Integrative taxonomy reveals hidden species within the western Atlantic

Callichirus major s. l. (Decapoda, Axiidea, Callichiridae)

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Running title: A new species of *Callichirus* from the southwestern Atlantic

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Abstract

The ghost shrimp *Callichirus major* (Say, 1818) is widely distributed in the Atlantic Ocean from ~23°N to ~26°S, and has also been reported from the tropical eastern Pacific. Evidence has been accumulating over many years that *C. major* is actually a species complex. Yet, the name *C. major* is widely and frequently used in many kinds of research. The current lack of clarity in the use of the name *C. major* has resulted in nomenclatural instability, but also in unreliability and miscommunication of the available ecological and distributional information. Existing morphological and molecular evidence is reviewed and new evidence presented for the specimens from the southern localities previously assigned to *C. major* s. l. actually being a new species. That new species is herein described based on morphological and molecular evidence. Additionally, a neotype is selected for *C. major* in order to settle the defining characters of *C. major* s. str. and, therefore, ensuring the correct use of this name.

Keywords. Burrowing shrimps, *Callichirus*, morphology, neotype, 16S DNA, sandy beaches, western Atlantic

Introduction

The ghost shrimp *Callinassa major* Say, 1818, was based upon a single specimen from the bay shore of the river St. John, east Florida, and subsequently transferred to *Callichirus* by Stimpson (1866) as the type species of his new genus. This species has been recorded over the years from many localities along the western Atlantic coasts where it has a broad distribution ranging from North Carolina (~23°N) to Santa Catarina (~26°S), Brazil, including the Gulf of Mexico, and the Caribbean coast of Colombia, but has also been reported from the eastern tropical Pacific (Hay and Shore 1918; Felder 1973; Rodrigues 1983; Williams 1984; Melo 1999; Felder 2001; Sakai 2011).

However, evidence has been accumulating over many years that *C. major* is actually a species complex (Rodrigues and Shimizu 1997; Strasser and Felder 1998, 1999a, b, c; Staton and Felder 1995; Felder and Robles 2009; Peiró 2012). Yet, the name continues to be widely and frequently used in ecological, distributional, morphological, checklists, and taxonomic researches carried out along its implied geographic distribution (Rodrigues 1966, 1971, 1983; Souza and Borzone 1996; Blanco-Rambla 1997; Souza et al. 1998; Coelho et al. 2007; Botter-Carvalho et al. 2007; Botter-Carvalho et al. 2012; Rio et al. 2019; Souza et al. 2020). The current lack of clarity in the use of the name *C. major* has hence resulted in nomenclatural instability, but also in unreliability and miscommunication of the available ecological and distributional information.

The study of a wealth collection of *Callichirus* assembled by the first author along the Brazilian coast from north to south along with the study of males and females from many different localities from the northwestern and southwestern Atlantic in Museum collections, prompted us to review the existing morphological and molecular evidence for the specimens from the southern localities previously assigned to *C. major*

actually being a new species. That new species is herein described. Additionally, a neotype is selected for *C. major* (Say, 1818) in order to settle the defining characters of *C. major* s. str. and, therefore, ensuring the correct use of this name.

Material and methods

Most specimens obtained along the Brazilian coast were collected during the low tide using a yabby pump (diameter = 77 mm, length = 100 cm) in the intertidal zone (< 1 m depth) (Fig. 1a–c). Otherwise, the studied material belongs to collections from the following institutions: MZUSP (Museu de Zoologia, Universidade de São Paulo), USNM (National Museum of Natural History, Smithsonian Institution, Washington D.C.), MZUC (Museo de Zoología, Universidad de Concepción), and MZUCR (Museo de Zoología, Universidad de Costa Rica). The holotype and the paratypes of the new species described herein are deposited at the MZUSP.

Measurements (mm) were made under a stereomicroscope (Zeiss® Stemi® SV-6) equipped with digital analysis image system (Zeiss® AxioCam® MRc5). Each image was digitized using an electronic tablet for graphic design (Wacom®). Size is expressed as postorbital carapace length (cl) measured along the middorsal line of the carapace from the anterior margin of rostrum to the posterior margin of the carapace.

