A morphological and molecular study of *Hydrodynastes gigas* (Serpentes, Dipsadidae), a widespread species from South America

Priscila S Carvalho Correspond., 1, 2, Hussam Zaher 3, Nelson J da Silva Jr. 4, Diego J Santana 1

1 Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil
2 Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista, São José do Rio preto, São Paulo, Brazil
3 Museu de Zoologia da Universidade de São Paulo, São Paulo, São Paulo, Brazil
4 Escola de Ciências Médicas, Farmacêuticas e Biomédicas, Pontifícia Universidade Católica de Goiás, Goiânia, Goiás, Brazil

Corresponding Author: Priscila S Carvalho
Email address: pricarvalho.bio@gmail.com

**Background.** Studies with integrative approaches (based on different lines of evidence) are fundamental for understanding the diversity of organisms. Different data sources can improve the understanding of the taxonomy and evolution of snakes. We used this integrative approach to verify the taxonomic status of *Hydrodynastes gigas* (Duméril, Bibron & Duméril, 1854), given its wide distribution throughout South America, including the validity of the recently described *Hydrodynastes melanogigas* Franco, Fernandes & Bentim, 2007. **Methods.** We performed a phylogenetic analysis of Bayesian Inference with mtDNA 16S and Cytb, and nuDNA Cmos and NT3 concatenated (1902 bp). In addition, we performed traditional morphometric analyses, meristic, hemipenis morphology and coloration pattern of *H. gigas* and *H. melanogigas*. **Results.** According to molecular and morphological characters, *H. gigas* is widely distributed throughout South America. We found no evidence to support that *H. gigas* and *H. melanogigas* species are distinct lineages, therefore, *H. melanogigas* is a junior synonym of *H. gigas*. Thus, the melanic pattern of *H. melanogigas* is the result of a polymorphism of *H. gigas*. Melanic populations of *H. gigas* can be found in the Tocantins-Araguaia basin.
A morphological and molecular study of *Hydrodynastes gigas* (Serpentes, Dipsadidae), a widespread species from South America

Priscila Santos Carvalho\(^1\), Hussam Zaher\(^3\), Nelson Jorge da Silva Jr.\(^4\), Diego José Santana\(^1\)

\(^1\)Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brasil.
\(^2\)Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista, São José do Rio Preto, SP, Brasil.
\(^3\)Museu de Zoologia da Universidade de São Paulo, São Paulo, SP, Brasil.
\(^4\)Escola de Ciências Médicas, Farmacêuticas e Biomédicas, Pontifícia Universidade Católica de Goiás, Goiânia, GO, Brasil.

Corresponding Author: Priscila Santos Carvalho
Av. Costa e Silva, S/N, Campo Grande, MS, 79070–900, Brasil.
Email address: pricarvalho.bio@gmail.com

Abstract

**Background.** Studies with integrative approaches (based on different lines of evidence) are fundamental for understanding the diversity of organisms. Different data sources can improve the understanding of the taxonomy and evolution of snakes. We used this integrative approach to verify the taxonomic status of *Hydrodynastes gigas* (Duméril, Bibron & Duméril, 1854), given its wide distribution throughout South America, including the validity of the recently described *Hydrodynastes melanogigas* Franco, Fernandes & Bentim, 2007.

**Methods.** We performed a phylogenetic analysis of Bayesian Inference with mtDNA 16S and Cytb, and nuDNA Cmos and NT3 concatenated (1902 bp). In addition, we performed traditional morphometric analyses, meristic, hemipenis morphology and coloration pattern of *H. gigas* and *H. melanogigas*.

**Results.** According to molecular and morphological characters, *H. gigas* is widely distributed throughout South America. We found no evidence to support that *H. gigas* and *H. melanogigas* species are distinct lineages, therefore, *H. melanogigas* is a junior synonym of *H. gigas*. Thus, the melanic pattern of *H. melanogigas* is the result of a polymorphism of *H. gigas*. Melanic populations of *H. gigas* can be found in the Tocantins-Araguaia basin.

Introduction

Species are considered lineages with distinct evolutionary histories (de Queiroz, 2007). Taxonomic studies are traditionally based on morphological descriptors to delimit species (e.g. ...
Franco et al. 2017; França et al. 2018; Meneses-Pelayo & Passos 2019). However, in many cases, species are difficult to delimit due to the limited number of morphological differences or the absence of them, preventing the recognition of valid cryptic species (Bickford et al., 2007). Morphology alone might result in more than one name being assigned to individuals belonging to the same evolutionary lineage (i.e. species) (Passos & Prudente, 2012; Passos, Martins & Pinto-Coelho, Mângia et al. 2020). Many species are described based solely on morphological patterns, which could merely reflect interpopulational variation, instead of evidence of lineage separation (e.g. Brusquetti et al., 2014; Mângia et al., 2020).

Currently, species delimitation must be based on the integration of more than one type of data set (e.g., DNA sequences, morphology, behavior, pheromone), which helps improve taxonomic understanding (Dayrat, 2005; Padial & De la Riva 2010; Padial et al., 2010; Pante, Schoelinck & Puillandre, 2014). An integrative approach contributes to taxonomic, phylogenetic and phylogeographic studies (Pante, Schoelinck & Puillandre, 2014), being thus useful in delimiting species and sorting possible interspecific variations and lineages with similar morphologies. Ultimately, integrative approaches are essential for testing taxonomic schemes and correcting nomenclatural inconsistencies (e.g., Recoder et al. 2014; Ruane et al. 2018; Mângia et al., 2020).

Among the morphological characteristics adopted in taxonomic studies that usually result in inaccurate nomenclatural decisions are coloration pattern variations (e.g. *Atractus* spp.: Passos, Martins & Pinto-Coelho, 2016; *Apostolepis* spp.: Entiauspe-Neto et al., 2019). Animal coloration has several adaptive functions, including thermoregulation, signaling and protection (Briolat et al., 2019). Variation in coloration patterns (polychromatism), often associated with ontogenetic dimorphism (e.g., *Corallus* spp.: Henderson, 1997; Henderson, Passos & Feitosa 2009; and *Drymoluber* spp.: Costa, Moura & Feio, 2013) or with chromatic anomalies such as leukism, albinism and melanism (the latter caused by the increase of epidermal pigments known as melanin; Kettlewell, 1973) can lead to erroneous nomenclatural decisions. Although there are some exclusively melanistic species (e.g., some species of the Pseudoboini tribe), melanism can also be the result of intraspecific polymorphism (e.g., Bernardo et al., 2012). The adaptive value of melanism may be related to predation, protection or thermoregulation (Andrén & Nilson, 1981; Forsman & Ås, 1987; Capula, Luiselli & Monney 1995; Briolat et al., 2019).

*Hydrodynastes* Fitzinger, 1843 is a genus of large semiaquatic snakes, which currently contains three species: *Hydrodynastes bicinctus* (Hermann, 1804) distributed in Colombia, Venezuela, French Guiana, Guyana, Suriname and Brazil (Murta-Fonseca, Franco & Fernandes, 2015); *Hydrodynastes gigas* (Duméril, Bibron & Duméril, 1854) distributed in French Guiana, Bolivia, Paraguay, Argentina and Brazil (Giraudo & Scrocchi, 2002; Pereira-Filho & Montingelli, 2006; Wallach, Williams & Boundy, 2014; Nogueira et al., 2019); and *Hydrodynastes melanogigas* Franco, Fernandes & Bentim, 2007 recorded only in the Tocantins-Araguaia Basin in the states of Tocantins, Mato Grosso and Maranhão, Brazil (Silva Jr. et al., 2012; Santos Jr. et al., 2017). *Hydrodynastes gigas* (Duméril, Bibron & Duméril, 1854) has a wide distribution throughout Brazil, and has been recorded in the states of Amapá, Amazonas, Pará, Rondônia, Roraima,
Tocantins, Maranhão, Piauí, Rio Grande do Norte, Mato Grosso, Mato Grosso do Sul, Minas Gerais, São Paulo, Paraná, Rio Grande do Sul (Nogueira et al., 2019). Given the wide distribution of *Hydrodynastes gigas* in South America, a level of intraspecific variation throughout its populations is to be expected, with some potential to represent still undescribed cryptic species. Indeed, Franco, Fernandes & Bentim (2007) considered one of these populations as a distinct species, describing *H. melanogigas* mainly through its differential color pattern and pointed out its similarity with *H. gigas* on meristic and hemipenial characteristics. Therefore, the distinction between *H. melanogigas* and *H. gigas* rests mainly on its melanistic color pattern and on its inferred allopatric distribution with *H. gigas*. The present study aims to evaluate the taxonomic validity of *Hydrodynastes gigas* and *Hydrodynastes melanogigas* using an integrative taxonomic approach inferred by molecular and morphological data.

