampa, Tampa, FL 33606, United States

ARTICLE INFO

Article history:

Received 8 July 2015

Received in revised form

2 October 2015

Accepted 7 October 2015

Keywords:

Introduced species

Cuban treefrog

Introduced parasites

Enemy release hypothesis

ABSTRACT

Because shifts in host–parasite relationships can alter host populations, attention should be given to the parasites that introduced species take with them or acquire in their introduced range. The Cuban treefrog, *Osteopilus septentrionalis*, is a successful invasive species in Florida with its parasites in the native range being well-documented, but there is a void in the literature regarding what parasites were lost or introduced in its expansion. We necropsied 330 *O. septentrionalis* from Tampa, FL and compared their macroparasites to those of *O. septentrionalis* in their native range and to the parasites of anurans native to the Tampa, FL area to determine the species *O. septentrionalis* likely introduced or acquired in Florida. At least nine parasite species (*Aplectana* sp., *Oswaldocruzia lenteixeirai*, *Cylindrotaenia americana*, *Physaloptera* sp., *Rhabdias* sp., *Centrorhynchus* sp., unidentified trematode metacercariae, unidentified larval acuariids, and unidentified pentastomids) were isolated. We found no differences in parasite communities of adult male and female frogs, which averaged 19.36 parasite individuals and 1.39 parasite species per adult frog, and had an overall prevalence of 77.52%. Acuariid larvae were likely acquired by *O. septentrionalis* in FL because they are not found in their native range. *O. lenteixeirai* was likely introduced because it is commonly reported in *O. septentrionalis*' native range but has never been reported in FL-native anurans. *Aplectana* sp. is also likely introduced because it has been reported in several anurans in Cuba but only reported once in Florida. *O. septentrionalis* tended to harbor fewer of its native parasites in the introduced range, which is consistent with the enemy release hypothesis and potentially creates an immunological advantage for this invasive host. Because native populations can be threatened by introduced parasites, there is a need to further explore the frequency and rate at which non-native hosts introduce parasites.

© 2015 Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Invasive species can be expensive pests, causing changes to local ecosystems that can interfere with ecosystem services and extirpate native organisms (Mack et al., 2000; Sakai et al., 2001). Invasive hosts can also disrupt native host–parasite relationships that are important to ecosystem productivity, stability, and biodiversity (Anderson and May, 1978). Host–parasite dynamics within the native populations can be altered by the introduction of nonindigenous parasites because native host populations might lack the defenses necessary to combat the introduced pathogens (Bar-David et al., 2006). Likewise, increases in the abundance of native

parasites can occur if populations of the introduced host are capable of being suitable hosts and/or reservoirs for parasites of native species (Hudson et al., 2006; Kelly et al., 2009). Given that invasives can alter native species diversity via their introduced parasites (Strauss et al., 2006), it is important to investigate the parasite–host interactions when areas become invaded (Peeler et al., 2011; Frankel et al., 2015; Scholz et al., 2015). The Cuban treefrog (CTF), *Osteopilus septentrionalis*, is an invasive amphibian species that is ideally suited to address any such loss or new acquisition of parasites because they are extremely abundant and easily captured.

The CTF is an invasive anuran native to Cuba, the Cayman Islands, Island de Pinos, and the Bahamas. It was introduced to Puerto Rico, various islands in the Lesser Antilles, Alabama, Georgia, Maryland, Minnesota, Hawaii, and Florida (Schwartz and Henderson, 1991; Meshaka, 2001; Johnson, 2004). The original

* Corresponding author.

E-mail address: nortegace@gmail.com (N. Ortega).

introduction of CTFs into Florida is believed to have occurred in the Keys and spread throughout Florida as a result of highway construction (Barbour, 1931; Meshaka, 2001; Krysko et al., 2011). Because *O. septentrionalis* is often larger than the Florida-native treefrogs, has a broad dietary niche (Meshaka, 1996), and can reproduce throughout the year with average clutch sizes of 2000–4000 eggs, it is displacing native treefrogs (Meshaka, 2001).

Although CTFs have inhabited Florida for nearly 100 years, we are unaware of any published surveys of its parasites in this introduced range. Thus, we quantified macroparasites in CTFs from a natural area near Tampa, Florida, USA where the CTFs have been established since 1992 (Campbell et al., 2010) and compared these parasites to those previously reported in the CTF from its native range, as well as the parasites of native anurans. We hypothesized that CTFs from Tampa harbor parasite species from their native range and that an exchange of parasites between native hosts and CTFs has resulted in CTFs acquiring new parasite species.

2. Materials and methods

2.1. Experimental details

During the summers of 2005–2008, 330 CTFs were collected from polyvinyl chloride (PVC) pipes encircling 37 wetlands spanning 4000 acres within or near the Morris Bridge Wellfield (MBWF) within the Flatwoods Wilderness Park in northeastern Hillsborough County, Florida (28°07'01.08"N 82°18'11.15"W). The MBWF is primarily second-growth pine flatwoods forest matrix with numerous borrow pits, hardwood swamps, freshwater marshes, and cypress domes (Guzy et al., 2006; Campbell et al., 2010).

