



Tadpoles of the dyeing poison dart frog *Dendrobates tinctorius* (Cuvier, 1797) from eastern Amazonia

MIQUÉIAS FERRÃO^{1,2,3*}, PEDRO HENRIQUE DOS SANTOS DIAS⁴, IGOR L. KAEFER⁵, ANTHONY SANTANA FERREIRA⁶, RODRIGO TAVARES-PINHEIRO⁷, ABDIEL PINHEIRO FREITAS⁷ & CARLOS EDUARDO COSTA-CAMPOS⁷

¹Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, United States of America

²Programa de Pós-Graduação em Biodiversidade Animal, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiânia, Goiás, Brazil

³Centro Nacional de Pesquisa e Conservação de Répteis e Anfíbios, Instituto Chico Mendes de Conservação da Biodiversidade, Goiânia, Goiás, Brazil

✉ uranoscodon@gmail.com; <https://orcid.org/0000-0001-7035-8276>

⁴Leibniz Institut zur Analyse des Biodiversitätswandels, Zoologisches Museum Hamburg, Martin-Luther-King-Platz, Hamburg, Germany

✉ pedrodiasherpeto@gmail.com; <https://orcid.org/0000-0002-6428-6496>

⁵Instituto de Ciências Biológicas, Universidade Federal do Amazonas, Manaus, Amazonas, Brazil

✉ kaefer@ufam.edu.br; <https://orcid.org/0000-0001-6515-0278>

⁶Coordenação de Biodiversidade, Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil

✉ anthonyferreira@gmail.com; <https://orcid.org/0000-0002-3718-8049>

⁷Laboratório de Herpetologia, Departamento de Ciências Biológicas e da Saúde, Universidade Federal do Amapá, Macapá, Brazil

✉ rodrigomcp@gmail.com; <https://orcid.org/0000-0002-8021-9293>

✉ abdiel_freitas@hotmail.com; <https://orcid.org/0000-0003-0830-2954>

✉ dududueducampos@gmail.com; <https://orcid.org/0000-0001-5034-9268>

*Corresponding author

The dendrobatid genus *Dendrobates* Wagler currently includes five species distributed from southern Nicaragua through Costa Rica, Panama, and Colombia to the Guianas and adjacent Brazil (Frost 2023). Two species are found in Brazil: *Dendrobates leucomelas* Steindachner and *D. tinctorius* (Cuvier). The latter was originally described from “Amérique”, and a neotype was designed by Silverstone (1975) from the Rivière Matarony, French Guiana. In Brazil, it is found in the States Amapá and Pará (Avila-Pires *et al.* 2010; Taucce *et al.* 2022). It is a diurnal species that inhabits the leaf litter of rainforests, and exhibits polymorphism with various distinct post-metamorphic color patterns (Rojas & Pašukonis 2019). Due to its popularity in the pet trade it is listed under Appendix II of CITES. Adults deposit clutches in the leaf litter, typically containing 2–14 eggs that hatch within 14–28 days. Males then transport the tadpoles to small water bodies, e.g., palm bracts, bromeliads, or tree holes (Born 1994; Born *et al.* 2010; Rojas & Pašukonis 2019). Tadpoles have a varied diet, including detritus, insect larvae and other tadpoles, often conspecific. Metamorphosis typically occurs between 90 and 120 days after oviposition (Masurat & WolfRudiger 1991; Born 1994; Rojas & Pašukonis 2019). The external morphology of a tadpole from the western slope of Vier Gebroeders Mountain in Surinam was briefly described by Hoogmoed (1969) as *D. azureus* Hoogmoed, which is now considered a synonym of *D. tinctorius* (Wollenberg *et al.* 2008). Silverstone (1975) provided additional descriptions and illustrations of the body and oral apparatus of specimens from Serra do Navio (Amapá, Brazil). Lescure (1984) provided a draft of the oral apparatus and body, but without any morphological description. Finally, the chondrocranium morphology of these tadpoles was described by Haas (2003). Despite previous studies addressing the external morphology of *D. tinctorius* larvae, there are still several aspects that have not been adequately described, and some character-states require further examination. Thus, in the present study, the external morphology of *D. tinctorius* is redescribed following current standards for larval descriptions.