**Materials & Methods**

We evaluated the taxonomic status of *Hydrodynastes gigas* and *Hydrodynastes melanogigas* by sequencing two mitochondrial and two nuclear genes for 32 individuals belonging to the two species. We further analyzed the external morphology of 186 specimens of *H. gigas* and *H. melanogigas*.

**Molecular analysis**

We extracted the DNA from muscle, liver or scale of 27 samples of *Hydrodynastes gigas*, five of *H. melanogigas* and 12 of *H. bicinctus*. Samples of *Hydrodynastes bicinctus* were added to our analysis in order to provide a complete species representation for the genus. We used the phenol-chloroform extraction protocol (Sambrook, Fritsch & Maniatis, 1989) (Fig.1a). We amplified the partial sequences of the mitochondrial 16S ribosomal (mtDNA) genes (16S rRNA, 326 pb) (Palumbi et al., 2002), Cytochrome b (Cytb, 618 pb) (Pook, Wüster & Thorpe, 2000), the nuclear genes (nuDNA) Oocyte maturation factor Mos (Cmos, 478 pb) (Lawson et al., 2005) and Neurotrophin-3 (NT3, 480 pb) (Townsend et al., 2008) using the standard Polymerase Chain Reaction (PCR) technique as described by Pook, Wüster & Thorpe, 2000 and Moraes-Da-Silva et al. (2019). We visually checked all nucleotide sequences and aligned the concatenated genes (1902 pb) using the Muscle algorithm (Edgar, 2004) in the Geneious v.9.1.8 program. We used the species *Pseudoboa nigra* and *Xenopholis scalaris* as outgroup of *Hydrodynastes* (Vidal, Dewynter & Gower, 2010; Zaher et al., 2019), and rooted the tree in *Xenopholis*. We used the sequences available in GenBank and deposited those generated in this study into the same database (Table 1).

We used PartitionFinder 2 to identify partitioning schemes and the most appropriate nucleotide replacement models (Lanfear et al., 2016). According to our concatenated alignment, we found five partitions evaluated by BIC (Table 2). For phylogenetic analysis, we used the Bayesian inference implemented in MrBayes v3.2.6 (Ronquist & Huelsenbeck, 2003) using the substitution models generated by PartitionFinder. We ran two independent runs of four Markov
chains for 20 million generations sampling every 5,000 generations and discarding 25% as burn-in. We evaluated the stability of the analysis in Tracer v1.6, ensuring that all ESS values were above 200 (Rambaut et al., 2014). We calculated the divergence between sequences (distance $p$) in Mega v10.0.5 (Tamura et al., 2013).

**Morphological analysis**

We examined 144 specimens of *Hydrodynastes gigas* and 42 specimens of *H. melanogigas* (Fig. 1b). Museum acronyms follow Sabaj Pérez (2014), except for Coleção Herpetológica da Universidade Federal da Paraíba (CHUFPB), João Pessoa, PB; Coleção Zoológica Delta do Parnaiba (CZDP), Parnaiba, PI; Universidade Luterana do Brasil (MZCEULP), Palmas, TO. The specimens examined are listed in the Appendix 1.

Franco, Fernandes & Bentim (2007) described *Hydrodynastes melanogigas* based on 17 specimens collected in the municipalities of Palmas (type locality), Porto Nacional, and Lajeado, which are all located in the state of Tocantins, Brazil. Unfortunately, most of the type series was lost in the 2010 fire that occurred at the Instituto Butantan. Currently, only three individuals remain from the type series: two at the proper Institute in São Paulo (paratypes IBSP 65978 and IBSP 66387) and one at the Museu Nacional do Rio de Janeiro (paratype MNRJ 15101). From the remaining type-series, we analyzed all the remaining individuals.

We examined 14 meristic characters (Table 3) and eight morphometric ones (Table 4), in addition to the coloration pattern and morphology of the hemipenis. Sex was determined by the presence or absence of hemipenes through a ventral incision at the base of the tail. We measured individuals with an electronic caliper (0.01 mm) and a flexible ruler (1 mm), on their right side whenever possible. In order to test morphometric differences between *H. gigas* and *H. melanogigas*, we conducted a principal component analysis (PCA) and took the first two principal components of the ordination to create a MANOVA. We ran this analysis with adult males and females separately, and performed the analysis in R software (R Core Team 2014) using the package Vegan (Oksanen 2007). We followed the terminology of Dowling (1951) for counting the ventral scales, and Peters (1964) and Vanzolini, Ramos-Costa & Vitt (1980) for pholidosis. We surveyed the geographic coordinates of the data catalogs of zoological collections using Google Earth software.

**Hemipenial morphology**

We prepared a hemipenis from a topotype of *Hydrodynastes melanogigas* and 19 from *H. gigas* from different localities in the Amazon, East Brazil and La Plata hydrobasins (Appendix 1). Whenever possible, we prepared the hemipenes on the right side according to the technique originally described by Manzani & Abe (1988), as modified by Pesantes (1994), Zaher (1999), and Zaher & Prudente (2003). We stained the external calcareous structures with alizarin red, as suggested by Nunes et al. (2012), for a better visualization of microstructures in the surface of the organ. Terminology follows Dowling & Savage (1960), Zaher (1999) and Myers & Cadle (2003).
Results

Molecular approach

We recovered the genus *Hydrodynastes* as monophyletic, and the topology of the concatenated gene tree showed two strongly supported clades with posterior probability (pp = 1.00). Our concatenated dataset tree grouped *Hydrodynastes melanogigas* within *H. gigas* (Fig. 2). The uncorrected p-distance for both the mtDNA 16S and Cytb showed low genetic differences (0.01% and 0.2%, respectively) between the lineages of *H. gigas* and *H. melanogigas*. However, the genetic differences between *H. gigas* and *H. bicinctus* were 0.43% for 16S and 13% for Cytb (Table 5). Intraspecific variation in *H. gigas* was 0.0% to 0.04% for 16S and 0.0% to 0.17% for Cytb, while in *H. bicinctus* it was 0.0% for 16S and 0.0% to 0.23% for Cytb (Supplementary Material).

Morphological approach

We found overlap in all meristic and morphometric characteristics between *Hydrodynastes gigas* and *H. melanogigas* (Table 3 and 4). The first principal components from both PCA analysis (males and females) recovered 99% of variation, and through the MANOVA of males (F = 2.2949; p = 0.1109) and females (F = 0.3463; p = 0.7095) we found no significant morphometric differences between both species (Fig. 3). In addition, we observed gradient levels of melanism in *H. melanogigas* (Figs. 4a-f). We examined fully melanic specimens (Fig. 4a) to specimens with clear visible bands along the body (Fig. 4f). We also observed that some *H. gigas* individuals from the Amazon, La Plata and Tocantins-Araguaia basins present darker coloration overlapping the gradient of melanism found in *H. melanogigas* (Figs. 5a-f). We did not find any morphological characteristics that differentiate the two species.

We did not observe coloration patterns within or between the populations of *Hydrodynastes gigas* (Figs. 6a-f). We observed ontogenetic variation in the color pattern of all populations analyzed, with no distinction between males and females. Juveniles in the early stages of life have well-defined rounded dark spots all over their backs to the end of their tails; these spots are outlined by a lighter line, while in adults rounded spots may or may not be well defined, and may not present a clear outline (Figs. 7a-e). Furthermore, we identified two young males of *H. gigas* (CHUNB 22053, Figs.7e; CHUNB 22068) from the type locality of *H. melanogigas*, as well as 18 more specimens from the Tocantins-Araguaia Basin. We provide more details in the “variation” section below.

Hemipenis morphology (Figs. 8a-j): When fully everted and expanded, hemipenes of *H. melanogigas* and *H. gigas* are undistinguishable (Figs. 8k-l). The hemipenis is deeply bilobed, semicaliculate and semicapitate, with three or four vertical rows of large spines arranged on each side of the body. The body of the hemipenis is covered by spikes on the sulcate and assulcate faces. The sulcus spermaticus bifurcates in the proximal region of the hemipenis body and each branch extends centrolinearly until it reaches the proximal region of the lobes, in which they follow a centrifugal position that ends at the lateral region of the tip of each. The capitulum, formed by papillate calyces and spikes, extends over most of the surface of the lobes, except in the region of the assulcate face that is occupied by two parallel rows of papillated and
conspicuous body calyces that extend to the distal region of the hemipenis body, where they converge on the lobular crest and continues to the middle portion of the hemipenis. We detected low intraspecific variation among *Hydrodynastes gigas* populations. Some hemipenes showed little size variation in lobes and body calyces, varying from slightly visible to conspicuous in the hemipenial body.