Each host was placed in a plastic bag and frozen until necropsied. Host snout-vent length (SVL) and wet weight were recorded. For hosts >41 mm, sex was determined by the presence/absence of nuptial pads and evidence of mature reproductive organs. All hosts <42 mm were considered juveniles due to a lack of discernable reproductive organs. The body cavity was opened by a longitudinal incision from vent to throat and all internal body organs were examined for macroparasites. Species of parasites were counted and preserved in 70% ethanol. Identification and confirmation of parasites were provided by Charles Bursley at Pennsylvania State University's Shenango Campus and by Dr. Omar M. Amin at Parasitology Center, Inc. (PCI) in Scottsdale, Arizona. The following voucher specimens were deposited in the United States National Parasite Collection Beltsville, Maryland: *Cylindrotaenia americana* (No. 105165), *Oswaldocruzia lenteixeirai* (No. 105160, 105161), *Centronynchus* sp. (No. 105166), *Physaloptera* sp. (No. 105162), acuariid larvae (No. 105163), and trematode metacercaria (No. 105164).

2.2. Statistical analyses

Statistical analyses were conducted using R statistical software (R Development Core Team, 2014). Prevalence, mean intensity, and mean abundance were calculated in accordance with definitions provided by Bush et al. (1997). We tested for differences among males, females, and juveniles in their parasite mean abundance and mean intensity based on a negative binomial error distribution using the "glm.nb" function ("MASS" package). Differences among males, females, and juveniles in their parasite prevalence and richness were determined using binomial and Poisson error distributions, respectively, by using the "glm" function ("MASS" package). Parasite evenness was calculated using Simpson's Diversity Index, and differences in parasite evenness among males, females, and juveniles were tested using a normal distribution and the "lm" function. A Bonferroni's alpha adjustment was used to

keep the experiment-wise error rate at 0.05, which means that a p value ≤ 0.017 was considered statistically significant when we tested for differences among males, females, and juveniles.

3. Results

Three hundred thirty *O. septentrionalis* were collected and necropsied (68 males, 150 females, and 112 juveniles; mean mass [g] \pm SE: males 6.28 ± 0.32 , females 10.79 ± 0.66 , and juveniles 2.14 ± 0.08 , respectively). The overall parasite prevalence was 74.2%, and the overall mean abundance was 14.7 ± 2.5 . At least nine species of parasites were isolated from the CTFs, which included six host records (Table 1). *Aplectana* sp., metacercariae, and acuariid larvae had the highest prevalences and mean abundances; the remaining helminths were relatively rare (Table 2). *Oswaldocruzia lenteixeirai* and *Aplectana* sp. were the only species that have been reported in the CTF's native and introduced ranges (Table 3).

Host type (male, female, or juvenile) significantly affected parasite mean abundance ($X^2 = 29.44$, $P < 0.001$) and mean intensity ($X^2 = 29.55$, $P < 0.001$) (Fig. 1) but did not affect prevalence ($X^2 = 3.55$, $P = 0.170$), evenness ($F_{2,201} = 0.632$, $P = 0.532$), or richness ($X^2 = 6.96$, $P = 0.031$) (Fig. 2). Juveniles had lower mean abundance and mean intensity, but the male and female adults did not significantly differ in these two categories ($P > 0.58$; Fig. 1). On the other hand, juveniles did not significantly differ from adults, in prevalence and evenness, but they did significantly differ from adult females for parasite richness ($X^2 = 6.71$, $P = 0.01$; Fig. 2). There was no significant difference between adult males and females in parasite evenness, richness, or prevalence ($P > 0.42$, Fig. 2). Adults averaged (\pm SE) 19.36 (± 3.67) parasite individuals, 1.39 (± 0.07) parasite species, and 77.52% (± 2.83) prevalence, whereas juveniles averaged 5.71 (± 1.57) individuals, 1.05 (± 0.09) species, and 67.86% (± 4.41) prevalence, respectively.

4. Discussion

4.1. Variation in parasites among juveniles and adult males and females

Our results indicate that there was no significant difference in abundance, intensity, evenness, richness, or prevalence between adult male and female CTFs despite males having greater home ranges because of extensive mate searching (Vargas-Salinas, 2006). However, adult CTFs had many more parasites than juveniles. For many host species, infections can accumulate with age (Raffel et al., 2009, 2010, 2011). Our results suggest that CTFs acquire parasites through time such that older hosts have more parasites than younger hosts.

Table 1

Parasites found in *Osteopilus septentrionalis* from Tampa, FL and whether they were introduced, acquired, and represent new host records.

Parasite	Status in <i>O. septentrionalis</i>
Acuariid larvae (nematode)	Acquired; new host record
<i>Oswaldocruzia lenteixeirai</i> (nematode)	Introduced; previously reported
<i>Aplectana</i> sp. (nematode)	Likely introduced; previously reported
<i>Physaloptera</i> sp. (nematode)	Undetermined; new host record
<i>Rhabdias</i> sp. (nematode)	Undetermined; new host record
<i>Physaloptera</i> sp. (nematode)	Undetermined; new host record
Digenean metacercaria (trematode)	Undetermined
<i>Cylindrotaenia americana</i> (cestode)	Undetermined; new host record
Pentastomid	Undetermined; new host record

Table 2Prevalence, mean intensity (range), mean abundance, and location of parasites found in 330 *Osteopilus septentrionalis* from Tampa, Florida.