Free-living tadpoles were collected from a palm bract filled with water in a transitional environment between savanna and rainforest in Matão do Piaçacá, State of Amapá, Brazil (0.05208° N, 51.16520° W; 0 m a.s.l.). The tadpoles were euthanized with a 2% aqueous benzocaine solution diluted in water and then fixed and stored in 10% formalin. Collected specimens were deposited in the herpetological collection of the Universidade Federal do Amazonas, Manaus, Brazil. Morphometric measurements were taken from nine tadpoles at different developmental stages (Gosner 1960), including

Stage 25 (n = 2), 26 (n = 5), 27 (n = 1) and 29 (n = 1), using a micrometric ocular lens (nearest 0.1 mm) coupled to a stereoscopic microscope. Morphometric measurements were taken according to Altig & McDiarmid (1999) for total length (TL), body length (BL), tail length (TAL), maximum tail height (MTH), internarial distance (IND), interorbital distance (IOD), tail muscle height (TMH) and tail muscle width (TMW). Other measurements, such as body height (BH), body width (BW), body width at eyes level (BWE), eye-nostril distance (END), nostril-snout distance (NSD), eye diameter (ED) and oral disc width (ODW) followed Lavilla & Scrocchi (1986), and spiracle length (SL) and vent-tube length (VTL) followed Lins *et al.* (2018). The terminology used in the description and proportions is based on Altig & McDiarmid (1999), and the detailed description is primarily derived from a tadpole at Stage 29. Additionally, for comparative purposes, we examined individuals of other *Dendrobates* species: *D. auratus* (Panama: Museo de Zoología Pontificia Universidad Católica del Ecuador QCAZ 37407), *D. leucomelas* (Brazil: Roraima: American Museum of Natural History AMNH 137308), and *D. truncatus* (Colombia: Córdoba: Pueblo Nuevo: Instituto de Ciencias Naturales, Universidad Nacional de Colombia ICN 48945, 48947; Santander: Sabana de Torres: ICN 54630. Magdalena: Santa Marta: ICN 45735). Intraspecific comparisons among dendrobatid larvae are rare and the taxonomic status of many populations of several species are still unclear. Thus, we also compared our data with one larva of *D. tinctorius* from French Guiana (Nouragues: Antoine Fouquet material).

External morphology (Table 1 and Fig. 1). Body depressed and elongated in lateral view, BH 80% and 52% of BW and BL, respectively; BL 37% of TL; oval-shaped in dorsal view, body width at eye level 93% of the BW, without any well-marked constriction anterior to the spiracle level (Fig. 1A–C). Snout rounded in lateral and dorsal views; NSD 75% of END. Anterolateral and anterodorsal regions of the body marked by well-developed muscles orbitohyoideus. Internarial region slightly convex; IND 67% of IOD. Eyes small, eye diameter 20% and 38% of IOD and END, dorsally located, laterally directed. Interorbital region slightly concave; IOD 50% of BWE. Nostrils small, elliptical, nearly rounded, dorsolaterally located, anterolaterally directed; border of the external nares surrounded by a fleshy, smooth marginal rim, prominent upward and with a small lateral projection on the anterior portion. Spiracle single, sinistral, and tubular; posterodorsally directed, visible in ventral view and partially from dorsal view; opening elliptical, almost the same diameter of spiracle width; inner wall present, with only the distal portion free from body. Vent tube open, medially positioned, ventrally directed, and parallel to the longitudinal body axis; short, as long as wide; opening large, elliptical, medially directed, with irregular margins; dorsal wall fully attached to the distal border of the ventral fin. Tail long, its length 63% of TL; low, MTH 95% of BH; tail tip rounded. Caudal muscle moderately robust with an acuminate tip; higher than wide near the body junction, TMW 93% of TMH; TMH 44% of MTH; myotomes do not reach tail tip. Dorsal fin arch-shaped; originated near the body/tail junction; maximum height at the mid-tail. Ventral fin arch-shaped, as high as the dorsal fin; maximum height at the mid-tail. Oral disc of moderate size, ODW 48% of BW, laterally emarginate and anteroventrally directed (Fig. 1D); upper and lower labium present but not expanded; upper labium with few short conical papillae on lateral regions, organized in a straight line, interrupted by a large medial anterior gap of ~70% of upper labium length; posterior labium projected anteriorly and surrounded by a single row of short conical alternate papillae. Submarginal papillae absent. Jaw sheaths massive, fully keratinized, serrated, >50% of oral disc height; upper sheath arch-shaped, lower sheath V-shaped and narrower than the upper one. Labial tooth row formula 2(2)/3(1); A1 slightly longer than A2; A2 gap ~17% of A2 length; P1 slightly longer than P2, and P2 slightly longer than P3; P1 gap very short, ~5% of P1 length.

