*Zootaxa* 2073: 1–21 (2009)
www.mapress.com/zootaxa/


**Article**

ISSN 1175-5326 (print edition)
**ZOOTAXA**
ISSN 1175-5334 (online edition)

# A new polyploid species of *Pleurodema* (Anura: Leiuperidae) from Sierra de Comechingones, Córdoba, Argentina and redescription of *Pleurodema kriegi* (Müller, 1926)

JULIÁN A. VALETTI[1,2], NANCY E. SALAS[1] & ADOLFO L. MARTINO[1,3]

[1]*National University of Rio Cuarto, Ecology, X5804BYA Rio Cuarto (Córdoba) Argentina*
[2]*CONICET*
[3]*Corresponding author: amartino@exa.unrc.edu.ar*

## Abstract

We describe a new anuran species of the genus *Pleurodema* from Sierra de Comechingones, Córdoba province, Argentina and redescribe *Pleurodema kriegi* with designation of neotype. The new species has lumbar glands and according to its external morphology is cryptic with *Pleurodema kriegi*. Morphometry, bioacoustics, erythrometry and cytogenetics have allowed differentiating the new species from *Pleurodema kriegi*. The new species, *Pleurodema cordobae*, is the single species of its genus with an octoploid chromosomic complement. Its distribution is only known from type locality and two temporary ponds located 5 kilometers away from the former.

**Key words:** Amphibia, *Pleurodema cordobae* **sp. nov.**, Octoploid, Bioacoustics, Morphometry, Cytogenetics, Erythrometry, Redescription, Neotype, *Pleurodema kriegi*

## Resumen

Nosotros describimos una nueva especie de anfibio del género *Pleurodema* de la Sierra de Comechingones, provincia de Córdoba, Argentina y redescribimos *Pleurodema kriegi* con la asignación de un neotipo. La nueva especie tiene glándulas lumbares y según su morfología externa es críptica con *Pleurodema kriegi*. La morfometría, la bioacústica, la eritrometría y la citogenética han permitido distinguir la nueva especie respecto de *Pleurodema kriegi*. La nueva especie, *Pleurodema cordobae*, es la única de su género con un complemento cromosómico octoploide. La distribución geográfica es solamente conocida para la localidad típica y dos charcas temporarias distantes 5 kilómetros.

## Introduction

Sierra de Córdoba constitutes the eastern group of the Sierras Pampeanas System embracing Sierra Grande, Sierra Norte and Sierra Chica. Sierra Grande includes Sierra de Achala to the north and Sierra de Comechingones to the south (Miró 1999; Ramos 1999). The previous tectonic processes and the current morphology from Sierra Grande favoured the topographical isolation that has produced a remarkable endemic flora and fauna due to allopatric speciation processes (Cei 1972). The endemic herpetofauna from Sierra Grande is represented by the anurans *Odontophrynus achalensis* di Tada, Barla, Martori & Cei, 1984, *Rhinella achalensis* (Cei 1972) and *Pleurodema kriegi* (Müller 1926) and the saurian *Pristidactylus achalensis* (Gallardo 1964), (di Tada *et al*. 1996; Cabrera 1996).

Although polyploidy is an important evolutionary force in some groups of plants (White 1973; Otto & Whitton 2000), this mechanism is less frequent in vertebrates (Orr 1990; Holloway *et al.* 2006). However,

*Accepted by M. Vences: 9 Feb. 2009; published: 16 Apr. 2009*

polyploidy in anuran amphibians has already been documented in numerous families (Bogart 1980; Kawamura 1984; Beçak & Beçak 1998; Otto & Whitton 2000; Martino & Sinsch 2002; Stöck *et al*. 2002; Rosset *et al*. 2006). There are several well-studied cases of diplo/tetraploid cryptic species pairs where bioacoustics has allowed to distinguish different *taxa* (e.g. Wasserman 1970; Ralin 1977; Gerhardt 1978; Martino & Sinsch 2002; Holloway *et al*. 2006). The genus *Pleurodema* Tschudi is distributed from Panama throughout South America to southern Chile and southern Argentina (Frost 2008) and currently is represented by thirteen species, nine of which are present in Argentina (Lynch 1971; Heyer 1975; Frost 1985; Cei 1987; McLister *et al*. 1991, Nascimento *et al*. 2006). The ploidy level of species of the genus *Pleurodema* is 2n=2X=22 excepting *Pleurodema kriegi* and *P. bibroni* which are 2n=4X=44 (Brum-Zorrilla & Sáez 1968; Barrio & Rinaldi de Chieri 1970; Veloso *et al*. 1972; Duellman & Veloso 1977; Schmid *et al.* 1993; Lourenço *et al.* 2006). The erythrocyte size in amphibians is correlated with the DNA content (Stöck & Grosse 1997). Therefore, and having the caution that a higher amount of nuclear DNA does not necessarily mean a different ploidy level - as was previously reported for other species of *Pleurodema* (Schmid *et al*. 1993) -, the erythrocyte size allows estimating the ploidy level in anurans in an indirect way (Martino & Sinsch 2002; Rosset *et al*. 2006).

In February 2006, during a field trip in the north area of Sierra de Comechingones we found individuals morphologically similar to *Pleurodema kriegi*. That locality is geographically located ca. 100 km south of La Posta, Pampa de Achala, Córdoba province. We compared the population from Sierra de Comechingones with *Pleurodema kriegi* from La Posta, Pampa de Achala and we determined that the specimens recently collected correspond to an undescribed species. The holotype of *Pleurodema kriegi* is actually "lost" (Glaw & Franzen 2006), and in this case, a name-bearing type is necessary to define the nominal taxon objectively. The aim of this article is describe and name a new species of genus *Pleurodema* from Sierra de Comechingones and redescribe *Pleurodema kriegi* on the basis of specimens from Pampa de Achala with designation of neotype.

## Material and methods

**Collection of specimens**: We collected 28 males and 1 female of the new species in Estancia Los Tabaquillos, Sierra de Comechingones, province of Córdoba (32º23'58.4" S, 64º55'35.1" W, approximately 2105 m elevation; Fig. 1). Specimens were collected during three field trips in the years 2006/07. Additionally, in the year 2006 we collected 15 males and 3 females of *Pleurodema kriegi* in La Posta, Pampa de Achala (31º36'46" S, 64º52'29" W, approximately 2151 m elevation; Fig. 1). Specimens were preserved in 70% ethanol and they are deposited in the herpetological collection of Museum of Miguel Lillo Foundation (FML). Specimens analyzed are listed in the Appendix.

We compared the populations from Estancia Los Tabaquillos and La Posta by means of bioacoustics, morphometry, erythrometry and cytogenetics.

**Morphometry**: Morphometric measurements of specimens were taken *in vivo* with digital caliper Mahr 16ES to the nearest 0.01 mm and then rounded to only one decimal to avoid pseudo precision (Hayek *et al*. 2001). All measurements were taken by one of us (JV) to avoid inter-observer differences. Sixteen standard measurements were recorded (Martino & Sinsch 2002): (1) snout-vent length (SVL); (2) maximal head width; (3) head length; (4) snout-eye distance; (5) internarinal distance; (6) interocular distance; (7) eye-narinal distance; (8) rostronarinal distance; (9) eye diameter; (10) arm length (from the elbow-joint up to the top of the third finger); (11) length of 3$^{rd}$ finger; (12) femur length; (13) tibia length; (14) foot length (including tarsal length); (15) length of 3$^{rd}$ toe, (16) length of 4$^{th}$ toe.

For morphometric analysis only males were used and all variables were $\log_{10}$-transformed, standardized and subjected to a discriminant analysis (Forward selection) to identify the minimum set of variables allowable in order to distinguish the new species from *Pleurodema kriegi*. To analyze the possible difference between *Pleurodema kriegi* and the new species in these morphometric variables, univariate analyses of variance were performed.

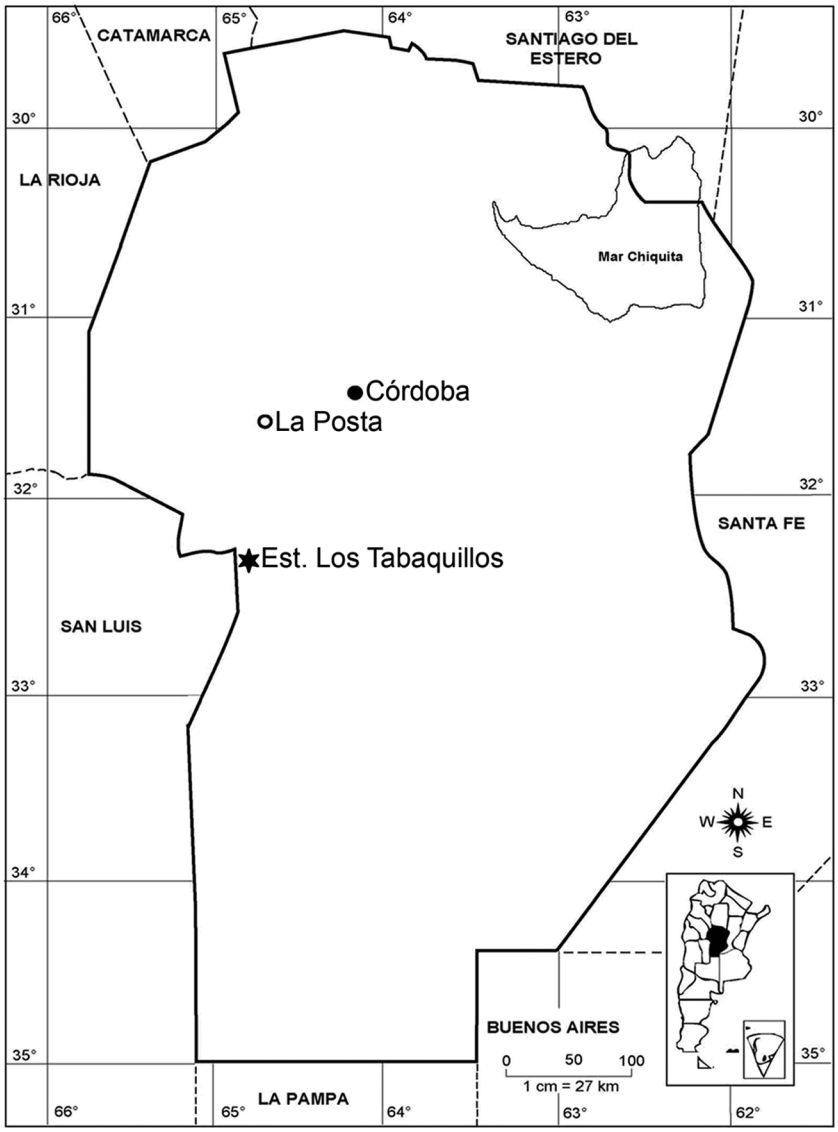


**FIGURE 1.** Geographic position of type locality of *Pleurodema* sp. nov. (Estancia Los Tabaquillos; asterisk) and locality of specimens of *Pleurodema kriegi* used in the redescription (La Posta, Pampa de Achala; circle).

