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## Journal of Natural History

Publication details, including instructions for authors and subscription information:

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### Reproductive biology of *Argenteohyla siemersi pedersenii* Williams and Bosso, 1994 (Anura: Hylidae) in northeastern Argentina

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Online publication date: 08 July 2010

**To cite this Article** Cajade, Rodrigo , Schaefer, Eduardo Federico , Duré, Marta Ines , Kehr, Arturo Ignacio and Marangoni, Federico(2010) 'Reproductive biology of *Argenteohyla siemersi pedersenii* Williams and Bosso, 1994 (Anura: Hylidae) in northeastern Argentina', Journal of Natural History, 44: 31, 1953 — 1978

**To link to this Article:** DOI: 10.1080/00222931003642590

URL: <http://dx.doi.org/10.1080/00222931003642590>

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## Reproductive biology of *Argenteohyla siemersi pedersenii* Williams and Bosso, 1994 (Anura: Hylidae) in northeastern Argentina

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(Received 4 June 2009; final version received 21 January 2010)

The present study describes the reproductive biology of *Argenteohyla siemersi pedersenii* in a modified “quebracho” forest of *Schinopsis balansae* in northeastern Argentina. Observations were made between January 2007 and March 2009 at El Perichón, Corrientes Province, Argentina. *Argenteohyla s. pedersenii* bred once per year over a 3-day period in a semi-permanent pond during the first spring rains in mid-September. Males called while floating on the water surface. The advertisement call was short and repeated at regular intervals in relatively fast succession, forming groups of multiple calls. Aggressive calls were evoked in two different agonistic interactions. Eggs were black, laid in three-dimensional arrangements interspersed within the submerged vegetation. Egg number per clutch ranged from 2638 to 8987. Tadpole redescription shows many differences and similarities with previous descriptions. The reproductive biology of *A. s. pedersenii* has several similarities with another pond-dwelling casque-headed frog species.

**Keywords:** Anura; *Argenteohyla siemersi pedersenii*; calls description; reproductive mode; tadpole redescription.

### Introduction

Many studies on reproductive biology have been conducted on anuran species (e.g. Crump 1974; Basso 1990; Rodrigues et al. 2007). Reproductive features provide the basis of comparative studies on the evolution of major natural history features (Giaretta and Facure 2008) and play an important role in formulating conservation strategies. Calls, eggs and ovipositional mode are involved in evolutionary processes and are essential reproductive features that contribute to understanding the evolutionary relationships among amphibians (Rand 1988; Halliday and Tejedo 1995; Sullivan et al. 1995; Altig and McDiarmid 2007). Other reproductive features, such as egg number per clutch and number of individuals that are capable of reproduction, are taken into account in anuran conservation categorizations (e.g. Lavilla et al. 2000; IUCN 2008).

The South American genus *Argenteohyla* Trueb 1970 is characterized by having a heavily ossified skull, and the skin of the dorsal surfaces of the skull partially co-ossified with the underlying bone (Trueb 1970). These characteristics presumably are associated with their taking refuge in bromeliads or tree holes as seen in *Aparasphenodon*,

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*Corythomantis*, *Itapotihyla*, *Nyctimantis*, *Osteocephalus*, *Osteopilus*, *Phyllodytes*, *Tepuihyla* and *Trachycephalus*. Frogs of these genera, and the genus *Argenteohyla*, are included in the tribe Lophiohylini (Miranda-Ribeiro 1926), which actually represents the monophyletic group known as “Casque-headed Frogs” as defined by Faivovich et al. (2005). The genus *Argenteohyla* comprises a single species, *Argenteohyla siemersi* Mertens 1937, with two allopatric subspecies: *A. s. siemersi* and *A. s. pedersenii*. The northern form, *A. s. pedersenii*, was described by Williams and Bosso (1994) from Corrientes, Argentina. It inhabits the forest of the Chacoan Domain, Oriental Chaco District (Cabrera and Willink 1980; Carnevali 1994) near San Roque, San Cosme, Corrientes Capital and Parque Nacional Mburucuyá (Williams and Bosso 1994; Céspedes et al. 1995). A single male of this subspecies was recorded from the Guairá Department, Paraguay (Trueb 1970). The conservation status of *A. s. pedersenii* has been evaluated as “Vulnerable Species” for Argentina (Lavilla et al. 2000) and the Paraguayan populations have not been evaluated (Brusquetti and Lavilla 2006). Recently the IUCN (2008) listed *A. siemersi* as “Endangered”.

The published data on this very poorly known species mostly represent systematic studies (Barrio 1966; Trueb 1970; De Sá 1983; Williams and Bosso 1994; Céspedes 2000), and there are very few data on the reproductive biology. The advertisement call has been described (Barrio 1966) for the southern form *A. s. siemersi*, and egg clutches and egg size have been reported for the northern form *A. s. pedersenii* (Diminich and Zaracho 2008). However, the reproductive biology of either subspecies of *A. siemersi* has not been studied in detail.

We compiled data over 2 years on the reproductive biology of *A. s. pedersenii* and report here the reproductive activity pattern and the calling behaviour, with a description of advertisement and aggressive calls; we analyse qualitative and quantitative data from the clutches and redescribe the tadpole.

## Materials and methods

Field work was carried out at El Perichón (27°25'55.6" S, 58°44'47.8" W) 10 km northeast from Corrientes City. This area is characterized by the presence of numerous temporary and semi-permanent ponds. Mean annual temperature is 21.5°C and the mean annual precipitation is 1500 mm without a pronounced dry season, although periods of rain shortages occur every 4–6 years (Carnevali 1994). The original vegetation was *Schinopsis balansae* “quebracho” forest, which is currently extremely degraded and largely replaced by sclerophyllous forest, with prevalence of *Acacia caven*, *Celtis* spp., *Prosopis affinis*, *Prosopis nigra*, and numerous colonies of *Aechmea distichantha* and *Bromelia* spp. (Carnevali 1994). Our observations were made in an elliptical semi-permanent pond with an estimated area of approximately 1960 m<sup>2</sup>. The water was clear and had a maximum depth of 50 cm. Emergent vegetation was abundant near the shore and consisted mainly of *Andropogon* sp., *Oxycaryum* sp., *Rhynchospora corymbosa* and *Vernonia* sp. The central area of the pond lacked vegetation. Other species of frogs in the pond included *Dendropsophus sanborni*, *Elachistocleis bicolor*, *Hypsiboas raniceps*, *Leptodactylus ocellatus*, *Physalaemus albonotatus*, *Physalaemus santafecinus*, *Pseudis limellum*, *Pseudis platensis*, *Rhinella bergi*, *Rhinella fernandezae*, *Rhinella schneideri*, *Scinax acuminatus*, *Scinax fuscomarginatus*, *Scinax nasicus* and *Trachycephalus venulosus*.

From January 2007 to March 2009, we sampled twice a week, as well as after any occurrence of rain, recording reproductive activity (calls, amplexant pairs and egg clutches) of *A. s. pedersenii*. Acoustic and visual surveys (19:00 to 24:00 h) were made during each period of fieldwork. When *A. s. pedersenii* individuals were reproductively active, we worked in the studied pond from 19:00 to 07:00 h. In the first year, we focused on recording advertisement calls, advertisement calling behaviour, and reproductive mode with quantitative reproductive data. In the second year, we focused on recording aggressive calls, observing calling behaviour, amplexant pairs, oviposition behaviour and redescribing the tadpole.

Advertisement and aggressive calls of *A. s. pedersenii* were recorded on 26 September 2007 and 30 September 2008, respectively, with an Audio-Technica® ATR55 directional microphone and a Panasonic® RQ-L31 tape recorder. We held the microphone approximately 60 cm above and 3 m in front of a calling male. Recordings were analysed with ADOBE AUDITION 1.0 software at a sample rate of 44100 Hz and 16 bits resolution. Frequency information was obtained through Fast Fourier Transformation (width 2408 points) at Blackmann–Harris window function. The sonograms and oscillograms were produced with SYRINX 2.2 b (Burt 2001). The analysed fragments of advertisement calls contained five call-groups for each specimen. We named call-groups according to the series of repeated simple vocalizations formed by a variable number of calls which are grouped in a structure that is repeated after variable periods of time. A total of eight different call variables were considered for the analysis: call duration(s), intercall interval(s), call period(s) (= call duration + intercall interval), call rate [(total number of calls in a call-group)/(time from the beginning of the first call to the beginning of the last call of a call-group's calling session)], call-group duration(s), number of calls in call-groups, intercall-group intervals(s), and dominant frequency (Hz) (Schneider and Sinsch 2006).

The analysed fragment of aggressive call contained five calls. A total of three variables were considered for the analysis: call duration(s), intercall interval(s) and dominant frequency (Hz). Means and standard deviations were calculated for each temporal variable. After recording the males, we collected and measured their snout–vent lengths (SVL) with calipers (0.01-mm scale) and their body mass (BM) with a pesola spring balance (0.1-g scale). We also measured water temperature (°C) and water depth (cm) at the calling sites. Call types were classified according to Wells (2007). Censuses of calling males and chorus durations were made in each breeding period. The chorus size was determined with acoustic and visual surveys in the first year, and one survey made in the second year involved collecting and marking males by toe-clipping. On the night of 26 September 2007, we marked the calling sites with stakes with vinyl flagging tape, and on the next day, we measured the distances between calling sites to describe the chorus spatial distribution.