Parasite	Prevalence (# infected/# examined, %)	Mean intensity \pm SE (range)	Mean abundance \pm SE	Habitat ^a
Nematoda				
<i>Aplectana</i> sp.	50	24.82 \pm 5.2 (1–150)	11.3 \pm 2.5	a–c
<i>Oswaldocruzia lenteixeirai</i>	4	1.4 \pm 0.2 (1–14)	0.06 \pm 0.02	b
<i>Physaloptera</i> sp. larvae	1	11.3 \pm 9.4 (1–3)	0.10 \pm 0.09	a–c
<i>Rhabdias</i> sp.	1	1.0 \pm 0 (1–3)	0.01 \pm 0.005	e
Unidentified larval acuariid (cysts)	32	2.49 \pm 0.07 (1–105)	1.2 \pm 0.3	a–d, i
Trematoda				
Unidentified metacercaria	36	5.2 \pm 0.71 (1–52)	1.9 \pm 0.3	a–g
Cestoda				
<i>Cylindrotaenia americana</i>	0.3	3.0 \pm 0 (1)	0.03 \pm 0.01	b
Acanthocephala				
<i>Centrorhynchus</i> sp. (cystacanth)	7	2.1 \pm 0.47 (1–11)	0.15 \pm 0.04	a–d, f, h, j
Pentastomid				
Unidentified pentastomid	1	1.0 \pm 0 (1–4)	0.01 \pm 0.006	e

^a a = stomach, b = small intestine, c = large intestine, d = body cavity, e = lungs, f = gallbladder mesenteries, g = kidney mesenteries, h = testes mesenteries, i = liver mesenteries, j = urinary bladder.

Table 3Parasite species previously reported in *Osteopilus septentrionalis* in its native range.

Parasite	Habitat	Locality	Reference(s)
Nematoda:			
Rhabditoidea			
<i>Strongyloides</i> sp.	c	Bahamas	Goldberg et al., 1994
Diactophymatoidea			
<i>Eustrongylides</i> sp. (larvae)	d	Cuba	Walton, 1940
Oxyuroidea			
<i>Parapharyngodon bassi</i>	d	Cuba	Walton, 1940; Baruš and Moravec, 1967; Coy Otero and Ventosa, 1984
<i>Parapharyngodon osteopilli</i>	c	Cuba	Adamson, 1981
Larval oxyurids	c,d	Bahamas	Goldberg et al., 1994
Unidentified oxyurid	b	Cuba	Coy Otero and Ventosa, 1984; Goldberg et al., 1994
	d	Cuba	Coy Otero and Ventosa, 1984
Cosmocercoidea			
<i>Aplectana</i> sp.	d	Cuba	Coy Otero and Ventosa, 1984
<i>Aplectana hamatospicula</i>	d	Cuba	Coy Otero and Ventosa, 1984
Ascaridoidea			
<i>Contraecaecum</i> sp. (larvae)	b	Cuba	Coy Otero and Ventosa, 1984
<i>Porrocaecum</i> sp. (larvae)	b	Cuba	Coy Otero and Ventosa, 1984
Physalopteroidea			
<i>Abbreviata</i> sp. (larvae)	b	Cuba	Coy Otero and Ventosa, 1984
<i>Physalopteroidea bahamiensis</i>	a–d	Bahamas	Goldberg et al., 1994
<i>Physalopteroidea valdesi</i>	b	Cuba	Coy Otero and Ventosa, 1984
Filarioidea			
<i>Foleyellides brachyoptera</i>	e	Cuba	Coy Otero and Ventosa, 1984
Trichostrongyloidea			
<i>Oswaldocruzia lenteixeirai</i>	b,c	Cuba	Baruš and Moravec, 1967
	c	Cuba	Coy Otero and Ventosa, 1984
	b,c	Bahamas	Goldberg et al., 1994
Thelastomatidae			
<i>Hammerschmidtella</i>	?	Cuba	Coy Otero et al., 1980
Tematoda:			
<i>Mesocoelium crossophorum</i>	b,c	Cuba	Baruš and Moravec, 1967

a = esophagus, b = stomach, c = small intestine, d = large intestine, e = body cavity.

4.2. Ecology of the observed parasites

Aplectana spp. are cosmopolitan cosmocercid nematodes that usually reside in the gastrointestinal tract of amphibians and reptiles (Baker, 1987; Anderson, 2000). Identity to species is based on males; since only juveniles and females were found in the present study, specific identity was not possible. Gravid females can be viviparous or ovoviviparous and shed eggs and larvae in feces. At this time, the complete life cycle of *Aplectana* spp. is unknown (Anderson, 2000), but a single study suggests that tadpoles can ingest juvenile nematodes, but that direct penetration of the skin of frogs is unsuccessful (Vhora and Bolek, 2013). In contrast, preliminary data from our lab show that post-metamorphic CTFs can be experimentally infected via skin penetration. Although there

have been three reported *Aplectana* spp. in North America, only one, *Aplectana hamatospicula*, has been found in both Cuba and the U.S.A. In Cuba, this species has been found in 14 species of anurans, but it only occurs in one species native to Florida (Baker, 1987; Vhora and Bolek, 2013).