**Bioacoustics**: We recorded 49 advertisement calls (22 individuals) in the population from Estancia Los Tabaquillos and 23 advertisement calls (10 individuals) from La Posta. The advertisement calls were registered using a DAT record Sony TCD-100 with stereo microphone ECM-MS907 Sony and tape TDK DAT-RGX 60. Water and air temperatures were registered immediately after each sound recording by means of a digital thermometer TES 1300 0.1°C. Water temperatures registered were between 17.5 and 20.9ºC. The recordings of advertisement calls used in this study were deposited in the Fonoteca of Ecology, Department of Natural Sciences, National University of Río Cuarto, Argentina.

The advertisement calls for bioacoustic analysis were digitized using the Canary 1.2 software (Charif *et al*. 1995) at a sample rate of 44100 Hz, and sample size of 16 bits. The temporal variables of each advertisement call were measured using oscillograms and sonograms. The analyses of dominant frequency were made using a spectrogram with the following settings: filter bandwidth 649.4 Hz, frame length 256 points, grid resolution 128 points, overlap 50%, frequency 43.07 Hz, FFT size 1024 points; window function hamming; amplitude logarithmic; clipping level -95 dB. The analyzed call parameters were: (1) call duration [ms]; (2) intercall interval [ms]; (3) dominant frequency [Hz]; (4) number of pulse groups per call [N]; (5) duration of pulse group [ms]; (6) interval between pulse groups [ms]; (7) pulse duration [ms]; (8) interpulse

interval [ms]; (9) pulse rate [pulses/ sec). Three calls per series of calls, three pulse groups per call and three pulses per pulse group were analyzed. The arithmetic means of these call parameters were calculated for each series and used for further analyses (Martino & Sinsch 2002). Low temperature range of advertisement calls recorded do not allow for temperature correction using regression. To evaluate possible differences in the acoustic properties between the new species and *Pleurodema kriegi*, a discriminant analysis with all acoustic variables was performed. In order to analyze which acoustic variables differ between both *taxa*, univariate analyses of the variance were performed.

**Cytogenetics**: Seven specimens of the new species and three specimens of *Pleurodema kriegi* were examined. Animals were intraperitoneally injected with 0.3% colchicine (0.01 ml/g of body weight) for 10 hours. Mitotic chromosomes were obtained from testicular cell suspensions treated for 30 minutes in 1% sodium citrate (weigth/volume) and then fixed in 3:1 methanol and acetic acid for 30 minutes (Schmid 1978). Chromosomes were stained with 10% Giemsa solution. Karyotypes were arranged following Levan *et al.* (1964).

**Erythrometry**: A blood sample of each specimen was obtained by angularis vein puncture (Nöller 1959) and blood smears were made. The largest and smallest diameter of 15 randomly chosen erythrocytes per individual were measured (to the nearest 1 µm) and surface area was calculated assuming an ellipsoid shape as $AB\pi/4$, where A and B are the largest and smallest diameter of the erythrocyte, respectively (Martino & Sinsch 2002). To compare the erythrocyte size between the new species and *Pleurodema kriegi*, univariate analysis of variance was performed.

All calculations were performed on an AMD Athlon XP 2200 PC using the program package STATGRAPHICS for Windows, version 5.0.

## Results

**Morphometry.** Discriminant analysis showed one significant function (Canonical correlation = 0.78224; W=0.38809 y $p<0.0000$), which used only four out of 15 measured morphometric distances. The discriminant function is: 61.1609 - 32.1778 * $Log_{10}$(SVL) - 16.3516 * $Log_{10}$(head length) + 29.6492 * $Log_{10}$(snout-eye distance) - 13.2596 * $Log_{10}$(rostronarinal distance).

Classification matrix correctly distinguished specimens from *P. kriegi* and the new species with high values (93.33% and 96.43%, respectively). Only one individual of *P. kriegi* and one individual of the new species were incorrectly classified. The specimens of the new species are generally bigger than *P. kriegi* and ten variables were statistically different (Table 1). However, all variables present a wide overlap and none of them is diagnostic.

**Bioacoustics.** The advertisement call of *Pleurodema kriegi* is a compound call formed by groups or trains of pulses. All pulse trains have three pulses. The new species has the same call type. (Fig. 2 and 3). The values of the analyzed acoustic variables are shown in Table 2.

Differences in the temporal pattern of the pulse groups between species were observed. Therefore, we carried out comparisons between the two taxa to evaluate possible differences in the temporal variables of the pulse groups.

All temporal variables of the advertisement call in both species were statistically different (Table 2), while the dominant frequency was not significantly different. Durations of each pulse of the pulse group and its corresponding intervals also showed highly significant differences between species (Table 3).

The discriminant analysis showed a segregation of two species. The canonical correlation showed a value of 0.93252 indicating a high discriminant power of the generated function and Wilks' lambda 0.1304068 ($p<0.0000$) indicate a high discriminant power of the variables. The most important variables in the discriminant function correspond to duration of pulse group and pulse duration (Fig. 4). The classification rate shows all cases correctly classified indicating a marked acoustic differentiation between *Pleurodema kriegi* from La Posta and the new species.

**TABLE 1.** Measurements in millimetres of specimens of *Pleurodema kriegi* and *Pleurodema* sp. nov. from Estancia Los Tabaquillos. Mean ± SD (Range).

| Morphometric variable | *Pleurodema kriegi* Mean ± SD (Range) | *Pleurodema* sp. nov. Estancia Los Tabaquillos Mean ± SD (Range) |
|---|---|---|
| Snout-vent length* | 33.24 ± 1.79 (29.37–36.80) | 35.41 ± 1.56 (32.74–38.73) |
| Maximal head width | 13.27 ± 0.77 (12.32–14.83) | 13.29 ± 0.68 (12.01–14.78) |
| Head length* | 12.36 ± 1.22 (10.48–14.30) | 13.05 ± 0.95 (10.49–14.44) |
| Snout-eye distance* | 5.36 ± 0.31 (4.89–5.90) | 5.60 ± 0.30 (4.97–6.10) |
| Internarinal distance | 2.08 ± 0.26 (1.71–2.69) | 2.20 ± 0.19 (1.95–2.64) |
| Interocular distance | 3.05 ± 0.22 (2.69–3.38) | 2.92 ± 0.34 (2.36–3.63) |
| Eye-narinal distance | 2.90 ± 0.16 (2.57–3.10) | 2.80 ± 0.24 (2.33–3.17) |
| Rostronarinal distance* | 3.04 ± 0.18 (2.71–3.33) | 3.22 ± 0.31 (2.82–4.22) |
| Eye diameter | 3.55 ± 0.30 (3.18–4.30) | 3.63 ± 0.30 (2.48–4.02) |
| Arm length * | 15.56 ± 0.97 (13.48–17.50) | 16.31 ± 0.92 (14.53–18.33) |
| Length of 3$^{rd}$ finger* | 4.91 ± 0.36 (4.60–5.89) | 5.27 ± 0.40 (4.45–6.07) |
| Femur length | 14.89 ± 0.90 (13.48–17.00) | 15.02 ± 0.76 (13.63–16.59) |
| Tibia length * | 15.51 ± 0.50 (14.59–16.50) | 16.09 ± 0.92 (13.28–18.21) |
| Foot length* | 23.16 ± 0.46 (22.40–24.09) | 24.56 ± 1.27 (22.21–27.28) |
| Length of 4$^{th}$ toe* | 9.08 ± 0.57 (8.30–10.60) | 9.96 ± 0.66 (8.44–11.48) |
| Length of 3$^{rd}$ toe* | 5.22 ± 0.42 (4.45–5.90) | 5.49 ± 0.37 (4.96–6.58) |

n= 15 males of *Pleurodema kriegi* and 28 males of *Pleurodema* sp. nov. from Estancia Los Tabaquillos.
* statistically different variables between populations (ANOVA, $p<0.05$)

**TABLE 2.** Features of the advertisement calls. Means, standard deviations and range refer to water temperature of 17.5-20.9°C in *Pleurodema kriegi* and 18.8–19.6°C in *Pleurodema* sp. nov. from Estancia Los Tabaquillos.

| | *Pleurodema kriegi* | | *Pleurodema sp. nov.* Estancia Los Tabaquillos | |
|---|---|---|---|---|
| Call variable | n | Mean ± SD (Range) | n | Mean ± SD (Range) |
| Call duration [ms] * | 10/23 | 16338.8 ±11990.0 (3701.0–38387.0) | 22/49 | 7853.83 ± 4480.53 (1607.0–16453.3) |
| Intercall interval [ms] * | 6/15 | 5925.41 ± 3355.82 (1669.0–10091.3) | 18/37 | 2720.38 ± 1355.56 (1082.5–5831.5) |
| Dominant frequency [Hz] | 10/23 | 1823 ± 91 (1660–1938) | 22/49 | 1793 ± 100 (1616–2002) |
| Number of pulse groups per call [N] * | 10/23 | 200.77 ± 164.38 (53–498) | 22/49 | 110.311 ± 61.66 (24–229) |
| Duration of pulse group [ms] * | 10/23/69 | 42.54 ± 3.03 (38.88–48.13) | 22/49/147 | 35.23 ± 1.59 (31.07–37.31) |
| Interval between pulse groups [ms] * | 10/23/69 | 30.42 ± 2.70 (26.75–36.88) | 22/49/147 | 35.61 ± 4.47 (29.02–45.49) |
| Pulse duration [ms] * | 10/23/69/ 207 | 5.42 ± 0.74 (4.09–6.14) | 22/49/147/441 | 3.89 ± 0.49 (2.89–4.90) |
| Interpulse interval [ms] * | 10/23/69/ 207 | 12.53 ± 1.38 (10.72–14.76) | 22/49/147/441 | 11.37 ± 1.20 (9.25 -13.62) |
| Pulse rate [pulses/ sec] * | 10/23 | 55.94 ± 3.71 (49.14–60.49) | 22/49 | 65.69 ± 3.74 (60.15–74.48) |

n= Individuals/Calls/ Number of pulse groups per call /Pulses)
* statistically different variables between populations (ANOVA, $p<0.05$).