Abbreviations include: A1 (antennule or first antenna), A2 (antenna or second antenna), tl (total length), coll. (collector or collected by), Mxp3 (maxilliped 3), Pl (pereopod 1), Plp 1-2 (pleopods 1 and 2), EP (eastern Pacific), WP (western Pacific), EA (eastern Atlantic), WA (western Atlantic).

Molecular data analysis

The partial sequences of the mitochondrial loci 16S rDNA were retrieved from the GenBank (Table 1). All *Callichirus* species with suitable 16S rDNA sequences available were included. Other Callichiridae and Axiidea species were included to test the monophyletic status of *Callichirus* (Table 1). Sampling locality information, GenBank accession numbers, museum voucher numbers and references for taxa included in the molecular analyses are provided in Table 1.

The phylogenetic analysis was inferred by using the Maximum Likelihood method and General Time Reversible model (Nei and Kumar 2000). The tree with the highest log likelihood (-2817.72) is presented. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to the matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4201)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 33.03% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis used 54 nucleotide sequences with a total of 455 positions in the final dataset. Phylogenetic analyses were conducted in MEGA X (Kumar et al. 2018). Branch support values were calculated using bootstrap analyses with 1,000 replicates (Felsenstein 1985). Bootstrap support values are shown on nodes of the phylogenetic tree, when support is greater than 50. Nucleotide divergence estimated from pairwise distance was calculated in MEGA X with the same best-fit model (Table 2).

Results

Taxonomy

Infraorder AXIDEA de Saint Laurent, 1979

Family CALLICHIRIDAE Manning & Felder, 1991

Genus *Callichirus* Stimpson, 1866

Type species – *Callichirus major* (Say, 1818), by original designation [Type locality: Bay shore of the river St. John, east Florida, USA].

Included species (genera of the original combination indicated within brackets) –

Callichirus adamas (Kensley, 1974) [*Callianassa*] (EA); *C. garthi* (Retamal, 1975) [*Callianassa*] (EP); *C. islagrande* (Schmitt, 1935) (WA); *C. meridionalis* sp. nov. (WA); *C. major* (Say, 1818) [*Callianassa*] (WA); *C. santarosaensis* Sakai & Türkay, 2012 (WA); and *C. seilacheri* (Bott, 1955) [*Callianassa*] (EP).

***Callichirus meridionalis* sp. nov.**

(Figures 1a, 2, 3, 4)

Callianassa (*Callichirus*) *major* – Rodrigues 1971: 192, figs 1-20 [not *Callichirus major* (Say, 1818)].

Callichirus major – Rodrigues 1976: 85, figs. 1-35; 1985: 195, figs. 1-30; Rodrigues and Höld 1990: 48; Borzone and Souza 1996: 67; Souza and Borzone 1996: 553; Rodrigues and Shimizu 1997: 155, fig. 1; Souza et al. 1998: 151; Coelho and Rodrigues 2001: 1447, figs. 13-21; Botter-Carvalho et al. 2002: 97; Souza and Borzone 2003: 625; Botter-Carvalho et al. 2007: 508; Peiró and Mantelatto 2011: 5; Peiró et al. 2011: 261; Botter-Carvalho et al. 2012: 89; Dworschak et al. 2012: 151, fig. 69.29a,b, 69.31t;

