An adult female Guatemalan Black Iguana, *Ctenosaura palearis* (Iguanidae) (PIT-tag 025.102.301; snout–vent length = 210 mm; total lenght = 221 mm; body mass =157.8 g) resting on a bromeliad (*Tillandsia xerographica*). This endangered, arboreal, spiny-tailed iguana is endemic to the seasonally dry tropical forest of eastern Guatemala. *Ctenosaura palearis* uses the hollow trunks of old Pitayo Organ Pipe Cacti (*Stenocereus pruinosus*), Quebracho (*Lysiloma divaricatum*), and Yaje (*Leucaena collinsii*) as shelters, and during the dry season frequently is seen feeding on the fruit of *S. pruinosus*. A conservation program for *C. palearis* and its habitat, spearheaded by locals, was established in 2010, with the support of Zootropic and the International Iguana Foundation. The photo was taken at Reserva Natural para la Conservación del Heloderma y el Bosque Seco del Valle del Motagua, El Arenal, Cabañas, Zacapa, Guatemala, elev. 625 m. © Daniel Ariano-Sánchez

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Endozoochory by the Guatemalan Black Iguana, *Ctenosaura palearis* (Iguanidae), as a germination trigger for the Organ Pipe Cactus *Stenocereus pruinosus* (Cactaceae)

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Abstract: *Ctenosaura palearis* is an endangered iguana endemic to dry forest in the Motagua Valley in Guatemala. In this study we assess the effect of consumption by *C. palearis* on the seed germination rate of the Pitayo Organ Pipe Cactus (*Stenocereus pruinosus*). We conducted *in vitro* germination experiments involving three different treatments and a control. The differences in germination percentage were extremely significant among all the treatments ($P < 0.0001$). The iguana-ingested seeds had the highest germination percentage (26.1%). Considering that seed germination in *S. pruinosus* is positively affected by ingestion by *C. palearis*, we suggest that *C. palearis* might be an effective seed disperser for *S. pruinosus*, and thus would play a fundamental role on the seed dispersal ecosystem service in the dry forest of the Motagua Valley, Guatemala.

Key Words: Dry forest, ecosystem services, Guatemala, germination assays, seed dispersal

Resumen: *Ctenosaura palearis* es una iguana endémica amenazada que habita el bosque seco del Valle del Motagua en Guatemala. En este estudio evaluamos el efecto del consumo por *C. palearis* en la tasa de germinación de las semillas del Tuno de Órgano (*Stenocereus pruinosus*). Llevamos a cabo experimentos *in vitro* de germinación que involucraban tres diferentes tratamientos, así como un control. Las diferencias en los porcentajes de germinación fueron extremadamente significativas entre todos los tratamientos ($P < 0.0001$). Las semillas consumidas por las iguanas fueron las que tuvieron el mayor porcentaje de germinación (26.1%). Tomando en cuenta que la germinación de semillas de *S. pruinosus* es afectada positivamente por la ingestión de *C. palearis*, nosotros sugerimos que *C. palearis* puede ser un dispersor de semillas efectivo para *S. pruinosus* y por lo tanto tiene un rol fundamental en el servicio ecosistémico de dispersión de semillas en el bosque seco del Valle del Motagua, Guatemala.

Palabras Claves: Bosque seco, dispersión de semillas, ensayos de germinación, Guatemala, servicios ecosistémicos

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INTRODUCTION

The Guatemalan Black Iguana, *Ctenosaura palearis* (Stejneger, 1899), is endemic to the dry forest of the Motagua Valley of Guatemala (Cotí and Ariano-Sánchez, 2008), and is listed as Endangered (EN) on the IUCN Red List (Ariano-Sánchez and Pasachnik, 2011). Although studies are available on the distribution and basic ecology of this species (Cotí and Ariano-Sánchez, 2008), in addition to its morphology (Köhler and Vesely, 1996; Ariano-Sánchez and Gil-Escobedo, 2016), conservation status (Pasachnik and Ariano-Sánchez, 2010), and systematics (Buckley and Axtell, 1997; Pasachnik et al., 2010), the overall ecology of *C. palearis* remains poorly known. *Ctenosaura palearis* frequently is seen feeding on the fruit of the Pitayo Organ Pipe Cactus, *Stenocereus pruinosus* (Fig. 1), and Cotí and Ariano-Sánchez (2008) suggested that *C. palearis* might be a seed disperser for *S. pruinosus*, and thus would contribute to forest cover regeneration in the dry forest of Guatemala. To date, however, no formal assessment of this hypothesis has been made.