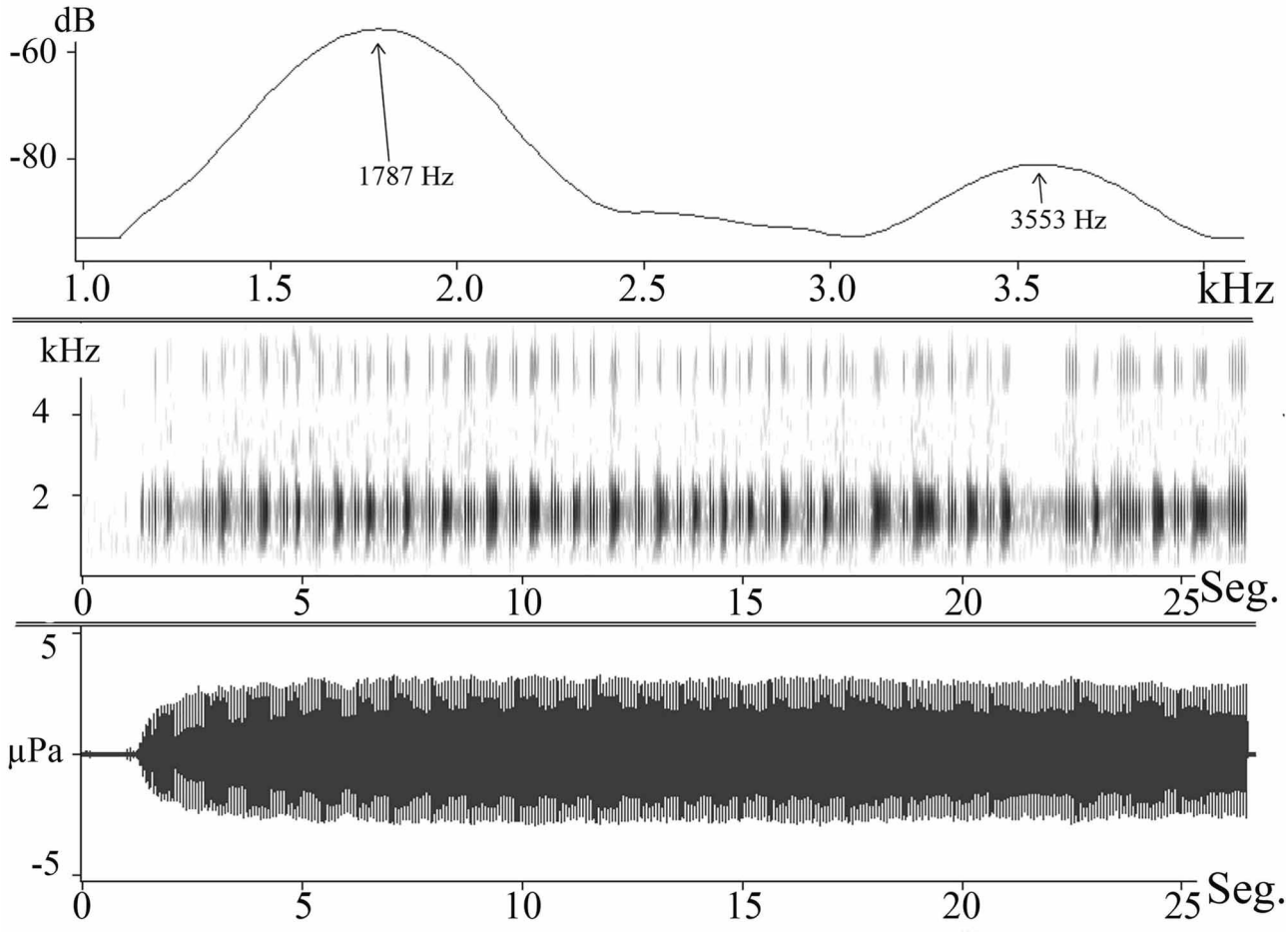


**FIGURE 2**. Advertisement call of *Pleurodema* sp. nov. from Estancia Los Tabaquillos. Power spectrum (above), Sonogram (middle) and Oscillogram (below). Water temperature: 19.3ºC.


**FIGURE 3**. Fragment of the advertisement call of *Pleurodema* sp. nov. from Estancia Los Tabaquillos showing three tri-pulsed pulse groups. Water temperature: 19.3ºC.

**Cytogenetics.** Forty-eight metaphases (seven individuals of the *Pleurodema* sp. nov. and three individuals of *Pleurodema kriegi*) were analyzed and the corresponding karyotypes were arranged (Fig. 5). The individuals of *Pleurodema kriegi* showed a tetraploid chromosomic complement (2n=4x=44), while the individuals of the new species showed an octoploid chromosomic complement (2n=8x=88).

**Erythrometry.** The erythrocyte size was significantly different between species (ANOVA, $F = 236.04$, $p < 0.0001$) with a bigger size in the specimens of the new species. (Table 4, Fig. 6 and 7).

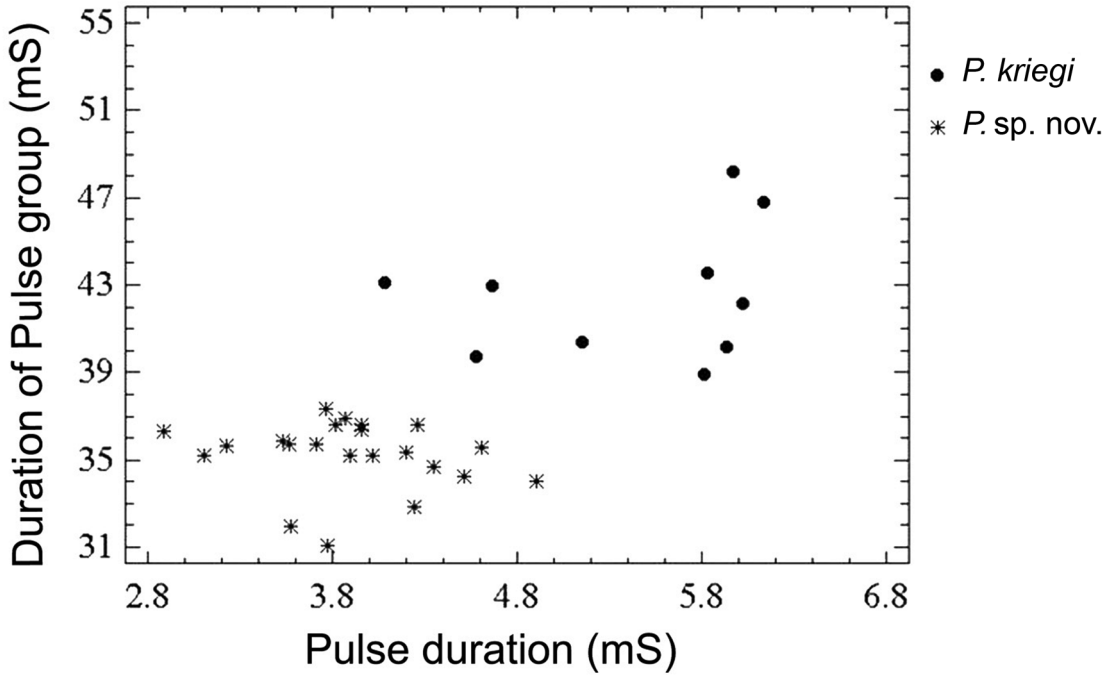


**FIGURE 4**. Scatter plot of duration of pulse group versus pulse duration. Individuals from La Posta (circles) and Estancia Los Tabaquillos (asterisks).

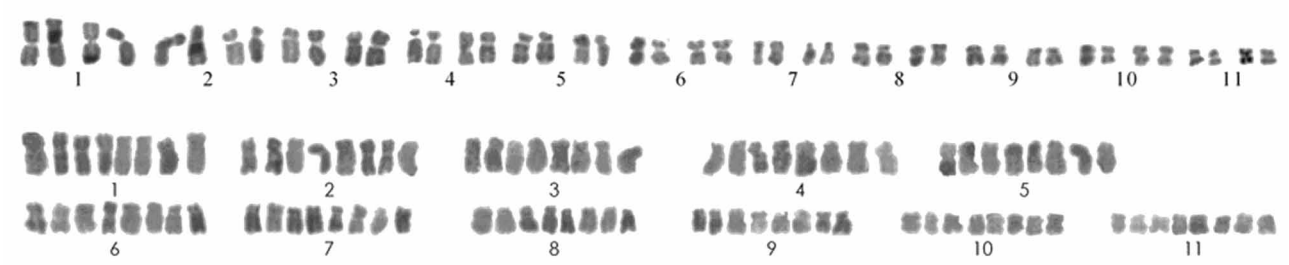


**FIGURE 5**. Karyotypes of two species of *Pleurodema* analyzed. *Pleurodema kriegi*, La Posta, Pampa de Achala, 2n=4x=44 (above) and *Pleurodema* sp. nov. from Estancia Los Tabaquillos, Sierra de Comechingones, 2n=8x=88 (below). Photo by N. Salas.

## Discussion

The external morphology of *Pleurodema kriegi* and the new species is quite similar although the morphometric comparisons showed significant differences. The discriminant analysis carried out with the morphometric variables showed a value of correct classification superior to 95% and 10 variables indicate statistically significant differences between both species (Table 1). However, unequivocal distinction between taxa based only on external morphology is not possible. Similar results were obtained by Martino and Sinsch (2002) in the morphometric differentiation between *Odontophrynus americanus* (tetraploid) and *O. cordobae* (diploid). Morphological differences could be interpreted as geographical variation (e.g. in other anuran species Heyer 1997; Schmalz & Zug 2002). However, further studies will be necessary to determine the interpopulation variability in *Pleurodema kriegi*.

The erythrometric analysis in both species helped us to detect differences at ploidy level. The octoploidy was confirmed by cytogenetic analysis. Thus, becoming the first discovery and report of an octoploid population to the genus *Pleurodema*.

