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3 **First record of biogenic silica in the stomach contents**

4 **of South American freshwater turtles**

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10 **This article has been accepted for publication and undergone full peer review but has not**
11 **been through the copyediting, typesetting, pagination and proofreading process, which**
12 **may lead to differences between this version and the Version of Record.**

13

14 **Please cite this article as:**

15

16 Maler de Oliveira, L., Parolin, M., Vargas Grou, C. E., Ratz Scoarize, M. M., Benedito, E.
17 (2026): First record of biogenic silica in the stomach contents of South American freshwater
18 turtles. *Acta Hepretol.* **21**. Doi: 10.36253/a_h-18063

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20 **First record of biogenic silica in the stomach contents of South American freshwater
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29 *Submitted on: 2025, 19th June; revised on: 2025, 9th December; accepted on: 2025, 10th
30 December.*

31 *Editor: Raoni Rebouças*

32 **Abstract.** Sponges of the phylum Porifera, especially those in the class Demospongiae, produce
33 siliceous spicules, while plants form phytoliths, both of which are sources of biogenic silica in
34 aquatic environments. Marine and freshwater turtles have diets that vary throughout their life
35 stages and may consume these organisms. However, there are still few studies investigating the
36 ingestion of biogenic silica by freshwater turtles. This study aimed to analyze the diversity of
37 biogenic silica bodies in the stomachs of *Phrynnops geoffroanus*. Specimens were captured in
38 Iguaçu National Park (PNI), Paraná, a vital remnant of the Atlantic Forest. Individuals
39 underwent biometric measurements, photographic documentation, and euthanasia using
40 Thiopental (93 mg/kg), following strict ethical protocols. Biological material was sent to the
41 State University of Maringá, where stomachs were extracted. Stomach contents were processed
42 at the Laboratory of Paleoenvironmental Studies (LEPAFE) at the State University of Paraná
43 (UNESPAR), treated with HNO₃ on a heating plate, and the resulting material was mounted on
44 slides for analysis. Three specimens of *Phrynnops geoffroanus* at different ontogenetic stages
45 were examined. Stomach analysis revealed the presence of biogenic silica, including phytoliths,
46 diatom frustules, and sponge spicules, with the highest concentration found in young individuals
47 and the lowest in juveniles. The predominant phytolith types suggest interactions with grasses
48 and Podostemaceae. Gemmulescleres of *Oncosclera navicella* were identified in young and
49 adult individuals, confirming predation on freshwater sponges. This study highlights the
50 interaction between *Phrynnops geoffroanus* and organisms that produce biogenic silica.

51
52 **Keywords.** Biomineralization, Phytoliths, Sponge Spicules, Freshwater Sponge, Turtles.

INTRODUCTION

54 Marine and freshwater turtles have a diverse diet that varies according to ontogenetic
55 stage. Juveniles tend to be predominantly carnivorous, young individuals display benthic
56 feeding behavior, and adults are primarily herbivorous. However, most species are considered
57 omnivorous, feeding on sponges, fish, crustaceans, medusae, and gastropods (Márquez, 1990).
58 In marine environments, these animals have been documented preying on biogenic silica-
59 producing organisms such as sponges (León BJORNDAL, 2002), while in freshwater habitats, they
60 consume plants (Esteves et al., 2021). Nevertheless, research on the presence of biogenic silica
61 in the stomachs of freshwater animals has not yet been conducted.

62 Sponges belong to the phylum Porifera and produce body structures composed of silica
63 and calcium carbonate, known as spicules (Volkmer-Ribeiro and Parolin, 2010). Species in the
64 class Demospongiae occur in both marine and freshwater environments, producing exclusively
65 siliceous spicules, which are considered biogenic silica. These structures are bound by organic
66 collagen filaments and are categorized into three types: megascleres (the most prominent
67 spicules forming the skeletal structure), microscleres (smaller spicules found in the outer surface
68 or pinacoderm of the sponge), and gemmules (the smallest spicules derived from
69 gemmae, with taxonomic value allowing identification at the family, genus, or species level)
70 (Kalinovsk et al., 2016). Sponges exhibit high biodiversity and can be found in both lotic and
71 lentic environments, attached to rocks, tree roots, and other substrates (Volkmer-Ribeiro and
72 Machado, 2017).

73 Biogenic silica can also be produced by certain plant species in the form of phytoliths.
74 Phytoliths are microscopic bodies formed by plants from silica absorbed from the soil and
75 deposited in plant tissues such as the lumen, intercellular spaces, and cell walls (Piperno, 2006).
76 This deposition process creates isometric molds of the original plant cells, which can sometimes
77 be taxonomically identifiable (ICPT et al., 2019; de Oliveira et al. 2023; 2024; 2025). Aerial
78 structures such as leaves, bracts, and fruits have the greatest affinity for phytolith production
79 (Piperno, 1991).

80 *Phrynosoma geoffroanus* (SCHWEIGER, 1812) exhibits the widest geographic
81 distribution among Chelidae turtles, occurring throughout most of Brazil, except in the states of
82 Roraima, Amapá, and Sergipe (Costa et al., 2022). Studies in recent decades indicate a wide
83 distribution of *P. geoffroanus* in South America, encompassing diverse environments and
84 biomes (Schneider et al., 2011; de Carvalho et al., 2017; Friol, 2019). Species of this genus of
85 freshwater turtles have a shovel-shaped jaw, a feature that allows them to feed on items
86 deposited on the bottoms of aquatic bodies (Rhodin and Mittermeier 1983). In contrast, *P.*

87 *geoffroanus* exhibits a more specialized feeding pattern, consuming exclusively plant fruits
88 during the rainy season (Fachín-Terán et al., 1995; Souza, 2004).

