









Morphological description and phylogenetic characterization of Hydra (Cnidaria: Hydrozoa) from São Paulo state, Brazil Deserti, MI¹, Durán-Fuentes, JA², Maronna, MM^{2,3}, Morandini, AC^{4,5}, Acuña, FH^{1,6} & Stampar, SN²

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INTRODUCTION & AIM

One of the biggest challenges in *Hydra* is accurately identifying specimens (1). Their taxonomy is riddled with synonyms and misapplied species names (2). The analysis established four distinct groups: *oligactis* (stalked hydra), *vulgaris* (common hydra), *viridissima* (symbiotic green hydra) and *braueri* (gracile hydra) (3). Each group may contain several closely related strains that were previously thought to be distinct species. According to (4), the *viridissima* and *vulgaris* groups were probably present before the breakup of the continents, whereas the *oligactis* and *braueri* groups were restricted to the northern continents, presumably arising after the separation of both landmasses.

The South American territory presents great variability in its continental aquatic environments. Brazil occupies a considerable percentage of this continental waters (5). Surprisingly, freshwater cnidarians in southern landmasses have received little attention. In Brazil, hydra polyps have been found only in five out of the 27 states. There are four described species, and three are endemic to this country: *H. iheringi* Cordero, 1939, *H. intermedia* Carvallo Wolle, 1978 and *H. salmacidis* Silveira et al, 1997. These works are relatively old and they were all based on classical systematics, using only external morphological features and cnidoma. In our study, we present the results of morphological and molecular analysis of specimens of the *vulgaris* and *viridissima* groups collected in seven different freshwater bodies of São Paulo state and one of Santa Catarina state.



METHOD

Sampling was carried out during August 2021 and April, May and June 2023. Floating and submerged macrophytes were collected at the sites shown in Figure 1, located in São Paulo (SP) and Santa Catarina states (SC), respectively SE and S Brazil. The collected polyps were maintained in the laboratory in Petri dishes with Cultural Solution M (6) and fed with freshly hatched Artemia nauplii three times a week. The medium was changed twice a week. The cultures were kept at constant T° (18° \pm 2°C) with 12 hs of light/dark cycle. The polyps were starved for three days prior to DNA extraction. The following data was recorded: coloration, length and width of the column, number of tentacles and relative length, number of buds and sexual structures. The length and width of the four types of cnidocysts was measured with Motic Images Plus v3.1 software. A pool of 5-10 whole live individuals clonally derived from a single hydra from each sampling site were used for molecular analysis. The protocol used in DNA extraction, PCR amplification, sequencing, bioinformatics and phylogenetic analysis can be found in (7).



RESULTS

are grouped in three different clades: (1) Rio Paraná, SP, occupy a higher position and are grouped with specimens from Thailand, (2) Mafra (SC) make an unique group, having a proximal relationship with specimens from Japan, (3) Rio Tietê and Prainha de Arealva, Lagoa Dos Patos and Lago Ness, occupy a more basal position than the previous two, grouping with specimens from Switzerland. The fact that all the specimens collected in different bodies of water in Brazil have been identified as H. vulgaris, supports the idea that this species is one of the most cosmopolitan of the genus, with a high phenotypic plasticity. The analysis also showed that the green hydras are Hydra sinensis, described for Dongjiang River, China (11) and found in Germany (2). Many freshwater hydrozoans have been identified based on specimens collected in artificial water bodies. An example of the implications that the transport of fish, aquatic invertebrates and plants around the world for agricultural, sports and aquaculture purposes can have on the taxonomy of the species and their geographical origins, is that reported by (12) for Craspedacusta sowerbii, a species native to China and considered invasive in the rest of the globe. The vegetal substrate, a potential dispersal vector of the artificial tank in Regent's Park in London from where the species was collected for its first description, did not come from China, but in fact from Brazil. This situation and the recent discovery of *H. sinensis*, we can guess if Brazil could be the original locality of both species and not China? Perhaps they are originally from Brazil, but were described based on specimens found in other parts of the world. This last statement would remain a question to be solved with future studies focusing on populational data based on specimens from many parts of the globe.



We identify two species: *Hydra vulgaris* Pallas, 1766 (*vulgaris* group) (Fig. 2a) and *Hydra sinensis* Wang, Deng, Lai and Li, 2009 (*viridissima* group) (Fig. 2b). Figure 3 shown distinct growth patterns tentacles on young buds. The phylogenetic reconstruction based on maximum likelihood analysis of genus *Hydra* within order Anthoathecata using COX are shown in Figure 4. Our specimens are highlighted in red.

DISCUSSION & CONCLUSION

The origin of freshwater cnidarians and their diversity presents intriguing biogeographic questions. Molecular phylogenetic studies of freshwater cnidarians can be an indispensable tool to help solve these problems (8). The morphological differences, added to the molecular results, allow us to discard the possibility that the specimens used in this analysis are H. iheringi, H. intermedia or H. salmacidis. As with the case of H. vulgaris pedunculata from Argentina (9), these variations could be related only to phenotypic plasticity (10). Our molecular analysis shows that the collected specimens

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