CONTRIBUCIÓN BREVE

BODY SIZE IMPLICATIONS ON THE MATING SUCCESS IN SOUTH AMERICAN REDBELLY TOADS (ANURA: BUFONIDAE: *MELANOPHRYNISCUS*) FROM THE PARANAENSE FOREST

Implicaciones del tamaño corporal en el éxito de apareamiento en Melanophryniscus de la Selva Paranaense

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ABSTRACT: *Melanophryniscus* species from the Selva Paranaense exhibit explosive reproductive strategies and larger males might have greater reproductive success in direct competition for females. The goal of this contribution was to analyze whether there is a difference in body size between males captured amplexing and non-amplexing, in the three *Melanophryniscus* species (*M. atroluteus, M.* aff. *devincenzii* and *M. krauczuki*) of Paranaense Forest (27°29'25.00 'S, 55°40'7.30 'W), Misiones, Argentina. For this, the following body traits were considered: snout-vent length, arm length and body mass. The differences were evaluated using Type III General Linear Models. In general, males in amplexus showed equal or lower mean values for the three body traits compared to non-amplexing males. Our findings suggest that body size is not under selective pressure and may not play a significant role in mating success in the studied population.

KEYWORDS: Sexual Selection, *Melanophryniscus*, Paranaense Forest, Argentina.

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RESUMEN: *Las especies de* Melanophryniscus *de la Selva Paranaense exhiben estrategias reproductivas explosivas y los machos más grandes podrían tener un mayor éxito reproductivo en la competencia directa por las hembras. El objetivo de esta contribución fue analizar si existe una diferencia en el tamaño corporal entre los machos capturados en amplexo y aquellos que no lo estaban, en las tres especies de* Melanophryniscus *(*M. atroluteus, M. aff. devincenzii *y* M. krauczuki*) de la Selva Paranaense (27°29'25.00 "S, 55°40'7.30 "W), Misiones, Argentina. Para ello, se consideraron las siguientes características corporales: longitud hocico-cloaca, longitud de los brazos y masa corporal. Las diferencias se evaluaron utilizando Modelos Lineales Generales de Tipo III. En general, los machos en amplexo presentaron valores medios iguales o más bajos para las tres características corporales en comparación con los no amplexados. Nuestros hallazgos sugieren que el tamaño corporal no está sujeto a presión selectiva y puede no desempeñar un papel significativo en el éxito reproductivo de la población estudiada.*

KEYWORDS: *Selección sexual, Melanophryniscus, Selva Paranaense, Argentina.*

Darwin's theory of sexual selection explains the evolution of morphological traits, such as body size in animals, predicting that in species with explosive reproduction, direct competition among males for females will be more significant than selection by females. Thus, large males will be more successful than small males (Darwin, 1871, but see Blanckenhorn, 2000). Many studies on sexual selection in anurans have focused on body size to investigate the morphological factors associated with male reproductive success (Halliday and Verrell, 1986, Gastón and Vaira, 2020). Thus, the differences in forelimb length between male and female frogs, as well as between amplexing and non-amplexing males, have been interpreted as outcomes of sexual selection acting on forelimb length (Howard and Kluge, 1985, but see Buzatto *et al*., 2017). On the other hand, the age at which an organism reaches sexual maturity and the minimum size required for reproduction are perhaps the most crucial aspects of its life cycle. This is why biologists focus on estimating both parameters when studying population dynamics (Morrison and Hero, 2003).

The body size variation of *Melanophryniscus* (Bidau *et al.,* 2011) has

implications on the reproductive traits, which are explosive breeders with a strong association between reproductive activity and rainfall (Vaira 2005; Goldberg *et al.,* 2006). Of 31 species described of *Melanophryniscus*, which are distributed in southern Brazil, southern Bolivia, Paraguay, Uruguay, and central and northern Argentina (Zank *et al.,* 2014; Deforel *et al.,* 2021), 11 have been recorded for Argentina, and three of them inhabit the Paranaense Forest (PF, Hereafter), in Misiones Province (Baldo and Basso 2004; Vaira *et al*., 2012). They are: *Melanophryniscus atroluteus*, from the *M. stelzneri* group, *M.* aff. *devincenzii*, from the *M. tumifrons* group and *M. krauczuki*, unassigned to any group, see Deforel *et al*., 2021; Fig. 1).