89 Some aquatic species such as fish and freshwater turtles rely on sponges and plants as
90 sources of energy, being classified as spongivorous and herbivorous (León and Bjorndal, 2002;
91 Esteves et al., 2021). These food resources contain silica incorporated into their body structures
92 (Piperno, 1988; Kalinovsk et al., 2016). The quantification of biogenic silica in the digestive
93 tract of freshwater turtles will advance the understanding of their ecological role in aquatic
94 ecosystems. Furthermore, the characterization and specific identification of freshwater sponges
95 through their spicules, also present in the digestive tract of these turtles, allows for an indirect
96 assessment of the distribution of freshwater sponges. In this context, this study aimed to analyze
97 the presence of biogenic silica (sponge spicules, phytoliths, and diatom frustules) in the
98 stomachs of *Phrynobius geoffroanus* (Schweigger, 1812), a turtle species widely distributed in
99 Brazil. This is the first study to investigate biogenic silica in the stomach contents of freshwater
100 turtles in South America and provides evidence of predation on freshwater plants and sponges
101 by the native species *P. geoffroanus*.

102

103 MATERIAL AND METHODS

104 Study area

105 The sampling area is located within the boundaries of Iguaçu National Park (PNI), in the
106 southwestern region of the state of Paraná, Brazil (25°05' to 25°41' S, 53°40' to 54°38' W). The
107 park covers a total area of 185,262.5 hectares and represents one of the largest and most
108 ecologically significant remnants of the Atlantic Forest biome in southern Brazil. The region is
109 characterized by a predominantly subtropical climate and comprises two main forest types:
110 Seasonal Semi-deciduous Forest and Ombrophilous Forest. These forest types are differentiated
111 based on the phytogeographic characteristics of the region, including floristic composition,
112 structure, and species distribution (Vogliotti, 2008; Alvares et al., 2013; Brocardo et al., 2019).

113 Several specimens of the freshwater turtle *Phrynobius geoffroanus* were captured, marked,
114 and subsequently released as part of a parallel study aimed at monitoring the species' population
115 in the lower Iguaçu River. For specific analyses in this study, individuals Pg1, Pg3, and Pg12
116 were selected for euthanasia, in accordance with current ethical protocols. The captures were
117 conducted in areas designated for public use and ecological restoration, as established in the
118 Management Plan of Iguaçu National Park (IBDF, 1981) (Fig. 1).

119

120 *Field procedures*

121 Morphological data (biometrics) and photographic records of the captured *P.*
122 *geoffroanus* individuals were collected (Fig. 2). The animals were then euthanized. Euthanasia
123 was conducted in the field using injectable agents (barbiturates), following the guidelines
124 recommended by CONCEA (2013). The drug used was Thiopental (diluted according to the
125 Euthanasia Guide for Animals Used in Teaching and Research), administered intravenously at
126 a lethal dose of 93 mg/kg. Biological specimens were sent to the State University of Maringá
127 (UEM) for processing and final preparation for deposition in the herpetological collection of the
128 Capão da Imbuia Natural History Museum (MHNCI). The study was approved by the Ethics
129 Committee on Animal Use (CEUA), protocol 9251160223, and authorized by the Instituto
130 Chico Mendes de Conservação da Biodiversidade (ICMBio) under license 86706-4.

131

132 *Animal analysis*

133 Three specimens of *Phrynops geoffroanus* (SCHWEIGGER, 1812) were analyzed at
134 different ontogenetic stages (Table 1). Sex determination was based on observations of shell
135 body plan (carapace height and plastron shape), tail length, and the position of the cloacal
136 opening relative to the distal portion of the tail (Molina, 1998; Almonacid et al., 2007). The
137 gonadal assessment could not be performed due to limited technical expertise (Fig. 3).

138

139 *Laboratory Procedures*

140 Only the stomachs and intestines of the turtles were selected, which were processed as
141 follows: 1) treatment with HNO₃ (65%) + H₂O₂ (v. 130) on a heating plate for 60 minutes at
142 100°C; 2) washing of the resulting material by centrifugation with distilled water (1000 rpm/3
143 min); 3) the resulting material, consisting of inorganic mineral residues and biomimetication,
144 was collected using a 50 µL mechanical pipette, deposited on microscope slides, the slides were
145 placed on a heated plate (~50°C) until dry and then covered with Entellan® and coverslipped.
146 The prepared slides were stored at the Laboratory of Paleoenvironmental Studies (LEPAFE) of
147 the State University of Paraná, UNESPAR – Campo Mourão Campus.

148

149 *Slide analysis*

150 Slides were analyzed under a biologic microscope, and images were captured using a
151 50-megapixel camera. The analysis involved counting and identifying biogenic silica bodies
152 along four randomly selected transects within the diameter of the optical field, evaluating from

153 the base to the top of the slide. Phytoliths were classified based on morphological description,
154 following the International Code for Phytolith Nomenclature (ICPN 2.0) (ICPT et al., 2019),
155 while freshwater sponge spicules were described according to the existing literature.

RESULTS

158 *Biogenic silica*

159 Stomach content analysis of *Phrynosoma geoffroanus* turtles revealed the presence of
160 biogenic silica bodies. These microremains were identified in all three individuals at different
161 ontogenetic stages (juvenile, young, and adult). In addition, diatom frustules were observed in
162 all analyzed specimens.