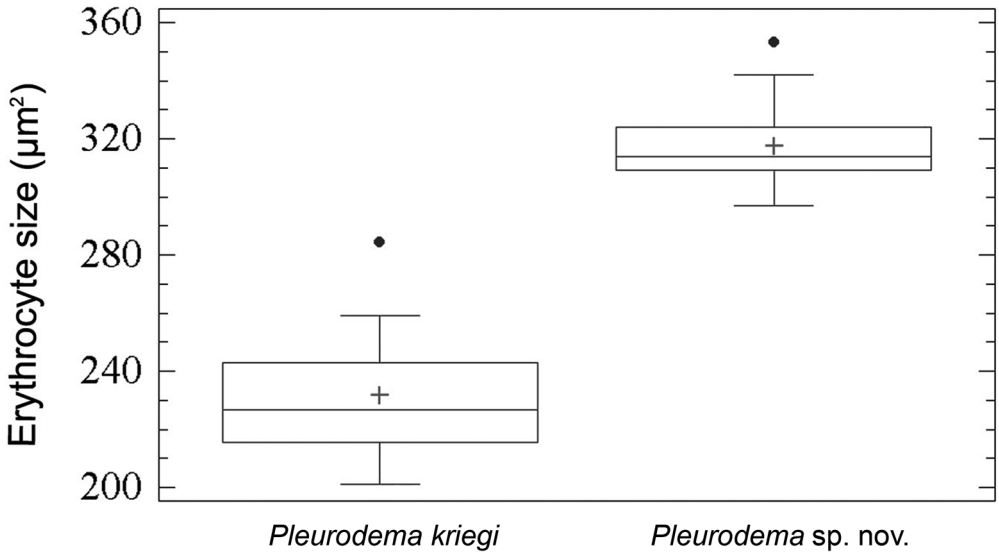


**FIGURE 6**. Box-and-Whisker plots of erythrocyte sizes for *Pleurodema kriegi* and *Pleurodema* sp. nov. from Estancia Los Tabaquillos. Upper and lower ends of boxes represent 75th and 25th percentiles.Whisker represent the minimum and the maximum values, except for outlier points. The center line within each box shows the locations of the sample median and the plus sign indicates the location of the sample mean.

**TABLE 3**. Features of pulse groups of the advertisement calls. Means, standard deviations and range refer to water temperature of 17.5-20.9°C in *Pleurodema kriegi* and 18.8–19.6°C in *Pleurodema* sp. nov. from Estancia Los Tabaquillos.

| | Pleurodema kriegi | | *Pleurodema* sp. nov Estancia Los Tabaquillos | |
|---|---|---|---|---|
| Pulse group variable | n | Mean ± SD (Range) | n | Mean ± SD (Range) |
| First pulse duration [ms] * | 10/23/69/207 | 4.87 ± 0.54 (4.05–5.70) | 22/49/147/441 | 3.56 ± 0.61 (2.49–4.71) |
| First pulse interval [ms] * | 10/23/69/207 | 14.17 ± 1.62 (12.15–16.94) | 22/49/147/441 | 12.86 ± 1.32 (10.72–15.93) |
| Second pulse duration [ms] * | 10/23/69/207 | 5.52 ± 0.75 (4.12–6.35) | 22/49/147/441 | 4.00 ± 0.55 (2.91–4.96) |
| Second pulse interval [ms] * | 10/23/69/207 | 10.88 ± 1.33 (8.97–12.91) | 22/49/147/441 | 9.88 ± 1.17 (7.33–11.48) |
| Third pulse duration [ms] * | 10/23/69/207 | 5.87 ± 1.26 (4.02–7.48) | 22/49/147/441 | 4.13 ± 0.67 (3.10–5.13) |

n= Individuals/Calls/ Number of pulse groups per call /Pulses)
* statistically different variables between populations (ANOVA, $p<0.05$)

**Table 4**: Mean, standard deviation and range of the erythrocyte areas ($\mu m^2$) of two species of *Pleurodema* analyzed.

| | | **n** | **Mean ± SD (Range)** |
|---|---|---|---|
| *Pleurodema kriegi* | (Tetraploid) | 14 | 231.82 ± 21.46 (201.19–284.19) |
| *Pleurodema* sp. nov. from Est. Los Tabaquillos | (Octoploid) | 25 | 317.46 ± 13.43 (296.81–353.12) |

n: sample size.

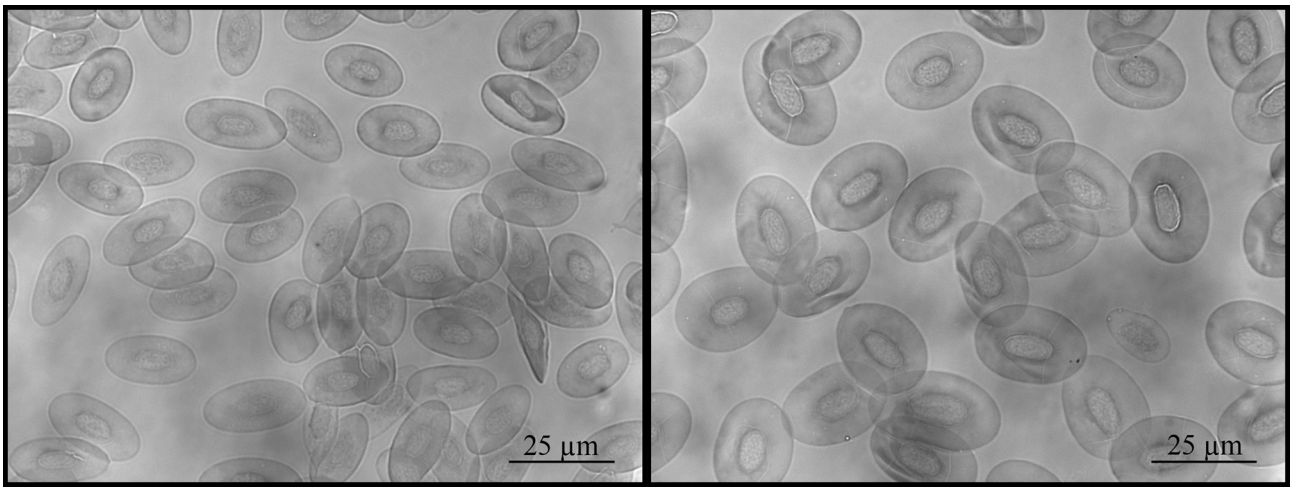


**FIGURE 7**. Erythrocyte sizes. *Pleurodema kriegi*, Pampa de Achala (left) and *Pleurodema* sp. nov. from Estancia Los Tabaquillos, Sierra de Comechingones (right).

It has already been shown that differences in the advertisement call among anuran populations could suggest reproductive isolation (e.g. Bogart & Wasserman 1972; Platz & Forester 1988; Martino & Sinsch 2002) and that the advertisement calls of anurans are thus species-specific (Duellman & Trueb 1986). Moreover, in morphologically similar species but genetically different, differences in the pulse rate could be an important measure of reproductive isolation in species (Platz & Forester 1988; Angulo & Reichle 2008), mainly in cryptic species with different ploidy levels (Bogart & Wasserman 1972; Martino & Sinsch 2002). There are well-studied examples of species complexes with different ploidy levels that differ in the pulse rate, e.g. *Hyla crhysoscelis* (2n)/*H. Versicolor* (4n) (Holloway *et al.* 2006); *Bufo viridis* complex 2n/4n (Stöck 1998); *Odontophrynus cordobae* (2n)/*O. americanus* (4n) (Martino & Sinsch 2002). Therefore, a differentiation of the acoustic properties of the advertisement call of octoploid individuals from *Pleurodema kriegi* could be expected if they represented a different species. We demonstrated that marked differences exist in the temporal properties of the advertisement call and the pulse rate is significantly higher in octoploid individuals than in *Pleurodema kriegi* (65.7 and 55.9 Pulses/sec., respectively). In our study, the octoploid individuals showed higher pulse rates, contrary to the *Bufo viridis* complex (Stöck 1998), *Odontophrynus cordobae/O. americanus* (Martino & Sinsch 2002)) and *Hyla crhysoscelis/H. versicolor* (Holloway *et al*. 2006) where the pulse rate in the tetraploid species are smaller than their diploid counterparts.

Although the specimens collected in Sierra de Comechingones are morphologically cryptic regarding *Pleurodema kriegi* from Pampa de Achala, we have demonstrated that both species clearly differ in bioacoustic, erythrometric and cytogenetic features and we conclude that the population of *Pleurodema* from Sierra de Comechingones should be recognized as new species of the genus *Pleurodema*.

*Pleurodema kriegi* was described and assigned to the genus *Paludicola* by Müller (1926) on the basis of a female specimen collected in "Fuß der Sierra Grande von Cordoba", Córdoba province, Argentine. Posteriorly, Parker (1927) includes it in the synonymy of *Pleurodema cinerea* (now *Pleurodema cinereum*). In 1968, after forty four years from the first discovery of *Pleurodema kriegi* in Sierra Grande of Cordoba by Krieg, *Pleurodema kriegi* is rediscovered in Pampa de Achala, Córdoba by Claudio and Mario Stiebel. Gallardo (1968) studied this material and it was synonymized with *Pleurodema darwinii* from Uruguay (now *Pleurodema bibroni*) and he made a redescription of the species on the basis of an adult male (MACN 24930), also he studied material from Uruguay and ten aditional individuals from Pampa de Achala. Barrio and Rinaldi de Chieri (1970) performed a cytogenetic analisis of eight species of the genus *Pleurodema* and they concluded that the entity from Córdoba is a different species from that of Uruguay. In addition, Laurent (1975) by means of biometric and coloration comparisons ratifies two different entities on the basis of individuals from Pampa de Achala (Córdoba province, Argentina) and Uruguay.

