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A new species of *Knodus* (Characiformes: Characidae), with deep genetic divergence, from the Mearim and Munim river basins, Northeastern Brazil, and evidence for hidden diversity in adjacent river basins

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A new species of *Knodus* from the Mearim and Munim River basins, Northeastern Brazil, is herein described based on integrative taxonomy, by using different molecular based species delimitation methods and independent approaches. The new species possesses the combination of character states that usually diagnoses the genus. The new species possesses a similar colour pattern to *K. victoriae*, which is also morphologically similar to it. The species described herein differs from *K. victoriae* by possessing more total vertebrae, more branched anal-fin rays, and fewer circumpeduncular scales. We also provide a detailed discussion of the morphological diagnostic features exhibited by *Knodus* species from adjacent river basins.

Keywords: Cryptic species, Integrative Taxonomy, Stevardiinae.

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Uma nova espécie de *Knodus* das bacias dos rios Mearim e Munim, Nordeste do Brasil, é descrita com base em taxonomia integrativa, utilizando diferentes métodos moleculares de delimitação de espécies e abordagens independentes. A nova espécie possui a combinação de estados de caráter que geralmente é utilizada para diagnosticar o gênero. A nova espécie possui um padrão de coloração semelhante a *K. victoriae*, que também é morfologicamente semelhante a ela. A espécie aqui descrita difere de *K. victoriae* por possuir mais vértebras totais, mais raios ramificados na nadadeira anal e menos escamas circumpedunculares. Nós também fornecemos uma discussão detalhada das características morfológicas diagnósticas exibidas por espécies de *Knodus* de bacias hidrográficas adjacentes.

Palavras-chave: Espécie críptica, Stevardiinae, Taxonomia Integrativa.

INTRODUCTION

Knodus Eigenmann, 1911 is one of the most species-rich characid genera within the subfamily Stevardiinae (Thomaz *et al*., 2015; Mirande, 2019; Ferreira *et al*., 2021), including 35 valid species (Menezes, Marinho, 2019; Sousa *et al*., 2020; Fricke *et al*., 2022). It is distributed among the Amazon, Tocantins-Araguaia, Orinoco, Paraná-Paraguay, Parnaíba, São Francisco, and Jequitinhonha river basins (Fricke *et al*., 2022), with its diversity peaking in the Amazon River basin (García-Melo *et al*., 2019; Fricke *et al*., 2022).

The genus *Knodus* is diagnosed only by the possession of two rows of pre-maxillary teeth – the inner row with four teeth – and a scaled caudal-fin (Eigenmann, 1918; Géry, 1972, 1977). This combination traditionally used to diagnose the genus has been questioned by some authors (*e.g*., Schultz, 1944; Taphorn, 1992; Román-Valencia, 2000; Román-Valencia *et al*., 2008) who consider *Knodus* a synonym of the closely related genus *Bryconamericus* Eigenmann, 1907.

Recent phylogenetic hypotheses based on molecular and morphological data have improved our knowledge on *Knodus* diversity and its intrageneric relationships (*e.g*., Thomaz *et al*., 2015; Mirande, 2019; García-Melo *et al*., 2019). However, although some species have been described within *Knodus* in recent years, the alpha taxonomy of most species and related genera is still somewhat confusing (García-Melo *et al*., 2019; Sousa *et al*., 2020).

Recent sampling efforts conducted in tributaries of the Mearim and Munin river basins (Northeast Brazil) revealed the existence of a new species morphologically similar to but still distinct from *Knodus victoriae* (Steindachner, 1907), whose type locality is in the upper Parnaiba River basin (Steindachner, 1907). Thus, we describe a new cryptic species of *Knodus sensu* Bickford *et al*. (2007), from the Mearim and Munim river basins, based on an integrative taxonomic approach.

MATERIAL AND METHODS

Taxon sampling, specimen collection, and preservation. Specimens were captured with a manual trail-net (2 m long × 1.8 m high; mesh size, 2 mm) and euthanized in a buffered solution of ethyl-3-amino-benzoat-methanesulfonate (MS–222) at a concentration of 250 mg/L until complete cessation of opercular movements, according to animal welfare laws and guidelines (Close *et al*., 1996; Leary *et al*., 2013). Specimens selected for morphological analysis were fixed in formalin for 10 days, after which they were preserved in 70% ethanol. Molecular data were obtained from specimens fixed and preserved in absolute ethanol. Specimens for morphological analysis are included in the lists of type and comparative material. Specimens for molecular approaches are listed in Tab. 1. Type material are deposited in the following ichthyological collections: Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista Júlio de Mesquita Filho, Botucatu (LBP); Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro (UFRJ); Coleção Ictiologica do Centro de Ciências Agrárias e Ambientais da Universidade Federal do Maranhão, Chapadinha (CICCAA); and Coleção Ictiológica da Universidade Estadual do Maranhão, São Luís (CIUEMA).

Morphological analysis. Measurements and counts were made according to Fink, Weitzman (1974), with exception of the scale rows below the lateral line, which were counted to the insertion of the pelvic fin. Vertical scale rows between the dorsal-fin origin and lateral line do not include the scale of the median predorsal series situated just anterior to the first dorsal fin ray. Counts of supraneurals, vertebrae, procurrent caudal fin rays, unbranched dorsal and anal fin rays, branchiostegal rays, gill-rakers, premaxillary, maxillary, and dentary teeth were taken only from cleared and stained paratypes (C&S), prepared according to Taylor, Van Dyke (1985). The four modified vertebrae which constitute the Weberian apparatus were not included in the vertebral counts and the fused PU1 + U1 was considered a single element. Osteological nomenclature follows Weitzman (1962). Comparisons with other species of *Knodus* were based on examined material, as well as information from literature (Zarske, Géry, 2006; Ferreira, Lima, 2006; Zarske, 2007, 2008; Ferreira, Carvajal, 2007; Román-Valencia *et al*., 2008; Menezes *et al*., 2009, 2020; Ferreira, Netto-Ferreira, 2010; Esguícero, Castro, 2014; Menezes, Marinho, 2019; Sousa *et al*., 2020; Deprá *et al*., 2021).

DNA extraction, amplification, and sequencing. DNA was extracted from fin clips using a saline buffer extraction protocol (Aljanabi, Martinez, 1997). Fragments of the mitochondrial gene cytochrome c oxidase subunit 1 (COI) were amplified with the universal primers designed by Ward *et al*. (2005) (FISHF1 5´-TCAACCAACCACAAAGACATTGGCAC-3´ and FISHR1 5´-TAGACTTCTGGGTGGCCAAAGAATCA-3´), *primers* from Melo *et al*. (2011) (COI L6252-Asn 5'-AAGGCG GGGAAAGCCCCGGCA G-3' and H7271-COXI 5'-TCC TATGTAGCCGAATGGTTC TTT T-3') and primers developed in the present study (KNODUS-TOF 5´-GGGCGATGACCAAATCTA-3´ and KNODUS-TOR 5´-AGGGTCGAAGAATGAGGTAT-3'). Polymerase chain reactions (PCR) for samples from *Knodus* sp. "Mearim", *Knodus* cf. *savannensis*, *Knodus* sp. "Maracaçumé", and *Knodus* cf. *victoriae* comprised a total volume of 15 μL containing 1x polymerase

buffer, 1.5 mM $MgCl_2$, 200 μM dNTP, 0.2 μM of each primer, 1U Taq polymerase (Invitrogen), 100 ηg DNA template, and ultrapure water. The PCR cycles were as follows: 2 min at 94°C, followed by 35 cycles of 94°C for 30s, 54°C for 30s and 72°C for 1 min and 10 min at 72°C. Polymerase chain reactions (PCR) for samples from the *Knodus* sp. "Munim", and *Knodus* sp. "Itapecuru" comprised a total volume of 15 μl containing 1x Polymerase buffer, 400 μM dNTP, 0,4 uM of each primer, 1U Taq Polymerase (Invitrogen), 100 ηg DNA template, and ultrapure water. The PCR cycles were as follows: 5 min at 94°C, followed by 35 cycles of 94°C for 45s, 48°-52°C for 45s, and 72°C for 1 min, and 10 min at 72°C. Amplicons were purified using ExoSAP-IT PCR Product Clean-up (Thermo Fisher Scientific) and Gel Purification Kit (GE Healthcare Systems), and sequenced using forward and reverse primers and the BigDye Terminator 3.1 Cycle Sequencing kit in a ABI 3730 DNA Analyzer (Thermo Fisher Scientific).

Data partitioning, evolutionary models, and alignment. The dataset included the COI sequences (401 base pairs, bp) of individuals from 10 *Knodus* species, including the species here described, specimens that we were not able to identify at the species level, as well as two *Bryconamericus* species. Many sequences were newly generated for this project, while others originated from the Barcode of Life Database (BOLD) and the National Center for Biotechnology Information (NCBI) (Tab. 1). Sequences were aligned using ClustalW (Chenna *et al*., 2003) and translated into amino acid residues using the program MEGA 7 (Kumar *et al*., 2016) to test if the sequences came from NUMTs (nuclear mitochondrial DNA sequences), in which case premature stop codons or indels would be expected. The best-fit evolutionary model (GTR+I+G) was selected using the Akaike Information Criterion (AIC) and the Corrected Akaike Information Criterion (AICc) by jModelTest 2.1.7 (Darriba *et al*., 2012), and used in all analyses, except for ABGD (Automatic Barcode Gap Discovery) which is a model-free approach based only on genetic distances.