Color in preservative. Dorsal surface of body greyish brown, with a pale brown region laterally to the anterior junction tail/body. Lateral surface brownish grey anteriorly, pale brown posteriorly; a distinct brown stripe at the level of posterior half of the body, runs from one side of the lateral body to the other side, through the belly; fleshy rim whitish grey. Margin of the spiracle aperture translucent grey. Vent tube translucent with a high amount of melanophores; opening also translucent. Ventral surface greyish brown anteriorly, translucent grey with scattered melanophores posteriorly. Tail uniformly pale brown, except for the translucent border of the ventral fin.

The phenotype of *Dendrobates* larvae is highly conserved. Still, tadpoles of *D. tinctorius* can be promptly differentiated from its congeners by the presence of a prominent fleshy projection on the marginal rim of the nostrils (absent in *D. auratus*, *D. leucomelas*, and *D. truncatus*). Additionally, it can be further differentiated from *D. leucomelas* and *D. truncatus* by the tail fin originating on the tail (vs. on the body/tail junction) and from *D. auratus* by the alternate marginal papillae (vs. aligned). Lastly, *D. tinctorius* tadpoles described in this study exhibit the same character-states as those from French Guiana, providing further support for the current taxonomic status of both populations.

TABLE 1. Morphometric measurements of tadpoles of the dyeing dart poison frog *Dendrobates tinctorius*. Measurements are presented in millimeters (mm) and values expressed as mean \pm SD (range) for development stages with more than two tadpoles, and only range for those with $n = 2$. Abbreviations are described in the text.

	Stage 25	Stage 26	Stage 27	Stage 29
<i>n</i>	2	5	1	1
TL	19.5–19.5	22.4 \pm 1.8 (21.0–25.6)	27.3	33.9
BL	7.9–7.9	8.7 \pm 0.4 (8.5–9.5)	10.3	12.4
TAL	11.6–11.6	13.7 \pm 1.4 (12.4–16.1)	17.0	21.5
MTH	3.2–3.2	3.7 \pm 0.2 (3.6–4.1)	4.5	6.2
IND	1.6–1.7	1.8 \pm 0.1 (1.6–2.0)	2.1	2.5
IOD	2.5–2.6	2.8 \pm 0.2 (2.7–3.2)	3.3	3.8
TMH	1.5–1.6	1.9 \pm 0.1 (1.7–2.0)	2.3	2.7
TMW	1.5–1.6	1.8 \pm 0.1 (1.8–2.0)	2.0	2.5
BH	4.0–4.0	4.6 \pm 0.3 (4.3–5.0)	5.4	6.5
BW	5.0–5.0	5.6 \pm 0.4 (5.1–6.2)	6.8	8.1
BWE	4.5–4.5	5.4 \pm 0.4 (5.0–5.8)	5.9	7.5
END	1.5–1.5	1.6 \pm 0.2 (1.5–1.9)	1.8	2.0
NSD	1.0–1.0	1.1 \pm 0.1 (1.1–1.4)	1.2	1.5
ED	0.6–0.6	0.6 \pm 0.0 (0.6–0.7)	0.6	0.8
ODW	2.2–2.2	2.4 \pm 0.1 (2.3–2.6)	2.8	3.1
SL	0.6–0.6	0.5 \pm 0.1 (0.4–0.6)	0.6	0.6
VTL	0.6–0.7	0.6 \pm 0.0 (0.6–0.6)	1.0	1.1