Fig. 1. Dorsal (A, C and E) and ventral (B, D and F) views of (A,B) *Melanophryniscus atroluteus*), (C, D), *M.* aff. *devincenzii*), and (E,F) *M. krauczuki*. (Photographed by Diego Baldo). Modified of Marangoni and Baldo (2023)

These three species of PF, bred during two or three consecutive days, in several explosive events that occurred between autumn and spring of the southern hemisphere. These events are determined by a trade-off between the air and water temperature, and the level of the water bodies (Marangoni and Baldo, 2023). During these events, we have documented male-male interactions, such as males of *M. krauczuki* attempting to separate another male in amplexus (a behavior proposed as a factor influencing selection on forelimb size), as well as fighting or calling interactions related to active defense of calling sites in *M. atroluteus* (Marangoni and Baldo, 2023). Based on this previous study, We hypothesized that, in the FP population, larger males of Melanophryniscus species (by considering snout-vent length, arm length or body mass), would achieve greater reproductive success compared to smaller males. In this brief contribution, we analyze whether there is a difference in body size between individuals captured amplexing and non-amplexing, in the three *Melanophryniscus* species of PF.

The study was conducted at Ñu Pyahú (27°29'25.00"S, 55°40'7.30"W), 30 km northeast of Posadas city, Misiones Province, Argentina (Fig. 2, see Marangoni and Baldo 2023, for a detailed description of the study area). We collected 510 mature *Melanophryniscus* from fall 2009 to spring 2012, in 20 reproductive events that occurred immediately after heavy rains (*M. atroluteus*, n = 290; *M.* aff. *devincenzii*, n = 97; and *M. krauczuki*, n = 123; see Table 1 and Appendix in Marangoni and Baldo, 2023). We collected either calling males or pairs in amplexus during chorusing mornings between 0900 and 1300 to ensure that all individuals were mature.

