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

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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.

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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

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 Bursey 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 lenteixeiria* (No. 105160, 105161), *Centrorynchus* 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 $\pm$ 0.32, females 10.79 $\pm$ 0.66, and juveniles 2.14 $\pm$ 0.08, respectively). The overall parasite prevalence was 74.2%, and the overall mean abundance was 14.7 $\pm$ 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 ($\pm$3.67) parasite individuals, 1.39 ($\pm$0.07) parasite species, and 77.52% ($\pm$2.83) prevalence, whereas juveniles averaged 5.71 ($\pm$1.57) individuals, 1.05 ($\pm$0.09) species, and 67.86% ($\pm$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 *O. septentrionalis*
Acuariid larvae (nematode)	Acquired; new host record
Oswaldocruzia lenteixeirai (nematode)	Introduced; previously reported
Aplectana sp. (nematode)	Likely introduced; previously reported
Physaloptera sp. (nematode)	Undetermined; new host record
Rhabdias sp. (nematode)	Undetermined; new host record
Physaloptera sp. (nematode)	Undetermined; new host record
Digenean metacercaria (trematode)	Undetermined
Cylindrotaenia americana (cestode)	Undetermined; new host record
Pentastomid	Undetermined; new host record