Volume 23 (October 2013), 193–202

Herpetological Journal

FULL PAPER

Published by the British Herpetological Society

Amphibian abnormalities: Historical records of a museum collection in Tucuman Province, Argentina

Regina G. Medina[1,2,3], María L. Ponssa[2,3], Cecilia Guerra[2,3] & Ezequiel Aráoz[1,3,4]

[1]Facultad de Ciencias Naturales e I.M.L, Universidad Nacional de Tucumán, San Miguel de Tucumán (4000), Argentina,

[2]Instituto de Herpetología, Fundación Miguel Lillo, San Miguel de Tucumán (4000), Argentina,

[3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina,

[4]Instituto de Ecología Regional, Universidad Nacional de Tucumán, CC 34, Yerba Buena (4107), Tucumán, Argentina

We analyze patterns of abnormalities in anurans from Tucumán, Argentina, deposited in the Fundación Miguel Lillo collection. We examined 1651 specimens collected between 1940 and 2010. Overall abnormality prevalence was 4.4% for post-metamorphic individuals. The most common types of abnormalities affected the hindlimbs, and concerned reductions in the number of phalanges. Abnormality prevalence for post-metamorphic individuals did not vary by species or region, but varied temporally over the 50 year dataset. Overall abnormality prevalence was higher for tadpoles (30%), and the most common types of abnormality were extraneous projections in the marginal papillae of oral discs. In tadpoles, abnormality types varied among species, development stages and affected oral disc regions.

Key words: abnormality, Anura, biological collection, longitudinal study, morphology

INTRODUCTION

Historically, established museum collections have been used to support taxonomic and systematic research. However, in the last 20 years there has been a rise of their application in ecological and environmental studies, since they preserve historical records that help us understand biological changes over time (Pyke & Ehrlich, 2010). In addition to the specimens themselves, collections offer valuable information about collection dates and sites.

Museum collections have proved valuable in the study of anuran morphological abnormalities (e.g., Gray, 2000; Johnson et al., 2003; McCallum & Trauth, 2003; Gridi-Papp & Gridi-Papp, 2005; Hoppe, 2005). Reports of amphibian abnormalities have appeared in the literature since 1860 (Lannoo, 2008). The frequency of these reports increased dramatically in recent years (Ouellet, 2000; García-Muñoz et al., 2010), causing considerable concern because abnormalities could exacerbate declines in already threatened or vulnerable populations (Johnson et al., 1999, 2001; Souder, 2000). Abnormality studies mainly focus on metamorphosed individuals and larvae at late stages, reporting limb malformations (Ouellet, 2000; Johnson et al., 2010). Other body areas commonly affected by abnormalities are the craniofacial region (e.g., Meteyer, 2000; Meteyer et al., 2000), tails (e.g., Hopkins et al., 2000) and oral discs of tadpoles (e.g., Drake et al., 2007). Parasitic cysts and predatory attacks cause missing and abnormal limbs (Johnson et al., 1999, 2001, 2002; Ankley et al., 2004; Ballengée & Sessions, 2009; Bowerman et al., 2010), and infection by *Batrachochytrium dendrobatidis* causes abnormalities in the oral discs of tadpoles and skin damage in adults (e.g., Fellers et al., 2001; Knapp & Morgan, 2006; Barrionuevo et al., 2008). UV-B can cause flexion of the tail and edema (Blaustein et al., 1997; Ankley et al., 2000, 2002) and chemical contaminants affect the skin and skeleton of limbs, and the normal development of reproductive organs and growth (Gardiner & Hoppe, 1999; Hayes et al., 2002, 2006). The action of multiple stressors are further referred to as causes of amphibian abnormalities (Reeves et al., 2010)

While reports of abnormalities are widespread in North America (e.g., Kaiser, 1997; Ouellet, 2000; Reeves & Trust, 2004; Johnson et al., 2010), they are less common for other regions. In Argentina, abnormalities have been reported in both adults (e.g., Peri & Williams, 1988; Fabrezi, 1999; Attademo et al., 2004; Peltzer et al., 2001, 2011) and tadpoles (e.g., Lajmanovich et al., 2003a, 2003b; Sandoval et al., 2006) from the Buenos Aires, Entre Ríos, Córdoba, Santa Fe and Salta provinces, but there is currently no information available about present or historical occurrences of abnormalities from Tucumán Province. Here, we present the first study on long-term historical records of amphibian malformations in Argentina based on museum records, and the first study on amphibian abnormalities for

Correspondence: R.G. Medina (regina.g.medina@gmail.com)

Tucumán Province. We classify and describe the types of abnormalities encountered, and quantify the prevalence of abnormalities through time across localities, species, and life stages.

MATERIALS AND METHODS

The sample consisted of 1227 specimens of post-metamorphic individuals (juveniles and adults) and 424 specimens of tadpoles (Gosner Stages 25–46) deposited in the Herpetology Collection of the Instituto de Herpetología of the Fundación Miguel Lillo (FML). Post-metamorphic individuals of the following 19 species were examined: *Ceratophrys cranwelli, Dendropsophus nanus, Gastrotheca gracilis, Hypsiboas riojanus, Leptodactylus bufonius*, *L. chaquensis*, *L. gracilis, L. latinasus*, *L. mystacinus*, *Odontophrynus americanus, Oreobates discoidalis, Phyllomedusa sauvagii, Physalaemus biligonigerus, Pleurodema borellii, P. tucumanum, Rhinella arenarum*, *R. schneideri, Scinax fuscovarius* and *S. nasicus.* Our sample included specimens collected from 1940–2010 at six localities in Tucumán Province (see Online Appendix). Tadpoles of the following four species were also examined: *H. riojanus, O. americanus, P. borellii* and *R. arenarum*. This sample corresponds to 50% of specimens collected at the Yerba Buena site between 1976 and 1997.

Tucumán Province is located in northwestern Argentina, a subtropical area within a transition between western montane rainforests (the Yungas biogeographic province) and eastern semiarid forests (the Chacoan biogeographic province; Burkart et al., 1999). The area is characterized by agriculture and crops such as soy bean, sugar cane, wheat, corn, citrus and tobacco

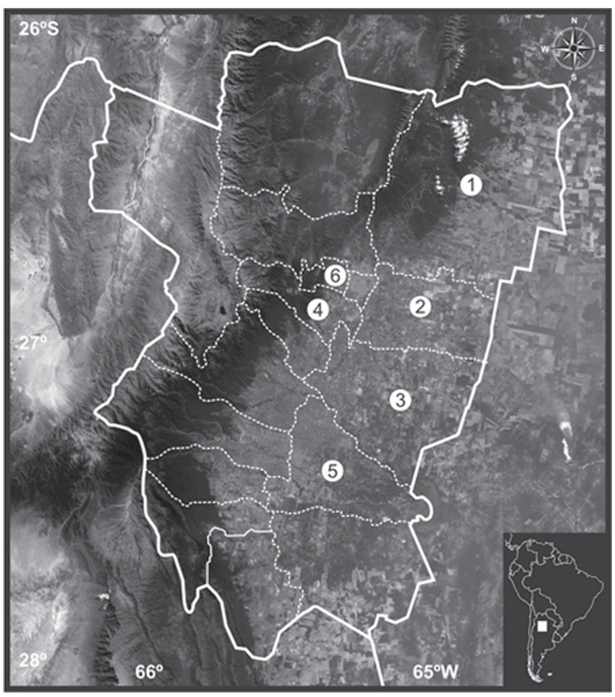

Fig. 1. Tucumán Province Departments where specimens were collected: 1) Burruyacu, 2) Cruz Alta, 3) Leales, 4) Lules, 5) Simoca, 6) Yerba Buena (see localities in Online Appendix).

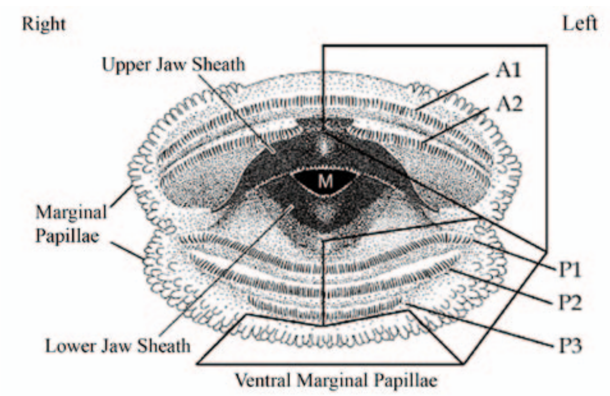

Fig 2. Oral disc illustration showing regions. A1–2=Anterior tooth rows 1 and 2; P1–3=Posterior tooth rows 1 through 3; M=mouth; Marginal Papillae, Ventral Marginal Papillae; Upper Jaw Sheath; Lower Jaw Sheath. Figure modified from Drake et al., 2007.

(Estación Experimental Agroindustrial Famaillá, 2010). We examined amphibians collected in six departments of Tucumán Province, including Yerba Buena and Lules located in the centre of the province, and Burruyacu, Cruz Alta, Leales and Simoca, located in the eastern portion of the province (Fig. 1). All sites have a long history of anthropogenic impacts.

The external morphology of specimens was analyzed to determine abnormalities. In post-metamorphic specimens, observed abnormalities were classified according to Meteyer (2000), USFWS (2008) and Rothschild et al. (2012). Digit amputations, a common marking practice of toe-clipping, were not considered as abnormalities. Oral disc abnormalities of tadpoles were classified according to Drake et al. (2007). Each abnormality type was classified by the affected area of the oral disc (Fig. 2). The areas considered were: A1, A2, P1, P2 and P3, depending on the tooth row; upper and lower, depending on the part of jaw sheath; and upper, lower or ventral, depending on the marginal papillae affected. For comparison with normal larval anatomy, we used descriptions of *Hypsiboas riojanus* (Kolenc et al., 2008), *Odontophrynus americanus* and *Pleurodema borellii* (Cei, 1980) and *Rhinella arenarum* (Vera Candioti, 2007).

Nine post-metamorphic specimens were eviscerated, cleared and double-stained for cartilage and bone following Wassersug (1976). These animals were dissected because they presented an abnormality not previously observed or not visible externally. The diaphanized specimens were also analyzed to assess the existence of parasites located in the joint areas. All observations were made with a Carl Zeiss (Discovery. V8) stereoscope. Photographs were taken with a digital camera (Nikon Coolpix P6000).

To test for associations across abnormality types we performed a permutation analysis based on 10,000 Monte Carlo randomizations of the six types of external abnormality found in adults (total n=58). The number of abnormality co-occurrences per specimen was recorded for every simulation. We then compared the results from the simulations with the observed counts of co-occurrence for each pair of abnormality type. The same

Table 1. Abnormality types of anuran specimens from six localities of Tucumán Province, Argentina (1940 to 2010), deposited at the Herpetology Collection, Instituto de Herpetología, Fundación Miguel Lillo. *n*: number of specimens studied; Abn: number of abnormal specimens; %: percentage of abnormal specimens.

