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Observations on reproduction in captivity of the endemic long-tailed snake *Philodryas chamissonis* (Wiegmann, 1835) (Reptilia, Squamata, Dipsadidae) from Chile

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Abstract

The long-tailed snake *Philodryas chamissonis* is an oviparous rear-fanged species endemic to Chile, whose reproductive biology is currently based on anecdotic reports. The characteristics of the eggs, incubation time, and hatching are still unknown. This work describes for the first time the oviposition of 16 eggs by a female in captivity at Zoológico Nacional in Chile. After an incubation period of 59 days, seven neonates were born. We recorded data of biometry and ecdysis of these neonates for 9 months. In addition, a review about parameters of egg incubation and hatching for *Philodryas* species is provided.

Key Words

Chile, colubrids, eggs, hatching, oviposition, rear-fanged snake, reproduction

Introduction

Philodryas is a genus composed of twenty-three oviparous species widely distributed in South America (Grazziotinet al. 2012; Zaher et al. 2014; Caccialiet al. 2016), which are geographically separated by the Andes Mountains forming two recognized groups of species (Thomas 1976, 1977; Zaher et al. 2014). Some authors have suggested that the reproductive biology of *Philodryas* species is phylogenetically conservative, showing characteristics such as a high number of vitellogenic follicles (Vitt 1980; Fowler et al. 1998; López and Giraudo 2008; Mesquita et al. 2011), the ability to simultaneously produce eggs and vitellogenic follicles (Mesquita et al. 2011, 2013; Loebenset al. 2018) and a similar size of sexual maturation (Fowler and Salomão 1995; Mesquita et al. 2013). Despite the aforementioned, the abundant data on the reproduction of the cis-Andean species of the genus, especially *P. aestiva* (Fowler and Salomão 1995; Fowler et al. 1998), *P. nattereri* (Fowler and Salomão 1995; Fowler et al. 1998; Passoset al. 2014), *P. olfersii* (Mesquita et al. 2012, 2013), *P. patagoniensis* (Hartmann and Marques 2005; López and Giraudo 2008; Loebenset al. 2018), and *P. trilineata* (Gómez-Alés et al. 2016) contrast with the lack of studies for the species of trans-Andean group, which is composed of *P. amaru*, *P. chamissonis*, *P.simonsii* and *P. tachymenoides*.

From these species, *P. chamissonis* is an endemic species to Chile with diurnal and terrestrial habits (Thomas 1976). This snake has a generalist diet, feeding on small lizards, anurans, birds, small mammals (Greene and Jaksic 1992; Skewes et al. 2013; Torres 2017) and frequently is found in areas close to human settlements (Sallaberry-Pincheira et al. 2011). Aspects of its reproductive biol-

ogy have only been barely reported in anecdotic observations. Regarding this, Donoso-Barros and Candiani (1950) mentioned an oviposition in captivity of six eggs by a female, Webb and Greer (1969) reported a female with eight eggs in her belly and Bozinovic and Rosenmann (1988) stated an oviposition of twelve eggs in captivity. Notably, information on requirements of temperature and humidity for the eggs, incubation time, hatching and biometric data for growing of neonates remains unknown.

In 2011, two specimens (male and female) of *P. chamissonis* from Santiago (Metropolitan Region, Chile) were received and maintained at Zoológico Nacional de Chile according to a rehabilitation program. During this period, the female deposited 16 eggs that were incubated, observing the hatching and growth of the neonates. In this work, we describe for the first time some details of reproductive biology of *P. chamissonis*.

Materials and methods

The *Philodryas chamissonis* specimens studied in this work were found in the city area of Santiago (Metropolitan Region, Chile). These snakes were sexed using a 2 mm intracloacal plastic probe, according to Schmidt (1994). Clinical condition, size and weight were recorded. Specimens were maintained in a 1400 mm × 1300 mm × 1700 mm container with an upper window (700 mm × 400 mm) to allow sunlight in. Vegetal substrates with litter and tree bark were inserted in the container to offer hiding places. According to Bozinovic and Rosenmann (1988), the adult snakes were fed with one adult house mouse (*Mus musculus*) once a week, which was provided by the bioterium of Zoológico Nacional. Temperature and humidity were measured using a thermal hydrometer (Veto, Chile), with an accuracy of ±1 °C and ±8 %, respectively.