163

164 *Phytolith Analysis*

165 The analysis of the stomach contents of the three specimens revealed 94 phytoliths
166 distributed across 12 distinct morphotypes. The juvenile individual presented three morphotypes
167 and 3 phytoliths (3.1% of the total), the young individual presented 6 morphotypes and 60
168 phytoliths (63.8%), and the adult presented 12 morphotypes and 31 phytoliths (31.9%) (Table
169 3).

170 The juvenile contained only three phytolith morphotypes: ELONGATE ENTIRE
171 (*ELO_ENT*) (33.3%, n=1), BLOCKY PSILATE (*BLO_PSI*) (33.3%, n=1) and ACUTE BULBOUS
172 (*ACU_BUL*) (33.3%, n=1).

173 In the young individual, seven distinct morphotypes were identified. Phytoliths from the
174 family Podostemaceae accounted for 48 out of 60 phytoliths (80%). The following morphotypes
175 were also observed: *ELO_ENT*, *BULLIFORM FLABELLATE (BUL_FLA)*, *BLOCKY PSILATE*
176 (*BLO PSI*), *ACU BUL* and *RECTANGULAR SINUATE (REC SIN)*.

177 The adult individual exhibited the highest diversity of phytoliths, with 12 distinct
178 morphotypes found in the stomach content: *BLO*, RECTANGULAR ROUGH (*REC_ROU*), SADDLE
179 (*SAD*), RECTANGULAR PSILATE (*REC_PSI*), *ELO_ENT*, SPHEROID ORNATE (*SPH_ORN*),
180 *BUL_FLA* and POLYHEDRAL (*POL*).

181

182 *Sponge spicules*

183 Spicules of the megasclere and gemmолосclere types were identified in the stomach
184 contents of the young and adult specimens but were absent in the juvenile. Three oxeas-type
185 megascleres were found in the young individual and five in the adult, along with two

186 gemmulescences attributed to the freshwater sponge species *Oncosclera navicella* (Carter,
187 1881), present in both individuals.

188

189 **DISCUSSION**

190 The stomach content analysis of three *Phrynops geoffroanus* individuals revealed the
191 presence of preserved sponge spicules in both juvenile and adult specimens, as well as phytoliths
192 and diatom frustules in all individuals, at different ontogenetic stages. This study constitutes the
193 first record of *Oncosclera navicella* in the Iguaçu River which had only previously been
194 recorded in lotic environments of the Piquiri River, Paraná, Brazil, as well as in lentic habitats
195 in Venezuela (Ribeiro and Pauls., 2000; Volkmer-Ribeiro and Parolin, 2005).

196 The stomach contents of the analyzed individuals showed varying amounts of phytoliths
197 across the different ontogenetic stages. The highest concentration was recorded in the young
198 individual, followed by the adult, while the juvenile exhibited the lowest amount. This pattern
199 indicates age-related differences in diet, feeding behavior, or resource selectivity, suggesting
200 that young individuals may consume food items with a higher proportion of plant material. This
201 diet shift according to the ontogenetic stage also occurs in the marine turtle *Caretta caretta*,
202 while the juveniles feed on zooplankton, adults consume predominantly pelagic tunicates,
203 bivalves, gastropods, and fish (Cardona et al., 2024). Thus, more studies are needed to verify
204 whether this pattern can be explained by specimen size and ability to forage.

205 The stomach material of the juvenile, young and adult individuals contained the
206 *ELO_ENT* morphotype, which is considered problematic in phytolith analysis. This phytolith
207 type has low taxonomic value, as it can be produced by numerous monocotyledonous and
208 eudicotyledonous species with different growth habits (Albert et al., 2018; de Oliveira et al.,
209 2024; 2025).

210 The stomach contents of the young individual revealed phytolith morphotypes
211 characteristic of the Podostemaceae family (da Costa et al., 2011; da Costa et al., 2021),
212 indicating that the young turtle consumed vegetation from this group. Podostemaceae species
213 are riparian plants adapted to anchor onto rocks and other solid substrates in fast-flowing
214 freshwater environments, such as rapids and waterfalls (Ruhfel et al., 2024). The presence of
215 these phytoliths in the stomach material therefore suggests that the young individual foraged in
216 areas associated with these habitats. The *ACU_BUL* type identified displayed two distinct
217 morphologies; the elongated and pointed variant resembled forms found in several eudicot
218 families (Cannabaceae, Boraginaceae, Ulmaceae, Moraceae), commonly referred to as "Hair"

219 (Wu et al., 2017; de Oliveira et al., 2024), and the short, pointed variant resembled the "Prickle"
220 morphotype commonly found in monocot grasses (Fig. 4 a2) (Tripathi et al., 2014; Wroth et al.,
221 2019).

222 The stomach contents of the adult individual showed the greatest diversity of phytoliths
223 (ICPT, 2019; Qader et al., 2024). The *BUL_FLA* morphotype is a significant taxonomic marker,
224 often associated with Poaceae species under water stress (Kondo et al., 1994). The *SAD*
225 morphotype is widely distributed in the Chloridoideae, Bambusoideae, and Arundinoideae
226 subfamilies (Lu e Liu, 2003). Woody species morphotypes were also identified in the adult
227 turtle's stomach content. The *SPH_ORN* morphotype is commonly found in leaf tissues of
228 eudicots (de Oliveira et al., 2024; 2025). When present in stems or trunks, this morphotype can
229 serve as a diagnostic marker for tree species (Bremond et al., 2008). The *POL* morphotype
230 observed in this specimen's stomach content resembles those found in the leaves of woody
231 species from Norway (Lisztes-Szabó et al., 2019). The large number of phytolith morphotypes
232 derived from grasses and riparian plants confirms the selective use of these plant resources by
233 freshwater turtles. This phytolithic interpretation is consistent with studies conducted in
234 Australia, where similar plants were also recorded in the stomach and fecal contents of
235 freshwater turtles (Kennett et al., 1996; Armstrong et al., 2005).