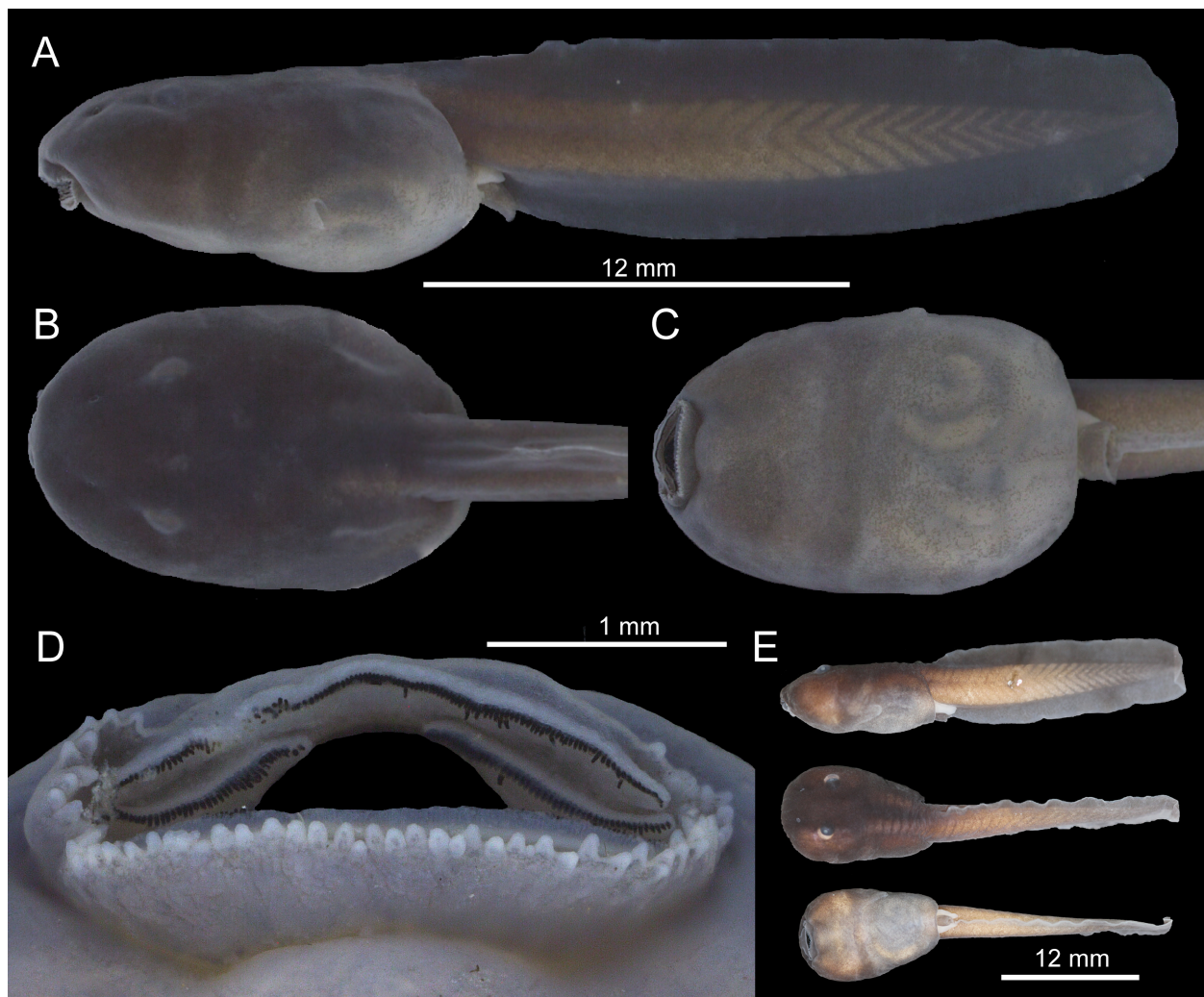


FIGURE 1. Preserved tadpoles of *Dendrobates tinctorius* (Gosner Stage 29) from (A–D) Matão do Piaçacá, state of Amapá, Brazil, and (E) Nouragues, French Guiana. (A) Lateral, (B) dorsal and (C) ventral views of the body, and (D) ventral view of the oral disc; (E) Lateral, dorsal and ventral views of the body.