The incubator was a foam box of 800 mm × 300 mm × 300 mm with small perforations that were open/closed to maintain the humidity. To provide temperature and humidity inside the incubator, an aquarium heater thermostat 50 W (Sera, USA) submerged in water was used. The artificial incubation of the eggs was carried out in a plastic container without upper lid with 30 mm vermiculite, which was sprayed with water twice a day (in the morning and afternoon). The incubator maintained a mean temperature of 28 °C and a mean humidity of 55 %. In the evening, the temperature decreased by 2 °C. The temperature and humidity of the room were not measured. The eggs were measured with a caliper (Veto, Chile, accuracy 0.03 mm). To prevent eventual damage to the egg cluster, the eggs were not separated. Neonates were weighed with a balance of 0.1 g precision (Iitrust, France) and the total length was measured with a flexible metric tape. Each neonate was maintained in an individual container.

The hatching rate (HR) was calculated using the formula HR = number of neonates/numbers of eggs. All statistical analyses were performed using Graph Pad Prism 4.03 (GraphPad Software, USA). The data are expressed as mean (X) ± standard deviation (SD). Statistical analysis was performed using Student´s *t*-test and data were considered statistically significant when $p < 0.05$.

Results

Adult specimens incorporated to Zoológico Nacional showed healthy corporal conditions and lacked physical injuries (Fig. 1A, B). The female had total length =1100 mm long with a weight = 220 g and the male, total length = 1020 mm and weight = 180 g.

In the container, the specimens received daily 6 h of natural light with small oscillations in the room temperature and humidity. Although the adult specimens were in the same container and the male was observed following the female, a possible breeding behavior that has been reported for other *Philodryas* species (Williams 1982), no mating was observed. After 5 months of lodging (July–November), a cluster of 16 eggs with smooth, oval and white shell was observed during the morning in a corner of the container. The conditions recorded at that moment were 24.8 °C and 55% humidity. No parental care was observed. The eggs, which had irregular lengths (range 40–60 mm), were placed in the plastic incubator described in Materials and Methods section (Fig. 1C, D).

After 59 days of incubation, from 16 eggs only 7 neonates (named P1–P7) were born whose coloration was reminiscent of adults (calculated hatching rate = 0,44). The complete hatching of the eggs lasted between 10 to 14 h and the neonates came out of the egg, breaking the shell by means of the hatching tooth, making 3 to 5 parallel cuts to open it (Fig. 2A). They repeatedly projected the first third of the body outwards, remaining attentive to any movement that might represent a threat, and hiding in the egg again (Fig. 2B). Three neonates (specimens P5, P6 and P7) emerged from the eggs with remains of yolk sac, which came off when they crawled through the container (Fig. 2C). The neonates were not sexed. Some non-hatching eggs exhibited dark coloration and dehydration evidence, whose dissection showed lack of snake embryos (6 eggs) and the other three non-hatched eggs had snake embryos in final stages of development (Suppl. material 1: Fig. S1A, B). The stillbirth snakes (N=3) had an average total length (X = 104.0 mm, SD = 12.17 vs X = 212.85 mm, SD = 4.88; $p< 0.001$) and weight (X = 2.30 g, SD = 0.27 vs X = 4.00 g, SD = 0.82; $p < 0.01$) significantly less than neonates (N=7; Fig. 3A, B).

During the first three months, the 7 neonates of *P. chamissonis* were maintained in individual containers (dimensions 290mm x 190mm x 100mm), using paper as substrate (Suppl. material 1: Fig. 1C, D). Then, they were changed to new individual containers (dimensions 390mm × 190mm × 300mm) with topsoil as substrate.

The environmental conditions for neonates were 20 to 25 °C and 50 % to 55% humidity in night and daytime, like the natural habitat during summer in Chile Central. Throughout the growing period, the neonates did not accept pieces of, or complete pinkie mice as feed; however they were fed with crickets on a weekly basis. Notably, the neonates with high length and weight (P1 and P2) were better eaters than other P3–P7. Information on four ecdysis events and biometry of *P. chamissonis* neonates was recorded for 9 months (Table 1). Neonates of *P. chamissonis* performed ecdysis every 64 to 66 days, which lasted between 6 and 8 days. Two neonates (P1 and P2; Fig. 3C) with a high initial total length (220 mm vs 210 mm) were observed. Interestingly, the increase in the average total length correlates (r^2 = 0.9979) with the ecdysis number (Fig. 3D) but not the average weight (r^2

Figure 1. Adult specimens of *Philodryas chamissonis*. **A** male. **B** female. **C, D** Incubator with *Philodryas* eggs.