**Discussion**

*Hydrodynastes gigas* is widely distributed throughout South America, occurring with low genetic variability throughout most of its extension range. Although the genetic structure of widely distributed species can be easily influenced by natural barriers (Patton, da Silva & Malcolm, 1994; Pellegrino et al., 2005; Rocha et al., 2015), this clearly is not the case for the genus *Hydrodynastes* (see Murta-Fonseca, Franco & Fernandes, 2015).

Here, we used an integrative taxonomic approach and adopted the species concept of one lineage with distinct evolutionary histories (de Queiroz, 2007), to test the taxonomic validity of *Hydrodynastes melanogigas*. Our results did not separate *H. gigas* and *H. melanogigas* based on molecular, meristic, morphometric and hemipenial characters. The hemipenis of *Hydrodynastes melanogigas* analyzed showed no differences from the hemipenis of *H. gigas* (n=19). In their description of *H. melanogigas*, Franco, Fernandes & Bentim (2007) also pointed out its similarity with *H. gigas* based on meristic characters and hemipenis morphology. The only superficial distinction that remains between these two taxa is the presence of melanism in the latter. Geographic or regional melanism has already been reported for several populations of Squamata (Pearse & Pogson, 2000; King, 2003; Bernardo et al., 2012). In addition, polychromatism can be a bias within taxonomy, if the revision and/or description of species does not take into account the organisms throughout their whole distribution (Bernardo et al. 2012; Ruane et al. 2018; Mângia et al. 2020). In fact, Franco, Fernandes & Bentim (2007) carried out an analysis covering almost the entire distribution of *H. gigas*, however we found degrees of melanism in some populations that were not identified by other authors. The variation of melanism found in *H. gigas* and the melanistic gradient observed in *H. melanogigas* (Figs. 4 and 5), along with genetic support, suggests that *H. melanogigas* is not a distinct species but rather a melanistic population of *H. gigas*. The distribution of *Hydrodynastes melanogigas* without sympatry with *H. gigas* in the Tocantins-Araguaia basin was an important factor for its description (Franco, Fernandes & Bentim, 2007). However, in this study, we analyzed two juveniles of *H. gigas* from the type locality of *H. melanogigas* (CHUNB 22053, Fig.7e; CHUNB 22068). All specimens analyzed by Franco, Fernandes & Bentim (2007) and herein were adults or juveniles and no neonates were observed. Therefore, we do not know whether the specimens considered as *H. melanogigas* could have been born melanic or if melanism occurs during their ontogeny. Still, some studies suggest that thermal melanism is associated with latitude and high altitudes, i.e., relatively cold environments (Capula, Luiselli & Monney, 1995), which does not agree with the present case. More studies are needed to confirm the adaptive meaning of...
melanism through studies of thermal biology. Therefore, due to the lack of any characteristic that sustains these two taxa as distinct species and their low genetic distance (0.04% 16S and 0.2% Cytb), we consider *H. melanogigas* Franco, Fernandez & Bentim, 2007 as a junior synonym of *H. gigas* (Duméril, Bibron & Duméril, 1854).

Systematic account

*Hydrodynastes gigas* (Duméril, Bibron & Duméril, 1854)

*Xenodon gigas* Duméril, Bibron & Duméril, 1854. Erpétologie générale vol. 7: 761.

*Lejosophis gigas* Jan, 1863. Elenco Sistematico degli Ofidi descriti e disegnati per l'Iconografia Generale. vol. 2: 56.

*Cyclagras gigas* Cope, 1885. Proceeding of the American Philosophical Society: 185.

*Cyclagras gigas* Boulenger, 1894. Catalogue of the snakes in the British Museum vol. 2: 144.

*Lejosophis gigas* Dunn, 1944. Caldasia: 69.

*Lejosophis gigas* Hoge, 1958. Papéis Avulsos de Zoologia: 222.

*Hydrodynastes gigas* Hoge, 1966. Ciência e Cultura: 143.

*Cyclagras gigas* Peters & Orejas-Miranda 1970. Catalogue of the Neotropical Squamata. Part I: 78.

*Hydrodynastes gigas* Dowling & Gibson, 1970. Herpetological Review (2): 38

*Hydrodynastes melanogigas* Franco, Fernandes & Bentim, 2007 Zootaxa (1613): 58. New Synonymy

Type material: syntype MNHN 3623

Type locality: Corrientes Province, Argentina.

Comments on the type series: Duméril, Bibron and Duméril’s (1854) description of *Xenodon gigas* did not refer to any voucher specimen. The authors only cited that M. A. d’Orbigny collected three individuals in Rio de La Plata, Corrientes Province, Argentina (without further information). The authors also mentioned a plate (Xénodon géant. Atlas, pi. 76, fig-5), which only presents a cranial picture. Overall, Wallach, Williams & Boundy (2014, p. 339) indicate that the types would be MNHN 2493 a-c, but according to Uetz et al., (2019), it would be an individual labeled MNHN 3623. Due to this conflicting information, we contacted the curator of the Herpetological Collection at the Muséum National d'Histoire Naturelle de Paris (Dr. N. Vidal) who confirmed that there is only one type specimen, a skin labeled MNHN 3623 (Fig. 9), deposited in the collection, and that the other two specimens appear to be lost. Since Duméril, Bibron & Duméril (1854) did not select any specific specimen from their type series, we therefore, designate the specimen MNHN 3623 as the lectotype of *Hydrodynastes gigas*.

Description of the lectotype MNHN 3623 (Fig. 9): Adult of undetermined sex; SVL 1570mm; TL 540mm; HL 62mm; HW 35mm; DN 10mm; two internasals; nasal divided; one loreal; one preocular; three suboculars; two postoculars; temporal 2+2/2+2; nine supralabials, none contacting the orbit; eleven infralabials, first to sixth contacting chin shields on the right side and...
first to fifth on the left side; two pairs of chin shields; dorsal 19/16/15 scales, smooth; two apical pits; ventral 153; subcaudals 74, paired and cloacal scale single.

**Color of the preserved lectotype (ethanol 70%)** (Fig. 9): Head brown with black ‘U’ shaped spot at the end of the parietal scale; post-ocular stripe that extends longitudinally on each side; supralabial brown with the last four scales stained black; infralabials and chin shields cream; dorsum of body brown with dark rounded spots that extend to the end of the tail; ventral body cream with three black stripes to the middle of the body.

**Diagnosis:** *Hydrodynastes gigas* is distinguished from its congener *H. bicinctus* by the following combination of characters: dorsal scales normally 19/19/15; ventral scales in males 150–168 and in females 152–172; subcaudal scales in males 58–88 and in females 49–84; maxillary teeth 15–17; two apical pits in the dorsal scales; post-ocular stripe that extends longitudinally (on each side); ventral body with three lines of continuous spots up to the middle of the body.

**Variation:** All variation in morphometric and meristic data are presented in Tables 3 and 4.

Regarding coloration patterns, a considerable degree of color variation can be found in *H. gigas* (Figs. 4a-f; Figs. 5a-f; Figs. 6a-f and Figs. 7a-e) dorsum ranging from yellow to dark brown or completely dark in melanic populations; rounded spots on the dorsum may vary in shape and size, in some individuals they may be hollow or filled; in neonates the dark rounded spots are well defined throughout the dorsum until the end of the tail, and these spots are outlined by a lighter line; darks spot in the shape of ‘V’ or ‘U’ at the end of the parietal scale, only visible in non-melanic populations; ventral body cream/brown with three black stripes that usually go up to the middle of the body, rarely to the cloaca, some individuals do not have these stripes, in melanic populations the belly is dark without spots or when present these spots are continuous on the sides.

**Distribution:** *Hydrodynastes gigas* is widely distributed throughout South America, east of the Andes, occurring in Amazon, East Brazil, La Plata, North Brazil, Northeast South America, Parnaiba and Tocantins-Araguaia hydrobasins (Fig. 10).

