

RESEARCH ARTICLE

Morphological and molecular evidence of Asian *Gyraulus* spp. (Gastropoda: Planorbidae) in artificial habitats in Brazil

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ABSTRACT. *Gyraulus* Charpentier, 1837 comprises small freshwater gastropods with an almost cosmopolitan distribution, recorded across Europe, Africa, Asia, Oceania, and the Americas. The genus is highly diverse in Asia, with only a single species previously recorded in South America, specifically in Ecuador. We conducted a morphological and molecular analysis on specimens of *Gyraulus* spp. obtained from aquarium shops in south-eastern Brazil. Morphological analyses included the characterization of the shell and reproductive system, while molecular identification was based on partial sequencing of the mitochondrial cytochrome c oxidase subunit I (COI) gene. The *Gyraulus* specimens analyzed did not present morphological differences among samples, sharing characteristics of the shell and reproductive system with species of the *Gyraulus chinensis* (Dunker, 1848) complex. On the other hand, molecular data revealed the presence of two species: *G. convexiusculus* (Hutton, 1849) and *G. chinensis*. Most of the sequences obtained were related to sequences of *G. convexiusculus* from Singapore, a country known for its intense ornamental aquatic organism trade. A single sequence—derived from a specimen originating from an aquarium shop in Rio de Janeiro and used in its entirety for DNA extraction—clustered in the phylogenetic tree with GenBank sequences of *G. chinensis* from the Czech Republic, South Africa, and Hong Kong. The phylogenetic relationships among these taxa and other representatives of the genus *Gyraulus* included in the present analysis were not resolved, evidencing the species complex already pointed out by other authors. Based on our findings, we report the occurrence of *G. convexiusculus* and *G. chinensis* in artificial environments in Brazil, and alert aquarists about the risks and the necessary precautions to prevent the dispersal of these *Gyraulus* species into natural environments.

KEYWORDS. Aquarium trade, exotic species, freshwater gastropod, planorbid.

INTRODUCTION

Gyraulus Charpentier, 1837 is the most speciose genus within the subfamily Planorbinae, exhibiting a broad geographic distribution across nearly all continents, including Africa, the Americas, Asia, Europe, and Oceania. In general, they inhabit lentic freshwater environments such as swamps, lakes, and slow-moving rivers, and typically feed on cyanobacteria, diatoms, detritus, and aquatic vegetation (Baker 1945). The genus comprises over 81 valid species, with the

highest diversity recorded in Asia (MolluscaBase 2025). Native representatives are found in North, South, and Southeast Asia, as well as in Mongolia and West Africa (Meier-Brook 1983). Meier-Brook (1983) also reports approximately five native species in Europe and six species endemic to lakes in Macedonia.

In the Americas, *Gyraulus* species have been documented in North, Central, and South America. In North America, Johnson et al. (2013) and Czaja et al. (2020) reported six species. In Central America, Paraense (2003) reported *Gyraulus*

percarinatus Paraense, 2000 from Panama. In South America, only one species has been reported: *Gyraulus hindsianus* (Dunker, 1848), from Ecuador, where it is considered endemic (Paraense 2004, Breure et al. 2022). However, Fernandez et al. (2020) emphasized that both records require further morphological assessment because they are based on old reports and have not been corroborated by recent studies, a concern previously raised by Brown et al. (1998) Brown (2001), Pointier et al. (2005), and Shu et al. (2013). Consequently, the presence and distribution of *Gyraulus* species in South America remain uncertain.

From a public health perspective, mollusks of the genus *Gyraulus* may pose a potential risk to human health by acting as intermediate hosts for parasitic trematodes that infect the gastrointestinal tract (Chai and Jung 2020). Several species within this genus have been identified as hosts for Echinostomatidae trematodes. Notably, *Gyraulus convexiusculus* (Hutton, 1849) was reported as the first intermediate host for *Echinostoma ilocanum* (Garrison, 1908) and *Artyfechinostomum malayanum* (Leiper, 1911), previously treated under *Echinostoma*, both of which are parasites of humans and animals (Lie and Nasemary 1973, Chai et al. 2009, Chai and Jung 2020). In addition, *Echinostoma lindoense* (Sandground & Bonne, 1940) has also been reported infecting *G. convexiusculus* (Joe 1968, Meier-Brook 1983), which is also susceptible to experimental infection by miracidia of *Echinostoma cinetorchis* Ando & Ozaki, 1923, according to Chung et al. (2001). *Gyraulus chinensis* (Dunker, 1848) has been identified as the first intermediate host of *Echinostoma macrorchis*, a trematode that infects humans and other vertebrates (Lo 1995, Sohn et al. 2013, Sohn and Na 2017). From the viewpoint of veterinary significance, *G. chinensis* is known as a natural and experimental first intermediate host of *Euparyphium albuferensis* Esteban et al., 1997, an intestinal trematode of rodents (Muñoz-Antolí et al. 2008, 2010).

Several species of *Gyraulus* have expanded their geographic ranges through the global aquarium trade, primarily via the unintentional transport of eggs or juveniles attached to aquatic plants, as well as through the intentional trade of live snails (Appleton and Miranda 2015). This pattern, also documented for other freshwater gastropod families (e.g., Ampullariidae, Lymnaeidae, Physidae, Planorbidae, Viviparidae; Cowie and Robinson 2003), underscores the aquarium trade as an important pathway for the dispersal of non-native species (Ng et al. 2016). Among *Gyraulus* species, the Asian *G. chinensis* is the most widely reported and extensively studied, having been introduced to Europe over five decades ago, where it has demonstrated high invasive

potential and ecological adaptability. Besides Europe, it has spread to multiple regions, including Africa, Australia, the Caribbean, and the Middle East. *G. convexiusculus* has been reported in major aquarium-exporting countries in Asia and has been introduced into the Middle East, especially within the Mesopotamian plain (Brown 2001, Ng et al. 2016). This species exhibits considerable morphological variability and belongs to a taxonomically complex group sometimes referred to as a “race circle” that includes *G. chinensis* and *G. spirillus*, with unresolved taxonomic boundaries among them (Brown 1981, Meier-Brook 1983, Shu et al. 2013). *Gyraulus parvus* has been introduced into Europe, particularly in Germany, where it has established populations in artificial habitats such as aquaria and water reservoirs (Meier-Brook 1983, Lorencová et al. 2015, 2021).

Despite the wide geographic distribution of *Gyraulus* species, no confirmed records of their occurrence in Brazil have been documented to date. Herein, we present morphological and molecular analyses of two *Gyraulus* samples received by the Laboratório de Malacologia, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, from two aquarium stores in the municipalities of Rio de Janeiro (Rio de Janeiro State) and Diadema (São Paulo State), southeastern Brazil.