Table 1. Biometric data of *Philodryas chamissonis* specimens recorded during four ecdysis. Data shown represent the mean ± SD (N=7).

	Born		First ecdysis		Second ecdysis		Third ecdysis		Fourth ecdysis	
Neonates	length (mm)	weight (g)	length (mm)	weight (g)	length (mm)	weight (g)	length (mm)	weight (g)	length (mm)	weight (g)
P1	220	5	222	5	225	5	227	6	230	7
P2	220	5	221	5	223	5	225	6	226	7
P3	210	4	211	4	213	4	214	6	216	7
P4	210	4	211	4	212	4	214	5	215	6
P5	210	4	211	4	212	4	213	5	214	6
P6	210	3	211	3	211	3	212	5	213	5
P7	210	3	211	3	211	3	212	4	213	5
X ± SD	212.85 ± 4.88	4.00 ± 0.82	214.00 ± 5.13	4.00 ± 0.82	215.29 ± 6.02	4.00 ± 0.82	216.70 ± 6.42	5.29 ± 0.76	218.14 ± 6.91	6.14 ± 0.89

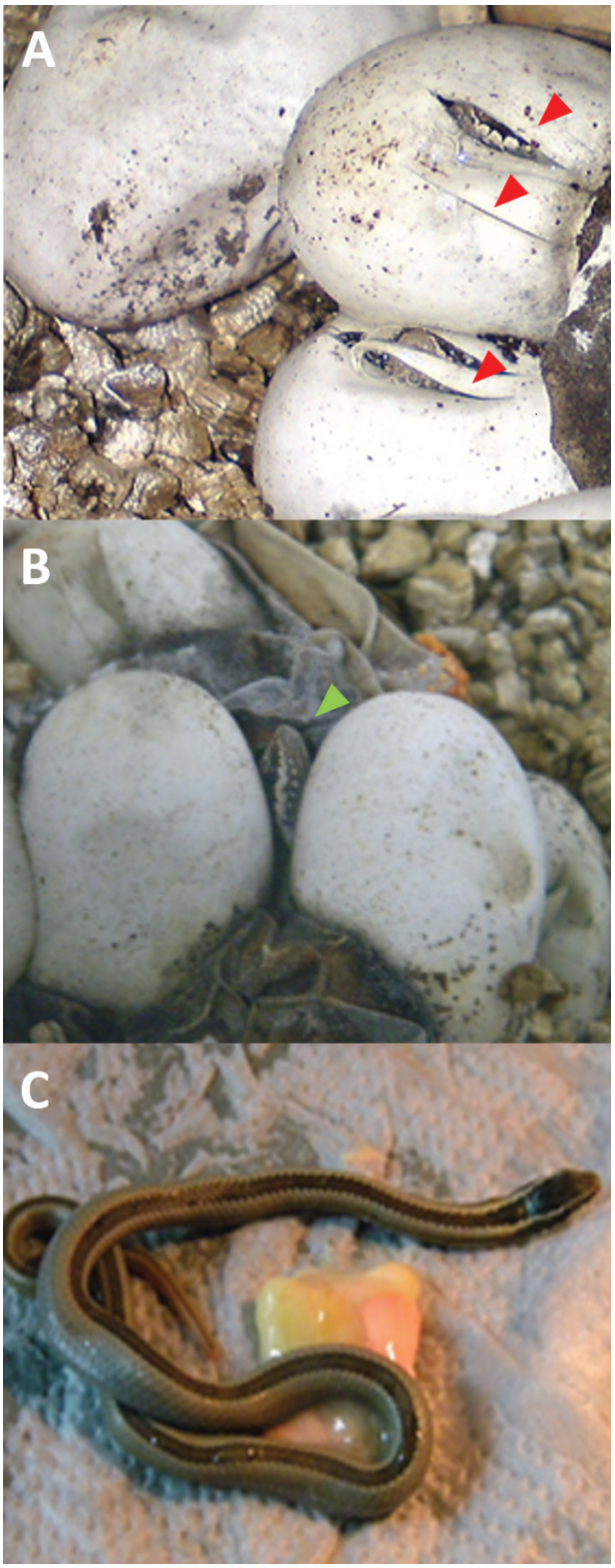

Figure 2. Hatching of *Philodryas chamissonis* eggs. **A** Neonates breaking the eggs. Red arrowheads indicate the cuts done with hatching tooth. **B** First neonate sticking his head (green arrowhead), called P1 in this work. **C** P5 neonate with yolk sac.