140 Alves-Junior et al. 2014a: 109; Alves-Junior et al. 2014b: 13; Alves-Junior et al. 2018:
141 166; Peiró et al. 2014: 294; Peiró and Mantelatto 2016: 103, pl. 1; Hernáez et al. 2018:
142 97; Rosa et al. 2018: 1; Hernáez et al. 2019: 1, fig. 1; Rio et al. 2019: 1, figs. 1A, 2;
143 Hernáez et al. 2020: 1, fig. 4c; Moschetto et al. 2020: 1; Laurino et al. 2020: 1 [not
144 *Callichirus major* (Say, 1818)].
145 *Callichirus* sp. – Strasser and Felder 1999a: 865.
146 *Callichirus macrotelsonis* – Peiró, 2012: 58, figs. 3-7 [*nomen nudum*].
147 *Callichirus brasiliensis* Rio, 2018: 20, figs. 1A, 2A, 3A, 3B, 4A [*nomen nudum*].
148
149 **Type material** – Brazil: São Paulo: holotype, male, cl 18.8 mm (MZUSP 41251), Praia
150 do Gonzaga, 23°58'13"S, 46°20'04"W, lower intertidal, Santos, São Paulo, P. Hernáez
151 coll., 1 September 2016. Paratypes: 1 female, cl 20.4 mm (MZUSP 41252), 4 males
152 (one dissected), cl: 15.4–19.4 mm, and 4 females, cl 16.4–22.2 mm (2 ovigerous
153 females) (MZUSP 41253), same data as holotype.
154
155 **Non type material** – Brazil: Pará: 8 males, cl: 10.1–18.3 mm and 15 females, cl: 10.4–
156 19.2 mm, Praia do Crispim, 00°34'58"S, 47°39'04"W, lower intertidal, Marapanim,
157 coll. P. Hernáez, 9 July 2017 (MZUSP 38995); 8 males, cl: 13.7–19.6 mm and 11
158 females, cl: 10.5–21.2 mm, Praia de Ajuruteua, 00°49'40"S, 46°36'20"W, lower
159 intertidal, Bragança, P. Hernáez coll., 7 July 2017 (MZUSP 38993). Maranhão: 10
160 males, cl: 10.8–17.6 mm and 15 females, cl: 12.5–19.8 mm, Praia Olho d'água,
161 02°28'44"S, 44°13'51"W, lower intertidal, São Luis, P. Hernáez coll., 6 July 2017
162 (MZUSP 38994); male, cl 12.4 mm and 2 females, cl: 15.6–18.7 mm, Praia de Tutoia,
163 02°45'40"S, 42°15'45"W, lower intertidal, Tutóia, P. Hernáez coll., 5 July 2017
164 (MZUSP 39001). Piauí: 9 males, cl: 9.6–15.6 mm and 14 females, cl: 10.2–17.7 mm,

165 Praia da Atalaia, 02°53'22"S, 41°37'41"W, lower intertidal, Luis Correia, P. Hernáez
166 coll., 3 July 2017 (MZUSP 38998). Ceará: 2 females, cl: 5.8–10.4 mm, close to the
167 marine lighthouse, 02°52'35"S, 40°55'24"W, lower intertidal, Camocim, P. Hernáez
168 coll., 3 July 2017 (MZUSP 39006). Rio Grande do Norte: 22 males, cl: 5.6–12.8 mm
169 and 19 females, cl: 7.6–13.3 mm, Praia do Pirangi, 05°58'28"S, 35°07'28"W, lower
170 intertidal, Pirangi, P. Hernáez coll., 11 June 2016 (MZUSP 39011). Pernambuco: male,
171 cl 10.5 mm and 4 females, cl: 13.9–16.1 mm, Praia de Baixa Verde, 07°45'17"S,
172 34°49'28"W, lower intertidal, Ilha de Itamaracá, P. Hernáez coll., 17 June 2016
173 (MZUSP 39012); 6 males, cl: 7.9–15.9 mm and 5 females, cl: 7.8–15.3 mm, Praia
174 Piedade, 08°10'01"S, 34°54'47"W, lower intertidal, Recife, P. Hernáez coll., 10 June
175 2016 (MZUSP 39013). Alagoas: 15 males, cl: 9.0–16.0 mm and 14 females, cl: 12.9–
176 17.7 mm, Praia do Sobral, 09°40'22"S, 35°33'44"W, lower intertidal, Maceió, P.
177 Hernáez coll., 9 June 2016 (MZUSP 39014). Sergipe: 9 males, cl: 11.5–15.8 mm and 18
178 females, cl: 10.3–18.0 mm, Praia Aruana, 11°00'50"S, 37°03'50"W, lower intertidal,
179 Aracajú, P. Hernáez coll., 8 June 2016 (MZUSP 39016). Bahia: 16 males, cl: 12.6–15.7
180 mm and 16 females, cl: 11.8–14.7 mm, Praia Malvinas, 18°04'48"S, 39°32'35"W,
181 lower intertidal, Mucuri, P. Hernáez coll., 20 June 2016 (MZUSP 39022). Espírito
182 Santo: 15 males, cl: 7.8–12.5 mm and 11 females, cl: 6.8–12.2 mm), Praia de Piúma,
183 20°50'37"S, 40°44'02"W, lower intertidal, Piúma, P. Hernáez coll., 3 June 2016
184 (MZUSP 39023). São Paulo: 14 males, cl: 5.7–11.9 mm and 08 females, cl: 7.4–11.5
185 mm, Praia de Barequeçaba, 23°49'39"S, 45°26'04"W, lower intertidal, Barequeçaba,
186 São Paulo, P. Hernáez coll., 2 June 2016 (MZUSP 39028); 28 males, cl: 10.1–19.1 mm
187 and 64 females, cl: 8.0–23.2 mm, Praia do Gonzaga, 23°58'13"S, 46°20'04"W, lower
188 intertidal, Santos, P. Hernáez coll., 1 September 2016 (MZUSP 39027); 3 males, cl:
189 8.6–15.5 mm and 3 females, cl: 7.9–8.3 mm, Cibratel, 24°12'04"S, 46°48'45"W, lower

