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## Captive Husbandry and Reproduction of *Phrynosoma asio* (Squamata: Phrynosomatidae) at the Los Angeles Zoo and Botanical Gardens

Since 2003, the Los Angeles Zoo and Botanical Gardens, along with San Diego Zoo, has participated in a cooperative, ongoing study of western Mexican herpetofauna conducted by Universidad Autonoma de Nuevo Leon (UANL). One species of particular interest is the Giant Horned Lizard, *Phrynosoma asio*, which ranges along Pacific coastal forests of western Mexico from Colima south to northern Guatemala. Little is known about the natural history and breeding behavior of Giant Horned Lizards. One of the primary goals of this collaboration is to develop a baseline for successful captive maintenance and propagation of this species. As the first institution to successfully breed this species, Los Angeles Zoo staff has established several important husbandry guidelines.

*Materials and methods.*—In August 2008, three Giant Horned Lizards (two males, one female) were collected in the Mexican state of Colima and exported to Los Angeles Zoo. Upon arrival they were placed into a three-month quarantine. Fecal material collected during transport was preserved and the contents analyzed in an effort to better understand the natural diet of the lizards. These samples indicated *P. asio* feeds on a variety of terrestrial insects including, but not limited to, ants and termites (Recchio et al. 2009). *Phrynosoma* species generally show a range in dietary habits, but most are strongly myrmecophagous, or ant consumers (Pianka and Parker 1975; Montanucci 1989; Lemos-Espinal et al. 2004; Sherbrooke and Schwenk 2008). The habitat and dietary observations in conjunction with known husbandry protocols for other *Phrynosoma* (N. Atteberry, pers. comm.; J. Judd, pers. comm.; Montanucci 1989) were used to develop a viable captive management plan for this species.

Weekly weighing indicated that the smallest male was not gaining weight at a rate comparable to the others. While the larger pair continued to eat vigorously, the small male refused to feed and began losing weight. This lizard was assist-fed using a small index card to gently open the mouth, which initiated a feeding response. Eventually this lizard began eating voluntarily, but in order to achieve a healthy weight he was assist-fed larger, pre-killed crickets for an additional six months. This assistance with larger prey was necessary apparently due to the unique stereotyped feeding behavior resulting from highly reduced dentition and extensive pharyngeal papillae that produce a mucilaginous coating. These adaptations effectively package ants and other small prey and deliver them directly into the esophagus alive without the need to expend time and energy chewing, a requirement of other iguanids (Sherbrooke and Schwenk 2008).

Fecal tests were run on all three lizards. Each was treated for endoparasitism (unspeciated flagellates and ascarid eggs) with 50 mg/kg oral fenbendazole (Panacur®), and each continued to gain weight throughout the quarantine period. Parasitic nematodes have been found in up to 75% of some wild horned lizard populations (Sherbrooke 2003). The life cycle of *Skrjabinoptera phrynosoma* involves the horned lizard as its definitive host and the harvester ant (*Pogonmyrex* spp.) as the intermediate host,


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FIG. 1. Adult wild-collected *Phrynosoma asio* basking in their enclosure.

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Fig. 2. Adult *Phrynosoma asio* utilizing artificial anthill feeding device.

ensuring a constant cycle of infection. This relationship can be seen in the Texas Horned Lizard (*P. cornutum*) and the Desert Horned Lizard (*P. platyrhinos*) but has not been established in *P. asio* (Sherbrooke 2003; Hilsinger et al. 2011). No other medical issues were observed. Although there is no pathology associated with this parasitism, it is possible that a significant load might adversely affect digestion (Babero and Kay 1967).

Upon release from quarantine, the lizards were placed in an opaque 378-liter Rubbermaid® plastic stock tank with approximately 25 cm of substrate consisting of 80% sand and 20% soil. The ambient temperature was 28°C ± 2°C with relative humidity of 70% ± 10%. A basking area was created utilizing a 160-watt Zoo Med PowerSun® mercury vapor bulb and a 100-watt Zoo Med Repti Halogen® bulb over large stones. These produced surface temperatures ranging from 30–37°C. A 10.0 T8 Zoo Med ReptiSun® fluorescent lamp was placed over the cooler side of the enclosure for additional UVB exposure. Clusters of both live and artificial bunch grasses were planted throughout the enclosure. This provided nesting and hiding places as well as variation in topography. A large shallow water dish was provided for soaking and drinking (Fig. 1).