Observations on amplexus were made from 29 September to 1 October 2008. Males and females in amplexus were marked by toe-clipping and their SVL was measured with calipers (0.01-mm scale) and their BM with a pesola spring balance (0.1-g scale). Sexual dimorphism both in SVL and BM was tested with a multivariate analysis of variance (MANOVA). All data were tested for normality. All statistical analyses were performed using STATISTICA 6.0 statistical package. Observations of egg clutches were made during the day on 25 September 2007. We measured the clutch diameter as viewed from above and water depth at the oviposition sites. Because of the limited number of clutches found and considering that *A. s. pedersenii* is an

endangered species (IUCN 2008), we collected only three clutches. They were selected visually by size, to obtain an understanding of the variation in the number of eggs per clutch. Eggs per clutch were counted in the laboratory and measured to the nearest 0.01 mm with an ocular micrometer in an Olympus® stereomicroscope. Tadpoles that hatched were raised to metamorphosis in the laboratory to confirm identity.

Tadpoles used for redescription ( $n = 8$ ) were collected in the study pond on 20 October 2008 and fixed in 5% formalin. Description of external morphology and drawings were based on tadpoles at stages 34–36 (Gosner 1960). Terminology of external morphology traits and measurements followed Altig and McDiarmid (1999); body and total lengths were measured with calipers (0.01 mm), and other measurements were made with an ocular micrometer (0.01 mm) in a Zeiss® stereomicroscope. Four individuals at stage 36 were prepared for scanning electron microscopy (SEM) of the internal buccal anatomy and keratinized teeth. Terminology of internal surface features followed Wassersug (1976). Observations on ontogenetic changes in colouration were based on tadpoles raised in the laboratory and from tadpoles observed in the field. Voucher specimens (CECOAL 006–013) and juveniles (CECOAL 014–016) are housed in Centro de Ecología Aplicada del Litoral (CECOAL-CONICET), Corrientes, Argentina. The tadpole that is illustrated corresponds to CECOAL 013.

Temperatures and daily rainfall were obtained from the meteorological station at the Centro de Información Meteorológica del Servicio Meteorológico Nacional, Comando de Regiones Aéreas de la Fuerza Aérea Argentina located 2 km from the study site.

## Results

### *Reproductive activity pattern*

The pond was completely dry from May to August in 2007 and 2008. The wet season began in the middle of September in both years (Figure 1 A), and the ponds remained full during the wet seasons. The breeding activity of *A. s. pedersenii* occurred immediately after the first heavy rains at the beginning of each wet season and was associated with a notable decrease in temperature in the previous days (Figure 1B,C). The first reproductive event began on 24 September and concluded on 26 September 2007, and the second began on 29 September and concluded on 1 October 2008. We detected occasional calling activity once in each wet season following heavy rain after the major breeding event. In both cases, this occasional calling activity lasted only one night and we found no evidence of reproduction.

### *Advertisement call and calling behaviour*

Three males were recorded and captured on 26 September 2007 from 21:20 to 22:40 h. Water temperature during recordings was 20°C. The SVL of the three males was 69.82 mm (male 1), 72.32 mm (male 2) and 73.82 mm (male 3). Depth of water at calling sites was 27 cm (male 1), 32 cm (male 2) and 27 cm (male 3). The short advertisement call of *A. s. pedersenii* (0.030–0.053 s) is repeated in groups at regular intervals (Figure 2). The

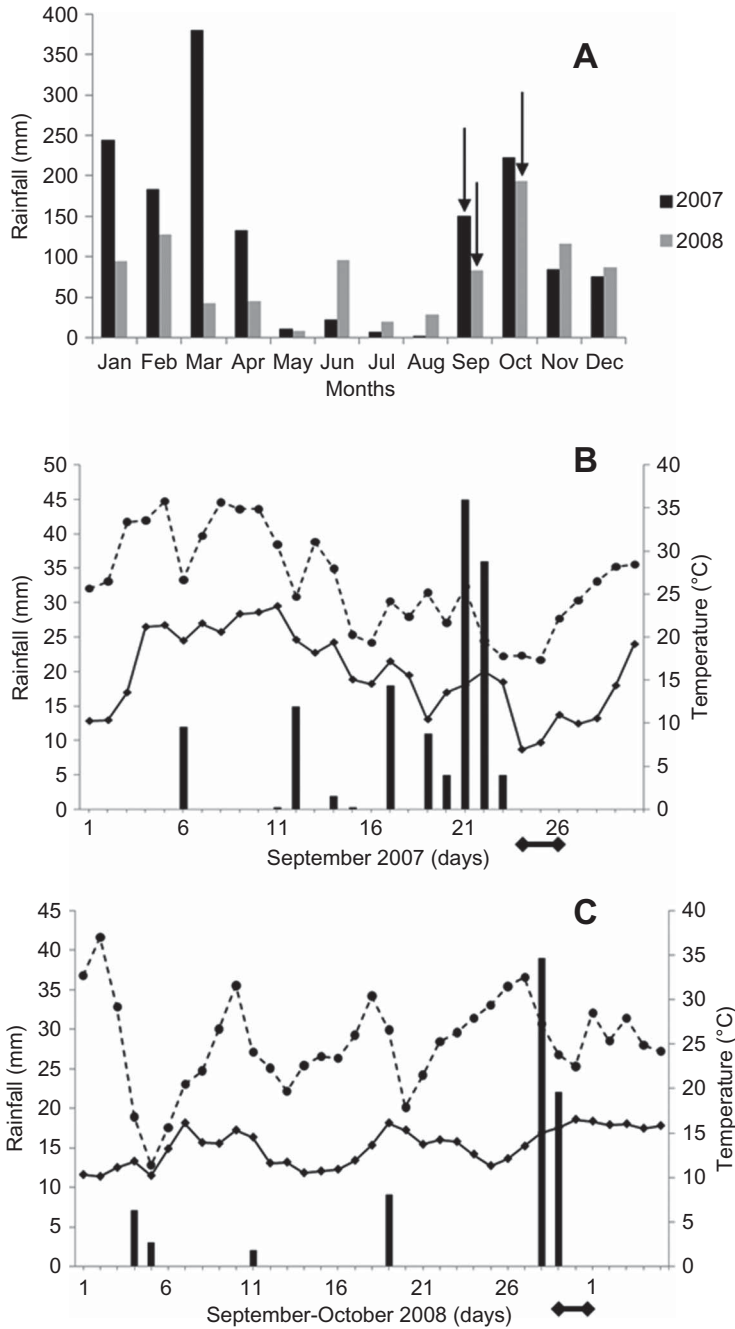


Figure 1. (A) Monthly accumulated (mm) rainfall during 2007 and 2008 (arrows indicate the reproductively active months of *Argenteohyla siemersi pederseni*). (B,C) Details of the average daily air temperatures (maximum and minimum) and accumulated daily rainfall registered during (B) September 2007 and (C) September and first 5 days October 2008. In (B) and (C), active breeding days of *A. s. pederseni* are those between the two dark diamonds.

