

## Preliminary Experiences with the Husbandry, Captive Breeding, and Development of the Hispaniolan Yellow Tree Frog, *Osteopilus pulchrilineatus* (Amphibia: Anura: Hylidae), with Ecological and Ethological Notes from the Wild

*Osteopilus pulchrilineatus* (Cope 1839) is the smallest of the four species of hylid frogs in Hispaniola. Females reach 43 mm SVL with males being smaller, at 39.5 mm (Henderson and Powell 2009). This frog is easily distinguished by having a dorsal pattern consisting of three conspicuous yellow lines on different tones and shades of yellow, tan, green or chocolate-brown (Fig. 1). Lower flanks are yellow. Dorsal lines are more or less evident depending on color phase. This species has a scattered distribution throughout the island, from sea level to moderate altitude, and seems to be locally abundant. This frog was considered Endangered (EN) by the IUCN Red List, due to the increasing loss and fragmentation of habitats (IUCN 2013; Stuart et al. 2008). The species was assessed as requiring additional *in situ* conservation action during the Caribbean Amphibian Ark Conservation Needs Assessment of 2011. More recently, *O. pulchrilineatus* has been considered highly vulnerable to climate change by Foden et al. (2013). Eggs and tadpoles of *O. pulchrilineatus* remain undescribed (Henderson and Powell 2009), although it was assumed that early development occurs in still water (IUCN 2013). Vocalizations have been reported (Schwartz and Henderson 1991), but no data on temporal and spectral acoustic parameters exist.

The importance of developing husbandry protocols and captive breeding programs for endangered species of amphibians have been discussed (Gascon et al. 2007; Reid and Zippel 2008; Zippel and Mendelson 2008; Zippel et al. 2011). Captive maintenance and breeding has the dual function of providing basic information on species biology (often absent or hard to obtain in the wild) and, most importantly, to favor conservation by combining complementary *ex situ* and *in situ* actions. This is crucial for amphibians in which populations are fast declining due to loss of suitable habitats, emerging infectious diseases, the impact of introduced species, over-collection, and other threats. It is also a preventive strategy for populations that might continue to decline in the near future due to these and other causes.

Herein, we discuss the first successful experiences with husbandry and breeding of *Osteopilus pulchrilineatus* in captivity, together with some ecological and ethological information obtained from the field. The limited observations and short term

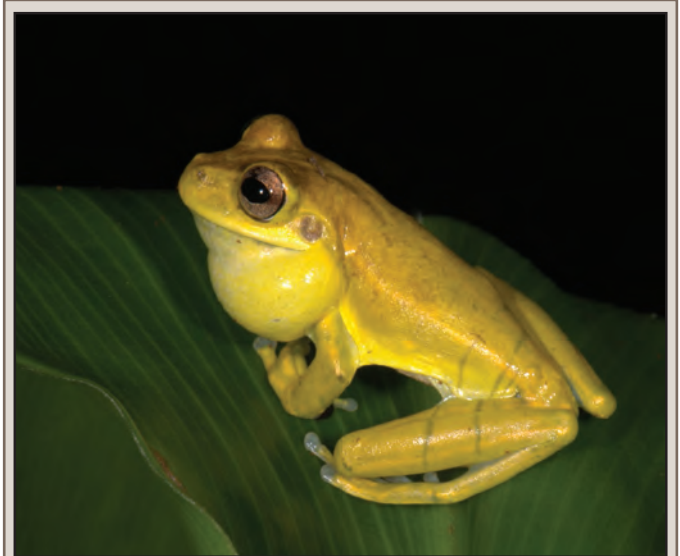


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FIG. 1. Adult male calling from a leaf in the wild, at surroundings of Río La Travesía, Cordillera Central, Loma La Canela, Duarte Province, Dominican Republic.

of this study determine its preliminary character. However, since this species is endangered and little is known about its biology, the information provided would be useful as a baseline to encourage future efforts to establish a standard protocol for long-term *ex situ* conservation programs.

**Terms and measurements.**—Terminology related to eggs and clutch structure follow Altig and McDiarmid (2007). The Gosner (1960) system was used for larval staging. Measurements were taken with a caliper (0.5 mm accuracy) under a dissecting microscope (Swift M27LED). Frogs were weighed with an AWS® AC-100 Digital Scale (100 × 0.01g). Calls were recorded with a Marantz Professional PMD 661 digital recorder and a Sennheiser ME 66 microphone. Acoustic signals were digitized at 48 kHz and a sample size of 32 bits. Acoustic analysis was performed with the software BatSound 2.1 (Pettersson Elektronik AB © 1996–1999). Sonogram was generated with a FFT (Fast Fourier Transform) of 512 points, using Hanning windows. Temporal variables were all measured on an oscillogram, and the dominant frequency was taken as the highest peak of energy in the power spectrum.