In this work, the presence of a new species of the genus *Pleurodema* that inhabits the Sierra de Comechingones, an orographic formation at Sierra Grande of Córdoba, is determined. This new species is cryptic and allopatric regarding *Pleurodema kriegi* of Pampa de Achala, an orographic formation at Sierra Grande, too (Ramos 1999). The holotype of *Pleurodema kriegi* originally deposited in the Zoologische Staatssammlung München (ZSM 138/1925) is actually "lost", may be destroyed during World War II (Glaw and Franzen 2006). Moreover, the *Pleurodema kriegi* decription (Müller 1926) is quite poor and the description of the type locality is somewhat vague. Therefore, according to the article 75 of The International Code of Zoological Nomenclature (ICZN 1999), it is necessary to carry out a description and to assign a neotype to *Pleurodema kriegi* to be able to solve the nomenclatural problem among the two species of *Pleurodema* of the Sierra Grande of Córdoba. Qualifying conditions established by the ICZN (1999) when describing a neotype include (art. 75.3.1) the existence of "a statement that it is designated with the express purpose of **clarifying the taxonomic status or the type locality** of a nominal taxon", (art. 75.3.5) the existence of "evidence that the neotype is consistent with what is known of the former name-bearing type from the original description and from other sources; however, a neotype may be based on a **different sex** or life stage if necessary or desirable to secure stability of nomenclature", and (art. 75.3.6) "evidence that the neotype came as nearly as practicable from the **original type locality**…".

Regarding to the type locality of the neotype, La Posta is a locality of Pampa de Achala comprehended within Sierra Grande, the broad type locality of *Pleurodema kriegi* given by Müller (1926). Müller indicated "Fuß der Sierra Grande von Cordoba", but this species was never found in Sierra Grande inferior to altitudes to 2000 m.a.s.l. (Barrio 1977; J. Valetti, I. di Tada and A. Martino, unpublished data). Most studies on *Pleurodema kriegi*, since the original description of Müller (1926), were realized with specimens from Pampa de Achala (e.g. Gallardo 1968; Barrio & Rinaldi de Chieri 1970; Laurent 1975, Barrio 1977), and for this reason we select specimens from La Posta, Pampa de Achala, to redescribe *Pleurodema kriegi* and design a neotype.

**Re-description of *Pleurodema kriegi* (Müller, 1926)**
Fig. 8

*Paludicola kriegi* Müller, 1926.

*Pleurodema kriegi* di Tada, Salusso and Martori, 1976.

**Material. Neotype**. FML 20460, adult male (Fig. 8) near La Posta (31º36'46" S, 64º52'29" W, approximately 2151 m elevation), Pampa de Achala, Córdoba province, Argentina (Fig. 1), collected during the night of 17 January 2006 by Julián A. Valetti.

**Referred specimens. (17 specimens).** FML 20459, 20461, 20463–20471, 20474-20476, fourteen adult males and FML 20462, 20472–20473, three adult females, all collected with the neotype.

**Diagnosis.** A small species of *Pleurodema* (33.24 mm SVL), characterized by: (1) relatively small size; (2) snout short, canthus rostralis rounded in dorsal view and truncate in profile; (3) lumbar glands present, 1 ½ eye diameter; (4) round tympanic annulus, almost concealed; (5) tympanum length half of the eye diameter; (6) vomerine teeth absent, (7) comissural gland prominent; (8) brilliant red-orange spots on the groin and around lumbar glands; (9) ploidy level 2n=4x=44; (10) axillary amplexus; (11) egg deposition in gelatinous nest attached to vegetation; (12) compound advertisement call with tri-pulsed pulse groups, average dominant frequency 1823 Hz.

According to external morphology, *Pleurodema kriegi, Pleurodema bibroni* and the new species described below are cryptic and the properties of their advertisement calls are similar (see above). *Pleurodema kriegi* differs from the new allopatric species from Sierra de Comechingones by having tetraploid chromosomic complement (octoploid in the new species) and by having a lower pulse rate (55.9 pulses/sec in *Pleurodema kriegi* and 65.7 pulses/sec in the new species). *Pleurodema kriegi* and *Pleurodema bibroni* are the only tetraploid species of the genus. They are morphologically very similar and although Laurent (1975) found some morphological differences between these entities, an unequivocal distinction between these taxa is not possible. The advertisement call of *Pleurodema kriegi* is similar to *Pleurodema bibroni*, too. Barrio (1977) detected difference in the pulse rate between these taxa, although the temperatures of recording are different and this environmental variable can affect this temporal variable of the call; Kolenc *et al.* (2009) found no acoustic differences between these two species. Barrio and Rinaldi de Chieri (1970) found secondary constriction chromosomes 12 only in *Pleurodema kriegi.* Ultimately, *Pleurodema kriegi* and *P. bibroni* were considered as distinct species by Barrio (1977) based mostly on biogeographic and ecologic differences.

*Pleurodema kriegi* is distinguished markedly from *P. tucumanum, P. nebulosum, P. guayapae, P. marmoratum* and *P. diplolister* by the presence of lumbar glands (lumbar glands absent in these species); from *P. bufoninum, P. borellii, P. cinereum* and *P. thaul* by the presence of advertisement call compound by tri-pulsed pulse groups (absent in *P. bufoninum*, Duellman & Veloso 1977; formed by a single pulse group in *P. borellii* and *P. cinereum*, McLister *et al*. 1991; and formed by pulse groups of 5-6 pulses in *P. thaul*, Barrio 1977); from *P. brachyops* by having lumbar glands bigger than eye diameter (smaller in *P. brachyops*), yellow lumbar glands with black central ocellus (black lumbar glands with whitish central blotchs in *P. brachyops*), tympanum size half of the eye diameter (smaller than half eye diameter in *P. brachyops*).

**Description of the neotype.** The neotype (FML 20460, Fig. 8) is an adult male of 34.4 mm, body robust; head triangular, slightly wider than long; snout short, canthus rostralis rounded and vertically truncated. Eyes protuberant; eye diameter larger than interocular distance; interocular distance larger than internarinal interval. Round tympanic annulus almost concealed, approximately half the size of eye diameter. Commissural glands present, oval, approximately the same size of eye diameter. Vomerine teeth absent. Dark vocal sac. Fingers free; relative length of fingers: 3>4>1>2; two not-darkened palmar tubercles (Fig. 8). Femur length less than tibia length; sum of femur length and tibia length longer than foot length. Toes free with cutaneous edge and rudimentary interdigital basal membrane; two metatarsal tubercles, inner metatarsal tubercle pointed and outer metatarsal tubercle shovel-shaped (Fig. 8); relative length of toes: 4>5=3>2>1. Lumbar glands large, oval, one and a half times the size of eye diameter. Vocal sacs single, median and subgular. In life, dorsally brownish with large almost symmetrical dark spots. Dark transverse bands on upper surface of arms and legs. Yellow lumbar glands with a black central ocellus covering 60% of the gland. Brilliant red-orange spots on the groin and around lumbar glands. Dark palms and soles, with whitish palmar tubercles. Dark vocal sac with the rest of ventral body surface whitish and mild dotted dark. Iris gold with black reticulations. The neotype is ventrally very infected by the sub-cutaneous acari *Hannemania achalai* Alzuet and Mauri, 1987.

Morphometric measures of neotype (mm): Snout-vent length 34.4 ; maximal head width 13.6; head length 11.5; snout-eye distance 5.0; internarinal distance 1.9; interocular distance 2.7; eye-narinal distance 2.7; rostronarinal distance 3.2; eye diameter 3.6; arm length 16.1; length of 3$^{rd}$ finger 4.6; femur length 15.0; tibia length 15.8; foot length (including tarsal length) 22.5; length of 3$^{rd}$ toe 5.2; length of 4$^{th}$ toe 9.1.

**Distribution.** The species is currently known from Pampa de Achala, Córdoba province (Fig. 1) (see Barrio 1977, Kolenc et al. 2009) and cited to near Alpa Corral, Córdoba province by Ávila and Priotto (1995). However , the last record should be corroborated because the locality is to the south of the new species with octoploid chromosomic complement and the specimens could be octoploid, too.

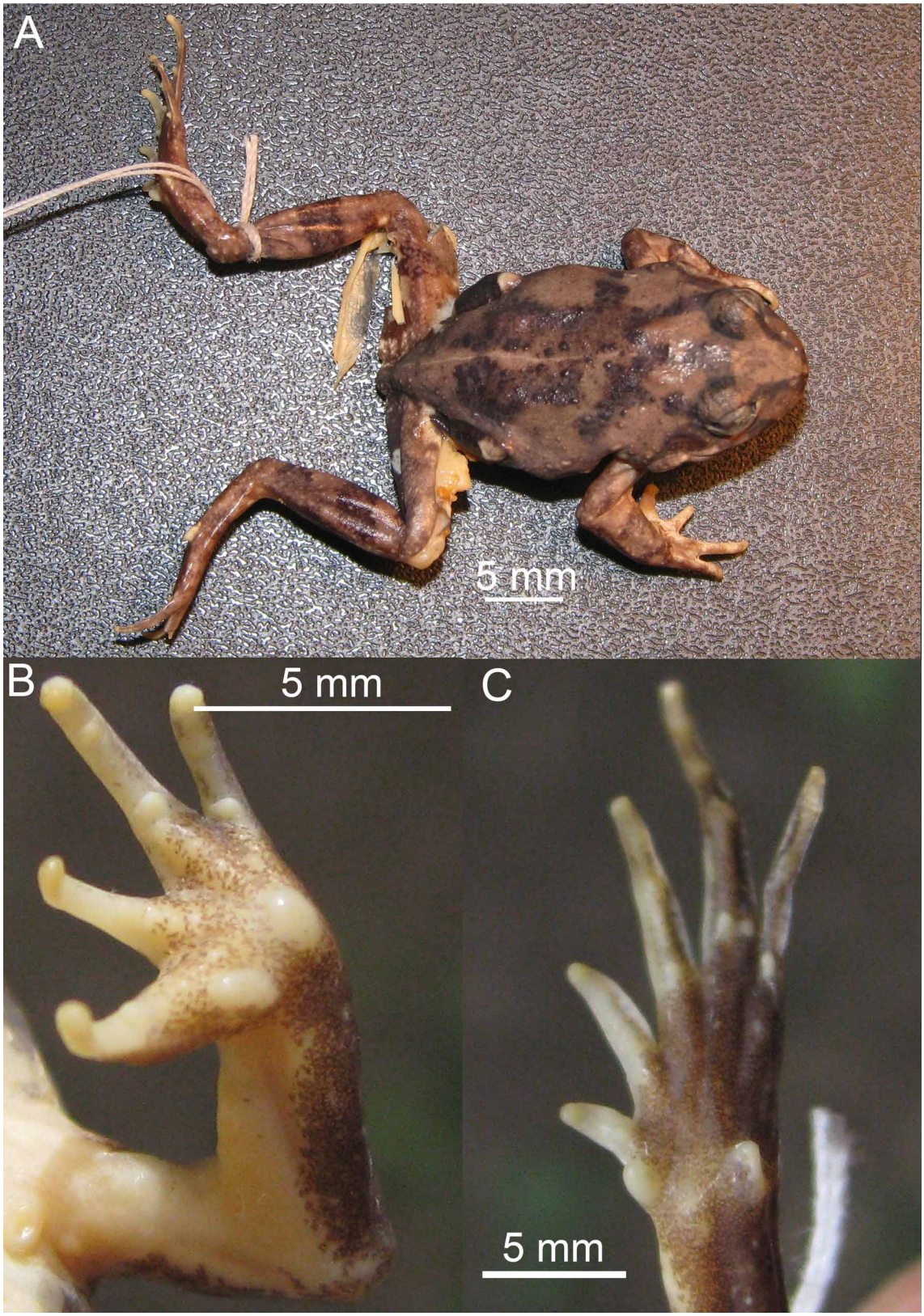


**FIGURE 8**. *Pleurodema kriegi*, neotype (FML 20460, SVL 34.4 mm), (A) dorsal view of specimen; ventral views of (B) hand and (C) foot. Photograph by J. Valetti.

