



## The tadpole of *Physalaemus nanus* (Boulenger, 1888) (Anura, Leptodactylidae) from Southern Brazil

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The genus *Physalaemus* Fitzinger, 1826 currently comprises 47 described species distributed from Central America (Guianas) to Uruguay (Frost 2016). The genus has been recently organized in two main clades based on molecular data, *Physalaemus signifer* Clade and *Physalaemus cuvieri* Clade (Lourenço *et al.* 2015). The *P. signifer* Clade, with 15 species, is composed by the *P. deimaticus* and *P. signifer* groups, plus *P. nattereri* (Steindachner, 1863) and *P. maculiventris* (Lutz, 1925) (Nascimento *et al.* 2005; Lourenço *et al.* 2015).

*Physalaemus nanus* (Boulenger, 1888) belongs to the *P. signifer* group, which contains nine species (Lourenço *et al.* 2015). Among these, *P. crombiei* Pombal & Madureira, 1997, *P. obtectus* Bokermann, 1966 and *P. nanus* do not have their tadpoles described. *Physalaemus nanus* occurs in forested areas in Paraná, Rio Grande do Sul and Santa Catarina states along the Atlantic Forest domain, at elevations from 0 to 1200 m a.s.l. (Silvano *et al.* 2004; Crivellari *et al.* 2014). Once that tadpole morphology provides an important source of characters for anuran taxonomy (Santana *et al.* 2016), in the present article we describe the external morphology of the tadpoles of *P. nanus*, in comparison with known tadpoles of the *P. signifer* group.

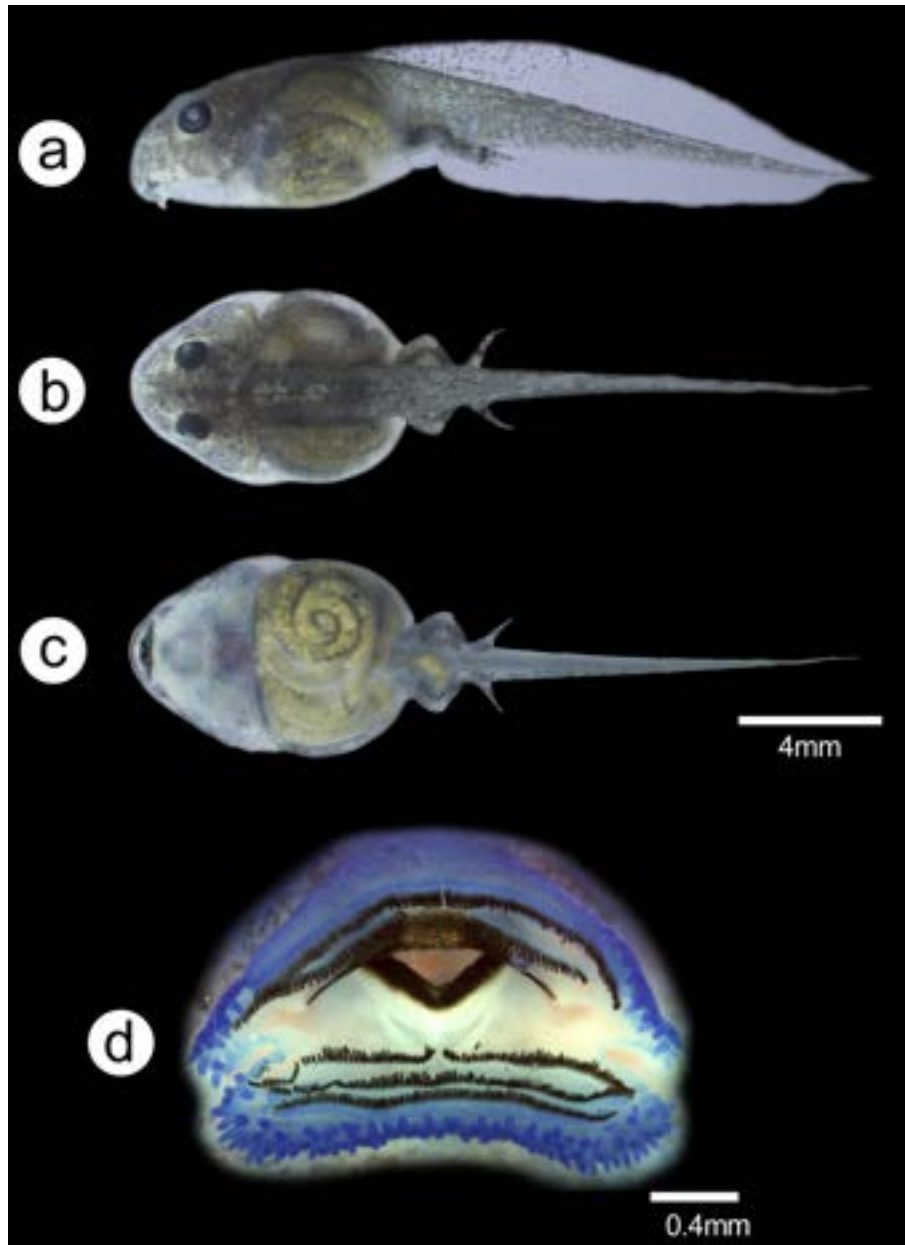
Tadpoles of *Physalaemus nanus* were collected on 31 December 2016 during fieldwork at Sítio Marioti, Urussanga municipality, Santa Catarina state, Brazil (28°28'48.98"S, 49°21'19.49"W; approximately at 190 m a.s.l.). One tadpole was reared to metamorphosis to confirm species identification. Specimens were killed in 5% lidocaine, preserved in 5% formalin and housed at Coleção Zoológica de Referência da Universidade Federal de Mato Grosso do Sul (lot ZUFMS-AMP05542 and ZUFMS-AMP5625). The pond where we collected the tadpoles has about 5m<sup>2</sup> in area, located in a forest edge, and formed by a water flow. The margins and interior of the pond were covered by herbaceous vegetation (*Brachiaria* spp.) located in a shadow of forest remnant.