= 0.7974). A remarkable increase in the average weight of 2 g was only observed after second ecdysis (Fig. 3E). When some specimens were manipulated, they exhibited defensive behavior, trying to bite.

Discussion

The reproductive biology of *P. chamissonis* is poorly known and is based on anecdotal observations, mentioning only the number of eggs (Donoso-Barros and Candiani 1950; Webb and Greer 1969; Bozinovic and Rosenmann 1988). This work reports the highest number of eggs observed in an oviposition by a *P. chamissonis* female and it describes for the first time some aspects about hatching and ontogenetic development of this species. Our records are similar to reproductive data and conditions in captivity available for other *Philodryas* species (i.e. *P. baroni*, *P. nattereri*, *P. olfersii*, *P. patagoniensis* and *P. viridissima*), which are summarized in Table 2. Notably, the number of eggs and incubation times are different for each study and even for the same species, reporting wide ranges of days (48–89 days; Table 2) and hatching rates (0.23–1.0; Table 2). Particularly, one female of *P. patagoniensis* has the highest number of eggs described for the genus (28 eggs; Campbell and Murphy 1984), *P. baroni* has the highest calculated hatching rates (1.0; 0.86; 0.54 in three reports) and the fungus infections and dehydration are the main causes of mortality of *Philodryas* eggs in captivity (Gudynas and Gamborotta 1981; Rivera et al 2009; this work). In wildlife, *Philodryas* species such as *P. patagoniensis* and *P. viridissima*, may find the requirements of humidity and temperature for incubation of their eggs in anthills (Vaz-Ferreira et al. 1970; Rivera et al. 2009); however, the thermic requirements and preferences of sites for oviposition are unknown for *P. chamissonis*.

As it has been described for other *Philodryas* species (Hartmann and Marques 2005; López and Giraudo 2008; Mesquita et al. 2011), Greene and Jaksic (1992) suggested a possible ontogenetic shift in the diet of adults of *P. chamissonis*. In fact, the larger specimens of this species prey on larger endotherms such as birds, rodents and rabbits (Greene and Jaksic 1992) and adult specimens with less snout-vent length (SVL) than 576 mm eat lizards and frogs (Greene and Jaksic 1992). In contrast to *P. viridissima* and *P. baroni* neonates that only fed on small amphibians and reptiles (Williams 1982; Rivera et al. 2009), we observed *P. chamissonis* neonates (average total length = 212.9 mm) eating invertebrates. To our knowledge, in *Philodryas* species this size-related shift in the diet including invertebrates has only been described for *P. patagoniensis* from Uruguay (Carreira-Vidal 2002), where specimens of 301–600 mm of total length fed on invertebrates, amphibians and reptiles.

Finally, data on the reproductive cycle, seasonal activity pattern and mating remain unknown for *P. chamissonis*. We expect that this report will stimulate more detailed studies involving the reproductive biology of this endemic species.

