UNIVERSIDADE ESTADUAL PAULISTA

"Júlio de Mesquita Filho"

INSTITUTO DE BIOCIÊNCIAS DE BOTUCATU

Vinícius Vilela Carvalho

Revisão taxonômica do complexo – *Ceriodaphnia cornuta* no Brasil: uma abordagem integrativa

BOTUCATU – SP

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Vinícius Vilela Carvalho

Orientador: Gilmar Perbiche Neves

Dissertação apresentada ao Instituto de Biociências, Universidade Estadual Paulista - Campus de Botucatu – como parte dos requisitos para obtenção do título de Mestre em Ciências Biológicas – Área de concentração: Zoologia

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RESUMO

Cladóceros são microcrustáceos encontrados em ambientes aquáticos continentais, constituindo um importante componente do zooplâncton em lagos e reservatórios. Existem aproximadamente 600 espécies descritas em todo o mundo, e sua taxonomia apresenta diversas lacunas. Muitos táxons são compostos por espécies crípticas descritas no século XIX com base em caracteres superficiais. Entre essas espécies, *Ceriodaphnia cornuta* é um complexo de espécies crípticas que apresentam alta plasticidade fenotípica. A literatura indica que essa espécie não pode ser diferenciada por métodos morfológicos convencionais, sendo necessárias abordagens mais recentes, como o estudo de machos e fêmeas efipiais e patas torácicas. O objetivo deste estudo foi realizar uma revisão taxonômica das populações brasileiras do complexo - *Ceriodaphnia cornuta* utilizando taxonomia integrativa. As populações analisadas foram coletadas em diferentes corpos d'água, dissecadas, e estruturas de alto valor taxonômico, como cabeça, apêndices torácicos e pós-abdômen, foram estudadas. Os resultados morfométricos revelaram diferenciação entre as populações. Os espinhos da carapaça apresentaram variação significativa e baixa correlação com os valores alométricos. A população JMED mostrou a maior diferenciação, principalmente devido a forma da carapaça, estrutura da pata II, e no número de dentículos anais. Sua morfologia efipial também se destacou por diferenças na forma e na ornamentação das colunas do efípio. Portanto, esse conjunto de caracteres é suficiente para definir uma nova espécie brasileira de *Ceriodaphnia*. As populações brasileiras também se diferenciaram quanto ao número de setas no IDL da pata I, e recomenda-se a realização de análises moleculares futuras com foco nessas populações para verificar sua diferenciação.

Palavras-Chave: biogeografia, efípios, espécies crípticas, polimorfismo, zooplâncton.

ABSTRACT

Cladocerans, are microcrustaceans found in continental aquatic environments, constituting an important component of the zooplankton in lakes and reservoirs. There are approximately 600 described species worldwide, and their taxonomy exhibits numerous gaps. Many taxa consist of cryptic species that were described in the 19th century based on superficial characters. Among these species, *Ceriodaphnia cornuta* is a complex of cryptic species exhibiting high phenotypic plasticity. The literature indicates that the species cannot be differentiated through conventional morphological methods, necessitating the use of novel approaches such as studying male, ephippial females and thoracic limbs. The objective of this study was to conduct a taxonomic revision of Brazilian *Ceriodaphnia cornuta* – complex populations using integrative taxonomy. The populations used in the study were collected from various water bodies, dissected, and structures with high taxonomical value such as head, thoracic limbs, and postabdomen were studied. The morphometric results revealed differentiation among the populations. Carapace spines exhibited significant variation and low correlation with allometric values. The JMED population showed the greatest divergence, primarily due to differences in carapace shape, limb II structure and anal teeth. Its ephippial morphology differed in both shape and the ornamentation of the ephippial columns. Thus, this set of characters is sufficient to define a new Brazilian *Ceriodaphnia* species. Brazilian populations also differed in the number of setae on the IDL of limb I, and future molecular analyses focusing on these populations are recommended to verify their distinctiveness.

Keywords: biogeography, cryptic species, ephippia, polymorphism, zooplankton.

SUMÁRIO

IDENTIFICAÇÃO

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TÍTULO: Revisão taxonômica do complexo – *Ceriodaphnia cornuta* no Brasil: uma abordagem integrativa.

APRESENTAÇÃO

A presente dissertação foi convertida em formato de artigo científico e traduzido para a língua inglesa. A formatação segue as normas da revista Hydrobiologia, Special issue: Cryptic Diversity.

INTRODUCTION

Among the microcrustaceans of continental aquatic environments, the superorder Cladocera is distinguished by its notable species richness and biomass. These organisms play a crucial role in the ecosystem of lakes and reservoirs, where they function as essential components of plankton. Beyond their presence in open waters, cladocerans have also been observed to inhabit association with vegetation, fine-grained sediments, and interstitial habitats (Smirnov, 1974; Elmoor-Loureiro, 1997; Kotov, 2006; Sousa et al., 2015). Preliminary estimates of global diversity suggest the presence of approximately 600 valid described species. However, it is important to note that this estimate may be two to four times higher than the actual number of species in existence. Of these, approximately 200 species (one third of the total) have been documented from the Neotropical region (Forró et al., 2008). According to the most recent findings and species redescriptions, the Brazilian cladoceran fauna comprises more than 84% of the described diversity in the Neotropics (Elmoor-Loureiro et al., 2023).

Taxonomic research on the group has advanced significantly in recent years; however, several Neotropical species already known in the literature require taxonomic revision. A significant number of these species were described in the 19th century based on a limited set of superficial and fragile morphological characteristics, which hinders accurate differentiation among them. Furthermore, the existence of cryptic species—defined as those that are morphologically indistinguishable—results in an underestimation of biodiversity within the group (Van Damme et al., 2011; Sousa et al., 2015).

Approximately 35 years ago, Frey (1987) proposed the concept of non-cosmopolitanism in cladocerans, establishing the foundation for taxonomists to conduct detailed analyses of species with restricted distributions on each continent. This shift facilitated the development of new methodologies in cladoceran research, including biogeographic studies. In the Neotropical region, several species were no longer considered cosmopolitan and underwent redescriptions (Sinev, 1998; Sinev & Elmoor-Loureiro, 2010; Van Damme et al., 2011). Despite these efforts, many taxa in Brazil are still regarded as cosmopolitan, which contradicts the concept advocated by Frey (e.g., *Moina macrocopa* Strauss, 1820; *Moina micrura* Kurz, 1874).

Among these groups, the one known as the *Ceriodaphnia cornuta* - complex stands out. The taxonomic history of *Ceriodaphnia cornuta* Sars, 1885 is complex, following the pattern seen in other cladoceran species. The species from Australia was originally described by Georg

Sars in 1885, based solely on the presence of carapace spines. These spines were located on the rostrum, head, fornice, and as bifurcated projections on the posterior region of the carapace. Almost a decade later, Richard described a second species of *Ceriodaphnia*, which he designated *Ceriodaphnia rigaudi* Richard, 1894. This species exhibited minimal differences from *C. cornuta*, primarily by the absence of a head spine and having a simple, non-bifurcated posterior spine.

Four years later, Daday (1898) published a study based on populations from Sri Lanka, where he identified both species of *Ceriodaphnia* occurring at the same locality. Additionally, he observed the presence of intermediate forms—some with a bifurcated posterior spine but lacking the head spine, and others with a simple posterior spine but possessing the head spine. Consequently, he concluded that the two species were not truly distinct, but rather local varieties of the same species, *C. cornuta*.

The controversy surrounding the classification of the group persisted throughout the first half of the 20th century. While some authors supported Daday's view that the two "species" were simply local forms (e.g., Gurney, 1916; Bär, 1924), others proposed that four forms should be recognized based on the presence of spines: *C. cornuta rigaudi*, *C. cornuta intermedia*, *C. cornuta cornuta*, and *C. cornuta paradoxa* (Jenkin, 1934).

Starting in the 1950s, studies on *Ceriodaphnia* began incorporating statistical and ecological approaches. The study of Rzoska (1956), based on populations from the Nile River (Africa), was particularly important, as he demonstrated that carapace spines are variable characters subject to phenotypic plasticity. Consequently, it was proposed that *C. rigaudi* be considered a junior synonym of *C. cornuta*. The phenotypic plasticity of the *C. cornuta* carapace has since been the focus of several studies, which indicate that spine formation is induced by kairomones released by predators into the water (Zaret, 1969; Rietzler, 2008; Gu et al., 2021). This type of plasticity has also been proposed and investigated in other daphniids (e.g., Dodson, 1974; Pijanowska, 1990), highlighting the role of morphological changes as a response to predation. The compound eye is another structure known to exhibit polymorphism. Zaret (1969) and Mort & Kerfoot (1988) reported forms with high coefficients of variation (75-89%) in the pigmented area of the compound eye in their studies on *C. cornuta* in Lake Gatún, Panama.

In the 1980s, Dorothy Berner utilized Scanning Electron Microscopy to investigate the comparative morphology of ephippia from *C. cornuta* and *C. rigaudi*, although without testing

any formal taxonomic hypotheses. She observed some differences in the ornamentation of these ephippia and, based on this, revived the controversy suggesting that the two species might indeed be distinct (Berner, 1985). However, this hypothesis proved inconsistent, given that the geographic distribution of *Ceriodaphnia cornuta* (Australasia) and *Ceriodaphnia rigaudi* (Afrotropical region) differs. Furthermore, other morphological characters and molecular data should be taken into account to verify this differentiation.

Currently, taxonomists have paid little attention to the issues involving *Ceriodaphnia* species, including those related to *Ceriodaphnia cornuta*. Sharma and Kotov (2013) verified Australian populations of the *cornuta*-complex using molecular methods. Mitochondrial markers COI and 16S, as well as the nuclear marker 28S, were employed in the study. Based on these analyses, they identified three distinct local populations with low levels of differentiation among them. However, other populations of *C. cornuta* have been identified outside its native range, such as in the Iberian Peninsula (Alonso, 1996), South Korea (Kotov et al., 2022a), Central and North America (Elías-Gutiérrez et al., 2008), and South America (Elmoor-Loureiro et al., 2023; Fuentes-Reinés et al., 2022). Thus, molecular data suggest that *C. cornuta* represents a complex of cryptic species that cannot be differentiated using traditional techniques, such as the morphology of parthenogenetic females. Therefore, alternative methods are required as proxies for species delineation.

A widely accepted method among taxonomists is the use of thoracic limbs. This approach was introduced in taxonomic studies only in the 1960s—nearly 100 years after the first monographs describing cladocerans were published (Smirnov, 1966). The morphology of these structures holds high taxonomic value for distinguishing species within various families, particularly the Chydoridae family (Smirnov, 1996; Kotov, 2013). Regarding the genus *Ceriodaphnia*, recent studies have demonstrated the taxonomic utility of thoracic limbs for species differentiation (Sharma, 2014; Alonso et al., 2021; Garibian et al., 2023). However, this approach has been underutilized for identifying species complexes such as *C. cornuta*, representing a gap that needs further investigation.

Three aspects of the *Ceriodaphnia cornuta* - complex taxonomy remain incomplete: the study of the limbs of parthenogenetic females, the study of males, and the knowledge of ephippial females, which is based on Berner's work. For many cladoceran groups, the presence of males is crucial for distinguishing species when females lack consistent morphological differences (Kotov, 2015). Male morphology has been successfully used to support the

description of *Ceriodaphnia smirnovi* Alonso, Neretina & Ventura, 2021 and *Ceriodaphnia nikolaii* Garibian, Andreeva & Kotov, 2023.

Regarding ephippia, Kotov et al. (2018) published an identification key based on the shape and ornamentation of ephippia in European *Ceriodaphnia laticaudata* O. F. Müller, 1867 group. While males and ephippial females are rare in natural environments, laboratory methods exist to induce these forms (Sinev & Sanoamuang, 2011). Addressing these knowledge gaps is therefore essential for advancing our understanding of taxonomic aspects related to *Ceriodaphnia cornuta*.

An additional modern approach, integrative taxonomy, can facilitate species recognition by incorporating multiple lines of evidence including morphological, biogeographic, and molecular data (Padial et al., 2010). Taxonomists can employ these tools to evaluate support for or against taxonomic hypotheses.

Thus, the objective of our study was to conduct a taxonomic review of the *Ceriodaphnia cornuta* - complex using populations from Brazilian freshwater bodies. Our goal was to investigate morphometric and morphological patterns, focusing on structures with taxonomic gaps such as spines, compound eye, thoracic limbs and ephippia. We also aim to describe a new species of *Ceriodaphnia* from Brazil. Additionally, we sought to evaluate how populations of *C. cornuta* s.l. vary morphologically according to their geographic distribution and to identify which morphological structures are most reliable for defining the group's taxonomy.

MATERIALS AND METHODS

Study Area and Sampling Locations

This study examined populations of *Ceriodaphnia cornuta* from 16 water bodies distributed across seven Brazilian Hydrographic Regions (as defined by CNRH Resolution 32, 2003) (Table 1; Figure 1). Some samples were obtained from research projects (e.g., Perbiche-Neves et al., 2014, 2015) and other sampling efforts in several river basins. Samplings were taken using conical plankton nets with mesh sizes opening of 68 µm.

All samples were cataloged in the zoological collections of the Laboratório de Taxonomia Animal (UFJ) and the Laboratório de Plâncton (UFSCar) and were preserved in

either 70% or 90% ethanol. Sampling was conducted across various types of aquatic environments, including lentic systems such as shallow ponds (IGA), lakes and lagoons (BAC, Bonita Pond, FOR, GUA, JMED, Mineiros, MAR), and large reservoirs (BBON, EMB, FUR, ITA, RCUR, SSIM), as well as two lotic sections (RPAG and Rio Correntes).

Figure 1: Geographic distribution of sampling sites for *Ceriodaphnia cornuta* Sars, 1885 populations examined in this study across Brazilian Hydrographic Regions.

Table 1: Sampling locations of *C. cornuta* populations verified in this study, including: site codes, Hydrographic Regions (Resolution 32 of CNRH, 2003) and geographic coordinates (decimal degrees) in Latitude and Longitude. (*): Populations not utilized for morphometrics analyses.