External larval morphology description of *Physalaemus nanus* was based on one tadpole at Stage 38 (Gosner 1960) and measurements were made on specimens from Stages 26 to 43 (n=35, Table 1). Morphological description follows Altig & McDiarmid (1999). Measurements were taken with a digital caliper to the nearest 0.01 mm under a stereomicroscope. The following measurements were taken: total length (TOL), body length (BL), body height (BH), body width (BW), tail length (TL), tail height (TH), tail musculature width (TMW), tail musculature height (TMH), dorsal fin height (DFH), ventral fin height (VFH), eye diameter (ED), eye–snout distance (ESD), interorbital distance (IOD), naris diameter (ND), naris–snout distance (NSD), internarial distance (IND), oral disc diameter (OD), upper jaw sheath width (UJSW), upper jaw sheath height (UJSH).

**Tadpole description.** Body ovoid in dorsal view and globular depressed in lateral view (Figure 1a–c). Body length less than half the total length (BL/TL=0.39±0.03; see Table 1 for measurements), body height half the body length (BH/BL=0.48±0.04), body width larger than body height (BH/BW= 0.77±0.05). Snout rounded in lateral and dorsal views. Nostrils small, rounded and dorsal, located about mid length between the eyes and the tip of the snout. Eyes small (ED/BW=0.21±0.03), dorsally positioned, located between first and second third of the body. Internarial distance less than half the interorbital distance (IND/IOD=0.37±0.06). Spiracle short and sinistral, directed posterodorsally, with the inner wall present, located below the median line; spiracle aperture rounded. Vent tube medial, fused to the ventral fin. Tail long, about 60% of the total length (TaL/TL=0.6±0.03), with the tail musculature not reaching the end of the rounded tail; dorsal fin originated on the body-tail junction and ventral fin lower than dorsal fin. Oral disc small, its width approximately 41% of body width, ventral, laterally emarginated, and bordered by one or two rows of small papillae, interrupted in a long dorsal gap. Labial tooth row formula 2(2)/3(1). Upper jaw sheath arc-shaped and lower jaw sheath V-shaped, with the upper jaw sheath being wider than the lower one.