Acuariids, nematodes belonging to the order Spirurida, have a heteroxenous life cycle, with birds and arthropods as definitive and intermediate hosts, respectively (Anderson, 2000). CTFs may only serve as a paratenic hosts. Meshaka (1996) showed that the CTFs diet consists of an array of arthropods, thus the most likely mode of infection is by consumption of an infected arthropod intermediate host.

CTFs most likely acquired metacercaria through infection by cercariae during the tadpole stage, when they are often found in

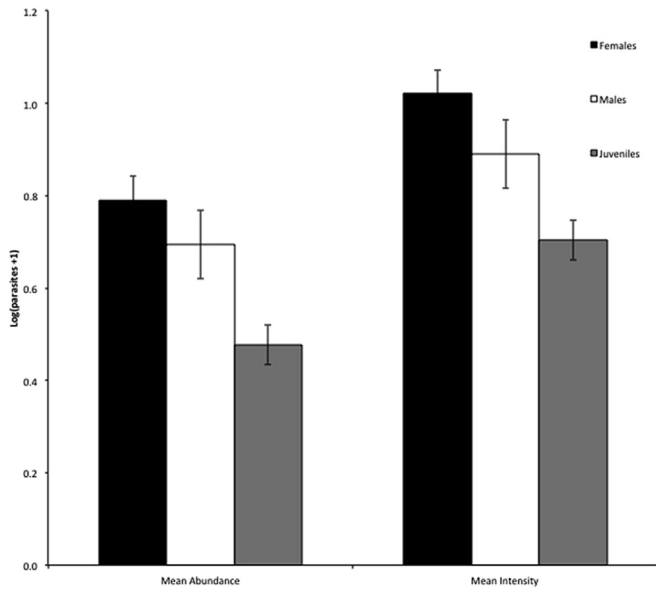


Fig. 1. Mean (\pm SE) abundance (log transformed) and mean intensity (log transformed) of parasites in male, female, and juvenile Cuban treefrogs, *Osteopilus septentrionalis*, collected from Tampa, FL.

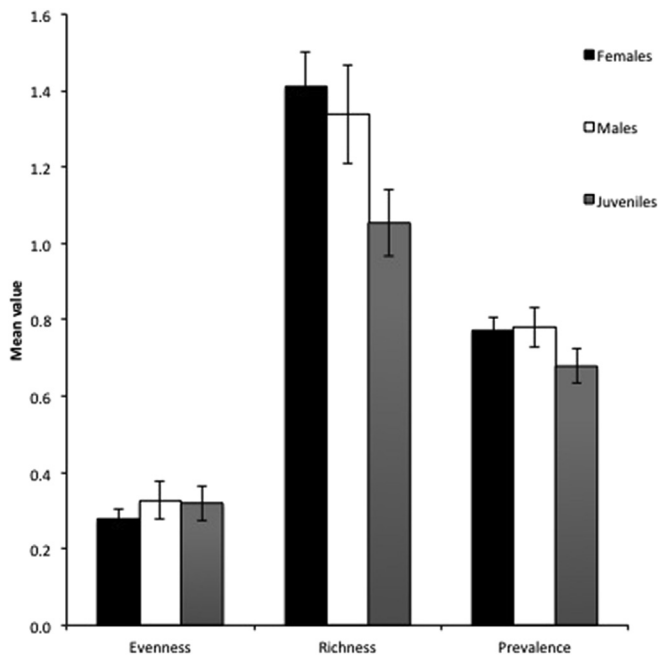


Fig. 2. Prevalence, richness, and evenness (Simpson's Diversity Index) of parasites in male, female, and juvenile Cuban treefrogs, *Osteopilus septentrionalis*, collected from Tampa, FL.

sympatry with trematode-infected snails. However, we cannot rule out the adults being infected with cercariae when they returned to the water to reproduce or by postmetamorphic frogs consuming trematode-infected terrestrial snails.

C. americana is a tapeworm that is found in at least 30 species of amphibians and reptiles in North America, three of which are native species in our study site. Because *C. americana* is also found in South America, Europe, Asia, and the Caribbean, it is impossible to determine whether or not this species was introduced to or acquired in Florida (McAllister, 1991; Goldberg et al., 2002). The life cycle of *C. americana* is not yet entirely understood, but transmission can occur in two ways: 1) directly, through coprophagy, or

2) indirectly, in which an infected invertebrate intermediate host (probably an insect) is ingested by the definitive host (Prudhoe and Bray, 1982).

O. lenteixeria is a common species of strongylid nematode reported from about forty species of amphibian and reptile final hosts, including CTFs, in the Caribbean (Coy Otero and Baruš, 1979; Baker, 1987; Moravec and Kaiser, 1995). The third stage larva penetrates the skin or is ingested by the final host where it matures into an adult in the intestines (Pérez Viguera, 1938). This species is also known to parasitize brown anoles, *Anolis sagrei*, in the Caribbean, but has never been reported from this common nonindigenous lizard in Florida (Goldberg and Bursey, 2000).