**Observations in the wild.**—This section has information obtained during fieldwork in the summers of 2011 and 2012. *Osteopilus pulchrilineatus* was observed in a wide range of ecological situations. Frogs were active at night at temperatures of 24–27°C and relative humidity of 82–90%. The species was associated with six types of breeding habitats (Fig. 2): 1) permanent ponds with grassy vegetation and ferns in open grassland; 2) small ponds in forested mountain areas; 3) pools along intermittent streams in

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FIG. 2. Some breeding habitats of *Osteopilus pulchrilineatus*. A) Pond about 100 m long in open grassland at the surroundings of Juana Vicenta, Samaná Province, where species is sympatric with *Osteopilus dominicensis*; B) puddle in grasslands that are often used for rice fields in Río Limpio, Reserva Natural Nalga de Maco, Elías Piña; C) forested edges of Cañada de Quilino, Reserva Natural Nalga de Maco, Elías Piña, breeding place of the four hylid frogs of Hispaniola: *Hypsiboas heilprini*, *Osteopilus dominicensis*, *O. pulchrilineatus* and *O. vastus*.

mountain forests; 4) small ephemeral pools along trails in forested areas; 5) pools and puddles in rice fields; and 6) margins of rivers and streams with gallery forests. Breeding sites were always lentic habitats of standing or slow-moving water. In some localities, the species was associated with other hylid frogs like *Osteopilus dominicensis* (lowland and highland habitats), *Hypsiboas heilprini*, and *O. vastus* (highlands). Tadpoles were found sympatrically with those of the other Hispaniolan hylid frogs at Río Limpio, Nalga de Maco Natural Preserve (Elías Piña Province).

Males generally called from small shrubs and grasses, usually less than 1.5 m high, rarely up to 3 m. Advertisement calls of ten males recorded in Loma La Canela, Cordillera Central (100 calls in total) were pulsatile in structure, having 11–19 pulses (Fig. 3) with pulse duration of 3–5 milliseconds. Pulses were narrowly spaced at the beginning of each call (8–9 milliseconds), becoming more spaced in the middle (24–62 milliseconds), and very narrowly spaced in the last 3–5 pulses (4–6 milliseconds). Call duration was 0.3–0.4 seconds. Call intervals were 0.8–1.5 seconds with a repetition rate of 47–64 calls per minute. The dominant frequency was 1.8–3.2 kHz.

Three egg clutches were observed in shallow water (Fig. 4), hidden among grasses at the edge of a forest pond, or fully exposed over beds of algae in pools along intermittent streams. Each clutch was a floating film of eggs. Clutch jelly matrix was somewhat thicker than those observed for *Osteopilus dominicensis* (pers. obs.). Tadpoles were seen swimming in all habitats, in depths that ranged from 2–60 cm. Larvae were exposed on the bottom, or hidden among grasses and algae.

*Acquisition of adults and husbandry.*— We began with four individuals: one male and a female were collected in the surroundings of Juana Vicenta, Samaná Province on 20 August 2011; one male was collected on 16 September 2011, at Río La Travesía, Loma La Canela, Ébano Verde Natural Preserve (Duarte Province), and an additional female was collected 9 July 2012 from Río Limpio, Nalga de Maco Natural Preserve (Elías Piña Province). After a quarantine period of two months, following general guidelines (Browne et al. 2007; Lynch 2001; Wright and Whitaker 2001), the frogs were accommodated in an all-glass silicone-glued aquarium (75 × 40 × 50 cm) that was modified as an indoor terrarium (Fig. 5). The bottom was drilled out, a bulkhead was installed, and a 19-mm-diameter plumbing system allowed for controlled drainage of the terrarium and the continuous discarding of overflowing water. A false bottom was made from a plastic light diffuser and plastic screening. It raised a basal layer of gravel (diameter ~4–5 mm) about 3 cm from the bottom glass. Half of the terrarium was a 7-cm-depth aquatic section ~17 liters in capacity, delimited from the land section by flat rocks. Water was filtered through the terrarium gravel by using a submerged water-pump (Exoterra®; model PT 2095) with a small waterfall output. The pump was placed inside a plastic chamber for easy access, and two cartridges for chemical filtration (activated carbon and zeolite) were situated in the pump inlet. The pump chamber was made from plastic mesh to allow water flow.