**TABLE 1.** Tadpole measurements in millimeters (mean, standard deviation) taken from *Physalaemus nanus* tadpoles Gosner Stages 26–43. Abbreviations: total length (TOL), body length (BL), body height (BH), body width (BW), tail length (TL), tail height (TH), tail musculature width (TMW), tail musculature height (TMH), dorsal fin height (DFH), ventral fin height (VFH), eye diameter (ED), eye–snout distance (ESD), interorbital distance (IOD), naris diameter (ND), naris–snout distance (NSD), intermarial distance (IND), oral disc diameter (OD), upper jaw sheath width (UJSW), upper jaw sheath height (UJSH).

Measures	Stages (n)																
	26 (1)	27 (2)	28 (3)	29 (1)	30 (2)	31 (3)	32 (1)	33 (2)	34 (1)	35 (1)	36 (5)	37 (3)	38 (3)	40 (3)	41 (2)	42 (1)	43 (1)
<b>TOL</b>	11.47	12.33 ± 0.66	13.04 ± 1.86	14.41	14.08 ± 1.20	15.17 ± 1.18	14.59	15.65 ± 0.29	17.5	18.34	18.97 ± 0.77	20.26 ± 3.11	19.84 ± 1.29	19.66 ± 2.06	19.76 ± 0.87	20.61	18.17
<b>BL</b>	4.59	4.51 ± 0.04	4.72 ± 0.10	5.49	5.20 ± 0.51	6.08 ± 0.93	5.9	6.69 ± 0.17	6.5	7.13	7.46 ± 0.39	8.46 ± 0.96	7.83 ± 0.56	8.29 ± 1.15	7.78 ± 0.03	8.67	6.55
<b>BH</b>	2	1.91 ± 0.04	2.36 ± 0.12	2.66	2.21 ± 0.16	2.99 ± 0.33	3.05	2.96 ± 0.03	3.18	3.48	3.83 ± 0.35	4.50 ± 0.75	3.74 ± 0.70	4.45 ± 0.77	3.63 ± 0.45	3.46	2.84
<b>BW</b>	2.52	2.64 ± 0.31	2.94 ± 0.25	3.12	3.00 ± 0.16	3.81 ± 0.44	4.33	4.13 ± 0.21	4.16	4.95	4.96 ± 0.25	5.47 ± 0.72	4.86 ± 0.63	5.64 ± 0.41	4.72 ± 0.04	4.69	3.49
<b>TL</b>	6.88	7.82 ± 0.71	8.32 ± 1.85	8.92	8.88 ± 0.69	9.09 ± 0.68	8.69	8.96 ± 0.46	11	11.21	11.51 ± 0.66	11.81 ± 2.48	12.01 ± 0.73	11.37 ± 0.98	11.98 ± 0.90	11.94	11.62
<b>TH</b>	2.48	2.01 ± 0.15	2.57 ± 0.06	2.79	2.48 ± 0.05	2.92 ± 0.19	3.46	2.89 ± 0.14	2.9	3.51	3.41 ± 0.36	4.46 ± 0.68	3.94 ± 0.34	3.41 ± 0.64	3.59 ± 0.01	3.8	3.53
<b>TMW</b>	0.68	0.63 ± 0.03	0.66 ± 0.11	0.81	0.71 ± 0.08	0.88 ± 0.03	1.04	1.03 ± 0.05	1.09	1.13	1.23 ± 0.13	1.60 ± 0.13	1.49 ± 0.30	1.48 ± 0.14	1.32 ± 0.46	1.59	1.16
<b>TMH</b>	0.87	1 ± 0.07	1.09 ± 0.04	1.28	1.07 ± 0.11	1.11 ± 0.10	1.28	1.30 ± 0.17	1.32	1.5	1.50 ± 0.19	1.84 ± 0.30	1.47 ± 0.10	1.78 ± 0.12	1.70 ± 0.03	1.79	1.48