Phylogenetic analysis. A Bayesian inference phylogenetic (BI) tree was estimated in MrBayes 3.2 (Ronquist *et al*., 2012) to reconstruct the evolutionary relationships among terminals using the General Time Reversible (GTR+I+G) evolutionary model. The BI analysis was conducted with the following parameters: two independent Markov chain Monte Carlo (MCMC) runs of two chains each for 10 million generations, with a tree sampling frequency at every 1,000 generations. The convergence of the MCMC chains and the proper burn-in value were assessed by evaluating the stationary phase of the chains using Tracer v. 1.6 (Rambaut *et al*., 2014). The final consensus tree and its posterior probabilities were generated with the remaining tree samples after removing the first 25% samples (burn-in). We used as outgroups sequences of *Bryconamericus exodon* Eigenmann, 1907 and *B. iheringii* (Boulenger, 1887). The remaining haplotypes were used as ingroups.

Species delimitation and molecular diagnoses. We implemented five distinct and independent single locus species delimitation methods based on molecular data, each of which rely on different operational criteria for species delimitation. DBC, DNA barcoding (hereafter Traditional DNA barcoding) was initially proposed by Hebert *et*

al. (2003a,b). Since then, it has improved and gained supporters due to its practicality and efficiency (*e.g*., Hajibabaei *et al*., 2007; Coissac *et al*., 2016; DeSalle, Goldstein, 2019). The premise of the method consists of standardized sequencing of a specific gene for each species, followed by the organization of the sequences in virtual reference libraries. Once a species is added to the library, any individual of that species, at any ontogenetic stage, or even fragments, can be identified by simple comparison, simply by sequencing the standard gene (Hebert *et al*., 2003a,b). For fish and other animals, the gene used is the mitochondrial protein cytochrome c oxidase I (COI) and its effectiveness has been frequently demonstrated (*e.g*., Costa-Silva *et al*., 2018; García-Melo *et al*., 2019). The methodology suggests that the maximum genetic distance between individuals of the same species, based on the COI sequences, can be defined for each taxonomic group. Any difference higher than this cut-off value would represent discontinuity between species (Hebert *et al*., 2003a,b). The other approaches were CBB, a character-based DNA barcoding method (DeSalle *et al*., 2005) adapted by Ottoni *et al*. (2019) and Guimarães *et al*. (2020b); GMYC, the General Mixed Yule Coalescent method, single-threshold version (Fujisawa, Barraclough, 2013); bPTP, the Bayesian implementation of the Poisson tree processes (Zhang *et al*., 2013); and ABGD, Automatic Barcode Gap Discovery (Puillandre *et al*., 2012).

Traditional DNA barcoding (DBC). We used the Kimura-2-parameter model (K2P) (Kimura, 1980) to estimate the pairwise genetic distances between species in MEGA 7 software (Kumar *et al*., 2016). We considered a cutoff of 2% as sufficient to discriminate species, since this threshold is commonly inferred by species delimitations among freshwater Neotropical fish species based on COI (Jacobina *et al*., 2018).

Molecular diagnosis (CBB). The molecular diagnosis approach delimited the new species by the presence of a unique combination of nucleotides at particular sites. In addition, the new species was diagnosed by nucleotide substitutions following Costa *et al*. (2014), Ottoni *et al*. (2019), and Guimarães *et al*. (2020b). Nucleotide substitutions among lineages were optimized on the Bayesian inference topology using PAUP version 4 (Swofford, 2002). Each nucleotide substitution is represented by its relative numeric position determined through sequence alignment with the complete mitochondrial COI gene of *Psalidodon paranae* (Eigenmann, 1914) (KX609386.1:5503-7062), followed by the specific nucleotide substitution in parentheses. Unique nucleotide substitutions in our analysis are marked with asterisk.

General Mixed Yule Coalescent (GMYC). The GMYC is a single locus coalescent-based species delimitation approach that relies on branch lengths to establish a threshold between speciation and coalescent processes (Fujisawa, Barraclough, 2013). Here we applied the single-threshold version of the method, which usually outperforms the multiple-threshold version (Fujisawa, Barraclough, 2013). A new reduced dataset was created for this analysis using DAMBE5 (Xia, 2013), including only unique haplotypes following the requirements of this method.

The ultrametric phylogenetic tree needed for input was inferred in BEAST version 1.8.4 (Drummond *et al*., 2012), with the following parameters: an uncorrelated relaxed clock with lognormal distribution, a Yule Process as tree prior with 10 million generations

and sampling frequency of 1,000. We used as outgroups sequences of *Bryconamericus exodon* and *B. iheringii*. The remaining haplotypes were used as ingroups. The GMYC analysis was performed on the Exelixis Lab's server https://species.h-its.org/gmyc.

Bayesian implementation of the Poisson tree processes (bPTP). The bPTP is another single locus coalescent-based species delimitation method that differs from other similar approaches, such as GMYC, by not requiring an ultrametric tree and thus not relying on branch lengths to delimit species (Zhang *et al*., 2013). The method assumes that more molecular variability (number of nucleotide substitutions) is expected between haplotypes from different species than within a species (Zhang *et al*., 2013), establishing a threshold between speciation and coalescent processes. The reduced dataset for performing the bPTP was the same at that used in GMYC, following the requirements of this method. The input phylogenetic tree was estimated in software Mrbayes 3.2 (Ronquist *et al*., 2012) to reconstruct the evolutionary relationships among terminals using the General Time Reversible (GTR+I+G) evolutionary model. The BI analysis was conducted with the following parameters: two independent Markov chain Monte Carlo (MCMC) runs of two chains each for 10 million generations, with a tree sampling frequency at every 1,000 generations. The convergence of the MCMC chains and the proper burn-in value were assessed by evaluating the stationary phase of the chains using Tracer version 1.6 (Rambaut *et al*., 2014). The final consensus tree and its posterior probabilities were generated with the remaining tree samples after removing the first 25% samples (burn-in). The remaining haplotypes were used as ingroups. The bPTP analysis was performed on the Exelixis Lab's web server http://species.h-its.org/ptp/, following the default parameters except for designation of a 20% burn-in. *Bryconamericus exodon* was chosen as the outgroup.

Automatic Barcode Gap Discovery (ABGD). The ABGD is a barcode species delimitation method that aims to establish a minimum gap that probably corresponds to the threshold between interspecific and intraspecific processes (Puillandre *et al*., 2012). The major advantage of ABGD when compared to the other barcode species delimitation methods is that the inference of the limit between interspecific and intraspecific processes (gap detection) is recursively applied to previously obtained groups to get finer partitions until there is no further partitioning, allowing a more refined search. Basically, the ABGD analysis indicates the number of groups (species) estimated relative to a large spectrum of p values (prior intraspecific values). A value of 0.1 indicates maximum intraspecific variability with all sequences belonging to a single species, whereas a 0.001 value indicates a small intraspecific variability with each distinct haplotype representing a different species. The reduced dataset for performing the ABGD was the same as that used in GMYC, following the requirements of this method. We ran ABGD on the ABGD server website https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html with default parameters, except for the X value (we used an X value of 1.0 as recommended by the server for our analysis). After running the ABGD, additional molecular, morphological, or ecological characters are needed to infer the correct number of species if the analysis will follow an integrative taxonomy paradigm.

RESULTS

Knodus guajajara Aguiar, Brito, Ottoni & Guimarães, new species

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(Figs. 1–3; Tabs. 2–3)

Knodus victoriae [non *Knodus victoriae* (Steindachner, 1907)]. —Guimarães *et al.* (2020a:90). —Oliveira *et al.* (2020:6–7).

Holotype. CICCAA 4883, 31.4 mm SL, Alto Alegre do Pindaré municipality, Igarapé Arapapá, Pindaré River drainage, Mearim River basin, 03°42'26"S 46°00'25"W, Nov 2016. E. C. Guimarães & P. S. Brito.

FIGURE 1 | *Knodus guajajara*, holotype, CICCAA 4883, 31.4 mm SL, Alto Alegre do Pindaré municipality, Igarapé Arapapá, Pindaré River drainage, Mearim River basin.