Species	*n*	Abn	%	Abnormality types (number of occurrences)
Ceratophrys cranwelli	19	1	5.3	Brachydactyly (1), Ectrodactyly (1).
Dendropsophus nanus	3	0	0	-
Gastrotheca gracilis	1	0	0	-
Hypsiboas riojanus	79	0	0	-
Leptodactylus bufonius	6	0	0	-
L. chaquensis	109	5	4.6	Brachydactyly (1), Ectrodactyly (1), Swollen phalangeal articulation (2), Digits expanded at the end (3).
L. gracilis	2	0	0	-
L. latinasus	184	9	4.9	Brachydactyly (6), Ectrodactyly (3), Ectromelia (1), Absence of sub-phalangeal tubercle (1).
L. mystacinus	37	2	5.4	Brachydactyly (1), Ectrodactyly (1).
Odontophrynus americanus	64	3	4.7	Brachydactyly (3).
Oreobates discoidalis	7	0	0	-
Phyllomedusa sauvagii	13	0	0	-
Physalaemus biligonigerus	54	1	1.8	Brachydactyly (1).
Pleurodema borellii	197	13	6.6	Brachydactyly (9), Ectrodactyly (2), Swollen phalangeal articulation (2), Digits expanded at the end (3), Hemimelia (1).
P. tucumanum	77	3	3.9	Brachydactyly (2), Ectromelia (2).
Rhinella arenarum	239	9	3.7	Brachydactyly (6), Ectrodactyly (1), Ectromelia (2), Swollen phalangeal articulation (2).
R. schneideri	25	0	0	-
Scinax fuscovarius	79	1	1.3	Brachydactyly (1).
S. nasicus	32	0	0	-
TOTAL	1227	47	3.8	

analysis was performed for abnormalities recorded in tadpoles.

To assess whether abnormality prevalence changed between time periods, localities or species, we performed independent chi-squared tests. All specimens were classified by three predictor variables: collection period (decade), locality and species. Because different types of abnormality were not independent, the frequency table was built using abnormal specimens rather than specific abnormalities. Due to the expected number of abnormal specimens being low, we estimated *p* values through 10,000 Monte Carlo simulations rather than contingency tables. For tadpoles and newly metamorphosed juveniles we used chi-squared tests to test for independence between species and abnormality prevalence. We also used chi-squared tests to evaluate the homogeneity of prevalence of abnormalities between different regions of the oral disc. Whenever expected abnormalities were below five, we used Monte Carlo simulations to evaluate the *p* value. We also evaluated the association of different types of abnormalities and the affected areas of the oral disc using the same procedure. To evaluate the assumption that abnormalities affect survival we fitted a logistic regression to abnormalities with life stage as a linear predictor variable using a logit transformation as a link function. Abnormal specimens within each stage were expressed as a proportion of the sample, and we used a binomial distribution for errors (Crawley, 2007). We fitted regressions using maximum likelihood methods (R v.2.13 software; R Core Team, 2012).

RESULTS

Types of abnormalities

Seven types of abnormality were observed in post-metamorphic individuals. The prevalence within species ranged from 0% to 6.6% with an overall mean of 3.8% (*n*=1227; Table 1). The specimens of each species affected are indicated in the Online Appendix. Four out of seven types of abnormality have been previously described: brachydactyly (reduction in the number of phalanges, but not in the number of metatarsal bones, Fig. 1 of the Online Appendix), ectrodactyly (complete missing digit including the metatarsal bone and phalanges), ectromelia (missing limb segment) and hemimelia (short bone, but distal limb and foot present).

In some cases of ectrodactyly, skeletal elements showed a peculiar morphology. *Leptodactylus latinasus* (FML 12256) showed four digits in the left hindlimb, with toe V being absent. Digit IV had a slight inward curvature

Table 2. Types of abnormalities in tadpoles from Yerba Buena, Tucumán Province, Argentina, deposited in the Herpetology Collection, Instituto de Herpetología, Fundación Miguel Lillo. *n*: number of specimens studied; Abn: number of abnormal specimens; %: percentage of specimens with abnormalities. TR1: missing teeth, TR2: missing teeth with disrupted supporting tissue, TR3: duplication of teeth, TR4: stunted or underdeveloped teeth, TR5: intersecting tooth rows, TR6: puckering (sharp convolutions) of tooth rows, TR7: overlapping rows, MP1: gaps or distortions in the edge, MP2: extraneous projections, JS1: lack of keratinization, JS2: breaks, gaps, or other deformities in the edge, FT: forked tail. TOTAL: total number of occurrences of types of abnormality.

Species	*n*	Abn	%	Ocurrence of types of abnormality												TOTAL
				TR1	TR2	TR3	TR4	TR5	TR6	TR7	MP1	MP2	JS1	JS2	FT	
Hypsiboas riojanus	211	84	39.8	12	21	0	3	4	5	2	3	60	8	2	1	121
Odontophrynus americanus	97	23	23.7	20	8	2	5	1	1	0	1	1	0	0	0	39
Pleurodema borellii	67	13	19.4	11	4	0	0	0	0	0	0	1	0	0	0	16
Rhinella arenarum	49	8	16.3	4	7	0	0	0	2	0	0	0	0	0	0	13
TOTAL	424	128	30.2	47	40	2	8	5	8	2	4	62	8	2	1	189

(clinodactyly) and only three phalanges (brachydactyly); the two more terminal phalanges were fused, and the remaining digits were normal. Another specimen of this species (FML 4809–9) showed four digits, with a reduced digit II (Fig. 2A of the Online Appendix). In the diaphanized specimen it was observed that digit II showed the metatarsal curved outward, and with only one phalange instead of two. Toe I presented only a metatatarsal bone and no phalanges, showing a brachydactyly case. This metatarsal is shorter than the normal bone, and this digit was externally not distinguishable (Fig. 2B of the Online Appendix). *Pleurodema borellii* (FML 02553–5) had the left hindlimb with four digits; toe V was absent (Fig. 2C of the Online Appendix). Internally, digit IV had two phalanges (brachydactyly) and the metatarsal bone was curved outward. Digits II and III were normal and digit I had a distal outward curvature (Fig. 2D of the Online Appendix). Externally, *L. chaquensis* (FML 01425–4) showed four digits; but scrutiny of the diaphanized specimen revealed the presence of five metatarsal bones, evidence of brachydactyly.

Three new types of abnormality were observed: (i) Absence of subphalangeal tubercle observed in digit I of the right hindlimb, whose skin had a smooth appearance (present in one specimen of *L. latinasus*, Fig. 3A of the Online Appendix); (ii) Swollen phalangeal articulation: the external anatomy showed a swelling between phalanges of the hindlimbs (Fig. 3B of the Online Appendix). Internally, the skeletal elements were not involved (Fig. 3C of the Online Appendix; this was found in *L. chaquensis* and *P. borellii*). (iii) Digits expanded at the end: observed in fore and hindlimbs, in *L. chaquensis* (Fig. 4A of the Online Appendix), *P. borellii* and *R. arenarum*. In this abnormality, the skeletal elements were not affected (Fig. 4B of the Online Appendix). However, in one specimen of *L. chaquensis*, with an apparent expansion of digit IV of both forelimbs (Fig. 4C of the Online Appendix), the osteological analysis demonstrated brachydactyly with

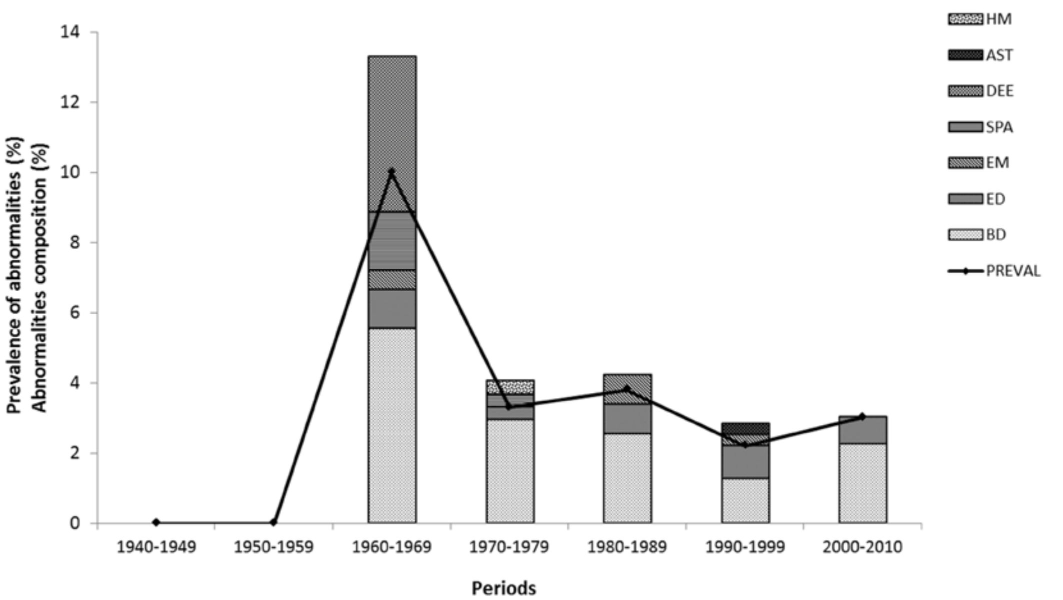

Fig. 3. Distribution of the abnormality prevalences (line) and of the types of abnormality (bars) expressed in percentages, through the historical records of the museum collection of the FML. The data were organized into seven periods of time. AST: Absence of subphalangeal tubercle; BD: Brachydactyly; DEE: Digits expanded at the end; ED: Ectrodactyly; EM: Ectromelia; HM: Hemimelia; SPA: Swollen phalangeal articulation.

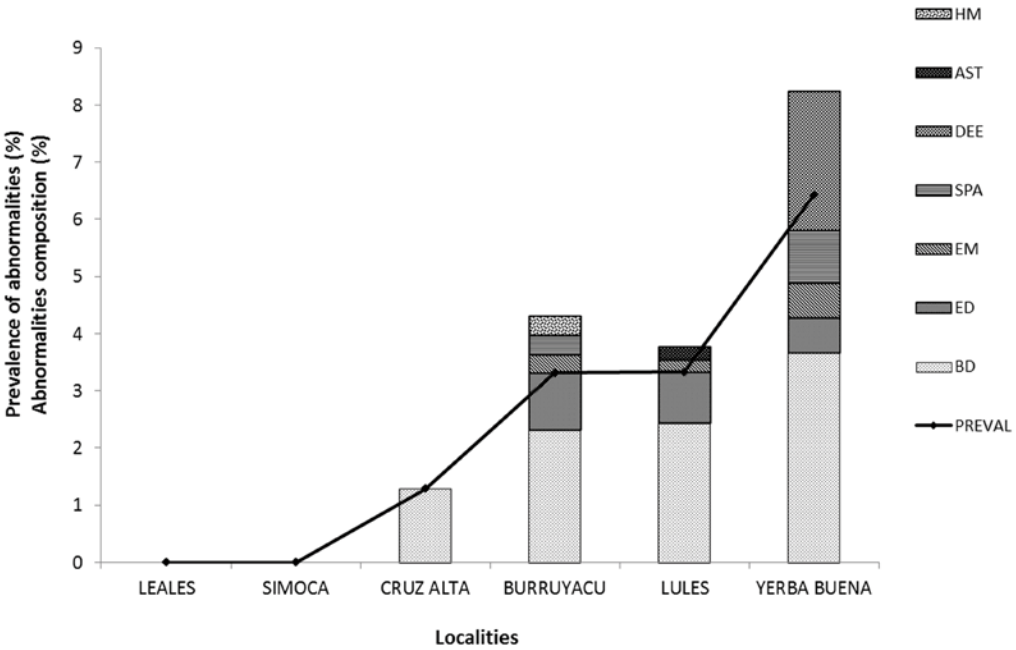

Fig. 4. Distribution of the abnormality prevalence (line) and of the types of abnormality (bars) as expressed in percentages, through the six studied localities. AST: Absence of subphalangeal tubercle; BD: Brachydactyly; DEE: Digits expanded at the end; ED: Ectrodactyly; EM: Ectromelia; HM: Hemimelia; SPA: Swollen phalangeal articulation.

absence of the terminal phalange (Fig. 4D of the Online Appendix). In all cleared and double-stained specimens, cysts of parasitic infections were absent.