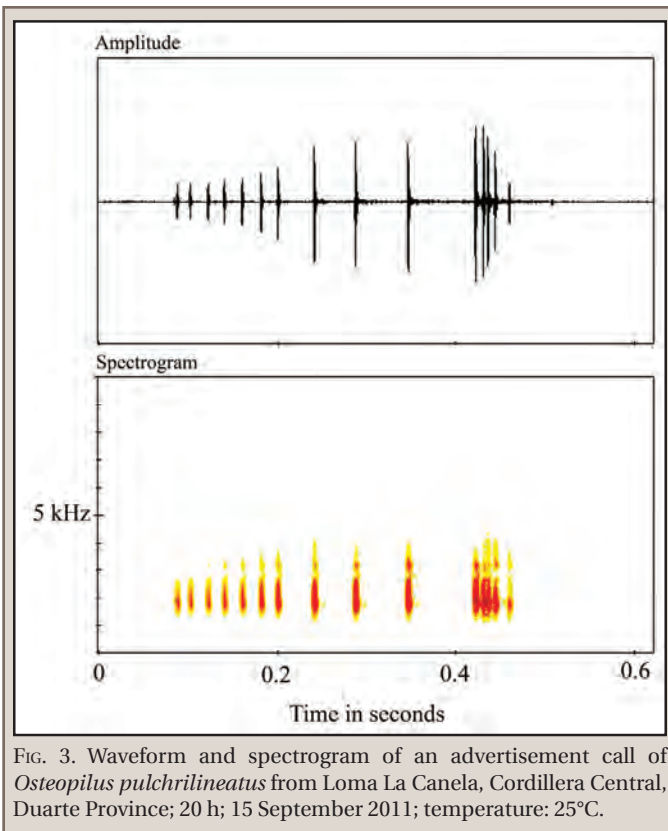


FIG. 3. Waveform and spectrogram of an advertisement call of *Osteopilus pulchrilineatus* from Loma La Canela, Cordillera Central, Duarte Province; 20 h; 15 September 2011; temperature: 25°C.

The water had a pH of 7.6, and 660  $\mu$ S of conductivity. In the land section, the underlying gravel was thicker, covered with tree-fern fiber substrate, and planted with *Scindapsus* sp. and *Calathea* sp. The first plant species became the dominant vegetation. Ammonium, phosphates, nitrites, and nitrates were regularly monitored and kept within safe ranges (Wright and Whitaker 2001). The terrarium cover had an access window and was built from a plastic light diffuser, PVC sheets, and a plastic mesh (3.5 mm). The mesh comprised 50% of the cover, to allow the placement of lights and ventilation. Cooling fans were installed on the terrarium cover to force ventilation, and to avoid over-heating and water condensation on the glass. An artificial rain system (made from a high pressure pump: Lucky Reptile® SR-1, and two nozzles near each end of the terrarium) was activated for one minute twice each day and controlled by a digital timer (Woods® model 59377). Two lamps were used: a Nisso® plant growing fluorescent tube and an Exoterra Repti Glo® 5.0 (15 W). Both fluorescent lamps promoted healthy plant growth; however, Lindgren et al. (2005) documented the low effectiveness of the UVB emission by Exoterra Repti Glo® 5.0 tubes compared with other lamps available for herpetoculture. In March 2012 two Eiko® Halogen lamps were installed over the terrarium cover, and were switched on for two hours at 0900 h and at 1600 h. We have no information on the UVB needs of this species, but for other hylid frogs this factor was demonstrated to be important for normal growth and health (Verschooren et al. 2011). The frog room was kept at ambient temperature and humidity fluctuations. The terrarium was placed in a naturally illuminated room, and lamps were programmed by a digital timer to duplicate a natural photoperiod. A data logger (HOBO® Pro V2 Onset) was installed and programmed to gather data of relative humidity (RH) and temperature every 30 minutes for 1.2 years (Fig. 6).