MATERIAL AND METHODS

Samples

The *Gyraulus* specimens analyzed in this study are deposited in the Coleção de Moluscos do Instituto Oswaldo Cruz (CMIOC), totaling 81 specimens in two lots. The mollusks (shells and live individuals) were received at the Laboratório de Malacologia, Instituto Oswaldo Cruz from two aquarium stores: one in Rio de Janeiro, state of Rio de Janeiro, in September 2022, and another in Diadema, state of São Paulo, in May 2024. The animals were fixed, photographed, measured, and deposited in the CMIOC under accession numbers CMIOC 15173 (Rio de Janeiro) and CMIOC 15520 (Diadema).

Morphological analyses and measurements

For fixation, all specimens received alive were anesthetized with sodium pentobarbital (0.03%) and immersed in water at 70 °C for 10 seconds, according to a protocol adapted from Thiengo et al. (2012). Subsequently, part of the material was preserved in 92.8% ethyl alcohol for molecular analyses and the remainder in Railliet-Henry solution for morphological analyses. The shells and soft parts of the specimens were analyzed under a stereomicroscope and

photographed in different positions using a Leica M205c equipped with a DMC2900 image capture system.

We evaluated all shells qualitatively and measured 20 specimens from each lot using the Distance Line Tool of the Leica Application Suite (LAS V4.8) and the ImageJ software (Schneider et al. 2012). Measurements of the following variables were obtained: total maximum diameter (Md), shell height (Sh), spire diameter (Sd), aperture height (Ah), aperture diameter (Ad) (Fig. 1), and the flatness index (Fi = Md/Sh).

Morphological analyses were performed on 10 specimens from each lot. These evaluations included dissection and detailed examination of the internal anatomy, with emphasis on parts of the male and female reproductive systems, based on the methodologies of Thiengo et al. (2012). The main morphological characteristics were described and compared (Table 1).

DNA extraction and COI amplification

Total DNA was obtained from three specimens from *Diadema* and three from Rio de Janeiro using the Qiagen DNeasy Blood and Tissue kit, following the manufacturer's

protocol. The extracted DNA was diluted 1:20 with type II ultrapure water. From this material, the mitochondrial gene COI (cytochrome c oxidase subunit I) was amplified using the universal primers of Folmer et al. (1994): LCO1490 (forward, 5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (reverse, 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). Each PCR mixture contained 12.5 µL of GoTaq Green Master Mix 2x (PROMEGA, Madison, USA), 1 µL of each primer [10 µM], 2 µL of DNA sample (10 ng/µL), and ultrapure water to reach a final volume of 25 µL. Thermocycling consisted of an initial denaturation cycle at 95 °C (5 min), annealing at 48 °C (1 min), and extension at 72 °C (1 min); 35 cycles of denaturation at 95 °C (1 min), annealing at 48 °C (2 min), and extension at 72 °C (1 min), followed by a final extension at 72 °C (5 min).

The amplified products were purified using the GFX PCR and Gel Band Purification kit (GE Healthcare) and sequenced bidirectionally using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, California, USA) according to the manufacturer's instructions. All samples were sequenced in an ABI 3730 automatic sequencer (Applied Biosystems) at the Genomics and DNA Sequencing Platform of the Instituto Oswaldo Cruz, Fundação Oswaldo

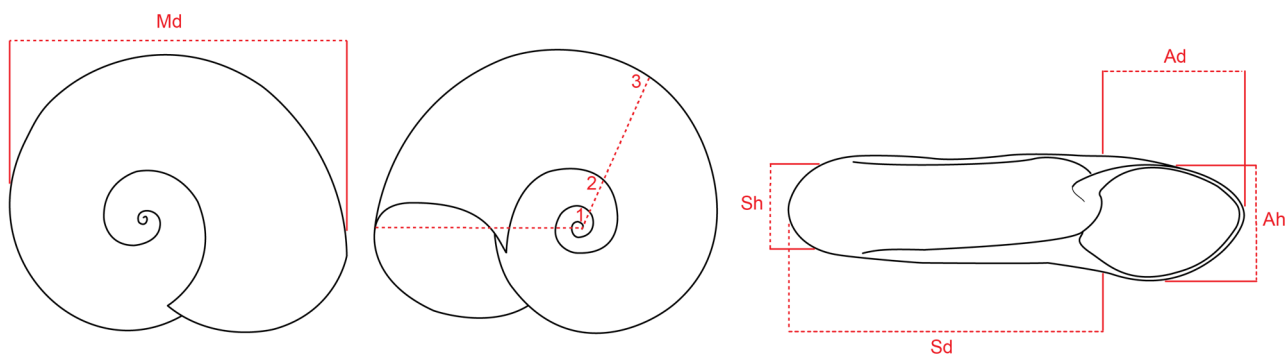


Figure 1. Diagram of the number of whorls counted and the measurements taken on the shells of specimens of the genera *Gyraulus*. (Md) maximum diameter, (Sh) shell height, (Sd) spire diameter, (Ad) aperture diameter, (Ah) aperture height.

Table 1. Morphological characteristics of the *Gyraulus* lots analyzed, from Rio de Janeiro and São Paulo.

Características	Bento Ribeiro, Rio de Janeiro/RJ (n = 10)	Taboão, Diadema/SP (n = 10)
Shell shape	Planispiral	Planispiral
Shell whorls	3¾	3¾
Apertura Shell	Oval-shaped, either rounded or angular	Oval-shaped rounded
Spiral lines	Faint; present or absent	Absent
Prostatic diverticula	Digitiform shape, long, arranged in a single row, not branched, 12–14 diverticula ($\bar{x}$ = 13.2 ± 0.6)	Digitiform shape, long, arranged in a single row, not branched, 14–16 diverticula ($\bar{x}$ = 14.3 ± 1.3), young specimens exhibit about 13 smaller, widely spaced diverticula
Spermatheca	Oval, more or less elongated	Oval, more or less elongated
Penile sheath x Penile prepuce	The penile sheath measures equal to or almost twice the length of the prepuce	The penile sheath measures equal to or slightly exceeds the size of the prepuce
Penile	Penial stylet present	Penial stylet present

Cruz (PDTIS/FIOCRUZ) subunit RPT01A–DNA Sequencing. The chromatograms were assembled and edited using the Geneious package 2024.2 (Kearse et al. 2012) (<http://www.geneious.com>) to produce a consensus sequence (contig). The Basic Local Alignment Search Tool (BLAST search) was used to find sequences matching the target organism. Additional sequences from extensive review studies or geographical areas concerning *Gyraulus* spp. and related genera were retrieved from GenBank for phylogenetic analyses. All sequences obtained in the present study were deposited in GenBank (PV832446–PV832450).

Phylogenetic analyses

Phylogenetic analyses were performed using an alignment comprising the five sequences generated for this study and 70 partial MT-CO1 sequences of *Gyraulus* spp. obtained from GenBank. These included species representing the Planorbini tribe according to Morgan et al. (2002): *Anisus spirorbis* (Linnaeus, 1758) and twelve species of *Gyraulus*: *G. acronicus* (Férussac, 1807), *G. albus* (Müller, 1774), *G. chinensis*, *G. circumstriatus*, *G. connollyi* Brown & Van Eeden, 1969, *G. convexiusculus*, *G. costulatus* (Krauss, 1848), *G. huwaizahensis* Glöer & Naser, 2007, and *G. laevis* (Alder, 1838), *G. luguhuensis* Shu, Köhler, Fu & Wang, 2013, *G. rossmaessleri* (Auerswald, 1852) e *G. takhteevi* Sitnikova & Peretolchina, 2018. As an out-group, we used sequences from the tribe Segmentinini Baker, 1945: *Hippeutis cantori* (Benson, 1850) and *Segmentina* sp.