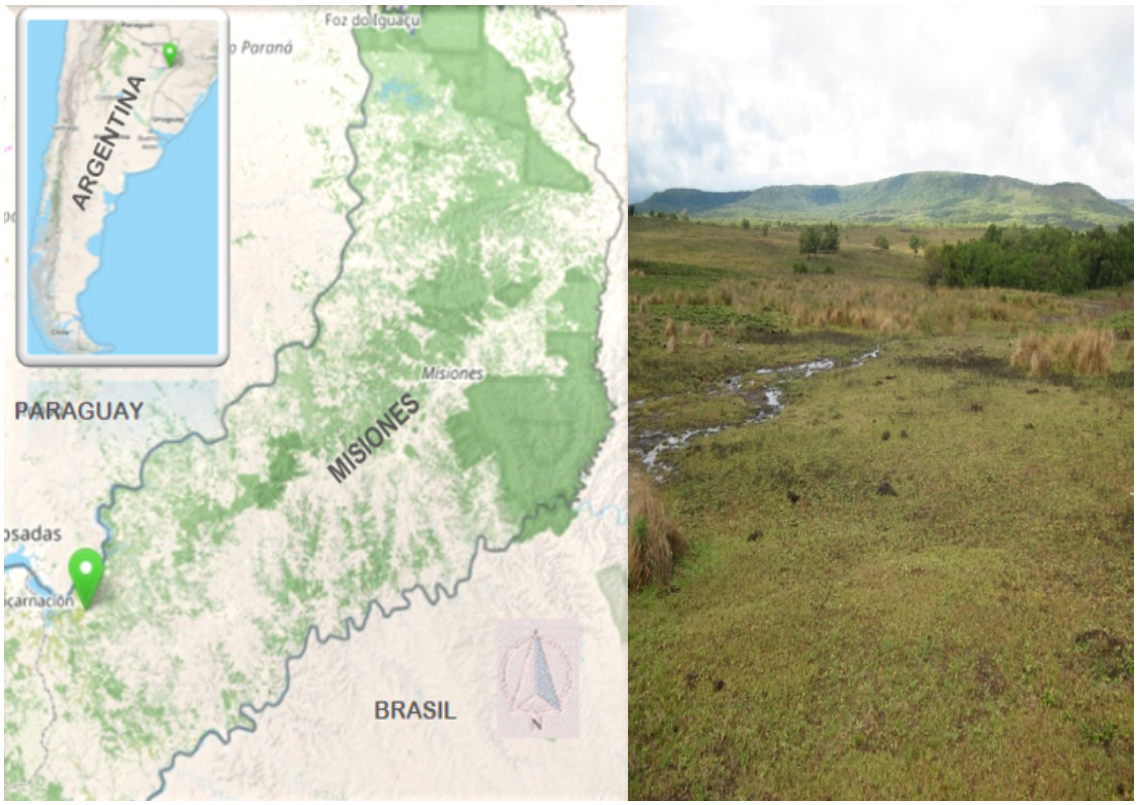

Fig. 2. (Left) Location of the population and (Right) characteristic semi-permanent streams used by the three species of *Melanophryniscus* to reproduce that we studied (©Federico Marangoni).

We recorded the following measurements based on Duellman (1970): snout-vent length (SVL) and arm length (AL) using a digital caliper (0.1 mm precision). We measured body mass (BM) to the nearest 0.0001 g, using an electronic balance (Denver Instrument Tp-214; Denver Instrument Company, Goettingen, Germany) (Fig.3). We released all individuals back into their original ponds within 24–48 h after us obtaining oviposition in the laboratory (see Marangoni and Baldo, 2003). We log-transformed all variables to achieve normality, and tested using Shapiro-Wilk. However, since this was not achieved and we made all analyses with type III General Linear Models using the STATISTICA 8.0 statistical package (StatSoft Inc.). We used multi- and univariate Analyses of Variance (MANOVA/ANOVA) to test for differences in the three body size variables measured between amplexing and non-amplexing (amplexing = A and non-amplexing = NA, hereafter), at α=0.05. Because we found positive and significant association between SVL and AL in the three species studied (see results), we then carried out ANCOVAs, using SVL as a covariate, to compare body size-adjusted AL between A and NA.

Fig. 3. Measurements. (Left) Snout-vent length (SVL) and (Right) body mass (BM), using a digital caliper and electronic balance, respectively.

MANOVA showed, significant body size differences between A and NA males of *M.* aff. *devincenzii* (Wilk's $\lambda = 0.784$, $F_{3,42} = 3.868$, $P < 0.016$) and *M. krauczuki* (Wilk's $\lambda = 0.794$, $F_{3,72} = 6.242$, $P < 0.001$), but these where no significant in *M. atroluteus* (Wilk's $\lambda = 0.951$, $F_{3,143} = 2.460$, $P < 0.065$). Univariate ANOVAs showed significant differences in AL between A and NA of *M.* aff. *devincenzii* ($F_{1,44} = 4.175$, $P = 0.0470$) and in BM of *M. krauczuki* ($F_{1,91} = 6.5491$, $P = 0.012$) (see also Table 1 and Fig. 4). The results of Linear regressions showed positive and significant co-variation between body size (SVL) and AL in the three species (*M. atroluteus*: $r^2 = 0.071$, $P = 0.0011$, n = 147; *M.* aff. *devincenzii*: $r^2 = 0.215$, $P = 0.0012$, n = 46, *M. krauczuki*: $r^2 = 0.168$, $P = 0.0002$, n = 77). ANCOVA showed even the significance of the difference is maintained when we compare the body size-adjusted AL between A and NA of *M.* aff. *devincenzii* ($F_{1,43} = 7.218$, $P = 0.01$; Cov.-SVL: $P < 0.001$).