Abnormalities recorded in tadpoles affected mainly the oral disc and, to a minor degree, the tail. Prevalence within species ranged from 16.3% to 39.8% with an overall mean of 30% (n=424, Table 2). Oral disc abnormalities were classified into three groups, affecting tooth rows, marginal papillae and jaw sheaths. Seven types of abnormality affecting tooth rows were found, six of which were previously described (Drake et al., 2007): (i) missing teeth (Fig. 5A of the Online Appendix), (ii) missing teeth with disrupted supporting tissue (Fig. 5B of the Online Appendix), (iii) duplication of teeth (e.g., double row, circular arrangement; Fig. 5C of the Online Appendix), (iv) stunted teeth, (v) intersecting tooth rows, and (vi) puckering (sharp convolutions) of tooth rows. A new type of abnormality, overlapping rows (Fig. 5D of the Online Appendix), was registered in the P3 tooth row for two specimens of *H. riojanus* at Stage 27. This abnormality implies a division of the P3 tooth row and the overlapping of the two resulting rows. Two types of abnormalities affected marginal papillae: (i) extraneous projections (Fig. 6A of the Online Appendix), and (ii) gaps or distortions in the edge (Fig. 6B of the Online Appendix). Two types of abnormality affected jaw sheaths: (i) lack of keratinization (Fig. 6C of the Online Appendix) and (ii) breaks, gaps or other deformities in the edge (Fig. 6D of the Online Appendix). Only one specimen, *H. riojanus* exhibited a forked tail.

There was an association between abnormality types of post-metamorphic individuals, implying that the presence of an abnormality in a specimen increased the probability of presenting a second abnormality. Brachydactyly in post-metamorphic specimens was associated with ectrodactyly (p<0.001) and with

Table 3. Types of abnormality by tooth rows affected. A1-A2: Anterior tooth rows; P1, P2, P3: posterior tooth rows; TR1: missing teeth, TR2: missing teeth with disrupted supporting tissue, TR3: duplication of teeth, TR4: stunted teeth or underdeveloped teeth, TR5: intersecting tooth rows, TR6: puckering (sharp convolutions) of tooth rows, TR7: overlapping rows; n: total types of anomalies in areas; %: percentage of types of anomalies in relation to total area anomalies.

Tooth rows	Types of abnormality														n TOTAL	% TOTAL
	TR1		TR2		TR3		TR4		TR5		TR6		TR7			
	n	%	n	%	n	%	n	%	n	%	n	%	n	%		
A1	27	62.8	11	25.6	0	0	2	4.6	0	0	3	7	0	0	43	38.4
A2	7	29	12	50	2	8.3	1	4	0	0	2	8.3	0	0	24	21.4
P1	3	33.3	3	33.3	0	0	2	22.2	0	0	1	11	0	0	9	8
P2	7	35	6	30	0	0	3	15	4	20	0	0	0	0	20	17.8
P3	3	18.7	8	50	0	0	0	0	1	6.2	2	12.5	2	12.5	16	14.3
TOTAL	47		40		2		8		5		8		2		112	

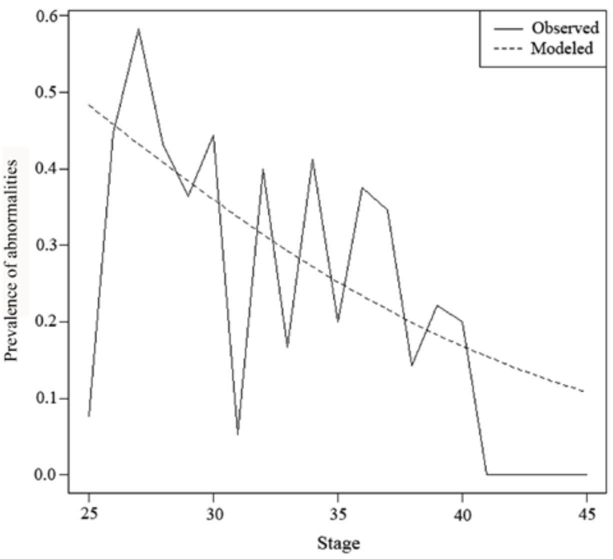

Fig. 5. Observed and modelled prevalence of abnormalities in amphibian tadpoles through developmental stages. Abnormalities were modelled as a binomial process with all the analyzed specimens per stage as the size and the prevalence of abnormalities as the probability.

digits expanded at the end (p<0.001). The swollen phalangeal articulation was associated with the digits expanded at the end (p<0.05). Only one adult presented three abnormalities in the same limb: brachydactyly, ectrodactyly and hemimelia (Fig. 2C,D of the Online Appendix). Also in tadpoles, there was a clustering of abnormalities across species (p<0.01; Fig. 7 of the Online Appendix).

Abnormality Presence

We found abnormalities in 47 post-metamorphic specimens belonging to ten species: *C. cranwelli, L. chaquensis*, *L. latinasus*, *L .mystacinus*, *O. americanus*, *P. biligonigerus*, *P. borellii*, *P. tucumanum*, *R. arenarum* and *S. fuscovarius.* A total of 58 abnormalities were observed; nine specimens presented more than one abnormality. Although abnormality rates in post-metamorphic individuals varied across species, they were not statistically different from each other.

Abnormality prevalence in post-metamorphic individuals was not uniform across decades (χ^2=25.1; p=0.001). The average prevalence of abnormalities over the entire study duration was 3.8%. Prevalence was highest in the 1960s, when 13.3% (n=24) of the observed specimens were affected respectively after no recorded abnormalities in the 1940s and 1950s (n=81 and 11 examined specimens). After the 1960s, abnormality prevalence varied between 2.8% and 4.2% (Fig. 3).

The prevalence of abnormalities in post-metamorphic individuals was uniform between localities (χ^2=10.66; p=0.061). Yerba Buena had the highest prevalence, with 8.2% of specimens affected. Neither the 55 specimens of Leales nor the 14 specimens of Simoca had external abnormalities. Abnormality rates in Cruz Alta and Burruyacu were 1.3% and 4.3%, respectively (Fig. 4).

Among larvae, we recorded 128 abnormal specimens with 189 abnormalities; 42 specimens presented more than one abnormality. Abnormality prevalence significantly varied across species (χ^2=19.36; df=3; p<0.001); rates were highest in *H. riojanus* (39.8%), followed by *O. americanus* (23.7%), *P. borellii* (19.4%) and *R. arenarum* (16.3%; Table 2). Abnormalities were not homogeneously distributed within the oral disc; 60% of abnormalities were in the anterior region and the remaining 40% in the posterior tooth rows. Tooth row A1 accounted for 38% of abnormalities, whereas P1 only accounted for 8% (χ^2=29.17; df=4; p<0.001; Table 3). Moreover, different rows were associated with specific types of abnormality (χ^2=53.92; p<0.001). A1 and P2 were affected mainly by missing teeth (62.8% and 35%, respectively), whereas A2, P1 and P3 were characterized by missing teeth with disrupted supporting tissue (50%, 33% and 50%, respectively; Table 3). In the marginal papillae region, 90% of the abnormalities were observed in the lower region, 7.6% in the upper region and 1.5% in the ventral region (n= 66; χ^2=93.54; df=2; p<0.001). There was a strong association between the type of abnormality and the affected region of the marginal papillae. Thus, 100% (n=59) of abnormalities in the lower region are extraneous projections; while 60% (n=3) of abnormalities in the upper region are gaps or distortions in the edge. Finally, in the ventral region two kinds of abnormality were present, gaps and extraneous projections, each one representing 50% (n=1) of abnormalities. In jaw sheaths, both the number of abnormalities and the association between abnormality types and their occurrence in each region were not significant.

Prevalence showed a peak in the early feeding stages (e.g., Stage 25 and 26), and decreased until stage 41, i.e. the onset of metamorphosis (p<0.001; Fig. 5).

DISCUSSION

Using museum specimens, we contribute comprehensive data on amphibian abnormality rates in South America. We found a low percentage of abnormalities, 4% in post-metamorphic individuals; studies in North American museums found 7% (McCallum & Trauth, 2003; Gridi-Papp & Gridi-Papp, 2005) and 1.63% (Johnson et al., 2003) of abnormalities. The only abnormality type in common between our study and that of Gridi-Papp & Gridi Papp (2005) was the shortened digit reported in specimens of Texas Memorial Museum. We assume that the abnormality rates that we document represent the rates found in those species at these sites at time of collection, but cannot rule out bias either towards or against collection of deformed individuals. In particular we document the first records of abnormalities in *C. cranwelli*, *O. americanus*, *P. borellii*, *P. tucumanum* and *S. fuscovarius*. Sample sizes of *D. nanus*, *G. gracilis*, *L. bufonius*, *L. gracilis* and *O. discoidalis* were low. This is also the first report of abnormalities in tadpoles of *H. riojanus*, *O. americanus* and *P. borellii*. The absence of previous reports can reflect the lack of studies focused on this topic.

In post-metamorphic individuals, abnormalities were confined to limbs, particularly hindlimbs, which is consistent with many studies (e.g., Gardiner & Hoppe, 1999; Meteyer et al., 2000; Eaton et al., 2004; Piha et al., 2006; García-Muñoz et al., 2010; Peltzer et al., 2011). Some authors suggest that the pelvic limb buds would be more vulnerable to environmental insult during critical cell-division stages and morphogenesis than their anterior counterparts, which are protected within the gill chamber until metamorphosis occurs (Sessions & Ruth, 1990; Stopper et al., 2002). This is accurate if the possible agent is something such as trematode parasites or sublethal predators (Sessions & Ruth, 1990; Ballengée & Sessions 2009; Bowerman et al., 2010; Johnson et al., 2012). However, if the hypothesized agent is a chemical contaminant, then the forelimbs are also exposed inside the gill chamber during feeding and respiration when water enters the buccal cavity and spiracles (Duellman & Trueb, 1986) and contaminants that may have been ingested or absorbed will be distributed systemically. Thus, the observed abnormalities in hindlimbs could be suggesting something other than chemical contamination.

Seven abnormality types were recognized externally but the analysis of skeletal anatomy permitted gaining more information on external abnormalities. For example an external configuration corresponding to ectrodactyly, internally implied other types of abnormality, such as phalangeal fusion, brachydactyly and clinodactyly. Thus, a comprehensive study of the whole morphological system, including both external and internal anatomy, would permit a more precise classification and complete description of the abnormalities reported.

New abnormalities (swollen phalangeal articulation, digits expanded at the end -hamartoma in the terminology of Rothschild et al., 2012 - and absence of subphalangeal tubercle) affected both skin and connective tissue. These abnormalities might be anatomical modifications produced by the preservation condition of specimens deposited in scientific collections (Reed, 2001).

