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DATA ON THE REPRODUCTIVE BIOLOGY OF THE SATANIC LEAF-TAILED GECKO, *UROPLATUS PHANTASTICUS* (SQUAMATA, GEKKONIDAE), AT THE BION TERRARIUM CENTER AS A CONTRIBUTION TO EX SITU OFFSPRING PROGRAMS

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Data on the Reproductive Biology of the Satanic Leaf-tailed Gecko, *Uroplatus phantasticus* (Squamata, Gekkonidae), at the BION Terrarium Center as a Contribution to ex situ Offspring Programs. Marushchak, O. Yu., Tkachev, D. O., Nekrasova, O. D., Marushchak, A. D. — This paper represents the results of a project on mass breeding of satanic leaf-tailed geckos (*Uroplatus phantasticus* Boulenger, 1888) in controlled laboratory conditions of BION Terrarium Center (Kyiv, Ukraine) in 2020. Given the growing popularity of *U. phantasticus* among hobbyists all over the world, it is important to develop a method of mass breeding of the species in conditions of herpetoculture. The work provides statistical data on the duration of gestation and incubation of eggs, obtained from a fairly large sample. The mean duration of gestation period for females (n = 119; exact counts of inter-clutch periods) was 34 days (min = 12, max = 64). The average duration of incubation was 94 days (min = 67, max = 130). The work also contains information on peculiarities of mating behavior, breeding ecology, copulation, percentage of infertile eggs and experience of maintaining a breeding stock with number of females exceeding that of males. Such information and its analysis are of particular scientific and practical value for the development of management plans for the conservation of the species, both in the wild and ex situ.
Key words: arboreal geckos, mass breeding, herpetoculture, eggs, incubation, gestation, Madagascar.

Introduction

As the matter of fact the island of Madagascar, is one of 30 recognized world biodiversity hotspots with more than 300 endemic reptile species (36 of 34 genera) with 21 currently recognized species of Leaf-tailed Gecko (*Uroplatus* Duméril, 1806) (Ratsoavina et al., 2020). Leaf-tailed geckos are known to be particularly dependent on pristine or only slightly modified habitats. Thus, their distribution area is dramatically suffering from irreversible degradation of original habitats. The main reasons are extensive agriculture, deforestation for the needs of charcoal harvesting, fire-cutting type of agriculture to establish new plantations of tea, rice, coffee and other crops (Jolly et al., 1984; Jenkins et al., 2014) and appearance of invasive species and their good adaptation to new environmental conditions due to climate change (Nekrasova et al., 2021). The slash-and-burn approach used by farmers also leads to uncontrolled bush fires that can be devastating for forests. In many areas, cleared land is abandoned after only a few years as its fertility is depleted and new areas of forest must be cleared for new agriculture (Harper et al., 2007; Rakotomanana et al., 2013). Usually the size of protected areas is initially small and provides no long-term perspective for the existence of many animal and plant species of (Stephenson, 1993). Specific living conditions in small isolates of a fragmented landscape lead to a gradual change in the structural and functional properties of the gene pool of protected populations and their extinction due to gene drift and inbreeding (Schierenbeck, 2017; Kliman et al., 2008). A decrease in the heterozygosity level of the population leads to a decrease in viability (Kliman et al., 2008). Last but not least is uncontrolled collection, which seems to be reduced in recent years due to limited CITES (Washington Convention on International Trade in Endangered Species of Wild Fauna and Flora, 1971) quotas, control over the collection of animals by state inspectors and local activists and thorough custom's control (Smith et al., 2011).

The negative anthropogenic changes on the island of Madagascar lead to the disappearance of unique geobotanical complexes, and hence the reduction of natural habitats of many endemic reptile species. A good example is leaf-tailed geckos of the *Uroplatus* genus with 21 species scientifically named for today (Ratsoavina et al., 2020). All of them are protected by CITES (Appendix II) (https://cites.org/eng/app/index.php). This Appendix consists of species that are not necessarily threatened with extinction at present but that may become so unless trade is closely controlled. In 2020 seven *Uroplatus* species (including *U. phantasticus* Boulenger, 1888) were available for official export according to annual quotas (https://cites.org/eng/resources/quotas/index.php), among them: spearpoint leaf-tailed gecko (*Uroplatus ebenaui* Boettger, 1879), satanic leaf-tailed gecko (*U. phantasticus*), common leaf-tailed gecko (*Uroplatus fimbriatus* (Schneider, 1792)), Günther's leaf-tailed gecko (*Uroplatus guentheri* Mocquard, 1908), lined leaf-tailed gecko (*Uroplatus lineatus* (Duméril & Bibron, 1836)), southern flat-tailed gecko (*Uroplatus sikorae* Boettger, 1913) and Sameit's flat-tailed gecko (*Uroplatus sameiti* Böhme & Ibisch, 1990).