**Conclusions**

Our results did not separate *H. gigas* and *H. melanogigas* based on molecular, meristic, morphometric and hemipenial characters. Therefore, the melanic pattern of *Hydrodynastes melanogigas* is characterized here as the result of polymorphism within *H. gigas*. Although our integrative approach helped elucidate the taxonomic status of *H. melanogigas*, we believe future, multi-loci phylogeographic studies are needed in order to better understand the evolutionary history of the populations belonging to the two remaining species *H. gigas* and *H. bicinctus*.

**Acknowledgements**

We are grateful to G. Puorto and F. Grazziotin (IBSP), G. Colli (CHUNB), D. Mesquita and G. Vieira (CHUFPB), J. Lima (IEPA), P. Manzani (ZUEC), G. Graccioli (ZUFMS), S. Cechin
(ZUFSM), C. Previero (MZCEULP), A. Guzzi (CZDP), F. Resende (FUNED), R. Oliveira (MCN), G. Pontes (MCP), JC. Leite (MNHCI), A. Prudente (MPEG), F. Curcio (UFMT), P. Passos (MNRJ), and N. Vidal (MNHN) for allowing access or loaning specimens under their care. We are indebted to Ana Bottallo Quadros for verifying and photographing the type specimen at the MNHN. We also thank Diego Cavalheri and Roberta Murta-Fonseca for verifying and taking photos from *H. melanogigas* type series specimens at the IBSP and MNRJ.

References

Andrén, C., & Nilson, G. 1981. Reproductive success and risk of predation in normal and melanistic colour morphs of the adder, *Vipera berus*. Biological Journal of the Linnean Society. 15(3): 235–246.

Bernardo, P.H., Machado, F.A., Murphy, R.W., & Zaher, H. 2012. Redescription and morphological variation of *Oxyrhopus clathratus* Duméril, Bibron and Duméril, 1854 (Serpentes: Dipsadidae: Xenodontinae). South American Journal of Herpetology. 7(2): 134–149. doi:10.2994/057.007.0203.

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K., Meier, R., Winker, K., Ingram, K.K., & Das, I. 2007. Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution. 22(3): 148–155. doi:10.1016/j.tree.2006.11.004.

Boulenger, G.A. 1894. Catalogue of the Snakes in the British Museum (Natural History), Vol. II. Trustees of the British Museum, London.

Briolat, E.S., Burdfield-Steel, E.R., Paul, S.C., Rönkä, K.H., Seymoure, B.M., Stankowich, T., & Stuckert, A.M. 2019. Diversity in warning coloration: selective paradox or the norm?. Biological Reviews. 94(2): 388–414. doi:10.1111/brv.12460.

Brusquetti, F., Jansen, M., Barrio-Amorós, C., Segalla, M., & Haddad, C.F. 2014. Taxonomic review of *Scinax fuscomarginatus* (Lutz, 1925) and related species (Anura; Hylidae). Zoological Journal of the Linnean Society. 171(4): 783–821. doi:10.1111/zoj.12148.

Capula, M., Luiselli, L., & Monney, J.C. 1995. Correlates of melanism in a population of adders (*Vipera berus*) from the Swiss Alps and comparisons with other alpine populations. Amphibia-Reptilia. 16(4): 323–330. doi.org/10.1163/156853895X00406.

Cope, E.D. 1885. Twelfth contribution to the herpetology of tropical America. Proceedings of the American Philosophical Society. 22(118): 167–194.

Costa, H.C., Moura, M.R., & Feio, R.N. 2013. Taxonomic revision of *Drymoluber* Amaral, 1930 (Serpentes: Colubridae). Zootaxa. 3716(3): 349–394. doi.org/10.11646/zootaxa.3716.3.3.

Dayrat, B. 2005 Towards integrative taxonomy. Biological Journal of the Linnean society. 85(3): 407–415. doi.org/10.1111/j.1095-8312.2005.00503.x.

De Queiroz, K. 2007. Species concepts and species delimitation. Systematic Biology. 56(6): 879–886. doi.org/10.1080/10635150701701083.
Dowling, H.G., & Savage, J.M. 1960. A guide to the snake hemipenis: a survey of basic structure and systematic characteristics. Zoologica. 45(1): 17–28.

Dowling, H.G. 1951. A proposed standard system of counting ventrals in snakes. British Journal of Herpetology. 1: 97–99.

Dowling, H.G. 1967. Hemipenes and other characters in colubrid classification. Herpetologica. 23(2): 138–142.

Dowling, H.G., & Gibson, F.W. 1970. Relationship of the Neotropical snakes Hydrodynastes bicinctus and Cyclagras gigas. Herpetological Review. 2(2): 37–38.

Duméril, A.M.C., Bibron, G., & Duméril, A. 1854. Erpétologie Generale ou Histoire Naturelle Complete des Reptiles. Vol. VII. Librairie Encyclopédique de Roret, Paris.

Dunn, E.R. 1944. Dugandia, a new snake genus for the Coluber bicinctus Hermann. Caldasia. 3(11): 69–70.

Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32(5): 1792–1797. doi.org/10.1093/nar/gkh340.

Entiauspe-Neto, O.M., de Sena, A., Tiutenko, A., & Loebmann, D. 2019. Taxonomic status of Apostolepis barrioi Lema, 1978, with comments on the taxonomic instability of Apostolepis Cope, 1862 (Serpentes, Dipsadidae). ZooKeys. 841: 71–78. doi: 10.3897/zookeys.841.33404.

Forsman, A., & Ås, S. 1987. Maintenance of colour polymorphism in adder, Vipera berus, populations: a test of a popular hypothesis. Oikos. 50(1): 13–16.

França, D.P., Barbo, F.E., Silva-Junior, N.J., Silva, H.L., & Zaher, H. 2018. A new species of Apostolepis (Serpentes, Dipsadidae, Elapomorphini) from the Cerrado of Central Brazil. Zootaxa. 4521(4): 438–552. doi 10.11646/zootaxa.4521.4.3.

Franco, F.L., Trevine, V.C., Montingelli, G.G., & Zaher, H. 2017. A new species of Thamnodynastes from the open areas of central and northeastern Brazil (Serpentes: Dipsadidae: Tachymenini). Salamandra. 53(3): 339–350.

Giraudo, A.R., & Scrocchi, G.J. 2002. Argentinian Snakes: An annotated checklist. Smithsonian Herpetological Information Service. 132: 1–53.

Henderson, R.W. 1997. A taxonomic review of the Corallus hortulanus complex of Neotropical tree boas. Caribbean Journal of Science. 33: 198–221.

Henderson, R.W., Passos, P., & Feitosa, D. 2009. Geographic variation in the emerald treeboa, Corallus caninus (Squamata: Boidae). Copeia. 3: 572–582.

Hoge, A.R. 1966. Notes on Hydrodynastes [Serpentes – Colubridae]. Ciência e Cultura. 18(2): 143.

Hoge, A.R. 1958. Três notas sobre serpentes brasileiras. Papéis Avulsos do Departamento de Zoologia. Secretaria da Agricultura–São Paulo–Brasil. 3: 221–225.

Jan, G. 1863. Elenco sistematico degli Ofidi descritti e disegnati per l’Iconographia Generale. A. Lombardi, Milan.

Kettlewell, H.B.D. 1973. The evolution of melanism. Clarendon Press, Oxford.
King, R.B. 2003. Mendelian inheritance of melanism in the garter snake *Thamnophis sirtalis*. Herpetologica 59(4): 484–489. doi.org/10.1655/02-93.

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., & Calcott, B. 2016. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution. 34(3): 772–773. doi.org/10.1093/molbev/msw260.

Lawson, R., Slowinski, J.B., Crother, B.I., & Burbrink, F.T. 2005. Phylogeny of the Colubroidea (Serpentes): New evidence from mitochondrial and nuclear genes. Molecular Phylogenetics and Evolution. 37(2):581–601. doi:10.1016/j.ympev.2005.07.016.

Mângia, S., Oliveira, E.F., Santana, D.J., Koroiva, R., Paiva, F., & Garda, A.A. 2020. Revising the taxonomy of *Proceratophrys* Miranda- Ribeiro, 1920 (Anura: Odontophrynidae) from the Brazilian semiarid Caatinga: Morphology, calls and molecules support a single widespread species. Journal of Zoological Systematics and Evolutionary Research. doi:10.1111/jzs.12365.

Manzani, P.R., & Abe, A.S. 1988. Sobre dois novos métodos de preparo do hemipenis de serpentes. Memórias do Instituto Butantan. 50(1): 15–20.