Fig. 1. Dry forest endozoochory-related species: (A) Guatemalan Black Iguana *Ctenosaura palearis*, (B) Pitayo Organ Pipe Cactus *Stenocereus pruinosus* fruits.
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Endozoochory among reptiles (saurochory) is not commonly studied, and few taxa, such as iguanids, have been reported to show this type of behavior (Iverson, 1979; Traveset, 1990; Benítez-Malvido et al., 2003; Beovides-Casas and Mancina, 2006; Blázquez and Rodríguez-Estrella, 2007; Celedón-Neghme et al., 2008; Cotí and Ariano-Sánchez, 2008; Piazzon et al., 2012; Moura et al., 2015; Burgos-Rodríguez et al., 2016). Endozoochory affects the germination of seeds primarily by inducing the removal of the pulp (Meyer and Witmer, 1998; Yagihashi et al., 1998; Traveset et al., 2007), by causing scarification of the seed coat (Traveset 1998; Traveset et al., 2001), and by depositing the seeds in feces this process would include the disperser's microbiota (Traveset et al., 2001; Cosyns et al., 2005; Couvreur et al., 2005). In this study we evaluate the effect of gut passage by *C. palearis* on the *in vitro* germination of *S. pruinosus* seeds. Specifically, we evaluate the effects on germination caused by scarification of the seed coat, germination disinhibition by removal of the pulp, and the effects of gut passage by *C. palearis.*

MATERIALS AND METHODS

We captured 20 adult individuals (10 males, 10 females) of *Ctenosaura palearis* from the Reserva Natural para la Conservación del Heloderma y el Bosque Seco, in Cabañas, Zacapa, Guatemala (14°53'N, 89°47'W; WGS 84) and maintained them in captivity. We fed each individual an exclusive diet of one-half a fruit of *Stenocereus pruinosus* per day for seven consecutive days. On the fourth day we collected feces from each individual, and stored the feces in separate sealable plastic bags and refrigerated them at ~4°C for no more than 15 days. We also collected fruits from the same *S. pruinosus* used to feed the *C. palearis*, to conduct germination experiments on seeds that were not ingested by the iguanas, and maintained the seed refrigerated in the same conditions as the feces.

Germination tests

We conducted four *in vitro* germination tests (control: hand-extracted inhibited seeds; treatment A: hand-extracted seeds; treatment B: gut-passed and later disinfected seeds; and treatment C: gut-passed seeds without disinfection) at the Laboratorio de Cultivo de Tejidos Vegetales at the Universidad del Valle de Guatemala. The control treatment (hand-extracted inhibited seeds) consisted of seeds manually extracted from the fruits, and planted them along with fruit pulp extract to simulate the conditions of the seed inside a fruit. To accomplish this, we washed all the pulp from the seeds, and supplemented them with 10 ml of a *S. pruinosus* pulp juice 5% solution to inhibit germination. We sterilized this solution in an autoclave at 121°C and 1.2kg/cm^2 for 10 min. Treatment A (hand-extracted seeds) consisted of seeds that we manually extracted from the fruits. We also hand-washed all the pulp from these seeds using demineralized water. Treatment B (gut-passed and later disinfected seeds) consisted of seeds that had passed through the gut of *C. palearis* and later were sterilized with a 30% ethanol solution for 5 min, followed by 15 min in a 25% calcium hypochlorite solution, following the protocol of Guillén et al. (2009). Finally, treatment C (gut-passed seeds) consisted of seeds that had passed through the gut of *C. palearis*, without further treatment.

Each germination assay consisted of 10 Petri dishes with 20 seeds per dish (200 seeds per assay), covered with sterilized white sand. For treatment A and the control, each Petri dish contained seeds obtained directly from different fruits. In treatments B and C, each Petri dish contained 20 gut passed seeds obtained from two different *C. palearis* of the same sex, 10 from each individual. We added 10 ml of distilled water to each Petri dish of experiments (A), (B), and (C). We placed all the Petri dishes in shelves with a fixed photoperiod of 12 h and a constant temperature of 25°C for 40 days (from 16 May to 24 June 2015). After that time, we counted the germinated seeds for each experiment. We considered seeds as germinated if any part of the seedling was visible over the sand surface. We calculated the germination percentage as the total number of seeds germinated in each Petri dish divided by 20, and provide the means as ± 1 SD. We then assessed differences in the germination percentages between groups using the Kruskal-Wallis test (α = 0.05) along with Dunn's multiple comparison post-hoc test using JMP 12.2.0 (SAS Institute).