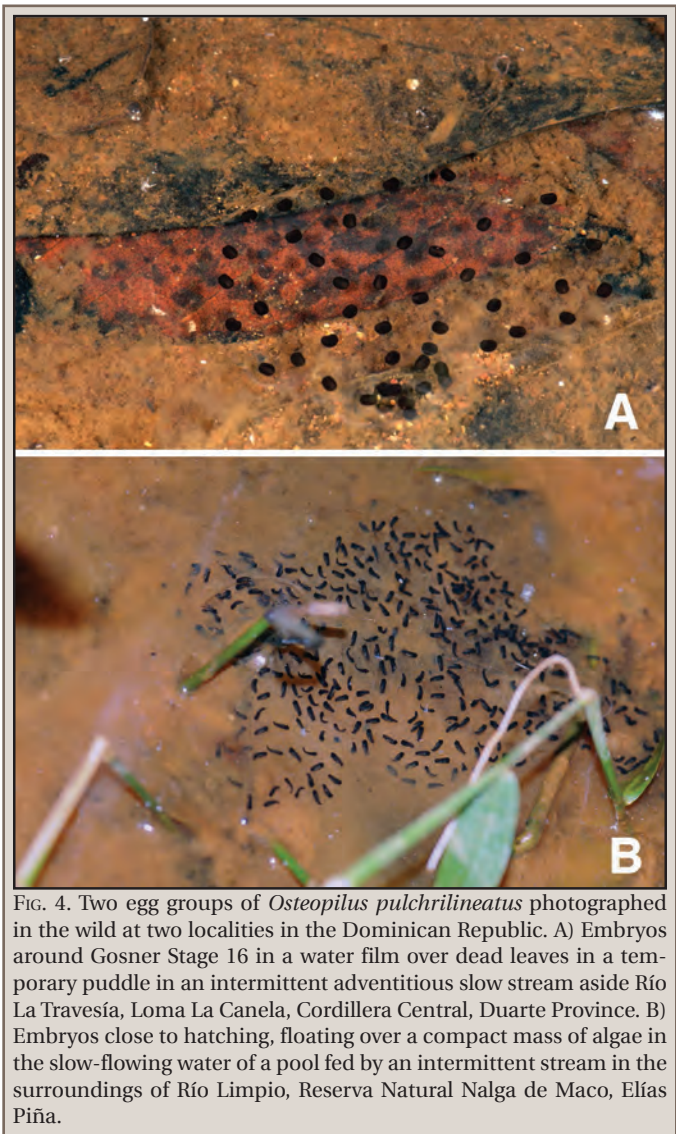


FIG. 4. Two egg groups of *Osteopilus pulchrilineatus* photographed in the wild at two localities in the Dominican Republic. A) Embryos around Gosner Stage 16 in a water film over dead leaves in a temporary puddle in an intermittent adventitious slow stream aside Río La Travesía, Loma La Canela, Cordillera Central, Duarte Province. B) Embryos close to hatching, floating over a compact mass of algae in the slow-flowing water of a pool fed by an intermittent stream in the surroundings of Río Limpio, Reserva Natural Nalga de Maco, Elías Piña.

**Terrarium maintenance.**—The water was changed twice weekly by partial to full draining of the aquatic section. Fluorescent lamps were replaced after one year. The water-pump filtering sponge was cleaned once weekly. Cartridges for chemical filtration were changed once a month. Dried plant leaves were removed and plants pruned as needed. Frog feces were regularly removed from the surface of leaves, rocks and glass, washing them with manual high-pressure water spraying. Water quality was monitored at least once weekly.

**Feeding.**—Basic food consisted of captive raised tropical house crickets (*Gryllobates sigillatus*) and cockroach nymphs (*Blattella orientalis*). Adult frogs were fed crickets three times a week and cockroaches only once per week. Prey insects did not exceed 1 cm in total length. Each frog ate 1–5 prey items at each feeding. Females often ate more than males. Insects were gut-loaded with a high protein and calcium food 48 h before being offered to the frogs. Prey items were dusted with Repashy Super Foods® Calcium plus ICB (Repashy Ventures, Inc.). Froglets were fed daily with pin-head crickets and once a week with tiny cockroach nymphs, all dusted with the same supplementation as the adults. Prey size and number was gradually adjusted as they grew.

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FIG. 5. Terrarium designed to house four adult individuals of *Osteopilus pulchrilineatus*.