Meneses-Pelayo, E., & Passos, P. 2019. New polychromatic species of *Atractus* (Serpentes: Dipsadidae) from the eastern portion of the Colombian Andes. Copeia. 107(2): 250–261. doi.org/10.1643/CH-18-163.

Moraes-Da-Silva, A., Amaro, R.C., Nunes, P.M.S., Strüssmann, C., Teixeira, M.J., Andrade, A.J., Sudré, V., Recoder, R., Rodrigues, M.T., & Curcio, F. F. 2019. Chance, luck and a fortunate finding: a new species of watersnake of the genus *Helicops* Wagler, 1828 (Serpentes: Xenodonta), from the Brazilian Pantanal wetlands. Zootaxa, 4651(3): 445–470. doi.org/10.11646/zootaxa.4651.3.3.

Murta-Fonseca, R.A., Franco, F.L., & Fernandes, D.S. 2015. Taxonomic status and morphological variation of *Hydrodynastes bicinctus* (Hermann, 1804) (Serpentes: Dipsadidae). Zootaxa. 4007(1): 63–81. doi.org/10.11646/zootaxa.4007.1.4

Myers, C.W., & Cadle, J.E. 2003. On the snake hemipenis, with notes on *Psomophis* and techniques of eversion: a response to Dowling. Herpetological Review. 34(4): 295–302.

Nogueira, C.C., Argôlo, A.J.S., Arzaendia, V., Azevedo, J.A., Barbo, F.E., Bénils, R.S., Bolochio, B.E., Borges-Martins, M., Brasil-Godinho, M., Braz, H., Buononato, M.A., Cisneros-Heredia, D.F., Colli, G.R., Costa, H.C., Franco, F.L., Giraudo, A., Gonzalez, R.C., Guedes, T., Hoogmoed, M.S., Marques, O.A.V., Montingelli, G.G., Passos, P., Prudente, A.L.C., Rivas, G.A., Sanchez, P.M., Serrano, F.C., Silva Jr. N.J., Strüssmann, C., Vieira-Alencar, J.P.S., Zaher, H., Sawaya, R.J., & Martins, M. 2019. Atlas of Brazilian snakes: verified point-locality maps to mitigate the Wallacean shortfall in a megadiverse snake fauna. South American Journal of Herpetology. 14(SI 1): 1–274. doi.org/10.2994/SAJH-D-19-00120.1

Nunes, P.M., Fouquet, A., Curcio, F.F., Kok, P.J., & Rodrigues, M.T. 2012. Cryptic species in *Iphisa elegans* Gray, 1851 (Squamata: Gymnophthalmidae) revealed by hemipenial morphology and molecular data. Zoological Journal of the Linnean Society. 166(2): 361–376. doi:10.1111/j.1096-3642.2012.00846.x.
Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M.H.H. 2007. Vegan: community ecology package, Department of Statistics and Mathematics, Vienna University of Economics and Business Administration. Vienna. Accessed at: 2016.11.20.

Padial, J.M., & De La Riva, I. 2010. A response to recent proposals for integrative taxonomy. Biological Journal of the Linnean Society. 101(3): 747–756. doi.org/10.1111/j.1095-8312.2010.01528.x.

Padial, J.M., Miralles, A., De la Riva, I., & Vences, M. 2010. The integrative future of taxonomy. Frontiers in zoology. 7(1): 1–14. doi.org/10.1186/1742-9994-7-16.

Palumbi, S., Martin, A., Romano, S., McMillan, W.O., Stice, L., & Grabowski, G. 2002. The simple fool’s guide to PCR, version 2.0. Honolulu, EUA.

Passos, P., and A. L. C. Prudente. 2012. Morphological variation, polymorphism, and taxonomy of the Atractus torquatus complex (Serpentes: Dipsadidae). Zootaxa. 3407(1): 1–21.

Passos, P., Martins, A., & Pinto-Coelho, D. 2016. Population morphological variation and natural history of Atractus potschi (Serpentes: Dipsadidae) in Northeastern Brazil. South American Journal of Herpetology. 11(3): 188–211. doi.org/10.2994/SAJH-D-16-00034.1.

Patton, J.L., Da Silva, M.N.F., & Malcolm, J.R. 1994. Gene genealogy and differentiation among arboreal spiny rats (Rodentia: Echimyidae) of the Amazon basin: a test of the riverine barrier hypothesis. Evolution. 48(4): 1314–1323. doi:10.1111/j.1558-5646.1994.tb05315.x.

Pearse, D.E., & Pogson, G.H. 2000. Parallel evolution of the melanic form of the California legless lizard, Anniella pulchra, inferred from mitochondrial DNA sequence variation. Evolution. 54(3): 1041–1046. doi.org/10.1111/j.0014-3820.2000.tb00104.x

Pellegrino, K., Rodrigues, M.T., Waite, A.N., Morando, M., Yassuda, Y.Y., & Sites, J.W. 2005. Phylogeography and species limits in the Gymnodactylus darwinii complex (Gekkonidae, Squamata): genetic structure coincides with river systems in the Brazilian Atlantic Forest. Biological Journal of the Linnean Society. 85(1): 13–26. doi.org/10.1111/j.1095-8312.2005.00472.x.

Pereira-Filho, G.A., & Montingelli, G.G. 2006. Geographic distribution Hydrodynastes gigas. Herpetological Review. 37: 497.

Pesantes, O.S. 1994. A method for preparing the hemipenis of preserved snakes. Journal of Herpetology. 28(1): 93–95. doi:10.2307/1564686.

Peters, J.A. 1964. Dictionary of Herpetology - a brief and meaningful definition of words and terms used in herpetology. New York Hafner Publishing Company.

Peters, J.A., & Orejas-Miranda, B. 1970. Catalogue of the Neotropical Squamata. Part I. Snakes. Bulletin United States National museum 1–347.

Pook, C.E., Wüster, W., & Thorpe, R.S. 2000. Historical biogeography of the western rattlesnake (Serpentes: Viperidae: Crotalus viridis), inferred from mitochondrial DNA sequence information. Molecular Phylogenetics and Evolution. 15(2): 269–282. doi.org/10.1006/mpev.1999.0756.
R Core Team. 2014. A language and environment for statistical computing. Vienna. Accessed at: 2016.11.20

Rambaut, A., Suchard, M.A., Xie, D. & Drummond, A.J. 2014. Tracer v1.6. – Disponível em http://beast.bio.ed.ac.uk/Tracer, acessado em 10 setembro de 2019.

Recoder, R.S., De Pinho Werneck, F., Teixeira Jr, M., Colli, G.R., Sites Jr, J.W., & Rodrigues, M.T. 2014. Geographic variation and systematic review of the lizard genus Vanzosa (Squamata, Gymnophthalmidae), with the description of a new species. Zoological Journal of the Linnean Society. 171(1): 206–225. doi: 10.1111/zoj.12128.

Rocha, R.G., Ferreira, E., Loss, A.C., Heller, R., Fonseca, C., & Costa, L.P. 2015. The Araguaia river as an important biogeographical divide for didelphid marsupials in central Brazil. Journal of Heredity. 106(5): 593–607. doi.org/10.1093/jhered/esv058.

Ronquist, F., & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 19(12): 1572–1574. doi:10.1093/bioinformatics/btg180.

Ruane, S., Myers, E.A., Lo, K., Yuen, S., Welt, R.S., Juman, M., Futterman, I., Nussbaum, R.A., Schneider, G., Burbrink, F.T & Raxworthy, C.J. 2018. Unrecognized species diversity and new insights into colour pattern polymorphism within the widespread Malagasy snake Mimophis (Serpentes: Lamprophiidae). Systematics and Biodiversity. 16(3): 229–244. doi.org/10.1080/14772000.2017.1375046.

Sabaj-Pérez, M.H. 2014. Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an Online Reference. Version 5.0. American Society of Ichthyologists and Herpetologists, Washington, DC. Available from: http://www.asih.org (accessed 24 November 2019).

Sambrook, J., Fritsch, E.F., & Maniatis, R. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York.

Santos Jr, A.P., Adams, G.B., Buhler, D., Ribeiro, S., & Carvalho, T.S. 2017. Distribution extension for Hydrodynastes melanogigas Franco, Fernandes & Bentim, 2007 (Serpentes: Dipsadidae: Xenodontinae) in the Araguaia-Tocantins basin, Brazilian Cerrado. Check List. 13(3): 2135–2136. doi:10.15560/13.3.2135.