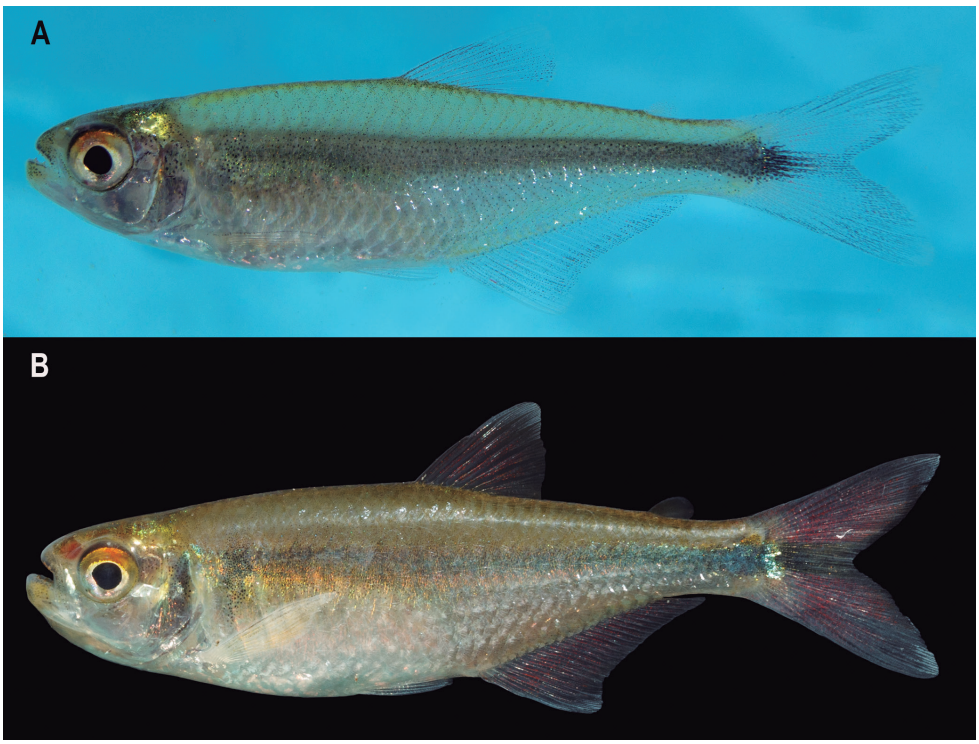

FIGURE 2 | *Knodus guajajara*, paratypes. **A.** CICCAA 1518, 22.8 mm SL, Brazil, Maranhão, Alto Alegre do Pindaré municipality, igarapé Jenipapo, Pindaré River drainage, Mearim River basin. **B.** CICCAA 2696, 40.2 mm SL, Brazil, Maranhão, Chapadinha municipality, stream in riparian forest on the road BR–222, Munim River basin.

TABLE 1 | Specimens and DNA sequence information included in the study. Sequences made available by this study follow with asteristic.

Nº	Species	Depositories of vouchers			Genbank ID
		Collection	Tissue code	Voucher	
1	*Bryconamericus exodon*	LBP	26191	5118	MH002960
2	*Bryconamericus iheringii*	LBP	60645	14473	MH002991
3	*Knodus alpha*	STRI	9564	531	MH003217
4	*Knodus alpha*	LBP	9561	663	MH003216
5	*Knodus borki*	LBP	53820	12472	MH003218
6	*Knodus borki*	LBP	–	53821	KT248111
7	*Knodus caquetae*	MPUJ	637	11073A	MH003221
8	*Knodus caquetae*	MPUJ	639	11073B	MH003222
9	*Knodus heteresthes*	LBP	57371	13870	MH003232
10	*Knodus heteresthes*	LBP	37317	7959	MH003233
11	*Knodus megalops*	LBP	54223	12566B	MH003234
12	*Knodus meridae*	LBP	15818	7569	MH003235
13	*Knodus tiquiensis*	LBP	–	33217	KT248096
14	*Knodus tiquiensis*	LBP	–	33218	KT248097
15	*Knodus tiquiensis*	LBP	–	33216	KT248098
16	*Knodus victoriae*	LBP	–	27366	KT248128
17	*Knodus victoriae*	LBP		27370	KT248129
18	*Knodus victoriae*	LBP	27342	5607	KT248130
19	*Knodus victoriae*	LBP	–	27254	KT248131
20	*Knodus victoriae*	LBP	–	27368	KT248132
21	*Knodus victoriae*	LBP	–	27295	KT248133
22	*Knodus victoriae*	LBP		27369	KT248134
23	*Knodus victoriae*	LBP	–	27253	KT248135
24	*Knodus victoriae*	LBP	–	27255	KT248136
25	*Knodus victoriae*	LBP	–	27367	KT248137
26	*Knodus* aff. *victoriae* (Balsas)*	CICCAA	4817.1	4817	MW556675
27	*Knodus* aff. *victoriae* (Balsas)*	CICCAA	4817.2	4817	MW556676
28	*Knodus* aff. *victoriae* (Balsas)*	CICCAA	4817.3	4817	MW556677
29	*Knodus* aff. *victoriae* (Balsas)*	CICCAA	4818.1	4818	MW556678
30	*Knodus* aff. *victoriae* (Balsas)*	CICCAA	4818.2	4818	MW556679
31	*Knodus* aff. *victoriae* (Balsas)*	CICCAA	4818.3	4818	MW556680
32	*Knodus* aff. *victoriae* (Itapecuru)*	CICCAA	2064.1	2064	MW556681
33	*Knodus* aff. *victoriae* (Itapecuru)*	CICCAA	2064.2	2064	MW556682
34	*Knodus* cf. *savannensis*	LBP	–	66360	KT248199

TABLE 1 | (Continued)

Nº	Species	Depositories of vouchers			Genbank ID
		Collection	Tissue code	Voucher	
35	*Knodus* cf. *savannensis*	LBP	–	66339	KT248200
36	*Knodus* cf. *savannensis*	LBP	–	66340	KT248201
37	*Knodus* cf. *savannensis*	LBP	–	66356	KT248202
38	*Knodus* cf. *savannensis*	LBP	–	66362	KT248203
39	*Knodus* cf. *savannensis*	LBP	–	66363	KT248204
40	*Knodus* cf. *savannensis*	LBP	–	66363	KT248218
41	*Knodus* cf. *savannensis*	LBP	–	62500	KT248219
42	*Knodus* cf. *savannensis* (Carolina-MA, Tocantins River basin)*	CICCAA	3609.1	3609	MW556695
43	*Knodus* sp. "Guamá"	LBP	43070	9141	KT248223
44	*Knodus* sp. "Guamá"	LBP	–	43718	KT248225
45	*Knodus* sp. "Guamá"	LBP	–	43067	KT248226
46	*Knodus* sp. "Guamá"	LBP	–	43068	KT248227
47	*Knodus* sp. "Tapajós"	LBP	57048	13750	KT248123
48	*Knodus* sp. "Tapajós"	LBP	–	57049	KT248124
49	*Knodus* sp. "Xingu"	LHGP	–	66314	KT248184
50	*Knodus* sp. "Xingu"	LHGP	–	66315	KT248185
51	*Knodus* sp. (Marabá)*	CICCAA	2087.1	2087	MW556692
52	*Knodus* sp. (Marabá)*	CICCAA	2087.2	2087	MW556693
53	*Knodus* sp. (Marabá)*	CICCAA	2087.3	2087	MW556694
54	*Knodus* sp. (Maracaçumé)*	CICCAA	2390.1	2390	MW556683
55	*Knodus* sp. (Maracaçumé)*	CICCAA	2390.2	2390	MW556684
56	*Knodus* sp. (Maracaçumé)*	CICCAA	2390.5	2390	MW556685
57	*Knodus guajajara**	CICCAA	2052.2	2052	MW556688
58	*Knodus guajajara**	CICCAA	2052.3	2052	MW556689
59	*Knodus guajajara**	CICCAA	2052.4	2052	MW556690
60	*Knodus guajajara**	CICCAA	2052.5	2052	MW556691
61	*Knodus guajajara**	CICCAA	2391.1	2391	MW556686
62	*Knodus guajajara**	CICCAA	2391.2	2391	MW556687

Paratypes. All from Brazil, Maranhão State: CICCAA 1535, 1, 29.2 mm SL, collected with holotype. CICCAA 1585, 3, 28.0–34.7 mm SL, Alto Alegre do Pindaré municipality, igarapé Arapapá, Pindaré River drainage, Mearim River basin, 03°42'26"S 46°00'25"W, Nov 2015, E. C. Guimarães & P. S. Brito. CICCAA 4860, 2 C&S, 29.9–31.6 mm SL, Alto Alegre do Pindaré municipality, igarapé Arapapá, Pindaré River drainage, Mearim River basin, 03°42'26"S 46°00'25"W, Nov 2015, E. C. Guimarães & P. S. Brito. LBP 31041, 16, 24.5–31.4 mm SL, Alto Alegre do Pindaré municipality, Igarapé Igarapá, Pindaré River drainage, Mearim River basin, 03°45'51"S 46°08'15"W, Nov 2015, E. C. Guimarães & P. S. Brito. CICCAA 4858, 5 C&S, 22.9–27.0 mm SL, Alto Alegre do