Localities - States	Code	HR	Latitude	Longitude
Araguari - MG				
1. Emborcação Reservoir	EMB	Paraná	-18.3779	-47.7343
Bacabal - MA				
2. Brisa's Pond	BAC	Western Northeast Atlantic	-4.2140	-44.8036
Barra Bonita - SP				
3. Barra Bonita Reservoir	BBON	Paraná	-22.6678	-48.3513
Formosa - GO				
4. Cabocla Pond	FOR	São Francisco	-15.8041	-47.2493
Guaramiranga – CE				
5. Guaramiranga's Lake	GUA	Easthern Northeast Atlantic	-4.2601	-38.9441
Igatu – BA				
6. Pond at side of BA-142	IGA	East Atlantic	-18.8211	-41.3219
Itaipu – PR				
7. Itaipu Reservoir	ITA	Paraná	-24.4863	-54.3284
Maragogi - AL				
8. Lake next to inn	MAR	Eastern Northeast Atlantic	-8.9767	-35.1852
Mineiros - GO				
9. Canto do Cerrado Lake	*	Paraná	-17.5641	-52.6383
Planaltina – DF				
10. Bonita Pond	*	Paraná	-15.5842	-47.6975
11. Joaquim Medeiros Pond	JMED	Paraná	-15.6377	-47.6915
Santa Cruz - MS				
12. Paraguai River	RPAG	Paraguay	-18.9688	-57.6488
Santarém - PA				
13. Curuá – Una Reservoir	RCUR*	Amazonic	-2.8116	-54.2991
São José da Barra – MG				
14. Furnas Reservoir	FUR	Paraná	-20.9765	-45.5233
São Simão – GO				
15. São Simão Reservoir	SSIM	Paraná	-18.6729	-50.0716
Sonora - MS				
16. Correntes River	*	Paraná	-17.5211	-54.7402

Samples were sorted using a Zeiss Stemi stereomicroscope. *Ceriodaphnia cornuta* s.l. individuals were identified under a light microscope (Olympus C3) using specialized taxonomic literature as a reference (e.g., Elmoor-Loureiro, 1997). The identification was based on the following diagnostic characters: (1) the presence of a rostral spine, (2) the presence of accessory spines on fornice, and (3) the presence of head spines.

Morphometric analyses

A total of 171 individuals from 12 populations of *Ceriodaphnia cornuta* were measured. Four morphometric measurements were obtained from the specimens under study: (1) Carapace Length, (2) Carapace Height, (3) Head Spine Length, and (4) Posterior Spine Length. Approximately 15 specimens per population were verified, though some populations had fewer individuals due to limited material availability (FOR, GUA). The specimens were mounted on slides with glycerin and measured using a Leica DMLB optical microscope equipped with a Leica Flexacam C3 camera. Measurements were taken using Leica Application Suite X software. Additionally, morphometric assessments of the compound eye (area and perimeter) were conducted. Ten specimens were mounted on slides in lateral view using glycerin, and an ellipse was drawn around the compound eye to measure the pigmented region and the ommatidia.

We calculated mean values, standard deviations, and coefficients of variation for allometric and compound eye measurements using RStudio (RStudio Team, 2020). Frequency distributions with Gaussian curves were generated for carapace length and height measurements to identify potential size-class discontinuities. We performed regression analysis to examine the dependence of (1) head spine length and (2) posterior spine length on allometric carapace dimensions (3) length and (4) height. We log-transformed all data to reduce variance heterogeneity and meet the assumptions of homoscedasticity.

A two-factor analysis of variance (ANOVA) was conducted to examine potential differences in allometric measurements between populations and disparities in population means. Comparative analyses of compound eye area and perimeter were also performed. All ANOVA data were log-transformed to meet the assumption of homogeneity of variance, as verified by Levene's test. Post-hoc Tukey HSD tests identified heterogeneous groups for significant results ($p < 0.001$). All plots were generated using the 'ggplot2' package (Wickham, 2016).

A multivariate canonical analysis of variance (MANOVA/CV) was also conducted to assess the separation of populations based on carapace length and height, as well as eye area and eye perimeter measurements. All data were log-transformed prior to analysis. This analysis was performed using the Candisc package (Friendly & Fox, 2017) in RStudio, and Wilk's λ was employed as the multivariate test. A clustering plot of the populations was also generated using the ggplot2 package.

Morphological analyses

The morphology of parthenogenetic females was examined using phase-contrast optical microscopy. Specimens from each population were mounted in drops of glycerine on glass slides, covered with coverslips and analyzed under an Olympus BX41 optical microscope. To facilitate better visualization of anatomical structures (antennules, spines, and postabdomen), individuals were dissected using fine tungsten wire needles. Head morphology was assessed to determine the presence or absence of spines on the rostrum, head and fornices. Postabdominal morphology was verified in accordance with Sharma's (2014) guidelines, which included evaluating the distribution pattern of spines on the postabdominal claw pecten and counting the number of teeth along the anal margin. The homologies of limb structures followed Garibian et al. (2023). All drawings were made using a *camera lucida* and digitally covered using a graphic tablet.

External morphology was also studied using Scanning Electron Microscopy (SEM) imaging. Specimens were fixed in 2.5% glutaraldehyde and 90% ethanol, then placed in perforated cylindrical polyethylene chambers. Within these supports, individuals were washed, fixed, and dehydrated. Washing was carried out in 0.1 M phosphate buffer at pH 7.3 (three times, 5 minutes each). Specimens were then fixed by immersion in 0.5% osmium tetroxide (in water) for 20 minutes. Dehydration was performed through a graded ethanol series in the following sequence: 7.5%, 15%, 30%, and 50% (twice each for 5 minutes), followed by 70% (three times, 10 minutes each), and finally 90% and 100% (twice each for 5 minutes). The material was then processed in a BALZERS UNION critical point dryer (model CTD-020), where ethanol within the specimens was replaced with liquid carbon dioxide, which was subsequently evaporated by raising the temperature to 40 °C under 70 bar pressure. Specimens were then mounted on stubs using adhesive tape and coated with a 15 nm layer of gold using a

BALZERS UNION sputter coater (model MED-010). Observations were carried out using a Phillips SEM (model SEM-515) equipped with a camera.

Abbreviations of the scientific collections. FDRS: Personal collection of Francisco Diogo Rocha Sousa tombed in Universidade Federal de Jataí.

Abbreviations used in the figures and the text. A1 = Antennule; A2 = Antenna; as = accessory seta; en = endite; ep = epipodite; ex = exopodite; gn = gnathobase; IDL = Inner Distal Lobe; il = Inner Lobe; ODL = Outer Distal Lobe; pep = pre-epipodite; s = sensillum.

RESULTS

Morphometrical analyses

The Gaussian curve in the frequency distribution graph showed a single peak for body length, whereas carapace height displayed two distinct frequency peaks (Figure 2, C–D). The highest body length frequence was observed around 440 µm, while the highest carapace height frequencies were concentrated around 230 µm and 330 µm (Table 2).

The presence of spines varied among the studied populations (Table 2). For example, individuals lacking spines on the head and posterior region of the valves were observed in BAC, JMED and MAR (small urban ponds). The IGA population (a small floodplain ponds adjacent to a river) exhibited only one individual with a head spine. The population with the highest mean head spine length was ITA (mean = 69.45 µm), while the EMB population showed the highest mean spine length on the valves (mean = 62.20 µm).

Table 2: Mean, standard deviation, and coefficient of variation of morphometric measurements: from Brazilian populations of *C. cornuta*. n: number of individuals analyzed per population. Population Codes: BAC = Bacabal; BBON = Barra Bonita; EMB = Emborcação; FOR = Formosa; FUR = Furnas; GUA = Guaramiranga; IGA = Igatu; ITA = Itaipu; JMED = Joaquim Medeiros; MAR = Maragogi; RPAG = Rio Paraguai; SSIM = São Simão.

Codes	Length (µm)	Height (µm)	Head Spine Length (µm)	Posterior Spine Length(µm)	Eye Area (µm^2)	Eye Perimeter (µm)
BAC	369.30 ± 23.48 (6.35%)	263.53 ± 26.23 (9.95%)	-	-	1126.58 ± 183.51 (16.28%)	120.46 ± 10.01 (8.31%)
BBON	343.53 ± 79.26 (23.07%)	242.17 ± 65.69 (27.12%)	37.43 ± 20.76 (55.47%)	44.26 ± 25.45 (57.49%)	609.25 ± 339.06 (55.65%)	84.97 ± 24.12 (28.38%)
EMB	417.74 ± 53.34 (12.76%)	302.80 ± 44.77 (14.78%)	64.93 ±24.50 (37.74%)	62.20 ± 21.66 (34.83%)	805.19 ± 152.86 (18.98%)	100.37 ± 9.57 (9.53%)
FOR	423.67 ± 82.50 (19.47%)	332.12 ± 71.71 (21.59%)	63.93 ± 15.84 (24.78%)	51.82 ± 23.71 (45.76%)	623.89 ± 105.22 (16.86%)	88.42 ± 7.74 (8.76%)
FUR	370.19 ± 54.48 (14.71%)	259.24 ± 52.25 (20.15%)	23.81 ± 14.03 (58.95%)	33.23 ± 12.53 (37.72 %)	542.13 ± 184.57 (34.04%)	81.95 ± 13.26 (16.18%)
GUA	327.68 ± 40.33 (12.30%)	244.23 ± 35.05 (14.35 %)	5.22 ± 0.45 (8.66%)	7.55 ± 2.01 (26.66%)	1065.12 ± 303.18 (28.46%)	115.82 ± 15.84 (13.68%)
IGA	319.74 ± 36.05 (11.27%)	228.95 ± 44.22 (19.31%)	8.95	14.96 ± 4.20 (28.11%)	927.37 ± 243.56 (26.26%)	108.72 ± 13.22 (12.16%)
ITA	387.57 ± 58.82 (14.66%)	287.70 ± 48.05 (16.70%)	69.45 ± 24.58 (35.39%)	61.40 ± 27.46 (44.73%)	614.18 ± 193.58 (31.51%)	90.22 ± 19.52 (21.64%)
JMED	419.4 ± 29.55 (7.04%)	341.68 ± 28.16 (8.24%)	-	-	1265.35 ± 251.91 (19.90%)	126.89 ± 11.81 (9.31%)
MAR	381.92 ± 39.68 (10.39%)	290.40 ± 46.11 (15.87%)	-	8.97 ± 1.25 (14.03%)	1511.09 ± 374.77 (24.80%)	138.41 ± 15.95 (11.52%)
RPAG	307.85 ± 68.74 (22.32%)	210.61 ± 55.73 (26.46%)	17.29 ± 12.59 (72.81%)	20.62 ± 11.27 (54.64%)	429.17 ± 188.87 (44.00%)	73.27 ± 15.13 (20.66%)
SSIM	448.51 ± 39.85 (8.88%)	323.75 ± 31.52 (9.73%)	55.97 ± 20.31 (36.29%)	60.39 ± 32.72 (54.18%)	669.08 ± 123.18 (18.41%)	91.67 ± 8.71 (9.50%)

Both ITA and EMB are large reservoirs in terms of area and volume. For populations exhibiting posterior carapace spines, the coefficient of variation was high (e.g. BBON = 57.49% and SSIM = 54.18%; see Table 2).

Carapace length differed significantly among the verified populations (F_{12-1} = 9.881; $p < 0.001$). The SSIM population from a large reservoir exhibited the highest mean length (mean = 448.51 µm), while the RPAG population from a lotic environment exhibited the lowest (mean = 307.85 µm) (Figure 2-A; Table 2).

The ANOVA results also indicated significant differences in carapace height among the populations (F_{12-1} = 10.75; $p < 0.001$). JMED had the highest mean carapace height (mean = 341.68 µm), while RPAG had the lowest (mean = 210.61 µm) (Figure 2-B; Table 2).

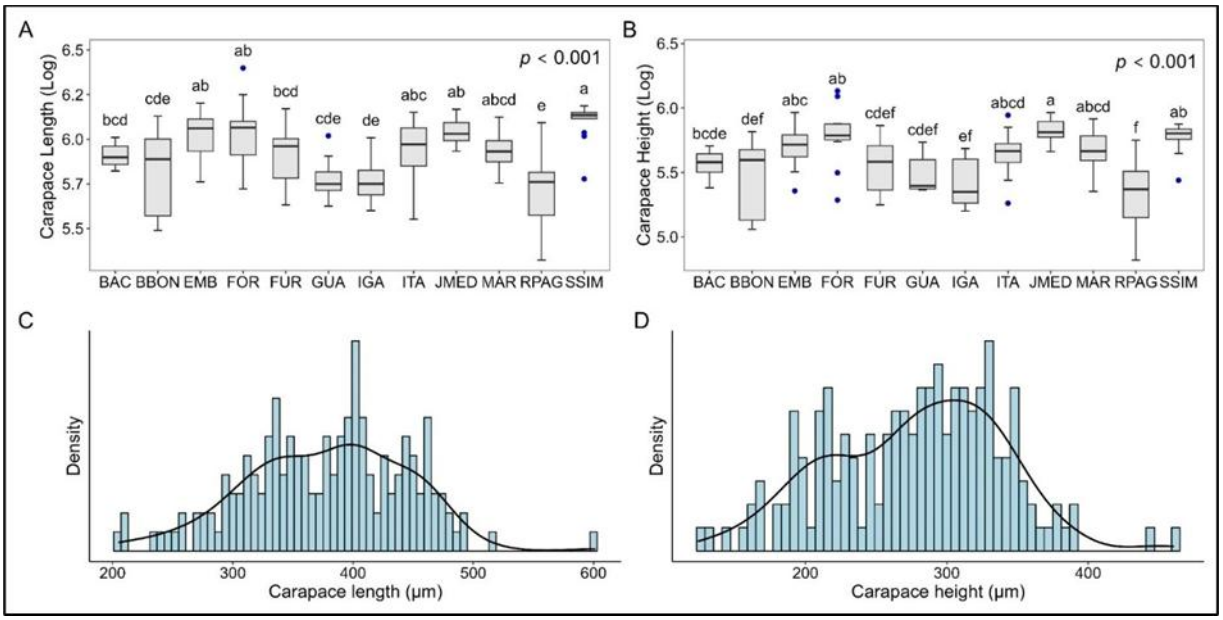

Figure 2: A) ANOVA for carapace length among populations. **B)** ANOVA for carapace height among populations. **C)** Frequency distribution of carapace length among populations. **D)** Frequency distribution of carapace height among populations.