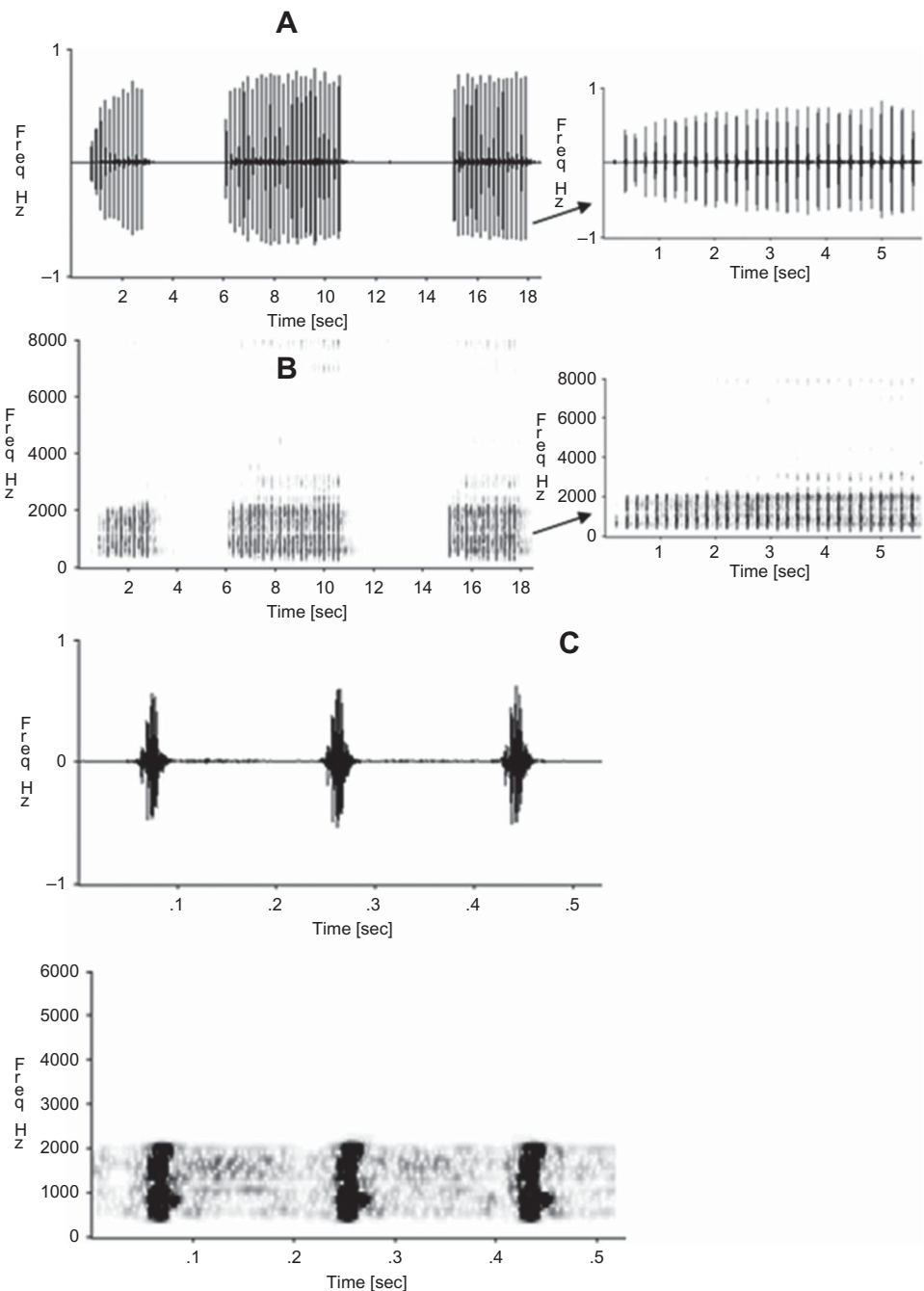


Figure 2. (A) Oscillograms and (B) sonograms for three call-groups of *Argenteohyla siemersi pedersenii*, Corrientes, Argentina and (C) detail of a single call-group, oscillogram (above) and sonogram (below).

Table 1. Calls variables used for quantitative description of *Argenteohyla siemersi pedersenii* courtship calls; means  $\pm$  SD.

	<i>Argenteohyla siemersi pedersenii</i>		
	No. 1	No. 2	No. 3
Number of call groups analysed	5	5	5
Number of calls analysed	63	75	75
Call duration (s)	0.039 ( $\pm$ 0.006)	0.039 ( $\pm$ 0.004)	0.036 ( $\pm$ 0.005)
Intercall interval (s)	0.120 ( $\pm$ 0.011)	0.140 ( $\pm$ 0.009)	0.136 ( $\pm$ 0.008)
Call period (s)	0.159 ( $\pm$ 0.011)	0.177 ( $\pm$ 0.019)	0.171 ( $\pm$ 0.008)
Call rate	6.180 ( $\pm$ 0.067)	5.469 ( $\pm$ 0.163)	5.712 ( $\pm$ 0.110)
Call group duration (s)	2.289 ( $\pm$ 0.746)	4.319 ( $\pm$ 1.339)	3.417 ( $\pm$ 1.409)
Number of calls per group	14.8 ( $\pm$ 4.55)	24.2 ( $\pm$ 6.943)	20.2 ( $\pm$ 7.727)
Intergroup interval (s)	3.721 ( $\pm$ 1.409)	37.685 ( $\pm$ 42.262)	20.2376 ( $\pm$ 18.775)
Maximum number of calls per group	20	29	30
Minimum number of calls per group	8	12	13
Dominant frequency (Hz)	1933 ( $\pm$ 108)	1943 ( $\pm$ 128)	1771 ( $\pm$ 201)

call is similar to the sound made by knocking two pieces of wood together and sounded like “tok”. The intercall interval ranged from 0.100 to 0.170 s. Each call-group was composed of 8–30 calls with duration of 1.180–5.420 s. The time between call-groups was highly variable with a very short call interval of 1.710 s and other truly long intervals that reached 92.860 s (Table 1). The dominant frequency ranged from 1700 to 2070 Hz.

Male *A. s. pedersenii* called from the water surface near emergent vegetation, while floating in a horizontal position with their vocal sacs inflated and their hind limbs extended. When the males stopped calling, their vocal sacs and lung remained semi-inflated so they continued to float. Frogs are totally exposed during vocalizations, but they submerged quickly at any disturbance. In the first year of reproduction, the chorus activity began in the evening between 19:00 and 20:00 h with maximum activity between 22:00 and 24:00 h; calling subsided in the predawn hours. No calling occurred during daylight. The number of males in the chorus was 12 on the first day and 14 on the remaining 2 days. Males were distributed around the perimeter near emergent vegetation and called in duets ( $n = 4$ ) or trios ( $n = 2$ ); each group was separated by 7–10 m (mean = 8.63, SD = 0.98,  $n = 6$ ). Within each group, the males were separated at about 1–2 m (mean = 1.62, SD = 0.38,  $n = 14$ ). Chorus activity was initiated by a single male that was subsequently joined by the rest of the members of the group. In a synchronized way, the males of nearby groups began to vocalize until all individuals within the chorus were calling. In the same way, the chorus was interrupted when a male in either duet or trio stopped calling.

In the second year, the chorus activity began in the evening between 19:00 and 20:00 h and continued for 3 days even intermittently during daylight hours. The chorus was composed of 51 males, distributed around the perimeter of the pond near the emergent vegetation. Males were separated by short distances and not organized into duets or trios; several males were observed swimming between the



emergent clumps of vegetation. Males did not synchronize their calling to start or end the chorus.

*Aggressive call and calling behaviour*

Aggressive calls (Figure 3A,B) were detected only among males in the larger chorus during the second year. One male recorded (20°C and 14°C, water and air temperature, respectively) and captured on 30 September 2008 weighed 15 g and measured 63.5 mm in SVL. The aggressive calls of *A. s. pedersenii* consist of a pulsated note that ranges from 0.153 to 0.192 s (mean = 0.175, SD = 0.015,  $n = 5$ ). The intercall interval

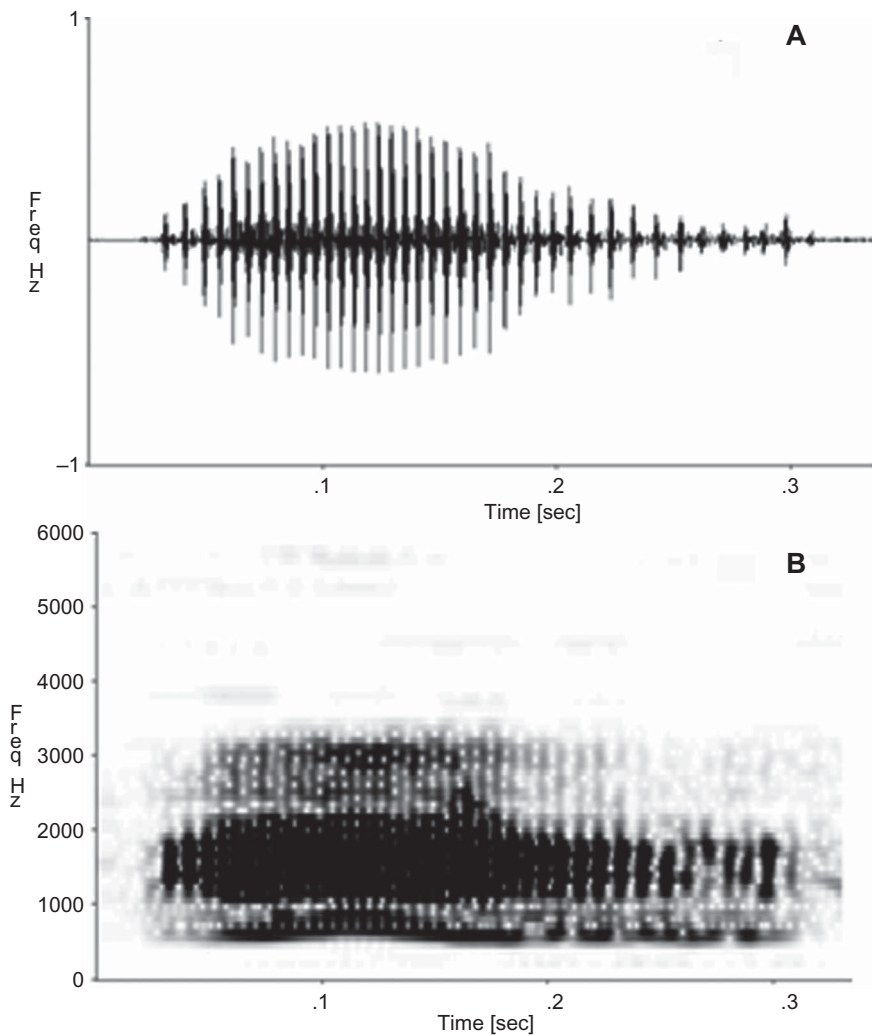


Figure 3. Oscillogram (A) and sonogram (B) of one aggressive call of *Argenteohyla siemersi pedersenii*.

ranged from 0.386 to 1.715 s (mean = 0.979, SD = 0.647,  $n = 4$ ), and the dominant frequency ranged from 1335 to 1421 Hz (mean = 1403, SD = 38.46,  $n = 5$ ). Amplitude modulation was irregular with maximum amplitude at 0.09 s, after which time it declined asymmetrically toward each end.