**FIGURE 9**. Typical habitat of *Pleurodema kriegi*. La Posta, Pampa de Achala. Córdoba province, Argentina. Photograph by J. Valetti and A. Martino.

**Ecology.** *Pleurodema kriegi* males were acoustically active from the first rains of austral spring (November) to March from 21.00 hs. to 3.00 hr. (sunset time: 21.00 – 21.30 hr) *Pleurodema kriegi* breeds in temporary and semipermanent ponds with vegetation at the edges, or aquatic vegetation, and 10 to 40 cm deep (Fig. 9). The males emit their calls floating in the surface of the water or underground near the edge of ponds. The eggs are deposited in semisubmerged eggs-masses and adhered to the aquatic vegetation. The amplexus is axillary. This species was observed in sympatry with *Rhinella achalensis*, and in syntopy with *R. arenarum, Odontophrynus achalensis* and *Hypsiboas cordobae*.

The tadpole of *Pleurodema kriegi* was recently redescripted by Kolenc *et al.* (2009) and a comparison with *Pleurodema bibroni* was performed.

## Species description

***Pleurodema cordobae* sp. nov.**
Fig. 10

**Material. Holotype**. FML 20490, adult male (Fig. 11) from Estancia Los Tabaquillos (32º23'58.4" S, 64º55'35.1" W, approximately 2105 m elevation), Sierra de Comechingones, Córdoba province, Argentina (Fig. 1), collected during the night of 05 February 2006 by Adolfo L. Martino and Julián A. Valetti.

**Paratypes (9 specimens).** FML 20492, adult female and FML 20484–20489, 20491, eight adult males, all collected with the holotype.

**Diagnosis.** A small species of *Pleurodema* (35.41 mm SVL) with lumbar glands and chromosomic complement octoploid. The new species is distinguished from other species of the genus *Pleurodema* by the

following combination of characters: (1) relatively small size; (2) snout short, canthus rostralis rounded in dorsal view and truncate in lateral view; (3) lumbar glands present, 1 ½ eye diameter; (4) round tympanic annulus, almost concealed; (5) tympanum length half of the eye diameter; (6) vomerine teeth absent, (7) comissural gland prominent; (8) brilliant red-orange spots on the groin and around lumbar glands; (9) ploid level 2n=8x=88; (10) axillary amplexus; (11) egg deposition in gelatinous nest attached to vegetation; (12) compound advertisement call with tri-pulsed pulse groups, average dominant frequency 1787 Hz.

According to external morphology, *Pleurodema kriegi, Pleurodema bibroni* and *Pleurodema cordobae* **sp. nov.** are cryptic and the properties of their advertisement calls are very similar. *Pleurodema cordobae* **sp. nov.** is the only octoploid species of the genus (*P. kriegi* and *P. bibroni* are tetraploid and the rest of species are diploid; Brum-Zorrilla & Sáez, 1968; Barrio & Rinaldi de Chieri, 1970; Veloso *et al*., 1972; Duellman & Veloso, 1977; Schmid *et al.*, 1993; Lourenço *et al.*, 2006). Morphometric comparisons indicate differences between *Pleurodema cordobae* **sp. nov.** and *P. kriegi* (Table 1). However, there are no morphometric measures that allow a precise identification between both *taxa*. The temporal variables of the advertisement call differ from *P. kriegi* (Table 2) and the pulse rate is higher than *P. kriegi*. The erythrocyte size of *Pleurodema cordobae* **sp. nov.** is significantly bigger than *P. kriegi* (Table 4) near 100 $\mu m^2$.

**FIGURE 10**. Adult male of *Pleurodema cordobae* **sp. nov.** from Estancia Los Tabaquillos, Sierra de Comechingones, Córdoba province, Argentina. Photograph by A. Martino and J. Valetti.

*Pleurodema cordobae* **sp. nov.** is distinguished markedly from *P. tucumanum, P. nebulosum, P. guayapae, P. marmoratum* and *P. diplolister* by the presence of lumbar glands; from *P. bufoninum, P. borellii, P. cinereum* and *P. thaul* by the presence of advertisement call compound by tri-pulsed pulse groups (absent in *P. bufoninum*, Duellman & Veloso 1977; formed by a single pulse group in *P. borellii* and *P. cinereum*, McLister *et al*. 1991; and formed by pulse groups of 5-6 pulses in *P. thaul*, Barrio, 1977); from *P. brachyops* by having lumbar glands bigger than eye diameter (smaller in *P. brachyops*), yellow lumbar glands with black central ocellus (black lumbar glands with whitish central blotchs in *P. brachyops*), tympanum size half of the eye diameter (smaller than half eye diameter in *P. brachyops*).

Sixteen morphometric distances of the type series describe the quantitative morphological features of the new species (Table 5).

**Description of the holotype.** The holotype (FML 20490, Fig. 11) is an adult male of 35.2 mm (Table 5), body robust; head triangular slightly wider than long; snout short, canthus rostralis rounded in dorsal view and truncated in lateral view. Eyes protuberant; eye diameter equal to interocular distance; interocular distance larger than internarinal interval. Round tympanic annulus almost concealed, approximately half the size of eye diameter. Commissural glands present, oval, approximately the same size of eye diameter. Vomerine teeth absent. Dark vocal sac. Fingers free; relative length of fingers: 3>4>1>2; two not-darkened palmar tubercles (Fig. 11). Femur length less than tibia length; sum of femur length and tibia length longer than foot length.

Toes free with cutaneous edge and rudimentary interdigital basal membrane; two metatarsal tubercles (Fig. 11); relative length of toes: 4>5=3>2>1. Lumbar glands large, oval, one and a half times the size of eye diameter. Vocal sacs single, median and subgular. In life, dorsally brownish with large almost symmetrical dark spots. Dark transverse bands on upper surface of arms and legs. Yellow lumbar glands with a black central ocellus covering 60% of the gland. Brilliant red-orange spots on the groin and around lumbar glands. Dark palms and soles, with whitish palmar tubercles. Dark vocal sac, with the rest of ventral body surface being whitish and mild dotted dark. Iris gold with black reticulations.

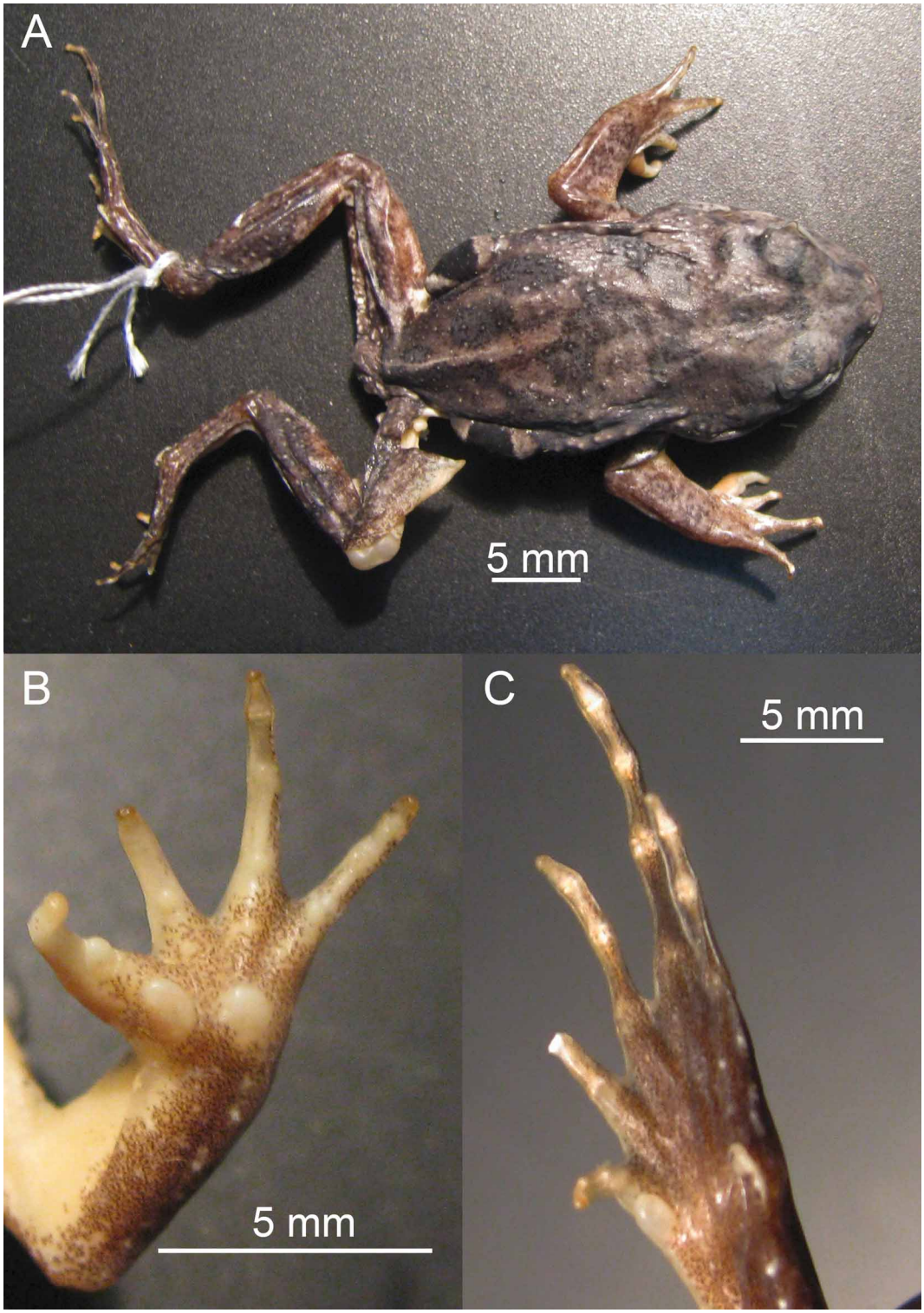


**FIGURE 11**. *Pleurodema cordobae* sp nov., holotype (FML 20490, SVL 32.22 mm), (A) dorsal view of specimen; ventral views of (B) hand and (C) foot. Photograph by J. Valetti.