Larval abnormalities found in marginal papillae, tooth rows and jaw sheaths of oral discs might compromise foraging and thus could lead to reduced growth, vigour and survival (Rowe et al., 1996; Venesky et al., 2010). The abnormal tadpoles appear to have a reduced ability to harvest some food types compared with normal tadpoles (Rowe et al., 1996). Additionally, any reduction in growth and development can reduce the ability to escape from predators (Venesky et al., 2010). Regarding anurans with abnormal limbs, studies postulate that these individuals do not survive to sexual maturity, resulting in fewer deformed adults (Johnson et al., 1999; Blaustein & Johnson, 2003; Eaton et al., 2004). With regards to the analysis of prevalence through developmental larval stages, the significant decrease through stages could be reflecting a differential susceptibility to the factors that produce abnormalities in these stages. Another explanation would be that the abrupt decline of abnormalities after Stage 41 is simply the result of the oral reconstruction that begins taking place at Stage 41 and is largely complete by Stage 46.

Abnormalities were more frequent in tadpoles than in post-metamorphic individuals, which is a common finding in other studies (Johnson et al., 2003). Thus, 100% of species whose larval stages were studied showed abnormalities and 30% of larval specimens were affected by abnormalities. In contrast, 50% of the post-metamorphic species reviewed presented abnormalities, and only 3.8% from total post-metamorphic specimens were affected by abnormalities. Despite this difference, types, localization and origin of abnormalities are different in each phase (larval and post-metamorphic), so they provide complementary information.

Differences in abnormality prevalence between species was only observed in tadpoles, i.e. *H. riojanus* and *O. americanus*, which had the highest rates. *Hypsiboas riojanus* and *O. americanus* present two reproductive periods, at the beginning and at the end of the breeding season. Tadpoles of the second reproductive period overwinter and metamorphose in the next suitable season (Lavilla & Rougès, 1992). Rowe et al. (1996) suggest that abnormalities are associated with a chronic exposure to contaminants, it being more likely to find abnormalities in species with long larval periods. It is also possible for species with long larval stages to be exposed to more parasites or predators. Thus, high prevalence of abnormalities in tadpoles of *H. riojanus* and *O. americanus* could be explained by the long development time for the clutches that overwinter.

Location of the larval abnormalities within the oral disc, and the locations of each type of abnormality among tooth rows as well as in the marginal papillae region were not homogeneously distributed. This pattern indicates differences in vulnerability of the oral disc regions. Tooth rows A1, A2 and P2 presented a higher prevalence of abnormalities, similar to the results found by Drake et al. (2007). Curiously, it has been generally reported that structures that appear early in oral disc development, like A1 and P2 tooth rows (Thibaudeau & Altig, 1988) are less susceptible than later structures in development (Drake et al., 2007). On the other hand, the occurrence of labial teeth in marginal papillae (extraneous projections) is a common abnormality in *Hypsiboas riojanus*, where these projections were reported as a variable feature on the oral disc (Kolenc et al., 2008).

Our results show that there was no clear historic trend in the prevalence of abnormalities observed; only a peak of abnormalities which was evident in the 1960s. In contrast, other studies that include museum data have reported a progressive increase of abnormality prevalence through time (e.g., McCallum & Trauth, 2003; Gridi-Papp & Gridi-Papp, 2005).

Specimens, both post-metamorphic and larvae, tended to have more than one abnormality type. In agreement with these results, other studies reported specimens with multiple abnormalities (Schoff et al., 2003; Taylor et al., 2005). This association could be related to one of the hypothetical causes proposed to explain the occurrence of abnormalities, the exposure to natural or anthropogenic chemicals that act as retinoids. The retinoic acid (RA) acts in the homeotic regulation of the development in the vertebrate brain, eye, mandible and limbs (Bryant & Gardiner 1992; Schoff et al., 2003; Müller et al., 1996). The homeotic effects of the genes activated by the RA have been described in amphibians and other vertebrates (Holder & Hill, 1991; Scott et al., 1994; Hill et al., 1995; Horton & Maden, 1995; Zile, 1999). Therefore, abnormalities found in amphibians could be an alarm, signalling the action of mutagenic agents that could be active on vertebrates as a whole. Further research is necessary to assess whether a relationship exists between the effect of the RA and the associated abnormalities reported here.

The main contribution of our work was the use of data derived from museum collections. This kind of information is new and contributes useful historical information to the literature, our study stands as an example of how these types of collections can be utilized to gain perspective on conditions of the populations that no longer exist or have changed over time.

ACKNOWLEDGEMENTS

We thank the Herpetology Collection-Instituto de Herpetología-FML curator E. Lavilla, for allowing access to the database and specimens deposited there; S. Kretzschmar and M. Cánepa from Herpetology Collection-Instituto de Herpetología; F. Zaffanella for English revision; D. Baldo for the map confection; A. Valeiro and J. Grignola from INTA Famaillá provided bibliographic materials; M. J. Lannoo for editorial assistance and helpful comments. MLP acknowledges CONICET (PIP 112–200801–00225) and FONCyT (PICT 2008–0578) for financial support. CG and MR acknowledge CONICET for a doctoral grant. EA acknowledges Bunge & Born Foundation for a post-doctoral grant.

REFERENCES

Ankley, G.T., Tietge, J.E., Holcombe, G.W., DeFoe, D. L., Diamond, S. A., et al. (2000). Effects of laboratory ultraviolet radiation and natural sunlight on survival and development of *Rana pipiens*. *Canadian Journal Zoology* 78, 1092–1100.

Ankley, G.T, Diamond, S.A., Tietge, J.E., Holcombe, G.W., et al. (2002). Assessment of the risk of solar ultraviolet radiation to amphibians. I. Dose-dependent induction of hindlimb malformations in the northern leopard frog (*Rana pipiens*). *Environmental Science Technology* 36, 2853–2858.

Ankley, G.T., Degitz, S.J., Diamond, S.A. & Tietge, J.E. (2004). Assessment of environmental stressors potentially responsible for malformations in North American anuran amphibians. *Ecotoxicology and Environmental Safety* 58, 7–16.

Attademo, A.M., Peltzer, P.M. & Lajmanovich, R.C. (2004). Nuevo caso de malformación en un ejemplar de rana (*Leptodactylus ocellatus*) (Amphibia: Anura) del litoral argentino. *Boletín Asociación Herpetológica Argentina* 15, 20–22.

Ballengée, B. & Sessions, S.K. (2009). Explanation for missing limbs in deformed amphibians. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* 312B, 770–779.

Barrionuevo, J.S., Aguayo, R. & Lavilla, E.O. (2008). First record of chytridiomycosis in Bolivia (*Rhinella quecha*; Anura: Bufonidae). *Diseases of Aquatic Organisms* 82, 161–163.

Blaustein, A.R., Kiesecker, J.M., Chivers, D.P. & Anthony, R.G. (1997). Ambient UV-B radiation causes deformities in amphibian embryos. *Proceedings of the National Academy of Sciences USA* 94, 13735–13737.

Blaustein, A.R. & Johnson, P.T.J. (2003). The complexity of deformed amphibians. *Frontiers in Ecology Environment* 1, 87–94.

Bowerman, J., Johnson, P.T.J. & Bowerman, T. (2010). Sublethal predators and their injured prey: Linking aquatic predators and severe limb abnormalities in amphibians. *Ecology* 91, 242–251.

Bryant, S.V. & Gardiner, D.M. (1992). Retinoic acid, local cell-cell interactions, and pattern formation in vertebrate limbs. *Developmental Biology* 152, 1–25.

Burkart, R., Bárbaro, N.O., Sánchez, R.O. & Gómez, D.A. (1999). Eco-regiones de la Argentina. *Secretaría de Recursos Naturales y Desarrollo Sustentable*, Buenos Aires.

Cei, J.M. (1980). Amphibians of Argentina. *Monitore Zoologico Italiano*, *Italian Journal of zoology Monografia* 2, 1–609.

Crawley, M.J. (2007). *The R Book*. Chichester, England: John Wiley and Sons Inc.

Duellman, W.E. & Trueb, L. (1986). *Biology of amphibians*. Baltimore: Johns Hopkins University Press.

Drake, D.L., Altig, R., Grace, J.B. & Walls, S.C. (2007). Occurrence of oral deformities in larval anurans. *Copeia* 2007, 449–458.

Eaton, B.R., Eaves, S., Stevens, C., Puchniak, A. & Paszkowski, C.A. (2004). Deformity levels in wild populations of the wood frog (*Rana sylvatica*) in three ecoregions of western Canada*.* *Journal of Herpetology* 38, 283–287.

Estación Experimental Agroindustrial Famaillá. (2010). Estimación de residuos de envases de fitosanitarios generados por los principales cultivos de la provincia de Tucumán. Informe, 33 pp.

Fabrezi, M. (1999). Duplicación de la extremidad anterior en *Lepidobatrachus llanensis* (Anura: Leptodactylidae). *Cuadernos de Herpetología* 13, 99–100.

Fellers, G.M., Green, D.D. & Longcore, J.E. (2001). Oral chytridiomycosis in the mountain yellow-legged frog *Rana muscosa*. *Copeia* 2001, 945–953.

García-Muñoz, E., Rato, F.J.C. & Carretero, M.A. (2010). Four types of malformations in a population of *Bufo boulengeri* (Amphibia, Anura, Bufonidae) from the Jbilet Mountains (Marrakech, Morocco). *Herpetology Notes* 3, 267–270.

Gardiner, D.M. & Hoppe, D.M. (1999). Environmentally induced limb malformations in mink frogs (*Rana septentrionalis*). *Journal of Experimental Zoology* 284, 207–216.

Gray, R.H. (2000). Historical occurrence of malformations in the cricket frog, *Acris crepitans,* in Illinois. *Transactions of the Illinois State Academic of Science* 93, 279–284.

Gridi-Papp, M. & Gridi-Papp, C.O. (2005). Abnormal digits in Strecker's chorus frogs (*Pseudacris streckeri*, Hylidae) from Central Texas. *The Southwestern Naturalist* 50, 490–494.

Hayes, T.B., Collins, A., Lee, M., Mendoza, M., et al. (2002). Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. *Proceedings of the National Academy of Sciences USA* 99, 5476–5480.

Hayes, T.B., Case, P., Chui, S., Chung, D., et al. (2006). Pesticide mixtures, endocrine disruption, and amphibian declines: Are we underestimating the impact? *Environmental Health Perspectives* 114, 40–50.

Hill, J., Clarke, J.D.W., Vargesson, N., Jowett, T. & Holder, N. (1995). Exogenous retinoic acid causes specific alterations in the midbrain and hindbrain of the zebrafish embryo including positional respecification of the Mauthner neuron. *Mechanisms of Development* 50, 3–16.

Holder, N. & Hill, J. (1991). Retinoic acid modifies development of the midbrain-hindbrain border and affects cranial ganglion formation in zebrafish embryos. *Development* 113, 1159–1170.

Hopkins, W.A, Congdon, J. & Ray, J.K. (2000). Incidence and impact of axial malformations in larval bullfrogs (*Rana catesbeiana*) developing in sites polluted by a coal-burning power plant. *Environmental Toxicology and Chemistry* 19, 862–868.

Hoppe, D.M. (2005). Malformed frogs in Minnesota: history and interspecific differences. In *Amphibian Declines: The Conservation Status of United States Species*, 103–108. Lannoo, M.J (Ed). Berkeley, California: University of California Press.