Rhabdias is a cosmopolitan nematode genus found as adults in lungs of amphibians and reptiles. These protandrous hermaphrodites typically infect anuran hosts via skin penetration. (Rune, 1978; Anderson, 2000; Langford and Janovy, 2009). At present, eight species of *Rhabdias* are known from North America, and at least two are from the insular Caribbean (Bursey et al., 2003; Martínez-Salazar and León-Règagnon, 2007). Since the few specimens found in our study could not be identified to species, it is impossible to determine whether this nematode was acquired or introduced.

Physaloptera spp. are known to occur in North America and in the Caribbean (Morgan, 1943; McAllister and Bursey, 2007). Generally, adult nematodes are found in the stomachs of mammals, reptiles, and birds that have ingested insect intermediate hosts. Although amphibians have not been reported as definitive hosts for *Physaloptera*, this group, as well as lizards, may harbor larvae that attach to the gastric mucosa, but do not encyst (Goldberg et al., 1993; Anderson, 2000). Some studies suggest that these hosts should not be considered paratenic since larvae do not encyst and often do not persist long in the host (Goldberg et al., 1993; Bursey and Goldberg, 1994). Therefore, CTFs may not serve as functional paratenic hosts for the unencysted *Physaloptera* larvae found in the present study as well as for another larval physalopterid, *Abbreviata* sp., reported from CTFs in Cuba (Coy Otero and Ventosa, 1984).

The developing larval stages of the acanthocephalan *Centrorhynchus* sp. are commonly found in terrestrial insects or isopods, but adults are found in the small intestines of carnivorous birds. Furthermore, amphibians and reptiles may serve as paratenic hosts by consuming arthropods infected with cystacanths (Nickol, 1985).

Pentastomids (worm-like arthropods bearing two pairs of retractable hooks in the mouth region) have a heteroxenous life cycle, and adults are commonly found in the respiratory tract of vertebrates (Paré, 2008). Although all classes of vertebrates and some invertebrates can serve as intermediate hosts, only fishes are reported to be unsuitable as final hosts (Riley, 1986; Barton and Riley, 2004). It is likely that intermediate hosts are infected by ingesting eggs that are shed in the feces of an infected final host. After the larva penetrates the gut, it continues to migrate in the abdomen/coelem, and often ends up in the visceral tissue where it undergoes several molts until it becomes an infective nymph (Paré, 2008). Although a few anurans can harbor adult pentastomids, most anurans are considered to be intermediate hosts with the final host being reptiles, a group that makes up approximately 90% of pentastomid species (Riley, 1986; Barton and Riley, 2004; Paré, 2008). Despite the fact that we found pentastomid nymphs in the lungs of the CTFs, we were unable to further identify this group because the pentastomid maturation was either incomplete or stunted in the CTF host.

4.3. Implications for enemy release

Perhaps the best-described effect of invasive species on host–parasite interactions is the enemy release hypothesis (ERH)

(Torchin et al., 2001; Mitchell and Power, 2003). ERH suggests that invasive species have a competitive advantage in their introduced range because they leave behind many of their natural enemies in their native range. This is a product of several phenomena. First, most of the parasites in a population are in a small proportion of the hosts (Shaw and Dobson, 1995; Shaw et al., 1998). Thus, by chance alone, it is unlikely that a heavily infected host will be introduced and successfully become established in a new environment; it is also unlikely that a heavily parasitized host would survive translocation. Second, most infected hosts are not infected with all the parasite species in their native range (Poulin, 2013). Therefore, even if parasites are introduced with the host and successfully establish, the parasites typically only represent a small portion of the host's parasites from its native range. Third, many parasites have complex life cycles requiring multiple host species. Hence, even if a parasite with a complex life cycle is introduced with the host, it is unlikely to establish because other required hosts are typically missing. In addition to the loss of natural enemies, introduced hosts are also thought to acquire few parasites in their introduced range because these parasites lack an evolutionary history with the introduced host; therefore, the parasites might not recognize or be capable of infecting a novel host (Mitchell and Power, 2003; Torchin et al., 2003).

In the present study, we found that CTFs harbored at least one parasite species from its native range but have also likely acquired parasites from herpetofauna native to the Tampa, FL area. Of the three types of parasites reported with the highest prevalences and mean abundances, the larval acuariids appear to have been acquired post invasion because they have never been reported in the CTF native range (Table 3) (Baker, 1987; Anderson, 2000). Only one parasite species, *O. lenteixeria*, appears to be a confirmed introduced species because it is found commonly in the native range of CTFs (Table 3), but there are no records of this species from frogs native to Florida. Because the *Aplectana* specimens were not identifiable to species, we cannot be certain whether it was introduced or acquired by the CTF because this genus has been reported in Cuba as well as in Florida. However, it is worth noting that only one species, *A. hamatospicula*, has ever been reported from native anurans of Florida, and it was found only in one species, *Gastrophryne carolinensis*, in 1939, which means that it has most likely been introduced (Walton, 1940).