**FIGURE 12**. Type locality of *Pleurodema cordobae* **sp. nov.** Estancia Los Tabaquillos, Sierra de Comechingones. Córdoba province, Argentina. Photograph by J. Valetti and A. Martino.

**TABLE 5.** Morphometric features of the type series of *Pleurodema cordobae* **sp. nov.** which were collected at Est. Los Tabaquillos, Córdoba. All data are given in [mm]. (1) snout-vent length (SVL); (2) maximal head width; (3) head length; (4) snout-eye distance; (5) internarinal distance; (6) interocular distance; (7) eye-narinal distance; (8) rostronarinal distance; (9) eye diameter; (10) arm length; (11) length of 3rd finger; (12) femur length; (13) tibia length; (14) foot length; (15) length of 3rd toe; (16) length of 4th toe. FML = Fundación Miguel Lillo, Tucumán, Argentina. M= male; F= female.

| | sex | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) |
|---|---|---|---|---|---|---|---|---|---|
| Holotype | | | | | | | | | |
| FML 20490 | M | 35.2 | 13.1 | 12.8 | 5.7 | 2.1 | 3.5 | 3.0 | 3.5 |
| | | | | | | | | | |
| Paratype | | | | | | | | | |
| FML 20492 | F | 42.9 | 15.5 | 15.5 | 6.7 | 2.6 | 3.9 | 3.5 | 4.4 |
| FML 20483 | M | 34.3 | 12.7 | 13.5 | 5.2 | 2.0 | 2.9 | 2.8 | 3.3 |
| FML 20484 | M | 35.1 | 14.0 | 14.4 | 5.4 | 2.3 | 2.6 | 2.8 | 3.1 |
| FML 20485 | M | 37.5 | 13.5 | 14.4 | 5.3 | 2.0 | 3.3 | 3.2 | 3.1 |
| FML 20486 | M | 34.0 | 12.0 | 13.2 | 5.0 | 2.0 | 2.9 | 2.7 | 2.9 |
| FML 20487 | M | 35.1 | 13.8 | 13.0 | 5.2 | 2.0 | 3.6 | 2.6 | 3.1 |
| FML 20488 | M | 35.0 | 12.6 | 13.0 | 5.7 | 2.0 | 3.1 | 2.5 | 2.8 |
| FML 20489 | M | 32.7 | 12.2 | 12.8 | 5.4 | 2.4 | 2.9 | 2.7 | 3.3 |
| FML 20491 | M | 35.2 | 13.7 | 11.7 | 5.6 | 2.2 | 3.1 | 2.7 | 3.3 |

continued.

| | sex | (9) | (10) | (11) | (12) | (13) | (14) | (15) | (16) |
|---|---|---|---|---|---|---|---|---|---|
| Holotype | | | | | | | | | |
| FML 20490 | M | 3.6 | 16.0 | 4.5 | 13.6 | 15.0 | 22.5 | 5.6 | 9.1 |
| | | | | | | | | | |
| Paratype | | | | | | | | | |
| FML 20492 | F | 4.0 | 20.5 | 6.6 | 18.3 | 19.9 | 30.0 | 6.5 | 12.1 |
| FML 20483 | M | 3.8 | 16.0 | 5.2 | 16.1 | 16.6 | 25.0 | 5.5 | 9.8 |
| FML 20484 | M | 3.7 | 15.0 | 5.0 | 15.3 | 16.6 | 25.0 | 5.4 | 10.3 |
| FML 20485 | M | 3.6 | 18.1 | 5.9 | 16.6 | 17.1 | 27.3 | 5.6 | 10.8 |
| FML 20486 | M | 3.3 | 16.2 | 5.2 | 14.7 | 15.3 | 23.8 | 5.1 | 8.4 |
| FML 20487 | M | 3.6 | 16.3 | 5.4 | 14.1 | 13.3 | 24.9 | 5.7 | 10.5 |
| FML 20488 | M | 2.5 | 16.2 | 4.6 | 15.5 | 15.7 | 23.5 | 5.0 | 9.5 |
| FML 20489 | M | 3.5 | 16.3 | 5.4 | 14.8 | 15.7 | 23.8 | 5.5 | 9.6 |
| FML 20491 | M | 3.6 | 15.8 | 5.3 | 15.6 | 16.5 | 25.0 | 5.5 | 10.3 |

**FIGURE 13**. Typical localization of calling male of *Pleurodema cordobae* **sp. nov.** Type locality, Estancia Los Tabaquillos, Córdoba province, Argentina. Photograph by A. Martino and J. Valetti.

**Distribution.** The species is currently known from *Terra typica*, Estancia Los Tabaquillos (Fig. 1) and two temporary ponds located 5 kilometers away from the former.

**Ecology.** *Pleurodema cordobae* **sp. nov.** males were acoustically active from December to March (Austral summer) from 21.00 hs. to 4.00 hr. (sunset time: 21.00 – 21.30 hr) *Pleurodema cordobae* breeds in temporary and semipermanent ponds with vegetation at the edges and a depth of 20 to 30 cm (Fig. 12). The

males emit their calls floating on the surface of the water near the edge of ponds (Fig. 13). The eggs are deposited in semisubmerged eggs-masses and adhered to the vegetation (Fig. 14). The amplexus is axillary. This species was observed in syntopy with *Rhinella achalensis*, *R. arenarum*, *Odontophrynus achalensis* and *Hypsiboas cordobae*.

**Etymology.** The specific name (a noun in the genitive case) refers to the currently known geographical distribution of the new species.

**FIGURE 14**. Egg deposition mode of *Pleurodema cordobae* **sp. nov.** Eggs mass and small tadpoles hatching. Estancia Los Tabaquillos, Córdoba province, Argentina. Photograph by A. Martino and J. Valetti.


## Acknowledgments

We thank Pablo Grenat and Pablo Brandolin for help in field work, Ricardo Martori for facilitating the microscope to measure the erythrocytes and Susan Vilor for her assistance with the English review. We are very grateful to Ulrich Sinsch, Frank Glaw and Miguel Vences for help us to solve the nomenclatural problem between *Pleurodema kriegi* and *Pleurodema cordobae* sp. nov. and we also acknowledge Miguel Vences and the anonymous reviewers for their useful comments and suggestions on the manuscript. The access and field work in Parque Nacional Quebrada de Los Condoritos were provided by Delegación Técnica de Parques Nacionales whose cooperation is greatly appreciated. The Secretary Research and Technology of National University of Río Cuarto (SECyT-UNRC) provided funds by Grant PPI 18/C288. The first author thanks CONICET – Argentina (Consejo Nacional de Investigaciones Cienctíficas y Tecnológicas) for postgraduate fellowship granted.

## Reference


Alzuet, A.B. de & Mauri, R.A. (1987) Ácaros Trombiculidae del género *Hannemania* Oudemans, endoparásitos de anfibios. *Revista de la Sociedad Entomológica Argentina*, 44 (2), 111–116.

Angulo, A. & Reichle, S. (2008) Acoustic signals, species diagnosis, and species concepts: the case of new cryptic species of *Leptodactylus* (Amphibia, Anura, Leptodactylidae) from the Chapare region, Bolivia. *Zoological Journal of the Linnean Society*, 152, 59–77.

Ávila, L.J. & Priotto, J.W. (1995) Geographic Distribution. *Pleurodema kriegi. Herpetological Review*, 26 (2), 106.

Barrio, A. (1977) Aportes para la elucidación del "status" taxonómico de *Pleurodema bibroni* Tschudi y *Pleurodema kriegi* (Müller) (Amphibia, Anura, Leptodactylidae). *Physis*, 37(93), 311–331.

Barrio, A. & Rinaldi de Chieri, P. (1970) Estudios citogenéticos sobre el género *Pleurodema* y sus consecuencias evolutivas (Amphibia, Anura, Leptodactylidae). *Physis*, 30(80), 309–319.

Beçak, M.L. & Beçak, W. (1998) Evolution by polyploidy in Amphibia: New insights. *Cytogenet Cell Genet*, 80, 28–33.

Bogart, J.P. (1980) Evolutionary significance of polyploidy in amphibians and reptiles. *In*: Lewis, W (Ed), *Polyploidy: biological relevance*. Plenum Press, New York, pp. 341–378.

Bogart, J.P. & Wasserman, A.O. (1972) Diploid-polyploid species pairs: a possible clue to evolution by polyploidization in anuran amphibians. *Cytogenetics*, 11, 7–24.

Brum-Zorrilla, N. & Sáez, F.A. (1968) Chromosomes of Leptodactylidae (Amphibia Anura). *Experienta*, 24, 969.

Cabrera, M.R. (1996) Lista y distribución geográfica de saurios, anfisbenas y tortugas (Amniota, Reptilia) de la provincia de Córdoba. *In*: di Tada, I.E. & Bucher, E.H. (Eds.), *Biodiversidad de la Provincia de Córdoba. Fauna. Vol. I*, Universidad Nacional de Río Cuarto, Argentina, pp. 215–238.

Cei, J.M. (1972) Segregación corológica y procesos de especiación por aislamiento en anfibios de la Pampa de Achala, Córdoba. *Acta Zoológica Lilloana*, 29, 233–246.

Cei, J.M. (1987) Additional notes to Amphibians of Argentina: An update, 1980-1986. *Monitore Zoologico Italiano*.(N.S.), 21, 209–272.