The morphometric results for eye area showed statistically significant differences among populations (F_{12-1} = 17.13; $p < 0.001$). On average, the eye area of the MAR population—a small marginal pond—was larger compared to the other populations

(1511.09 µm²), while the RPAG population exhibited the lowest mean values (429.17 µm²). The greatest statistical differences ($p < 0.001$) were also observed between the BAC and MAR populations (Figure 3-A; Table 2).

There was significant variation in eye perimeter among populations ($F_{12-1} = 16.53$; $p < 0.001$). The MAR population had the highest mean eye perimeter (mean = 138.41 µm), and the RPAG population had the lowest mean (mean = 73.27 µm). The most significant differences in mean eye perimeter occurred among the BAC, JMED, and MAR populations (Figure 3-B; Table 2).

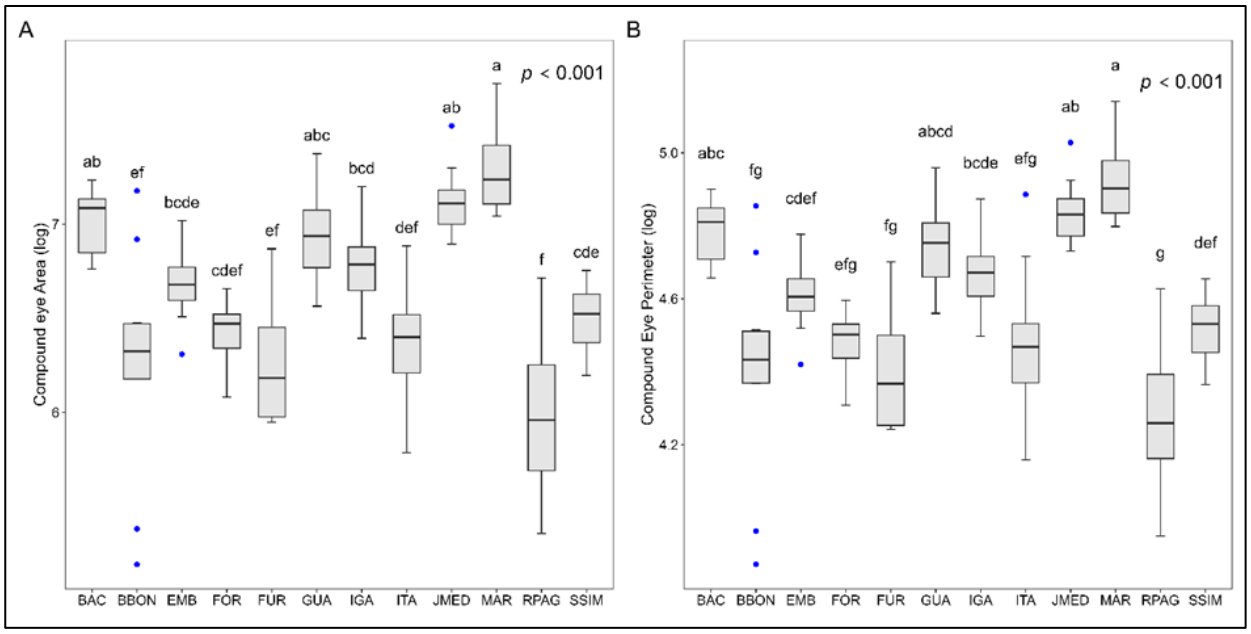

Figure 3: A) ANOVA for eye area among *C. cornuta* populations ($p < 0.001$); **B)** ANOVA for eye perimeter among populations ($p < 0.001$).

Regression analyses comparing carapace spine measurements with allometric values revealed no significant correlation between these two variables. The R^2 values were 0.29 (head spine length × carapace length), 0.24 (head spine length × carapace height), 0.34 (posterior spine length × carapace length), and 0.32 (posterior spine length × carapace height). These values indicate that spine measurements are not influenced by allometric factors (see Figure 4, A–D).

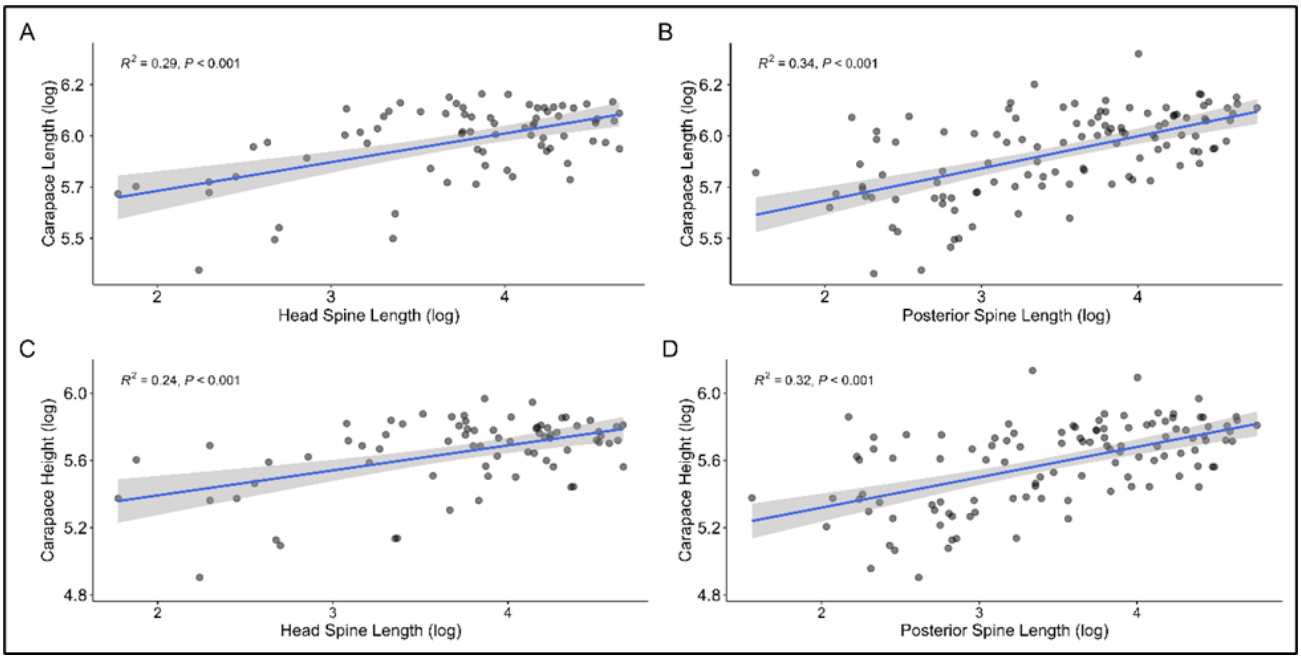

Figure 4: Regression analyses between spine length and allometric values. **A)** Head spine length × Carapace length; **B)** Posterior valve spine length × Carapace length; **C)** Head spine length × Carapace height; **D)** Posterior valve spine length × Carapace height.

Canonical multivariate analysis of variance (MANOVA) revealed differences among the studied populations (Wilk's lambda = 0.09905, F = 7.2544, $p < 0.001$). The JMED population was strongly separated from all other populations except MAR. The BAC, GUA, and IGA populations also exhibited tighter clustering relative to the others. (Figure 5).

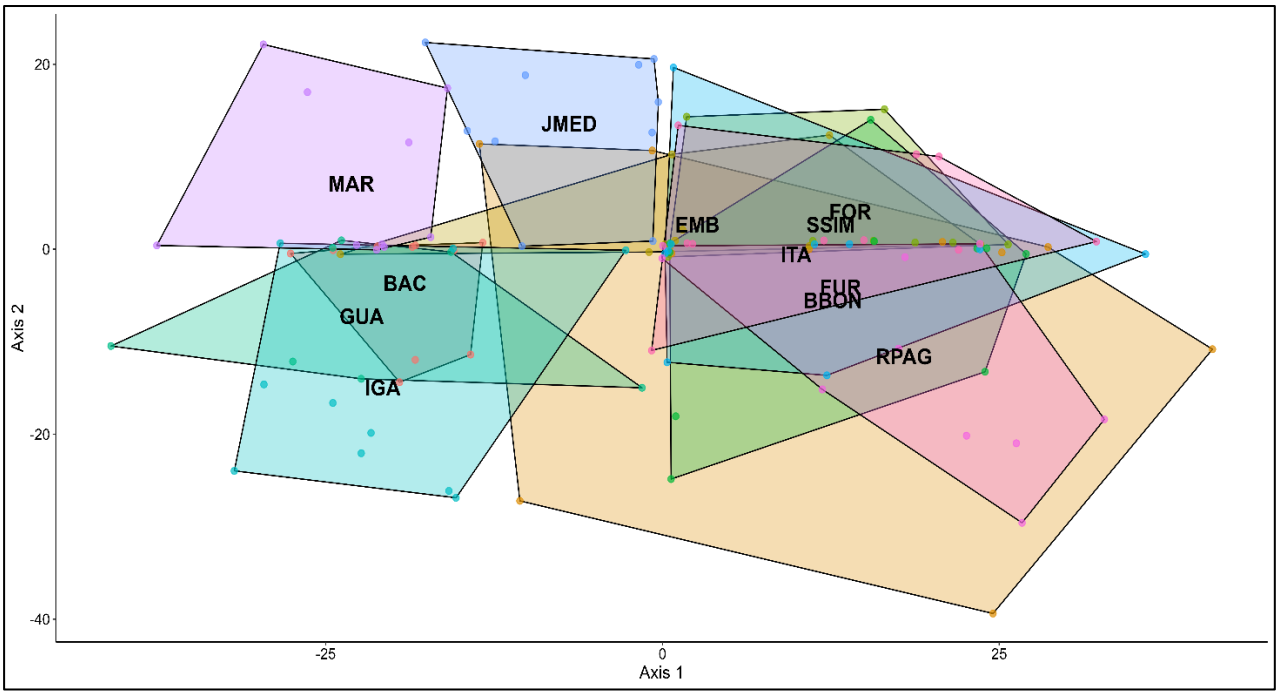

Figure 5: Clustering analysis on the four morphometric variables used in the Canonical Variate MANOVA, showing the separation among *Ceriodaphnia* populations (Wilk's $\lambda = 0.09905$; $F = 7.2544$; $p < 0.001$). For localities codes, see Table 1.

Taxonomy

Class Branchiopoda Letreille, 1817

Order Anomopoda Sars, 1865

Family Daphniidae Straus, 1865

Genus *Ceriodaphnia* Dana, 1853

Ceriodaphnia cornuta Sars, 1885 s.l. from Brazil

(Figure 7-8)

Material examined. Fifteen parthenogenetic females from Brisa's Pond, Bacabal, Maranhão, Brazil (4° 13' 53" S, 44° 45' 20" W), material collected by Arielly de Sousa Santos on 2023. Fifteen parthenogenetic females from Barra Bonita Reservoir, Barra Bonita, São Paulo, Brazil (22° 30' 26" S, 48° 32' 49" W), material collected by Gilmar Perbiche Neves on 2010. Fifteen parthenogenetic females from Emborcação Reservoir, Araguari, Minas Gerais, Brazil (18º 22' 40.4 S, 47º 44' 3.58" W), material collected by

Gilmar Perbiche Neves on 2010. Twelve parthenogenethic females from Cabocla Pond, Formosa, Distrito Federal, Brazil (15°48'15.00" S, 47°14'57,50" W), material collected by Lourdes Maria Abdul Elmoor-Loureiro on december, 2009. Fifteen parthenogenetic females from Furnas Reservoir, São José da Barra, Minas Gerais, Brazil (20º 58' 35.58" S, 45º 31.24' 18" W), material collected by Gilmar Perbiche Neves on 2010. Ten parthenogenetic females from Guaramiranga Lake, Guaramiranga, Ceará, Brazil (2° 48' 58" S 54° 17' 57" W), material collected by Lourdes Maria Abdul Elmoor Loureiro. Fifteen parthenogenetic females from a Pond at side of BA-142, Igatu, Bahia, Brazil (12° 53' 55" S 41° 19' 01" W). Fifteen parthenogenetic females from Itaipu Reservoir, Itaipu, Paraná, Brazil (25° 23' 43" S 54° 33' 59" W), material collected by Gilmar Perbiche Neves on 2010. Three parthenogenetic females from Canto do Cerrado Lake, Mineiros, Goiás, Brazil (17°33'51"S 52°32'18"W), material collected by Vinícius Vilela Carvalho on 2023. Fifteen parthenogenetic females from a marginal pond near of Maragogi, Alagoas, Brazil (9° 00' 14" S 35° 12' 53" W), material collected by Lourdes Maria Abdul Elmoor Loureiro. Two parthenogenetic females from Correntes River, lotic portion, Sonora, Mato Grosso do Sul, Brazil (17° 31' 16" S 54° 44' 25" W). Fourteen parthenogenetic females from Curuá-Una Reservoir, Santarém, Pará, Brazil (2°48'58"S 54°17'57"W), material collected by Ciro Yoshio Joko in 1978. Fifteen parthenogenetic females from a lotic portion on Rio Paraguai, Santa Cruz, Mato Grosso do Sul, Brazil (20° 59' 47" S 57° 49' 52" W), material collected by Gilmar Perbiche Neves on 2010. Fifteen parthenogenetic females from São Simão Reservoir, São Simão, Goiás, Brazil (18º 40' 22.54" S, 50º 4' 17.76" W), material collected by Gilmar Perbiche Neves on 2010.

Description of parthenogenetic female

General Habitus (Figure 8-A). Body is rounded in lateral view, not laterally compressed, lateral projections as fornices present. Length ranging between 230-570 µm, height/length ratio about 0.7315 µm.

Carapace (Figure 8-D). Ornamented with several polygons. Several pores distributed along the carapace (Figure 7, Table 3). Ventral margin of valve with up 17 short denticles on the external face; inner face presenting short spines and up five setulae (Figure 8-E). Dorsal margin of valve presenting posterior part convex. Posterodorsal margin with one or two spines. *Head* (Figure 6-A, Figure 8 F-G) with dorsal margin with one spine,

marginal line concave behind the spine, interrupted by a deep depression; a dorsal head pore present); fornices as a short or long spines.