The phylogenetic matrix of COI was aligned using the MAFFT (Multiple Alignment using Fast Fourier Transform) algorithms (Katoh and Standley 2013) implemented in the Geneious 2024.0.2 package (Kearse et al. 2012). Phylogenetic analyses were performed based on the GTR + I + G evolutionary model calculated for each codon position using MrModelTest v2.4 (Nylander 2004), selected using the Akaike criteria (AIC), and run in PAUP 4.0. Markov chain sampling (MCMC) was performed for 10,000,000 generations. Node robustness was assessed by Bayesian posterior probabilities (BPP), calcu-

lated from trees sampled every 100 generations, with 25% of the first generations removed as “burn-in”. The phylogenetic tree was inferred using MrBayes v.3.2.7 (Ronquist et al. 2012), implemented through XSEDE on the CIPRES Science Gateway (Miller et al. 2010). The resulting tree was visualized in FigTree v.1.4.4 (Rambaut 2012). Effective sample size (ESS) values were assessed in Tracer v.1.7.1 (Rambaut et al. 2018).

RESULTS

Morphological analysis

The specimens from both samples received from Rio de Janeiro and Diadema showed no visible morphological or conchological differences and were consistent with Asian species of the *G. chinensis* complex (Fig. 2, Table 1). The shell is planispiral, with 3 to 3¾ whorls that rapidly increase in diameter, with the body whorl being significantly wider. The maximum total diameter of the shell (Dt) was 4.784 mm, with a variable height reaching a maximum of 2.0 mm (Ac). The sutures are not very deep, with the dorsal side slightly concave and the ventral side more markedly concave. The body whorl was significantly deflected in some specimens. The shell aperture varies from rounded to more angled, with a rounded periphery and no apparent angulation. The periostracum is light yellow. On the surface of the shell, transverse growth lines can be seen and, in some specimens, faint spiral lines form a slightly reticulated surface, which is sometimes less conspicuous.

The results of the measurements and descriptive statistical analyses of the shells from each lot—including mean, median, and standard deviation—are presented in Table 2. The populations from Rio de Janeiro presented an average total shell diameter of 2.781 ± 0.3 mm, indicating a smaller size compared to the specimens from Diadema, whose average was 3.135 ± 1.0 mm (Table 2).

The body is minute, with some internal organs visible through the translucent mantle. It possesses a pair of pigmented, filiform tentacles with eyes situated at their bases.

Table 2. Results of descriptive statistical analyses based on measurements (mm) of shells analyzed from Rio de Janeiro and Diadema. (SD) Standard deviation.

Characteristics	Specimens	Rio de Janeiro					Diadema				
		Mean	Median	SD	Minimum	Maximum	Mean	Median	SD	Minimum	Maximum
Maximum diameter	20	2.781	2.764	0.319	2.303	3.356	3.135	3.175	1.013	1.186	4.784
Shell height	20	0.825	0.833	0.060	0.707	0.935	0.855	0.883	0.360	0.392	2.079
Spiral diameter	20	1.852	1.880	0.195	1.492	2.147	2.083	2.064	0.771	0.793	3.332
Aperture diameter	20	0.896	0.898	0.072	0.740	1.014	1.010	1.077	0.307	0.386	1.456
Aperture height	20	0.881	0.887	0.062	0.747	1.013	0.844	0.864	0.234	0.443	1.165
Flatness index	20	0.279	0.255	0.096	0.191	0.644	0.299	0.292	0.026	0.255	0.340

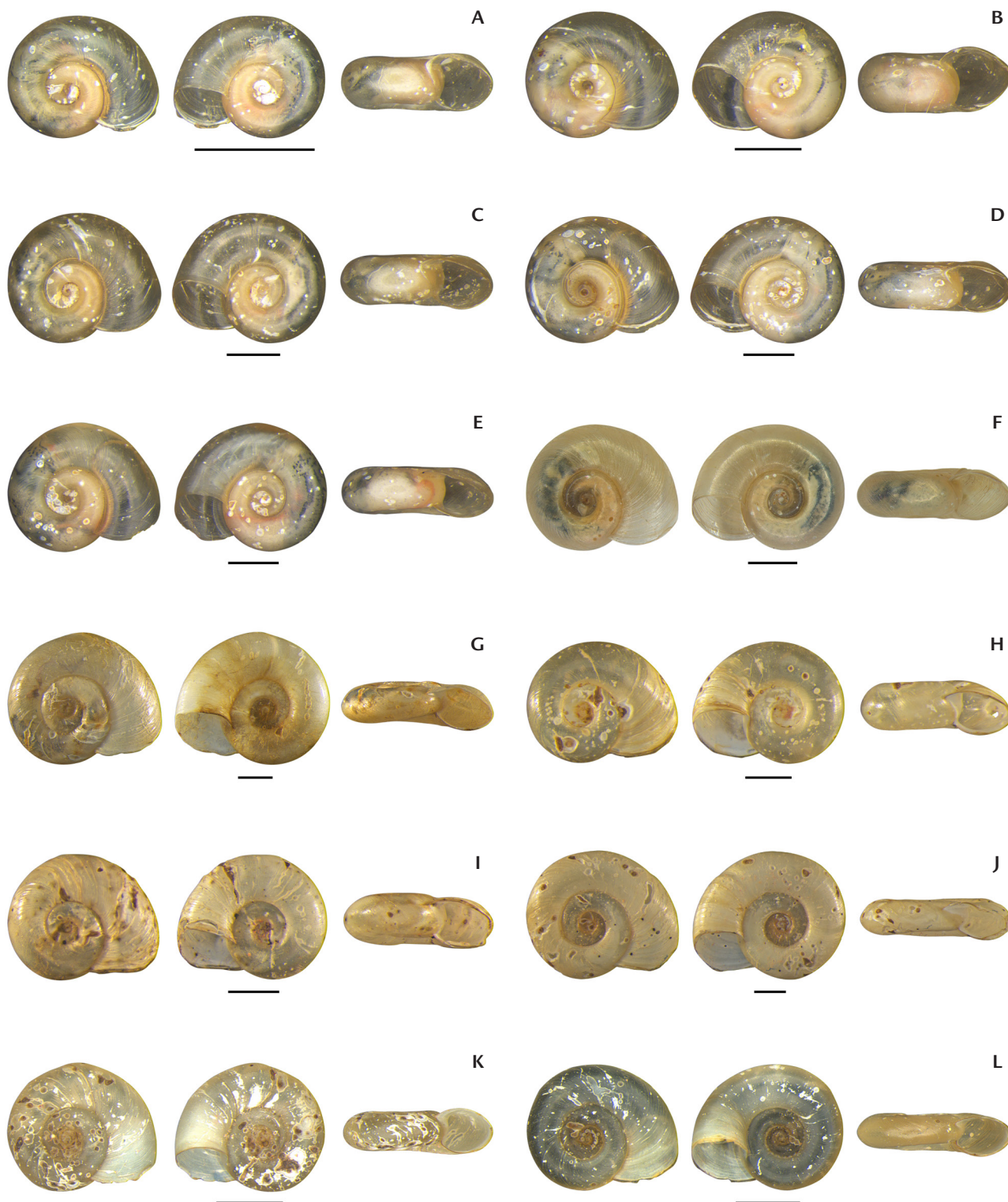


Figure 2. Shell variability in different specimens of *Gyraulus* cf. *convexusculus* analyzed: (A–F) Rio de Janeiro, CMIOC 15173; (G–L) Diadema, CMIOC 15520. Scale bars: 1 mm.