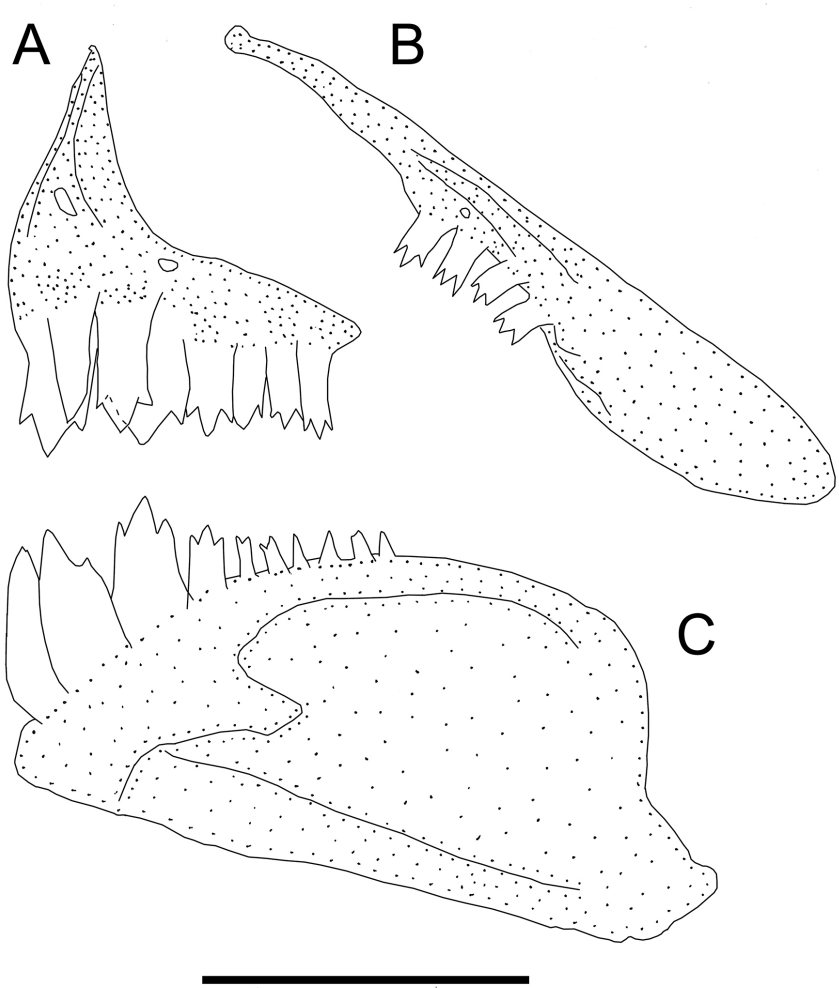

FIGURE 3 | *Knodus guajajara*, CICCAA 4861, paratype, 31.9 mm SL, jaw suspensorium. **A.** Premaxillary. **B.** Maxilla. **C.** Dentary. Scale bar = 1 mm.

Pindaré municipality, Igarapé Igarapá, Pindaré River drainage, Mearim River basin, 03°45'51"S 46° 08'15"W, Nov 2015, E. C. Guimarães & P. S. Brito. CICCAA 1517, 2, 21.8–24.4 mm SL, Alto Alegre do Pindaré municipality, igarapé Caititu, Pindaré River drainage, Mearim River basin, 03°42'30"S 46°01'19"W, Jul 2017, E. C. Guimarães & P. S. Brito. CICCAA 4859, 2 C&S, 23.5–27.6 mm SL, Alto Alegre do Pindaré municipality, igarapé Caititu, Pindaré River drainage, Mearim River basin, 03°42'30"S 46°01'19"W, Jul 2017, E. C. Guimarães & P. S. Brito. CICCAA 1536, 2, 28.8–29.8 mm SL, Buriticupu municipality, Buritizinho River, Pindaré River drainage, Mearim River basin, 04°11'53"S 46°28'41"W, Nov 2016, E. C. Guimarães & P. S. Brito. CICCAA 4861, 1 C&S, 31.9 mm SL, Buriticupu municipality, Buritizinho River, Pindaré River drainage, Mearim River basin, 04°11'53"S 46°28'41"W, Nov 2016, E. C. Guimarães & P. S. Brito. CICCAA 1227, 1, 29.6 mm SL, Buriticupu municipality, Buritizinho River, Pindaré River drainage, Mearim River basin, 04°19'45"S 46°29'46"W, 27 Jan 2017, E. C. Guimarães & P. S. Brito. UFRJ 7023, 10, 22.8–29.3 mm SL, Alto Alegre do Pindaré municipality, igarapé Jenipapo, Pindaré River drainage, Mearim River basin,

03°51'20"S 46°11'09"W, Jul 2017, E. C. Guimarães & P. S. Brito. CICCAA 4862, 5 C&S, 23.9–29.3 mm SL, Brazil, Maranhão State, Alto Alegre do Pindaré municipality, igarapé Jenipapo, Pindaré River drainage, Mearim River basin, 03°51'20"S 46°11'09"W, Jul 2017, E. C. Guimarães & P. S. Brito. CICCAA 1321, 39, 19.4–29.2 mm SL, Miranda do Norte municipality, Mearim River basin, 04°19'45"S 46°29'46"W, Nov 2016, E. C. Guimarães & P. S. Brito. CICCAA 4863, 11 C&S, 19.7–24.4 mm SL, Miranda do Norte municipality, Mearim River basin, 04°19'45"S 46°29'46"W, Nov 2016, E. C. Guimarães & P. S. Brito. CIUEMA 1021, 8, 21.2–26.9 mm SL, Alto Alegre do Pindaré municipality, Igarapé Timbira, Pindaré River drainage, Mearim River basin, 03°32'57"S 44°39'38"W, Nov 2015, E. C. Guimarães & P. S. Brito. CICCAA 3423, 42, 19.5–30.21 mm SL, CICCAA 3424, 57, 22.35–26.9 mm SL,, Chapadinha municipality, Riacho da Raiz, RESEX Chapada Limpa, Munim River basin, 03°53'45"S 43°29'21"W,

TABLE 2 | Morphometric data (N = 110) of the holotype and paratypes of *Knodus guajajara* from the Mearim River basin. SD = Standard deviation.

	Holotype	Paratypes	Mean	SD
Standard length	31.4	19.4–34.7	25.3	–
Percents of standard length				
Depth at dorsal-fin origin (body depth)	29.8	20.1–30.2	24.7	3.3
Snout to dorsal-fin origin	55.3	47.8–57.1	52.2	5.5
Snout to pectoral-fin origin	27.1	21.7–29.32	24.9	3.0
Snout to pelvic-fin origin	45.7	37.6–49.3	42.2	4.9
Snout to anal-fin origin	60.2	44.0–61.6	53.9	6.1
Caudal peduncle depth	10.5	7.6–11.7	9.3	1.2
Caudal peduncle length	15.3	7.1–15.2	11.1	1.9
Pectoral-fin length	20.5	14.8–23.8	18.7	2.6
Pelvic-fin length	13.5	9.8–15.4	12.6	1.7
Dorsal-fin base length	9.1	8.7–15.2	11.5	1.7
Dorsal-fin height	26.9	17.7–27.0	21.3	2.8
Anal-fin base length	29.4	25.5–36.3	30.5	3.8
Anal-fin lobe length	13.0	9.8–20.3	15.3	2.4
Eye to dorsal-fin origin	42.5	33.6–43.8	38.9	4.5
Dorsal-fin origin to caudal-fin base	52.5	39.4–56.7	46.9	5.8
Percents of head length				
Head length	23.9	21.3–26.5	23.3	2.5
Horizontal eye diameter	41.3	32.6–45.6	39.5	4.8
Snout length	24.9	15.8–29.6	21.8	3.3
Least interorbital width	35.1	23.7–36.8	32.3	4.0
Upper jaw length	47.5	31.5–48.8	38.3	5.3

TABLE 3 | Morphometric data (N = 136) for the paratypes of *Knodus guajajara* from the Munim River basin. SD = Standard deviation.

	Range	Mean	SD
Standard length	19.5–37.7	25.3	–
Percents of standard length			
Depth at dorsal-fin origin (body depth)	20.4–37.2	24.7	2.06
Snout to dorsal-fin origin	47.4–53.4	50.3	1.13
Snout to pectoral-fin origin	21.9–27.1	24.4	0.79
Snout to pelvic-fin origin	39.4–45.7	42.5	1.24
Snout to anal-fin origin	53.1–59.8	56.2	1.38
Caudal peduncle depth	8.3–10.4	9.3	0.41
Caudal peduncle length	9.2–12.8	10.4	0.75
Pectoral-fin length	18.5–23.4	20.9	0.74
Pelvic-fin length	12.0–15.7	13.8	0.68
Dorsal-fin base length	8.4–12.6	10.1	0.76
Dorsal-fin height	20.1–24.6	22.1	0.86
Anal-fin base length	22.8–28.7	26.4	1.17
Anal-fin lobe length	16.1–20.2	18.1	0.82
Eye to dorsal-fin origin	33.1–39.2	36.1	1.12
Dorsal-fin origin to caudal-fin base	47.4–54.3	49.5	1.14
Percents of head length			
Head length	21.5–25.7	23.4	0.82
Horizontal eye diameter	33.8–42.6	37.4	1.56
Snout length	20.1–25.9	22.5	1.34
Least interorbital width	27.1–35.3	31.7	1.77
Upper jaw length	34.5–41.2	37.9	1.47

11 Aug 2019, J. Reis, L. Oliveira, F. Ottoni, R. Fernandes & A. Silva. Chapadinha municipality, Bandeira River, Povoado Mata do Jeroca, RESEX Chapada Limpa, Munim River basin, 03°59'40"S 43°29'24"W, 11 Aug 2019, J. Reis, L. Oliveira, F. Ottoni, R. Fernandes & A. Silva. CICCAA 3425, 22, 20.22–26.71 mm SL, CICCAA 3426, 6, 28.87–32.6 mm SL, Chapadinha municipality, stream at Bairro Aldeia, Munim River basin, 03°44'53"S 43°21'32"W, 28 Jan 2019, F. Carvalho, H. Silva, L. Oliveira & I. Gôuvea. CICCAA 3467, 2, 21.22–21.43 mm SL, Anapurus municipality, stream on the road at Povoado de Paços, Munim River basin, 03°33'44"S 43°03'52"W, Sep 2019, D. Campos, J. Reis & F. Ottoni. CICCAA 4808, 3, 25.19–34.2 mm SL, CICCAA 4822, 1, 36.89 mm SL, Chapadinha municipality, stream at balneário Repouso do Guerreiro, Bairro Independência, Munim River basin, 03°44'57"S 43°20'26"W, Nov 2019, B. Furtado, M. Paiva, A. Bezerra, M. Coelho & I. Gouvêa. CICCAA 4490, 5, 24.73–29.11 mm SL, Chapadinha municipality, Riacho Fundo, Munim River basin, 03°42'20"S

43°31'46"W, 23 Sep 2018, L. Sousa, L. Oliveira & I. Gouvêa, CICCAA 4491, 3 C&S 23.26–28.19 mm SL, Chapadinha municipality, Riacho Fundo, Munim River basin, 03°42'20"S 43°31'46"W, 23 Sep 2018, L. Sousa, L. Oliveira & I. Gôuvea.