Table 1. Descriptive statistics (mean ± SE (CI 95%)) of the variables measured: snout-vent length (SVL), arm length (AL), and body mass (BM), of *Melanophryniscus* from the Paranaense Forest. ANOVAs.: A = amplexing vs NA = non-amplexing. * = denote statistics significance between both compared group A and NA.

Specie /body trait	*M. atroluteus*		*M.* aff. *devincenzii*		*M. krauczuki*	
	NA (n =113)	A (n = 34)	NA (n = 31)	A (n = 15)	NA (n = 53)	A (n = 23)
SVL (mm)	22.39 ± 0.410 (21.58 - 23.20)	23.35 ± 0.184 (22.98 - 23.73)	23.50 ± 0.157 (23.18 - 23.82)	23.46 ± 0.289 (22.86 – 24.06)	21.05 ± 0.171 (20.7 - 21.4)	20.49 ± 0.221 (20.03 - 20.94)
AL (mm)	5.99 ± 0.032 (5.92 - 6.05)	5.96 ± 0.051 (5.86 - 6.06)	6.44 ± 0.068 (6.30 - 6.58)	6.69 ± 0.093* (6.49 - 6.89)	5.67 ± 0.046 (5.57 - 5.76)	5.78 ± 0.075 (5.63 - 5.94)
BM (g)	1.03 ± 0.011 (1.01 - 1.05)	0.99 ± 0.030 (0.93 - 1.05)	0.93 ± 0.019 (0.89 - 0.96)	0.91 ± 0.036 (0.84 - 0.99)	0.69 ± 0.008 (0.67 - 0.71)	0.65 ± 0.013* (0.62 - 0.67)