Between the tentacles are the muffle and the labial palps positioned above the mouth. The overall body coloration is pale with a slight grayish tone, exhibiting localized pigment concentrations, particularly on the head over the muffle region and along the dorsal surface of the foot as small, scattered spots, with the sole remaining unpigmented. A thin, lightly pigmented line is present along the collar of the mantle. Mantle pigmentation varies in intensity, with the anterior region showing larger, more densely pigmented

patches, while the posterior area presents lighter and more diffuse spots. Two distinct pigmented bands are visible along the roof of the pallial cavity, extending over the renal tube from its origin to the posterior end of the mantle. The tentacles are pigmented from base to tip. The foot is short and oval-shaped, with genital openings located on the animal's left side (Figs 3, 4). Dissection allowed observation of the buccal bulb and the salivary glands, with the latter crossing internally through the periesophageal ganglion ring.

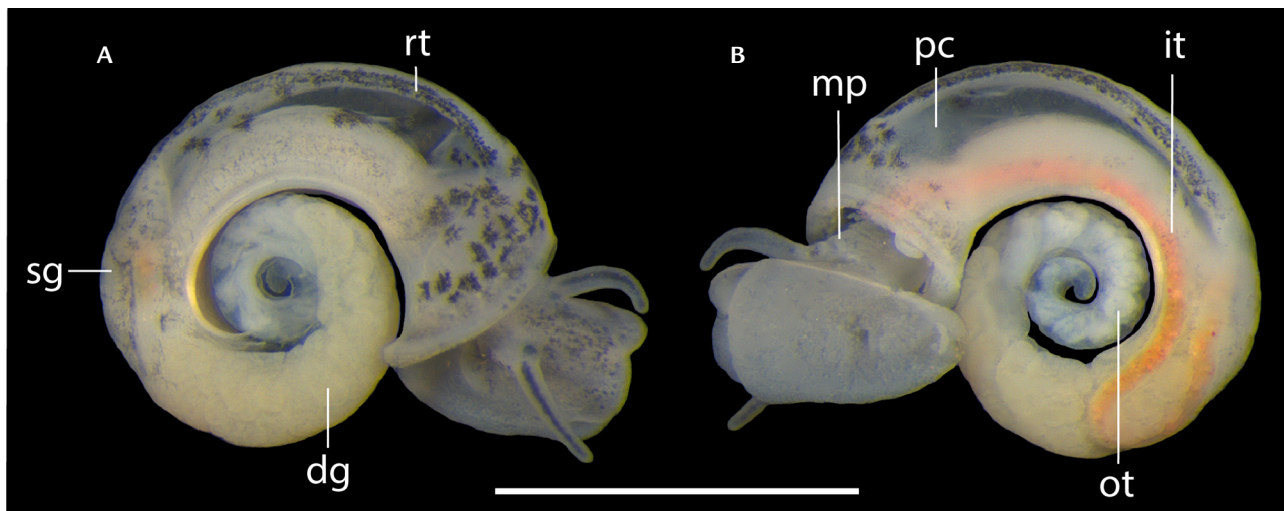


Figure 3. Specimen of *Gyraulus* cf. *convexiusculus* from Rio de Janeiro, out of the shell, CMIOC 15173, ex. 23: (A) right side; (B) left side. (mp) Male genital pore, (pc) pallial cavity, (sg) stomach, (dg) digestive gland, (it) intestine, (ot) ovotestis, (rt) renal tube. Scale bars: 1 mm.

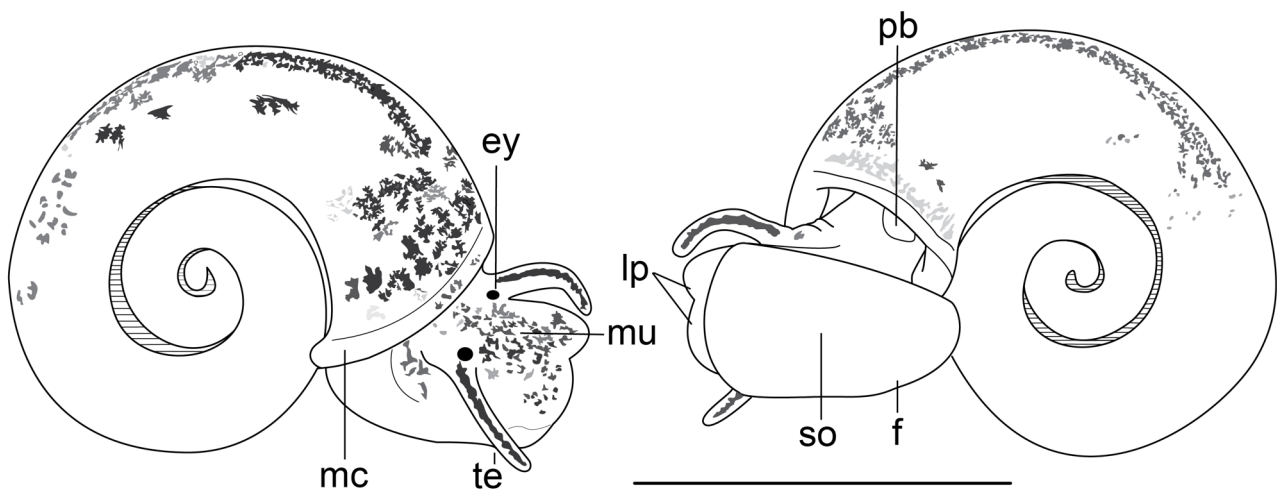


Figure 4. Schematic representation of coloration patterns in specimens identified as *Gyraulus* cf. *convexiusculus*, CMIOC 15173, ex. 23. (mc) Mantle collar, (mu) muffle, (ey) eyes, (f) foot, (lp) labial palps, (pb) pseudobranch, (so) sole, (te) tentacle. Scale bar: 1 mm.