Many character states in *Dendrobates tinctorius* are likely associated with a macrophagous feeding behavior. The fully keratinized and strong jaw sheaths can be used to prey on insect larvae and other tadpoles. These tadpoles also exhibit a well-developed m. orbitohyoideus, which is externally noticeable. This muscle is responsible for lowering the buccal floor (Satel & Wassersug 1981) and is commonly well-developed in macrophagous forms such as *Dendropsophus* tadpoles (Dias *et al.* 2019). This trophic specialization is likely linked to the colonization of phytotelmata; although water-filled plants can be safer than ponds or streams in terms of predators and competitors, they are also limited in food resources (Lannoo *et al.* 1987; Caldwell & Araújo 2004). In fact, with few exceptions (e.g., *Ololygon perpusilla* species group; Dias & Pie 2021), tadpoles that develop in such environments often exhibit macrophagous, oophagous, or endotrophic feeding strategies (Lehtinen *et al.* 2004 and references therein). Predator-like character-states of *D. tinctorius* are considered plesiomorphic and are present in most lineages of Dendrobatinae (e.g., Duarte-Marín *et al.* 2020); however, the exact evolutionary history of these traits remains controversial. Recently, Grant *et al.* (2017) revisited the phylogenetic relationships of Dendrobatoidea and recovered “*Colostethus*” *ruthveni* nested within Dendrobatinae. Tadpoles of this species have a pond-like phenotype and inhabit small pools in riverbanks (Kaplan 1997). The unexpected position of “*Colostethus*” *ruthveni* as the sister taxon to all Dendrobatini raises questions about the evolution of the macrophagous phenotype and the colonization of phytotelmata. It is unclear whether phytotelma usage evolved in the common ancestor of all Dendrobatinae and was subsequently lost in “*Colostethus*” *ruthveni*, or if it evolved independently in *Phylllobates* and Dendrobatini. Further studies are still necessary to fully understand the evolution of macrophagy and phytotelma development in poison frogs.

We are thankful to Marcelo Menin (in memoriam) for participating in the initial stages of this study, and to Antoine Fouquet for loaning the tadpole of *Dendrobates tinctorius* from French Guiana. Miquéias Ferrão received a fellowship from the David Rockefeller Center for Latin American Studies of Harvard University and from GEF Pró-Espécies, and a postdoctoral fellowship (PDPG) from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; Proc. 88887.927982/2023-00). Pedro H. S. Dias thanks Marie Skłodowska-Curie Actions (MSCA-IF-2020, MEGAN; 101030742) and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP Procs. 2013/20420-4 and 2015/11239-0) for supporting this study. Igor L. Kaefer (Proc. 310078/2022-9) and Carlos E. Costa-Campos (Proc. 307697/2022-3) thank Brazilian Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for productivity grants. Anthony S. Ferreira thanks CNPq for the PDJ scholarship (Proc. 151729/2022-0).

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.
- Avila-Pires, T.C.S., Hoogmoed, M.S. & Rocha, W.A. (2010) Notes on the vertebrates of northern Pará, Brazil: a forgotten part of the Guianan Region, I. Herpetofauna. *Boletim do Museu Paraense Emílio Goeldi Ciências Naturais*, 5, 13–112. <https://doi.org/10.46357/bcnaturais.v5i1.647>
- Born, M.G. (1994) *The poison arrow frog Dendrobates tinctorius in the tropical rainforest of French Guiana, microhabitat and feeding ecology*. Unpublished M.Sc. Dissertation. Forest Ecology and Forest Management Group, Center for Ecosystem Studies, Wageningen University, Wageningen. [unknown pagination]
- Born, M.G., Bongers, F., Poelman, E.H. & Sterck, F.J. (2010) Dry-season retreat and dietary shift of the dart-poison frog *Dendrobates tinctorius* (Anura: Dendrobatidae). *Phyllomedusa*, 9, 37–52. <https://doi.org/10.11606/issn.2316-9079.v9i1p37-52>
- Caldwell, J.P. & Araújo, M.C. (2004) Historical and ecological factors influence survivorship in two clades of phytotelma-breeding frogs (Anura: Bufonidae, Dendrobatidae). *Miscellaneous Publications Museum of Zoology University of Michigan*, 193, 11–21.
- Dias, P.H.S., Araujo-Vieira, K., Carvalho-e-Silva, A.M.P.T. & Orrico, V.D. (2019) Larval anatomy of *Dendropsophus decipiens* (A. Lutz 1925) (Anura: Hylidae: Dendropsophini) with considerations to larvae of this genus. *PlosOne*, 14, e0219716. <https://doi.org/10.1371/journal.pone.0219716>
- Dias, P.H.S. & Pie, M.R. (2021) Buccopharyngeal morphology of the tadpoles of *Scinax v-signatus*, with comments on larval characters of the *S. perpusillus* species group (Amphibia: Anura: Hylidae). *Zootaxa*, 4694 (1), 195–200. <https://doi.org/10.11646/zootaxa.4964.1.12>
- Duarte-Marín, S., González-Acosta, C.C., Santos Dias, P.H., Arias-Álvarez, G.A. & Vargas-Salinas, F. (2020) Advertisement call, tadpole morphology, and other natural history aspects of the threatened poison frog *Andinobates daleswansonii* (Dendrobatidae). *Journal of Natural History*, 54, 3005–3030. <https://doi.org/10.1080/00222933.2021.1889068>
- Frost, D.R. (2023) Amphibian species of the world: an online reference. Version 6.0. Available from: <http://research.amnh.org/herpetology/amphibia/index.html> (accessed 3 March 2023)