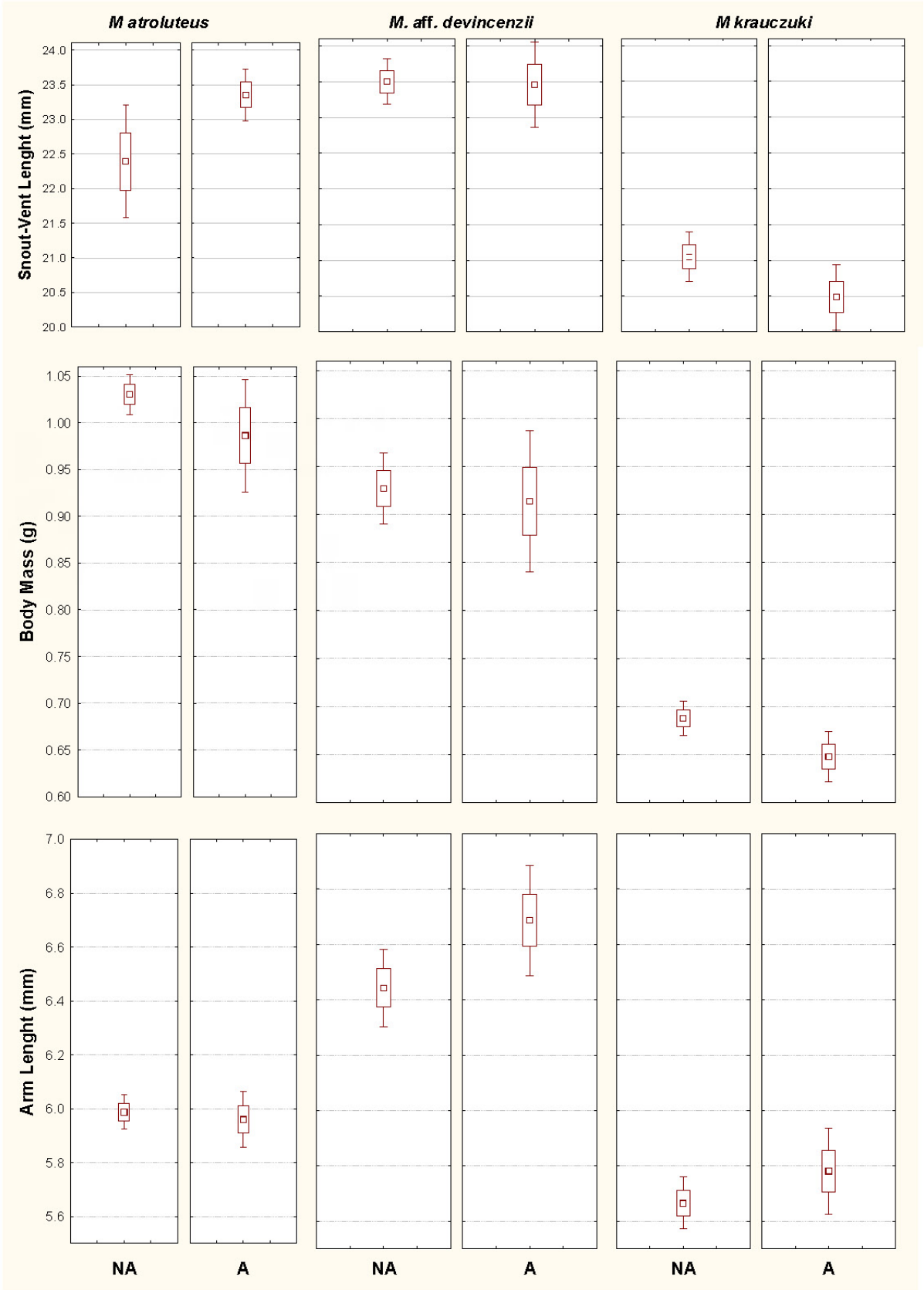

Fig. 4. Body size variation between non-amplexing (NA) and amplexing (A) males of the three *Melanophryniscus* species studied. Middle point = mean, box = mean + standard error and whisker = mean + 95% confidence interval.

Based on the three morphological variables analyzed across the three *Melanophryniscus* species, our results showed significant differences in AL for *M.* aff. *devincenzii*, with amplexing males exhibiting higher mean values. No differences were found in AL for the other two species, nor in snout-vent length (SVL) for either of the species studied. Our results suggest, in line with those obtained in *M. rubriventris* from the Yungas forest (Vaira, 2000; Gastón and Vaira, 2020), that the body size (SVL and AL) are not good predictors of male mating success in these explosive breeders. In relation to the body weight, we only found significant differences in BM in *M. krauczuki*, with lower average values in amplexing males compared to no-amplexing. This result differ from those obtained in *M. rubriventris* where males in pairs had significantly higher body mass and higher body condition indices value than those single or in mating balls (Gaston and Vaira, 2020). Despite we no analyze the body conditions, we suggest that body mass does not playing a significant role in mating success in the studied population. However, ecological or behavioral traits could explain this discrepancy between Yungas and PF species studies. In our previous study we have observed that PF species employ a strategy of spatial segregation at reproductive sites when they reproduce synchronously and sympatrically at the studied site (see “field observation” in, Marangoni and Baldo, 2023). We suggested that this strategy helps them avoid aggressive intra- and interspecific interactions, which might lead to evolve size differences between A and NA males. We also think that, besides body size, other causes would be acting to achieve reproductive success, since on average the non-amplexing individuals have reached the minimum size required to reproduce (Marangoni and Baldo, 2023). However, further studies are needed, increasing the number of A specimens from the three species, to enable more robust analyses and to determine whether large males have greater reproductive success than small males. Even, carry out a field and laboratory experimental design and age estimation, to test the implication of body size and age on the mating success. As well as, the implication of direct interactions, observed among males of *M. atroluteus,* on mating success.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

FM: developed the theory, field work and performed the statistical analyses. Wrote the manuscript with support from ***DB***, who also collaborated in the field work.