Cephalic structures (Figure 8). Compound eye large. *Rostrum* (Figure 8 F-G) with a spine. *Labrum* (Figure 8 F-G). With margin evenly curved in lateral view, setulated distal plate. *Antennules* – A1 (Figure 8 - H). Short, about 1.3 times longer than wide, antennular body naked; antennular sensory seta with thick base, about 1.3 times longer than the length of the antennular body, inserted at 3/3 of antennular body; nine terminal aesthetascs, which one similar in length to antennular body. *Antenna* – A2 (Figure 8 - I). Basipodite thick; coaxal setae of different length, proximal one with length about 0.2-0.3 of distal one; distal sensory seta length about 1.9 of distal coaxal seta. Branches of similar length; exopodite with four segments, endopodite with three segments. Exopodite with a row of long lateral setulae on the second segment; third segment armed with a bisegmented plumose seta; fourth segment armed with three apical bisegmented plumose setae, which one shorter than others. Endopodite first segment about two times longer than second segment, armed with a bisegmented plumose seta; second segment armed with a bisegmented plumose seta; third segment with a row of long lateral setulae, three apical bisegmented plumose setae. Antennal formula (exo/endo): spines 0000/000, setae 0033/113.

Thoracic limbs (Figure 9): Five pairs of thoracic limbs.

First limb (Figure 9-A). Epipodite oval. Accessory setae not studied. ODL armed with two setae; first seta markedly short and naked, about of 0.06 of length of the second seta; second seta naked. IDL (en4) with one-three setae. Endite 3 with two posterior setae (a-b), anterior seta 1 might be present or absent. Endite 2 with two posterior setae (c-d), anterior seta 2 might be present or absent. Endite 1 with four posterior setae (e-h), anterior seta 3 with a tip sharp. Ejector hooks of different length, armed with short spines.

Second limb (Figure 9-D). Exopodite elongated, without a row of setulae laterally inserted, armed, with two setulated setae of similar length. Endite 5 armed with two setulated posterior setae (a-b), anterior seta absent. Endite 4 armed with one setulated posterior seta (c), anterior seta absent. Endite 3 armed with one setulated seta (d), anterior seta presenting tip sharp. Endite 2 armed with a short seta. Endite 1 (gnathobase) with two rows of setae; on the anterior row the first seta is absent, seta 2 markedly longer,

about 1.7 times longer than seta 3 and 4.3 longer than seta 4; posterior row with six setae (a-f), setae (f) longer than others setae on the row.

Third limb (Figure 9-F). Pre-epipodite elongated, setulated. Epipodite oval. Exopodite flat, wide, armed with four distal (1-4) and two lateral setae (5-6), both setae are plumose; setae 1-3 similar in length; setae 4 and 6 similar in length, about 2.3 times longer than seta 5. Inner distal portion of limb with five endites; fifth endite armed with a single anterior seta (a); fourth endite armed with a single anterior seta (b); third endite armed with a single anterior seta (c); second endite armed with a single seta (d); first endite as a large lobe armed with a single anterior seta (e), two elements on the inner face which has different length and numerous posterior setae forming a filter plate (Figure 9-G).

Fourth limb (Figure 9-H). Pre-epipodite elongated, setulated. Epipodite oval. Exopodite flat, wide, armed with four distal (1-4) and two lateral setae (5-6), both setae are plumose; setae 3 and 4 similar in length; seta 2 about of 0.7 of first seta length; seta 5 about 0.3 of sixth seta length. Inner distal portion of limb without lobes clearly separated, armed with two relatively short setae (1-2); filter plate with numerous posterior setae.

Fifth limb (Figure 10-A). Pre-epipodite subquadrangular and densely setulated. Epipodite large, oval. Exopodite short, rectangular shaped, armed with three setae; seta 2 slightly longer than seta 1; seta 2 about 1.8 times shorter than seta 3. Inner lobe portion elongated, armed with a seta about two times longer than exopodite seta 3, about two times longer than exopodite seta 2.

Abdominal and postabdominal structures: *Abdomen* (Figure 10-B). With two transverse rows of setulae present at its dorsal surface; two short process present. *Postabdomen* (Figure 10-B). Elongated, relatively narrowing distally. Preanal margin straight without prominent angles. Postanal margin about 2.9 times shorter than preanal margin, bearing 4-7 pairs of marginal teeths which increasing in length distally (Figure 10-C). *Postabdominal seta* slightly longer than postabdomen length. *Postabdominal claw* (Figure 10-D). Markedly curved, about 1.5 times longer than anal margin, with tip sharp; pecten organized in two groups, proximal one armed with 10-16 spines relatively long, distal one presenting minute and fine spines not reaching the tip of claw.

Male. Not studied here. Deng et al. (2022), studied Chinese *Ceriodaphnia cornuta* populations and found that morphology of males is similar in habitus. Body rounded and not laterally compressed, though smaller in size, with an approximate length of 300 µm. Antennular accessory seta is short and thin, smaller than the antennule. First Limb bear a curved copulatory hook and a long, bi-segmented seta that exceeds 100 µm in length.

Ephippial female. Not studied here. Berner studied populations of the *cornuta* group from the United States and reported differences between the ephippial females of *Ceriodaphnia rigaudi* from the South Africa and *C. cornuta* s.l. The ephippium of *C. cornuta* s.l. has columns covering the surface more granular and more spaced between them. (Berner et al., 1985).

Remarks and variability. Within material studied, we observed variation in length of spines on the rostrum, head and fornices. The postabdomen presented 4-7 marginal teeth. On the thoracic limbs the main variation observed is related to the number of setae on the IDL and the number of anterior setae in the first limb (Figure 9 A-C). Morphological variations are summarized in Table 3.

Distribution and biology. *Ceriodaphnia cornuta* s. l. is a widely reported species in studies encompassing nearly all Brazilian Hydrographic Regions. It has been recorded as a common component of various freshwater systems in the following states: São Paulo (Rocha et al., 2011), Rio de Janeiro (Macêdo et al., 2022), Minas Gerais (Santos-Wisniewski, 2011), Federal District (Sousa & Elmoor-Loureiro, 2012), Goiás (Carvalho et al., 2024), Mato Grosso (Brito et al., 2020), Mato Grosso do Sul (Zanata et al., 2017), Pernambuco (Soares et al., 2011), Bahia (Macêdo et al., 2021), and Maranhão (Santos, 2021). It is also frequently cited in Neotropical studies in Paraguay (Duré et al., 2023), Colombia (Fuentes-Reinés et al., 2015), Venezuela (Rivas et al., 2023), and Mexico (Elías-Gutiérrez et al., 2006). Globally, *C. cornuta* s.l. has been recorded on all continents, preferring tropical and oligotropical waters. Africa (Smirnov, 2008), Asia (Deng et al., 2023; Kotov et al., 2018), Europe (Armengól, 1980). Should be considered a natural area of distribution of *Ceriodaphnia cornuta* Oceania (Timms, 1988; Sharma & Kotov, 2013). It is a species with broad tolerance to low oxygen concentrations and temperature fluctuations. It exhibits a filter-feeding trophic habit and prefers diatoms and green algae as food sources (Villalobos & González, 2006). In a study on populations from Madurai,

India, Murugan (1990) reported a short life cycle for species of 21 days, with reproduction primarily occurring through parthenogenesis under favorable environmental conditions.

Table 3: Comparison of morphological characters among *Ceriodaphnia cornuta* populations. **Carapace**: Height/length ratio; presence or absence of pores. **Head**: presence or absence of rostral and fornice spines. **Postabdomen**: Number of anal teeth and morphology of the pecten. **Thoracic Limbs**: Variation of each limb setaes. Codes: BAC = Bacabal; BBON = Barra Bonita; EMB = Emborcação; FOR = Formosa; FUR = Furnas; GUA = Guaramiranga; IGA = Igatu; ITA = Itaipu; JMED = Joaquim Medeiros; MAR = Maragogi; RCUR = Rio Curuá-Una; RPAG = Rio Paraguai; SSIM = São Simão.

	BAC	BBON	EMB	FOR	FUR	GUA	IGA	ITA	JMED	MAR	RCUR	RPAG	SSIM
Carapace													
Height/Length	0.64-0.77	0.61-0.83	0.66-0.78	0.64-0.88	0.61-0.86	0.64-0.86	0.63-0.91	0.65-0.88	0.73-0.93	0.61-0.83	-	0.56-0.77	0.67-0.78
Pores	+	+	+	+	+	+	+	+	+	+	+	+	+
Head													
Rostral Spine	+	+	+	+	+	+	+	+	+	+	+	+	+
Fornice Spines	-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	-	+/-	+/-	+/-	+/-
PostAbdomen													
Nº Anal Teeth	4-6	4-6	5-7	6	5-6	4-5	5	6-7	2	5-7	5-6	5-6	5-6
Thoracic Limbs													
Limb 1- Nº IDL Setae	2	2	1	2	1	2	2	1	2	2	1 or 3	1	1
Limb 1- Nº Anterior Setae	3	3	1	3	1	3	3	1	3	3	1 or 3	1	1
Limb 2- E3 Anterior Setae	Tip Sharp	Tip Sharp	Tip Sharp	Tip Sharp	Tip Sharp	Tip Sharp	Tip Sharp	Tip Sharp	Tip Rounded	Tip Sharp	Tip Sharp	Tip Sharp	Tip Sharp
Limb 3 Nº Filter Plate Setae	27	30	33	33	30	26	28	32	27	27	30	31	31
Limb 4 Filter Nº Plate Setae	14	18	20	17	17	14	16	19	17	15	16	18	19

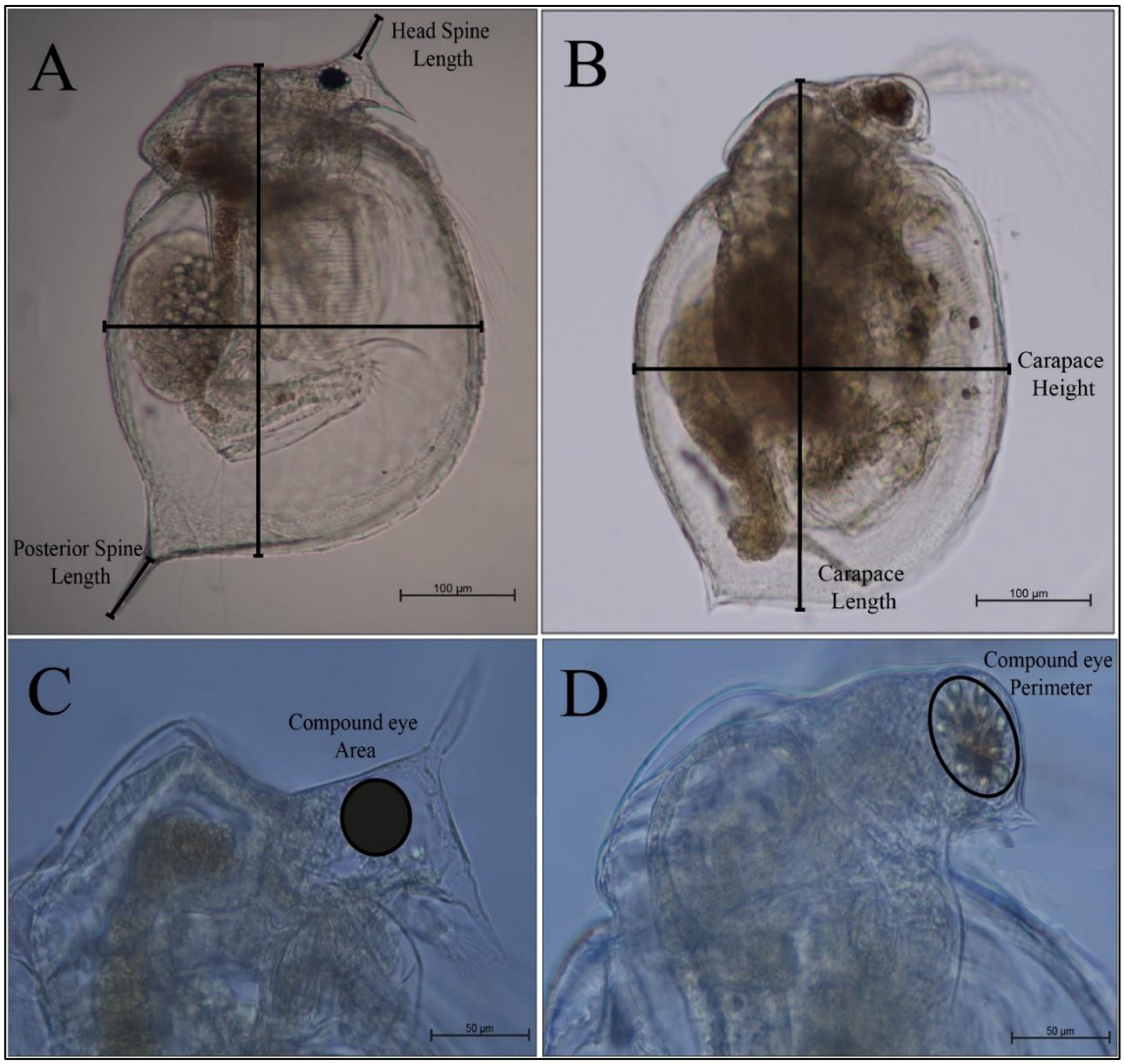

Figure 6: Measurements utilized in morphometric analyses**. A**) *Ceriodaphnia cornuta* s.l., EMB population, habitus (magnification 10x). **B)** *Ceriodaphnia* sp.nov., JMED population. **C)** *Ceriodaphnia cornuta* s.l., head morphology. **D)** *Ceriodaphnia* sp. nov., head morphology.