Specimens from both localities presented elongated spermathecae, varying in length. Its duct is visible and without apparent structural modifications. The prostate has approximately 14 well-defined, finger-shaped prostatic diverticula, regularly spaced and close in only one direction. The number of prostate diverticula in specimens from Diadema ranged from 14 to 16 ($\bar{x} = 14.3 \pm 1.3$), while those from Rio de Janeiro ranged from 12 to 14 diverticula ($\bar{x} = 13.2 \pm 0.6$). The vas deferens is narrow; the penis sheath is longer or almost as long as the prepuce in some specimens; and a rounded spiny seminal vesicle is present, as well as a stylet in the papilla of the penis, which is easily visible due to the transparency of the penis sheath (Fig. 5).

Molecular analysis of *Gyraulus* spp.

Sequences were obtained from five specimens of *Gyraulus* for the partial MT-CO1 gene, two from Rio de Janeiro and three from Diadema, with 600–700 bp. The

phylogenetic matrix included 75 sequences (602 bp), also including COI sequences obtained from GenBank for *Gyraulus* (Table 3).

Four of the five sequences generated in the study were positioned in a well-supported clade (BPP: 100%) together with a sequence of *G. convexiusculus* from Singapore (KU318340), with which it was 100% similar (Fig. 6), confirming the morphological identification of the specimens. Specimen 3, from the Rio de Janeiro population, represented a very young individual and was used in its entirety in the DNA extraction process due to its small size; it presented high similarity (100%) with sequences of the species *G. chinensis* (OQ121101, PP228855, PP228842), forming a clade with specimens from South Africa, the Czech Republic, and other European and Asian regions.

On the other hand, sequences identified as “*G. convexiusculus*” from GenBank also appear in other clades, such as the one that includes sequences from *G. chinensis* and another

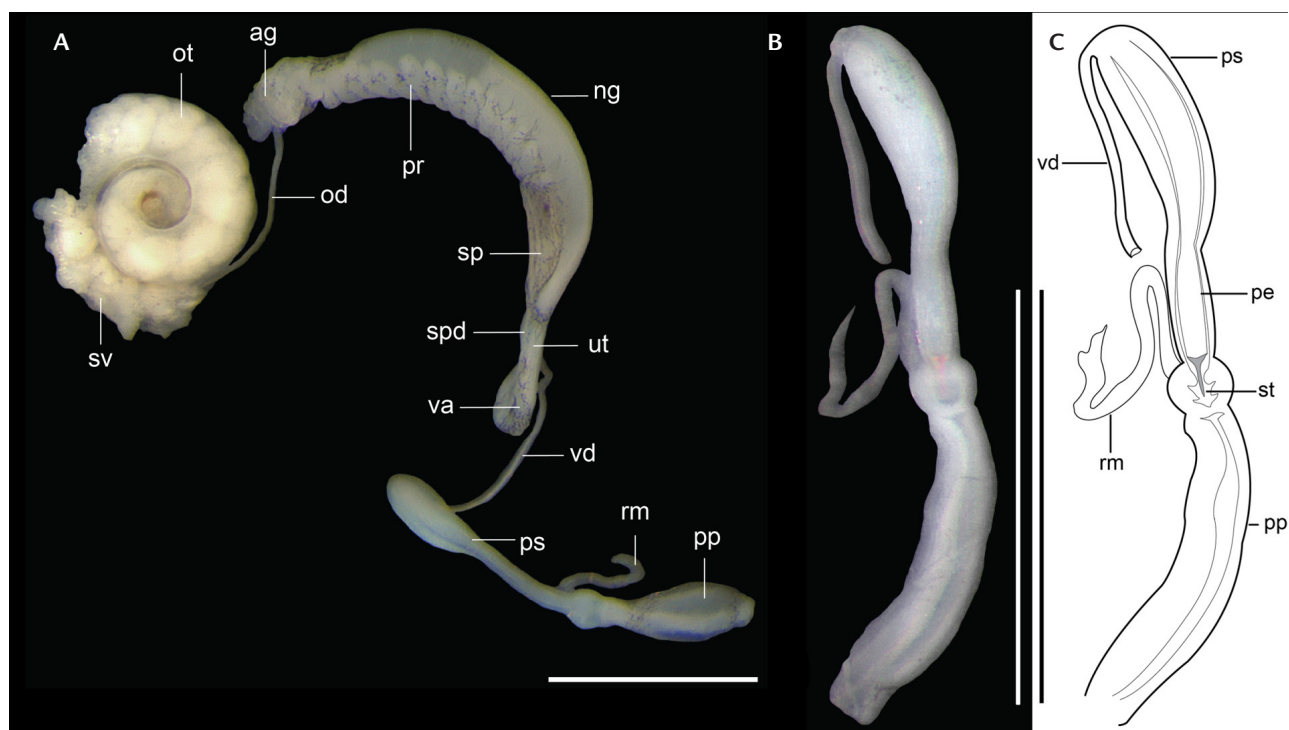


Figure 5. Details of the reproductive system of specimens of *Gyraulus* cf. *convexiusculus*: (A) complete reproductive organ, CMIOC 15520, ex. 32; (B) penile complex evidencing the presence of stylet (orangish structure) at the apex of the penis located inside the penis sheath, seen through the transparency of the penis; (C) schematic representation of the penile structure from CMIOC 15173, ex. 32. (ag) Albumen gland, (ng) nidamental gland, (od) ovispermiduct, (ot) ovotestis, (pe) penis, (pp) prepuce, (pr) prostate, (ps) penis sheath, (rm) retractor muscle, (sp) spermatheca, (spd) spermatheca duct, (sv) seminal vesicle, (st) stylet, (ut) uterus, (va) vagina, (vd) vas deferens. Scale bars: 1 mm.