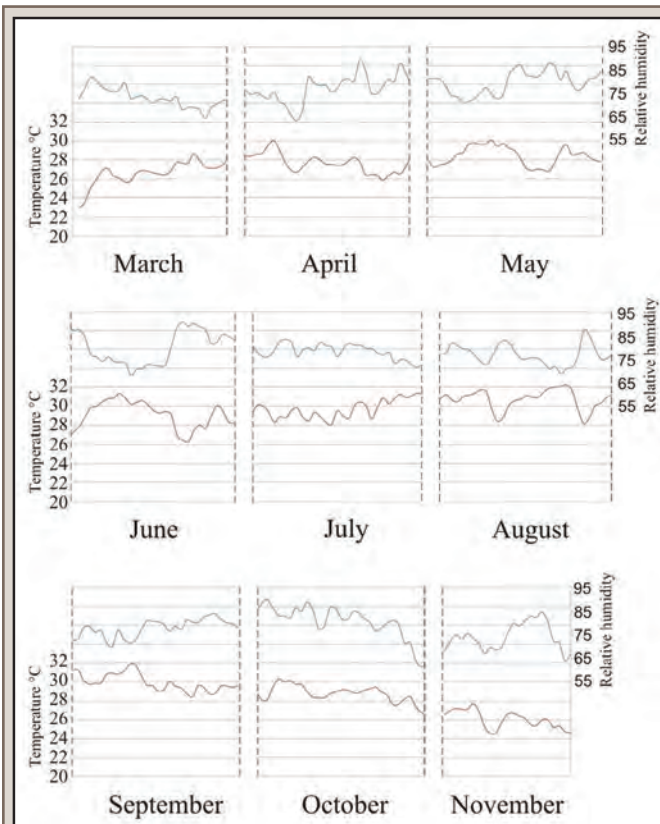


FIG. 6. Variation in temperature and relative humidity from March to November 2012.

**Captive behavior.**—Captive specimens were nocturnal. Soon after frogs began waking up they shed and ate the outer layer of skin, moving their legs and opening their mouths to swallow the tissue. During the day, frogs rested on leaves or inside rolled leaves (Fig. 7). Very often frogs were observed sleeping during the day on the concealed side of the leaves away from direct light. Other refuges were among rocks or in pockets at the base of plants. Males called from plant leaves, rocks, or the terrarium glass, distending a single, somewhat laterally lobed, yellow vocal sac (Fig. 1). Calling males sometimes interacted by jumping toward one another, displacing each other from perches, while



FIG. 7. Female *Osteopilus pulchrilineatus* sleeping in a rolled leaf of *Calathea* sp. (top). Amplexing pair in the water section of the terrarium (bottom).

producing clicking calls. Amplexus occurred at night (Fig. 7). On a few occasions, amplexant pairs were observed resting during the day. Like other hylid frogs, this species was observed catching prey by jumping on them from some distance.

**Captive breeding and early development.**—In our experience, *Osteopilus pulchrilineatus* was an opportunistic breeder, avoiding reproduction at temperatures that were below 24°C for an extended period and the driest periods. Frogs tended to be lethargic at temperatures that were near 32°C for a long time. Females produced oocytes throughout the year. Frogs were particularly active on warm, cloudy, or rainy days, but reproduction was observed under very dissimilar conditions. They usually bred at temperatures between 25–29°C, and a relative humidity over 75–80%. A decrease in atmospheric pressure seemed to be another stimulus for this species. Oviposition was always preceded by intense vocal activity, with adult males usually changing to a vivid yellow coloration. Amplexus was axillary (Fig. 7). The first egg clutch was found on 2 May 2012, after heavy outdoor rain. Seven egg clutches were obtained from 2 May 2012 to 18 May 2013. The egg clutch laid on 2 May 2012 had 327 eggs and one obtained on 11 February 2013 had 463 eggs. The exact number of eggs from the other clutches was undetermined. Clutches usually consisted of two or three groups of eggs that were apart from each other. Each group often had approximately 100–200 eggs, or less frequently as low as 50 eggs.