190 intertidal, Itanhaém, P. Hernández coll., 12 August 2016 (MZUSP 39026); 3 males, cl:
191 7.3–10.8 mm and 3 females, cl: 7.4–14.4 mm, Rio Peruíbe, 24°19'47"S, 46°59'59"W,
192 estuary, Peruíbe, P. Hernández coll., 1 September 2016 (MZUSP 39025); 8 males, cl: 6.4–
193 18.7 mm and 4 females, cl: 12.6–15.9 mm, Praia Ilha Comprida, 24°45'22"S,
194 47°33'34"W, lower intertidal, Ilha Comprida, P. Hernández & J. Rio coll., 1 July 2016
195 (MZUSP 39029). Paraná: 15 males, cl: 7.9–16.7 mm and 22 females, cl: 10.0–16.4 mm,
196 Praia de Leste, 25°37'50"S, 48°25'16"W, lower intertidal, Pontal de Paraná, P. Hernández
197 & J. Rio coll., 2 July 2016 (MZUSP 39037). Santa Catarina: 10 males, cl: 11.0–16.7
198 mm and 27 females, cl: 8.9–22.3 mm, Balneário Camboriú, 26°59'20"S, 48°37'45"W,
199 lower intertidal, Camboriú, P. Hernández & J. Rio coll., 3 July 2016 (MZUSP 39039).

200

201 **Comparative material examined** –*Callichirus garthi* (Retamal, 1975): Chile:
202 holotype, male, cl 35 mm, Lenga, 36°45'S, 73°10'W, Concepción, M.A. Retamal coll.,
203 May 1975 (MZUC-UCCC 7311); paratype, male, cl 30 mm, same site as holotype,
204 March 1974, coll. M.A. Retamal (MZUC-UCCC 7313); 66 males, cl: 7.6–21.3 mm and
205 68 females, cl: 8.6–20.0 mm, Lenga, 36°46'00"S, 73°10'18"W, Concepción, P. Hernández
206 coll., 9 March 2011 (MUAPCD 0432/2011). *Callichirus islagrande* (Schmitt, 1935):
207 USA: Gulf of Mexico: holotype, male, cl 19 mm, Grand Isle, Louisiana, W.W.
208 Anderson coll., summer of 1930 (USNM 69362). *Callichirus major* (Say, 1818): USA:
209 North Caroline: male, cl 20.6 mm, Onslow Bay, 34°36'23"N, 77°12'35"W, New River,
210 R.B. Manning & D.B. Bixler coll., 30 July 1989 (USNM 266227); female, cl 11.9 mm,
211 Onslow Bay, 34°36'23"N, 77°12'35"W, New River, R.B. Manning & D.B. Bixler coll.,
212 7 August 1990 (USNM 266232). Georgia: male, cl 21.2 mm, Tybee Island,
213 31°59'58"N, 80°50'29"W, G.A. Bishop coll., 1 April 1988 (USNM 266247).
214 Louisiana: 2 males, cl: 15.0– 24.4 mm, Grand Island, 30°08'N, 89°25'W, Gulf of