CTFs at our study site were infected with fewer species of parasites than in their native range (Table 2) With the exception of *O. lenteixeria* and possibly *Aplectana* sp., CTFs at our study site were devoid of the parasite species found in their native range (Table 3). Moreover, the prevalence of *O. lenteixeria* is eight to fifteen times higher in its native than introduced range (Table 3); however, caution must be taken when making this comparisons between the parasites being reported in the native and introduced ranges because it is likely that several populations were surveyed across extensive areas ranging from the Bahamas to the Cuban Archipelago. As a result, we may be comparing fewer populations of CTFs or populations over a smaller spatial extent in Florida to more populations or at least populations distributed over a greater spatial extent in its native range. Because several of the previous studies do not list their sites of collection, we can not be sure as to how many populations of Cuban treefrogs were surveyed in their native range. However, we know that we collected frogs from thirty-seven wetlands over a 4000 acre park.

According to the enemy release hypothesis, a loss of enemies (in this case, parasite species) could, to some extent, facilitate the establishment and subsequent invasiveness of introduced species (Torchin et al., 2001; Mitchell and Power, 2003; Torchin et al., 2003). Nevertheless, Colautti et al. (2004) advocate further investigation of the ERH because very few introduced species exhibit

evidence of an enemy release; rather, they undergo an exchange or reduction of enemies. For example, in comparison to an overall mean parasite abundance of 4.6 calculated from Coy Otero and Ventosa (1984) and 12.7 calculated from Goldberg et al. (1994) in the CTF native range, this study indicates that the CTF has a higher mean abundance of 14.7 in the introduced range. Thus, careful consideration should be given to the vulnerability of introduced species to all of its enemies in native and invaded locations before attributing the colonization success to ERH. Though ERH is a plausible explanation for the colonization success of introduced species, research is still needed address whether or not shifts in host–parasite dynamics of introduced species aid in their colonization or in the decline of natives.

Although comparison to natives is not imperative to substantiate the ERH because the hypothesis is strictly based on the abundance and richness of parasites in an introduced species in its native versus introduced range, we acknowledge that this study would have been much stronger with the inclusion of a comparison of the parasites of the native treefrogs in Florida to parasites found in the invasive Cuban treefrogs in Florida. This might have suggested that exposure to parasites is higher in the introduced than native range of CTFs, which might help to explain their higher parasite abundance in their introduced range. Unfortunately, at the time of these collections, the Southwest Florida Water Management District prohibited collection of native frogs from this county park. Understanding how diseases shift between introduced and native populations can be imperative to prioritizing efforts in the conservation of species, and future work should focus on collecting CTFs as well as native treefrogs from various locations throughout the introduced range of CTFs to determine if their success is at least partially attributable to a loss or introduction of parasites.

Acknowledgments

Frog collections were authorized by Florida Fish and Wildlife Conservation Commission Conditional/Prohibited Species Special Permit ESC 06-07, ESC 06-07A, ESC 07-02, and ESC 08-04, and by yearly Southwest Florida Water Management District Special Use Authorization Letters. Flatwoods Park personnel provided site access. The University of Tampa Dana and Delo grants provided partial funding to TSC. K. R. Campbell and S. A. Johnson provided equipment and logistical support. Field and lab assistants included S. Bell, N. Briend-Smith, E. Brown, B. Buckingham, M. Dykes, M. Friedman, J. Guzy, A. Gordon, A. Harding, M. Hawk, K. Hoffmann, M. Hudson, J. Ipock, P. Irvin, L. Jordan, E. Langan, M. McGarrity, S. Nelson, L. Oliver, N. Szczecinski, and B. Walters. I would like to thank Charles Bursey and Omar Amin for their assistance in identifying parasites. Funds for this project were provided by a USDA National Needs Training (2012-38420-30195) Grant to J.R.R.

References

- Adamson, M.L., 1981. *Parapharyngodon osteopilii* n.sp. (Pharyngodonidae: Oxyuroidea) and a revision of *Parapharyngodon* and *Thelandros*. Syst. Parasitol. 3 (2), 105–117.
- Anderson, R.C., 2000. Nematode Parasites of Vertebrates: Their Development and Transmission, second ed. CABI Publishing, Wallingford, New York, NY.
- Anderson, R.M., May, R.M., 1978. Regulation and stability of host–parasite population interactions: I. regulatory processes. J. Animal Ecol. 47, 219–247.
- Baker, M.R., 1987. In: Synopsis of the Nematoda Parasitic in Amphibians and Reptiles, 11. Memorial University of Newfoundland Occasional Papers in Biology, pp. 54–60; 171–176.
- Bar- David, S., Lloyd-Smith, J.O., Getz, W.M., 2006. Dynamics and management of infectious disease in colonizing populations. Ecology 87, 1215–1224.
- Barbour, T., 1931. Another introduced frog in North America. Copeia 1931, 140.
- Barton, D.P., Riley, J., 2004. *Raillietiella indica* (Pentastomida) from the lungs of the Giant toad, *Bufo marinus* (Amphibia), in Hawaii, U.S.A. Comp. Parasitol. 71, 251–254.
- Baruš, V., Moravec, F., 1967. Systematic studies of parasitic worms found in the hosts