Morphological diagnosis. *Knodus guajajara* differs from *K. borki* Zarske, 2008 and *K. delta* Géry, 1972 by having a complete lateral line (*vs*. incomplete lateral line) and from *K. cupariensis* de Sousa, Silva-Oliveira, Canto & Ribeiro, 2020 and *K. geryi* Lima, Britski & Machado, 2004 by having caudal fin lobes with sparse chromatophores and lacking basal blotches (*vs.* a dark basal blotch on each caudal fin lobe, Sousa *et al*., 2020; fig. 1); from *Knodus borki*, *K. diaphanus* (Cope, 1878), *K. victoriae*, *K. heteresthes* (Eigenmann, 1908), *K. deuterodonoides* (Eigenmann, 1914), *K. longus* Zarske & Géry, 2006, *K. septentrionalis* Géry, 1972, *K. figueiredoi* Esguícero & Castro, 2014, *K. geryi*, *K. meridae* Eigenmann, 1911, *K. nuptialis* Menezes & Marinho, 2019, *K. orteguasae* (Fowler, 1943), *K. tiquiensis* Ferreira & Lima, 2006, *K. angustus* Menezes, Ferreira & Netto-Ferreira, 2020, *K. rufford* Deprá, Ota, Vitorino-Júnior & Ferreira, 2021 and *K. obolus* Deprá, Ota, Vitorino-Júnior & Ferreira, 2021 by having 20–25 branched rays in the anal-fin (mode 23) (*vs.* 12–19, combined); and from *K. tiquiensis* by having a single humeral spot (*vs.* two). *Knodus guajajara* is distinguished from *K. breviceps* (Eigenmann, 1908) and *K. savannensis* Géry, 1961 by having a conspicuous round humeral blotch (*vs.* inconspicuous and vertically elongate); from *K. dorsomaculatus* Ferreira & Netto-Ferreira, 2010 by having a hyaline dorsal-fin (*vs.* dark blotch on the base of the first five branched dorsal fin rays); from *K. alpha* (Eigenmann, 1914), *K. chapadae* (Fowler, 1906), *K. geryi*, *K. hypopterus* (Fowler, 1943), *K. mizquae* (Fowler, 1943) and *K. shinahota* Ferreira & Carvajal, 2007 by having 4 or 5 rows of scales between the lateral line and the dorsal-fin origin (*vs.* 6 rows of scales); from *K. cinarucoensis* (Román-Valencia *et al*., 2008), *K. gamma* Géry, 1972 and *K. longus* Zarske & Géry, 2006 by having 12 or 13 scales in the median series between the tip of the supraoccipital spine and the dorsal-fin origin (*vs.* 10 or 11 rows of scales in *K. gamma* and 17 to 18 in *K. longus*); from *K. jacunda* (Fowler, 1913), *K. moenkhausii* (Eigenmann & Kennedy, 1903), *Knodus cismontanus* (Eigenmann, 1914), *Knodus caquetae* Fowler, 1945, *K. tanaothoros* (Weitzman, Menezes, Evers & Burns, 2005), *K. weitzmani* (Menezes, Netto-Ferreira & Ferreira, 2009) and by having 3 to 5 maxillary teeth (*vs.* absence in *K. jacunda*, one in *K. caquetae*, and 2 in *K. moenkhausii*, *K. tanaothoros, K. weitzmani* and *K. cismontanus*); from *K. megalops* Myers, 1929 by having 3 or 4 tricuspid teeth in the premaxillary outer row (*vs.* 5); from *K. jacunda* by having 3 to 5 maxillary teeth (*vs*. absence); from *K. smithi* (Fowler, 1913) by having 3 to 5 cusps on the teeth of the inner row of the premaxilla (*vs.* 7); and from *K. figueredoi*, *K. heteresthes*, *K. meridae*, *K. mizquae*, *K. moenkhausii*, *K. victoriae*, *K. pasco* Zarske, 2007 by having 12 circumpeduncular scales (*vs.* 13–14 combined). Furthermore, *Knodus guajajara* differs from *K. victoriae* by having more total vertebrae 33–35, mode 34 (*vs.* 30–33, mode 32).

Description. Morphometric data presented in Tabs. 2–3. Body comparatively small, with largest specimen examined measuring 37.7 mm SL. Greatest body depth at dorsal-fin origin. Dorsal profile of head convex from upper lip to vertical through middle portion of eye; slightly concave from this point to tip of supraoccipital spine; straight to slightly convex from posterior tip of supraoccipital spine to dorsal-fin origin; dorsal-

fin base straight; slightly convex to straight from end of dorsal-fin base to adipose fin and concave from latter point to anterior dorsal-procurrent ray. Ventral profile of body convex from lower lip to anal-fin origin; straight, posterodorsally inclined along anal-fin base. Dorsal and ventral profile of caudal peduncle slightly concave.

Mouth sub-terminal; jaws isognathous. Posterior terminus of maxilla extending beyond anterior margin of orbit. Premaxillary teeth in two rows; outer row with 3(18) and 4(8) tricuspid teeth, inner row with 4(26) tri-to pentacuspid teeth. Maxilla with 3(11), 4(13), 5(2) tri-to pentacuspid teeth. Dentary with three anteriormost teeth large, tri and pentacuspid teeth, followed by 4(2), 5(2), 6(4), 7(13) and 8(5) smaller, uni, tricuspid or pentacuspid teeth (Fig. 3).

Scales cycloid, moderately large, with 6–8 well-marked radii; circuli only present proximally. Lateral line complete, slightly curved ventrally along its anterior third, with 36(8), 37(61), 38*(137), 39(13), or 40(7) perforated scales. Longitudinal series of scales between dorsal-fin origin and lateral line 4(30) or 5*(202). Longitudinal series of scales between lateral line and pelvic-fin origin 4*(63), or 5(184). Predorsal scales 11(3), 12(106) or 13*(122). Circumpeduncular scales 12*(220). Single series of scales on anal-fin base.

Dorsal fin rays ii,7(45) or 8*(191). Dorsal-fin origin slightly posterior to middle of body, and slightly posterior to vertical through pelvic-fin origin. First unbranched ray approximately half length of second unbranched ray. Pectoral-fin rays i,9(9), 10(142) or 11*(94); tip of pectoral fin reaching or exceeds pelvic fin origin when adpressed. Pelvic-fin rays i,5,i(41) or i,6,i*(189); tip of pelvic fin usually reaching first anal fin rays when adpressed. Anal-fin rays iv, 20(2), 21(8), 22(40), 23*(140), 24(37) or 25(15). Anal-fin origin located at vertical through middle of dorsal fin. Anal-fin rays decreasing gradually in length. Caudal fin forked, lobes equal in size. Principal caudal-fin rays i,9,8,i(219). Dorsal procurrent caudal-fin rays 8(1), 9(1), 10(18), 11(3) or 12(3) and ventral procurrent caudal-fin rays 9(3), 10(2), 11(15), 12(3) or 13(3). Adipose-fin origin approximately at vertical through base of 19th to 21st branched anal-fin rays. Gill rakers on first gill arch 15(2), 16(4) or 17(2): hypobranchial 2(4) or 3 (4), ceratobranchial 7(8), cartilage between epibranchial and ceratobranchial 1(8), epibranchial 5(4) or 6(4). Gill rakers setiform. Branchiostegal rays 4(8), 3(8) on anterior ceratohyal and 1(8) on posterior ceratohyal. Total vertebrae 33(3), 34(18) or 35(8). First dorsal fin pterygiophore inserted between 10th and 12th vertebrae. First anal fin pterygiophore inserted between 16th and 17th vertebrae. Supraneurals 4(2), 5(12) or 6(9).

Color in alcohol. Ground of body and head light brown, with dorsal region of body (especially dorsal profile), dorsal portion of head (just above eyes), and upper snout area (upper jaw and adjacent areas) darker. Ventral region of trunk and head with light brown coloration, especially on scales adjacent to anal-fin base. Dark colored chromatophores concentrated on scales of dorsal region (from both body and head), on opercle, and lateral band of flank (especially on posterior region, near caudal peduncle and caudal-fin base). Conspicuous vertically elongated humeral spot, with some dark scattered chromatophores above and below this blotch. Lateral band formed by chromatophores located above lateral line, extending gradually from humeral blotch to middle rays of caudal fin, where they become thicker and more concentrated. All fins including adipose hyaline, with chromatophores on inter-radial membranes of dorsal fin. Anal and caudal fins with posterior margins dark grey.

Color in life. Yellowish coloration in dorsolateral region and silver coloration in ventrolateral region of body (Fig. 2). Gray lateral band located in middle portion of trunk, with bright pigment extending from operculum to caudal-fin base. Dorsal portion of eye yellowish-orange and lower portion silver; mouth and dorsolateral portion of head yellowish with concentrated chromatophores; operculum with silver coloration. Fins hyaline with chromatophores present and especially concentrated in medial region of dorsal fin, distal region of anal and caudal fins, on first pectoral-fin ray, and just posterior to medial lateral band on caudal fin. Adipose fin with yellowish-orange pigmentation; yellowish-orange pigments also present in middle region of caudal fin.