Silva Jr, N.J., Hamdan, B., Tonial, I., Silva, H.L.R., and Cintra, C. (2012). Hydrodynastes melanogigas Franco, Fernandes and Bentim, 2007 (Squamata: Serpentes: Colubridae): range extension and new state record. Check List 8(4): 813–814. doi:10.15560/8.4.813.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution. 30(12): 2725–2729. doi.org/10.1093/molbev/msr197.

Townsend, T.M., Alegre, R.E., Kelley, S.T., Wiens, J.J., & Reeder, T.W. 2008. Rapid development of multiple nuclear loci for phylogenetic analysis using genomic resources: an example from squamate reptiles. Molecular Phylogenetics and Evolution. 47(1): 129–142. doi:10.1016/j.ympev.2008.01.008.

Uetz, P., Cherikh, S., Shea, G., Ineich, I., Campbell, P.D., Doronin, I.V., Rosado, J., Wynn, A., Tighe, K.A., Medlarmid, R., Lee, J.L., Köhler, G., Ellis, R., Doughty, P., Raxworthy, C.J.,
Scheinberg, L., Resetar, A., Sabaj, M., Schneider, G., Franzen, M., Glaw, F., Böhme, W.,
Schweiger, S., Gemel, R., Couper, P., Amey, A., Dondorp, E., Ofier, G., Meiri, S., & Wallach, V.
2019. A global catalog of primary reptile type specimens. Zootaxa. 4695(5): 438–450.
doi.org/10.11646/zootaxa.4695.5.2.
Vanzolini, P.E., Ramos-Costa, A.M.M., & Vitt, L.J. 1980. Répteis das caatingas. Academia
Brasileira de Ciências.
Vidal, N., Dewynter, M., & Gower, D.J. 2010. Dissecting the major American snake radiation: A
molecular phylogeny of the Dipsadidae Bonaparte (Serpentes, Caenophidia). Comptes Rendus
Biologies. 333(1): 48–55. doi:10.1016/j.crvi.2009.11.003.
Wallach, V., Williams, K.L., & Boundy, J. 2014. Snakes of World: A catalogue of living and
extinct species. CRC Press, London New York.
Zaher, H. 1999. Hemipenial morphology of the South American xenodontine snakes, with a
proposal for a monophyletic Xenodontinae and a reappraisal of colubroid hemipenes. Bulletin of
the American Museum of Natural History. 240: 1–168.
Zaher, H., & Prudente, A.L.C. 2003. Hemipenes of Siphlophis (Serpentes, Xenodontinae) and
techniques of hemipenial preparation in snakes: a response to Dowling. Herpetological Review.
34(4): 302–306.
Zaher, H., Murphy, R.W., Arredondo, J.C., Graboski, R., Machado-Filho, P.R., Mahlow, K.,
Montingelli, G.G., Quadros, A. B., Orlov, N.L., Wilkinson, M., Zhang, Y.P., & Grazziotin, F.G.
2019. Large-scale molecular phylogeny, morphology, divergence-time estimation, and the fossil
record of advanced caenophidian snakes (Squamata: Serpentes). PloS One, 14(5): 1–82.
doi.org/10.1371/journal.pone.0216148.
Figure 1

Distribution of analyzed data in this study.

Sample localities for (a) molecular and (b) morphology of *Hydrodynastes* analyzed in this study.
Figure 2

Concatenated tree (16S, Cytb, C-mos and NT3) of the genus *Hydrodynastes* recovered by Bayesian analysis in MrBayes.

Numbers near the nodes correspond to support values indicate by posterior probability (pp). Asterisks indicate samples identified as *H. melanogigas*. Photo credit: Karoline Ceron.
Figure 3

Results of a Principal Component Analysis (PCA) on the morphometric variables.

Males (a) and Females (b) of *Hydrodynastes gigas* and *H. melanogigas*. Black circle corresponds to *H. gigas* and red circle to *H. melanogigas*. SVL: snout-vent length; HL: head length; HW: head width; DN: distance between nostrils; EN: distance between eye and nostril; ED: eye diameter; HH: head height.
Figure 4

Dorsal and ventral view of the melanism gradient in *Hydrodynastes melanogigas*.

MZCEULP 1218 (a, b); MZCEULP 516 (c, d); MZCEULP 1046 (e, f); MZCEULP 1273 (g, h); MZCEULP 758 (i, j); MZCEULP 938 (k, l). All specimens from Tocantins-Araguaia basin. Scale of 50mm.
Figure 5

Dorsal and ventral view of the coloration gradient in *Hydrodynastes gigas*.

La Plata basin UFMT 9076 (a, b); Amazon basin CHUNB 15159 (c, d); Tocantins-Araguaia basin MPEG 18012 (e, f), MPEG 18071 (g, h), MPEG 18046 (i, j), MPEG 18070 (k, l). Scale of 50mm.
Figure 6

Dorsal and ventral view of the body and lateral view of the head in *Hydrodynastes gigas*.

Amazon basin MPEG 22652 (a, b, c), CHUNB 56729 (d, e, f), MPEG 18674 (g, h, i); East Brazil CHUFPB 4611 (j, k, l); La Plata Basin UFMT 026 (m, n, o), ZUFMS 2389 (p, q, r). Scale of 50mm for body view and scale of 20mm head view.
Manuscript to be reviewed
Figure 7

Dorsal and ventral view of the ontogenetic variation in *Hydrodynastes gigas*.

Amazon basin CHUNB 66534 (a, b); Parnaiba basin CZDP 077 (c, d); East Brazil CHUFPB 14837 (e, f); La Plata basin ZUFMS 1603 (g, h); Tocantins-Araguaia basin CHUNB 22053 (i, j).

Scale of 30mm.
Figure 8

Hemipenial morphology.

*Hydrodynastes gigas*: Amazon basin MZUSP 18572 (a) asulcate and (b) sulcate side. Northeast South America basin MPEG 25438 (c) asulcate and (d) sulcate side. La plata basin ZUFMS 1910 (e) asulcate and (f) sulcate side; MHNCI 4511 (g) asulcate and (h) sulcate side; UFSM 1937 (i) asulcate and (j) sulcate side. *Hydrodynastes melanogigas* Tocantins-Araguaia basin CHUNB 12802 (k) asulcate and (l) sulcate side. Scale of 10mm.
Figure 9

Dorsal and ventral views of the body and head.

Lectotype MNHN 3623 *Hydrodynastes gigas*. Credit: Hussam Zaher. Scale of 50mm for body view and scale of 10mm head view.
Figure 10

Geographic distribution of *Hydrodynastes gigas* in South America.

We compiled data from the literature, specimens and tissues analyzed.
Table 1 (on next page)

Voucher information and GenBank numbers.