Horton, C. & Maden, M. (1995). Endogenous distribution of retinoids during normal development and teratogenesis in the mouse embryo. *Developmental Dynamics* 202, 312–323.

Johnson, P.T.J, Lunde, K. B., Ritchie, E.G. & Launer, A.E. (1999). The effect of trematode infection on amphibian limb development and survivorship. *Science* 284, 802–804.

Johnson, P.T.J., Lunde, K.B., Haight, R.W., Bowerman, J. & Blaustein, A.R. (2001). *Ribeiroia ondatrae* (Trematoda: Digenea) infection induces severe limb malformations in western toads (*Bufo boreas*). *Canadian Journal of Zoology* 79, 370–379.

Johnson, P.T.J., Lunde, K.B., Thurman, E.M., Ritchie, E.G., et al. (2002). Parasite (*Ribeiroia ondatrae*) infection linked to amphibian malformations in the western United States. *Ecological Monographs* 72, 151–168.

Johnson, P.T.J., Lunde, K.B., Zelmer, D.A. & Werner, J.K. (2003). Limb deformities as an emerging parasitic disease in amphibians: evidence from museum specimens and resurvey data. *Conservation Biology* 17, 1724–1737.

Johnson, P.T.J., Reeves, M.K., Krest, S.K. & Pinkney, A.E. (2010). A decade of deformities: advances in our understanding of amphibian malformations and their implications. In *Ecotoxicology of Amphibians and Reptiles*, 2nd edition, 511–536. Sparling, D.W, Linder, G., Bishop, C.A. & Krest, S. (Eds). Pensacola FL: Ecotoxicology of Pensacola, Society for Environmental Toxicology and Chemistry.

Johnson, P.T.J., Rohr, J.R., Hoverman, J.T., Kellermanns, E., et al. (2012). Living fast and dying of infection: host life history drives interspecific variation in infection and disease risk. *Ecology Letters* 15, 235–242.

Kaiser, J. (1997). Deformed frogs leap into spotlight at health workshop. *Science* 278, 2051–2052.

Knapp, R.A. & Morgan J.A.T. (2006). Tadpole mouthpart depigmentation as an accurate indicator of chytridiomycosis, an emerging disease of amphibians. *Copeia* 2006, 188–197.

Kolenc, F., Borteiro, C., Alcalde, L., Baldo, D., et al. (2008). Comparative larval morphology of eight species of *Hypsiboas* (Amphibia, Anura, Hylidae) from Argentina and Uruguay, with a review of the larvae of this genus. *Zootaxa* 1927, 1–66.

Lajmanovich, R.C., Sandoval, M.T. & Peltzer, P. (2003a). Induction of mortality and malformation in *Scinax nasicus* tadpoles exposed to glyphosate formulations. *Bulletin of Environmental Contamination and Toxicology* 70, 612–618.

Lajmanovich, R., Lorenzatti, E., Maitre, M.I., Enrique, S. & Peltzer P. (2003b). Comparative acute toxicity of the commercial herbicides glyphosate to neotropical tadpoles *Scinax nasicus* (Anura: Hylidae). *Fresenius Environmental Bulletin* 4, 364–367.

Lannoo, M.J. (2008). Resolutions. In *Malformed Frogs*, 111–177. California: University of California Press.

Lavilla, E.O & Rougès M. (1992). Reproducción y desarrollo de anfibios argentinos. *Asociación Herpetológica Argentina*, *Serie de Divulgación* N° 5.

McCallum, M.L. & Trauth S.E. (2003). A forty-three year museum study of northern cricket frog (*Acris crepitans*) abnormalities in Arkansas: upward trends and distributions. *Journal of Wildlife Diseases* 39, 522–528.

Meteyer, C.U. (2000). Field guide to malformations of frogs and toads with radiographic interpretations. *Biological Science Report* USGS/BRD/BSR–2000–0005.

Meteyer, C.U., Loeffler, I.K., Fallon, J.F., Converse, K.A., et al. (2000). Hind Limb Malformations in free-living northern leopard frogs (*Rana pipiens*) from Maine, Minnesota, and Vermont Suggest Multiple Etiologies. *Teratology* 62, 151–171.

Müller, G.B., Streicher, J. & Müller, R.J. (1996). Homeotic duplication of the pelvic body segment in regenerating tadpole tails induced by retinoic acid. *Development Genes and Evolution* 206, 344–348.

Ouellet, M. (2000). Amphibian deformities: current state of knowledge. In *Ecotoxicology Amphibians and Reptiles* 617–646. Sparling D.W., Linder G., Bishop C.A. (Eds). Florida: Ecotoxicology of Pensacola, Society for Environmental Toxicology and Chemistry.

Peltzer, P.M., Ponssa, M.L. & Lajmanovich, R.C. (2001). Caso de malformación en *Leptodactylus mystacinus* (Anura: Leptodactylidae). *Natura Neotropicalis* 32, 165–168.

Peltzer, P.M., Lajmanovich, R.C., Sanchez, L.C., Attademo, A.M., et al. (2011). Morphological abnormalities in amphibian populations from the mid-eastern region of Argentina. *Herpetological Conservation and Biology* 3, 432–442.

Peri, S. & Williams, J. (1988). Anomalías osteológicas en *Hyla pulchella pulchella* y *Pseudis paradoxus platensis* (Amphibia: Anura). *Boletín Asociación Hepertológica Argentina* 4, 4–5.

Piha, H., Pekkonen, M. & Merila, J. (2006). Morphological abnormalities in amphibians in agricultural habitat: a case study of the common frog *Rana temporaria*. *Copeia* 2000, 810–817.

Pyke, G.H. & Ehrlich, P.R. (2010). Biological collections and ecological/environmental research: a review, some observations and a look to the future. *Biological Reviews* 85, 247–266.

R Core Team. (2012). *R: A language and environment for statistical computing*. Vienna, Austria: *R Foundation for Statistical Computing*. Available from: http://www.R-project.org.

Reed, R.N. (2001). Effects of museum preservation techniques on length and mass of snakes. *Amphibia-Reptilia* 22, 488–491

Reeves, M.K., Dolph, P.J, Holyoak, M. & Trust, K.A. (2010). Multiple stressors and the cause of amphibian abnormalities. *Ecological Monographs* 80, 423–440

Reeves, M.K. & Trust, K.A. (2004). National abnormal amphibian study, FY2002: National wildlife refuges in Alaska. Annual Progress Report. *U.S. Fish and Wildlife Service Technical Report*. AFWFO-TR-04–02. 22 pp.

Rothschild, B.M., Schultze, P. & Pellegrini, R. (2012). *Herpetological Osteopathology, Annotated Bibliography of Amphibians and Reptiles*. New York: Springer Science Business Media, LLC.

Rowe, C.L., Kinney, O.M., Fiori, A.P. & Congdon, J.D. (1996). Oral deformities in tadpoles (*Rana catesbeiana*) associated with coal ash deposition: effects on grazing ability and growth. *Freshwater Biology* 36, 723–730.

Sandoval, M.T., Aronzon, C., Herkovits, J. & Pérez-Coll, C.S. (2006). Embriotoxicidad del herbicida 2,4-D y Esternon Ultra® sobre anfibios anuros. *Comunicaciones Científicas y Tecnológicas, Universidad Nacional del Nordeste.*

Schoff, P.K., Johnson, C.M., Schotthoefer, A.M., Murphy, J. E., et al. (2003). Prevalence of skeletal and eye malformations in frogs from north-central United States: estimations based on collections from randomly selected sites. *Journal of Wildlife Diseases* 39, 510–521.

Scott, W.J., Walter, R., Tzimas, G., Sass, J.O., et al. (1994). Endogenous status of retinoids and their cytosolic binding proteins in limb buds of chick vs. mouse embryos. *Developmental Biology* 165, 397–409.

Sessions, S.K. & Ruth, S.B. (1990). Explanation of naturally occurring supernumerary limbs in amphibians. *Journal of Experimental Zoology* 254, 38–47.

Stopper, G.F., Hecker, L., Franssen, R.A. & Sessions, S.K. (2002). How trematodes cause limb deformities in amphibians. *Journal of Experimental Zoology* 294, 252–263.

Souder, W. (2000). *A Plague of Frogs: The Horrifyingly True Story*. New York: Hyperion Press.

Taylor, B., Skelly, D., Demarchis, L.K., Slade, et al. (2005). Proximity to pollution sources and risk of amphibian limb malformation. *Environmental Health Perspectives* 113, 1497–1501.

Thibaudeau, D.G. & Altig, R. (1988). Sequence of ontogenetic development and atrophy of the oral apparatus of six anuran tadpoles. *Journal of Morphology* 197, 63–69.

U.S. Fish & Wildlife Service (USFWS). (2008). Standard operating procedures for abnormal amphibian surveys: Abnormality classification SOP. Annapolis, Maryland: U.S. Fish and Wildlife Service.

Venesky, M.D., Wassersug, R.J. & Parris, M.J. (2010). How does a change in labial tooth row number affect feeding kinematics and foraging performance of a ranid tadpole (*Lithobates sphenocephalus*)? *Biological Bulletin* 218, 160–168.

Vera Candioti, M.F. (2007). Anatomy of anuran tadpoles from lentic water bodies: systematic relevance and correlation with feeding habits. *Zootaxa* 1600, 1–175.

Wassersug, R. (1976). A procedure for differential staining of cartilage and bone in whole formaline fixed vertebrates. Stain. *Technology* 51, 131–134.

Zile, M.H. (1999). Avian embryo as a model for retinoid function in early development. In *Retinoids*, *the Biochemical and Molecular Basis of Vitamin A and Retinoid Action*, 443–464. *Series: Handbook of Experimental Pharmacology* Vol. 139. Nau, H. & Blaner, W. (Eds). Heidelberg: Springer.

Accepted: 20 June 2013

Please note that the APPENDIX containing Figures 1–7 for this article is available online via the Herpetological Journal website (http://http://www.thebhs.org/pubs_journal_online_appendices.html)

Herpetological Journal ONLINE APPENDIX

Published by the British Herpetological Society

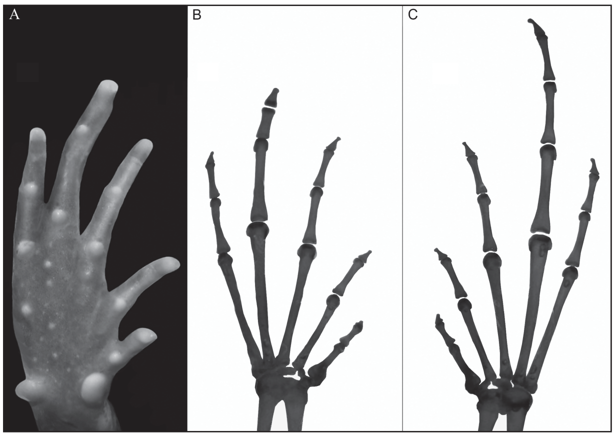

Fig. 1. *Pleurodema borellii* (FML 02235–64) showing brachydactyly in digit IV of the right hind limb. A) External morphology, showing digit IV shorter than the normal digit. B) Skeletal morphology, digit IV has only three phalanges, the last two phalanges are shorter than the normal phalanges. C) Normal morphology of the feet showing four phalanges.