215 Mexico, E.R. Willis coll., 26 June 1939 (USNM 79171). Texas: ovigerous female, cl
216 16.4 mm, Heald Bank, 29°43'36"N, 93°42'29"W, Sabine, W.G. Hewatt coll., 1967
217 (USNM 97653). Florida: 2 ovigerous females, cl: 9.7–20.2 mm, Indian River,
218 27°52'48"N, 80°27'24"W, R.B. Manning, W. Lee, M. Schotte & C. King coll., 20 April
219 1988 (USNM 266125); female, cl 21.0 mm, Indian River, 27°38'11"N, 80°21'50"W,
220 North Hutchinson Island, Fort Pierce, D.L. Felder & W. Lee coll., 14 August 1987,
221 USNM 266118; male, cl 11.1 mm, Fort Pierce area, 27°28'18"N, 80°17'48"W, R.B.
222 Manning coll., 2 March 1987 (USNM 266127); female, cl 15.4 mm, Indian River,
223 27°28'18"N, 87°17'48"W, North Hutchinson Island, Fort Pierce, R.B. Manning & W.
224 Lee coll., 2 March 1987 (USNM 266126); female, cl 16.3 mm, Indian River,
225 27°11'00"N, 80°09'30"W, Seminole Shores, R.B. Manning & L.K. Manning coll., 7
226 July 1984 (USNM 266111); female, cl 16.3 mm, Indian River, 27°10'30"N,
227 80°10'24"W, Flat Just Inside Saint Lucie Inlet, R.B. Manning coll., 11 February 1983
228 (USNM 228087); topotypic, male, cl 13.2 mm, Indian River, 27°10'14"N, 80°10'24"W,
229 R.B. Manning coll., 9 February 1983 (USNM 228086); male, cl 18.6 mm, Lake Worth
230 Inlet, 26°46'17"N, 80°02'14"W, Peanut Island, R.B. Manning & D.L. Felder coll., 11
231 August 1987 (USNM 266114). *Callichirus* aff. *major*: Colombia: Caribbean coast:
232 male, cl 22.2 mm, La Boquilla, 10°28'19"N, 75°29'59"W, Cartagena, R. Lemaitre coll.,
233 10 August 1980 (USNM 266208); male, cl 13.9 mm and ovigerous female, cl 13.7 mm,
234 La Boquilla, 10°28'19"N, 75°29'59"W, Cartagena, R. Lemaitre coll., 10 August 1988
235 (USNM 266225); female, cl 23.6 mm, Castillo Grande, 10°23'43"N, 75°33'02"W,
236 Cartagena Bay, Cartagena, R. Lemaitre coll., 7 July 1988 (USNM 266211). *Callichirus*
237 *seilacheri* (Bott, 1955): El Salvador: male, cl 16.3 mm and female, cl 24.1 mm, Los
238 Blancos, topotypic, 13°19'38"N, 88°58'10"W, P. Hernández & A. Gamboa-González
239 coll., 22 July 2013 (MZUCR 3335–01); male, cl 8.4 mm and 5 females, cl: 9.7–20.2

mm, Los Blancos, topotypic, 13°19'38"N, 88°58'10"W, P. Hernández & A. Gamboa-González coll., 22 July 2013 (MZUCR 3336–01). Costa Rica: Pacific coast: 15 males, cl: 8.2–14.5 mm and 16 females, cl: 8.6–14.9 mm, Mata de Limon, 09°55'12"N, 84°42'37"W, Puntarenas, P. Hernández & A. Gamboa-González coll., 10 June 2012 (MZUCR 3337–01).