Sexual dimorphism. Hooks present on pelvic fins and anal fin of sexually mature males. Pelvic fins with 5 to 14 hooks per branched ray; anal fin with 2 to 8 hooks per ray. Hooks concentrated on anterior anal fin rays (Fig. 4).

Geographical distribution. *Knodus guajajara* occurs in the rivers, streams, and lagoons of the Mearim and Munim river basins, in the State of Maranhão, Northeast Brazil (Fig. 5).

Etymology. The specific epithet honors the Guajajara indigenous tribe, which is one of the most numerous indigenous peoples in Brazil. They inhabit more than 10 Indigenous Lands on the eastern margin of the Amazon, all located in Maranhão. A noun in apposition the language spoken by them is the Teneteara, from the Tupi-Guarani linguistic family.

Conservation status. *Knodus guajajara* occurs in two distinct river basins of Maranhão, the Mearim and Munim river basins. It was found in several collecting sites along these basins, and it is always abundant. Therefore, according to the guidelines given in the International Union for Conservation of Nature (IUCN) categories and criteria (IUCN, 2019), we recommend that *Knodus guajajara* should be categorized as Least Concern (LC).

Molecular species delimitation

Molecular diagnosis. *Knodus guajajara* is diagnosed molecularly by a combination of 19 nucleotide substitutions, four of them unique* (listed below):

COI 279 (C→A), COI 282 (C→T), COI 306 (C→T), COI 327 (C→T), COI 357 (A→G*), COI 372 (A→G*), COI 378 (A→G), COI 414 (T→C), COI 450 (T→C), COI 459 (C→T), COI 510 (T→C), COI 528 (C→T), COI 537 (C→T), COI 565 (C→T), COI 585 (T→C), COI 589 (C→T), COI 597 (C→T), COI 627 (A→G*), COI 645 (T→A*).

Traditional DNA Barcoding. COI sequences support the existence of a new species of *Knodus* inhabiting the Mearim and Munim River basins. After trimming, the final alignment yielded 401 base pairs with 132 variable sites and 62 haplotypes. *Knodus guajajara* is 14% divergent, on average, from the other taxa, with a minimum genetic distance of 11% to *K. borki*, *K. caquetae* and *Knodus* sp. "Maracaçumé", and a maximum

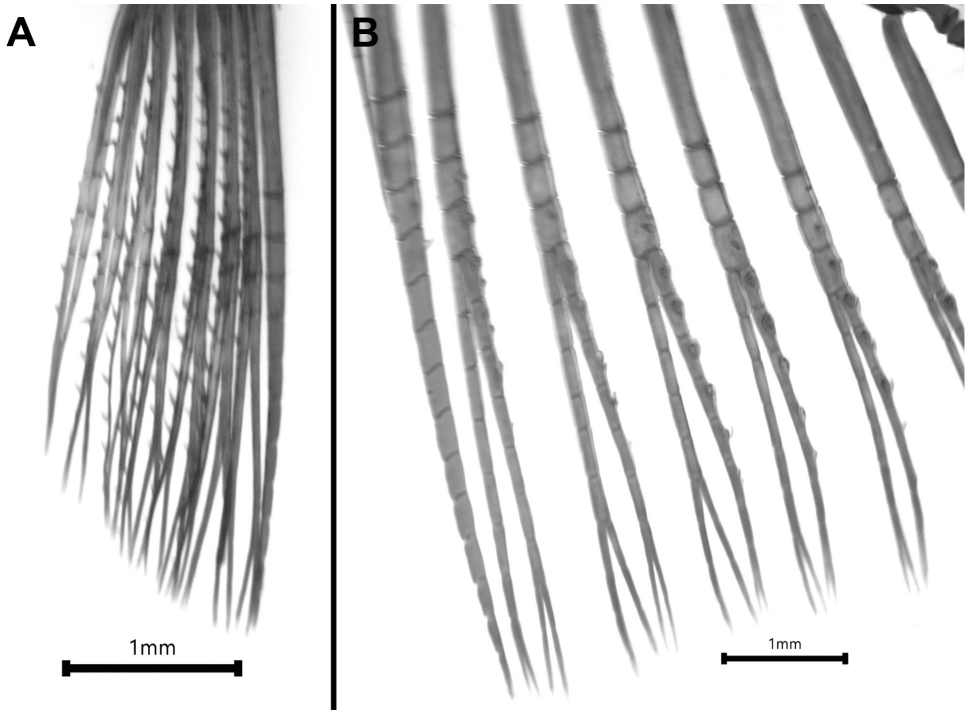

FIGURE 4 | *Knodus guajajara*, CICCAA 4861, paratype, male, 31.9 mm SL Maranhão, Mearim River basin. **A.** Hooks on pelvic fin. **B.** Hooks on anal fin. (Photographed by F. P. Ottoni).

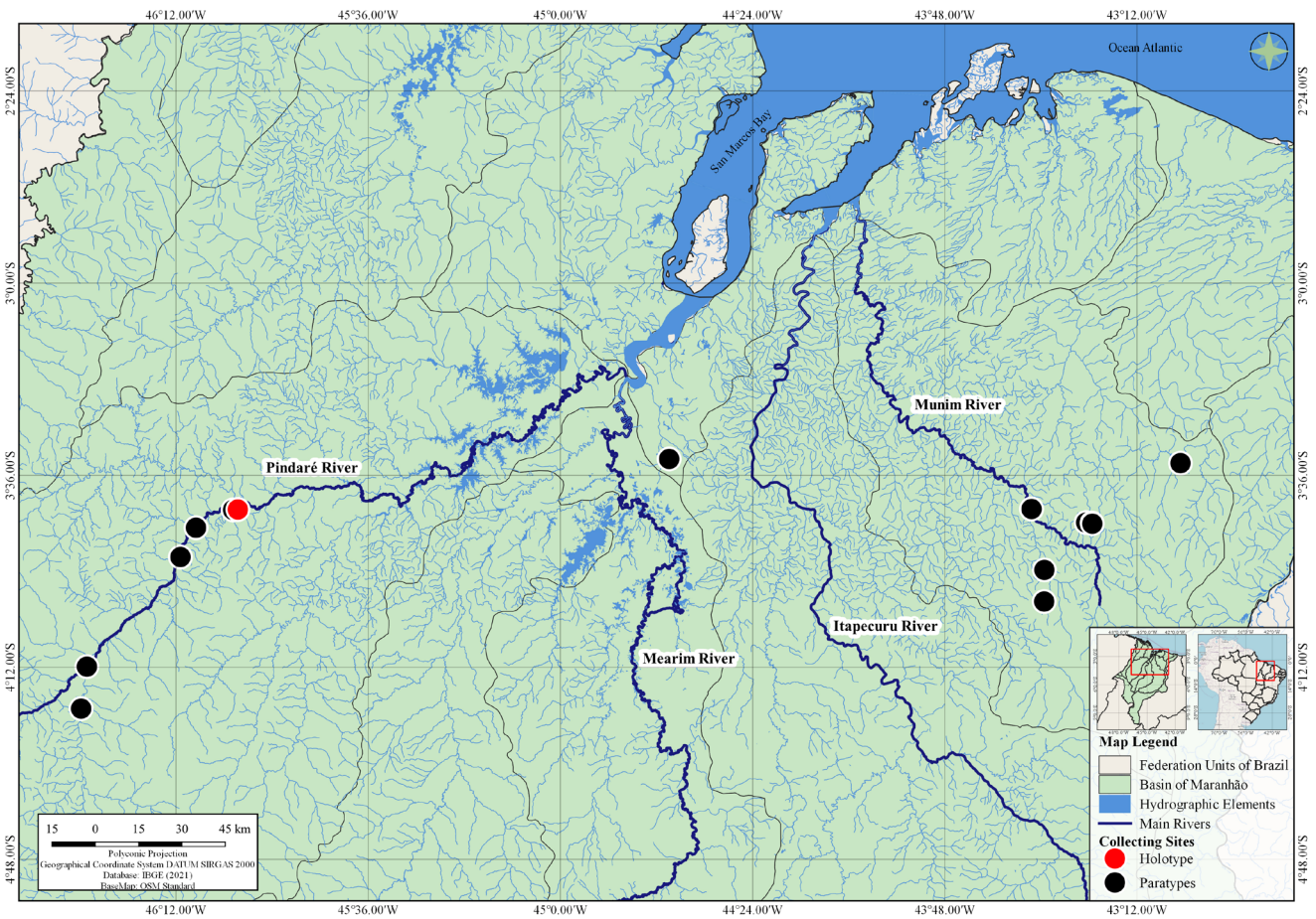

FIGURE 5 | Geographical distribution of *Knodus guajajara*.