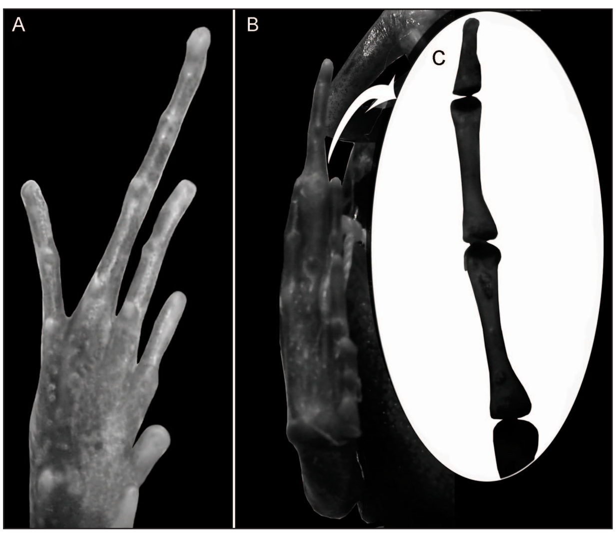

Fig. 3. A) *Leptodactylus latinasus* (FML 12186) showing absence of subphalangeal tubercle in digit I of the right hindlimb. B, C) *Pleurodema borellii* (FML 01434–7) showing swollen phalangeal articulation. B) External morphology, showing a swelling between phalanges on toe IV of the right hindlimb. C) Skeletal morphology exhibits that the bones are not affected.

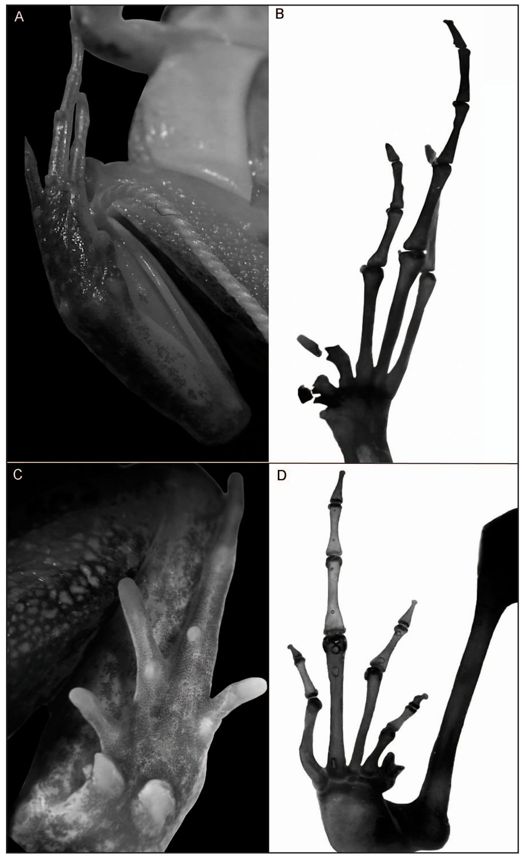

Fig. 2. *Leptodactylus latinasus* (FML 04809–9) showing ectrodactyly in the left hindlimb. A) External morphology, showing a very small digit II. B) Skeletal morphology, showing digit II with the metatarsal curved inward and with one phalange; digit I presents only a short metatatarsal bone.
Pleurodema borellii (FML 02553–5) showing ectrodactyly, brachydactyly and hemimelia in the left hindlimb. C) External morphology, showing four digits. D) Skeletal morphology, digit V is absent; digit IV has two phalanges (brachydactyly), and the metatarsal bone is curved outwardly; digit I has a distal inward curvature. The tibial and fibular bones are very short and curved, the joints involved are strongly fused and immobilize the autopodio.

Correspondence: R.G. Medina (regina.g.medina@gmail.com)

Fig. 4. *Leptodactylus chaquensis* (FML 01661–7) showing digits expanded at the end. A) External morphology of left hindlimb, showing toe IV expanded at the end. B) Skeletal morphology of left hindlimb showing that the bones are not involved. C) External morphology of the left forelimb showing an apparent expansion of finger IV. D) Skeletal morphology of the left forelimb showing brachydactyly of finger IV with two phalanges instead of three. Note the normal shape of the epiphyses of the second phalange at the end.

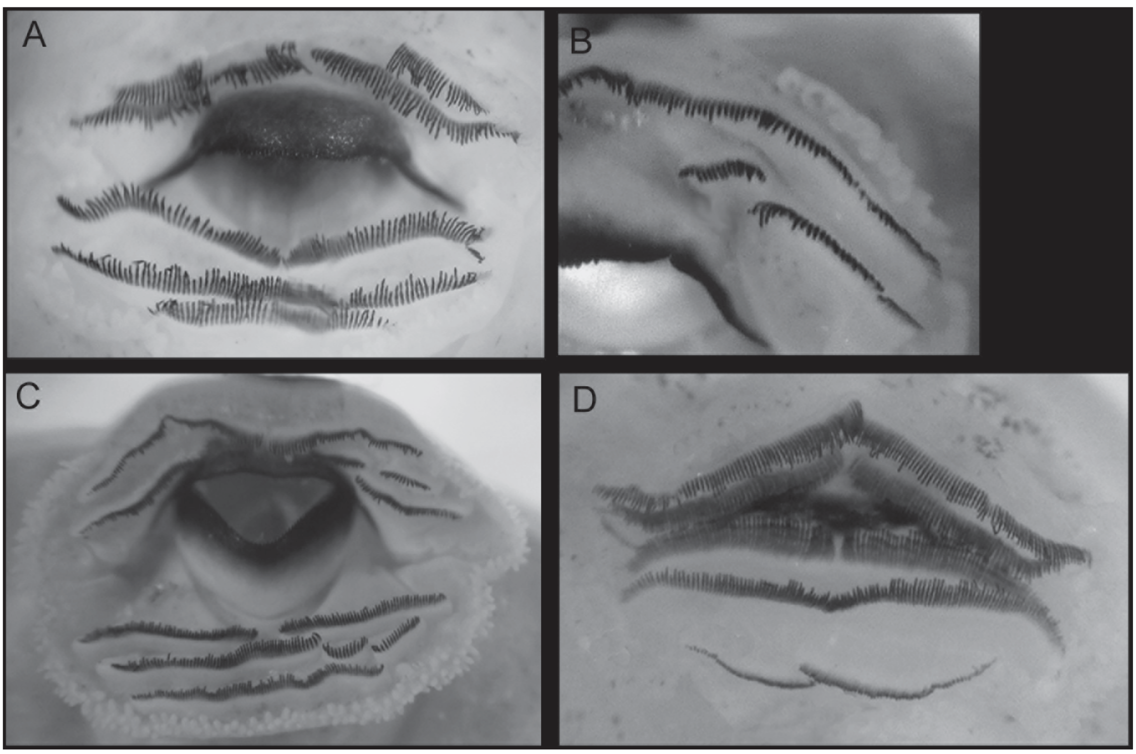

Fig. 5. A) *Odontophrynus americanus* (FML 4525), Stage 40, showing missing teeth in rows A1 and A2. Note that the underlying tissue is present. B) *Hypsiboas riojanus* (FML 16591), Stage 27, showing missing teeth with disrupted supporting tissue. Note that underlying tissue in left row A2 is absent. C) *Odontophrynus americanus* (FML 5007), Stage 35, showing duplication of teeth in a portion of the left row A2, moreover it shows missing teeth with disrupted supporting tissue in left rows A2 and P2. D) *Hypsiboas riojanus* (FML 16630), Stage 27, showing overlapping rows in row P3.

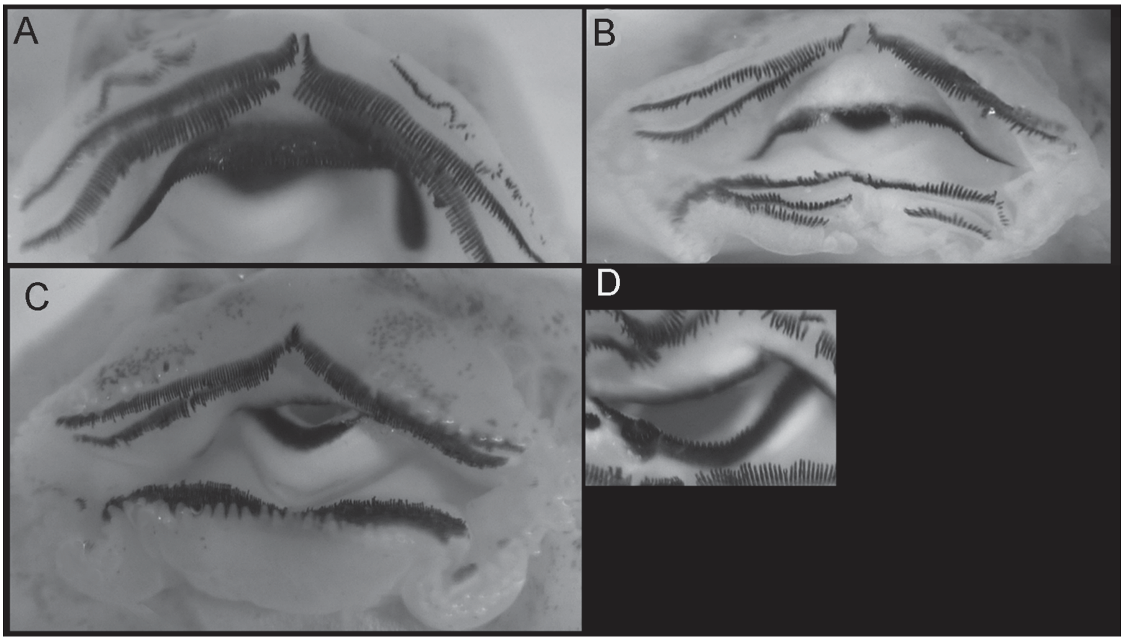

Fig. 6. A) *Hypsiboas riojanus* (FML 16584), Stage 28, showing teeth in left and right side of upper marginal papillae. B) *Hypsiboas riojanus* (FML 16583), Stage 26, showing a cleft in ventral marginal papillae. C) *Hypsiboas riojanus* (FML 16636), Stage 37, showing lack of keratinization in the upper jaw sheath. D) *Hypsiboas riojanus* (FML 16596), Stage 36, showing upper jaw sheath with abnormal disposition (breaks, gaps, or other deformities in the edge).