Aggressive calls were emitted in two different situations. In a single case, two nearby males were emitting courtship calls, and suddenly both males stopped calling and swam toward each other while emitting aggressive calls. The males continued emitting aggressive calls while trying to move the other by pushing with their vocal sacs inflated. The males did not fight with their forelimbs, finished rapidly, and returned to their calling sites. In three other situations, we observed an amplexant pair out of water on an emergent plant and one male floating on the water surface beneath the pair emitting an advertisement call. This male called while facing the pair and the male in amplexus emitted aggressive calls. This interaction stopped when the male lost visual contact with the amplexant pair, which moved to another position.

### *Amplexus and clutches*

Twenty-five pairs of *A. s. pederseni* in axillary amplexus were found during the three nights of the second year, between 21:00 and 02:00 h. Twenty-four amplexant pairs were observed on the second night (30–31 September 2008) and one pair was found on 31 September–1 October 2008. At first, the pairs were observed out of water on emergent vegetation where they rested for about 2 h. At this time all females in amplexus were gravid. Next, the pairs were floating at the water surface with an egg clutch nearby. We did not observe spawning. In females, SVL ranged from 69 to 83 mm (mean = 74.76, SD = 4.00,  $n = 25$ ) and BM ranged from 16.50 to 44 g (mean = 25.24, SD = 5.77,  $n = 25$ ). In males, SVL ranged from 60 to 76 mm (mean = 68.95, SD = 4.20,  $n = 25$ ) and BM ranged from 11 to 28 g (mean = 18.46, SD = 4.03,  $n = 25$ ). Sexual dimorphism both in SVL and BM was verified (Wilk's  $\lambda = 0.613$ ,  $F_{2,47} = 13.71$ ,  $p < 0.001$ ) where females were larger ( $F_{1,48} = 24.06$ ,  $p < 0.001$ ) and heavier ( $F_{1,48} = 24.80$ ,  $p < 0.001$ ) than males.

Qualitative and quantitative descriptions of eight clutches were made on 27 September 2007, after reproductive activity had ceased. Water depth at the oviposition sites was 20–31 cm (mean = 24.860, SD = 3.280,  $n = 8$ ). Each clutch was composed of several groups of eggs arranged in three-dimensions from the surface to near the bottom among submerged parts of the emergent plants. Eggs were not included in a common matrix, and the three-dimensional arrangements were formed by the adhesion of the outer jelly layers of adjacent eggs. The groups of eggs in each clutch were composed of a few to many series of eggs which can have an irregular or longitudinal shape and varied in size. Several single eggs were observed between the egg groups. The clutch diameter ranged from 40 to 63 cm (mean = 50.380, SD = 8.480,  $n = 8$ ). The eggs were black with diameters of 1.67–1.83 mm (mean = 1.790, SD = 0.060,  $n = 10$ ); the number of eggs per clutch ranged from 2638 to 8987 (mean = 4887, SD = 3556.280,  $n = 3$ ).

### *Tadpole redescription*

Tadpole measurements are given in Table 2. Body oval in lateral, dorsal and ventral views (Figure 4A–C) representing 42% of total length; maximum body height near

Table 2. Measurements (mm) of the tadpoles of *Argenteohyla siemersi pedersenii*; mean values for tadpoles at stage 34–36 (Gosner, 1960), the SD, maximum and minimum values and coefficient of variation.

Character	Mean	SD	Min	Max	CV
Total length	54.64	3.05	49.75	57.1	5.59
Body length	22.74	1.32	20.68	24.35	5.79
Body width	14.11	1.59	11.20	16.35	11.24
Body height	13.33	1.01	11.47	14.45	7.57
Tail length	31.92	2.03	29.10	34.26	6.35
Tail height	15.25	1.20	11.25	15.24	9.25
Dorsal fin height	4.75	0.75	3.75	6.25	15.80
Ventral fin height	4.62	0.36	4.16	5.41	7.89
Caudal muscle height	3.58	0.42	3.16	4.33	11.73
Nostril diameter	0.42	0.05	0.33	0.50	12.26
Eye diameter	1.76	0.12	1.58	1.92	6.76
Internostril distance	4.89	0.29	4.41	5.16	6.02
Interorbital distance	7.44	0.35	6.99	7.83	4.75
Snout-nostril distance	3.55	0.44	2.92	4.16	12.28
Nostril-eye distance	1.75	0.20	1.50	2.08	11.67
Oral disc width	5.18	0.30	4.75	5.58	5.80
Rostral gap	1.64	0.24	1.17	1.99	14.51
Upper jaw width	2.44	0.31	2.08	2.92	12.60

beginning of dorsal fin; maximum body width at middle third of body; body about 1.1 times higher than wide; snout truncated in lateral and dorsal views; eyes dorsolateral, located above the midline of body; nostrils oval, dorsolateral, closer to eyes than snout, with openings directed anterolaterally; interorbital distance about 2.5 mm wider than internostril distance; spiracle single, medium size, sinistral, located below midline of body and at limit between the anterior third and middle third of body, opening directed upward and backward; vent tube dextral, shifted slightly to right; caudal musculature tapering to pointed tip; dorsal fin originating at middle third of body; ventral fin originating near origin of vent tube; both fins arched, with margins undulated and maximum caudal height at limit between anterior and middle third; tail height about 1.9 mm higher than body height; ventral fin slightly higher than dorsal fin (about 0.14 mm); caudal tip ends abruptly; oral disc subterminal, elliptical, not emarginate, body 2.72 times wider than oral disc. Labial tooth row formula (LTRF) 2(2)/5(1) (Figure 4D); final lower tooth row fragmented, and submarginal papillae with labial teeth present; three specimens with LTRF of 2(2)/4(1) at stage 34; marginal papillae pigmented, present on most of oral disc, except for a wide gap on the upper labium; papillae triserial throughout except tetraserial ventrolaterally; jaw sheaths serrate; upper jaw long and arched; lower jaw narrow, wide V-shaped. In SEM photomicrographs, serrations on jaw sheaths cone-shaped (Figure 5A). Teeth of all rows with spatulate, convex heads bearing 12–14 well-marked cusps (Figure 5B).

Buccal floor (Figure 6 A) triangular, wider than long; 12 small conical papillae anterior to infralabial papillae; one pair of broadly-based infralabial papillae, medium-sized, compressed and U-shaped, without any projections and with rough margins; two simple, cylindrical, lingual papillae; buccal pockets transverse to the axial

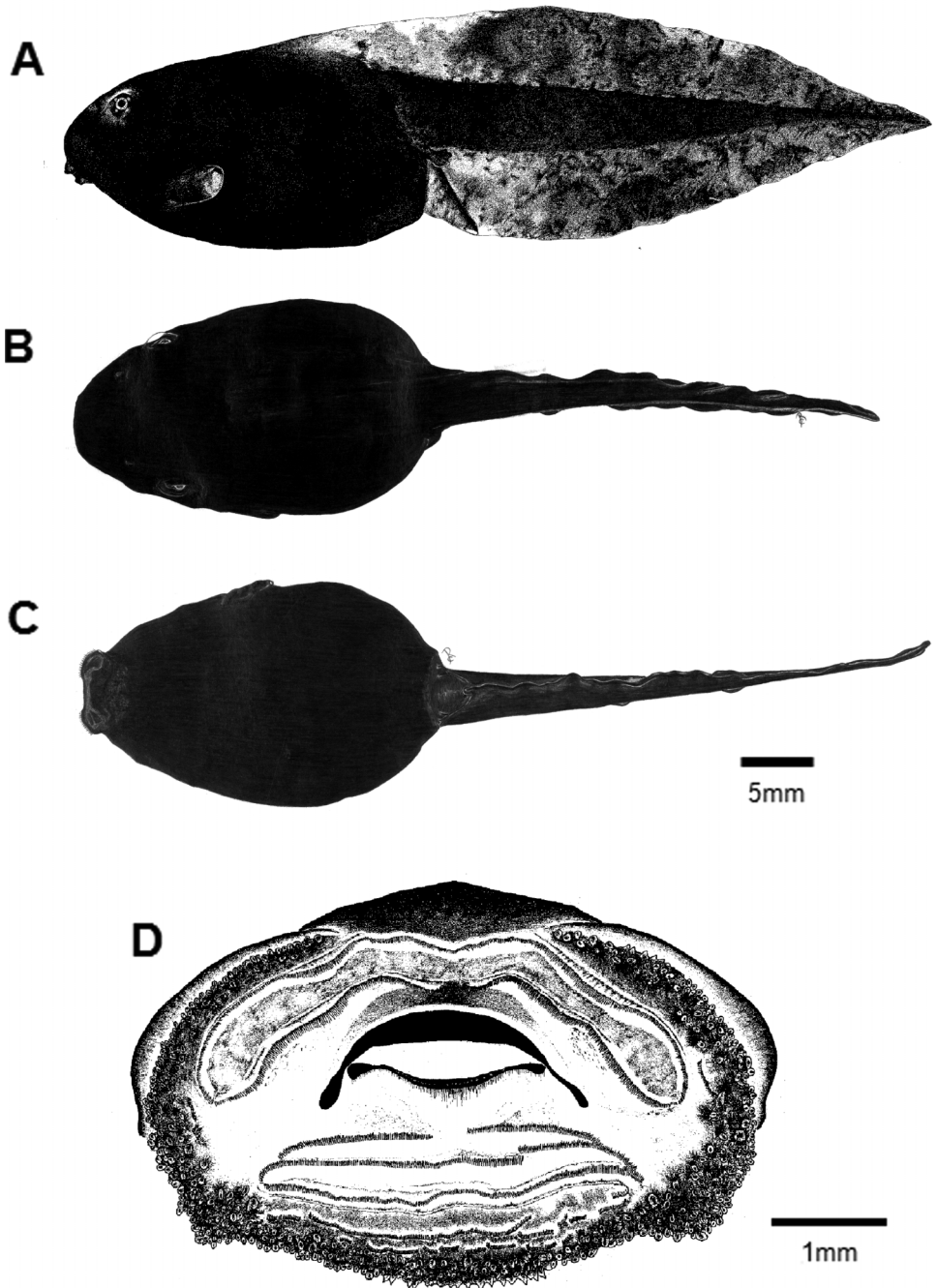


Figure 4. Lateral (A), dorsal (B) and ventral (C) views, and the oral disc (D) of *Argenteohyla siemersi pedersenii* tadpole at stage 35 (Gosner 1960).