Acknowledgements

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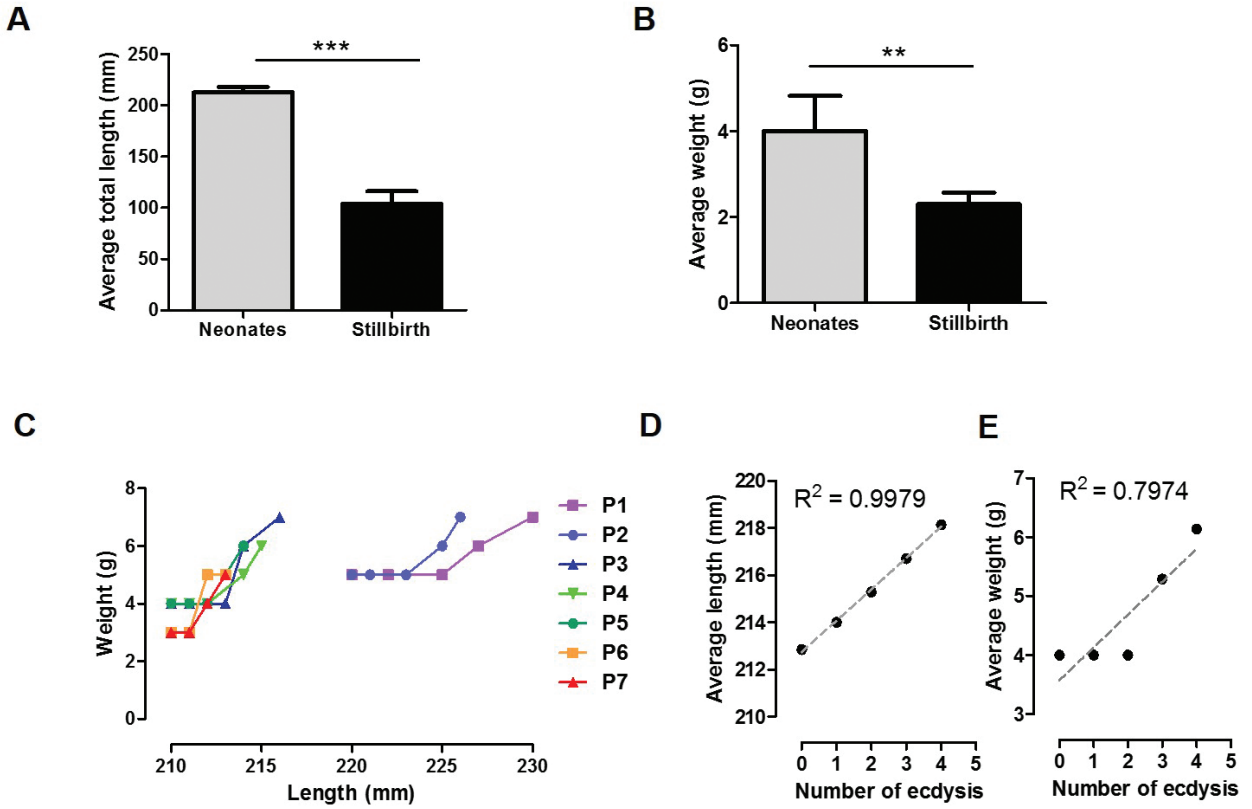

Figure 3. Biometric data of *Philodryas chamissonis* specimens recorded for 9 months. **A, B** Average total length and weight for neonates (N = 7) and stillbirths (N = 3). Data shown represent the mean ± SD. **p<0.01, ***p< 0.001. **C** Weight-length dot-plot for each neonate, which were identified as P1–P7, during the first four ecdysis. **D, E** lineal regression for average length and weight (N = 7) and number of ecdysis.

Table 2. Summary of reproductive data and conditions in captivity reported for *Philodryas* species. HR = hatching rate, SVL = snout-vent length, N.D. = not determined.

Species	Country	Total length female	Number of eggs/ Incubation time/ Hatching rate	Comments on incubation and hatching	Reference
P. baroni	Argentina	1 female, 1750 mm.	21 eggs / 63–66 days / HR= 0.86	Conditions: temperature 31.15 °C (min 27 °C/ max 38 °C), humidity 93%.	Williams 1982
				Eggs: 47.0 × 30.9 mm.	
				First ecdysis of neonates: 8–15 days after birth.	
				Average total length of neonates (N = 18): 410 mm.	
P. baroni	No declared	1 female, 1800 mm.	12 eggs / 83 days / HR = 1.0	Conditions: temperature 25 °C during the night and 22 °C during the days, humidity: 65–90%.	Golder 1973
				Eggs: average length: 56.3 mm, average weight: 25.46 g.	
				Average length of neonates: 375 mm.	
P. baroni	Argentina	Not reported	13 eggs / 76–78 days / HR = 0.54	Conditions: incubation in plastic bag with vermiculite at room temperature, humidity: 90 %.	Gallardo and Scrocchi 2006
				Neonates: range total length: 562–632 mm, average weight: 19.73 g (N = 7).	
P. nattereri	Brazil	1 female, SVL: 980 mm.	14 eggs / 65–70 days / HR = 1.0	Conditions of incubator: 100 mm x 230 mm x 340 mm, humid substrate and leak-litter.	Passos et al. 2014
				Temperature: 26 °C, Humidity: 65 %, light regime: 12 h/day.	
				Eggs: average length: 36.4 ± 2.1 mm, mass: 8.76 ± 0.35 g	
				Average snout-vent length of neonates (N = 14): 238.0 mm	
P. olfersii	Imported from Paraguay	1 female, 880 mm.	8 eggs / 89 days / HR = N.D.	Conditions: temperature 25 °C during the night and 22 °C during the days, humidity: 65–90 %.	Golder 1973
				Eggs: average length: 38.8 mm, average diameter: 16.5 mm and average weight: 7.87 g.	
				Average length of neonates: 280 mm	
P. olfersii	Brazil	1 female, 987 mm.	7 eggs / 64 days / HR = 1.0	-Eggs were kept in the plastic box filled with vermiculite. Temperature: 25–32 °C, humidified daily.	Rocha and Viana 2019
				-Average SVL neonates (N = 7): 250 mm.	