U. phantasticus is one of the smallest Leaf-tailed Geckos, all of which are endemic to Madagascar (Ratsoavina et al., 2013). The snout-to-vent length (SVL) is 55–70 mm, tail length (TL) is about 40 mm (total length 100–110 mm). The head is not flattened and ends with a rounded snout. The tail is flattened and has a leaf-like shape. The body is laterally compressed and a great example of phytomimesis, even within the genus (Bauer and Russell, 1989; Svatek and van Duin, 2001; Glaw and Vences, 2007; Gehring, 2020).

Although many taxonomical ambiguities within the *U. ebenaui* species complex could have been solved within the last decade (e. g. Ratsoaviana et al., 2012; Ratsoavina et al., 2019; Ratsoaviana et al., 2020) many aspects of the natural history of leaf-tailed geckos, especially their reproduction biology are only little known and empirical data based on meaningful unit numbers are missing, although there are some indications based on breeding experience in captivity (e. g. Gehring, 2020). For example there is still a lack of data on actual duration of gestation in females, unified approach to organizing of breeding season and data on incubation of eggs at certain temperatures. The proper research in captivity can provide invaluable information about the reproductive biology, systematics, genetics and the possibility of establishing reserve populations of rare species therefore providing opportunities for their conservation (Mattioli et al., 2006). In this regard, cooperation between herpetologists and herpetoculturists is of great potential.

In this study we aim to provide data on reproduction cycles, maximum clutch numbers of females and egg incubation of *U. phantasticus*, in order to maximize the reproduction success without risking overloading the resources of the females. In the following we present data on the reproduction cycle and the breeding season of *U. phantasticus* in 2020. The initial parental stock was legally obtained and kept at BION Terrarium Center (Kyiv, Ukraine). The algorithm of organizing of breeding season for *U. phantasticus* is described for laboratory conditions.

Materials and methods

All studies were carried out at the BION Terrarium Center base. The parental stock of *U. phantasticus* consisted of 115 mature animals (40 males and 75 females). 54 animals were legally exported from Madagascar according to CITES quotas through export permits (#939C-EA12/MG14 dd 24.12.2014; #475C-EA06/MG15 dd 15.06.2015; #472C-EA06/MG15 dd 15.06.2015; #448C-EA05/MG16 dd 31.05.2016; 450C-EA05/MG16 dd 31.05.2016; #680C-EA08/MG16 dd 16.08.2016; #1321C-EA12/MG16 dd 28.12.2016) in the period from 2015 to 2017 while remaining 61 individuals were bred in captivity at BION Terrarium Center during this time.

Fig. 1. Laboratory for breeding stock of *U. phantasticus* (A) and individual breeze-like minimally equipped terrariums (B).

Following an established protocol (Miller, 1996), all wild-collected animals were quarantined for 30 days and treated for helminth parasites (treating with Fenbendazole at 20–25 mg/kg by mouth every 7 days (3 dozes) and metronidazole at 20 mg/kg by mouth once) before introduction in the breeding colony and weed out weak individuals. The animals were kept individually, in conditions identical to those used for the breeding stock, but with minimal decoration (paper towel as substrate, shallow water dish, 1 thin birch branch and an artificial plant branch as a shelter).

The data was collected in the period between March 7th 2020 (moment of forming of the first pairs) and December 21st 2020 (hatching of the last baby gecko).

All females that laid at least one egg (fertile/infertile one (slug)) were considered as entering the reproductive cycle — a period starting from development of oocytes to the very last clutch in the season and hatching of the first babies (Williams, 2018). After pairs had been formed, the geckos were observed for the first night in order to register copulation.