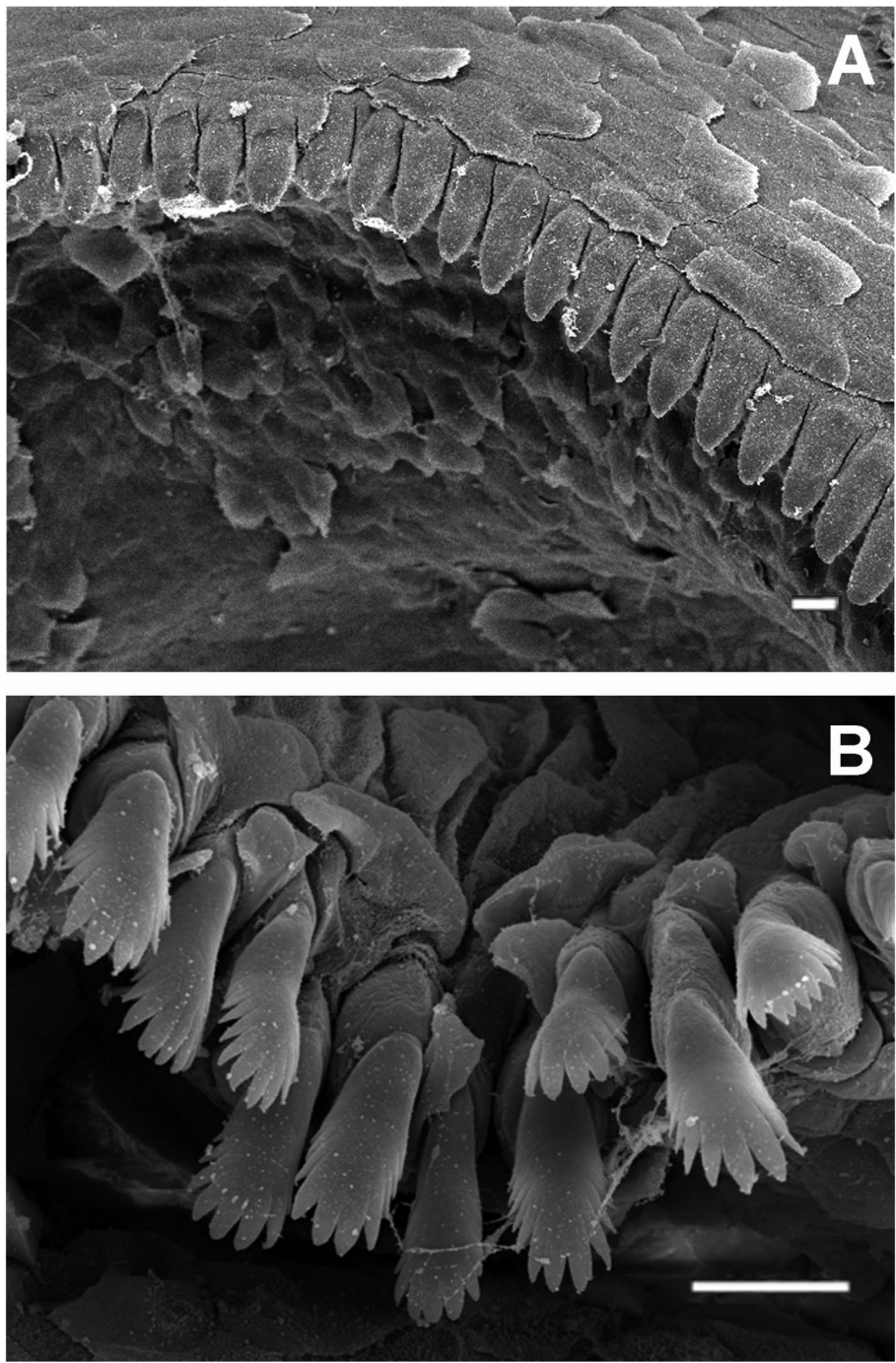


Figure 5. Scanning electron micrographs of edge of jaw sheath (A), and (B) keratodonts from the highest row. Scale bar represents 20  $\mu$ m.

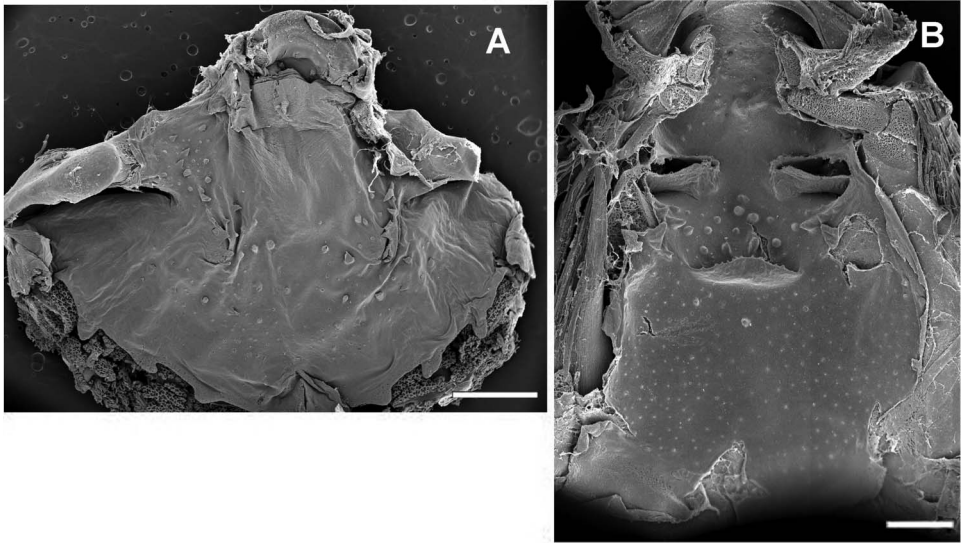


Figure 6. Scanning electron micrographs of buccal floor (A) and buccal roof (B). Scale bar represents 1 mm.

axis; buccal floor arena with centre bare, some pustules and conical papillae concentrated in a lateral line from buccal pocket to posterior margin of arena; three or four small papillae on each side of buccal floor arena, arising as flaps from a common base; long free velar surface with conspicuous spicular support; posterior velar margin semi-circular, with three long marginal projections on each side of well-marked median notch; dense secretory pits along margin of velar surface, glottis distinct, totally exposed.

Buccal roof (Figure 6B) longer than wide; prenarial arena broad with few small scattered conical prenarial arena pustulations; nares moderate sized, oriented 85–90° from transverse plane, with uneven edges; postnarial arena with three to five conical postnarial papillae below each nares arranged in anteriorly concave arch and some pustulations; median ridge large, wider than long, semicircular with rugose margin; lateral-ridge papillae triangular with irregular margins; buccal roof arena densely pustulated; three triangular lateral arena roof papillae aligned; large conspicuous, glandular zone with dense secretory pits.

In life, body uniformly black with blue reflections. Anterior dorsal fin less pigmented and translucent. Red spots present in fins from stage 23 tend to disappear gradually at stages 34–35 (Figure 7). Specimens darken gradually from first stages to reach invariable black colour by stages 34–36. Internal nostril surface and iris red. Preserved specimens retain uniformly black colouration but less intense. Red spots lose intensity with time. Juvenile colouration resembles that of adults.