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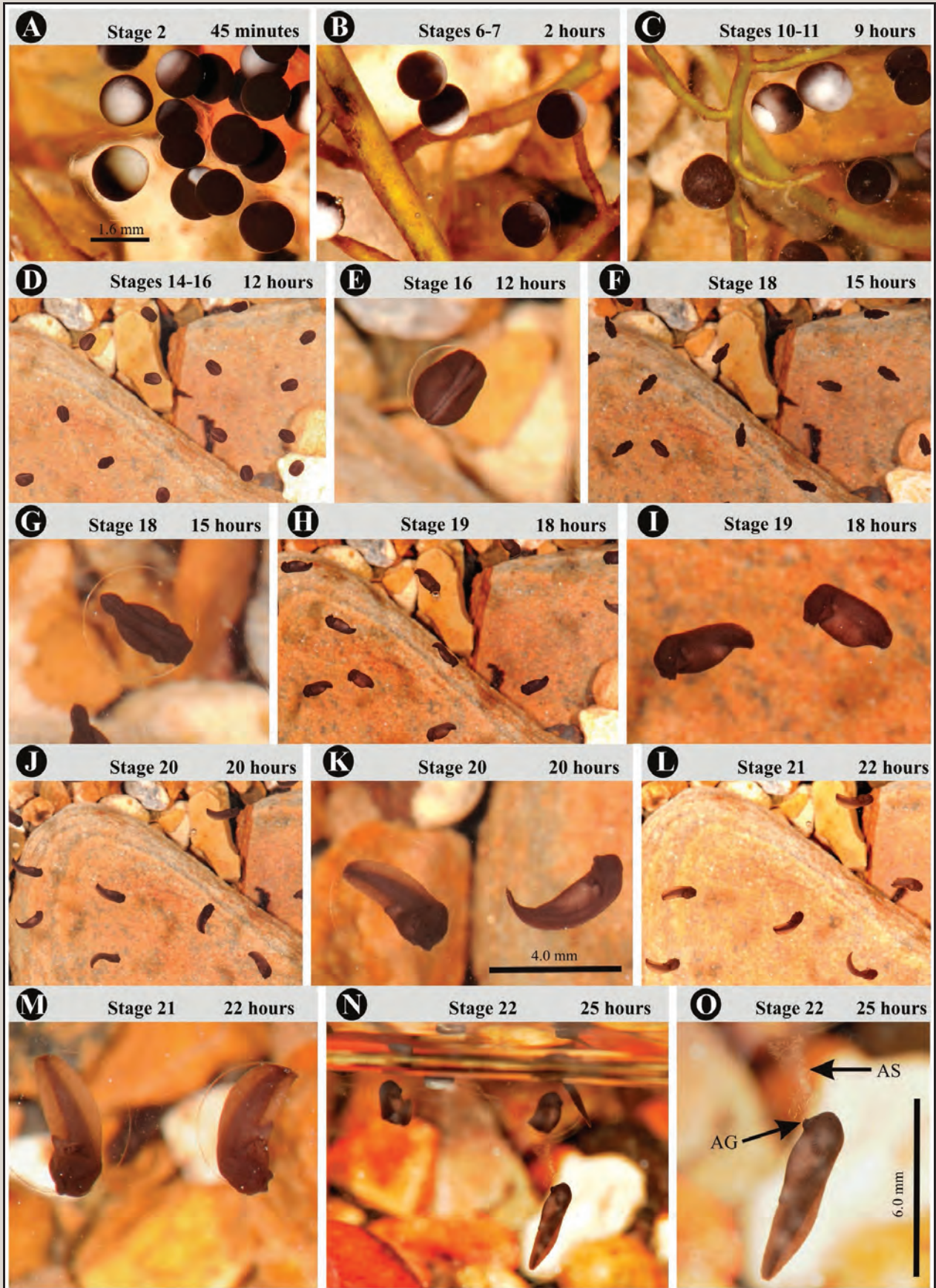


FIG. 8. Larval stages, from recently laid eggs (A) to hatchlings (N–O), in captive *Osteopilus pulchrilineatus*. Each frame specifies developmental timing. In O, AG is the adhesive gland, and AS is the adherent secretion.