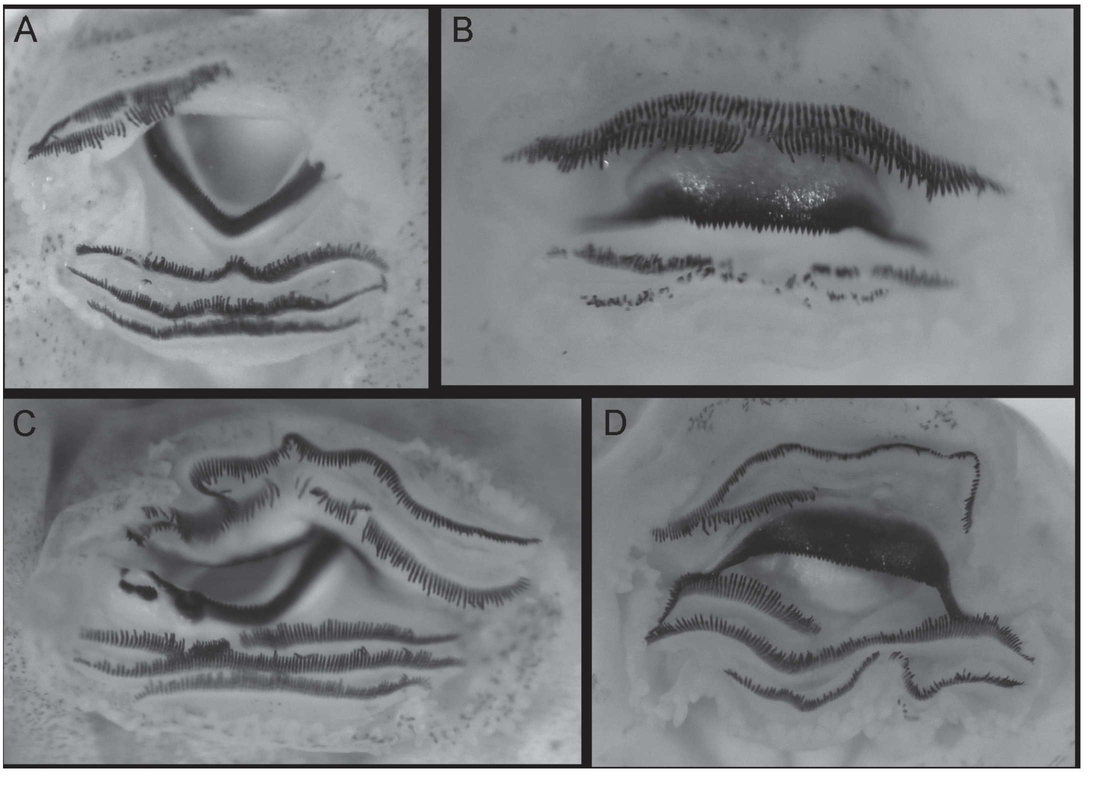

Fig. 7. A) *Rhinella arenarum* (FML 15535), Stage 40, showing total loss of teeth with disrupted supporting tissue in the left of rows A1 and A2, and lack of keratinization in upper jaw sheath. B) *Odontophrynus americanus* (FML 4525) Stage 34, showing stunted teeth in rows P1 and P2, and missing teeth in row P3. C) *Hypsiboas riojanus* (FML 16596), Stage 30, showing row A1 puckered, missing teeth in row A2 and jaw sheaths with irregular edge. D) *Odontophrynus americanus* (FML 4525), Stage 39, showing row A1 with irregular disposition and missing teeth with disrupted supporting tissue in the left of rows A1 and P1.

List of studied specimens with locations

Abbreviations: FML, Fundación Miguel Lillo; L, private collection of María Laura Ponssa; SB, private collection of Sebastián Barrionuevo. [bd] Denotes specimens with brachydactyly. [ed] Denotes specimens with ectrodactyly. [em] Denotes specimens with ectromelia. [h] Denotes specimens with hemimelia. [ast] Denotes specimens with absence of subphalangeal tubercle. [dee] Denotes specimens with digits expanded at the end. [spa] Denotes specimens with swollen phalangeal articulation. [t] Denotes specimens that were double stained and cleared.

Ceratophrys cranwelli: Departamento Lules: San Pablo: FML 05474/8761. Departamento Burruyacu: El Cadillal: FML 00041/08760/07790/08961/08962/08963/08964/08965/08966/08967/08968/08969/08970/09340/08260[bd, ed]. Departamento Cruz Alta: San Andres: FML 02063. Banda del Río Salí: FML 02653. *Dendropsophus nanus*: Departamento Lules: San Pablo: FML 0938/20939/20940. *Gastrotheca gracilis:* Argentina: Provincia de Tucumán: Departamento Burruyacu: Río Nío: FML 02544. *Hypsiboas riojanus*: Departamento Lules: Quebrada de Lules: FML 07607. Villa Nougués: FML 07944. Departamento Yerba Buena: Horco Molle: FML 01493–1/01493–2. San Javier: FML 04549–1/04549–2/06777/06778/07754/07755. Departamento Burruyacu: El Duraznito: FML 00471. 7 de Abril: FML 01426–1/01426–2/01426–3/01426–4/01426–5/01426–6/01426–7/01426–8/01426–9/01426–10/01426–11/01426–12/01426–13/01426–14/01426–15/01426–16/01426–17/01426–18/01426–19. Río Nío: FML 01698/02542–1/02542–2/02542–3/02542–4/02542–5/02542–6/02542–7/02542–8/02542–9/02542–10/02542–11/02542–12/02542–13/02542–14/02542–15/02542–16/02542–17/02542–18/02542–19/02542–20/02542–21. Sierra de Medina: FML 02551–1/02551–2/02551–3/02551–4/02551–5/02551–6/02551–7/02551–8/02551–9/02551–10/02606–1/02606–2/02606–3/02606–4/02606–5/02606–6/02606–7/02606–8/02606–9/02606–10/02606–11/02606–12/02606–13/02606–14/02606–15/02606–16/03929. *Leptodactylus bufonius:* Departamento Burruyacu: 7 de Abril: FML 01761–1/01761–2/01761–3. Departamento Leales: Los Gómez: FML 00634–1/00634–2/00634–3. *Leptodactylus chaquensis:* Departamento Lules: San Pablo: FML 03881/03882–1/03882–2/03888/03889/03894/03896–1/03896–2/03896–3/03899/12156/12157. Departamento Yerba Buena: San Javier: FML 01661–1/01661–2/01661–3/01661–4[dee]/01661–5/01661–6[spa]/01661–7[dee, spa, t]/01661–8/01661–9[bd, dee]/01661–10. Horco Molle: FML 05995. Yerba Buena: FML 01609/06958. Departamento Burruyacu: El Duraznito: FML 00470. 7 de Abril: FML 01425–1/01425–2/01425–3/01425–4[ed, t]/01425–5/01425–6/01425–7. El Cadillal: FML 02299–1/02299–2. Departamento Leales: Cañada el Arenal: FML 00609–1/00609–2/00609–3/00609–4/00609–5. Los Gómez: FML 00610–1/00610–2/00610–3/00610–4/00610–5/00610–6/00610–7/00610–8/00610–9/00610–10/00610–11/00610–12/00610–13/00610–14/00610–15/00610–16/00610–17/00610–18/00610–19/00610–20/00610–21/00610–22/00610–23. Nueva España: FML 00866–1/00866–2/00866–3/00866–4/00866–5/00866–6. Mixta: FML 08227. 2) Departamento Cruz Alta: Lastenia: FML 02170. Ranchillos: FML 07715/07716/07717/07718/07719/2097/12098/12099/12100/12101/17261/17262/17263. El Bracho: FML 8206/08207/08208/08209/08210/08211/08212/08213/08214/08215/08216. 5) Departamento Simoca: Simoca: FML 00631–1/00631–2/00631–3/00631–4/00631–5/00631–6/00631–7/00631–8/00631–9/00631–10/00631–11/00631–12/00631–13/00631–14. *Leptodactylus gracilis*: Departamento Lules: Potrero de las Tablas: FML 00607. Departamento Burruyacu: Río Nío: FML 02550. *Leptodactylus latinasus:* Departamento Lules: San Pablo: FML 03886–1/03886–2/04809–1/04809–2[bd] /04809–3/04809–4/04809–5/04809–6/04809–7/04809–8/04809–9[bd, ed, t]/04809–10/04809–11/04809–12/04809–13/04809–14/04809–15/04809–16/04809–17/04809–18/04809–19/04809–20/04809–21/11900/11901/11902/12046/12047/ 12048/12049/12050/12166/12167/12168/12169/ 12170/12171/12184/12185/12186[ast]/12187/12188/12189/12190/12191[ed]/12192/12193/12194/12195[bd]/12196/12205/12206/12207/12208/12209/12210/12240/12241/12242/12243/12244/12245/12246/12247/12255/12256[bd, ed, t] /12257/12258/SB251/252/L658/659/660/661/662/663/664/666/669/670/672/673/676/474/475/680/681/682/683/688/689/692/694/695/701/702/703/704/705/706/715/716/717/718/719/720/722/723/724/725/726/728/729[em]/735/737/738/739/740/741/742/743/754[bd]/755/756/758/759/760/761/763[bd]/764/765/767/768. Lules: FML 02543. Quebrada de Lules: FML 07338. Departamento Yerba Buena: San Javier: FML 01437/05496. Yerba Buena: FML 06954. Departamento Burruyacu: 7 de Abril: FML 01429–1/01429–2/01429–3/01429–4. El Cadillal: FML 02297/03891–1/03891/12051/12052/12053/12054/ 12055/12056/12057/12058/12059/12060/12061/ 07798/07799/02410–1/02410–2/02410–3/02410–4/02410–5/02410–6/02410–7/02410–8/02410–9/02410–10/02410–11/02410–12/02410–13/02410–14/02410–15/02410–16. La Mesada: FML 02935–1/02935–2. El Cajón: FML 09378. Departamento Leales: Mixta: FML 08226. Departamento Cruz Alta: El Bracho: FML 08196/08582/08583. Ranchillos: FML 12041/12042/12043. *Leptodactylus mystacinus*: Departamento Lules: San Pablo: FML 02769/03255–1/03255–2/03255–3/03255–4/03256[ed]/03290–1/03290–2/03290–3/03893/03538/03529–1/03529–2/03657–1/03657–2/03661–1/03661–2[bd]/03662–1/03662–2/03662–3/03662–4/03662–5/03662–6/04806–1/04806–2/04806–3/04806–4/03883/12343/12344/12345/12346/12347. Departamento Burruyacu: 7 de Abril: FML 01428. El Cadillal: FML 15945/15946. Departamento Cruz Alta: Ranchillos: FML 12315. *Odontophrynus americanus*: Departamento Lules: San Pablo: FML 03826/20668/20669/20670. Villa Nougués: 02236–1/02236–2/02236–3/02236–4/02236–5/02236–6/02236–7/02236–8/02236–9/02236–10/02236–11/02236–12/02236–13/02236–14/02236–15/02236–16/02236–17/02236–18/02236–19/02236–20/05862–1/05862–2/05887/07576/07579/07580. Departamento Yerba Buena: San Javier: FML 01362–1/01362–2/01436[bd]/01657–1/01657–2/01657–3/01657–4/01657–5/01657–6/01657–7/01657–8/01657–9/01657–10[bd]. Yerba buena: 01646/09746/02457/02158–1/02158–2/02158–3[bd]/02158–4/02158–5/02158–6/02158–7/02158–8/02158–9/02158–10. Departamento Burruyacu: Gobernador Piedrabuena: FML 00790–1/00790–2/00790–3. Sierra de Medina: FML 02554–1/02554–2/02554–3/02554–4. Departamento Cruz Alta: El Bracho: FML 08225. *Oreobates discoidalis*: Departamento Yerba Buena: San Javier: FML 05498–1/05498–2/02628. Horco Molle: FML 01895–1/01895–2/04405–1/04405–2. *Physalaemus biligonigerus*: Departamento Lules: San Pablo: FML 06052/ 07695/07696. Potrero de las Tablas: FML 08971. Departamento Yerba Buena: La Rinconada: FML 05863. Horco Molle: FML 05987/05992–1/05992–2/05992–3/05992–4. Yerba Buena: FML 06956/06957/07730/07731/07732/09759/14996. Departamento Burruyacu: 7 de Abril: FML 17561/17562. El Cadillal: FML 02278–1/ 02278–2/02278–3/02278–4/02278–