The duration of gestation (number of days, n = 119 counts) was counted exclusively between clutches (fertile/infertile eggs). The cases when it was impossible to tell the exact date of laying (slugs looked dried, or daily check was missed) were not taken into account. Therefore, the number of females for which the gestation period is calculated (n = 70) is smaller than the total number of females participating in the study (n = 75). Duration of incubation was counted (n = 134) as a number of day between the egg's laying and baby's hatching. The gestation data was recorded and charted using Microsoft Excel 2010 and Statistica v 10.0 (Descriptive Statistics, Box & Wiskers plot) (Weiß, 2007).

Initial keeping conditions were created according to the available literature resources (Svatek, van Duin 2001; Jensen, 2004), but significantly improved according to large scale keeping in BION's laboratories (Dubyna et al., 2019). As a result keeping conditions are as follows: all mature animals were kept in laboratory conditions using climate control systems (Split Air Conditioner Indoor Unit model CH-S09PL/R y Cooper & Hunter International Corporation; Electric Temperature Controller model TK-3 by DigiTop and Mechanical Daily Timer "TIMER-1" by Horoz Electric) to establish a climatic regime that correlated to data from the natural area of distribution (Svatek and van Duin, 2001). The animals were kept in plastic vertical terrariums (20*40*50 cm length*depth*height) with wire meshed screen door and roof providing flow-through ventilation and opaque white walls (fig. 1) pre-disinfected with "Agri'Germ 1000" water solution. Temperature ranges from +22.0 °C at night to +25.5 °C at day. Temperature never exceeds +26 °C or becomes lower than +21 °C during breeding season as a period of the year when the animals mate, lay eggs and recover before entering the cooler season. In order to simulate seasonal oscillations of temperature and humidity necessary for stimulation of proper breeding behavior and developing of germ cells changing daylight hours and temperature regimes was made according to the scheme shown in the table (table 1). Lighting (12–14 hours) included series of "Zoomed" tubes 10.0 UVB time relay. We use no additional heating elements (lamps, mats, cords). The indoor equipment of the terrariums was pre-disinfected as well. Inner decoration included thin birch branches, paper instead of substrate (in order to detect feces), a moist chamber (1 l plastic box with humid coconut substrate) and a shallow Petri dish refilled with fresh water daily. The necessary moist level (70–80 %) was achieved by 10-sec light spraying two times a day: in the morning (9:30–10:30) and in the evening (17:00–18:00). Diet consisted of insects (crickets (two-spoted cricket *Gryllus bimaculatus* Thunberg, 1815, house cricket *Acheta domesticus* (Linnaeus, 1758), Jamaican field cricket *Gryllus assimilis* (Fabricius, 1775)) and cockroaches (Turkestan cockroach *Shelfordella tartara* (Saussure, 1874)) of appropriate size (3–5 pieces per head 2–3 times a week depending on the animals' appetite and physical state) and juvenile snails

Table 1. Temperature-lighting scheme of provided winter dormancy

Date	T ,°C	Photoperiod hours
1–7 December 2019	21–25	8
8–12 December 2019	20–23	6
13–20 December 2019	18–20	4
21 December 2019–26 January 2020	16–18	3
27 January–5 February 2020 *	16–18	2
6–12 February 2020	16–18	4
13–21 February 2020	20–23	6
22–28 February 2020	20–23	8
29 February–5 March 2020	22–24	10–12
6 March 2020	23–26	

(garden snail *Cornu aspersum* (O. F. Müller, 1774)) as appropriate source of calcium for egg development in *Uroplatus* (Gehring, 2020), that are available on a regular base inside the terrarium.

Forming of breeding pairs took place approximately one week before the increase in daylight duration and temperature. Only animals of approximately same size were chosen to form a pair since size difference may lead to suppression of a smaller individual. After the pairs are formed using all available males, the animals were carefully monitored to identify possible signs of aggression and temporary separation of aggressive pairs and the selection of appropriate partners. After one week the males were transferred to another females and the process went on until every female had been placed with a male. Of all available females 2 were placed with a male 7 times; 7 females — 5 times; 21 female — 4 times; 21 females — 3 times; 19 females — 2 times and only 5 females were introduced to a male only once (then they were left solitary due to visual signs of poor health after oviposition).