## Discussion

### *Reproductive pattern*

Reproduction occurred in the middle of September with rainfall and a marked decrease of maximum temperatures (Figure 1B,C). This period includes the reproductive

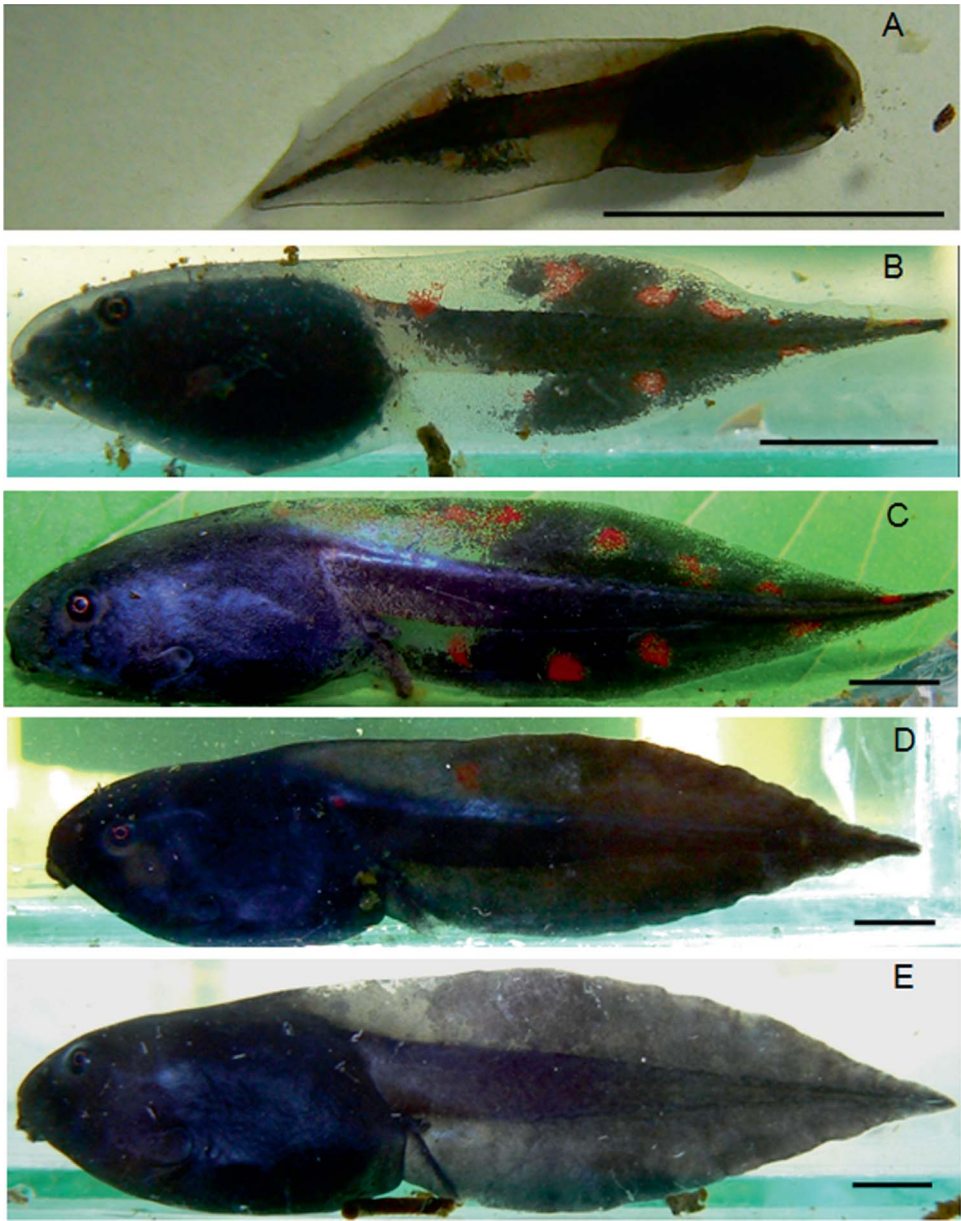


Figure 7. Ontogenetic colouration changes of the tadpoles of *Argenteohyla siemersi pederseni* at stages 23 (A), 29 (B), 33 (C), 34 (D) and 35 (E). Scale bar represents 5 mm.

period of July–September proposed by Céspedes (2000) for the same subspecies, but differs from the reproductive period of *A. s. siemersi*, which breeds during November (Barrio 1966). The short reproductive period suggests that this subspecies is an explosive breeder (Wells 1977a), and such behaviour has been reported for *A. s. siemersi* (Barrio, 1966) and *Aparasphenodon venezolanus* (Paolillo and Cerda 1981), *Osteocephalus*



*lepriurii* and *Osteocephalus taurinus* (Hödl 1990; Jungfer and Hödl 2002), *Osteopilus septentrionalis* (Savage 2002; Vargas Salinas 2006), *Trachycephalus venulosus* (De La Riva et al. 1995; Savage 2002; Rodrigues et al. 2005), *Trachycephalus coriaceus* (Schiesari and Moreira 1996), *Trachycephalus mesophaeus* (Prado et al. 2003; Da Silva 2006) and *Trachycephalus dibernardoi* (Kwet and Solé 2008). It has been hypothesized that explosive breeding is a strategy for reducing the risk of predation in the early instances of development and for the assurance of full ponds for larval metamorphosis (Wells 1977a; Duellman and Trueb 1994). The breeding pond used by *A. s. pedersenii* was dry both years before the rains began in September. Once they filled, common water predators in this pond were negligible. Fish were not present for the first 20 days, after which the pond was gradually colonized by fish. The pond was full until April when the rain became scarce and it started to dry up. Explosive breeding in *A. s. pedersenii* immediately after the pond filled could be a strategy to avoid loss by predation rather than to avoid the mortality risk caused by water evaporation and the pond drying up.

### **Advertisement call and calling behaviour**

The advertisement call plays a fundamental role in anuran reproduction and is involved in mate recognition, reproductive isolation and speciation (Rand 1988; Halliday and Tejedo 1995; Sullivan et al. 1995; Wells 1977a, b). Analyses of advertisement calls have played an important role in taxonomic decisions, especially in the families Hylidae, Ranidae, Bufonidae and Leptodactylidae (Schneider and Sinsch 2006). Studies that compare the calls of the subspecies of *A. siemersi* were suggested by Williams and Bosso (1994) as necessary to verify their correct taxonomic status. More recently, Lavilla and Cei (2001) suggested that a more complete evaluation of *A. s. pedersenii* would probably verify it as a distinct species. In view of this hypothesis, we had hoped to find consistent differences in the general call structure and call variables between the calls of the two subspecies of *A. siemersi*, but none was found. The overall call structure of *A. s. pedersenii* consisted of a short note repeated in groups at regular intervals, with a dominant frequency of 1700–2070 Hz. This call structure is similar to that observed in the audiospectrogram given by Barrio (1966) for *A. s. siemersi*. The values of dominant frequency, intercall interval, call duration and call number per call group are similar in both subspecies (2000 Hz; 0.12–0.18 s; 0.04 s; and 8–30 calls; Barrio 1966 and Table 2 for *A. s. siemersi* and *A. s. pedersenii*). Although we observed similarity between the calls of *A. s. pedersenii* and those of *A. s. siemersi* reported by Barrio (1966), a definitive conclusion about the taxonomic status is not warranted. Barrio (1966) described the advertisement call of *A. s. siemersi* from the Isla Talavera, Buenos Aires province, Argentina but only provided values for a single call without consideration of call variability and did not assign the call to a voucher specimen. His sonogram figured one call group of nine calls and does not have good resolution. Furthermore, he did not provide the methods used for his recordings and analysis. Conclusions could be biased by differences in the methods used by each author in the acoustic analysis and could be limited by the degree of detail in the description of each call. New recordings of vocalizations with more detailed analysis of the call of *A. s. siemersi* are needed.

Jungfer and Hödl (2002) hypothesized that species of *Osteocephalus* and *Trachycephalus*, that have a single subglular vocal sac and a small body, are adapted to breeding



and calling from leaf-axils and treeholes as opposed to traditional pond-breeding species, which have paired lateral vocal sacs. The males of *A. s. pedersenii*, breed in ponds, have paired vocal sacs, reach 76 mm in body size (Cajade, personal observation) and call from the water's surface. In the environment where *A. s. pedersenii* occurs, bromeliads with water reservoirs are available throughout the wet season. Despite the fact that *A. s. pedersenii* use these bromeliads as refuges, they are not used as breeding or calling sites. The paired vocal sacs and the large body size in *A. s. pedersenii* could be an impediment to males using bromeliads as a breeding or calling site.

The behaviour of males in breeding aggregations varies with density. At low densities, the mating system is the typical stationary calling male. With increases in density, the distance between calling males is reduced and agonistic interactions occur. Also males can change their mating system from stationary calling to active searching for females. In intermediate densities, males may alternate between systems (Wells 1977a). In the first year, male densities of *A. s. pedersenii* were low, and the chorus was organized in small groups of males that called in duets and trios; the start and end of chorus activity seemed to be organized. However, we could not distinguish a chorus organization in the larger chorus observed during the second breeding season, when male behaviour was more consistent with that at intermediate densities. We observed agonistic interactions and males swam continuously from one emergent plant to another. These swimming males could represent males actively searching for approaching females. Large reproduction aggregations with actively searching males have been reported for *Trachycephalus mesophaeus* (Da Silva 2006). However, we did not observe females being intercepted by swimming males, and these searching behaviours in *A. s. pedersenii* could also reflect searches for free calling sites.

### *Aggressive call and calling behaviour*

An aggressive call is evoked during any kind of aggressive encounter between males, including long-range and short-range signals. Aggressive calls are often modifications of advertisement calls (Wells 2007). In *A. s. pedersenii* the aggressive call consisted of a long pulsated note, whereas its courtship call was a short knock-like "tok" repeated at regular intervals in a relatively fast succession of groups. Despite this difference between calls, the pulses within the long pulsated note in the aggressive call of *A. s. pedersenii* have a similar structure (knock-like tok) to its courtship call. This suggests that the aggressive call of *A. s. pedersenii* could be a modification of the advertisement call caused by a much higher advertisement call repetition rate. Aggressive calls with a structure similar to that of advertisement calls, but with a much higher pulse repetition rate, have been reported for several hylids such as *Dendropsophus ebraccatus* (Wells and Greer 1981), *Dendropsophus microcephala* (Schwartz and Wells 1985) *Dendropsophus minutus* (Cardoso 1981) *Dendropsophus nanus* and *Dendropsophus sanborni* (Martins and Jim 2003), and *Dendropsophus phlebodes* (Schwartz and Wells 1984). The aggressive and advertisement calls of *A. s. pedersenii* not only differ in temporal structure but also in dominant frequency. The aggressive call had a lower dominant frequency than the advertisement call. Several frogs lower the dominant frequency of their advertisement calls when responding to the calls of other males and this has been interpreted as an aggressive response (Wells 2007). Lowering the dominant frequency in aggressive compared with advertisement calls could be

interpreted as a means of conveying information about the size of the caller. Thus, in an aggressive encounter between two males, each assesses the probability of being successful in a fight through an evaluation of their respective sizes.