- Lepisosteus tristoechus (Ginglymodi: Lepisosteidae) and Hyla insula (Ecaudata: Hylidae) from Cuba. Vestnik Ceskoslovenske Spolecnosti Zoologicke. Acta Soc. Zool. Bohem. 31, 1–14.
- Burse, C.R., Goldberg, S.R., 1994. Physalopteroides bahamensis n. sp. (Nematoda: Spiruroidea) from the Cuban treefrog *Osteopilus septentrionalis* (Hylidae) from San Salvador island, Bahamas. Trans. Am. Microsc. Soc. 113, 169–176.
- Burse, C.R., Goldberg, S.R., Telford Jr., S.R., 2003. *Rhabdias anolis* n. sp. (Nematoda: Rhabdiasidae) from the lizard, *Anolis frenatus* (Sauria: Polychrotidae), from Panama. J. Parasitol. 89, 113–117.
- Bush, A.O., et al., 1997. Parasitology meets ecology on its own terms: margolis et al revisited. J. Parasitol. 83 (4), 575–583.
- Campbell, K.R., Campbell, T.S., Johnson, S.A., 2010. The use of PVC refugia to evaluate spatial and temporal distributions of native and introduced treefrogs at a natural area in West-central Florida. Fla. Sci. 73, 78–88.
- Colautti, R.I., Ricciardi, A., Grigorovich, I.A., MacIsaac, H.J., 2004. Is invasion success explained by the enemy release hypothesis? Ecol. Lett. 7, 721–733.
- Coy Otero, A., Barus, V., 1979. Nematodes parasitizing Cuban nematodes. Prirodoved. Pr. Ustavu Ceskoslovenske Akad. Ved. V. Brne 13, 1–43.
- Coy Otero, A., Ventosa, L., 1984. Nematodos parasitos de anfibios cubanos. Poeyana 269, 1–20.
- Coy Otero, A., Ventosa, L., Quintana, A., 1980. Nuevo record de nematodo para Cuba. Misc. Zool. (Havana) 9 (1), 1–2.
- Frankel, V.M., Hendry, A.P., Rolshausen, G., Torchin, M.E., 2015. Host preference of an introduced 'generalist' parasite for a non-native host. Int. J. Parasitol. 45 (11), 703–709.
- Goldberg, S.R., Bursey, C.R., 2000. Transport of helminths to Hawaii via the brown anole, *Anolis sagrei* (Polychrotidae). J. Parasitol. 86, 750–755.
- Goldberg, S.R., Bursey, C.R., Tawil, R., 1993. Gastrointestinal helminths of the western brush lizard, *Urosaurus graciosus graciosus* (Phrynosomatidae). Bull. South. Calif. Acad. Sci. 92, 43–51.
- Goldberg, S.R., Bursey, C.R., Tawil, R., 1994. Gastrointestinal nematodes of the Cuban treefrog, *Osteopilus septentrionalis* (Hylidae) from San Salvador Island, Bahamas. J. Helminthol. Soc. Wash. 61, 230–233.
- Goldberg, S.R., Bursey, C.R., Trujillo, J.D., Kaiser, H., 2002. Intestinal helminths of seven frog species from Trinidad and Tobago. Caribb. J. Sci. 38, 147–150.
- Guzy, J.C., Campbell, T.S., Campbell, K.R., 2006. Effects of hydrological alterations on frog and toad populations at Morris Bridge Wellfield, Hillsborough County, Florida. Fla. Sci. 69, 276–287.
- Hudson, P.J., Dobson, A.P., Lafferty, K.D., 2006. Is a healthy ecosystem one that is rich in parasites? Trends Ecol. Evol. 21, 381–385.
- Johnson, S.A., 2004. Geographic distribution: *Osteopilus septentrionalis* (Cuban treefrog). USA: FL: Gadsden Co. Herpetol. Rev. 35, 405.
- Kelly, D.W., Paterson, R.A., Townsend, C.R., Poulin, R., Tompkins, D.M., 2009. Parasite spillback: a neglected concept in invasion ecology? Ecology 90, 2047–2056.
- Krysko, K.L., Burgess, J.P., Rochford, M.R., Gillette, C.R., Cueva, D., Enge, K.M., Somma, L.A., Stabile, J.L., Smith, D.C., Wasilewski, J.A., Kieckhefer, G.N., Granatosky, M.C., Nielsen, S.V., 2011. Verified non-indigenous amphibians and reptiles in Florida from 1863 through 2010: outlining the invasion process and identifying invasion pathways and stages. Zootaxa 3028, 1–64.
- Langford, G.J., Janovy Jr., J., 2009. Comparative life cycles and life histories of North American *Rhabdias* spp. (Nematoda: Rhabdiasidae): lungworms from snakes and Anurans. J. Parasitol. 95, 1145–1155.
- Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M., Bazzaz, F.A., 2000. Biotic invasions: causes, epidemiology, global consequences, and control. Ecol. Appl. 10, 689–710.
- Martínez-Salazar, E.A., León-Règagnon, V., 2007. New species of *Rhabdias* (Nematoda: Rhabdiasidae) from *Bufo occidentalis* (Anura: Bufonidae) from Sierra Madre del Sur, Mexico. J. Parasitol. 93, 1171–1177.
- McAllister, C.T., 1991. Protozoan, helminth, and arthropod parasites of the spotted chorus frog, *Pseudacris clarkii* (Anura: Hylidae), from north-central Texas. J. Helminthol. Soc. Wash. 58, 51–56.
- McAllister, C.T., Bursey, C.R., 2007. First report of the nematode, *Physaloptera squamatae* (Spirurida : Physalopteridae) in Oklahoma, with a summary of hosts. Proc. Okla. Acad. Sci. 87, 65–67.