Charif, R.A., Mitchell, S. & Clark, C.W. (1995). *Canary 1.2 user's manual*. Ithaca, New York: Cornell Laboratory of Ornithology, 229 pp.

di Tada, I.E., Saluso, M.M. & Martori, R. (1976). Lista de los batracios de Córdoba. *Boletín de la Academia Nacional de Ciencias*, 51(3-4), 325–362.

di Tada, I.E., Barla, M.J., Martori, R.A. & Cei, J.M. (1984) *Odontophrynus achalensis*. Una nueva especie de anfibio de la Pampa de Achala (Córdoba, Argentina). *Historia Natural*, 4(17), 149–155.

di Tada, I.E., Zavattieri, M.V., Bridarolli, M.E., Salas, N.E. & Martino, A.L. (1996) Anfibios Anuros de la Provincia de Córdoba. *In*: di Tada, I.E. & Bucher, E.H. (Eds.), *Biodiversidad de la Provincia de Córdoba. Fauna. Vol. I*, Universidad Nacional de Río Cuarto, Argentina, pp. 191–213.

Duellman, W.E. & Trueb, L. (1986) *Biology of Amphibian*. McGraw Hill Inc., USA, 670 pp.

Duellman, W.E. & Veloso, A. (1977) Phylogeny of *Pleurodema* (Anura: Leptodactylidae): A biogeographic model. *Occasional papers, Museum of Natural History, University of Kansas, Lawrance, Kansas*, 64, 1–46.

Frost, D.R. (1985) *Amphibian species of the world. A review of amphibian species of the world: A taxonomic and geographical reference*. Allen Press, Inc. and The Association of Systematic Collection. Lawrence, KA. 732 pp.

Frost, D.R. (2008) Amphibian Species of the World: an online reference,. version 5.2, American Museum of Natural History, New York, USA. Available from http://research.amnh.org/herpetology/amphibia/index.php (accessed 1 January 2009).

Gallardo, J.M. (1964) Los géneros *Urostrophus* Dumeril et Bibron y *Cupriguanos* gen. nov. (Sauria, Iguanidae) y sus especies. *Neotropica*, 10, 125–136.

Gallardo, J. M. (1968) Sobre la validez de algunas especies Argentinas de *Pleurodema* (Anura, Leptodactylidae). *Physis*, 28(76), 135–144.

Gerhardt, H.C. (1978) Temperature coupling in the vocal communication system of the gray tree frog *Hyla versicolor. Science*, 199, 992–994.

Glaw, F & Franzen, M. (2006) Type catalogue of amphibians in the Zoologische Staatssammlung München. *Spixiana*, 29(2), 153–192.

Hayek, L.C., Heyer, R.W. & Gascon, C. (2001) Frog morphometrics: a cautionary tale. *Alytes*, 18, 153–177.

Heyer, W.R. (1975) A preliminary analysis of the intergeneric relationships of the frog family Leptodactylidae. *Smithsonian Contributions to Zoology*, 199, 1–55.

Heyer, W.H. (1997) Geographic variation in the frog genus *Vanzolinius* (Anura: Leptodactylidae). *Proceedings of the Biological Society of Washington*, 110, 338-365.

Holloway, A.K., Cannatella, D.C., Gerhardt, H.C. & Hillis, D.M. (2006) Polyploids with different origins and ancestors form a single sexual polyploid species. *The American Naturalist*, 167, E88-E101.

ICZN (1999) *International Code of Zoological Nomenclature*, International Trust for Zoological Nomenclature, London,

306 pp.
Kawamura, T. (1984) Polyploidy in amphibians. *Zoological Science*, 1, 1–5.
Kolenc, F., Borteiro, C., Baldo, D., Ferraro, D. & Prigioni, C. (2009) The tadpoles and advertisement calls of *Pleurodema bibroni* Tschudi and *Pleurodema kriegi* (Müller), with notes on their geographic distribution and conservation status (Amphibia, Anura, Leiuperidae). *Zootaxa*, 1969, 1–35.
Laurent, R.F. (1975) Diferenciación biométrica entre dos poblaciones similares de *Pleurodema*. *Acta Zoológica Lilloana*, 31(10), 99–106.
Levan, A., Frega, K. & Sandberg, A. (1964) Nomenclature of centromeric position on chromosomes. *Hereditas*, 52, 201–220.
Lourenço, L.B., Nascimento, J.A., Andrade, G.V., Rossa-Feres, D.C., Recco-Pimentel, S.M. (2006) Chromosomal analysis of the leptodactylids *Pleurodema diplolistris* and *Physalaemus nattereri* (Amphibia, Anura). *Amphibia-Reptilia*, 27(4), 481–489.
Lynch, J.D. (1971) *Evolutionary relationships, osteology, and zoogeography of leptodactyloid frogs*. University of Kansas. Museum of Natural History. Miscellaneous publication no. 53, 238 pp.
Martino, A. & Sinsch, U. (2002) Speciation by polyploidy in *Odontophrynus americanus Journal of Zoology* (London), 257, 67–81.
McLister, J.D., Lougheed, S.C. & Bogart, J.P. (1991) Electrophoretic and vocalization comparisons among three leptodactylid frogs (*Pleurodema* spp) from northwestern Argentina. *Canadian Journal of Zoology*, 69, 2397–2403.
Miró, R.C. (1999) El basamento Precámbrico-Paleozoico Inferior de las Sierras Pampeanas, Famatina, Cordillera Oriental y Puna. *In*: Caminos, R. (Ed.), *Geología Argentina*. Instituto de Geología y Recursos Minerales. Anales 29, Buenos Aires, Argentina, pp. 133–167.
Müller, L. (1926) Neve reptilien und Batrachier der Zoologischen Sammlung des bayrischen Staate. *Zoologischer Anzeiger, Leipzig*, 65, 193–200.
Nascimento, L.B, Pimenta, B.V.S., Cruz, C.A.G. & Caramaschi. U. (2006) Taxonomic status of *Gomphobates marmoratus* Reinhardt and Lütken, 1862 1861 and *Eupemphix fuscomaculatus* Steindachner, 1864 (Amphibia, Anura, Leptodactylidae). *South American Journal of Herpetology*, 1(3), 166–174.
Nöller, H.G. (1959). Eine einfache Technik der Blutentnahme beim Frosch. *Pflügers Arch Gesamte Physiol Menschen Tiere*. 269: 98–100.
Orr, H.A. (1990) Why polyploidy is rarer in animals than in plants. Revisited. *The American Naturalis,*. 136, 759–770.
Otto, S.P. & Whitton, J. (2000) Polyploid incidence and evolution. *Annual Review of Genetics*, 34, 401–437.
Parker, H.W. (1927) A revision of the frogs of the genera *Pseudopaludicola*, *Physalaemus* and *Pleurodema*. *Annals and Magazine of Natural History* 9, 450–478.
Platz, J.E. & Forester, D.C. (1988) Geographic variation in mating call among the four subspecies of the chorus frog: *Pseudacris triseriata* (Wied). *Copeia*, 1988, 1062–1066.
Ralin, D.B. (1977) Evolutionary aspects of mating call variation in a diploid–tetraploid species complex of tree-frogs (Anura). *Evolution*, 31, 721–736.
Ramos, V.A. (1999) Rasgos estructurales del territorio Argentino. I. Evolución tectónica de la Argentina. *In*: Caminos, R. (Ed.), *Geología Argentina*. Instituto de Geología y Recursos Minerales. Anales 29 , Buenos Aires, Argentina, pp. 715–786.
Rosset, S.D., Baldo, D., Lanzone, C. & Basso, N.G. (2006) Review of the geographic distribution of diploid and tetraploid populations of the *Odontophrynus americanus* species complex (Anura: Leptodactylidae). *Journal of Herpetology*, 40 (4), 465–477.
Schmalz, T.D. & and Zug, G.R. (2002) Observations on geographic variation in the Asian frog, *Hoplobatrachus rugulosus* (Anura: Ranidae). *Hamadryad*, 27 (1), 90–98.
Schmid, M. (1978) Chromosome banding in amphibia. 1. Constitutive heterochromatin and nucleolus organizer regions in *Bufo* and *Hyla*. *Chromosoma*, 66, 361–388.
Schmid, M., Steinlein, C., Feichtinger, W. Poot, M. (1993) Chromosome banding in Amphibia. XVIII. Karyotype evolution and genomic size variation in *Pleurodema* (Anura, Leptodactylidae). *Cytogenetics and Cell Genetics*, 62, 42–48.
Stöck, M. (1998) Mating call differences between diploid and tetraploid green toads (*Bufo viridis* complex) in middle Asia. *Amphibia-Reptilia*, 19, 29–42.
Stöck, M. & Grosse, W.R. (1997) Erythrocyte size and ploidy determination in green toads (*Bufo viridis* complex) from Middle Asia. *Alytes*, 15(2), 72–90.
Stöck, M., Lamatsch, D.K., Steinlein, C., Epplen, J.T., Grosse, W.R., Hock, R., Klapperstück, T., Lampert, K.P., Scheer, U., Schmid, M. & Schartl, M. (2002) A bisexually reproducing all-triploid vertebrate. *Nature Genetics*, 30, 325–328.
Veloso, A.M., Diaz, N.P., Galleguillos; R.G. (1972) Revisión cariosistemática de las especies del género *Pleurodema* en Chile (Anura: Leptodactylidae). *Anales del Museo de Historia Natural de Valparaíso*, 6, 49–55.
Wasserman, A.O. (1970) Polyploidy in the common tree toad *Hyla versicolor* Le Conte. *Science*, 167, 385–386.
White, M.J.D. (1973). *Animal cytology and evolution*. Cambridge University Pr Press, London, 961 pp.

## Appendix: Specimens analyzed

*Pleurodema cordobae* **sp. nov.**

ARGENTINA: **Córdoba province**: Estancia Los Tabaquillos, Sierra de Comechingones (approximately 2105 m elevation); FML 20490 (Holotype); FML 20483–20489, FML 20491–20492 (nine Paratypes); FML 20477–20482, FML 20493–20505 (19 specimens).

*Pleurodema kriegi*

ARGENTINA: **Córdoba province**: La Posta, Pampa de Achala (approximately 2151 m elevation); FML 20460 (Neotype), FML 20459, 20461–20476 (17 specimens)