Physical combat usually follows the aggressive calls in male–male interactions at short range. Fights involving physical contact can be quite variable and can include different levels of aggression, such as jumping at one another, claspings, kicking and pushing with the forelimbs (Wells 1977a), and also, as was observed in our study, pushing with the vocal sacs inflated. However, aggressive behaviour does not always occur in response to aggressive calls given at short-range. Males in amplexus can emit the aggressive call in response to other males that try to clasp the female or clasp the male in amplexus, or in response to a male calling near an amplexant pair. This behaviour has been reported in other anuran species (e.g. *Pachymedusa dacnicolor* and *Agalychnis callidryas*, Pyburn 1970; *Rana temporaria*, Walkowiak and Brzoska 1982; and *Physalaemus fernandezae*, Cajade, personal observation) and *A. s. pedersenii* in this study. Besides being a message to an intruding male, an aggressive call of an amplexant *A. s. pedersenii* male could also cause the female to move.

### *Amplexus and clutches*

The fact that pairs in amplexus remain motionless for a few hours, presumably to give the females time to ovulate before they move down to the water to spawn, has been documented in many hylids (Carvalho-e-Silva and Carvalho-e-Silva 1994; Bastos and Haddad 1996; Scarlata and Murphy 2003; Toledo and Haddad 2005). Amplexant pairs remaining motionless may also be a strategy to avoid intruder males (Bastos and Haddad 1996; Bertoluci 1998; Brasileiro and Martins 2006) or related to predator abundances (Brasileiro and Martins 2006). In *A. s. pedersenii*, several amplexant pairs remained motionless on the emergent vegetation for over an hour before they moved to the water. In our observation between a male calling from the water and an amplexant pair remaining motionless on the emergent vegetation, the pair seemed to have afforded themselves protection by being beyond the reach of the intruder male. This suggests that climbing out of the water and remaining motionless could be a way for amplexant pairs to avoid intruder males in addition to providing the time needed for ovulation.

*Argentohyla s. pedersenii* displayed Mode 1 reproductive mode of Duellman and Trueb (1994) – eggs and feeding tadpoles in lentic water. Diminich and Zaracho (2008) gave a brief description of *A. s. pedersenii* egg clutches and mentioned that the eggs were laid in gelatinous ribbons floating on the water surface. Their picture shows an egg mass in very turbid water. Based on our observations, the eggs were dispersed from the surface to near the bottom. We think that this described difference may be the result of water clarity with each observation. Also, the eggs sometimes seemed to be dispersed as ribbons which could account for the interpretation provided by Diminich and Zaracho (2008). However, when the egg masses were examined closely, we determined that they comprised groups of few or several eggs with distinct outer jelly layers. Even single eggs were sometimes observed. In addition, the groups of eggs lacked a common surrounding matrix and were attached to each other by the adhesion of the sticky outer jelly layers. This type of grouping fits clearly with the ovipositional mode “clump” as described by Altig and McDiarmid (2007). The size and shape of the clumps in *A. s. pedersenii* seem to be determined by the spawning

behaviour. Although we did not observe the spawning process, we saw a pair located at different sites at different times, and then found a clutch as described above, which suggested that the pair moved during the egg-laying process. We hypothesize that the amplexant pair laid their eggs both while moving and in a stationary position. When the pair were moving during egg laying, narrow and longitudinal clumps with few eggs and even single eggs were deposited, and when the pair laid its eggs while moving slowly or in a stationary position, larger clumps with more eggs were deposited. Actually, three ovipositional modes are known for the species of casque-headed frogs of Faivovich et al. (2005). A film of eggs laid on the water surface is known in some species of the genus *Trachycephalus* (Zweifel 1964; Schiesari and Moreira 1996; Prado et al. 2003), *Osteocephalus* (Martin and Watson 1971; Jungfer and Hödl 2002), and *Osteopilus* (Vargas Salinas 2006) and is suspected in *Itapotihyla* (Izecksohn and Carvalho-e-Silva 2001); single eggs and groups laid in treeholes or bromeliads are known in some species of *Phyllodytes*, (Bokermann 1966; Caramaschi et al. 1992; Giaretta 1996), *Osteocephalus* (Lannoo et al. 1987; Jungfer and Schiesari 1995; Jungfer et al. 2000), *Osteopilus* (Lannoo et al. 1987) *Trachycephalus* (Schiesari et al. 2003) and is suspected in *Nyctimantis* (Crump 1974; Duellman and Trueb 1976). Eggs adhere to rocky walls above puddles in *Corythomantis* (Jared et al. 1999). Eggs laid as clumps in ponds as in *A. s. pedersenii* represent the first record of this mode within this group of frogs.

The number of eggs per clutch in *A. s. pedersenii* was highly variable (from 2638 to 8987). This fact could be the result of the great variation in SVL and particularly in BM of the gravid females measured. There is usually a positive relationship between female body size and fecundity in frogs (Crump 1974; Basso 1990; Duellman and Trueb 1994; Prado and Haddad 2005; Schaefer 2007). Larger body size allows females to increase the number and size of eggs (Crump 1974). Eggs per clutch counted in this study were more numerous than observed by Diminich and Zaracho (2008). Eggs were similar in colour and size (mean diameter 1.79 mm) to those reported by these authors.

Females of *A. s. pedersenii* were, on average, larger and heavier than males. Females larger than males are present in 90% of anuran species (Shine 1979) and several studies found the causes of this phenomenon in: the capacity of larger females to produce larger clutches and eggs (Crump 1974), the mortality rate caused by higher predation pressure on larger males or in the age at first reproduction (Howard 1981), in restrictions on the growth of males because of the energy demand linked to reproductive activity (Woolbright 1989), or also in faster growth rates and delayed reproduction in females (Monnet and Cherry 2002).

### ***Tadpole redescription***

The tadpole of *A. s. pedersenii* resembles those of other pond-dwelling casque-headed frogs, such as *Aparasphenodon brunoii* (Wogel et al. 2006), *Itapotihyla langsdorffii* (Pimenta and Canedo 2007), *Trachycephalus venulosus* (Schiesari and Moreira 1996), and *Trachycephalus nigromaculatus* (Wogel et al. 2006). These tadpoles are generally large, have robust bodies, many tooth rows and a gap in the marginal papillae on the upper labium. Ontogenetic changes on the labial tooth row formula are characteristic and have been documented in *Aparasphenodon brunoii*, *Itapotihyla langsdorffii*, most species of *Osteocephalus* and *Trachycephalus* (McDiarmid and Altig 1990; Schiesari

and Moreira 1996; Schiesari et al. 2003; Wogel et al. 2006; Pimenta and Canedo 2007), and in *A. s. pederseni* (present study). The black colouration with red spots in the caudal fins of tadpoles of *A. s. pederseni* and ontogenetic darkening are particularly notable (Figure 7). Ontogenetic darkening in tadpoles was interpreted as disruptive selection to avoid differential predation pressures (Dixon et al. 1995). This interpretation could also be possible for tadpoles of *A. s. pederseni*; however, this hypothesis needs to be tested. Black body colour may have an aposematic function (Heursel and Haddad 2002). Experimental data show that tadpoles with aposematic colouration are distasteful to certain vertebrates (Caldwell 1989; Heursel and Haddad 1999). Spots highlighted by bright contrasting colouration on the body or tail have been associated with integumentary glands that presumably secrete noxious substances (Altig and McDiarmid 1999). It is interesting to note that these tadpoles do not form aggregations and have a very slow and smooth swimming motion (Céspedes 2000; this paper). This swimming behaviour is contrary to that usually observed in tadpoles with black aposematic colouration, which form large schools in which the tadpoles move quickly and continuously (for examples see Altig and McDiarmid 1999). Slow swimming in tadpoles of *A. s. pederseni* may be conditioned by feeding behaviour and may increase the risk of predation, but also could be a behaviour to exhibit their red spots, thereby increasing the effect of their black aposematic colouration.

The internal buccopharyngeal features in *A. s. pederseni* are similar to those found in other pond-dwelling casque-headed frogs such as *Aparasphenodon brunoii*, *Osteocephalus taurinus*, *Osteopilus septentrionalis* and *Trachycephalus venulosus* (Schiesari and Moreira 1996). These tadpoles have more differentiated buccopharyngeal features (such as papillae, pustulations, glandular zone) compared to the overall reduction of the internal buccopharyngeal structures observed in arboreal tree-hole-dwelling and bromeliad-dwelling casque-headed frogs (Tables 3 and 4). Schiesari and Moreira (1996) pointed out that tadpoles of pond-dwelling species that were microphagous feeders had a greater differentiation of internal buccopharyngeal features than did macrophagous feeders. The buccopharynx of *A. s. pederseni* lacks papillae in the prenarial and buccal roof arenas, and papillae were scarce on the buccal floor arena. All these papillae operate as a sieve to prevent clogging of the filters or the food traps by large particles (Wassersug 1976). The low number of papillae in tadpoles of *A. s. pederseni* suggests that they may consume both small and large particles. Preliminary analyses of gut contents showed large pieces (macrophyte fragments) up to 2357 µm in length and 25 µm in wide, as well as smaller items such as diatoms, chlorophytes, ciliates, euglenoids and sand grains (Cajade, personal observation).