Diagnosis – Carapace with small triangular rostrum and two anterolateral rounded projections. Ocular peduncle with obtuse tip, distal margin not reaching to second article of antennular peduncle. A1 peduncle stouter, longer than A2 peduncle; second article of antennular peduncle slightly exceeding fourth article of antennal peduncle. Maxilliped 3 merus with distal and proximal margins not parallel, strongly oblique distally, not projecting beyond carpo-meral articulation. Male major cheliped with prominent hook on flexor margin of merus; fixed finger with small triangular tooth on midlength of cutting edge; dactylus strongly arched with tip curved downward and bifid, longer than fixed finger, cutting edge with large bifid tooth proximally, otherwise unarmed. Male pleopod 2 with endopod and exopod well developed. Telson slightly broader than long, tapering distally, emarginate posteriorly.

Description – Carapace length of adults up to 22 mm. Carapace smooth, dorsally oval, cervical groove and linea thalassinica distinct, cardiac prominence absent, lateral margin emarginate on central part; rostrum small, short and triangular, rounded anterolateral projections on each side (Figs. 2a, b). Branchial formula as shown in Table 3.

Ocular peduncle (Fig. 2b) with obtuse tip, slightly separated distally, distal margin not reaching second article of antennular peduncle (A1). Cornea subterminal, darkly pigmented and located distolaterally. A1 peduncle stouter, longer than A2

peduncle; lower margin of antennular peduncle setose. Second article of antennular peduncle slightly longer than fourth article, distal article about twice as long as penultimate article; flagellum with dorsal and ventral ramus of similar length, each one with 21-23 smaller articles, both ramus with sparse, long tufts of setae. A2 peduncle with distal and penultimate article of similar length; basal article with excretory pore protruded; second article with tuft of setae distolaterally; third article shorter than second; fourth article with sparse and long setae, fifth article with tuft of long setae distolaterally, scaphocerite reduced.

Mandible (Fig. 2c) with molar process developed, consisting of one calcareous blunt tooth projected upward; incisor process weakly calcified, distal margin armed with seven acute corneous teeth; palp trisegmented, terminal article longest, external surface covered with long setae from two-third to articulation with penultimate article, with dense, short, stiff setae at distal third. Maxillule (Fig. 2d) with narrow palp (endopod), terminal article deflected proximally at articulation; proximal endite kidney-shaped, external margin with row of short setae; distal endite elongate, hatchet-shaped, distal margin with short, sharp stiff setae at proximal half. Maxilla (Fig. 2e) foliaceous; proximal and distal endite deeply bilobed, external surface of both heavily setose; endopod narrowing terminally, slightly flexed distally; exopod forming large, broad, scaphognathite.

Maxilliped 1 (Fig. 2f) foliaceous; proximal endite reduced, triangular, setose distally; distal endite developed, subrectangular, external surface heavily setose; endopod rounded, reduced; exopod large, with marked notch on mesial margin, setose marginally; epipod large, with posterior and anterior lobes developed, both narrowing terminally. Maxilliped 2 (Fig. 2g) with long, narrow endopod; dactylus short, less than half of propodus, with terminal brush of short, thick, stiff setae; propodus less than half

of merus, strongly arcuate; carpus subtriangular, short; merus more than five times as long as broad, flexor margin with dense fringe of long, simple setae; exopod sword shaped, reaching distal margin of carpus, lateral margin setose; epipod narrow, strongly reduced. Maxilliped 3 (Fig. 2h) without exopod and epipod; ischium-merus operculiform, length about 1.2 times its width, marginally setose; ischium without crest or teeth on mesial surface, 1.4 times length of merus; distal and proximal margin of merus not parallel, strongly oblique distally, not projecting beyond carpo-meral articulation; carpus and propodus expanded ventrally; dactylus digitiform, shorter than propodus.