- Gosner, K.L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16, 183–190.
- Grant, T., Rada, M., Anganoy-Criollo, M., Batista, A., Dias, P.H., Jeckel, A.M., Machado, D.J. & Rueda-Almonacid, J.V. (2017) Phylogenetic systematics of dart-poison frogs and their relatives revisited (Anura: Dendrobatoidea). *South American Journal of Herpetology*, 12, 1–90.
<https://doi.org/10.2994/SAJH-D-17-00017.1>
- Haas, A. (2003) Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura). *Cladistics*, 19, 23–89.
[https://doi.org/10.1016/S0748-3007\(03\)00006-9](https://doi.org/10.1016/S0748-3007(03)00006-9)
- Hoogmoed, M.S. (1969) Notes on the herpetofauna of Surinam III. A new species of *Dendrobates* (Amphibia Salientia, Dendrobatidae). *Zoologische Mededelingen, Leiden*, 44, 133–141.
- Kaplan, M. (1997) A new species of *Colostethus* from the Sierra Nevada de Santa Marta (Colombia) with comments on intergeneric relationships within Dendrobatidae. *Journal of Herpetology*, 31, 369–373.
<https://doi.org/10.2307/1565665>
- Lannoo, M.J., Townsed, D.S. & Wassersug, R.J. (1987) Larval life in the leaves: arboreal tadpoles types, with special attention to the morphology, ecology, and behavior of oophagous *Osteopilus brunneus* (Hylidae) larvae. *Fieldiana*, 38, 1–31.
<https://doi.org/10.5962/bhl.title.2932>
- Lavilla, E.O. & Scrocchi, G.J. (1986) Mofometría larval de los géneros de Telmatobiinae (Anura: Leptodactylidae) de Argentina y Chile. *Physis*, 44, 39–43.
- Lehtinen, R.M., Lannoo, M.J. & Wassersug, R.J. (2004) Phytotelm-breeding anurans: past, present and future research. *Miscellaneous Publications. Museum of Zoology, University of Michigan*, 193, 1–9.
- Lescure, J. (1984) Las larvas de Dendrobatidae. *Reunión Iberoamericana de Conservación y Zoología de Vertebrados, Actas II*, 1984, 37–45.
- Lins, A.C., De Magalhaes, R.F., Costa, R.N., Brandão, R.A., Py-Daniel, T.R., Miranda, N.E., Maciel, N.M., Nomura, F. & Pezzuti, T.L. (2018) The larvae of two species of *Bokermannohyla* (Anura, Hylidae, Cophomantini) endemic to the highlands of central Brazil. *Zootaxa*, 4527 (4), 501–20.
<https://doi.org/10.11646/zootaxa.4527.4.3>
- Masurat, G. & Wolf-Rudiger, G. (1991) *Lurche. Vermehrung von Terrarientieren*. Urania-Verlag, Leipzig, 164 pp.
- Rojas, B. & Pašukonis, A. (2019) From habitat use to social behavior: natural history of a voiceless poison frog, *Dendrobates tinctorius*. *PeerJ*, 7, e7648.
<https://doi.org/10.7717/peerj.7648>
- Satel, S.L. & Wassersug, R.J. (1981) On the relative size of buccal floor depressor and elevator musculature in tadpoles. *Copeia*, 1981, 129–137.
<https://doi.org/10.2307/1444047>
- Silverstone, P.A. (1975) A revision of the poison-arrow frogs of the genus *Dendrobates* Wagler. *Natural History Museum of Los Angeles County Science Bulletin*, 21, 1–55.
- Taucce, P.P., Costa-Campos, C.E., Carvalho, T.R. & Michalski, F. (2022) Anurans (Amphibia: Anura) of the Brazilian state of Amapá, eastern Amazonia: species diversity and knowledge gaps. *European Journal of Taxonomy*, 836, 96–130.
<https://doi.org/10.5852/ejt.2022.836.1919>
- Wollenberg, K.C., Lötters, S., Mora-Ferrer, C. & Veith, M. (2008) Disentangling composite colour patterns in a poison frog species. *Biological Journal of the Linnean Society*, 93, 433–444.
<https://doi.org/10.1111/j.1095-8312.2007.00906.x>