Many *Phrynosoma* are seasonally crepuscular—diurnal in spring and fall, and bimodal in the summer (Pianka and Parker 1975). To elicit the best feeding response, food items were offered at least twice daily during periods of highest activity. The lizards were offered harvester ants (*Pogonomyrmex* sp.) daily from a specialized feeding device buried in the substrate so as to mimic an anthill (Fig. 2). This was constructed by staff using a 12-cm diameter by 12-cm tall cylindrical plastic storage container. A 5-cm hole was drilled in the lid and a 20-cm long wooden dowel rod was inserted and secured to the bottom of the container, protruding through the lid. The ants were able to climb up the rod but not escape. *Phrynosoma asio* are less sensitive to the stings of *Pogonomyrmex* venom due to a blood plasma factor that aids in detoxifying the venom. However, the venom can become fatal if the lizard is stung too many times (Sherbrooke 2003; Sherbrooke and Schwenk 2008). Therefore, we monitored and removed any uneaten ants daily. If suitable and sustainable local sources for harvester ants are not available, they can be obtained from commercial suppliers, in this case, Life Studies (www.lifestudies.com). Other species of ants such as *Iridomyrmex pruinosus* can be fatal to horned lizards due to a defensive chemical (methyl-n-amyl ketone) that is aerosolized when the ant is disturbed (Blum et al. 1963; Montanucci 1989).

A varied diet is important not only from a nutritional standpoint, but also for enrichment. One study concluded that termites make up the most numerically important single prey item based on stomach content analysis (Lemos-Espinal et al. 2004). These results are consistent with *P. asio* having a more generalized diet than other *Phrynosoma*. Due to insufficient and seasonal availability of termites, crickets (*Acheta domestica*) became the staple daily food item, with wax worms (*Galleria mellonella*) offered several times weekly. Keepers also collected insects from zoo grounds in an effort to mimic natural dietary variation. Prey items offered included carpenter ants (*Camponotus* sp.), termites (Isoptera), red-legged earwigs (*Euborellia annulipes*), European earwigs (*Forficula auricularia*), and the occasional juvenile multicolored centipede (*Scolopendra polymorpha*). One feeding per day was lightly dusted with ReptiVite™ vitamin and calcium powders with D3. Feces were frequently inspected to ensure the lizards were both ingesting ants and properly digesting food items. *Phrynosoma* have a very large stomach in comparison to other iguanids as a function of their dietary specialization on ants, requiring a large volume to meet nutritional needs (Montanucci 1989; Pianka and Hodges 1998; Sherbrooke 2003; Sherbrooke and Schwenk 2008).

*Observations and results.*—*Phrynosoma asio* were often seen licking each other, head bobbing, and basking on top of or near each other. No aggressive behaviors have been observed in mixed gender and bachelor groups. These lizards appeared to thrive in a communal environment, potentially due to the need to live in close proximity to anthills and termite mounds. While this species is capable of ocular-sinus blood squirting (Hodges 2004), this behavior has never been observed in this group, which has become accustomed to daily handling.

Every effort has been made to mimic conditions of photoperiod, temperature, humidity, and diet found in *P. asio*'s natural habitat. In the tropical deciduous forests of southwestern Mexico, the dry season lasts typically from November through April and the arrival of rain stimulates breeding. Approximately one year after acquisition, the lizards had reached a sexually mature size: minimum 91 mm snout-to-vent length and weight greater than 70 g (J. Judd, pers. comm.; Pianka and Parker 1975), and were cycled through a winter brumation. This process first entailed lowering basking temperatures and reducing the frequency of misting. Conditions were gradually changed from 28°C with 60–70% humidity to conditions ranging from 24°C during the day to 18°C at night with 40–50% humidity, mimicking natural winter conditions. Hot spots were maintained at approximately 29°C during the day and 26°C at night. A hibernaculum of 8 cm of clean, dry leaves was provided.

During brumation, the *P. asio* were semi-active, utilizing hot spots intermittently. Primarily, the lizards buried themselves under the leaf litter layer on the cool end of the enclosure. When the lizards showed activity, harvester ants were offered, utilizing the feeding device. However, feeding was observed on only a few occasions. The lizards were misted only when active to induce drinking. Due to a significantly reduced metabolic rate during brumation, the lizards may drink, but rarely eat (Mayhew 1965). Water is still necessary and was made available at all times although the substrate was allowed to dry out. The lizards' weights and body condition were closely monitored. During the four-month brumation period, which ended in April 2010, the lizards lost an average of 10.7% of their body weight.