REFERENCES

Baldo, D. and N.G. Basso (2004). A new species of *Melanophryniscus* Gallardo, 1961 (Anura: *Bufonidae*), with comments on the species of the genus reported for Misiones, northeastern Argentina. *Journal of Herpetology* 38: 393-403.

Bidau, C.J., D.A. Martí and D. Baldo (2011). Inter and intraspecific geographic variation of body size in South American redbelly toads of the genus *Melanophryniscus* Gallardo, 1961 (Anura: Bufonidae). *Journal of Herpetology* 45: 66-74.

Blanckenhorn, W.U. (2000). The evolution of body size: what keeps organisms small? *Quarterly Review of Biology* 75: 385-407.

Buzatto, A.B., Thyer, E.M., Roberts, J.D. and L.W. Simmons (2017). Sperm competition and the evolution of precopulatory weapons: Testis size and amplexus position, but not arm strength, affect fertilization success in a chorusing frog. *Evolution* 71: 329-341.

Darwin, C. (1871). The descent of man, and Selection in relation to sex, Vol. 1. John Murray.

Deforel, F., A.S. Duport-Bru, S. D. Rosset, D. Baldo and F. Vera Candioti (2021). Osteological atlas of *Melanophryniscus* (Anura, Bufonidae): a synthesis after 150 years of skeletal studies in the genus. *Herpetological Monographs* 35: 1-27.

Duellman, W.E. (1970). The hylid frogs of Middle America. Monographs, Museum of Natural History, University of Kansas 1: 1-753.

Gastón, M.S. and M. Vaira (2020). Male mating success is related to body condition and stress-induced leukocyte response in an anuran with scramble competition. *Canadian Journal of Zoology* 98: 391-398.

Goldberg, J., S.I. Quinzio and M. Vaira (2006). Oviposition-site selection by the toad Melanophryniscus rubriventris in an unpredictable environment in Argentina. *Canadian Journal of Zoology* 84: 699-705.

Halliday, T. and P. Verrell (1986). Review: Sexual Selection and Body Size in Amphibians. *Herpetological Journal* 1: 86-92.

Howard, R.D. and A.G. Kluge (1985). Proximate mechanisms of sexual selection in wood frogs. *Evolution* 39: 260-277.

Marangoni, F. and D. Baldo (2023). Life-history traits of three syntopic species of the South American Redbelly toads (Anura: Bufonidae: *Melanophryniscus*) from the Atlantic Forest of Argentina. *Herpetological Conservation and Biology* 18: 213-228.

Morrison, C. and J.M. Hero (2003). Geographic variation in life-history characteristics of amphibians: a review. *Journal of Animal Ecology* 72: 270-279.

Smirina, E. M. (1972). Annual layers in bones of *Rana temporaria*. *Zoologichesky Zhurnal* 51: 1529-1534.

StatSoft, Inc. (2007). STATISTICA (data analysis software system), version 8.0. www.statsoft.com.

Vaira, M. (2005). Annual variation of breeding patterns of the toad, *Melanophryniscus rubriventris* (Vellard, 1947). *Amphibia-Reptilia* 26: 193-199.

Vaira M., M. Akmentins, M. Attademo, D. Baldo, D.A. Barrasso, S. Barrionuevo, N., Basso, B. Blotto, S. Cairo, R. Cajade, et al. (2012). Categorización del estado de conservación de los anfibios de la República Argentina. *Cuadernos de Herpetología* 26:131-159.

Zank C., F.G. Becker, M. Abadie, D. Baldo, R. Maneyro, and M. Borges-Martins (2014). Climate Change and the Distribution of Neotropical Red-Bellied Toads *(Melanophryniscu*s, Anura, Amphibia): How to Prioritize Species and Populations? PLoS ONE 9(4): e94625.