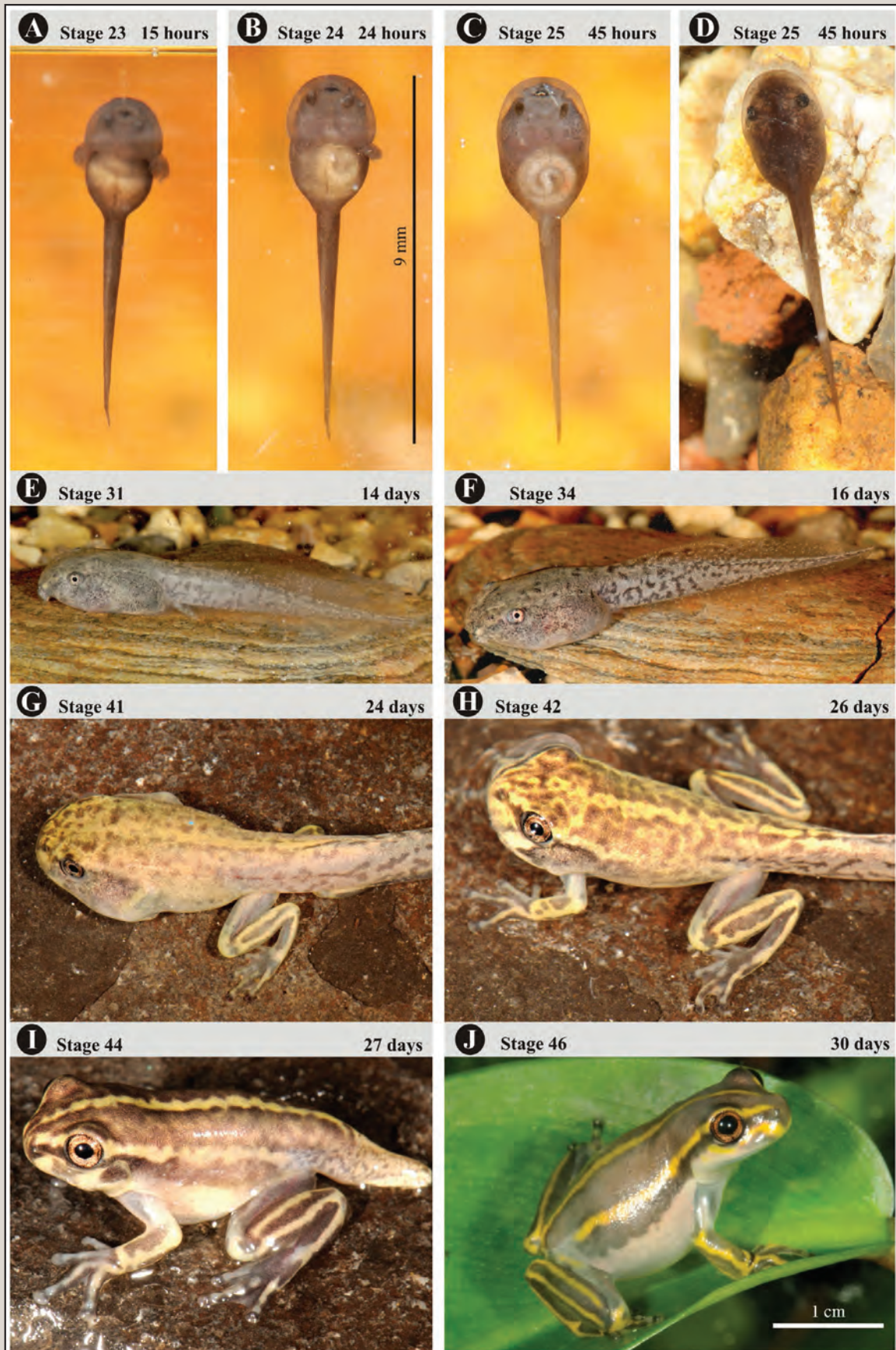


FIG. 9. Larval stages and metamorphs of *Osteopilus pulchrilineatus*. C and D are tadpoles in the same stage of development but in ventral (C) and dorsal (D) views.

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FIG. 10. Tadpole in Gosner Stage 31 with scoliosis.

At least three of the six observed clutches were laid around sunrise.

Clutches were laid on the surface of the water. At the beginning, the egg masses were somewhat compact but soon thereafter expanded as a one-egg-thick layer. Eggs were black (animal pole) and white (vegetal pole) as in other hylid frogs, and measured 1.4–1.6 mm in diameter. The eggs hatched after 25–30 h at 28–30°C. Embryonic development occurred very quickly at these temperatures but might be longer at lower temperatures. Representative embryonic stages in a chronological order are shown in Fig. 8. Hatchlings remained fixed to the vivarium glass, different objects, or even other eggs by means of a mucous secretion from the adhesive glands (Fig 8). After 15–16 h the larvae still had external gills, but 24 h later only one remained (Fig. 9A–B). After 40–48 h from hatching, larvae started swimming and searching for food (Fig. 9C–D).

Tadpoles metamorphosed in 26–40 days at 28–32°C. After 14 days from hatching, tadpoles were usually at Gosner Stages 30–31 and measured 29.3–33.6 mm (mean = 31.4 mm) in total length (Fig. 9E). After 16 days (Fig. 9F) they reached stages 33–35, measuring 33.7–38.4 mm (mean = 35.6 mm). Stages 36–37 were reached in 18 days, and the tadpoles were 36.1–40.8 mm (mean = 38.4 mm) in length. After 24 days (Fig. 9G) most tadpoles were in stages 40–41, and reached 40.0–48.4 mm (mean = 44.2 mm). The first individuals in stage 42 were usually found after 26 days. A tadpole in stage 44 is illustrated in Fig. 9H, and another in stage 45 in Fig. 9I. Two days later, recently metamorphosed frogs measured 13.6–15.1 mm (mean = 14.4 mm) snout–vent length (Fig. 9J). Tadpoles from different clutches, and even from different females, grew at a similar rate and in a quite synchronous way, but a few showed delayed metamorphosis. We estimated a tadpole survival rate of 90–95%, excepting those from the first clutch that developed scoliosis (see tadpole diseases below).