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Fig. 3. *Phrynosoma asio* pipping in incubator.

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Fig. 4. Male *Phrynosoma asio* showing enlarged post-anal scales (left) Female with no scale enlargement (right).

This was quickly regained and further growth was observed once normal activity and feeding resumed. In June, the summer light cycle was increased from 8 to 12 h.

In wild populations, breeding usually occurs in May and June (Davis and Dixon 1961) or June and July in captivity (Baur 1979). In our group, breeding took place on 20 July 2010. The female displayed to the males by holding her tail up vertically and waving it side to side; head bobbing and arm waving were also seen. Males experienced decreased appetite during the breeding season. The smaller male initiated copulation by chasing the female and licking at the base of her tail. The male then mounted the female, grasping the nuchal folds around her neck with his mouth and then swung his tail under hers to align the cloacae. This entire process took place in a matter of minutes.

Prior to this observed copulation, the female had increased her fat reserves. After breeding she exhibited a decrease in appetite, and her abdomen became distended. She was restless during gestation, which can range from 30–60 days. As she grew closer to oviposition, her abdomen became visibly lumpy. On 18 August, the female laid 20 eggs in a nest about 7 cm deep directly underneath the heat lamp, which was turned off during the laying process to decrease the potential for egg desiccation. The following year, the same female laid 19 eggs. These numbers are consistent with the previously recorded average clutch size for *P. asio* of 16.9 with a standard deviation of 4.9 (Pianka and Parker 1975; Pianka and Hodges 1998).

During the 2010 egg deposition, the female was left in privacy; in 2011 she was monitored closely and video-recorded throughout the process by staff in attendance. The female began excavating a nest in the same location as the previous year, using her forelegs to dig and her hind limbs to push the substrate further away. She was observed rolling individual eggs under her tail so as to coat each with substrate. This may serve both to camouflage the white eggs and to prevent them from sticking together (Sherbrooke 2003). When oviposition was completed, the female covered the eggs with soil. She would scoop a small amount of soil over the nest, then with a jackhammer-like motion pounce rapidly with her forelegs while her hind end remained stationary. The motion was so quick that it appeared as a blur on video recordings. She repeated the scooping and tamping process for several hours. When finished, she resumed basking and consumed a small meal. Her feedings were increased and supplemented heavily with powdered vitamins and calcium. Within 30 days she had returned to her pre-gravid weight. The weight loss observed was consistent with other species in the genus, where the Relative Clutch Mass (RCM) constitutes 13–35% of the female's weight (Pianka and Hodges 1998). All lizards quickly returned to their pre-breeding body condition and activity levels.

The eggs laid in 2010 were excavated and placed on fine vermiculite substrate inside a 3-liter, clear, snap-top plastic container with series of six 4-mm holes punched around the rim of the container. Due to a lack of available baseline data, an initial vermiculite-to-water ratio of 4:1 by weight was chosen to avoid oversaturation with water. This container was placed in an Avey RCAB200 Reptile Incubator™ at 31°C and 92% humidity. After a few days, the eggs began to desiccate. Under these conditions 11 of the 20 eggs withered, appeared nonviable and were discarded. The incubation temperature was reduced to 26.5°C and ambient humidity decreased to 70% in order to compensate for increased substrate moisture. The vermiculite-to-water ratio was doubled to 2:1 by weight, a small water bowl was added to the container, and the lid was gently misted to maintain light condensation. Specific humidity levels were not determined. Under these conditions the remaining nine eggs began swelling and appeared to thrive.

The first egg hatched after 100 days; the last egg after 106 days. The neonates took several days to fully emerge and frequently appeared static and lifeless. As with other reptile species, this is a period of transition during which the neonates absorb their yolk into their abdomen and switch from embryonic breathing to lung breathing. Therefore it is important to avoid interfering with the hatching process (Fig. 3). All nine neonates appeared normal and weighed between 1.8–2.5 g with an average weight of 2.2 g. They remained in the incubator for 24 h in order to fully absorb the yolk sac and gain strength. Preliminary sexing once the male hatchlings' enlarged post-anal scales were large enough to examine with a hand lens (approximately 5 g) suggested that the clutch consisted of eight males and one female (Fig. 4).