5/02296–1/02296–2/02296–3/02296–4/02296–5/02296–6/02296–7/02296–8/02296–9/02296–10/07795/07796/07797. Departamento Leales: Mixta: FML 08228. Santa Rosa de Leales: FML 16733/16734/16735. Departamento Cruz Alta: El Bracho: FML 08217/08218/08219/ 08220/08221/08222/08223/08224/08580[bd]/08581. Ranchillos: FML 07720/07721 /07722. *Phyllomedusa sauvagii*: Departamento Lules: San Pablo: FML 03658/04803/07738. Lules: FML 00020. Departamento Yerba Buena: Yerba Buena: FML 02097/06855. Horco Molle: FML 15304. Departamento Burruyacu: El Cadillal: FML 08758/08759/ 07800/07801/08917. Departamento Cruz Alta: Mancopa: FML 02607. *Pleurodema borellii:* Departamento Lules: Villa Nougués: FML 02235–1/02235–2/02235–3/02235–4/02235–5/02235–6/02235–7[bd]/02235–8/02235–9/02235–10/02235–11/02235–12/02235–13/02235–14/02235–15/02235–16/02235–17/02235–18/02235–19/02235–20/02235–21/02235–22/02235–23/02235–24/02235–25/02235–26/02235–27/02235–28/02235–29/02235–30/02235–31/02235–32/02235–33/02235–34/02235–35/02235–36/02235–37/02235–38/02235–39/02235–40/02235–41/02235–42/02235–43/02235–44/02235–45/02235–46/02235–47/02235–48/02235–49/02235–50/02235–51/02235–52/02235–53/02235–54/02235–55/02235–56/02235–57/02235–58/02235–59/02235–60/02235–61/02235–62[bd]/02235–63/02235–64[bd, t]/02235–65/02235–66/02235–67/02235–68/02235–69/02235–70/02235–71/02235–72/02235–73/02235–74/02235–75/02235–76/02235–77/02235–78/02235–79/02235–80/02235–81/02235–82/02235–83/02235–84[bd]/02235–85/02235–86/02235–87/02235–88/02235–89/02235–90/02235–91 /02235–92/02235–93/02235–94/02235–95/02235–96/02235–97/02235–98 /02235–99/02235–100/02235–101/02235–102/02235–103/02235–104. Departamento Yerba Buena: San Javier: FML 01361–1[dee]/01361–2/01361–3/ 01434–1[bd, dee]/01434–2/01434–3/01434–4[bd, dee, t]/01434–5/01434–6/01434–7[spa]/01434–8/ 01434–9[bd]/01434–10/01434–11/01658–1/01658–2/01658–3/05768–1/05768–2. Horco Molle: FML 07875/15297/15298[ed, t]/ 05990–1/05990–2/05988. Yerba Buena: FML 07900/07901/07902/01497–1/01497–2/01497–3/01497–4/01497–5/01497–6/01763. Departamento Burruyacu: Villa Padre Monti: FML 00544–1/00544–2. Sierra de Medina: FML 01638–1/01638–2/01638–3/ 02553–1/02553–2/02553–3/02553–4[bd]/02553–5[bd, ed, hm, t]/02553–6/02553–7[spa]/02553–8/02553–9/02553–10/02553–11/ 02553–12/02553–13/02553–14/02553–15/02553–16. El Cadillal: FML 03829/03830/07791/07792. Río Nío: FML 05506. El Cajón: FML 09346/09348/09349/09350/09351/09352/09353/09354/09355/09356/09357/09358/09359/09360/09361/09362/09363/09364/09365/09366/09367/09368/09369/09370/09371/09372/09373/09374/09375/09376/09377. Departamento Leales: Santa Rosa de Leales: FML 18089. *Pleurodema tucumanum*: Departamento Lules: San Pablo: FML 07687/07688/07689/07690/07691/07692/07693/07694. Departamento Burruyacu: 7 de Abril: FML 01423–1/01423–2/01423–3/01423–4[em]/01423–5/01423–6/01423–7/01423–8/01423–9/01423–10/01423–11/01423–12/01423–13/01423–14/01423–15/01423–16/01423–17/01423–18/01423–19/01423–20/01423–21/01423–22/01423–23/01423–24/01423–25/01423–26/01423–27/01423–28/01423–29/01423–30/01423–31/01423–32/01423–33[bd]/01423–34/01423–35/01423–36/01423–37/01423–38/01423–39/01423–40/01423–41. El Cadillal: FML 02279/03831/09747/09748/09749/09750/09751/09752/09753/09754[bd]/09755. Departamento Cruz Alta: Ranchillos: FML 07723/07724/07725. El Bracho: FML 08197/08198/08199/08200/08201/08202/08203/ 08204/08205/08584/08585/08586/08587/08588. *Rhinella arenarum*: Departamento Lules: Quebrada de Lules: FML 08630/08631/08632/08633/07341. San Pablo: FML 18073/18074/18075/18076/18077/18078/ 18079/18080/18081/18082/18083/20664. San Isidro de Lules: FML 18224. Potrero de las Tablas: FML 7327/07337/08648/08649/08650/08651/08867–1/08867–2/08867–3/08867–4/08867–5/08867–6/08867–7/08868/08869. Departamento Yerba Buena: San Javier: FML 01357–1/01357–2/01357–3/01357–4/01359–1/01359–2/01359–3/01359–4[bd]/01359–5/01359–6/01360–1[bd, ed]/01360–2/01360–3/01360–4/01433–1[dee]/01433–2/01433–3[bd, dee]/01433–4/01660–1/01660–2/01660–3/01660–4/01660–5/02156–1/02156–2/02156–3/02156–4/02156–5/02156–6/02156–7/02156–8/02156–9/05769–1/05769–2. Horco Molle: FML 4423[bd, em, t] (146 especímenes)/15302. Yerba Buena: 04905–1/04905–2/06098. Departamento Burruyacu: Villa Benjamín Aráoz: FML 00348–1/00348–2. Sierra de Medina: FML 02552–1/02552–2*/02552–3/02552–4/ 04516. Río Nío: FML 05507. El Cadillal: FML 07794. El Cajón: FML 09379. Piedra Tendida: FML 22274/22275/22276. Departamento Leales: Los Gómez: FML 00615–1/00615–2/00615–3/00615–4. Nueva España: FML 00867–1/00867–2/00867–3/00867–4. Santa Rosa de Leales: FML 18088. *Rhinella schneideri*: Departamento Lules: San Pablo: FML 00327–1/00327–2/00327–3/00327–4/00327–5/00327–6/00327–7/00327–8/00327–9/00327–10/00327–11/00327–12/00327–13/00327–14/00327–15/00327–16/00327–17/00327–18/00327–19. Departamento Burruyacu: Gobernador Piedrabuena: FML 00753. Departamento Leales: Los Gómez: FML 00628. Santa Rosa de Leales: FML 16732. Departamento Cruz Alta: Lastenia: FML 02168. Los Bulacio: FML 02642–1/02642–2. *Scinax fuscovarius*: Departamento Lules: Villa Nougués: FML 05864–1/05864–2/05864–3/05864–4/05865–1/05865–2/05865–3/05865–4/05865–5/05888–1/05888–2/05888–3/05888–4/05888–5/05888–6/05888–7/05888–8/05888–9/05888–10/05888–11/05888–12/05888–13/05888–14/05888–15/05888–16/05888–17/05888–18/05888–19/05888–20/05888–21/05888–22/05888–23/05888–24/07574/07575/07578. San Pablo: FML 07945/07946/07947. Departamento Yerba Buena: San Javier: FML 01435–1/01435–2/01598/01662–1/01662–2/01662–3/01662–4/01662–5/01662–6/01662–7/01662–8/01662–9/01662–10/01662–11/02563/05497/05770–1/05770–2/05770–3/05770–4/05770–5/05770–6/07577. Yerba Buena: FML 01607/02352–1/02352–2/02531/02862–1/02862–2/03807–1/03807–2. Departamento Burruyacu: Sierra de Medina: 04515–1/04515–2/04515–3[bd]/04515–4. El Cadillal: FML 07802/07803. Departamento Cruz Alta: La Soledad: FML 01494–1/01494–2/01494–3. *Scinax nasicus*: Departamento Lules: San Pablo: FML 03737–1/03737–2/03737–3/03737–4/03737–5/03737–6/04787/07686/07948/07949/07950/07951/07952/07953/07954/07955/07956/07957/18517/18518. Villa Nougués: FML 05861/05885. Departamento Yerba Buena: San Javier: FML 02562. Horco Molle: FML 05991/18071/18072. Departamento Cruz Alta: Las Cejas: FML 02643. Las Talas: FML 02532–1/02532–2/02532–3/02532–4/02532–5.

Specimens of tadpoles analyzed from Departamento Yerba Buena, Tucumán, Argentina. (*n*): number of specimens per lot. [tr1] Denotes specimens with missing teeth. [tr2] Denotes specimens with missing teeth with disrupted supporting tissue. [tr3] Denotes specimens with duplication of teeth. [tr4] Denotes specimens with stunted teeth or underdeveloped teeth. [tr5] Denotes specimens with intersecting tooth rows. [tr6] Denotes specimens with puckering (sharp convolutions) of tooth rows. [tr7] Denotes specimens with overlapping rows. [mp1] Denotes specimens with gaps or distortions in the edge in the marginal papillae. [mp2] Denotes specimens with the marginal papillae with extraneous projections. [js1] Denotes specimens with lack of keratinization in the jaw sheaths. [js2] Denotes specimens with breaks, gaps or other deformities in the edge of the jaw sheaths. [ft] Denotes specimens with a forked tail.

Hypsiboas riojanus: Laguna de San Javier: FML 15533 [tr2, mp1,] (4), 15535 (23); Rio muerto detrás del cementerio San Agustín: FML 16577[tr1, tr2, mp2] (18), 16583 [tr2, tr5, tr6, mp1, mp2,] (18), 16584 [tr1, tr2, tr6, mp2] (21), 16586 [tr2, mp2,] (15), 16587 [tr1, tr2, tr4, tr5, mp2] (16), 16588 [tr7, mp2, js1] (11), 16590 [mp2] (10), 16591 [tr2, mp2, js1] (12), 16592 [tr1, mp2] (11), 16596[tr1, tr6, js2], (3), 16630 [tr7, js1, ft] (10), 16636 [tr2, tr5, mp1, mp2, js1] (15), 16638 (7), 16639 [tr2, tr6, mp2.] (17). *Odontophrynus americanus*: Horco Molle: FML 4525 [tr1, tr2, tr3,tr4, tr5, mp1, mp2](80), 4988 [tr1] (10); Villa Marcos Paz: FML 5007 [tr1, tr2, tr3, tr4, tr5, tr6, mp1, mp2](7), *Pleurodema borellii*: Villa Marcos Paz: FML 4422 [tr1] (23); Yerba Buena: FML 4665 [tr2, mp2] (20), 5053 [tr1, tr2] (19); Horco Molle (Reserva experimental Horco Molle): FML 5461 [tr2, mp2] (5). *Rhinella arenarum*: Laguna de San Javier: FML 15533 [tr2] (11), 15534 [tr1, tr2, tr6] (37), 15535 [tr1, tr2, tr6] (1).