Specimens used for the molecular analyses, including GenBank numbers for mitochondrial 16S and Cytb, nuclear Cmos and NT3 sequences. * data not available in the original references.
| Species               | Voucher        | Locality                                      | Genbank Accession number         | Reference |
|----------------------|----------------|-----------------------------------------------|----------------------------------|-----------|
| Hydrodynastes bicinctus | CHUNB52057   | Brazil, Maranhão, Carolina                    | MT192271 MT224977 MT328069 MT328103 | This study |
| Hydrodynastes bicinctus | CHUNB47129  | Brazil, Mato Grosso, Alta Floresta             | MT192270 MT224976 MT328070 MT328104 | This study |
| Hydrodynastes bicinctus | CHUNB63637  | Brazil, Mato Grosso, Nova Xavantina            | MT192274 MT224980 MT328072 MT328105 | This study |
| Hydrodynastes bicinctus | CHUNB17831  | Brazil, Mato Grosso, Itáuba                    | MT192276 MT224981 MT328073 MT328107 | This study |
| Hydrodynastes bicinctus | UFMT7550     | Brazil, Mato Grosso, Tangará da Serra          | MT192277 MT224982 MT328074 MT328108 | This study |
| Hydrodynastes bicinctus | UFMT7551     | Brazil, Mato Grosso, Tangará da Serra          | MT192279 MT224973 MT328066 MT328109 | This study |
| Hydrodynastes bicinctus | 1817 (MZUSP) | Brazil, Mato Grosso, Sapezal                   | MT192267 MT224972 MT328065 MT328100 | This study |
| Hydrodynastes bicinctus | MZUSP20580   | Brazil, Rondônia, Porto Velho, Mutum          | MT192260 MT224973 MT328066 MT328106 | This study |
| Hydrodynastes bicinctus | CHUNB38982   | Brazil, Tocantins, Arraias                    | MT192267 MT224973 MT328066 MT328107 | This study |
| Hydrodynastes bicinctus | CHUNB40618   | Brazil, Tocantins, Mateiros                   | MT192267 MT224973 MT328066 MT328108 | This study |
| Hydrodynastes bicinctus | MZUSP15560   | Brazil, Tocantins, UHE Peixe Angical         | MT192267 MT224973 MT328066 MT328109 | This study |
| Hydrodynastes gigas    | INALI6573    | Argentina, Chaco, San Fernando               | MT192283 MT224989 MT328071 MT328114 | This study |
| Hydrodynastes megas    | INALI4779    | Argentina, Corrientes, General San Martín    | MT192282 MT224986 MT328072 MT328113 | This study |
| Hydrodynastes gigas    | INALI6867    | Argentina, Corrientes, Mburucuyá             | MT192285 MT224991 MT328072 MT328116 | This study |
| Hydrodynastes gigas    | LGE7992      | Argentina, Formosa, Piranè                   | MT192287 MT224995 MT328072 MT328119 | This study |
| Hydrodynastes gigas    | INALI6731    | Argentina, Formosa, Pilcomayo                | MT192284 MT224990 MT328072 MT328120 | This study |
| Hydrodynastes gigas    | MZUSP11704   | Brazil, Amapá                                 | MT192289 MT225000 MT328072 MT328124 | This study |
| Hydrodynastes gigas    | INPA-HT5513  | Brazil, Amazonas, Careiro                    | MT215328 MT224994 MT328080 MT328118 | This study |
| Hydrodynastes gigas    | INPA-HT190   | Brazil, Amazonas, Manaus                     | MT215327 MT224992 MT328079 MT328117 | This study |
| Hydrodynastes gigas    | MTR19444     | Brazil, Amazonas, Manacapuru                 | MT215333 MT224999 MT328086 MT328123 | This study |
| Hydrodynastes gigas    | CHUNB65028   | Brazil, Mato Grosso, Nossa Senhora do Livramento | MT192281 MT224986 MT328077 MT328111 | This study |
| Hydrodynastes gigas    | MAP-T3894    | Brazil, Mato Grosso do Sul, Porto Murtinho   | MT215330 MT224997 MT328083 MT328121 | This study |
| Hydrodynastes gigas    | MAP4050      | Brazil, Mato Grosso do Sul, Porto Murtinho   | MT215329 MT224996 MT328082 MT328120 | This study |
| Hydrodynastes gigas    | ZUFMS-REP2392| Brazil, Mato Grosso do Sul, Anastácio       | MT192296 MT225007 MT328092 MT328130 | This study |
| Species                   | Catalogue Number | Location                           | Museum Numbers                                        | This study     |
|--------------------------|------------------|------------------------------------|-------------------------------------------------------|----------------|
| Hydrodynastes gigas      | ZUFMS-REP 2393   | Brazil, Mato Grosso do Sul, Corumbá | MT192297, MT225008, MT328093, MT328131                 | This study     |
| Hydrodynastes gigas      | ZUFMS-REP 2395   | Brazil, Mato Grosso do Sul, Miranda | MT192298, MT225009, MT328094, MT328132                 | This study     |
| Hydrodynastes gigas      | ZUFMS-REP 2476   | Brazil, Mato Grosso do Sul, Corumbá | MT192299, MT225010, MT328095, MT328133                 | This study     |
| Hydrodynastes gigas      | ZUFMS-REP 2389   | Brazil, Minas Gerais, Fronteira    | MT192295, MT225006, MT328091, MT328129                 | This study     |
| Hydrodynastes gigas      | MPEG21864        | Brazil, Pará, Melgaço              | MT192288, MT224998, MT328084, MT328122                 | This study     |
| Hydrodynastes gigas      | AAGARDA8745      | Brazil, Rio Grande do Norte, Nisia Floresta | MT192278, MT224983, MT328075, MT328109               | This study     |
| Hydrodynastes gigas      | AAGARDA12357     | Brazil, Rio Grande do Norte, Canguaretama | MT192279, MT224984, –, – | This study     |
| Hydrodynastes gigas      | INPA-HT5427      | Brazil, Rondônia, Porto Velho, Teotônio | MT192286, MT224993, MT328080, – | This study     |
| Hydrodynastes gigas      | MZUSP18572       | Brazil, Rondônia, Porto Velho, Mutum | MT192290, MT225001, MT328087, MT328125                 | This study     |
| Hydrodynastes gigas      | MZUSP18573       | Brazil, Rondônia, Porto Velho, Abunã | MT192291, MT225002, MT328087, MT328126                 | This study     |
| Hydrodynastes gigas      | MZUSP19710       | Brazil, Rondônia, Porto Velho, Abunã | MT192292, MT225003, MT328089, MT328127                 | This study     |
| Hydrodynastes gigas      | MZUSP20449       | Brazil, Rondônia, Porto Velho, Abunã | MT192293, MT225004, MT328090, MT328128                 | This study     |
| Hydrodynastes gigas      | AF2382           | French Guiana, Matoury             | MT192280, MT224985, MT328076, MT328110                 | This study     |
| Hydrodynastes gigas      | PINV1580254      | Paraguay, Alto Paraguay            | MT192294, MT225005, –, MT424769 | This study     |
| Hydrodynastes melanogigas| MPEG24383        | Brazil, Maranhão, Carolina         | MT215331, MT225012, –, MT328097 | This study     |
| Hydrodynastes melanogigas| MPEG24384        | Brazil, Maranhão, Carolina         | MT215332, MT225013, MT328085, MT328098                 | This study     |
| Hydrodynastes melanogigas| MZUSP19557       | Brazil, Maranhão, Carolina         | MT215334, MT225014, –, MT328099 | This study     |
| Hydrodynastes melanogigas| IBSP65144        | Brazil, Tocantins, Lajeado         | –, MT224987, MT328078, MT328112 | This study     |
| Hydrodynastes melanogigas| UFMS-REP3446     | Brazil, Tocantins, Palmas          | MT215335, MT225011, MT328096, MT328134                 | This study     |
| Pseudoboa nigra          | MZUSP13278       | *                                  | *                                                      | –              |
| Xenopholis scalaris      | KU222204         | *                                  | JQ598915, GQ895897, JQ599002 | –              |

Zaher et al. 2009; Grazziotin et al. 2012; Pyron et al. 2009; Grazziotin et al. 2012
Table 2 (on next page)

PartitionFinder 2 model of nucleotide substitution.

Best-fitting partitioning scheme model of nucleotide substitution for 16S, Cytb, Cmos and NT3 genes.
| Partitioning scheme       | Model  |
|---------------------------|--------|
| Cytb1, 16S               | GTR+G  |
| Cytb2, Cmos2             | HKY+I  |
| Cytb3                    | HKY+G  |
| NT32, Cmos3, Cmos1       | JC     |
| NT31, NT33               | K80+I+G|
Table 3 (on next page)

Meristic characters in *Hydrodynastes gigas* and *Hydrodynastes melanogigas*.