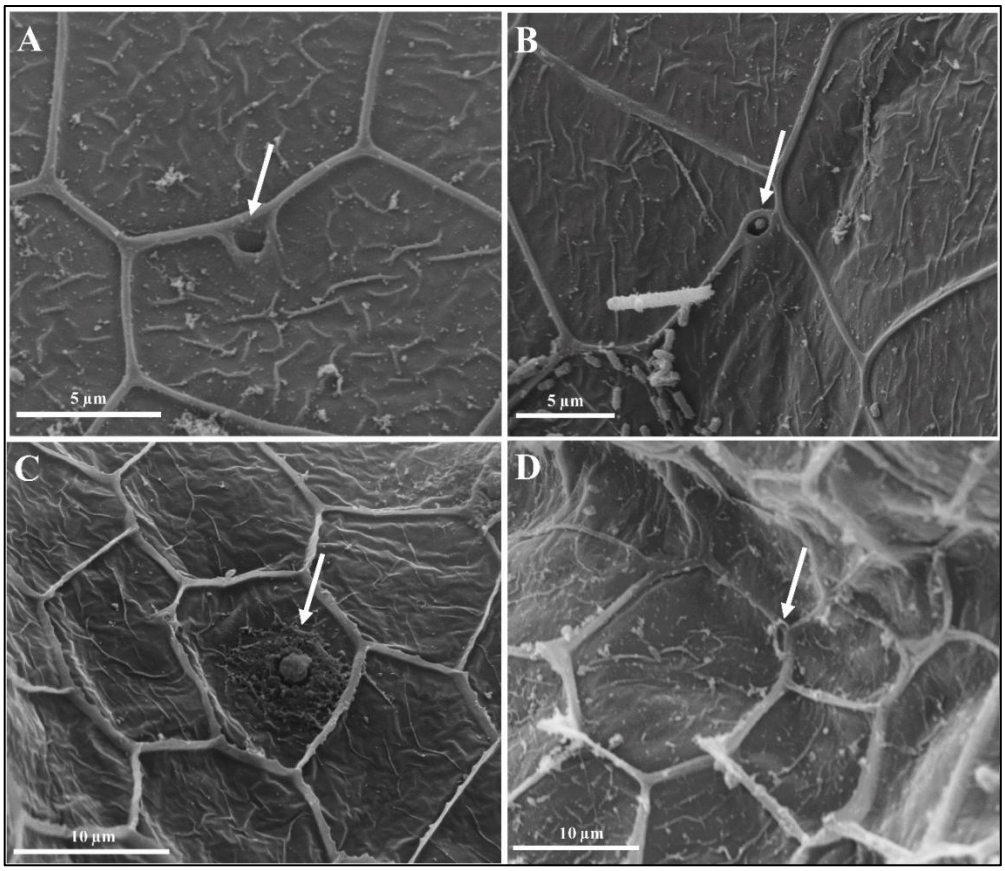

Figure 7: Ultrastructure of pores through SEM images along the carapace, indicated by arrows. **A)** 10,396× magnification; **B)** 10,969× magnification; **C)** 8,583× magnification; **D)** 7,000× magnification.

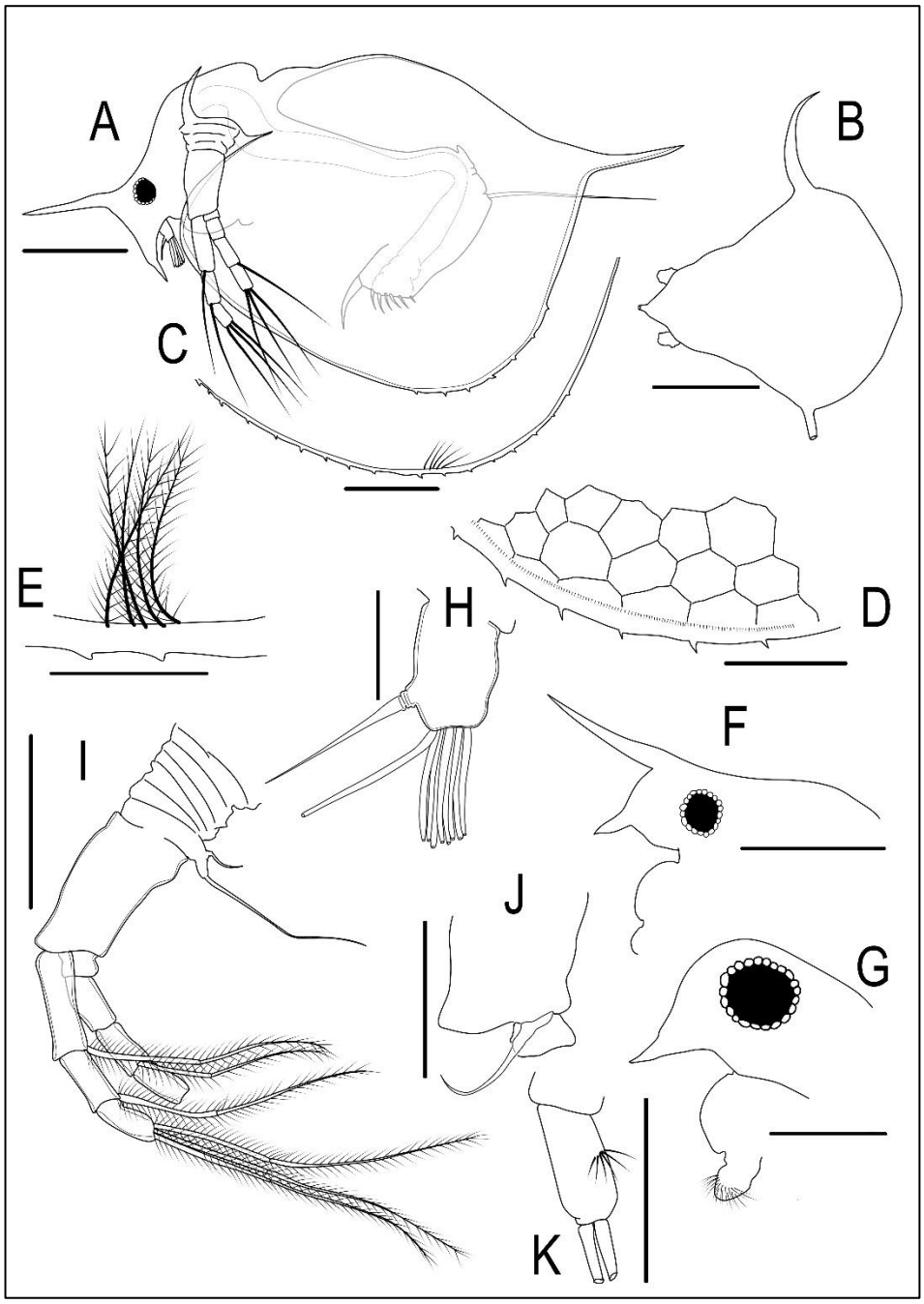

Figure 8: *Ceriodaphnia cornuta* s.l. parthenogenetic female from São Simão Reservoir, São Simão, Goiás, Brazil. A) Adult parthenogenetic female, lateral view. B) Headshield, dorsal view. C) Valve ventral margin, inner view. D) Valve ventral margin, denticles and ornamentation. E) Armature of valves. F) Head with spine, lateral view. G) Head without spine, lateral view. H) Antenna I. I) Antenna II. J) Coxopodite of antenna II. K) Distal part of antenna II basipodite and first two segments of exopodite. Scale bars (A, B, C, E, F, G, J, K) 100 µm. (D, H) 50 µm. (I) 25 µm.

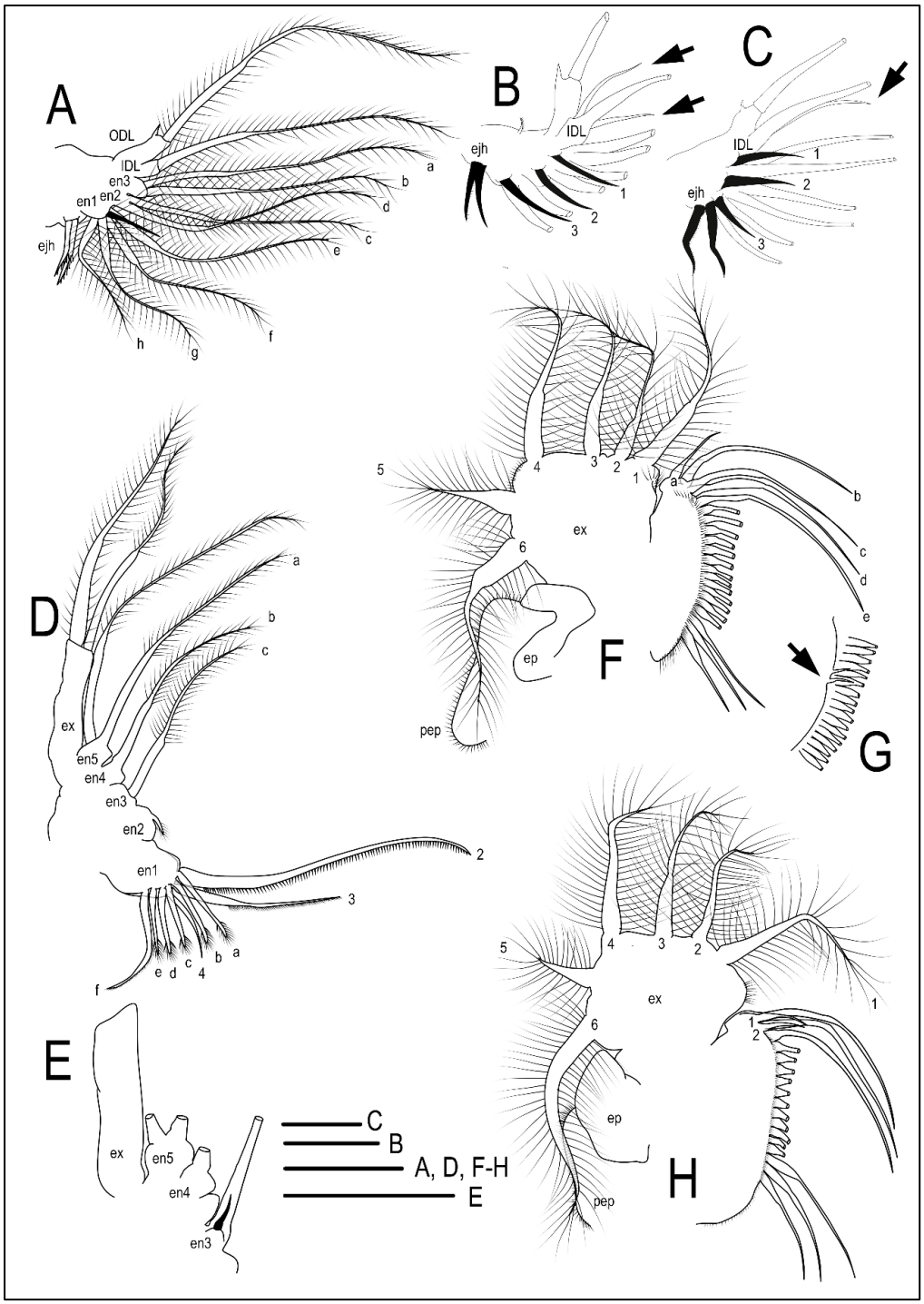

Figure 9: (A, D-H) *Ceriodaphnia cornuta* s.l. parthenogenetic female from São Simão Reservoir, São Simão, Goiás, Brazil. (B) *C. cornuta* s.l. parthenogenetic female from Curuá-Una Reservoir, Santarém, Pará, Brazil. (C) *C. cornuta* s.l. parthenogenetic female from shallow pond, Igatu, Bahia, Brazil. A. First Limb. B-C, idem, arrow showing variation on the number of IDL setae. D) Second Limb. E) Second Limb, Endites and Exopodite. F) Third Limb. G) Third Limb, filter plate inner face. H) Fourth Limb. Scale bars 100 µm.

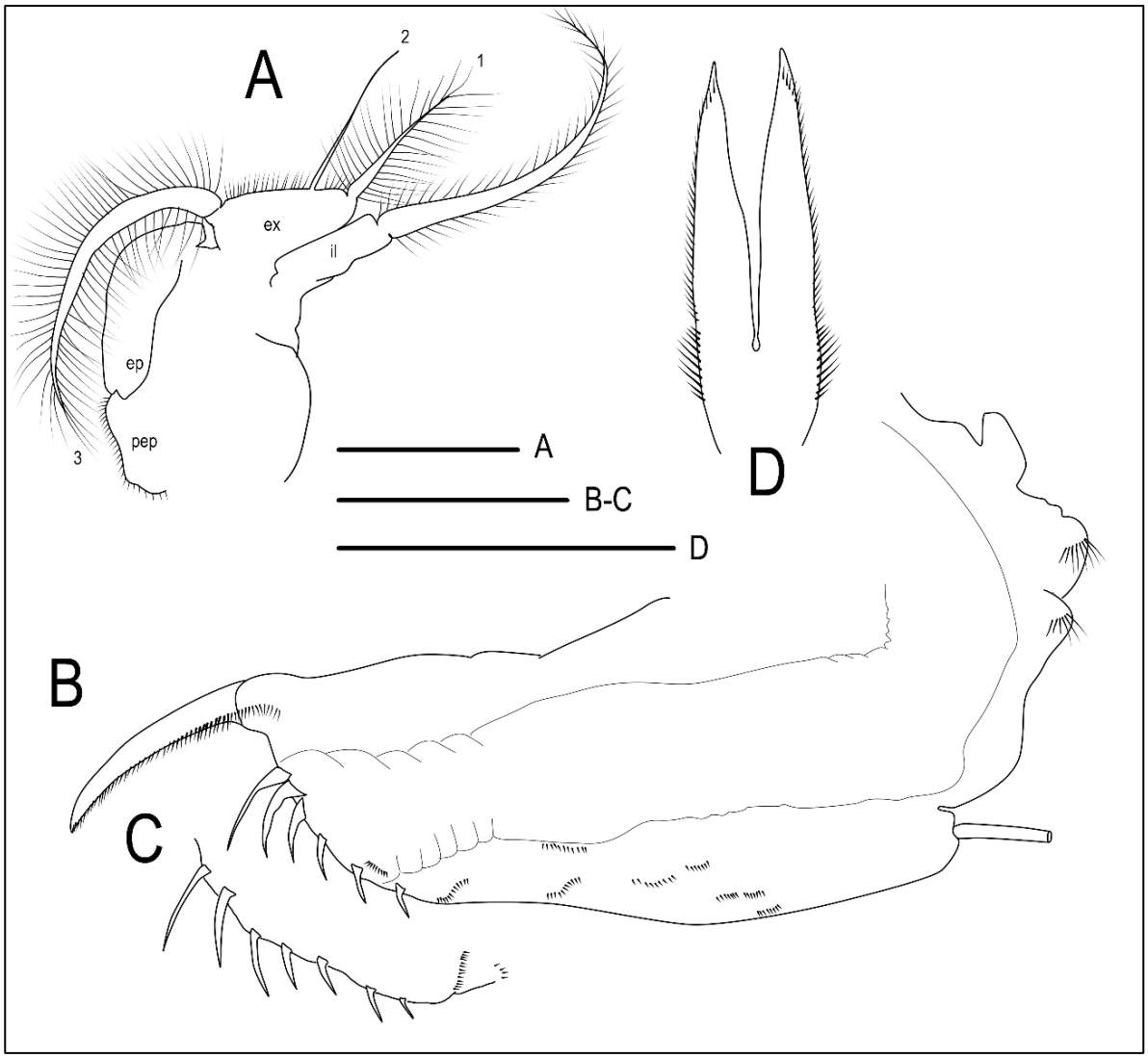

Figure 10: *Ceriodaphnia cornuta* s.l. parthenogenetic female from São Simão Reservoir, São Simão, Goiás, Brazil. A) Fifth Limb. B) Abdomen and Postabdomen, lateral view. C) Postabdomen, anal margin teeth. D) Postabdominal claw, dorsal view. Scale bars 100 µm.