- Meshaka Jr., W.E., 1996. Diet and the colonization of buildings by the Cuban treefrog, *Osteopilus septentrionalis* (Anura: Hylidae). Caribb. J. Sci. 32, 59–63.
- Meshaka Jr., W.E., 2001. The Cuban Treefrog in Florida: Life History of a Successful Colonizing Species.
- Mitchell, C.E., Power, A.G., 2003. Release of invasive plants from fungal and viral pathogens. Nature 421, 625–627.
- Moravec, F., Kaiser, H., 1995. Helminth parasites from West Indian frogs, with descriptions of two new species. Caribb. J. Sci. 31, 252–268.
- Morgan, B.B., 1943. The Physalopterinae (Nematoda) of Aves. Trans. Am. Microsc. Soc. 62, 72–80.
- Nickol, B.B., 1985. Epizootiology. In: Nickol, B.B., Crompton, D.W.T. (Eds.), Biology of the Acanthocephala. Cambridge University Press, NY, pp. 307–346.
- Paré, J.A., 2008. An overview of Pentastomiasis in reptiles and other vertebrates. J. Exot. Pet Med. 17, 285–294.
- Peeler, E.J., Oidtmann, B.C., Midtlyng, P.J., Miossec, L., Gozlan, R.E., 2011. Non-native aquatic animals introductions have driven disease emergence in Europe. Biol. Invasions 13, 1291–1303.
- Pérez Vigueras, I., 1938. Nota sobre algunos nemátodos parásitos nuevos de Cuba. Livro jubilar Prof. Travassos, Rio de Janeiro, pp. 501–508.
- Poulin, R., 2013. Explaining variability in parasite aggregation levels among host samples. Parasitology 140, 541–546.
- Prudhoe, S., Bray, R.A., 1982. Platyhelminth Parasites of the Amphibia. British Museum (Natural History).
- R Development Core Team, 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Raffel, T.R., LeGros, R.P., Love, B.C., Rohr, J.R., Hudson, P.J., 2009. Parasite age-intensity relationships in red-spotted newts: does immune memory influence salamander disease dynamics? Int. J. Parasitol. 39, 231–241.
- Raffel, T.R., Michel, P.J., Sites, E.W., Rohr, J.R., 2010. What drives chytrid infections in newt populations? Associations with substrate, temperature, and shade. Eco-Health 7, 526–536.
- Raffel, T.R., Sessions, S.K., Lloyd-Smith, J., Hudson, P.J., Rohr, J.R., 2011. Does the early frog catch the worm? Disentangling potential drivers of a parasite age-intensity relationship in tadpoles. Oecologia 165 (4), 1031–1042.
- Riley, J., 1986. The biology of pentastomids. Adv. Parasitol. 25, 45–128.
- Runey, W.M., Runey, G.L., Lauter, F.H., 1978. Gametogenesis and fertilization in *Rhabdias ranae* Walton 1929: I. The parasitic hermaphrodite. J. Parasitol. 64, 1008–1014.
- Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S., Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E., O'Neil, P., Parker, I.M., Thompson, J.N., Weller, S.G., 2001. The population biology of invasive species. Annu. Rev. Ecol. Syst. 32, 305–332.
- Scholz, T., Tavakol, S., Halajian, A., Luus-Powell, W.J., 2015. The invasive fish tapeworm *Atractolytocestus huronensis* (Cestoda), a parasite of carp, colonises Africa. Parasitol. Res. 114 (9), 3521–3524.
- Schwartz, A., Henderson, R.W., 1991. Amphibians and Reptiles of the West Indies: Descriptions, Distributions, and Natural History.
- Shaw, D.J., Dobson, A.P., 1995. Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. Parasitology 111, S111–S133.
- Shaw, D.J., Grenfell, B.T., Dobson, A.P., 1998. Patterns of macroparasite aggregation in wildlife host populations. Parasitology 117, 597–610.
- Strauss, S.Y., Lau, J.A., Carroll, S.P., 2006. Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? Ecol. Lett. 9, 354–371.
- Torchin, M.E., Lafferty, K.D., Dobson, A.P., McKenzie, V.J., Kuris, A.M., 2003. Introduced species and their missing parasites. Nature 421, 628–630.
- Torchin, M.E., Lafferty, K.D., Kuris, A.M., 2001. Release from parasites as natural enemies: increased performance of a globally introduced marine crab. Biol. Invasions 3, 333–345.
- Vargas-Salinas, F., 2006. Breeding behavior and colonization success of the Cuban treefrog *Osteopilus septentrionalis*. Herpetologica 62, 398–408.
- Vhora, M.S., Bolek, M.G., 2013. New host and distribution records for *Aplectana hamatospicula* (Ascaridida: Cosmocercidae) in *Gastrophryne olivacea* (Anura: Microhylidae) from the Great Plains U.S.A. J. Parasitol. 99, 417–420.
- Walton, A.C., 1940. Notes on amphibian parasites. Proc. Helminthol. Soc. Wash. 7, 87–91.