The 2011 clutch was incubated in a different manner. Of the 19 eggs, four appeared undersized. As with the 2010 clutch, the eggs were not weighed; however, based on the female's post-oviposition weight loss, the assumed average egg weight was 1.7 g. Two incubation containers were set up with a vermiculite-to-water ratio of 2:1 and a small water dish for added humidity. Six of the eggs were placed in a Percival I-36NL™ incubator set to 28°C. Three of these eggs remained undersized (less than one gram each), and began to shrivel and mold. They were therefore discarded. The remaining 13 eggs were incubated in an Avey RCAB200 Reptile Incubator™ at 26.5°C; after one week, one undersized egg began to shrivel and mold. It, too, was discarded. The three eggs in the 28°C incubator began hatching between 85–92 days. Individuals ranged in weight from 1.4–1.6 g, with the average weight of 1.5 g. The remaining 12 eggs in the 26.5ºC incubator hatched between 107–112 days. These individuals ranged in weight from 1.7–2.1 g, with an average weight of 1.9 g. The clutch incubated at a lower temperature took an additional 16–21 days to hatch and the hatchlings were, on average, 23% larger by weight at hatching than the higher temperature clutch. Once the hatchlings reached approximately 5 g in weight, preliminary sexing revealed that the three incubated at 28°C were females. The 12 hatchlings from the 26.5°C incubator consisted of four males and eight females. Because the smaller male was observed breeding the female, and because we have noted that distinctive skin patterns appear to be hereditary, we conclude that the smaller male sired the 2010 clutch and the larger male (which was the only observed copulation the following year) sired the 2011 clutch.

The 2010 neonates were housed in groups of four and five individuals, divided between two 38-liter glass aquariums. They were placed on moist paper towels with Petri® dishes for water. A large smooth rock was provided for basking and sleeping. A 50-watt Zoo Med Repti Halogen® bulb was placed over the basking rock to achieve 30–37°C. A 10.0 T8 Zoo Med ReptiSun® fluorescent lamp was placed evenly over both tanks to provide additional light and UVB exposure. The neonates were housed in the same room as the adults. No aggression was observed between the neonates and they often basked and slept on top of or next to each other. They were often observed licking each other and their surroundings. When the lizards were housed separately, their activity levels decreased. The neonates from the second clutch were treated in an identical manner as the first hatchings.

The neonates were offered as wide a variety of insects as possible, two to four times daily to ensure equal access for all individuals. The neonates had access to termites throughout the day when available. Pinhead crickets were offered twice daily in quantities that the lizards could consume within 10–15 minutes. The lizards demonstrated definite food preferences and specific techniques were developed to increase consumption of less desirable items to ensure varied and complete nutrition. Although the lizards showed a strong aversion to harvester ants (which could bite and sting them), the formic acid they contain may be an important component of *P. asio*'s diet. Formic acid is known to be an important gut flora component because it lowers pH levels, initiates protein synthesis, and aids in water extraction from consumed ants.

Feeding harvester ants to the lizards was best accomplished as the first feeding of the day and by placing a few in a container with vitamin powder to disguise them, then chilling them for 30 minutes to slow their movement. The smallest ants were then fed to the lizards one or two at a time until the ants become too active. It was not uncommon for the lizards to eat a single ant every few days. If more than a few ants were eaten at a time the lizards snapped their mouths open and shut repeatedly as if they were experiencing irritation inside of their mouths. Neonates appeared to consume ants more readily than their wild-caught parents.

Termites of any variety were the most preferred food items. The termites with their frass and other debris with which they were collected were presented to the lizards on a small dish or on a piece of wood. They often watched the debris or wood for extended periods of time waiting for the termites to emerge, though they did not actively dig to uncover them. If diligent collection efforts fail, termites are available from commercial biological supply companies. Pinhead and crickets up to 5 mm comprised the majority of the captive *P. asio* diet. Black carpenter ants were highly favored compared with harvester ants. Juvenile earwigs were also occasionally eaten. Small wax worms were fed to neonates, though they often tasted but did not consume them.

Neonates were rarely seen drinking from dishes, so, as with the adults, they were misted on their heads with a hand sprayer three to six times daily (and always after feedings) to ensure proper hydration. It was important to avoid oversaturation of their substrate to eliminate the potential for contact sores. They were also soaked twice weekly in shallow water (no more than 5 mm deep) during routine enclosure cleaning to prevent shed skin from remaining on their digits. Under these conditions the neonates thrived and developed adult behaviors as they grew and matured. These lizards require a high level of attention and care in a captive environment.