Description of a new species

***Ceriodaphnia* sp. nov.**

Ceriodaphnia cornuta in Sousa & Elmoor-Loureiro (2012) and Sousa et al. (2018)

(Figure 11)

Type locality. Joaquim Medeiros Pond, Planaltina, Distrito Federal, Brazil (15° 38' 15.9" S, 47° 41' 29.5" W).

Material examined. Eleven parthenogenetic females and one ephippial females from Joaquim Medeiros Pond, Planaltina, Distrito Federal, Brazil (15° 38' 15.9" S, 47° 41' 29.5" W), material colected by Lourdes Maria Abdu Elmoor-Loureiro on 13.v.2017 (FDRS0818); Seven parthenogenetic females from the Bonita Pond, Estação Ecológica de Águas Emendadas, Planaltina, Distrito Federal, Brazil (15° 35' 22.1" S, 47° 41' 50.1" W), material collected by Francisco Diogo Rocha Sousa on 12.ix.2008 (FDRS0822).

Material Type. Holotype: a parthenogenetic female deposited in a tube with ethanol at Museu de Zoologia da Universidade de São Paulo, Brazil, under access number MUZUSPXXX. **Paratypes**: see material examined.

Description of parthenogenetic female

General Habitus (Figure 11 A-B). Body is rounded in lateral view, not laterally compressed, without lateral projections. Length ranging between 381-477 µm, height/length ratio about 0.815 µm.

Carapace (Figure 11 C-D). Ornamented with several polygons. Ventral margin of valve evenly curved, with up 19 short denticles on the external face; inner face presenting short spines and up five setulae. Dorsal margin of valve evenly curved. Posterodorsal margin with one a short projection, tip sometimes acute (Figure 11 F). *Head* (Figure 11 G-I) with dorsal margin smooth, without spine, marginal line concave behind the eye, interrupted by a deep depression; fornices obtuse, without projections or spines.

Cephalic structures (Figure 11 G-I). Compound eye large occupying a significant portion of domo supraocular. *Rostrum* (Figure 11 G) with a spine. *Labrum* (Figure 11 G-H). With margin evenly curved in lateral view. *Antennules* – A1 (Figure 12 A). Short, about 1.6 times longer than wide, antennular body naked; antennular sensory seta with thick base, about 1.5 times longer than the length of the antennular body, inserted at 3/3 of antennular body; nine terminal aesthetascs, which one similar in length to antennular body. *Antenna* – A2 (Figure 12 B). Basipodite thick; coaxal setae of different length, proximal one with length about 0.-0.3 of distal one; distal sensory seta about middle length of distal coaxal seta. Branches of different length; exopodite with four segments, endopodite with three segments. Exopodite with third segment armed with a bisegmented plumose seta; fourth segment armed with three apical bisegmented plumose setae, which one shorter than others. Endopodite first segment about two times longer than second

segment, armed with a bisegmented plumose seta; second segment armed with a bisegmented plumose seta; third segment with a row of long lateral setulae, three apical bisegmented plumose setae. Antennal formula (exo/endo): spines 0000/000, setae 0033/113.

Thoracic limbs (Figure 14): Five pairs of thoracic limbs.

First limb (Figure 13 A). Epipodite oval. Accessory setae plumose, about 3 times longer than ODL first seta. ODL armed with two setae; first seta markedly short and naked, about of 0.05 of length of the second seta; second seta plumose. IDL (en4) with two setae of different length. Endite 3 with two posterior setae (a-b) and one anterior seta (1). Endite 2 with two posterior setae (c-d) and one anterior seta (2) might. Endite 1 with four posterior setae (e-h) and one anterior seta (3); anterior setae sclerotized; tip acute. Ejector hooks of different length, armed with short spines.

Second limb (Figure 13 B). Exopodite elongated, without a row of setulae laterally inserted, armed, with two setulated setae of different length; naked seta about 0.7 of plumose seta. Endite 5 armed with two setulated posterior setae (a-b), anterior seta absent. Endite 4 armed with one setulated posterior seta (c), anterior seta absent. Endite 3 armed with one setulated seta (d), anterior seta modified in an element long which presenting tip rounded. Endite 2 armed with a short seta. Endite 1 (gnathobase) with two rows of setae; on the anterior row the first seta is absent, seta 2 markedly longer, about 1.3 times longer than seta 3 and 3.3 longer than seta 4; posterior row with six setae (a-f) of similar length.

Third limb (Figure 13 C). Pre-epipodite elongated, setulated. Epipodite oval. Exopodite flat, wide, armed with four distal (1-4) and two lateral setae (5-6); setae 1 and 3-6 plumose; seta 2 clearly bisegmented, segment proximal plumose, segment distal armed with short and fine spines; setae 1 and 5 of similar length, markedly short; setae 3 and 6 of similar length; seta 4 about 0.8 of seta 2 length. Inner distal portion of limb with five endites; fifth endite with a long plumose seta without homology, a single anterior seta (a) present; fourth endite armed with a single anterior seta (b); third endite armed two anterior setae (c-d); second endite armed with two anterior setae (e-f); first endite as a large lobe armed anterior seta (g), two elements on the inner face which has different length and numerous posterior setae forming a filter plate.

Fourth limb (Figure 13 D). Epipodite oval. Exopodite flat, wide, armed with four distal (1-4) and two lateral setae (5-6), both setae are plumose; setae 1 and 3 similar in length; setae 4 and 6 similar in length; seta 2 about two times longer than seta 5. Inner distal portion of limb without lobes clearly separated, armed with two relatively short setae (1-2); filter plate with numerous posterior setae.

Fifth limb (Figure 13 E). Pre-epipodite subquadrangular and densely setulated. Pre-epipodite short, setulated. Epipodite large, oval. Exopodite subquadrangular shaped, armed with three setae; seta 1 relatively long, about 1.3 longer than seta 2; seta 2 about two times shorter than seta 3. Inner lobe portion elongated, armed with a seta about 2.3 times longer than exopodite seta 3, about three times longer than exopodite seta 2.

Abdominal and postabdominal structures: *Abdomen* (Figure 14 D). With two transverse rows of setulae present at its dorsal surface; two process present, proximal shorter than distal process. *Postabdomen* and *Postabdominal claws* (Figure 14 D-E). Similar to *Ceriodaphnia cornuta* s.l., however, the postanal margin has two pairs of marginal teeth.

Male. Not studied.

Ephippial female (Figure 15). Body is rounded in lateral view. Length ranging up 423.12 µm, height/length ratio about 0.7894 µm. Head and rostrum similar to parthenogenetic female. Valves ornamented with polygons. In lateral view the ephippium is narrowing posteriorly, dorsal margin straight (Figure 15 B). Ventral margin of ephippium regularly curved from the middle part, a narrow depression separating the sculptured portion from the border of ephippium. Ephippium bearing one egg. Locule of egg not clearly differentiated. Ultrastructure is organized in several columns bearing several short hairs on the border (Figure 15 C); some columns near to ventral and dorsal margins of ephippium might be shorter and naked. Postabdomen similar to parthenogenetic female.

Variability. It was observed one adult females without spines in rostrum and dorsal margin of head (Figure 11 H). In juveniles were observed short spines in rostrum and dorsal margin of head (Figure 11 B-I).

Diagnosis differential. *Ceriodaphnia* sp. nov. differ from the any species within genus, including *Ceriodaphnia cornuta* s.l., and the Neotropical *Ceriodaphnia silvestrii*, Daday, 1902 in presence of two pairs of marginal teeths the postanal margin of postabdomen. On the second limb, the endite 3 is armed with one anterior seta modified in an element long which has tip rounded. In *Ceriodaphnia* sp. nov. the basipodite of A2 has coaxal setae with different length being proximal one markedly larger (slightly shorter than length of basipodite) while in *Ceriodaphnia dubia* s.l. and *Ceriodaphnia smirnovi*, the proximal one seta is relatively short; in *Ceriodaphnia quadrangula* (O. F. Müller, 1785) and *Ceriodaphnia nikolaii* Garibian, Andreeva & Kotov, 2023 distal and proximal setae are short and it has similar length (Alonso et al., 2021; Garibian et al., 2023). The morphology of A2 was not described or documented to *Ceriodaphnia turkestanica* Berner & Rakhamattullaeva, 2001 (Berner & Rakhamattullaeva, 2001). Looking at the ultrastructure of ephippium *Ceriodaphnia* sp. nov. differ from the *C. laticaudata* O. F. Müller, 1867, *Ceriodaphnia megops* Sars, 1862, *C. quadrangula*, *C. reticulata* (Jurine, 1820), *C. pulchella* Sars, 1862, *C. smirnovi* and *C. nikollai*. *Ceriodaphnia rotunda* (Straus, 1820) has columns bearing several short hairs on the borders only on the locule egg position; such armature is covering of surface of *Ceriodaphnia* sp. nov. ephippium, however, the shape of ephippium of *C. rotunda* is very different when compared to *Ceriodaphnia* sp. nov. (Kotov et al., 2018). The morphology of ephippium of *Ceriodaphnia cornuta* Sars, 1885 and *Ceriodaphnia rigaudi* Richard, 1894 were studied by Berner (1985) showing differences in shape and ornamentation of columns covering the surface when compared to *Ceriodaphnia* sp. nov. We did not find ephippial females in material of *C. cornuta* s.l. studied here.

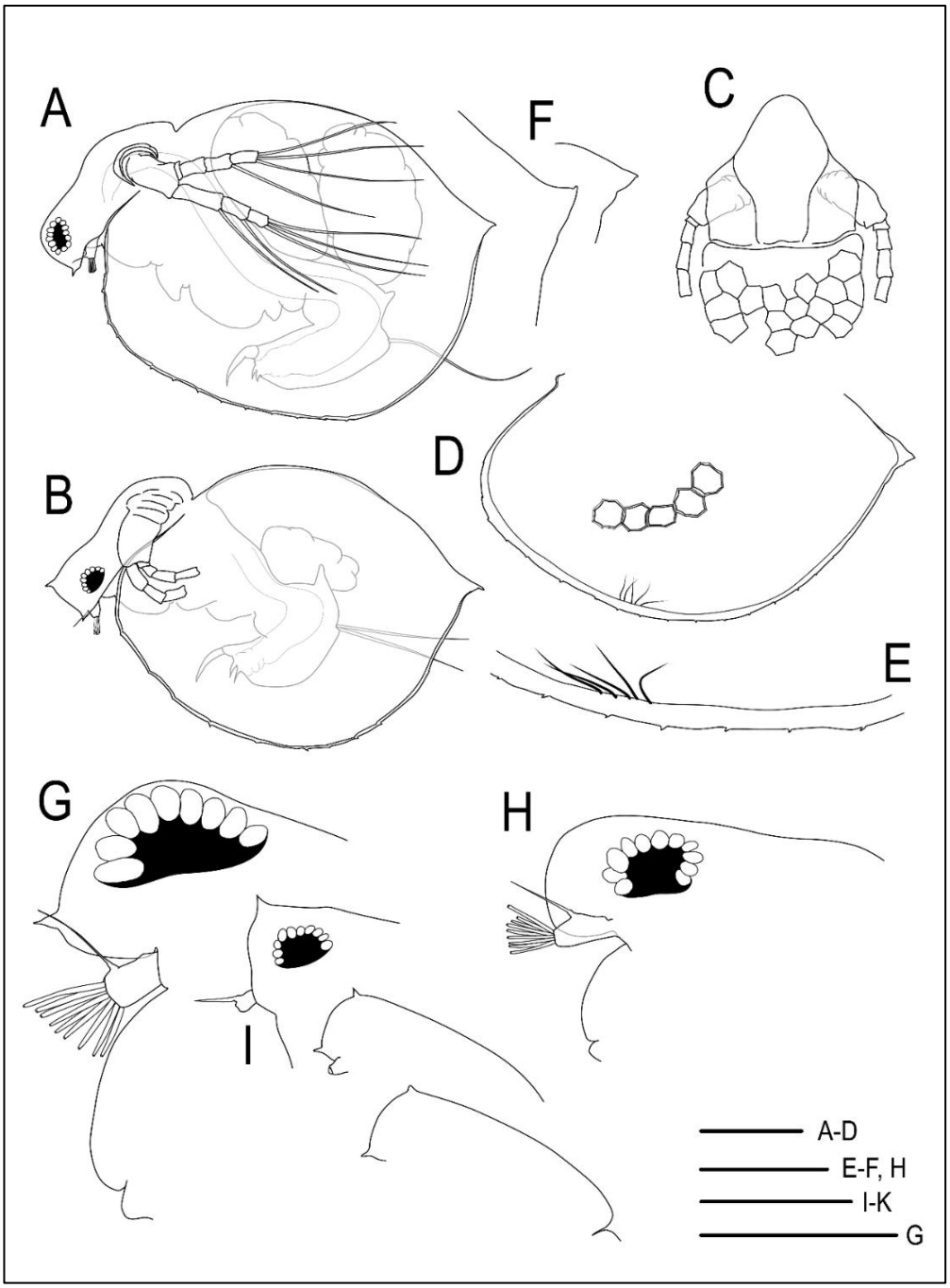

Figure 11: *Ceriodaphnia* sp. nov parthenogenetic female from Joaquim Medeiros Pond, Planaltina, Distrito Federal, Brazil. A) Adult parthenogenetic female, lateral view. B) Adult parthenogenetic female with head spines, lateral view. C) Headshield, dorsal view. D) Valve ventral margin and ornamentation, inner view. E) Armature of inner portion of valve ventral margin. F) Carapace dorsal posterior angle. G) Head (with rostrum spine) and antenna I, lateral view. H) Head (without rostrum spine), lateral view. I) Variations in rostrum and head shield, lateral view. Scale bars 100 µm.