Eggs were transferred to incubation containers without changing their polarity. If the eggs were indicated immediately after laying, they were transferred after 2–3 hours to allow the shells to harden. The eggs were placed in the incubation substrate no deeper than 2/3 of the diameter of the egg (Köhler, 2005). Porous fine-grained ceramic substrate “Seramis” was used as an incubation substrate. The containers were set in the laboratory according to the required incubation temperatures: +23 °C per day with a night drop to +21 °C. The air was changed daily by opening the lid of the containers.

Fig. 2. Copulation process of *U. phantasticus*.

Fig. 3. A part of all fertile eggs laid by *U. phantasticus* females during 2020 breeding season; incubation boxes are filled with "Seramis" medium.

Results

A total of 70 females (93.33 %) of the parental stock were considered to have entered the reproductive cycle. There were cases when females laid first one unfertilized egg (slug), and then a fertile one in a relatively short period of time. 20 cases of such laying were registered during the 2020 breeding season. On average, the interval between laying of each of the two eggs was six days (min 2, max 15).

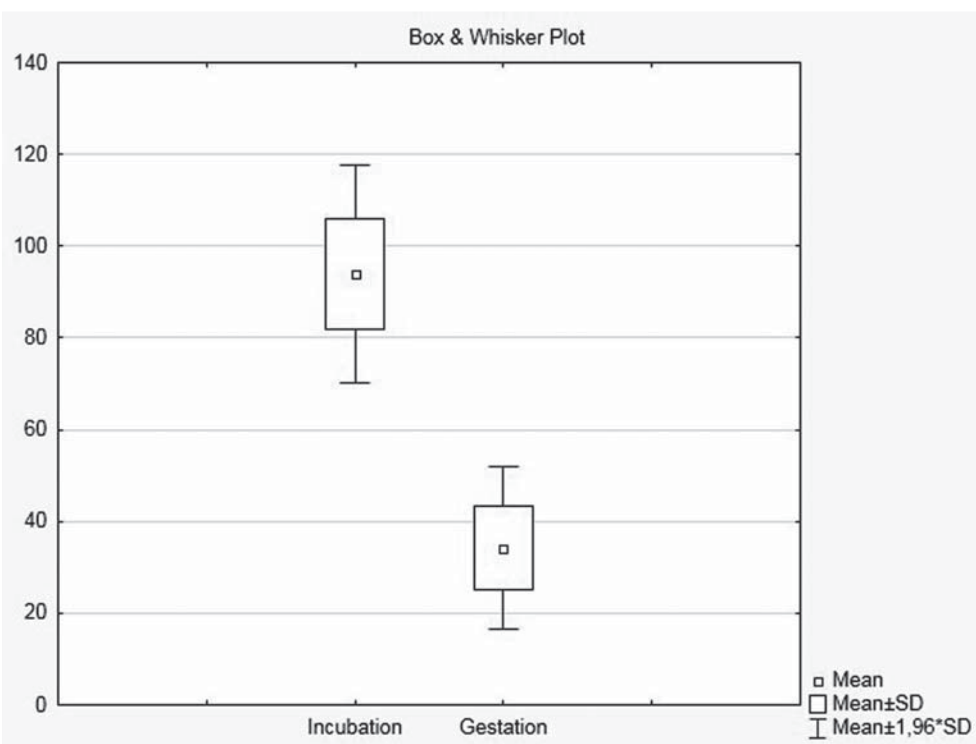

Fig. 4. Duration of gestation and incubation periods of *U. phantasticus*.