De Sá (1983) gave a brief description of the tadpole of *A. s. siemersi* based on one tadpole at stage VI (Rugh 1955=stage 33 of Gosner 1960; De Sá personal communication) from Uruguay. Céspedes (2000) described the tadpole of *A. s. pederseni* based on six tadpoles at stages 37–40 (Gosner 1960) from a population located in Parque Nacional Mburucuyá, Argentina. Our description of a tadpole of *A. s. pederseni* illustrates some differences and similarities with these previous descriptions, principally in nostril position, dorsal fin origin and dimensions. Wogel et al. (2006) evaluated the differences between the tadpoles of the subspecies of *A. siemersi* and hypothesized a specific rank of these taxa. We did not observe differences in nostril position and the origin of the dorsal fin between our tadpole description and the tadpole description

Table 3. Comparison of internal morphology of buccal floor among casque-headed frog species.

	<i>Argentohyla siemersi pedersenii</i>	<i>Aparasphenodon brunoi</i>	<i>Trachycephalus venulosus</i>	<i>Trachycephalus resnificatrix</i>	<i>Osteocephalus oophagus</i>	<i>Osteocephalus taurinus</i>	<i>Osteopilus brunneus</i>	<i>Osteopilus septentrionalis</i>
Habitat	pond dwelling	pond dwelling	pond dwelling	tree hole dwelling	bromeliad dwelling	pond dwelling	bromeliad dwelling	pond dwelling
Region pre-infralabial papillae	twelve small conical papillae	twelve small conical papillae	some pustulations and stout papillae	some pustulations and stout papillae	bare, without pustulations or papillae	some pustulations and stout papillae	absent	unspecified
Infralabial papillae	one pair, medium size, compressed, broad-based, with rough margins	one pair, small and compressed, margins without any projections	one pair, medium size, broad-based, margins with some lobulated projections finger-like	one pair, medium size, broad-based, margins with numerous lobulated projections finger-like	one pair, medium size, broad-based, margins with numerous lobulated projections finger-like	one pair, medium size, broad-based, margins with some lobulated projections finger-like	absent	two pairs, a small dorsal pair and a large ventral pair
Lingual papillae	one pair, simple and cylindrical	one pair, simple and conical	one pair, simple and cylindrical	one pair, simple and cylindrical	one pair, simple and cylindrical	one pair, simple and cylindrical	absent	two, small
Buccal pockets	perpendicular to the axial axis	perpendicular to the axial axis	oblique to the axial axis	oblique to the axial axis	perpendicular to the axial axis	oblique to the axial axis	unspecified	unspecified
Buccal floor arena (BFA)	centre bare; some pustules; conical papillae in a lateral line from buccal pocket to posterior margin	centre bare, some pustules in a lateral line from buccal pocket to posterior margin of arena; small papillae as flaps on each side of BFA	centre bare, some pustules; conical papillae in a lateral line from buccal pocket to posterior margin of arena; small papillae as flaps on each side of BFA	centre bare, some pustules; conical papillae in a lateral line from buccal pocket to posterior margin of arena; small papillae as flaps on each side of BFA	centre bare, some pustules; conical papillae in a lateral line from buccal pocket to posterior margin of arena; small papillae as flaps on each side of BFA	centre bare, some pustules; conical papillae in a lateral line from buccal pocket to posterior margin of arena; small papillae as flaps on each side of BFA	with 20 pustules scattered, without papillae	10 to 12 papillae per side
Free velar margin	with secretory pits	with secretory pits	with secretory pits	with dense secretory pits	with secretory pits	with secretory pits	with secretory pits	with secretory pits
Median notch	well marked	deeply marked	well marked	well marked	poorly marked	well marked	well marked	deeply marked
Glottis	fully exposed	fully exposed	fully exposed	fully exposed	fully exposed	fully exposed	fully exposed	fully exposed
Source	present study	Wogel et al. (2006)	Schiesari et al. (1996)	Schiesari et al. (1996)	Schiesari et al. (1996)	Schiesari et al. (1996)	Lannoo et al. (1986)	Lannoo et al. (1986)

Table 4. Comparison of internal morphology of buccal roof among casque-headed frog species.

	<i>Argenteohyla siemersi pedersenii</i>	<i>Aparasphenodon brunoi</i>	<i>Trachycephalus venulosus</i>	<i>Trachycephalus resinfictrix</i>	<i>Osteocephalus oophagus</i>	<i>Osteocephalus taurinus</i>	<i>Osteopilus brunneus</i>	<i>Osteopilus septentrionalis</i>
Habitat	pond dwelling	pond dwelling	pond dwelling	tree hole dwelling	bromeliad dwelling	pond dwelling	bromeliad dwelling	pond dwelling
Nares orientation	85–90°	25°	10–20°	30–45°	40–45°	40–45°	unspecified	unspecified
Prenarial arena	few small and scattered pustulations	Y-shaped ridge	tuberos	tuberos, scattered or fused to a median knob	tuberos, scattered or fused to a median knob	tuberos, scattered or fused to a median knob	unspecified without any structure	arch-shaped ridge
Postnarial papillae	3–5 arranged in two anterior concave arch	3–5 arranged in two anterior concave arch	3–4 papillae arranged in an anterior convex arch	3–4 papillae arranged in an anterior convex arch	3–4 papillae arranged in an anterior convex arch	3–4 papillae arranged in an anterior convex arch	absent	three papillae pairs, in oblique rows; scattered
Median ridge	medium size, semi-circular, broad-based, distant from lateral roof papillae	medium size, semi-circular, broad-based, and distant from lateral roof papillae	medium size, semi-circular, broad-based, laterally extended towards the lateral roof papillae	large size, semi-circular, broad-based and distant from lateral roof papillae	medium size, triangular, small-based, distant from the lateral roof papillae	medium size, semi-circular, broad-based, laterally extended towards the lateral roof papillae	absent	pustulations medium size, semicircular
Lateral ridge papillae	one per side, triangular, with irregular edges	one per side, triangular, with irregular edges	one per side, triangular, with irregular edges	one per side, triangular and with irregular edges	one per side, triangular, with irregular edges	one per side, triangular and with irregular edges	absent	one per side, small, with irregularly sculptured margins without pustules
Buccal roof arena	densely pustulated	densely pustulated	densely pustulated	poorly pustulated	moderately pustulated	densely pustulated	20 pustules scattered	pustules absent
Lateral roof papillae	3 triangular, aligned	6 small, aligned	scattered (shape unspecified)	scattered (shape unspecified)	scattered (shape unspecified)	scattered (shape unspecified)	absent	absent
Glandular zone	large	large	large	small	large	large	indistinct	unspecified
Source	present study	Wogel et al. (2006)	Schiesari et al. (1996)	Schiesari et al. (1996)	Schiesari et al. (1996)	Schiesari et al. (1996)	Lannoo et al. (1986)	Lannoo et al. (1986)

of *A. s. siemersi*, but the tadpoles of *A. s. pederseni* described here were larger than of *A. s. siemersi* (49.75–57.1 mm; 42 mm; De Sá 1983). Our description differs from that of Céspedes (2000) in nostril position, origin of caudal fin, maximum body width, snout shape in dorsal view, and dimensions. These differences between descriptions could be attributed to differences in the stages analysed by each author (33, De Sá 1983; 37–40, Céspedes, 2000; 34–36, present study) or could result from phenotypic plasticity in response to a host of biotic and abiotic environments resulting in population variations (Kehr 1989; Reylea and Werner 2000; Reylea 2002a, b) and not be associated with specific rank. Particularly, the nostril position equidistant to the snout and the dorsal fin originating on the posterior third of body as observed by Céspedes (2000) are consistent with a more advanced development of the tadpoles described by this author, where the nostril begins to get closer to the snout, as in the adults, and the dorsal fin begins to be reabsorbed. In addition, the smallest size of the tadpoles of *A. s. siemersi* may also be caused by an earlier stage of development.

We consider it extremely important to conduct molecular and comparative life history studies of both *Argenteohyla siemersi* subspecies. New records of vocalizations with more detailed analysis of the *A. s. siemersi* call and detailed descriptions of the reproductive mode, tadpole and other ethological features will be indispensable for subsequent appropriate comparisons and a definitive statement about the taxonomic status of the two *Argenteohyla siemersi* subspecies.

### Acknowledgements

We are grateful to D.A. Barrasso and G. Agostini for assistance in the field, L. Alcalde for helping with the SEM examination technique, Diego Baldo and Julian Faivovich for their comments, R. Altig for critical reading and valuable comments about reproductive modes, three anonymous reviewers who provided helpful comments on an earlier draft of the manuscript, and the Centro de Información Meteorológica del Servicio Meteorológico Nacional, Comando de Regiones Aéreas de la Fuerza Aérea Argentina for providing the meteorological data. This project was partially supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

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