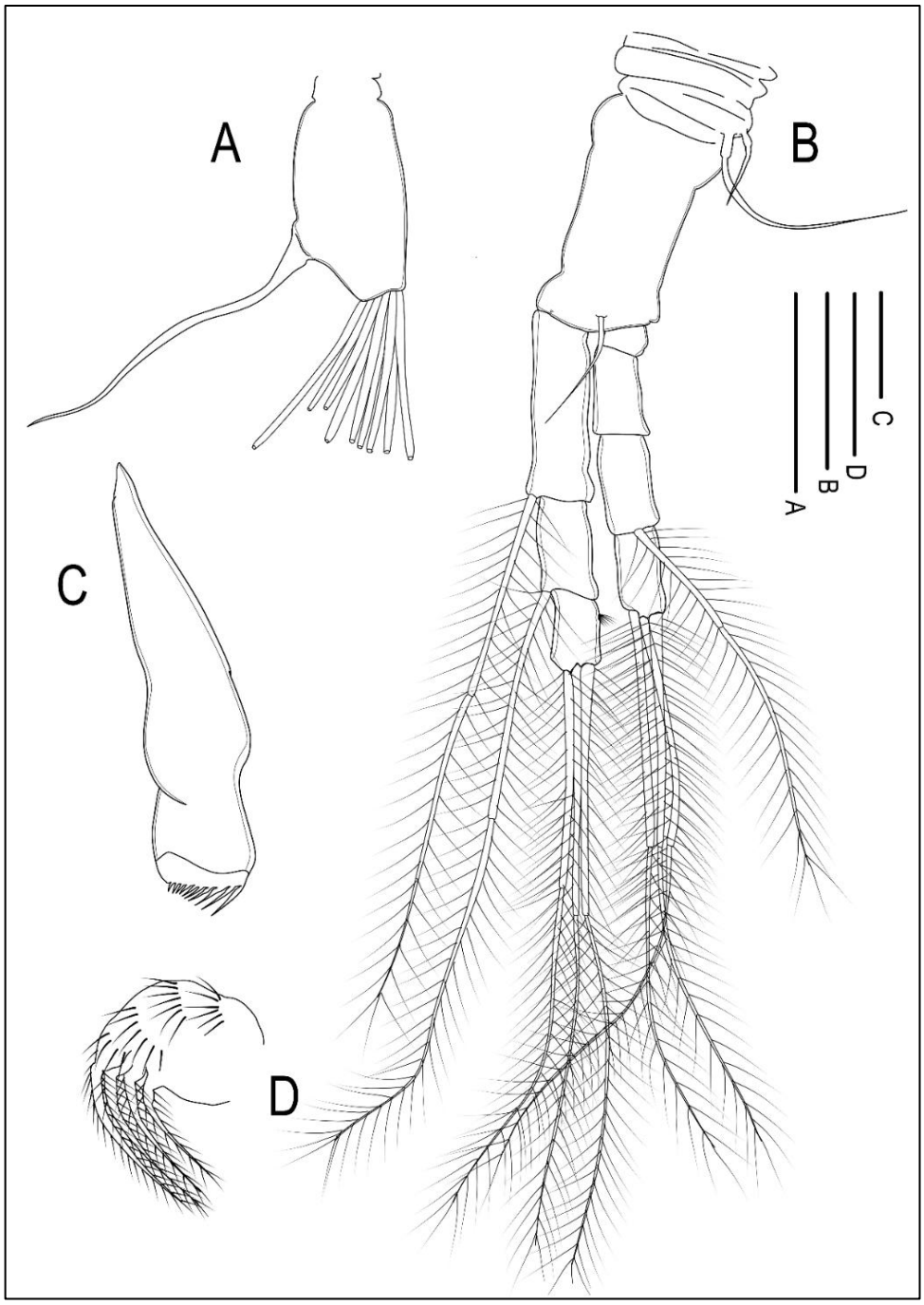

Figure 12: *Ceriodaphnia* sp. nov. parthenogenetic female from Joaquim Medeiros Pond, Planaltina, Distrito Federal, Brazil. A) Antenna I, lateral view. B) Antenna II, lateral view. C) Mandibles. D) Maxilla. Scale bars 100 µm.

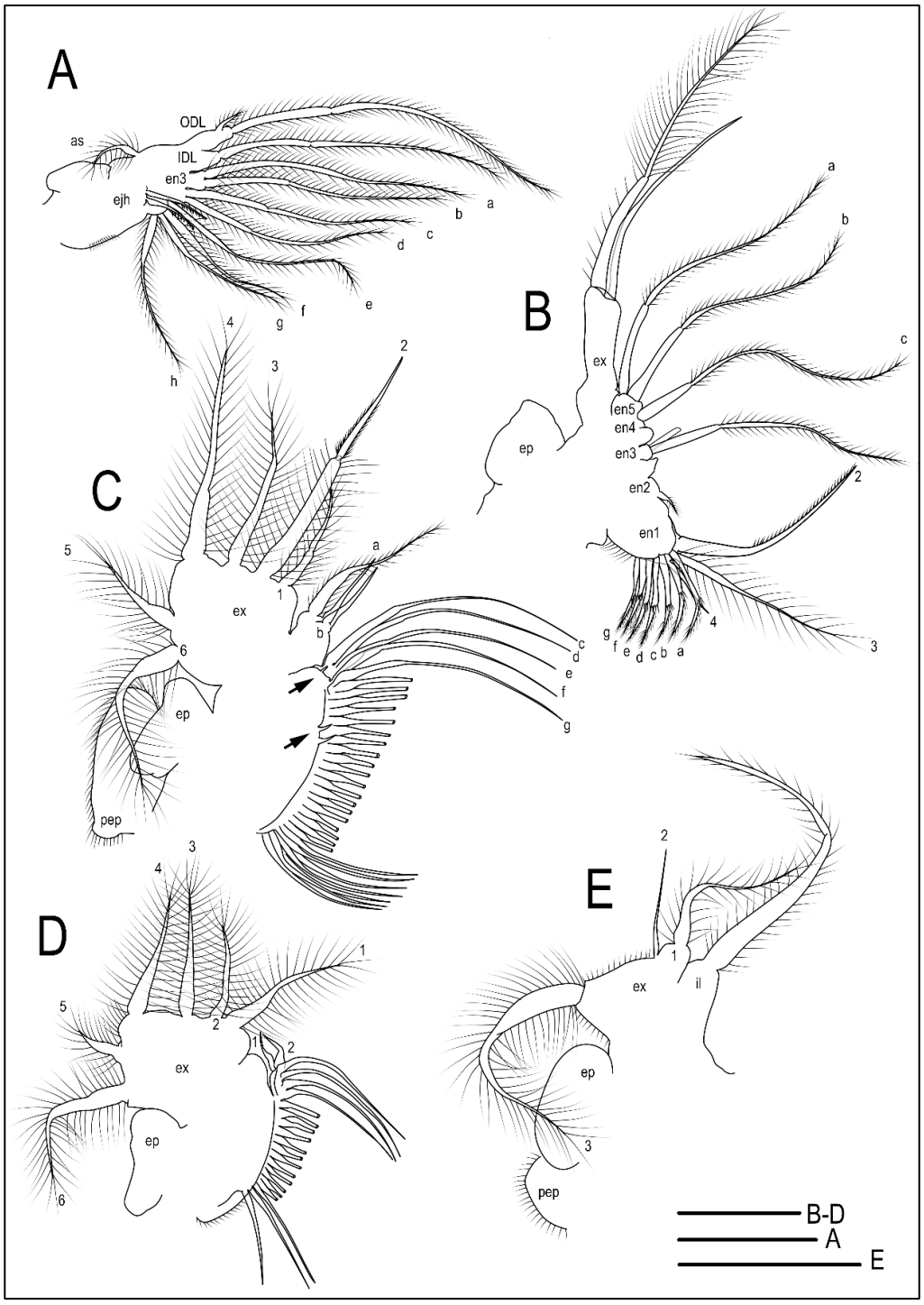

Figure 13: A) *Ceriodaphnia* sp. nov. parthenogenetic female from Joaquim Medeiros Pond, Planaltina, Distrito Federal, Brazil. A) First Limb. B) Second Limb. C) Third Limb. D) Fourth Limb. E) Fifth Limb. Scale bars 100 µm.

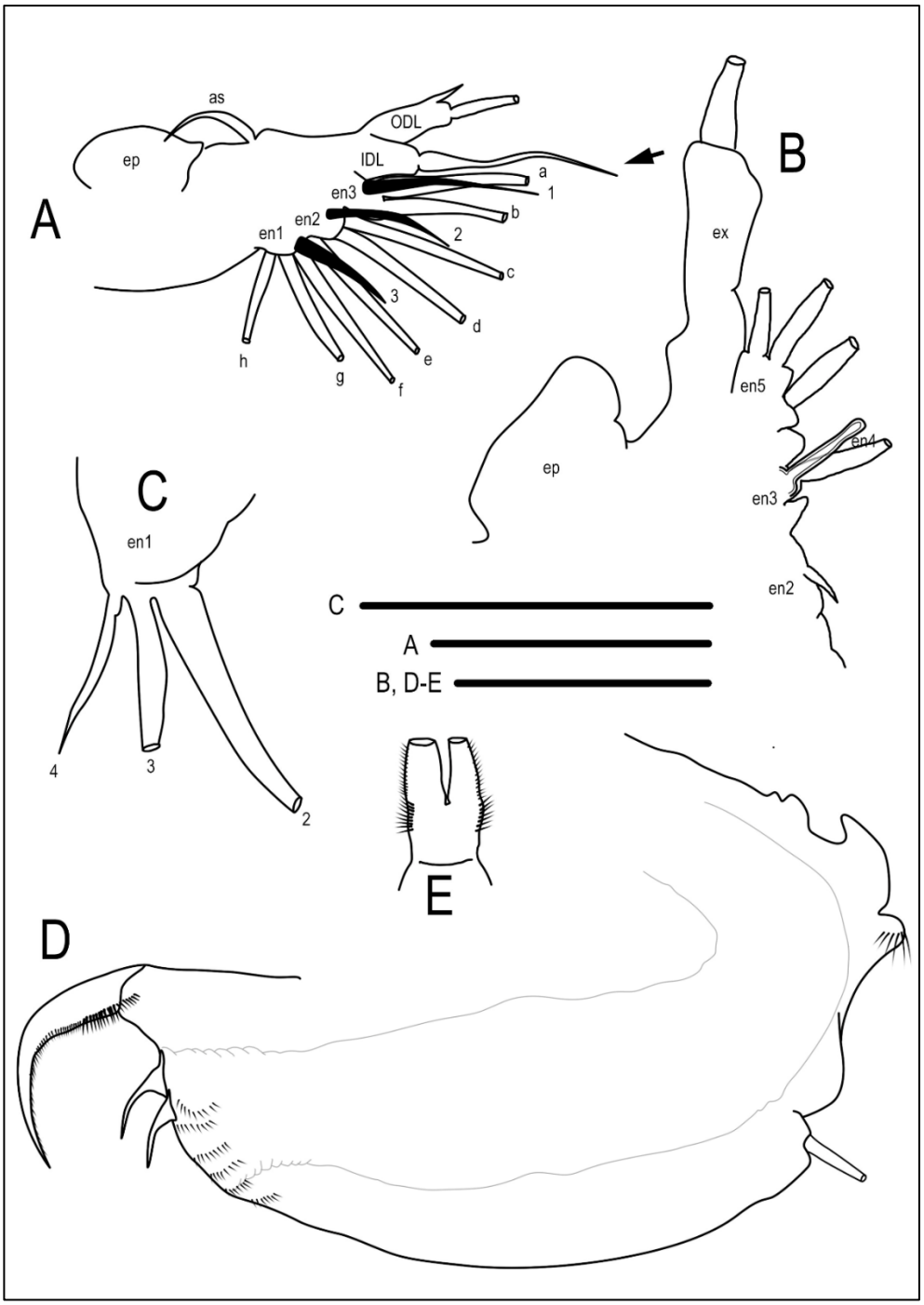

Figure 14: A) *Ceriodaphnia* sp. nov. parthenogenetic female from Joaquim Medeiros Pond, Planaltina, Distrito Federal, Brazil. A) First Limb. B) Second Limb. C) Endite I of Second Limb, anterior setae. D) Postabdomen, lateral view E) Postabdominal pecten, dorsal view. Scale bars 100 µm.