Species	Country	Total length female	Number of eggs/ Incubation time/ Hatching rate	Comments on incubation and hatching	Reference
P. patagoniensis	Argentina	No reported	9, 10 eggs / 56–60 days / HR = 0.44 and 0.40	-Conditions: incubation in plastic bag with vermiculite at room temperature, humidity: 90 %.	Gallardo and Scrocchi 2006
				-From each clutch, only 4 neonates born (total neonates = 8), with range of total length: 280–342 mm and range total weight: 4.62–5.70 g.	
P. patagoniensis	Uruguay	1 female, 920 mm.	13 eggs / 57–58[1] days / HR = 0.23	-Clutch in plastic bag with damp sawdust.	Gudynas and Gamborotta 1981
				-Artificial heating was not provided.	
				-Most eggs were attacked by fungus, only 3 neonates hatched.	
P. patagoniensis	Paraguay	9 females (lengths no reported)	7, 7, 8, 9, 10, 14, 14, 28 eggs / 48–60 days / HR = 0.69	-Eggs incubated in plastic bags in a medium of vermiculite at a constant temperature (27 °C).	Campbell and Murphy 1984
				-From these clutches with a total of 97 eggs, 67 neonates born.	
P. patagoniensis	Uruguay	No reported	13 eggs / 54–56 days / HR = N.D.	-Temperature of incubation: 30.3 °C.	Orejas-Miranda and García 1967
				-Eggs: 33.9 × 21.9 mm.	
				-Neonates: length: 167–192 mm, weight: 2.1–3.5 g.	
P. patagoniensis	Brazil	No reported	3–19 eggs / 65 days / HR = N.D.	-From 1990 to 1996, 7 clutches were observed and neonates (4 females and 9 males) exhibited a SVL = 230 – 289 mm and a weight = 5.1 – 7.0 g.	Fowler et al. 1998
P. chamissonis	Chile	No reported	12 eggs / incubation time not declared / HR = N.D.	-Adults were kept in the laboratory at room temperature and natural photoperiod. No data of incubation time and conditions were reported.	Bozinovic and Rosenmann 1988
				-Eggs: mean clutch weight: 84.6 ± 19.7 g	
P. chamissonis	Chile	1 female, 1100 mm.	16 eggs / 57–59 days / HR = 0.44	-Conditions: mean temperature 28 °C (min 26 °C/ max 30 °C), mean humidity 55% (min 52% / max 59%)	This work
				-First ecdysis of neonates: 20–26 days	
P. viridissima	Bolivia	1 female, 1.319 mm, weight: 136 g.	9 eggs / 77–80 days / HR = 0.78	-Incubation was done using a bag (800 mm x 500 mm x 800 mm) within another container. Range of incubation temperature: 25–28 °C	Rivera et al. 2009
				-Eggs: clutch weight: 7.35 g.	
				-Only seven neonates born. Non-eclossioned eggs exhibited fungus infection and dehydration. Neonates were fed with small frogs (*Hyla* and *Eleutherodactylus* sp.).	

[1] In an opened egg, two individuals joined by their umbilical cords to a common anexus were found. One of the specimens had everted hemipenes.

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Supplementary material 1

Figure S1

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Data type: JPG file

Explanation note: **Figure S1.** A, B Stillbirths and C, D neonates of *Philodryas chamissonis* in individual containers (https://doi.org/10.34691/FK2/IL1VMH).

Link: https://doi.org/10.3897/herpetozoa.32.e36705.suppl1