RESULTS

As shown in Figure 2, the differences in germination percentage were extremely significant among all the experiments ($\chi^2 = 22.4$, $P < 0.0001$) with no effects shown by the sex of the iguanas that had ingested the seeds ($\chi^2 = 0.54$, $P = 0.46$). None of the control seeds germinated. Treatment A had a mean germination percentage of 6.67 ± 7.91%, treatment B had a mean germination percentage of 13.33 ± 8.29%, and treatment C had a mean germination percentage of 26.11 ± 11.93%. Dunn's post hoc tests showed a significant increase in germination percentage between the control and treatment B ($Z = -3.04$, $P = 0.014$). We also found an extremely significant increase in germination percentage between the control and treatment C ($Z = -4.49$, $P < 0.0001$). Both treatments (B and C) consisted of gut-passed seeds. The hand-extracted seeds (treatment A) showed no difference in germination percentage compared to the control (Z = -1.62, P = 0.63). We found that the gut-passed seeds of treatment C showed a significant difference in germinability when compared to the hand-extracted seeds of treatment A (Z = 2.85, P = 0.026).

The scarification of the seed coat by passing through the gut passage of *Ctenosaura palearis* showed no effect on the seed germination of *S. pruinosus*, as there was no significant difference (Z = 1.40, P = 0.98) between the hand-extracted seeds of treatment A and the gut-passed and later disinfected seeds of treatment B. The results also showed a significant increase in germination percentage between the gut-passed seeds of treatment C and the hand-extracted seeds of treatment A (Z = 2.85, P = 0.026). The germination percentage of gut-passed seeds of treatment C was not significantly different (Z = 1.43, P = 0.91) from the germination percentage of gut-passed and later disinfected seeds (treatment B).