236 Another type of biogenic silica identified in the stomach content of *P. geoffroanus*
237 consisted of freshwater sponge spicules. These structures were observed as megascleres, which
238 lack taxonomic value, and gemmulescleres, which originate from gemmules and have
239 taxonomic significance, allowing species-level identification (Kalinovsk et al., 2016). The
240 gemmulescleres found in the young and adult individuals were confirmed to be from *O.*
241 *navicella*.

242 Predation of freshwater sponges by freshwater turtles has previously been documented
243 in different regions of the world, including the Burnett River basin in Australia (Armstrong et
244 al., 2005), seasonally ephemeral freshwater pools on coastal floodplains of the wet-dry tropics
245 of northern Australia (Kennett et al., 1996), and the Kawai Nui wetlands in Hawaii, USA (Works
246 et al., 2018). Furthermore, the detection of gemmulescleres in the stomach contents of young
247 and adult freshwater turtles, and their absence in juveniles has not previously been observed,
248 with other studies even describing an exclusively spongivorous diet for juveniles (León and
249 Bjorndal, 2002). This highlights a similarity in predation patterns between freshwater and
250 marine turtle species. The marine turtle *Eretmochelys imbricata*, for example, feeds almost
251 exclusively on sponges, including toxic ones, and its diet is geographically uniform (Meylan,
252 1988). Thus, further studies with *Phrynos geoffroanus* could investigate whether its diet is

253 geographically uniform and whether juveniles could be less resistant to toxic components than
254 young and adults, thus elucidating the absence of sponges in juveniles. However, the diet of
255 *Caretta caretta* juveniles differ from adults (Cardona et al., 2024), hence, the reasons for this
256 shift should be studied for *Phrynos geoffroanus*.

257

258 CONCLUSION

259 The results of this study confirmed the ingestion of phytoliths by *Phrynos geoffroanus*,
260 with differences in abundance and diversity across ontogenetic stages. The young individual
261 presented the highest concentration of phytoliths, while the juvenile had the lowest. The
262 predominance of morphotypes characteristic of grasses and Podostemaceae suggests a strong
263 feeding interaction with aquatic and riparian environments. Furthermore, the identification of
264 gemmulescieres of *O. navicella* in young and adult specimens indicates that this turtle species
265 consumes freshwater sponges. Thus, the record of *O. navicella* presented in this study for the
266 Iguaçu River reinforces previously documented evidence of the species' occurrence in both lotic
267 and lentic environments in South America.

268

269 ACKNOWLEDGMENTS

270 The authors thank the Comparative Biology Graduate Program (PGB-UEM), the
271 Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the grant
272 308093/2023-2 to M.P. and C.E.V.G., 141691/2020-4 to M.M.R.S., 308522/2021-4 to E.B.; the
273 Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the grant
274 88887.905477/2023-00 to L.M.O.

275

276 AUTHORIZATIONS

277 The study was approved by the Ethics Committee on Animal Use (CEUA), protocol
278 9251160223, and authorized by the Instituto Chico Mendes de Conservação da Biodiversidade
279 (ICMBio) under license 86706-4.

280 REFERENCES
281

282 Albert, R.M., Bamford, M.K., Stanistreet, I.G., Stollhofen, H., Rivera-Rondón, C.A., Njau, J.K.,
283 Blumenschine, R.J. (2018): River-fed wetland palaeo-vegetation and palaeoecology at the
284 HWK W site, Bed I, Olduvai Gorge. *Rev. Palaeobot. Palynol.* **259**: 223-241.

285 Almonacid, J.V.R., Mittermeier, C., Carr, J.L., Mittermeier, R.A., Malecha, J.V.R., Mast, R.,
286 Rueda, J.N. (2007): Las tortugas y los cocodrilianos de los países andinos del trópico. **6**.

287 Alvares, C.A., Stape, J.L., Sentelhas, P.C., Gonçalves, J.D.M., Sparovek, G. (2013): Köppen's
288 climate classification map for Brazil. *Meteorol. Z.* **22**: 711-728.

289 Armstrong, G., Booth, D.T. (2005): Dietary ecology of the Australian freshwater turtle (Elseya
290 sp.: *Chelonia*: *Chelidae*) in the Burnett River, Queensland. *Wildlife Research.* **32**: 349-353.

291 Brasil. Decreto nº 86.676, de 1º de dezembro de 1981 (1981): Dispõe sobre a proteção de
292 espécies da fauna silvestre e dá outras providências. Diário Oficial da União: seção 1,
293 Brasília, DF, 2 dez.

294 Bremond, L., Alexandre, A., Hély, C., Guiot, J. (2005): A phytolith index as a proxy of tree
295 cover density in tropical areas: calibration with Leaf Area Index along a forest-savanna
296 transect in southeastern Cameroon. *Glob. Planet. Change.* **45**: 277-293.