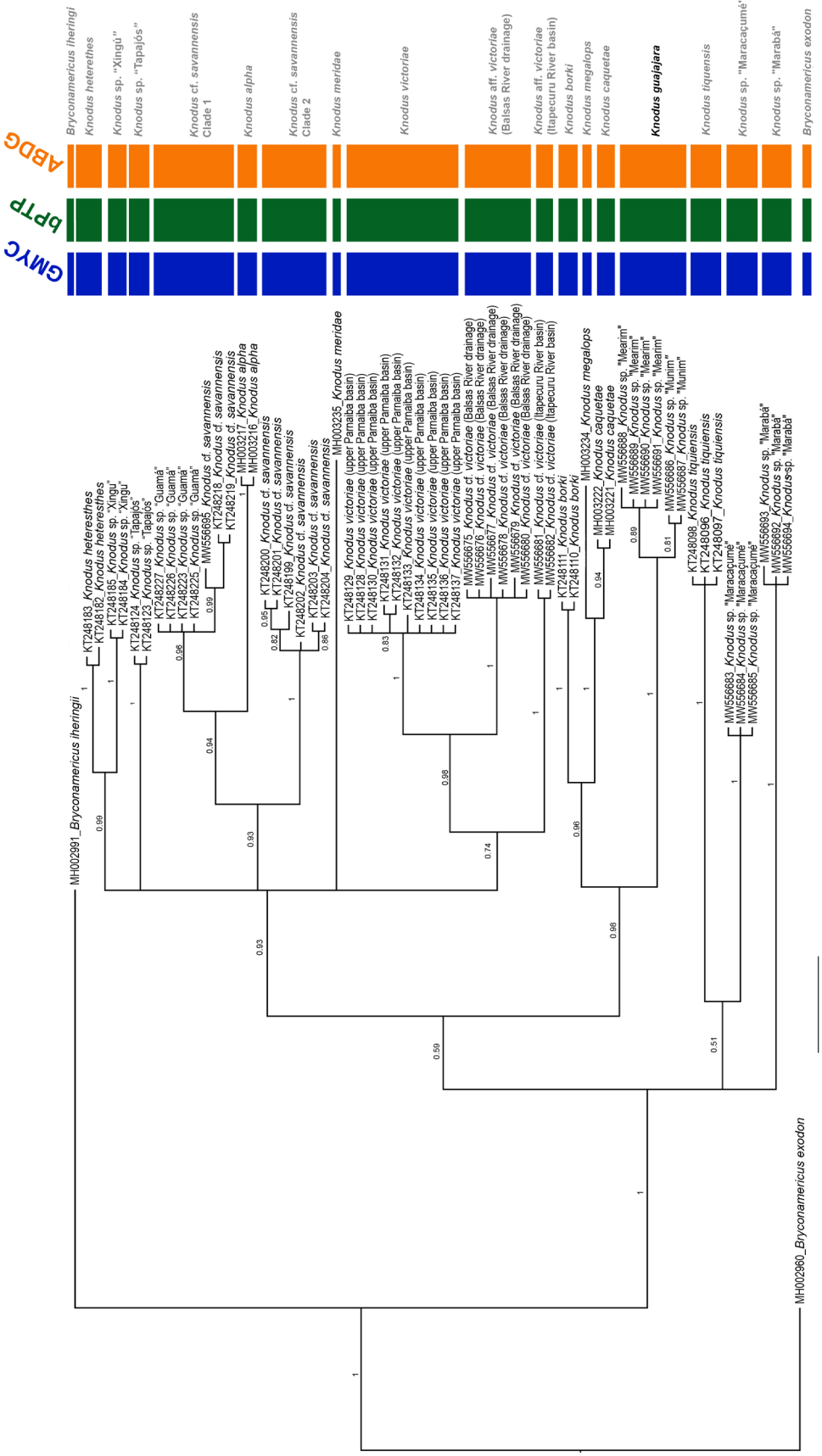

FIGURE 6 | Bayesian Inference phylogenetic tree generated by this study. The results of GMYC, bPTP and ABGD are included. Numbers above and below branches are posterior probability values. The gray bars correspond to the congruent results of the different species delimitation methods (Con.).

genetic distance of 16% to *Bryconamericus exodon* Eigenmann,1907 and *Knodus tiquiensis.* The genetic distance between the new species and *K. victoriae* is 13% (Tab. 4).

Phylogenetic analysis. The BI phylogenetic analysis delimited 19 lineages: two of them are outgroups (*Bryconamericus* species) and the remaining correspond to *Knodus* species (Fig. 6). One of the 17 lineages of *Knodus* represents the new species described herein (*Knodus guajajara*) with haplotypes from the Mearim and Munim river basins forming an exclusive clade supported by the highest possible support value (PP = 1) (Fig. 6). The BI phylogenetic analysis also delimited new lineages of *Knodus* inhabiting the Tocantins River (*Knodus* sp. "Marabá") and the Maracaçumé River (*Knodus* sp. "Maracaçumé"). In addition, two well supported clades were recovered within *Knodus* cf. *savannensis*: the first one containing *Knodus* cf. *savannensis* (Tocantins River, Carolina, MA), *Knodus* "Guamá" and *Knodus* cf. *savannensis* (KT248218 and KT248219) (posterior probability = 0.96). The second clade of *Knodus* cf. *savannensis* corresponds to six haplotypes (KT248199, KT248200, KT248201, KT248202, KT248203, and KT248204) of *Knodus* cf. *savannensis* (PP = 1). Two other lineages closely related to *K. victoriae* were detected: *Knodus* cf. *victoriae* (Balsas River drainage) and *Knodus* cf. *victoriae* (Itapecuru River basin) (PP = 1).

TABLE 4 | Kimura-2-parameters pairwise genetic distances among species.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	*Bryconamericus exodon*																		
2	*Bryconamericus iheringii*	0.12																	
3	*Knodus heteresthes*	0.15	0.16																
4	*Knodus* cf. *savanensis* (Clade 1)	0.13	0.17	0.09															
5	*Knodus* cf. *savanensis* (Clade 2)	0.13	0.16	0.08	0.06														
6	*Knodus* sp. "Tapajós"	0.13	0.18	0.08	0.10	0.09													
7	*Knodus* sp. "Xingu"	0.15	0.16	0.05	0.09	0.10	0.09												
8	*Knodus tiquiensis*	0.14	0.18	0.13	0.11	0.11	0.12	0.14											
9	*Knodus borki*	0.11	0.15	0.12	0.12	0.11	0.11	0.12	0.15										
10	*Knodus megalops*	0.13	0.14	0.14	0.14	0.14	0.12	0.13	0.15	0.08									
11	*Knodus caquetae*	0.11	0.14	0.14	0.14	0.13	0.12	0.14	0.15	0.07	0.04								
12	*Knodus meridae*	0.16	0.16	0.12	0.11	0.10	0.12	0.13	0.15	0.11	0.13	0.13							
13	*Knodus alpha*	0.14	0.18	0.10	0.06	0.09	0.13	0.09	0.15	0.14	0.14	0.15	0.15						
14	*Knodus* sp. "Marabá"	0.14	0.16	0.16	0.13	0.12	0.14	0.15	0.14	0.15	0.15	0.15	0.17	0.15					
15	*Knodus* sp. "Maracaçumé"	0.12	0.14	0.12	0.13	0.11	0.12	0.13	0.11	0.11	0.12	0.10	0.12	0.15	0.14				
16	*Knodus victoriae*	0.15	0.18	0.07	0.08	0.08	0.10	0.08	0.13	0.11	0.15	0.14	0.12	0.10	0.16	0.12			
17	*Knodus* aff. *victoriae* (Balsas)	0.13	0.19	0.09	0.09	0.09	0.10	0.10	0.13	0.12	0.13	0.15	0.10	0.10	0.14	0.14	0.05		
18	*Knodus* aff. *victoriae* (Itapecuru)	0.14	0.15	0.10	0.09	0.09	0.10	0.11	0.13	0.12	0.11	0.14	0.12	0.09	0.14	0.12	0.08	0.06	
19	***Knodus guajajara***	**0.16**	**0.13**	**0.15**	**0.13**	**0.13**	**0.14**	**0.14**	**0.16**	**0.11**	**0.13**	**0.11**	**0.13**	**0.13**	**0.13**	**0.11**	**0.13**	**0.15**	**0.13**

GMYC, bPTP and ABGD. The results of the three species delimitation methods (GMYC, bPTP, and ABGD) were congruent (Fig. 6), and all three delimited the same 19 lineages. Two of them are outgroups (*Bryconamericus* species), and 17 are *Knodus* lineages (species) (Fig. 6). The new species herein described (*K. guajajara*) was recovered in all three species delimitation methods. Furthermore, some potential undescribed species were detected: *Knodus* "Marabá", *Knodus* "Maracaçumé", *Knodus* cf. *victoriae* (Itapecuru River basin) and *Knodus* cf. *victoriae* (Balsas River drainage). *Knodus victoriae* (upper Parnaiba River basin) was also recovered, as well as two *K.* cf. *savannensis* clades.

DISCUSSION

Knodus guajajara is the first species of the genus described using a combination of morphological and genetic data. The new species has the traditional morphological characteristics that define *Knodus*, especially the small scales covering the procurrent rays of the caudal fin (Eigenman, 1918; Géry, 1972, 1977; Román-Valencia *et al*., 2008).