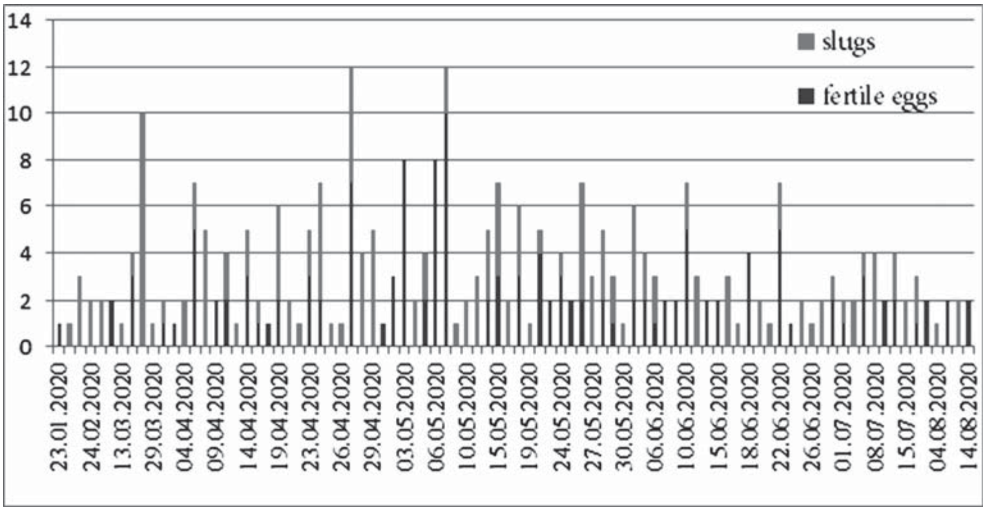

Fig. 5. A total number of eggs (pcs.) obtained from *U. phantasticus* females by dates during 2020 breeding season.

Eating infertile eggs was once observed for *U. phantasticus* and according to this information, approaches to pairwise shrinkage during the breeding season were adjusted. Every time a female made a clutch of two eggs, on the same day, she was placed with a male. During the observations, 43 copulations were recorded (fig. 2). 100 % of them occurred within the first two hours after turning off (21:00–23:20) the lighting in the room — the phase of the highest activity of these geckos. The average copulation lasted 37 minutes (min 15, max 67 minutes). Females that laid only one egg did not allow males to mate until the laying of the second fertile or infertile egg.

Of the 43 registered copulations, 36 (83.72 %) took place in the evening on the day of introducing a female, 4 (9.03 %) — on the second evening, 1 (2.32 %) — on the third, 1 (2.32 %) — on the fourth and one (2.32 %) — on the fifth day after introducing. Females, placed with males (due to lack of their number) later than on the 3rd day, gave, despite the registered acts of mating, slugs during the next laying.

Fig. 6. *U. phantasticus* hatchlings; the one in the right corner has not molt yet.

A total of 290 eggs were obtained from the parental stock, of which 137 (47.24 %) were classified as fertile and put for incubation (fig. 3). Other eggs were classified as slugs, having a drop-like shape, decalcified and attached to the walls or branches instead of normally be hidden under the substrate or foliage.

The mean duration of gestation period for females (n = 119 exact counts of inter-clutch periods) was 34 days (min = 12 days, max = 64 days, Std. Dev. = 9,10060) (fig. 4). Since females are able to make more than one clutch during the season, to develop a method and predict the annual efficiency of the breeding stock, it is important to visualize the peak periods of eggs' laying at certain conditions of previously provided winter dormancy (fig. 5).

134 eggs were successfully incubated (fig. 6) (out of 137 only 3 eggs were lost during incubation). The average duration of incubation was 94 days (min = 67 days, max = 130 days).

Unfortunately, ten (25.0 %) of 40 males died during breeding season in June–August, 2020. The results of autopsy showed no visual sign of health problems with any organ. The only thing that united these dead males was the absence of body fat. After the loss of the males we ended the breeding season in order to save the breeding stock. On average our females made three clutches which indicates that potentially the number of eggs could have been bigger.

Discussion

The results of 2020 breeding season for *U. phantasticus* show that for the needs of large scale reproduction maximum ergonomic approaches to maintenance of parental stock can be organized. This means that animals are able to successfully breed in to some extent minimized conditions, still sufficient for their well-being, maintaining the influence of necessary factors (light cycle, UV-light, temperatures, humidity level, terrarium decoration, winter dormancy).

A key factor in obtaining fertilized eggs during introducing of female to male at due time at the end of the winter period and after each subsequent laying of fertilized or unfertilized eggs, because according to our observations, females remain receptive for one to five days after laying. It is probably in this time range that the egg cell is capable of fertilization. According this observation we can conclude that the development of eggs in geckos is not always simultaneous, and while one unfertilized egg is already moving through the fallopian tubes, the second still has a chance to become a fertilized egg in the case of mating at this time.