297 Brocardo, C.R., da Silva, M.X., Ferracioli, P., Cândido Jr, J.F., Bianconi, G.V., Moraes, M.F.D.,
298 Crawshaw Jr, P. (2019): Mamíferos do Parque Nacional do Iguaçu. *Oecol. Aust.* **23**: 165-
299 190.

300 Cardona, L., Aznar, F.J., Bas, M., Tomás, J. (2024): The contribution of fish to the diet of
301 loggerhead sea turtles in the western Mediterranean revisited. *Mar. Biol.* **171**: 215.

302 Costa, F.G.C.M.D., Bove, C.P., Arruda, R.D.C.O., Philbrick, C.T. (2011): Silica bodies and
303 their systematic implications at the subfamily level in Podostemaceae. *Rodriguésia.* **62**: 937-
304 942.

305 da Costa, F.G.C.M., Pellegrini, M.O.O., Bove, C.P. (2021): Diversidade de corpos de sílica em
306 *Mourera Aublet* (Podostemaceae) e suas aplicações filogenéticas, paleobotânicas e
307 taxonômicas. *Rev. Palaeobot. Palynol.* **295**: 104509.

308 da Costa, H.C., Guedes, T.B., Bérnils, R.S. (2022): Lista de répteis do Brasil: padrões e
309 tendências. *Herpetol. Bras.* **10**: 110-279.

310 de Carvalho, V.T., Martínez, J.G., Hernández-Rangel, S. M., Astolfi-Filho, S., Vogt, R. C.,
311 Farias, I. P., Hrbek, T. (2017): Giving IDs to turtles: SNP markers for assignment of
312 individuals to lineages of the geographically structured *Phrynobatrachus geoffroanus* (Chelidae:
313 Testudines). *Conserv. Genet. Resour.* **9**: 157-163.

314 de Oliveira, L.M., Parolin, M., dos Santos, J.C.A., Caxambu, M.G. (2023): Fitólitos de
315 *Andropogon bicornis* L. e *Andropogon leucostachyus* Kunth in Humb., Bonpl. &
316 Kunth. (Poaceae). *Observatorio* **21**: 7880-7890.

317 de Oliveira, L.M., Calegari, M.R., Leli, I.T., Romagnolo, M.B. (2024): Production of phytoliths
318 in woody plants of the Atlantic Forest in islands of the Paraná River, Brazil. *Quat. Int.* **681**:
319 24-32.

320 de Oliveira, L.M., Leli, I.T., Ferrreira, M.L., Romagnolo, M.B., Calegari, M.R. (2025):
321 Phytolith production in non-woody plants from the Atlantic Forest on islands of the Paraná
322 River, Brazil. *Quat. Int.* **738**: 109858.

323 Esteves, K.E., Aranha, J.M.R., Albrecht, M.P. (2021): Ecologia trófica de peixes de riacho: uma
324 releitura 20 anos depois. *Oecol. Aust.* **25**: 282-282.

325 Fachín-Terán, A., Vogt, R.C., Gomez, M.F.S. (1995): Food habits of an assemblage of five
326 species of turtles in the Rio Guaporé, Rondônia, Brazil. *Journal of Herpetology* **29**: 536-547.

327 Friol, N.R. (2019): Revisão taxonômica e filogenia das espécies sul-americanas de Chelidae
328 (Testudines, Pleurodira). Unpublished doctoral dissertation. Universidade de São Paulo.

329 International committee for phytolith taxonomy (ICPT), Neumann, K., Strömberg, C.A.E., Ball,
330 T., Albert, R.A., Vrydaghs, L., Cummings, L.S. (2019): International code for phytolith
331 nomenclature (ICPN) 2.0. *Ann. Bot.* **124**: 189-199.

332 Kalinovsk, E.C.Z., Parolin, M., Souza Filho, E.E. (2016): Esponjas de água doce na América do
333 Sul: O estado da arte da produção científica no Brasil. *Terra e didat.* **12**: 4-18.

334 Kennett, R., Tory, O. (1996): Diet of two freshwater turtles, *Chelodina rugosa* and *Elseya*
335 *dentata* (Testudines: Chelidae) from the wet-dry tropics of northern Australia. *Copeia*. 409-
336 419 p.

337 Kondo, R., Childs, C.W., Atkinson, I.A.E. (1994): Opal Phytoliths of New Zealand. (No Title).

338 León Y, Bjorndal, K. (2002): Selective feeding in the hawksbill turtle, an important predator in
339 coral reef ecosystems. *Mar. Ecol. Prog. Ser.* **245**: 249-258.

340 Lisztes-Szabó, Z., Braun, M., Csík, A., Pető, Á. (2019): Phytoliths of six woody species
341 important in the Carpathians: characteristic phytoliths in Norway spruce needles. *Veg. Hist.*
342 *Archaeobotany*. **28**: 649-662.

343 Lu, H., Liu, K.B. (2003): Phytoliths of common grasses in the coastal environments of
344 southeastern USA. *Estuar. Coast. Shelf Sci.* **58**: 587-600.

345 Márquez, R.M. (1990): FAO species catalogue. Vol. 11: Sea turtles of the world. An annotated
346 and illustrated catalogue of sea turtle species known to date. Roma FAO.

347 Meylan A. (1988): Spongivory in hawksbill turtles: a diet of glass. *Science* **239**: 393-395.

348 Molina, F.B. (1998): Comportamento e biologia reprodutiva dos cágados *Phrynopsgeoffroanus*,
349 *Acanthochelys radiolata* e *Acanthochelys spixii* (Testudines, Chelidae) em cativeiro. *Rev.*
350 *Etol.* **1998**: 25-40 p.