<b>DFH</b>	0.5	0.7 ± 0.06	0.89 ± 0.03	0.74	0.91 ± 0.00	1.05 ± 0.14	1.14	0.96 ± 0.19	0.89	1.03	1.14 ± 0.10	1.65 ± 0.47	1.32 ± 0.10	1.19 ± 0.03	1.36 ± 0.05	0.76	1.19
<b>VFH</b>	0.76	0.6 ± 0.03	0.81 ± 0.08	0.77	0.67 ± 0.01	0.84 ± 0.17	1.17	0.72 ± 0.02	0.8	0.94	1.06 ± 0.29	1.72 ± 1.07	1.09 ± 0.22	0.85 ± 0.24	0.94 ± 0.12	0.9	0.86
<b>ED</b>	0.56	0.64 ± 0.06	0.78 ± 0.14	0.69	0.72 ± 0.06	0.82 ± 0.09	0.86	0.86 ± 0.03	0.87	0.84	0.89 ± 0.09	1.02 ± 0.16	1.06 ± 0.14	1.04 ± 0.06	1.07 ± 0.03	1.18	0.91
<b>ESD</b>	1.66	1.56 ± 0.11	1.43 ± 0.21	1.8	1.63 ± 0.01	1.91 ± 0.26	1.77	2.10 ± 0.10	2	2.26	1.89 ± 0.38	1.72 ± 0.15	1.95 ± 0.18	1.97 ± 0.45	1.55 ± 0.17	1.57	1.39
<b>IOD</b>	1.76	1.57 ± 0.07	1.81 ± 0.05	1.84	1.85 ± 0.08	2.14 ± 0.23	2.11	2.23 ± 0.03	2.44	2.44	2.46 ± 0.28	2.77 ± 0.46	2.36 ± 0.30	2.78 ± 0.40	2.47 ± 0.13	3.15	2.33
<b>ND</b>	0.22	0.17 ± 0.06	0.21 ± 0.04	0.24	0.24 ± 0.01	0.30 ± 0.04	0.15	0.26 ± 0.04	0.24	0.38	0.27 ± 0.02	0.34 ± 0.06	0.27 ± 0.04	0.25 ± 0.05	0.29 ± 0.06	0.32	0.29
<b>NSD</b>	0.83	0.68 ± 0.01	0.70 ± 0.05	0.9	0.77 ± 0.01	0.90 ± 0.11	0.44	1.15 ± 0.06	0.99	1.18	0.93 ± 0.24	0.60 ± 0.16	0.70 ± 0.32	0.92 ± 0.42	0.46 ± 0.28	0.32	0.35
<b>IND</b>	0.98	0.95 ± 0.04	0.96 ± 0.05	1	0.91 ± 0.07	1.14 ± 0.14	1.06	1.13 ± 0.05	1.24	1.15	1.16 ± 0.15	1.07 ± 0.13	1.06 ± 0.10	1.22 ± 0.22	1.14 ± 0.17	0.91	0.93
<b>OD</b>	1.29	1.13 ± 0.13	1.28 ± 0.10	1.62	1.41 ± 0.18	1.64 ± 0.18	1.52	1.68 ± 0.06	1.88	2.01	1.98 ± 0.22	2.11 ± 0.07	1.95 ± 0.16	2.14 ± 0.13	2.02 ± 0.05	1.32	1.46
<b>UJSW</b>	0.14	0.33 ± 0.26	0.13 ± 0.01	0.13	0.15 ± 0.01	0.14 ± 0.02	0.18	0.24 ± 0.01	0.24	0.18	0.25 ± 0.04	0.36 ± 0.32	0.21 ± 0.05	0.24 ± 0.05	0.25 ± 0.06	0.3	0.11
<b>UJSH</b>	0.45	0.28 ± 0.25	0.49 ± 0.18	0.59	1.03 ± 0.54	0.75 ± 0.06	0.63	0.70 ± 0.13	0.83	0.88	0.81 ± 0.19	0.47 ± 0.32	0.82 ± 0.06	0.87 ± 0.14	1.02 ± 0.25	0.8	0.52



**FIGURE 1.** The tadpole of *Physalaemus nanus*: (a) lateral, (b) dorsal (c) ventral views, and (d) oral disc.

**Coloration in live.** Body and caudal musculature light brown with little dark spots. Venter transparent showing golden spots in lateral regions. Fins translucent.