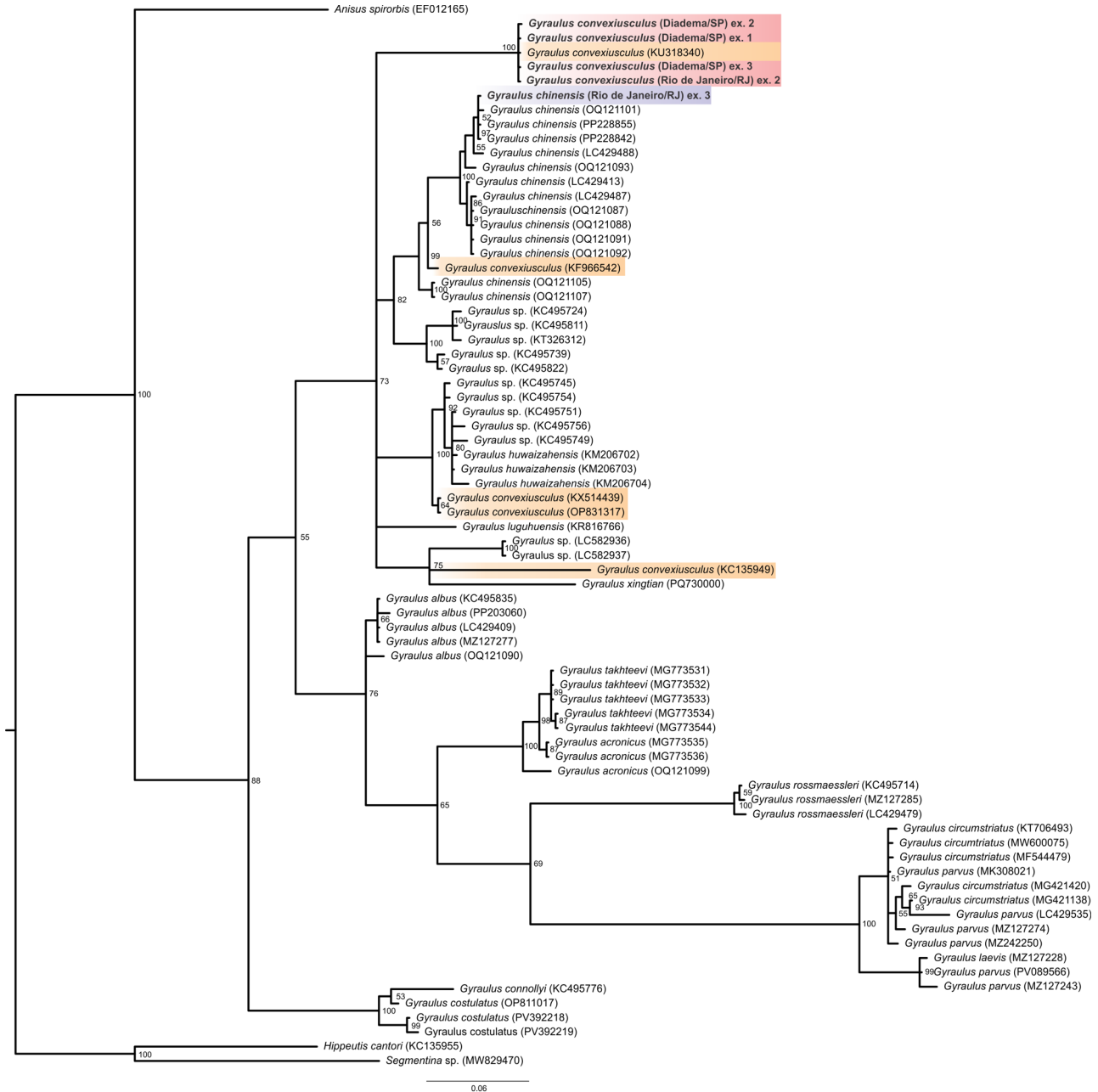


Figure 6. Bayesian Inference Tree (BI) of the partial COI sequences of *Gyraulus* spp. (602 bp). The branch support values are Bayesian posterior probabilities (BPPs). The trees sampled by MCMC, with a burn-in of 25%, exhibited mean scores of LnL = 4018.6615 and median LnL = 4018.26 with ESS values above 200. *Hippeutis cantori* and *Segmentina* sp. were used as outgroup.

that includes sequences from *G. huwaizahensis* and *Gyraulus* sp. These data demonstrate the complexity involved in identifying these species, which can lead to erroneous identifications. Moreover, the relationship between these two clades and other larger clades within *Gyraulus* is not yet resolved,

and there is a polytomy grouping different species of *Gyraulus* (*G. convexusculus*, *G. chinensis*, *Gyraulus* sp., *G. huwaizahensis*, and *G. luguensis*) (BPP: 73%; Fig. 6). Even so, the polytomy formed did not compromise the robustness of the internal groups, which delimited *G. convexusculus* and *G. chinensis*

Table 3. Tribe, species, Genbank accession number and geographic locality of MT-CO1 sequences used for this study.

Tribe	Species	Accession number	Geographic locality	Reference
Planorbini	<i>Anisus spirorbis</i>	EF012165	Germany: Ilmkreis, Riechheim	Albrecht et al. (2007)
	<i>G. acronicus</i>	MG773535	Russia: Lake Baikal	Sitnikova and Peretolchina (2018)
		MG773536	Russia: Lake Baikal	Sitnikova and Peretolchina (2018)
		OQ121099	Russia	Saito et al. (2025)
	<i>G. albus</i>	KC495835	Germany: Thuringia, Königsee	von Oheimb et al. (2013)
		LC429409	Poland: Lodz, Widawa	Saito et al. (2018)
		MZ127277	Austria: Niederösterreich, Lunz am See	Lorencová et al. (2021)
		OQ121090	Serbia: Bac	Saito et al. (2025)
		PP203060	Belgium	Schols et al. (2024)
	<i>G. chinensis</i>	PV832447	Brasil: RJ, Rio de Janeiro (aquarium)	Present study
		LC429487	Taiwan: Ylian County, Jiaoxi	Saito et al. (2018)
		LC429488	Hong Kong: Yuen Long	Saito et al. (2018)
		LC429413	Japan: Kagoshima, Kikai Island	Saito et al. (2018)
		OQ121087	Albania: Elbasan	Saito et al. (2025)
		OQ121088	Albania: Belsh	Saito et al. (2025)
		OQ121091	Albania: Belsh	Saito et al. (2025)
		OQ121092	Albania: Belsh	Saito et al. (2025)
		OQ121093	Czech Republic: Brno	Saito et al. (2025)
		OQ121101	Czech Republic: Dvur Kralove	Saito et al. (2025)
		OQ121105	Italy: Venaria	Saito et al. (2025)
		OQ121107	Italy: Arenzano	Saito et al. (2025)
		PP228842	South Africa	Outa et al. (2024)
		PP228855	South Africa	Outa et al. (2024)
	<i>G. circumstriatus</i>	KT706493	Canada: Ontario, Cambridge	Telfer et al. (2015)
		MF544479	Canada: Ontario, Rouge National Urban Park	Dewaard et al. (2019)
		MG421138	Canada: Manitoba, Churchill	Dewaard, unpublished
		MG421420	Canada: Manitoba, Churchill	Dewaard, unpublished
		MW600075	Romania	Menabit, unpublished
	<i>G. connollyi</i>	KC495776	South Africa: Stillerus	von Oheimb et al. (2013)
	<i>G. convexiusculus</i>	PV832446	Brasil: RJ, Rio de Janeiro (aquarium)	Present study
		PV832448	Brasil: SP, Diadema (aquarium)	Present study
		PV832449	Brasil: SP, Diadema (aquarium)	Present study
		PV832450	Brasil: SP, Diadema (aquarium)	Present study
		KC135949	South Korea	Park and Oh, unpublished
		KF966542	South Korea	Park and Oh, unpublished
		KX514439	India: Andhra Pradesh, Ananthapuram-District, Puttaparthi	Ayyagari & Sreerama (2020)
		KU318340	Singapore: aquarium	Ng et al. (2016)
		OP831317	Índia: Andhra Pradesh, distrito de Sri Sathya SaiPrasanthi	Ayyagari, unpublished
	<i>G. costulatus</i>	OP811017	Senegal	Gaye et al., unpublished
		PV392218	Gabon: Lastoursville	Kouna et al., unpublished
		PV392219	Gabon: Lastoursville	Kouna et al., unpublished
	<i>G. huwaizahensis</i>	KM206702	Iraq: Al-Qadisiyah, Sumer	Al-Bdairi, unpublished
		KM206703	Iraq: Al-Qadisiyah, Sumer	Al-Bdairi, unpublished
		KM206704	Iraq: Basra, Garmat Ali	Al-Bdairi, unpublished
	<i>G. laevis</i>	MZ127228	Czech Republic: Moravia, Haluzice	Lorencová et al. (2021)
	<i>G. luguhuensis</i>	KR816766	China: Lake Lugu	Clewing et al. (2015)
	<i>G. parvus</i>	LC429535	USA: Alaska State	Saito et al. (2018)
		MK308021	USA: Maryland, Baltimore County	Aguilar et al., unpublished
		MZ127243	Croatia: Adriatic Croatia, Ljubacki	Lorencová et al. (2021)
		MZ127274	USA: Sullivan County, NY	Lorencová et al. (2021)
		MZ242250	United Kingdom: Northern Ireland, County Down	Lorencová et al. (2021)