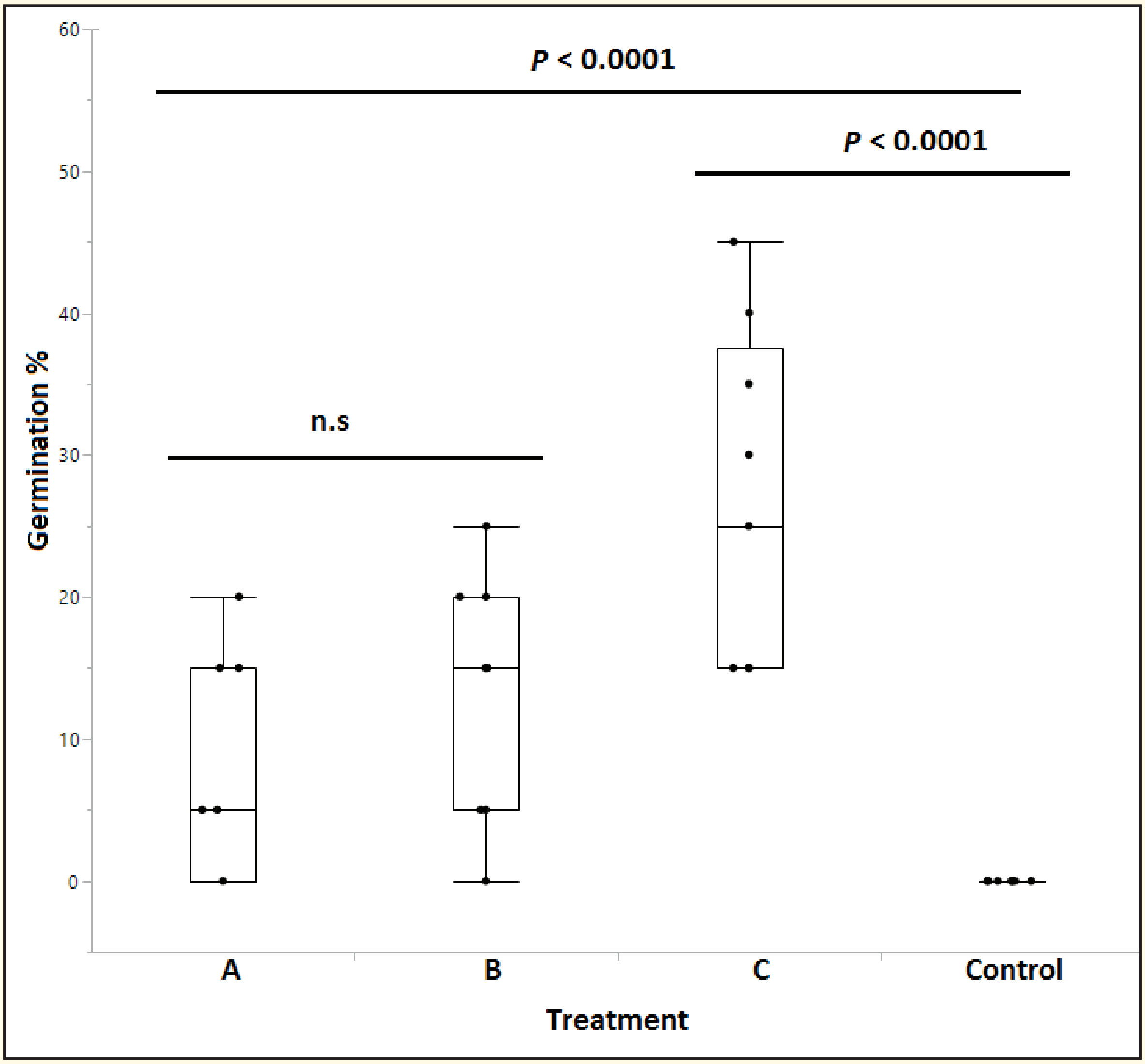

Fig. 2. Germination percentages of petri dishes containing 20 Pitayo Organ Pipe Cactus (*Stenocereus pruinosus*) seeds under four treatments: (A) hand-extracted seeds, (B) *Ctenosaura palearis* gut-passed and later disinfected seeds, (C) *Ctenosaura palearis* gut-passed seeds without further treatment and control (n = 40 petri dishes, Kruskal-Wallis test, P < 0.001, Dunn's multiple comparison post-hoc test).

DISCUSSION

Removal of the pulp was necessary for the germination of *Stenocereus pruinosus* seeds under any treatment. *Ctenosaura palearis* accomplishes this, as the pulp was not present on the excreted seeds of *S. pruinosus* (treatments B and C), and in treatment A we manually removed the pulp. Scarification of the seeds of *S. pruinosus* by passing through the gut of *C. palearis* did not significantly increase their germination percentage. The treatment of non-inhibited manually extracted seeds did not show a significantly different germination percentage than the sterilized scarified seeds (Fig. 2). Previously, Guillén et al., (2009) successfully germinated seeds of *S. pruinosus* without manual scaring of their seed coats. According to this study, scarification by itself is not necessary for *S. pruinosus* seeds to germinate.

The most important factor in increasing germination was biological additions through gut passage in *C. palearis*, which nearly doubled the germination percentage of the disinfected seeds (Fig. 2). This suggests that the microbiota in the gut of *C. palearis* is composed of at least one species of microorganism that promotes the germination of seeds of *S. pruinosus* by an unknown mechanism. As each individual of *C. palearis* was fed exclusively on fruits of *S. pruinosus*, this result is not conclusive for individuals that ingested a more diverse diet. Therefore, future studies should focus on identifying and describing the microbiota of *C. palearis*, and also in comparing the effect of different diets by analyzing the composition of feces of wild *C. palearis* and its effect on the germination percentages of seeds of *S. pruinosus*.

Considering that seed germination in *S. pruinosus* is positively affected by *C. palearis* ingestion, we suggest that *C. palearis* might be an effective seed disperser and consequently would play a fundamental role on seed dispersal in the dry forest of the Motagua Valley, Guatemala. *Stenocereus pruinosus* is a dominant component of several vegetation types in arid and semi-arid areas, and has been used as an important plant resource since humans first occupied the Motagua Valley (Véliz, 2008). The fruits of *S. pruinosus* are of the highest quality and produce a greater economic value compared to those of other members of *Stenocerus* along its distribution, and they are commonly gathered in areas partially consisting of thorn-scrub and in tropical dry forests (Parra et al*.,* 2010). Local villagers in Guatemala consider the fruit of *S. pruinosus* as a highly valuable food item that provides food security for most people living in the valley. The ability of *C. palearis* to disperse seeds will contribute to the welfare of the local human populations that uses *S. pruinosus* as a valuable dietary resource. Consequently, we encourage including *C. palearis* as a key element in local conservation efforts of the seasonally dry tropical forest of the Motagua Valley in Guatemala.

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