**Coloration in preservative.** Body and caudal musculature light brown with dark spots. Ventral region translucent, with viscerae evident. Dorsal and ventral fins translucent.

**Comparison with other species.** Comparisons were made with tadpoles of 7 species of the *P. signifer* group, namely *P. atlanticus* (Haddad & Sazima 2004), *P. bokermanni* (Cardoso & Haddad 1985), *P. caete* (Pombal & Madureira 1997), *P. camacan* (Pimenta *et al.* 2005), *P. moreirae* (Provete *et al.* 2011), *P. signifer* (Weber & Carvalho-e-Silva 2001), and *P. spiniger* (Haddad & Pombal 1998). Tadpoles of *P. nanus* present body size similar to *P. atlanticus* and *P. moreirae*, are smaller than *P. caete* and larger than *P. bokermanni*, *P. camacan*, *P. signifer*, and *P. spiniger*. The ovoid body of *P. nanus* differs in dorsal view from the hexagonal body of *P. bokermanni*. In lateral view, the body of *P. nanus* is similar to the depressed globular shape of *P. atlanticus*, *P. caete*, *P. moreirae*, *P. spiniger*, and differs from the ovoid body of *P. bokermanni* and *P. camacan* and from the elliptical body of *P. signifer*. Nostrils are similar in size and position among the species of the group; exceptions are *P. camacan* with larger nostrils, and *P. moreirae* and *P. signifer* with nostrils closer to the eyes. *Physalaemus nanus* eyes are larger than those of *P. moreirae*, *P. signifer*, and *P. camacan*, and smaller than those of *P. bokermanni*; similar eye sizes occur in *P. atlanticus*, *P. caete*, and *P. spiniger*. The configuration of the tail in *P.*

*nanus*, with a dorsal fin higher than the ventral, is similar to all species excepting *P. caete* that shows an inverse pattern. Oral discs are ventral and emarginated in *P. nanus* as in most species; exceptions are the not emarginate disc in *P. caete* and the anteroventral disc in *P. signifer*. The tadpole of *P. nanus* shares the regular coloration pattern among the species in the *P. signifer* group with dorsum light to dark brown with dark spots, with exception of the *P. caete* and *P. spiniger*, which have the body grey.

The tadpole of *P. nanus* can be diagnosed and distinguished from other species of *P. signifer* group by a combination of external morphological traits, except from *P. atlanticus* tadpole, which does not show morphological differences from *P. nanus*. However, adults of this species do not occur in sympatry, and they are easily distinguishable by acoustic parameters (Haddad & Sazima 2004).

Considering the currently necessity of delimiting species based on integrative taxonomy approaches, the descriptions of anuran larvae are imperative to solve taxonomic issues in the absence of adult morphological differences as in *P. signifer* species group (Haddad & Pombal 1998). Besides, once *P. crombiei* and *P. obtectus* share similar distribution patterns, and are considered cryptic species (Heyer & Wolve 1989; Peres & Simon 2012), the description of their tadpoles may help to infer species diagnoses because it has proved to be a good source of characters for the taxonomy of the group.