Continues

Tribe	Species	Accession number	Geographic locality	Reference
<i>G. rossmaessleri</i>		PV089566	Křižanov, Tchéquia	Klobusicka, unpublished
		KC495714	Alemanha: Neumark	Von Oheimb et al. (2013)
		LC429479	Poland: Mazovia; Kostrzyn River	Saito et al. (2018)
		MZ127285	Czech Republic: Moravia; Stupava	Lorencová et al. (2021)
<i>Gyraulus</i> sp.		KC495724	n/a	von Oheimb et al. (2013)
		KC495739	n/a	von Oheimb et al. (2013)
		KC495745	Nepal: Mahadevpuri.	von Oheimb et al. (2013)
		KC495749	Nepal: Mahendranagar	von Oheimb et al. (2013)
		KC495751	India: Muna Kote; Uttarakhand	von Oheimb et al. (2013)
		KC495754	Nepal: Latinath	von Oheimb et al. (2013)
		KC495756	Nepal: Bharatpur	von Oheimb et al. (2013)
		KC495811	n/a	von Oheimb et al. (2013)
		KC495822	China: Marmê; Gêrzê County; Ngari Prefecture; Tibet	von Oheimb et al. (2013)
		KT326312	China: Qinghai	von Oheimb et al. (2013)
		LC582936	Japan:Tokyo, Chichijima Island	n/a
		LC582937	Japan:Tokyo, Chichijima Island	n/a
		PQ730000	China: Yunnan, Lago Dianchi	Chen et al. (2025)
	<i>G. takhteevi</i>	MG773534	Russia: Lake Baikal	Sitnikova and Peretolchina (2018)
Segmentinini	<i>Hippeutis cantori</i>	KC135955	South Korea	n/a
	<i>Segmentina</i> sp.	MW829470	Sweden	Hobbs et al. (2021)

n/a: not available.

into distinct and distant clades. Therefore, despite previous discussions regarding the synonym of these two species, both taxa were herein treated as independent species, *G. chinensis* (BPP: 100%) and *G. convexiusculus* (BPP: 100%).

DISCUSSION

The conchological and anatomical characteristics of the specimens from Rio de Janeiro and Diadema were consistent with those described by Meier-Brook (1983) for *G. convexiusculus* and *G. chinensis*, both of which are Asian species. In fact, these two species of *Gyraulus*, common in Asian regions, have been considered part of a species complex or “race circle”. The term “Rassenkreis” (or “race circle”) was used by Meier-Brook (1983) to describe a group of geographically distributed populations that present morphological and anatomical differences but are not reproductively isolated, with the possibility of crossbreeding between them. In this case, the forms distributed from Iran to Japan and New Guinea would have as their central form *G. chinensis* and its synonyms, *G. convexiusculus* and *G. spirillus*, thus forming a single polytypic species according to Meier-Brook (1983). The author attributed all forms of *Gyraulus* from southern,

southeastern, and central mainland Asia to the *Gyraulus chinensis* species complex, even suggesting that previous reports of *Gyraulus albus* (Müller, 1774) in China were the result of mistaken identification and referred to *G. chinensis*. However, Shu et al. (2013), citing Zhang et al. (1997), indicated that *G. albus*, *Gyraulus compressus* (Hutton, 1849), and *G. convexiusculus* are valid species in China.

The specimens of both samples studied here presented rounded shell whorls without a keel, as well as a rounded aperture, except for some specimens from Rio de Janeiro that presented a slight angle at the aperture. The presence of spiral and growth lines, forming a slightly reticulated surface on the shell, was observed in some specimens from Rio de Janeiro, in agreement with what is described for *G. chinensis* (Meier-Brook 1983, Brown 2001). In the specimens from Diadema, only growth lines were observed, as described for *G. convexiusculus*. However, Meier-Brook (1983) pointed out that the original description by Dunker (1848) makes no mention of spiral lines and that this feature is not unique to *G. chinensis*, as it may also occur in other species, including members of different planorbis genera.

In the descriptive statistical analyses, the average total shell diameters were 2.78 mm for Rio de Janeiro and 3.14

mm for *Diadema*. The specimens of *Gyraulus* from Rio de Janeiro and *Diadema* showed the expected size for *G. chinensis*, which can reach up to 4 mm. According to Meier-Brook (1983), in *G. convexusculus* this measurement ranges from 4 to 5 mm; however, other authors report sizes up to 8 mm (Chen et al. 2024, Li et al. 2024). The mean flatness index was slightly higher in the *Diadema* specimens (0.299 ± 0.026) than in those from Rio de Janeiro (0.279 ± 0.096).

The presence of body and mantle pigmentation was a characteristic shared by specimens from both samples. According to Meier-Brook (1983), *G. convexusculus* and *G. chinensis* present high-contrast pigmentation, especially on the mantle. In Brown (1981, 2001), *G. convexusculus* is reported from South and East Asia, including northeastern New Guinea, with irregular pigmentation. The presence of mantle pigmentation is suggested as a possible synapomorphy of the Asian species (Meier-Brook 1983). Some authors have reported that the emergence of mantle pigmentation occurred in an ancestral form with a darker and more diffuse pattern, typical of some species from the western Palearctic region. However, most species of *Gyraulus* have distinct pigmentation and more clearly defined patterns, a derived trait transmitted eastward that reflects an evolutionary adaptation (Meier-Brook 1984). The presence of a population with a pigmentation pattern on the mantle was indicative of the potential for the introduction of *G. chinensis* into Australia by human activity, since this trait is rare in the native Australian fauna (Brown 2001).

According to Meier-Brook (1983), *G. convexusculus* has 10 to 15 prostatic diverticula ($\bar{x} = 13.1 \pm 1.7$), varying from 20 to 24 for specimens from Bangalore. In *G. chinensis*, the number of prostatic diverticula ranges from 11 to 15 (Hong Kong), and in the dichotomous key for specimens from Europe (except Macedonia), it varies from 8 to 20, with exceptions of up to 24. Both samples in the present study presented 12 to 16 diverticula, and the penis sheath of the specimens is slightly larger than the prepuce, which is consistent with the descriptions of both species (*G. convexusculus* and *G. chinensis*) in Meier-Brook (1983).