351 Piperno, D.R. (1988): Phytolith analysis. An Archaeological and Geological Perspective.
352 Academic Press. San Diego.

353 Piperno, D.R. (1991): The status of phytolith analysis in the American tropics. *J. World*
354 *Prehist.* **5:** 155-191.

355 Piperno, D.R. (2006): Phytoliths: a comprehensive guide for archaeologists and paleoecologists.
356 Rowman Altamira.

357 Qader, W., Dar, R.A., Rehman, I.U., Rashid, I., Sheikh, S.H. (2024): Assessing phytolith
358 preservation in a Late Quaternary loess-paleosol sequence from the Kashmir Valley,
359 Northwest Himalaya, India. *Quat. Sci. Adv.* **16:** 100238.

360 Ribeiro, C.V., Pauls, S.M. (2000): Esponjas de agua dulce (Porifera, Demospongiae) de
361 Venezuela. *Acta Biol. Venez.* **20:** 1-28.

362 Rhodin, A.G.J., Mittermeier, R.A. (1983): Description of *Phrynos williamsi*, a new species of
363 chelid turtle of the South American *P. geoffroanus* complex. Pp. 58-73 in A. Rhodin e K.
364 Miyata (eds.), *Advances in Herpetology and Evolutionary Biology – essays in honor of E.E.*
365 *Williams*. Museum of Comparative Zoology, Cambridge.

366 Ruhfel, B.R., Larson, D.A., Koenig, N., Rutishauser, R., Bove, C.P., Philbrick, C.T. (2024):
367 Plastid Phylogenomic Analysis of Podostemaceae with an Emphasis on Neotropical
368 Podostemoideae. *Syst. Bot.* **49:** 580-616.

369 Souza, F.L. (2004): Uma revisão sobre padrões de atividade, reprodução e alimentação de
370 cágados brasileiros (Testudines, Chelidae). *Phylomedusa*, **3:** 15-27.

371 Schneider, L., Ferrara, C.R., Vogt, R.C., Guilhon, A.V. (2011): Nesting ecology and nest
372 predation of *Phrynos geoffroanus* (Testudines, Chelidae) in the Guaporé River of the
373 Brazilian and Bolivian Amazon. *Chelonian Conserv. Biol.* **10:** 206-212.

374 Souza, F.L. (2004): Uma revisão sobre padrões de atividade, reprodução e alimentação de
375 cágados brasileiros (Testudines, Chelidae). *Phylomedusa*, **3:** 15-27.

376 Tripathi, D.K., Prasad, R. Chauhan, D.K. (2014): An overview of biogenic silica production
377 pattern in the leaves of *Hordeum vulgare* L. *Indian J Plant Sci*, **3:** 167-177.

378 Vogliotti, A. (2008): Partição de habitats entre os cervídeos do Parque Nacional do Iguaçu.
379 Unpublished doctoral dissertation. Universidade de São Paulo.

380 Volkmer-Ribeiro, C. Machado, V.D.S. (2017): Checklist das esponjas do Estado do Mato
381 Grosso do Sul, Brasil. *Iheringia Ser. Zool.* **107:** e2017102.

382 Volkmer-Ribeiro, C., Parolin, M (2010): As esponjas. In: *Abordagem ambiental em bacias*
383 *hidrográficas no Estado do Paraná*, 105-130 p. Parolin, M., Volkmer-Ribeiro, C., Leandrini,
384 J.A.(Org.), Eds., Campo Mourão. Fecilcam.

385 Volkmer-Ribeiro, C., Parolin, M. (2005): Segundo registro de *Sterrastrolepis brasiliensis*
386 Volkmer-Ribeiro e de Rosa-Barbosa (Demospongiae, Potamolepidae) com descrição do
387 habitat e de assembléia, Bacia do Rio Paraná, Brasil. *Rev. Bras. Zool.* **22:** 1003-1013.

388 Works, A.J., Olson, D.H. (2018): Diets of two nonnative freshwater turtle species (*Trachemys*
389 *scripta* and *Pelodiscus sinensis*) in Kawai Nui Marsh, Hawaii. *Journal of Herpetology* **52:**

390 444-452.

391 Wroth, K., Cabanes, D., Marston, J.M., Aldeias, V., Sandgathe, D., Turq, A., Dibble, H.L.
392 (2019): Neanderthal plant use and pyrotechnology: phytolith analysis from Roc de Marsal,
393 France. *Archaeol. Anthropol. Sci.* **11**: 4325-4346.

394 Wu, Y., Guo, X., Wang, W., Chen, X., Zhao, Z., Xia, X., Yang, Y. (2017): Red pigments and
395 Boraginaceae leaves in mortuary ritual of late Neolithic China: A case study of Shengedaliang
396 site. *Microsc. Res. Tech.* **80**: 231-238.

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423 **TABLE**

424 **Tab. 1.** Physical characteristics of *Phrynpops geoffroanus* specimens captured in Iguaçu National
 425 Park, Brazil. Abbreviations: ID – animal identification, OE – ontogenetic stage, WBM – weight
 426 (body mass) in grams, SC – size in centimeters, CL – carapace length in millimeter, CW –
 427 carapace width in millimeter, PL – plastron length in millimeter, PW – plastron width in
 428 millimeter, PH – carapace height in millimeter.