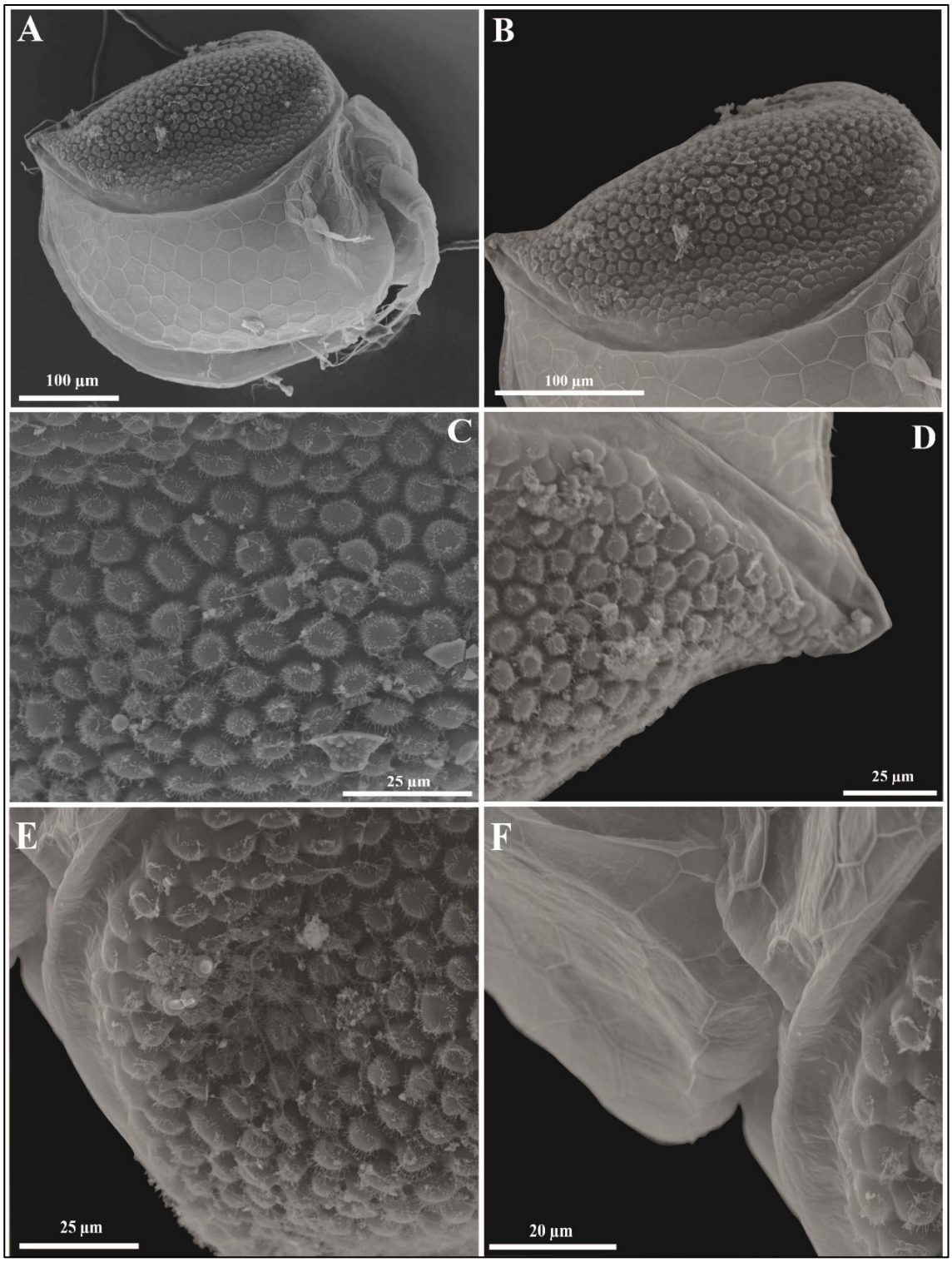

Figure 15: Ultrastructure of the ephippial female obtained through SEM. A) Female in lateral view (552x magnification); B) Ephippium in lateral view (887x magnification); C) Ornamentation of the ephippium (2901x magnification); D) Posterior region (2226x magnification); E) Anterior dorsal region (2768x magnification); F) Cervical notch region (3762x magnification).

Distribution and biology. *Ceriodaphnia* sp. nov. seems to be endemic, with geographic distribution restricted to shallow and oligotrophic ponds in central zone of Brazilian Cerrado above 1500m of altitude. Joaquim Medeiros (Figure 16 A-D) Pond is a temporary urban ecosystem which not exceeding 3m deep in rainy season. In this season, macrophytes of Cyperaceae family are very abundant and it might be connected to Caras Pond, another temporary pond. During dry season, ephippium of *Ceriodaphnia* sp. nov. can be found in superficial sediment. The Bonita Pond is a perennial shallow pond located in an area which preserve a large fragment of Cerrado (Estação Ecológica de Águas Emendadas). Bonita Pond is permanently covered by macrophytes of Cyperaceae and Pontederiacea families.

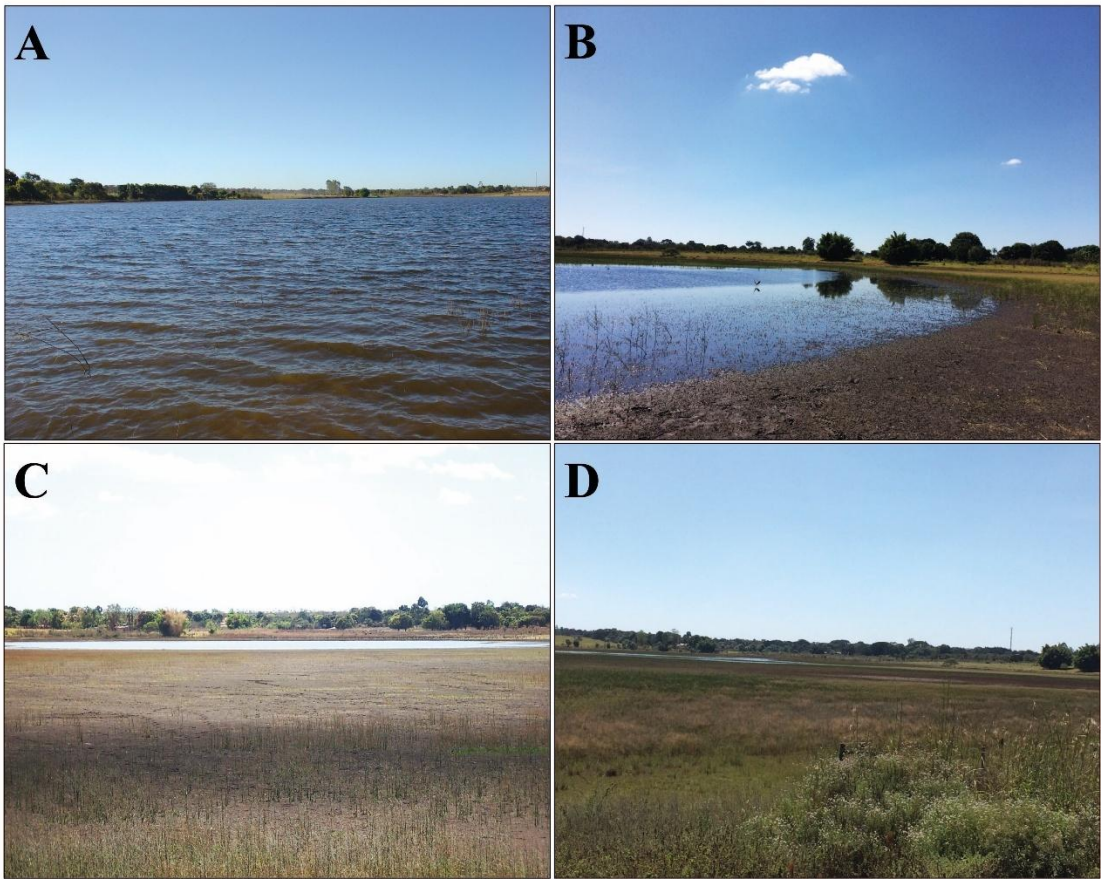

Figure 16: Joaquim Medeiros Pond and its seasonality. A) Rainy season; B) Onset of the dry season; C) Dry season; D) Peak of the dry season.

DISCUSSION

The *Ceriodaphnia cornuta* s.l. populations analyzed in this study do not differ from other global populations in terms of carapace length and height (Berner, 1985; Timms, 1988; Kotov et al., 2022b). Although the frequency distribution results showed wide variation in these measurements, few individuals exhibited larger sizes (Fig 3-A; Table 2).

The wide variation in spine size observed in *C. cornuta* s.l. can be explained by strong environmental predation pressure combined with phenotypic plasticity. The presence of predators or associated chemical compounds in the water can induce the development of defensive structures, such as spines (Tollrian & Dodson, 1999). Several studies have quantified the levels of spine polymorphism associated with predation (Zaret, 1969; Rietzler, 2008). In an experimental study on spine induction and reversibility, Gu et al. (2021) examined how spine plasticity responds to different concentrations of *Chaoborus* Lichtenstein, 1880 kairomones. The study found that posterior valve spines emerged during the early instars, followed by fornice spines and, finally, head spines in the later instars. Spine reversibility (under low kairomone concentrations) occurred in reverse order: head spines were reduced first, followed by fornice spines; posterior spines persisted longest.

Some studies have analyzed the size of the compound eye as a potential trait for differentiating *Ceriodaphnia* populations (Alonso, 2021; Kotov et al., 2022b). However, other authors regard the compound eye as a polymorphic trait influenced by selective predation pressure (Zaret, 1969; Berner & Rakhmatullaeva, 2001). The populations examined in this study exhibited wide variations of compound eye size (Figure 6 A-B, Table 2): individuals from large reservoirs (BBON, FUR, and SSIM) had smaller eyes and longer carapace spines. In contrast, the opposite pattern was observed in individuals from smaller ponds (BAC, JMED, and MAR): larger eyes and smaller spines. Individuals from RPAG, the only lotic environment analyzed, also had small eyes. Future studies may benefit from an approach that correlates compound eye size with selective predation pressure.

Regression analyses showed that spine length is not influenced by allometric values. Similar results were reported by Mort and Kerfoot (1988) when they compared the same variables in *C. cornuta* s.l.. Once again, the polymorphism observed within

populations appears to be the main factor, given that two or more morphotypes with variations in the presence and length of these spines can coexist within a single population. Therefore, using carapace spines as diagnostic characters could lead to incorrect identifications.

The separation of the JMED population observed in the Canonical Multivariate Analysis (MANOVA) may be attributed to eye size, as this trait notably diverges in the JMED population compared to the others. The proximity of the JMED and MAR populations in the clustering analysis is explained by their similarities in eye size and carapace height. However, the JMED population exhibits other morphological differences not observed in the MAR population. The northeastern populations (BAC, GUA, and IGA) were grouped together in the clustering analysis, likely due to carapace size and the absence of spines on the fornice. These three populations also share the trait of having individuals with two setae on the IDL. While this feature may be taxonomically relevant, further investigation is required in a broader context.

The postabdomen of the verified populations exhibited no differences in shape or size. Pecten morphology was consistent across populations and matched the characteristics of other populations in the *C. cornuta*-complex studied by Sharma et al. (2014). *C. cornuta* populations from artificial lakes in the United Arab Emirates exhibited the same pecten features as those observed in Brazilian populations, with the pecten subdivided into two groups of fine spines. However, the *C. laticaudata* group, which includes *C. dubia*, *C. laticaudata*, *C. silvestrii*, *C. smirnovi*, and *C. nikolaii*, differs from the *C. cornuta*-complex by bearing a pecten subdivided into three regions: a proximal pecten with fine, short spinules, a middle pecten with 3-5 long spines, and a distal pecten with fine, short spinules (Berner, 1986; Abreu et al., 2010; Garibian et al., 2023).

The number of teeth along the anal margin of *Ceriodaphnia* varies across the literature. Most studies report between five and seven teeth (Berner & Rakhmatullaeva, 2001; Garibian et al., 2023), though some studies have reported up to nine teeth (Kotov et al., 2022b). In this study, however, we observed a population (JMED) with only two teeth on the anal margin (Figure 6-B, Figure 14-D). This finding is unprecedented for the genus *Ceriodaphnia*, which including the *C. cornuta*-complex (Berner, 1986; Garibian et al., 2023).

Besides that, the parthenogenetic females from other ponds next to JMED, such as Bonita Pond, has distinctive morphometry of compound eye and body, the shape of carapace is different when compared to the other populations of *C. cornuta* s.l. studied, absence of prominent fornices and, the most relevant morphological trait: endite 3 on the second limb armed with one anterior seta modified in an element long which has tip rounded. The ephippial female has shape and columns ornamentation covering the ephippium different from the *C. laticaudata*, *C. megops*, *C. quadrangula*, *C. reticulata*, *C. pulchella*, *C. smirnovi*, *C. nikolaii* and *C. rotunda* (Kotov et al., 2018; Alonso et al., 2021; Garibian et al., 2023). Even when compared to *C. cornuta* s.l. and *C. rigaudi* studied by Berner (1985), the morphology of ephippium is quite different. Altogether, these morphological traits give support to description of *Ceriodaphnia* sp. nov..

Despite the large range of morphological variability observed in external morphology and on the first limb of *C. cornuta* s.l. studied here, some differences with *Ceriodaphnia* sp. nov. should be indicated. For instance, the sensorial seta on the antennule of *Ceriodaphnia* sp. nov. is larger than the observed in *C. cornuta* s.l.. On the gnathobase (en1) of second limb, the proportion between setae 1-2 is different when comparing *Ceriodaphnia* sp. nov. and *C. cornuta* s.l.. The second seta on the fourth limb exopodite of *Ceriodaphnia* sp. nov. is markedly shorter than third seta, while in *C. cornuta* s.l.. setae 2-3 has similar length. On the fifth limb exopodite, setae 1-2 has different length in *Ceriodaphnia* sp. nov. while in *C. cornuta* s.l such setae has similar length. We suggested that new studies involving *C. cornuta*-complex take in account the boundaries of variation on the morphology of limbs of the parthenogenetic females.

CONCLUSION

We conclude that Brazilian populations of *Ceriodaphnia cornuta* s.l. differ significantly from one another in terms of carapace and compound eye measurements. Some populations exhibited greater variation than others (MAR, BBON, RPAG). Carapace spine length showed no significant correlation with allometric values; therefore, we recommend that spines no longer be used as diagnostic characters in future taxonomic assessments. Based on allometric measurements and compound eye size, the northeastern populations (BAC, GUA and IGA) and the Joaquim Medeiros Pond (JMED) population appear to diverge from the others. Brazilian *C. cornuta* s.l. populations do not differ from

global populations in terms of allometric values. However, the JMED population likely represents a new species based on morphological differences in carapace shape, number of teeth in anal margin, ephippial morphology, morphology of coaxal setae in the basipodite of A2, and anterior seta of endite 3 of limb II. New studies based in molecular evidences, and study of males should be conducted to support this new species.

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