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## References

- Altig, R. & McDiarmid, R.W. (1999) Body plan: development and morphology. In: McDiarmid, R.W. & Altig, R. (Eds.), *Tadpoles: The Biology of Anuran Larvae*. The University of Chicago Press, Chicago, Illinois, pp. 24–51.
- Cardoso, A.J. & Haddad, C.F.B. (1985) Nova espécie de *Physalaemus* do grupo *signiferus* (Amphibia, Anura, Leptodactylidae). *Revista Brasileira de Biologia*, 45, 33–37.
- Crivellari, L.B., Leivas, P.T., Leite, J.C.M., Gonçalves, D.D.S., Mello, C.M., Rossa-Feres, D.D.C. & Conte, C.E. (2014) Amphibians of grasslands in the state of Paraná, southern Brazil (Campos Sulinos). *Herpetology Notes*, 7, 639–654.
- Frost, D.R. (2016) Amphibian species of the world: an online reference. Version 6. American Museum of Natural History, New York, USA. Electronic Database. Available from: <http://research.amnh.org/herpetology/amphibia/index.html> (accessed 2 Mar. 2016)
- Gosner, K.L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16, 183–190.
- Haddad, C.F.B. & Pombal, J.P. (1998) Redescription of *Physalaemus spiniger* (Anura: Leptodactylidae) and description of two new reproductive modes. *Journal of Herpetology*, 32, 557–565.  
<https://doi.org/10.2307/1565210>
- Haddad, C.F.B. & Sazima, I. (2004) A new species of *Physalaemus* (Amphibia; Leptodactylidae) from the Atlantic forest in southeastern Brazil. *Zootaxa*, 479 (1), 1–12.  
<https://doi.org/10.11646/zootaxa.479.1.1>
- Heyer, W.R. & Wolf, A.J. (1989) *Physalaemus crombiei* (Amphibia: Leptodactylidae), a new frog species from Espírito Santo, Brazil with comments on the *P. signifer* group. *Proceedings of the Biological Society of Washington*, 102, 500–506.
- Lourenço, L.B., Targueta, C.P., Baldo, D., Nascimento, J., Garcia, P.C., Andrade, G. V., Haddad, C.F.B. & Recco-Pimentel, S.M. (2015) Phylogeny of frogs from the genus *Physalaemus* (Anura, Leptodactylidae) inferred from mitochondrial and nuclear gene sequences. *Molecular Phylogenetics and Evolution*, 92, 204–216.  
<https://doi.org/10.1016/j.ympev.2015.06.011>
- Nascimento, L.B., Caramaschi, U. & Cruz, C.A.G. (2005) Taxonomic review of the species groups of the genus *Physalaemus* Fitzinger, 1826 with revalidation of the genera *Engystomops* Jiménez-de-la-Espada, 1872 and *Eupemphix* Steindachner, 1863 (Amphibia, Anura, Leptodactylidae). *Arquivos do Museu Nacional*, 63, 297–320.
- Peres, J. & Simon, J.E. (2012) *Physalaemus maximus* Feio, Pombal Jr. and Caramaschi, 1999 (Anura: Leiuperidae): distribution extension and advertisement call. *Check List*, 8, 507–509.  
<https://doi.org/10.15560/8.3.507>
- Pimenta, B.V., Cruz, C.A.G. & Silvano, D.L. (2005) A new species of the genus *Physalaemus* Fitzinger, 1826 (Anura, Leptodactylidae) from the Atlantic Rain Forest of southern Bahia, Brazil. *Amphibia-Reptilia*, 26, 201–210.  
<https://doi.org/10.1163/1568538054253483>
- Pombal, J.P. & Madureira, C.A. (1997) A new species of *Physalaemus* (Anura, Leptodactylidae) from the Atlantic rain forest of northeastern Brazil. *Alytes*, 15, 105–112.

- Provete, D.B., Garey, M.V., Dias, N.Y.N. & Rossa-Feres, D.C. (2011) The tadpole of *Physalaemus moreirae* (Anura: Leiuperidae). *Herpetologica*, 67, 258–270.  
<https://doi.org/10.1655/HERPETOLOGICA-D-11-00004.1>
- Santana, D.J., Magalhaes, F.M., São-Pedro, V.A., Mângia, S., Amado, T.F. & Garda, A.A. (2016) Calls and tadpoles of the species of *Pseudis* (Anura, Hylidae, Pseudae). *Herpetological Journal*, 26, 139–148.  
<https://doi.org/10.1163/15685381-00002885>
- Silvano, D., Garcia, P.C.A. & Kwet, A. (2004) *Physalaemus nanus*. The IUCN Red List of Threatened Species 2004. Available from: <http://www.iucnredlist.org/details/57266/0> (accessed 13 January 2017)
- Weber, L.N. & Carvalho-e-Silva, S.P. (2001) Descrição da larva de *Physalaemus signifer* (Girard, 1853) (Amphibia, Anura, Leptodactylidae) e informações sobre a reprodução e a distribuição geográfica. *Boletim do Museu Nacional*, 462, 1–6.