The sequences generated from both species analyzed here clustered into two distinct clades in the constructed phylogenetic tree. Specimen 3 from Rio de Janeiro showed high identity with sequences from GenBank identified as *G. chinensis* originating from the Czech Republic, South Africa, and Hong Kong, forming a well-supported clade (100%). The sequences from the Czech Republic and Hong Kong were obtained by Saito et al. (2025), who highlighted a close relationship with other Asian sequences near the type

locality of the species. Additionally, those authors found high haplotypic variability in the sequences for this species. The specimens from Rio de Janeiro (specimen 2) and *Diadema* (specimens 1, 2, and 3) grouped with a sequence identified as *G. convexusculus* from an aquarium population in Singapore. Singapore is considered the main exporting country and the seventh largest importer in the ornamental fish trade. Moreover, it acts as the primary redistribution center for global markets, including North America, Asia, Europe, and Oceania (Cheong 1996), making it a likely hub for the spread of accidentally introduced species (Duggan 2010). Part of this trade also extends to different tropical countries, expanding such dispersal to broader scales (Dudgeon and Yipp 1983 apud Madsen and Frandsen 1989). However, other sequences obtained from GenBank were identified as *G. convexusculus* and positioned in distinct clades, highlighting the possible existence of misidentifications for these *G. convexusculus* sequences, as also suggested by Chen et al. (2025).

Gyraulus chinensis and *G. convexusculus* are native to Asia and were described from Hong Kong and Afghanistan, respectively (Meier-Brook 1983). *Gyraulus chinensis* is known to be invasive on the European continent, and it has an introduced and potentially invasive status on the African continent (Appleton and Miranda 2015). In Europe, this species is present in drainage facilities, natural water bodies, greenhouses, fish tanks, and artificially heated habitats. In the Czech Republic, for example, *G. chinensis* is found in greenhouses (Beran and Glöer 2006, Appleton and Miranda 2015). In southern Europe, such as Italy, southern France, and the Iberian Peninsula, *G. chinensis* is recorded in rice crops (Brown 2001), and similarly on the African continent in Guinea-Bissau (Brown et al. 1998, Brown 2001). In Australia, Brown (2001) recorded *G. chinensis* for the first time, suspecting that colonization was the result of human action. The species was also recorded inhabiting lakes (Brown 2001), swamps (Pointier 2008 apud Appleton and Miranda 2015), and facilities for supplying fish and plants in the moving trade (Appleton and Miranda 2015). There are reports of invasion by *G. convexusculus* in Japan and the Middle East, probably through the trade in ornamental animals (Ng et al. 2016). In Israel, both species (*G. chinensis* and *G. convexusculus*) have also been introduced (Roll et al. 2009). In the European Union, incidental fauna showed a greater potential for species of *Gyraulus* to establish themselves in the environment when compared to other ornamental species, such as *G. chinensis* and *G. parvus*, which, based on probabilistic calculations, showed an extreme probability of establishment in new environments; that is, there is a high adaptive and survival potential for these species in the region (Patoka et al. 2017).

The presence of *Gyraulus* species, namely *G. convexiusculus* and *G. chinensis*, is recorded here for the first time in Brazil in aquarium shops. The aquarium industry is undoubtedly an important route for the introduction of aquatic ornamental species of mollusks, considering they can transport small species, juveniles, or eggs. Many of these organisms are moved around the globe and can establish populations due to accidental escape followed by environmental release (Corrêa et al. 1980, Thompson 1997, Cowie 2001, Vigliano and Darrigran 2002, Cowie and Robinson 2003, Letelier et al. 2007, Roll et al. 2009, Duggan 2010, Appleton and Miranda 2015, Ng et al. 2016, Patoka et al. 2017).

The introduction of non-native species has become one of the greatest contemporary environmental challenges. Therefore, it is crucial to identify the factors that contribute to this invasive process and understand the strategies to reduce its impact on ecosystems, which can result in significant changes in biodiversity and the functioning of natural environments (Saito et al. 2025), as well as the socio-ecological effects of biological invasions in aquatic habitats (Carranza et al. 2023). On the Hawaiian Islands, for instance, several freshwater gastropod species have become widespread, mainly cryptogenic ones, resulting in a predominance of alien over native freshwater snails, whereby unique native faunas have largely been replaced (Cowie 2001). In addition to replacing the native snail fauna, many of these species cause other ecological, agricultural, and medical problems (Cowie and Robinson 2003).

Although no species of *Gyraulus* have yet been found in the natural environment, this lack of records in the country may be due to the small size of the mollusk, which may cause it to go unnoticed in active collections or be confused with local species, such as the native *Antillorbis nordestensis* (Lucena, 1954) and *Drepanotrema* spp. Considering the recognized invasive potential of this species complex, it becomes evident how important it is to draw the attention of regulatory agencies, as well as aquarists and consumers, to the precautions necessary to avoid introducing exotic species into the environment. The present study warns of the introduction of non-native species through the commercial trade of ornamental animals and aquatic products and the impacts attributed to this trade.

ACKNOWLEDGEMENTS

We would like to thank all colleagues at the Laboratório de Malacologia (LMALAC), especially Marta C. Pinto for her support and guidance on the dissection of planorbids,

and Alexandre B. Pinheiro for his support in collecting and fixing mollusks. We also thank designer Eduardo Cinilha for processing and creating the image plates; and Walther Y. Ishikawa, a physician at the Instituto do Coração, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo (Incor – HCFMUSP) and founder/editor of the Planeta Invertebrados Brasil website, who kindly sent us the specimens provided by Cindy P. Lopes, an aquarist and science graduate from the Universidade Federal de São Paulo (UNIFESP).

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Submitted: July 2, 2025

Accepted: April 17, 2026

Editorial responsibility: Walter A.P. Boeger

Author Contributions

JPS: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Visualization, Validation. RTN, JRS: Formal analysis, Writing – review & editing. SRG: Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration. SCT: Writing – review & editing, Supervision, Resources, Project administration.

Competing Interests

The authors have declared that no competing interests exist.

Data Availability Statement

All data generated and/or analyzed are included in this article. GenBank accession numbers are provided in the manuscript.

Funding

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Finance Code 001) through a Master's scholarship awarded to the first author.

Ethical Statement

This study did not involve live vertebrate animals and therefore did not require approval by an ethics committee. Field activities were conducted under collection permits issued by Sisbio (permit no. 48371-2).

AI Statement

No artificial intelligence tools were used in the preparation

of this manuscript.

How to cite this article

Silva JP, Nogueira RT, Ramos-de-Souza J, Gomes SR, Thien-go SC (2026) Morphological and molecular evidence of Asian *Gyraulus* spp. (Gastropoda: Planorbidae) in artificial habitats in Brazil. *Zoologia* 43: e25046. <https://doi.org/10.1590/S1984-4689.v43.e25046>

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