The purpose of this paper is to establish baseline criteria for the captive management and propagation of *P. asio*. Research continues to improve the husbandry for this charismatic lizard. Captive studies allow for unique insights that would be difficult to obtain under wild and often hostile conditions. The Los Angeles Zoo aims to improve on previous hatching success and to further insure healthy captive populations. Through continued efforts and shared knowledge, Giant Horned Lizards may one day thrive in other facilities, allowing for broader exposure, a greater knowledge base, and a healthy assurance population of this unique species.

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# HERPETOCULTURE NOTES

## TESTUDINES — TURTLES

***SIEBENROCKIELLA LEYTENSIS* (Philippine Forest Turtle). ARTIFICIAL INCUBATION AND HATCHLING SIZE.** *Siebenrockiella leytensis* is the largest and heaviest Philippine geoemydid turtle attaining > 300 mm in median caparace length and weighing > 3.5 kg. As currently understood, it is the only endemic Philippine turtle and its natural distribution is on Palawan and Dumaran Island, Philippines (Diesmos et al 2012. *In* Rhodin et al [eds.], Conservation Biology of Freshwater Turtles and Tortoises. Chel. Res. Monogr. No. 5:066.1–9).

Since 2008, a few commercial turtle traders in the United States and Europe have offered for sale apparently captive bred *S. leytensis* on the Internet with prices ranging from US $2500–4500 per specimen (pers. obs.). In the Philippines, there were also anecdotal claims made by turtle enthusiasts and private zoological parks of successful hatching of *S. leytensis* eggs from wild-caught specimens and/or captive breeding. One private zoological park in the Philippines claimed to have produced > 100 *S. leytensis* hatchlings in 2010 (S. Schoppe, pers. comm.). Interestingly, no credible evidence is forthcoming such as photographs of hatchling emerging from egg to authenticate purported captive hatching or breeding successes. Herein, I report on the artificial incubation and a full-termed hatchling of *S. leytensis*.

On 10 January 2013, a *Siebenrockiella leytensis* egg was laid by a wild-caught female (SCL = 199 mm) and donated by a private individual to me on the same day for artificial incubation. The female *S. leytensis* was in captivity with no contact with conspecifics for at least 45 days prior to laying the egg. The egg measured 50.35 mm in length × 28.75 mm in width and weighed 26.8 g. The egg size and mass were within the range previously documented for captive *S. leytensis*, which ranged from 41.0–57.8 mm in length × 19.7–29.4 mm in width and weighed 18–30 g (Diesmos et al 2012, *op. cit.*). No data are available on *S. leytensis* eggs from the wild. The egg was immediately placed in a plastic container with a ~5-cm thick layer of moistened vermiculite as incubation medium. Temperature during the entire incubation period ranged from 27°C–31.5°C. Recorded daily variation of incubation temperature was 0°C–3°C.

On 27 April 2013 (day 107), water beads on top of and a slight crack on one end of the egg were observed. Two days later (day 109), a lateral crack was observed, but no attempt was made to assist the hatchling. The following day, the hatchling failed to emerge on its own and upon opening the egg on the same day, a deceased hatchling was observed. The hatchling had the following measurements: 43.2 mm carapace length × 27.1 carapace width × 21.4 carapace height, and mass of 15.3 g. Hatchlings observed in the wild were 41–44.7 mm in median caparace length and weighed 12–15 g (Schoppe 2009. *Siebenrockiella leytensis* over time—are populations stable? Katala Foundation, Palawan, Philippines. 13 pp.). In comparison, the mean size and mass of eggs laid in captivity by the congeneric *S. crassicollis* were 52.1 mm in length × 28.0 mm in width and 27.2 g, respectively (Honegger 1986. Salamandra 22:1–10). *Siebenrockiella crassicollis* eggs incubated at 29°C–30°C hatched after 68–84 days; hatchlings were 43–46 mm carapace length and weighed 14–15 g (Honegger, *op. cit.*). To my knowledge, this is the first recorded artificial incubation of a *S. leytensis* egg to full term.

PHOTOS BY EMERSON Y. SY

Fig. 1. Lateral (top) and ventral view (bottom) of a full-termed *Siebenrockiella leytensis* hatchling.