In parenthesis the sampled number (n). SLr = right supralabials; SLl = left supralabials; ILr = right infralabials; III = left infralabials; LO = loreal; PE = preoculars; PO = postoculars; SO = suboculars; AT = anterior temporals; PT = posterior temporals; NA = nasal; IL+G1 = infralabials in contact with first pair of genials; IL+G2 = infralabials in contact with second pair of genials; AP = apical pits; AD = anterior dorsal scale rows; MD = midbody dorsal scale rows; PD = posterior dorsal scale rows; PV = preventrals; VE = ventral; SC = subcaudal. *75 specimens present autotomized tail. **specimens present only the head or other body part.
| Variables | Hydrodynastes gigas | Hydrodynastes melanogigas |
|-----------|---------------------|-------------------------|
|           | Male | Female | Undetermined** | Male | Female |
| SLr       | 7 (n=1); 8 (n=62); 9 (n=2) | 7 (n=1); 8 (n=68) | 8 (n=6) | 8 (n=22) | 7 (n=1); 8 (n=15); 8 (n=1) |
| SLl       | 8 (n=64); 9 (n=1) | 8 (n=61); 9 (n=5) | 8 (n=6) | 8 (n=22) | 8 (n=17) |
| ILr       | 9 (n=5); 10 (n=37); 11 (n=21); 12 (n=1) | 9 (n=2); 10 (n=29); 11 (n=31); 12 (n=5) | 10 (n=3); 11 (n=2) | 8 (n=1); 9 (n=2); 10 (n=17); 11 (n=2) | 9 (n=1); 10 (n=14); 11 (n=2) |
| III       | 9 (n=4); 10 (n=32); 11 (n=26) | 9 (n=3); 10 (n=24); 11 (n=35); 12 (n=4) | 10 (n=3); 11 (n=3) | 9 (n=4); 10 (n=16); 11 (n=2); 10 (n=10); 11 (n=6); 12 (n=1) |
| TO        | 1 (n=68) | 1 (n=69) | 1 (n=7) | 1 (n=22) | 1 (n=17) |
| PE        | 1 (n=66); 2 (n=1) | 1 (n=68); 2 (n=1) | 1 (n=7) | 1 (n=20); 2 (n=2) | 1 (n=16); 2 (n=1) |
| PO        | 2 (n=48); 3 (19) | 1 (n=1); 2 (n=64); 3 (n=4) | 2 (n=7) | 2 (n=19); 3 (n=3) | 2 (n=17) |
| SO        | 2 (n=3); 3 (n=64) | 2 (n=4); 3 (n=65) | 3 (n=7) | 3 (n=22) | 3 (n=17) |
| AT        | 1 (n=5); 2 (60); 3 (n=2) | 1 (n=5); 2 (n=62); 3 (n=2) | 2 (n=5); 3 (n=1) | 2 (n=16); 3 (n=5); 4 (n=1) | 2 (n=17) |
| PT        | 1+2 (n=5); 1+3 (n=6); 2+1 (n=1); 2+2 (n=3); 2+3 (n=49); 2+4 (n=2); 3+3 (n=2) | 2 (n=1); 3 (n=1); 1+2 (n=3); 1+3 (n=9); 2+2 (n=7); 2+3 (n=45); 2+4 (n=1); 3+2 (n=1); 3+3 (n=1) | 2+2 (n=2); 2+3 (n=4); 2+4 (n=1) | 1+3 (n=3); 2+1 (n=1); 2+2 (n=2); 2+3 (n=12) |
| NA        | 2 (n=68) | 2 (n=69) | 2 (n=7) | 2 (n=22) | 2 (n=17) |
| IL+G1     | i-iv (n=15); i-v (n=48); i-vi (n=3) | i-ii (n=1); i-iv (n=10); i-v (n=54); i-vi (n=3) | i-iv (n=2); i-v (n=5) | i-iv (n=1); i-v (n=19); i-vi (n=2) | i-v (n=16); i-iv (n=1) |
| IL+G2     | 0 (n=2); v-vi (n=7); v | 0 (n=3); v-vi (n=5); v | v (n=2); vi (n=5) | v (n=1); vi (n=19); vii (n=2) | vi (n=15); v-vi (n=1); vi-
| Variables | Hydrodynastes gigas | Hydrodynastes melanogigas |
|-----------|----------------------|---------------------------|
|           | Male | Female | Undetermined* | Male | Female |
|           | (n=8); vi (n=46); vii (n=3) | (n=4); vi (n=56); vii (n=1) | | vii (n=1) |
| AP        | 2 (n=67) | 2 (n=69) | 2 (n=4) | 2 (n=22) | 2 (n=17) |
|           | 17 (n=1); 18 (n=1); 19 (n=9) | 18 (n=3); 19 (n=54); 20 (n=5); 21 (n=6) | 19 (n=4) | 19 (n=22) | 19 (n=17) |
| AD        | (n=59); 20 (n=3); 21 (n=4) | | | |
| MD        | 17 (n=2); 18 (n=1); 19 (n=64) | 17 (n=4); 19 (n=65) | 17 (n=1); 19 (n=3) | 17 (n=3); 19 (n=19) | 18 (n=1); 19 (n=16) |
| PD        | 14 (n=1); 15 (n=67) | 14 (n=1); 15 (n=67); 17 (n=1) | 15 (n=4) | 14 (n=3); 15 (n=18); 16 (n=1) | 15 (n=17) |
| PV        | 1 (n=22); 2 (n=42); 3 (n=4) | 1 (n=18); 2 (n=45); 3 (n=6) | 1 (n=2); 2 (n=1) | 1 (n=10); 2 (n=12) | 1 (n=4); 2 (n=13) |
| VE        | 150–164 | 152–169 | | 154–168 | 168–172 |
| SC*       | 58(9) – 88 | 49(30) – 84 | | 71–86 | 70(24) – 79 |
Table 4 (on next page)

Morphometric measurement in *Hydropnastes gigas* and *Hydropnastes melanogigas*.

In parenthesis the sampled number (*n*). SVL = snout-vent length (from the tip of the snout to the cloaca); TL = tail length; HL = head length (from the tip of the snout to the quadratemandibular articulation); HW = head width (length of the widest part of head); DN = distance between nostrils (maximum distance between the nostrils); EN = distance between eye and nostril; ED = eye diameter; HH = head height (maximum distance between the base of the mandible and the parietal surface.)
| Variables | Hydrodynastes gigas | Hydrodynastes melanogigas |
|-----------|---------------------|--------------------------|
|           | Male | Female | Male | Female |
| SVL       | 249 – 1747 (n= 68) | 277 – 1879 (n= 69) | 468 – 1548 (n= 22) | 524 – 2198 (n= 17) |
|           | 998 ± 1059,25 | 988.5 ± 512.2 | 1100.4 ± 396.1 | 1048.3 ± 381.4 |
| TL        | 84 (25) – 580 (n= 68) | 71 – 543 (n= 69) | 180 – 548 (n= 22) | 105 – 532 (n= 17) |
|           | 302,5 ± 392,44 | 276.6 ± 143.6 | 351.5 ± 141.5 | 355.5 ± 143.6 |
| HL        | 20.57 – 67.94 (n= 63) | 21.65 – 78.61 (n= 66) | 30.04 – 55.48 (n= 22) | 32.63 – 69.21 (n= 17) |
|           | 44,24 ± 33,49 | 44,5 ± 15,6 | 47,4 ± 7,3 | 46,7 ± 10,3 |
| HW        | 7.24 – 21.54 (n= 67) | 7.19 – 21.77 (n= 69) | 9.12 – 17.89 (n= 22) | 9.80 – 47.25 (n= 17) |
|           | 10,0 ± 0.17 | 13.9 ± 4.4 | 14.9 ± 2.6 | 15.5 ± 8.5 |
| DN        | 2.89 – 12.93 (n= 68) | 3.51 – 11.97 (n= 69) | 4.17 – 9.47 (n= 22) | 4.78 – 10.68 (n= 17) |
|           | 10,24 ± 0,71 | 7.6 ± 4.9 | 7.5 ± 1.4 | 7.0 ± 1.6 |
| EN        | 3.19 – 12.55 (n= 68) | 3.70 – 14.01 (n= 69) | 5.09 – 9.44 (n= 22) | 4.88 – 11.58 (n= 17) |
|           | 9,98 ± 0,18 | 7.3 ± 2.5 | 7.8 ± 1.2 | 7.3 ± 1.8 |
| ED        | 4.19 – 8.55 (n= 68) | 4.12 – 8.41 (n= 69) | 4.85 – 7.64 (n= 22) | 5.08 – 7.95 (n= 17) |
|           | 6,37 ± 3,08 | 6.1 ± 1.3 | 6.4 ± 0.8 | 6.2 ± 0.8 |
| AC        | 7.17 – 25.63 (n= 61) | 8.03 – 26.76 (n= 62) | 11.39 – 21.26 (n= 22) | 9.98 – 27.64 (n= 17) |
|           | 10,21 ± 0,33 | 16.3 ± 6.0 | 16.8 ± 2.8 | 15.9 ± 4.4 |
Table 5 (on next page)

Uncorrected \textit{p-distance} of 16S (Lower left) and Cytb (Upper right) mitochondrial fragment gene for the genus \textit{Hydrodynastes}.

Bold the \textit{p-distance} between \textit{H. gigas} and \textit{H. melanogigas} for the two genes.
|      |                | 1  | 2  | 3  | 4  | 5  |
|------|----------------|----|----|----|----|----|
| 1    | *Xenopholis scalaris* | 0.367 | 0.347 | 0.376 | 0.366 |
| 2    | *Pseudoboa nigra*    | 0.084 | 0.366 | 0.313 | 0.341 |
| 3    | *Hydrodynastes bicinctus* | 0.126 | 0.118 | 0.129 | 0.123 |
| 4    | *Hydrodynastes gigas* | 0.128 | 0.097 | 0.043 | **0.020** |
| 5    | *Hydrodynastes melanogigas* | 0.130 | 0.099 | 0.042 | **0.011** |