Cases of an extremely short registered periods of gravidity (12 days) are very rare in this group of geckos. However, the same cases were reported also for *U. ebenaui* (Gehring, 2020), *Norops sagrei* McCranie & Köhler, 2015 (Cox and Calsbeek, 2010) and *Paroedura picta* Peters, 1854 (Kubička and Kratochvil, 2009) with interclutch intervals as short as one to two weeks. It is believed that by shortening inter clutch intervals these can compensate for small clutch size and hence substantively increase total reproductive effort (Weiser et al., 2012). Short inter clutch intervals could be enabled by just small egg size (smaller eggs would need less time for formation) or more rapid growth of follicles (Weiser et al., 2012). Particularly for *P. picta* it was reported that females kept under optimal conditions can have a temperature dependent duration of gravidity of 9, 10, and 15 days at the temperatures +30 °C, +27 °C, and +24 °C, respectively (Starostova et al., 2012; Kubička et al., 2012). However, the cases on short inter clutch intervals are still rare in reports and cooperation with private breeders on this matter is needed for gathering more data.

The comparable high mortality rates of males may be explained by the fact, that in a terrarium with minimal interior decoration constant visual contact with a female induce the male staying sexually active and causing refusal of taking food. Moreover the females compete with the males for food items even if there are plenty of them. Consequently this leads to excessive exhaustion of males and their deaths. This effect is usually not observed in private collections due to presence of live or artificial plants that provide a lot of hiding places and visual barriers, however probably the number of males' deaths can be bigger than the ones being reported. After considering these results we decide complete pairs together for maximum of five days or until the copulation during 2021 breeding season. So far there were no deaths of males and the amount of fertile eggs is similar to those in 2020.

At the temperatures of incubation (+21 °C +23 °C) the eggs showed good hatching rate (97.81 %). Of other 134 hatchlings, only 7 babies (5.22 %) died within the following month as they appeared to be very weak at the moment of hatching.

The above mentioned results of 2020 breeding season prove that using ergonomic approach to imitation of important natural factors and taking into account biological and behavioral patterns of *U. phantasticus* could work well.

According to long term experience in BION Terrarium Center (Dubyna et al., 2019) it is quite possible to organize stable large scale breeding of *U. phantasticus* ex situ and make it fully established in the herpetoculture worldwide, creating the reserve populations for successful conservation in situ.

In a future perspective we aim to collaborate with scientists and experts in conservation and provide breeding statistics, photo, and video documentation of the reproduction biology of leaf-tailed geckos. In order to achieve that we keep at BION Terrarium Center 25 males and 48 females of *U. phantasticus* as main stock with 63 captive bred specimens (35 males and 28 females) in 2020 to be included in breeding process next year. In May 2021 we received additional 30 males and 30 females of legally wild caught *U. phantasticus* from Madagascar according to the available quotas which enables us to enhance breeding stock and ensure genetic diversity.

Conclusions

Leaf-tailed geckos are known for their bizarre appearance, especially in *U. phantasticus* the coloration is rather variable, which makes them very popular among reptiles' hobbyists worldwide. For today the progress of herpetoculture in Europe, Japan and North America has created a steady trend for captive bred specimens (Robinson et al., 2015).

The concept of responsible herpetoculture (https://bion.com.ua/basic-principles/) is the creation and management of populations of amphibians and reptiles by private breeders worldwide in the context of the global destruction of natural ecosystems with the aim of forming a reserve bank of genes in the form of live, breeding animals which are genetically, morphologically, physiologically and behaviorally to the maximum extent possible identical to individuals from specific natural populations with the prospect of their subsequent reintroduction. Ex situ populations should have a gene pool, being as similar as possible to that of the corresponding natural populations. Therefore in order to build up a long-term stable and genetically diverse ex situ population, it is essential to identify the genotypes of the individuals, to identify as many haplogroups as possible and to have them available in the gene pool of the ex situ population, in order to establish studbooks and to build up a coordinated breeding community.

Creating a sustainable, genetically diverse ex situ population to save natural populations or provide a reserve stock of individuals should play one of the important roles in preventing the extinction of species. Of course preserving of geckos' population in the wild, especially in nature reserves is much more important, but at least the demand for offspring worldwide can be met by the breeding program. Consequently this will reduce the need in wild caught animals for breeding stock and poaching and give a potential material for strengthening of wild populations and upholding of rewilding projects if conditions mentioned in the previous paragraph would be met. The project on *U. phantasticus* breeding held by BION Terrarium Center is just an example of such ex situ approach to saving species.

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