Species	ID	Sex	OE	WBM	SC	CL	CW	PL	PW	PH
<i>Phrynpops geoffroanus</i>	Pg1	Undermined	Juvenile	13	4.9	49,4	43	42,5	32,5	17
<i>Phrynpops geoffroanus</i>	Pg3	Female	Young	455	16.9	162,7	128,7	147,7	105,2	58,7
<i>Phrynpops geoffroanus</i>	Pg12	Female	Adult	1.298,3	33.4	334,5	147,5	298,9	210,1	98,3

429 **Tab. 2.** Morphological description of the main phytolith morphotypes identified in the stomach
 430 contents of *Phrynpops geoffroanus* captured in Iguaçu National Park, Brazil.

Morphotype	Code	Morphology
ACUTE BULBOSUS	ACU BUL	Elongated and pointed
BULLIFORM FLLABELLATE	BUL FLA	Elongated in a fan shape.
ELONGATE ENTIRE	ELO ENT	Elongated with smooth margins and surface
ELONGATE DENTATE	ELO DEN	Elongated with pointed margins
ELONGATE IRREGULAR	ELO IRR	Irregularly elongated
BLOCKY	BLO	Form parallelepipeds similar to those typically found in Poaceae
BLOCKY PSILATE	BLO PSI	Smooth-surfaced blocky
RECTANGULAR ROUGH	REC ROU	Rectangular with wrinkled surface
RECTANGULAR SINUATE	REC SIN	Rectangular with sinuous margins
SADDLE	SAD	Saddle-shaped
SPHEROID ORNATE	SPH ORN	Spheroidal with a rough and warty surface
POLYEHDRAL	POL	Polyhedral form

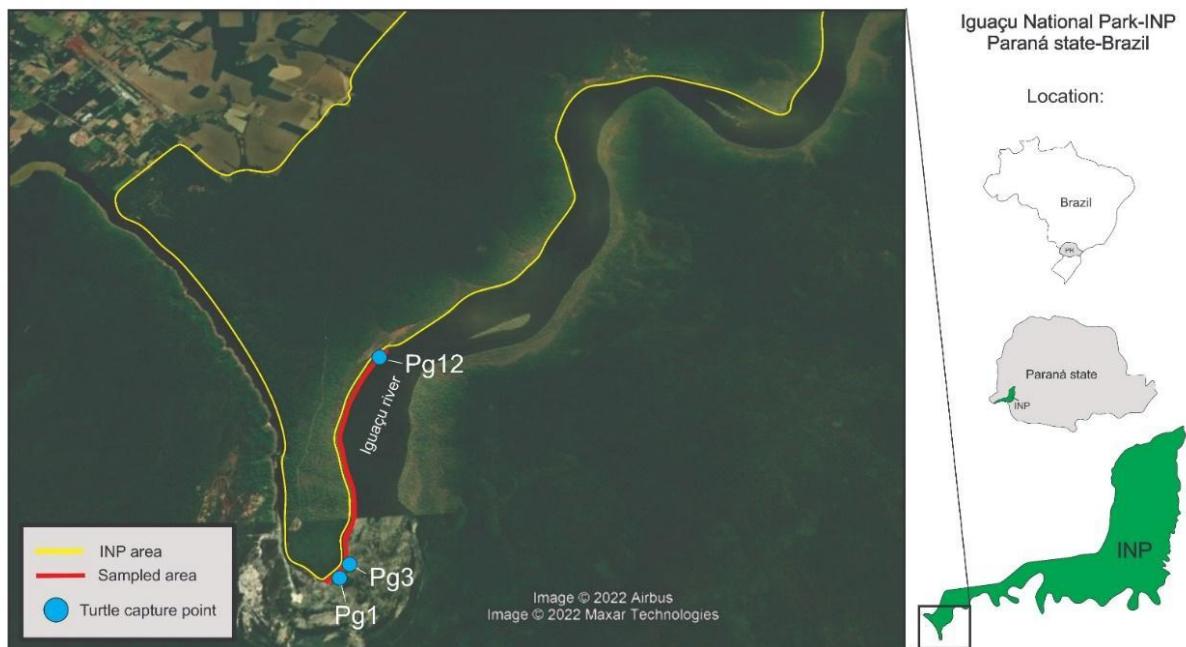
432 **Tab. 3.** Quantidade e diversidade de fitólitos encontrados no material estomacal de *Phrynpops*
 433 *geoffroanus* captured in Iguaçu National Park, Brazil.

Animal	Total phytoliths	Diversity and quantity of phytoliths
Juvenile	3	<i>ELO_ENT</i> (33.3%, n=1), <i>BLO_PSI</i> (33.3%, n=1), <i>ACU_BUL</i> (33.3%, n=1).
Young	60	<i>POD</i> (80%, n= 60), <i>ACU_BUL</i> (33.3, n=2), <i>BLO_PSI</i> (6.6%, n=4), <i>SAD</i> (5%, n=3), <i>BUL_FLA</i> (1.6%, n=1), <i>ELO_ENT</i> (1.6%, n=1) and <i>REC SIN</i> (1.6%, n=1).
Adult	31	<i>BLO</i> (38.7%, n=12), <i>REC_ROU</i> (12.9%, n=4), <i>SAD</i> (12.9%, n=4), <i>REC_PSI</i> (9.6%, n=3), <i>ELO_ENT</i> (9.6%, n=3), <i>SPH_ORN</i> (6.4%, n=2), <i>BUL_FLA</i> (6.4%, n=2), <i>SPH_ORN</i> (6.4%, n=2) and <i>POL</i> (3.2%, n=1).

435

436 **FIGURE**

437 **Fig. 1.** The study area and collection points in Parque Nacional do Iguaçu, Brazil.