There are some congeners that occur geographically close to the new species described here: *Knodus cupariensis*, *K. dorsomaculatus* and *K. heteresthes* (from the Tapajós River basin); *K. nuptialis* and *K. weitzmani* (from the Xingu River basin); *K. savannensis* (from the lower/middle Tocantins River basin); and *K. victoriae* (from the upper Parnaiba River basin) (Aguiar *et al*., 2021; Fricke *et al*., 2022; and this study). Furthermore, *Knodus septentrionalis* from Ecuador's upper Pastaza River basin was previously considered by Géry (1972) as a subspecies of *Knodus victoriae*, but was elevated to the species category by Chang, Ortega (1995). The new species is morphologically distinguished from these aforementioned species, as well as all the other congeners, by a unique combination of character states (see diagnosis). Of the geographically proximate species to *Knodus guajajara*, *K. savannensis* and *K. victoriae* are the closest ones (lower/middle Tocantins River and Parnaiba River basins, respectively). However, *Knodus guajajara* is distinguished from *K. savannensis* in the form of its conspicuous and slightly rounded humeral patch, which is inconspicuous and vertically elongated in *K. savannensis*. It is morphologically most similar to *K. victoriae*, having been previously misidentified as that species in the Munim and Mearim river basins (Oliveira *et al*., 2020; Guimarães *et al*., 2018a, 2020a). However, some morphological characteristics differ between these two species, such as the presence of fewer circumpeduncular scales, more branched rays in the anal fin and more vertebrae in *Knodus guajajara* (see diagnosis).

Accurate discussions regarding phylogenetic relationships, as well as the biogeography of the species in the studied area, would be speculative due to the lack of available data, poor taxonomic resolution of some species and knowledge gaps for *Knodus* species in this region, as well as for the coastal river systems of Maranhão.

Using only morphological characteristics to delimit species in *Knodus* has caused identification errors in taxonomic studies (García-Melo *et al*., 2019). For this reason, García-Melo *et al.* (2019) did not solely consider the presence of scales at the base of the tail fin as a convincing feature to delimit the genus, but rather emphasized the use of DNA sequences to delimit *Knodus* species more precisely.

In accordance with that recommendation, in addition to morphological evidence, the description of *Knodus guajajara* is also supported by molecular data (see Fig. 6 and

Tab. 4) from five different and independent single locus species delimitation methods. It has a high genetic distance value (13%) (Tab. 4) relative to *K. victoriae*, the most morphologically similar species, and appears to be phylogenetically distant (Fig. 6). *Knodus guajajara* is the sister group to a clade comprising *K. borki*, *K. caquetae* and *K. megalops*, possessing 11%, 11% and 13% of genetic distance, respectively, to these species. It is important to emphasize that *K. guajajara* is more genetically similar to these three species than to several geographically closer congeners (see Tab. 4). *Knodus borki* is known from near Iquitos, in Peru, *K. caquetae* occurs in the Amazon and Caquetá River basins of Colombia, and *K. megalops* is distributed in the Upper Amazon River basin, in Peru and Ecuador (Fricke *et al*., 2022). All three of these regions are far away from the known geographical distribution of *Knodus guajajara*. This pattern may be an artifact of the gap of knowledge for *Knodus* or of the scarcity of genetic data for the genus.

In addition to the new species described here, our results reveal the presence of even more potentially undescribed species for the genus *Knodus* (Fig. 6) than García-Melo *et al*. (2019), indicating that a knowledge gap for the group still likely exists and that more undescribed species await discovery. Studies that incorporate both molecular and morphological data, as well as, different operational criteria (integrative taxonomy) have shown success in delimiting and describing species and complexes of cryptic or hidden species (*e.g*., Mazetti *et al*., 2012; Guimarães *et al*., 2018b, 2019, 2020b; Brito *et al*., 2019, 2021; Ottoni *et al*., 2019; Faria *et al*., 2020). The same result is observed for the present study, in which the different species delimitation methods based on molecular data demonstrate congruence with each other. All these methods supported the validity of the new species, the hidden diversity in *K. victoriae* and the possible presence of new species of *Knodus* in the Maracaçumé and Tocantins river basins (Fig. 6).

Knodus savannensis is distributed throughout the Tocantins basin and has two possible type-localities according to Lima, Géry (2001): *"Brazil, state of Tocantins, municipality of Itacajá, between the Manoel Alves Pequeno river and the Vermelho river, Tocantins river basin approx. 8°19'S, 47°25W"*, or *"Brazil, Tocantins river basin, Javaés river, Bananal Island, approx. 11°S, 51°W"*. A more comprehensive study focusing on *K. savannensis* is necessary to solve the taxonomy complexity of this species and its type locality.

Our results point to another lineage (*Knodus* sp. "Marabá") in the Tocantins River that is apparently not closely related to the *Knodus savannensis* complex, suggesting two distinct colonization or speciation events in the basin. The occurrence of geographically close species in the genus was recently recorded by Deprá *et al.* (2021), in which two new species (*K. rufford* and *K. obolus*) share the same type locality in the upper Tocantins river basin, thus demonstrating another case of hidden diversity within *Knodus*.

Still another case of cryptic diversity occurs in the Maracaçumé river basin (Maranhão State). In this river a divergent lineage occurs (*Knodus* sp. "Maracaçumé", see Tab. 4), but despite its geographic proximity, it possesses a high genetic distance (11%) to *Knodus guajajara*, suggesting that is a new species according to the species delimitation methods conducted. The occurrence of new characids has been demonstrated for the Maracaçumé River basin, including cryptic species (*e.g*., Brito *et al*., 2019; Guimarães *et al*., 2020b). The description of new fish species for this basin is expected since according to Abreu *et al.* (2019, 2020), biogeographic processes favored the isolation of drainages in Maranhão, providing opportunities for the emergence of new species.

We also identified cryptic diversity occurring in *Knodus victoriae* with the detection

of two additional lineages: one from the Balsas River drainage of the upper Parnaiba River basin [*Knodus* aff. *victoriae* (Balsas)]; and the other from the upper Itapecuru River basin [*Knodus* aff. *victoriae* (Itapecuru)] (Fig 6; Tab. 4), totaling three lineages under the name *Knodus victoriae*. A noteworthy aspect is that two of these lineages, *Knodus* aff. *victoriae* (Balsas) and *Knodus victoriae*, occur in upper sections of the Parnaiba River basin (Aguiar *et al*., 2021; this study). It is likely that the presence of these sister lineages in the upper Parnaiba and upper Itapecuru river basins is due to past headwater captures between these river systems.

In addition to contributing to the description of a new species (*Knodus guajarara*), the results of our present work revealed that a hidden diversity of species of the genus *Knodus* inhabits the hydrographic basins of Maranhão and adjacent systems. The use of different data sources (morphology, molecular, geographic distribution, etc.) can help to identify genetically distinct groups with high morphological similarity, thereby avoiding identification errors and helping to solving problems of validity and taxonomic placement within Characidae.

Comparative material examined. *Knodus borki*: **Peru.** MTDF 31357, 1, 33.99 mm SL, holotype, Umgebung von Iquitos. *Knodus longus*: **Bolivia.** MTDF 28853, 1, 46.1 mm SL, holotype, río Warinilla (oder Harinilla) nahe Choiro. *Knodus pasco*: **Peru.** MTDF 30634, 1, 55.4 mm SL, holotype, Departamento Pasco, erste Quebrada bei San Antonio de Cacazú (eigentlich nach dem Vorort Baja Cacazú) auf der Straße, nach Puerto Bermúdez. *Knodus savannensis*: **Brazil.** USNM 196088, 1, 30.4 mm SL, holotype, Tocantins, Tocantins basin. *Knodus savannensis* (Clade 1): **Brazil.** CICCAA 2397, 108, 36.83–48.67 mm SL; CICCAA 2399, 73, 31.46–38.52 mm SL; CICCAA 2400, 27, 38.37–61.08 mm SL, Maranhão, Carolina. *Knodus* sp. "Marabá": **Brazil.** CICCAA 1522, 2, 29.16–33.92 mm SL; CICCAA 1306, 14, 22.92–36.22 mm SL; CICCAA 1503, 3, 34.36–38.24 mm SL, Pará, Marabá. CICCAA 1525, 9, 24.4–37.74 mm SL, Pará, Curinópolis. CICCAA 1436, 20, 17.74–27.51 mm SL, Pará, Paragominas. CICCAA 1409, 6, 30.7–38.9 mm SL; CICCAA 1521, 4, 29.85–37.67 mm SL; CICCAA 1516, 1, 29.62 mm SL; CICCAA 1524, 5, 20.68–20.82 mm SL;CICCAA 1509, 3, 34.86–38.24 mm SL, Maranhão, São Pedro da Água Branca. CICCAA 1295, 27, 20.36–28.27 mm SL; CICCAA 1523, 8, 17.82–22.58 mm SL, Maranhão, Vila Nova dos Martírios. *Knodus victoriae*: **Brazil.** NMW 57823, 16 syntypes; NMW 57824, 13 syntypes; NMW 57825, 18 syntypes of *Tetragonopterus victoriae*, Maranhão, Parnaíba basin; CICCAA 2708, 18; CICCAA 2709, 17; CICCAA 2710, 7 C&S, Maranhão, Alto Parnaíba municipality, Parnaíba river basin; *Knodus* aff. *victoriae*: **Brazil.** CICCAA 2842, 1, 29.5 mm SL, Maranhão, Itapecuru River basin. CICCAA 4809, 9, 15.57–28.47 mm SL; CICCAA 4810, 31, 20.73–43.48 mm SL; CICCAA 4811, 29, 17.18–42.22 mm SL; CICCAA 4812, 34, 17.32–42.18 mm SL; CICCAA 4892, 8 C&S, CICCAA 4893, 2 C&S, Maranhão, Parnaíba basin. *Knodus* cf. *victoriae*: **Brazil.** CICCAA 2395, 6, 25.6–36.08 mm SL; CICCAA 2391, 5 C&S, 27.51–31.4 mm SL, Maranhão, Itapecuru River basin.

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The authors declare no competing interests.

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