Tadpoles were reddish brown or grayish brown, with a profusion of iridocytes. The caudal muscle was somewhat lighter than the body and speckled or with a vermiculated pattern. Caudal fins had the same pattern as the caudal muscle or were immaculate. The eyes were dark brown from above; golden colored in the lateral view; the pupil was bordered by a yellow ring. Ventral coloration did not differ substantially from dorsal coloration, and was somewhat translucent or had scattered iridocytes. The tadpoles raised in spartan tanks were grayish colored and exhibited more conspicuously vermiculated tails than those raised in tanks with gravel on the bottom, which were

more reddish-brown in color with more speckled tails. The second condition is almost identical to that of tadpoles found in the wild. Tadpoles after Gosner Stage 36 gradually showed the yellow striping on the dorsum and hind limbs typical of the species (Fig. 9).

*Tadpole husbandry, feeding and diseases.*—Hatchlings were left in the aquatic section until they started swimming actively for food. At that time they were carefully siphoned and removed to separate tanks. If tadpoles were left in the aquatic section of the terrarium they ate eggs and hatchlings of new clutches. Tadpole rearing tanks were 55-liter Sterilite® plastic boxes (58.7 × 42.9 × 31.8 cm) filled with 30 L of water. Artificial lighting (programmed for 8 hours) was provided by means of Eiko® Supreme halogen lamps hung 55 cm above the water. Tanks were placed near a glass window through which they received diffuse natural light and a natural photoperiod. We kept medium densities (sensu Browne and Zippel 2007) of 5–8 tadpoles per liter. Two husbandry methods were used:

*Method 1.*—In the first attempt (May 2012), flat rocks were dispersed on each tank bottom to provide a natural substrate, but the tank was essentially spartan for ease of cleaning. Water was filtered by using a synthetic sponge and active carbon. Water quality was monitored every day for nitrites, nitrates, and phosphates. Water temperature ranged from 28–29°C, and pH was 7.8. Partial water changes (50%) were made twice daily, always removing debris from the tank bottom. Tadpoles were fed 3–4 times per day, with a combination of tropical marine fish flake food (Nutrafin® Max) and calcium (~1%, Tetra® Rep Cal). Artificially raised brown algae were also added. Algae remained for a longer time in the tank to allow grazing. After 12 days about 75% of surviving larvae in Gosner Stages 30–31 developed scoliosis (Fig. 9). Immediately, extra supplementation was provided (Repashy Superfoods® Calcium plus ICB). Tadpoles that started with problems became worse and were sacrificed. The remaining tadpoles metamorphosed normally after 30–40 days. A sample of 30 apparently healthy frogs was reared until they reached adult condition.

*Method 2.*—For all the other experiences, a gravel biological filter was installed on the bottom of tanks. Flat rocks were also added. Partial (50%) water changes were made daily. Active carbon was not used. Food was changed to a formula based on 50% Nutrafin® Basix + New Life Spectrum® color enhancing marine pellets, and 50% Spirulina powder. A homogeneous and compact mass of food was prepared, and small pieces were dispersed in the tank. Repashy Superfoods® Calcium plus ICB was added to the formula. Tadpoles were fed 3 times daily. Nitrite levels were very low, but nitrates increased after 48 h. Under these rearing conditions we do not recommend partial water changes more than two days apart. This husbandry method proved to be excellent for tadpoles; they showed a natural coloration and behavior and metamorphosed within the range of 26–40 days. No sign of scoliosis was observed.

Food consumption increased with larval growth. The turgid shape of tadpoles and activity patterns were used as indicators that they were eating enough. Tadpoles showed cannibalism on growth-retarded individuals. This behavior was observed until the second week after hatching, but greatly diminished later, when differences in size between larvae were smaller. Cannibalism has been observed to be common in tadpoles of the Cuban Tree Frog, *Osteopilus septentrionalis* (Crump 1986).

*Development and sexual maturity.*—The 30 metamorphosed frogs obtained from the first clutch were split into three groups of 10 frogs each in small (40 × 32.5 × 31 cm) plastic vivaria (Exoterra® Faunarium). The only furnishing was a potted *Scindapsus aureum* that served as refuge and provided perching surfaces. One corner of each terrarium was drilled to provide drainage. The vivaria were inclined to form a small aquatic section on one side. Containers received the same lighting as the adult enclosures and were cleaned three times weekly. In two months, frogs reached 22.2–30.1 mm (mean = 26.7 mm) and weighed 0.6–1.5 g. The first calling males with nuptial excrescences were observed two months post-metamorphosis, and females with evident black oocytes through the groin skin were observed after three months. This suggested reproductive maturity was reached quickly at the temperatures provided. At six months, females measured 32.7–36.2 mm (mean = 34.5 mm) snout–vent length and weighed 2.5–3.8 g (mean = 3.2 g); males 28.4–31.0 mm (mean = 29.3 mm), and weighed 1.3–1.9 g (mean = 1.7 g). In this sample, sexual proportion was 47% females and 53% males.

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