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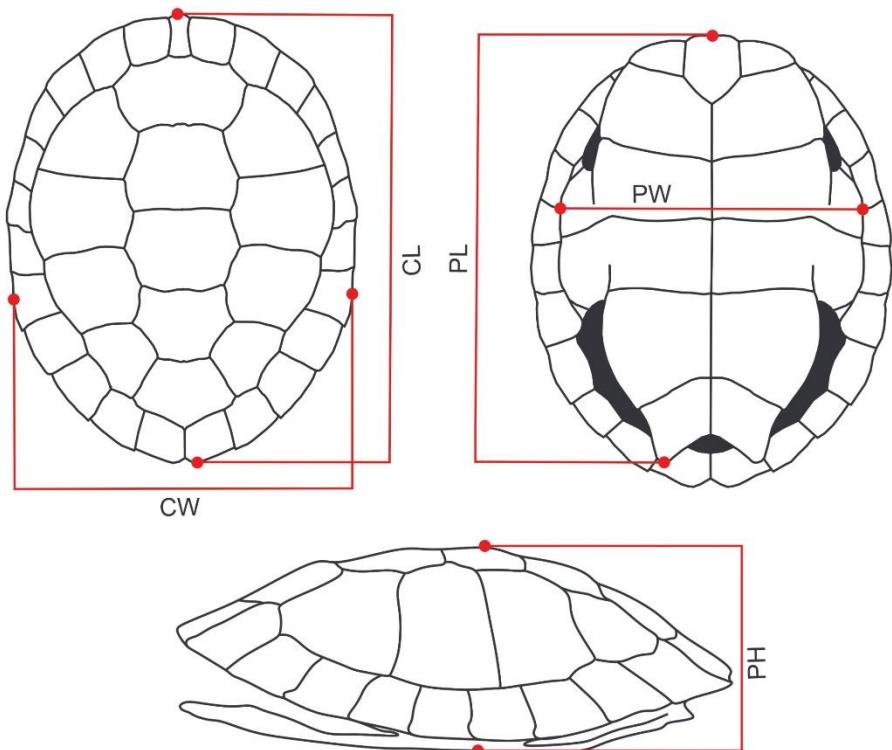
440 **Fig. 2.** Specimen of *Phrynobatrachus geoffroanus* captured in the Iguaçu National Park, Brazil.
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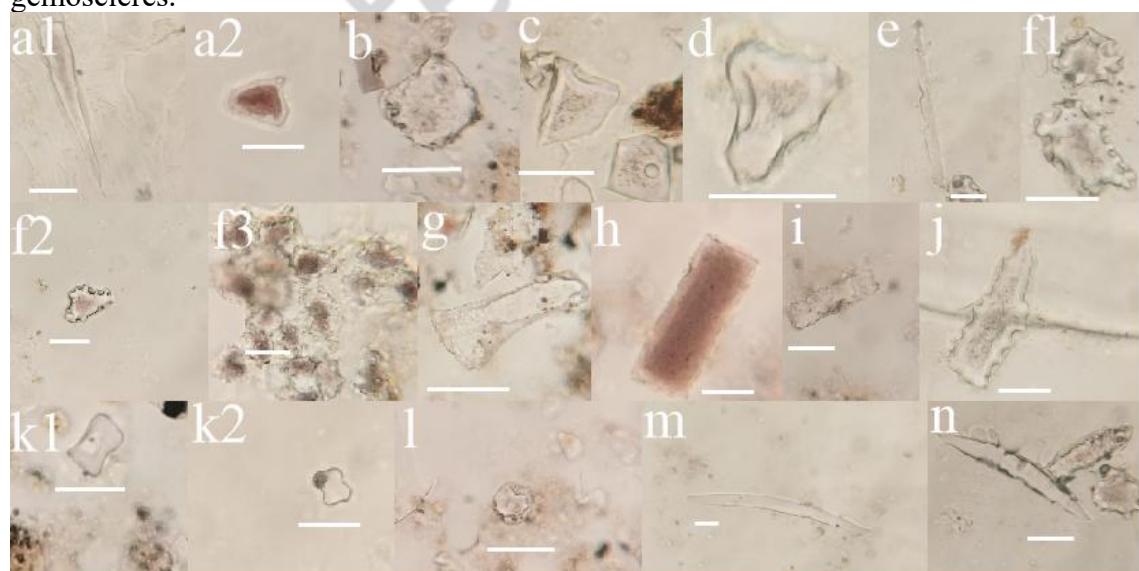
444 **Fig. 3.** Description of the morphometric measurements performed on the individuals: CL: length
445 between the nuchal scute and the 12th marginal scute; CW: width between the 8th and 17th
446 marginal scutes; PL: length between the intergular scute and the anal scute; PW: total width of
447 the pectoral scutes; PH: distance from the 3rd vertebral scute to the base of the carapace (lateral
448 view).



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451 **Fig. 4.** - The main morphotypes of phytoliths and sponge spicules found in turtles' stomach
 452 material from the Lower Iguaçu River. Scale (10 μ m). Sige a1 and a2 - *ACU_BUL*, b - *BLO*, c
 453 - *BLO_PSI*, d - *BUL_FLA*, e - *ELO_ENT*, f1, f2 e f3 - Podostemaceae phytoliths, g - *POL*, h -
 454 *REC_PSI*, i - *REC_ROU*, j - *REC_VEL*, k1 e k2 - *SAD*, o - *SPH_ORN*, m - megascleres, n
 455 gemoscleres.



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