Chelipeds (pereopods 1) unequal and dissimilar in males and females (Figs. 3a, b). Male major cheliped (Fig. 3a) extremely elongated and strongly calcified; ventral margin of ischium with row of well-spaced blunt teeth along surface, dorsal margin slightly straight; merus with prominent hook on flexor margin, margin of hook strongly serrate, acute distally, remainder of ventral margin with irregular rounded denticles, dorsal margin slightly denticulate and concave; carpus length about 1.5 times than palm, carpus about two times longer than wide, dorsal margin straight, unarmed, ventral margin slightly expanded medially, with rounded denticles at proximal third, sparse setae; palm rectangular, longer than width, dorsal and ventral margins smooth, with sparse setae on ventral margin; fixed finger with triangular tooth on midlength of cutting edge; dactylus strongly arcuate with tip curved downward, bifid, longer than fixed finger, cutting edge with large bifid tooth proximally, otherwise unarmed. Male minor cheliped (Fig. 3c) slender; ischium longer than merus, dorsal and ventral margin smooth; merus narrower than carpus, dorsal margin smooth, slightly curved, ventral margin with spaced tufts of short setae; carpus longest, lateral sulcus, with dorsal margin slightly curved, ventral margin slightly expanded, with spaced tufts of short

setae; length of palm about half of carpus length, dorsal margin unarmed, ventral margin with spaced tufts of setae, fixed finger approximately as long as dactylus, cutting edge minutely pectinate; dactylus slightly arcuate, minutely pectinate, with tuft of setae dorsally.

Female major cheliped (Fig. 3b) not as long as in males, differing from male larger cheliped as follows: dorsal and ventral margin of ischium straight; merus with less prominent hook on flexor margin, margin of hook weakly serrate, dorsal margin smooth, curved; carpus as long as palm; cutting edge of fixed finger with two acute teeth at proximal third, as long as dactylus; dactylus slightly curved, cutting edge with small acute teeth. Female minor cheliped (Fig. 3d) as in males, differing only in having a dorsal and ventral margin of merus curved, with cutting edge of fixed finger and dactylus unarmed.

Pereopod 2 (Fig. 3e) chelate, densely setose ventrally; length of ischium about 1.4 times its width; merus slightly longer than carpus, proximo-ventral expansion with long setae, dorsal margin slightly curved; carpus subtriangular, widening distally, dorsal and ventral margin with sparsely long setae; palm less than half of carpus, fixed finger subtriangular, cutting edge smooth; dactylus lanceolate, densely setose dorsally.

Pereopod 3 (Fig. 3f) pediform; ischium subsquare, unarmed; merus about two times longer than ischium, ventral margin setose, with weak expansion on central region, dorsal margin non-setose; carpus subtriangular, widening distally, margins setose; propodus expanded ventrally, keel-like, setose, length of dorsal margin shorter than ventral margin; dactylus broad, with two small notches on upper border, surface densely covered with setae. Pereopod 4 (Fig. 3g) not chelate; ischium more than half of merus, unarmed, margins non-setose; merus slightly wider than ischium, arcuate ventrally; carpus conical distally, almost equal in length with propodus, margins non-setose;

propodus wider than carpus, surface densely covered with short setae; dactylus reduced, digitiform, with two small notches on the upper border, surface densely covered with short setae. Pereopod 5 (Fig. 3h) chelate, fingers not gaping; ischium rectangular, unarmed; merus longest, about 2.7 times longer than ischium, unarmed; carpus subtriangular, widening distally, unarmed; inner and outer surface of propodus densely setose in distal half; surface of dactylus covered with dense setae.

Pleon (Fig. 2a) smooth, glabrous dorsally except for subcircular patches of setae on lateral margins of somites 3 to 5 representing integumental glands. Somite 2 longest; somite 3-5 similar in length. Somite 6 saddle-like, longer than telson. Telson (Fig. 4a) slightly broader than long, tapering distally, emarginate posteriorly; lateral margin with small lobe proximally, followed by deep notch and another large lobe at midlength to lateral margin; dorsal surface separated into three lobes, one large anterior and two smaller posterolateral separated by deep central notch.

Pleopod 1 (Figs. 4b, c) uniramous in both sexes; two-segmented in males, tri-segmented in females. Pleopod 2 (Figs. 4d, e) biramous in both sexes; in males and females endopod and exopod well developed, exopod slightly shorter than endopod; in males endopod without appendices interna and masculina; in females endopod with well-developed and terminal appendix interna, surface of appendix interna covered with patch of short hooked setae. Pleopods 3 to 5 (Fig. 4f) biramous, leaf-like, endopod broadened, with subtriangular appendix interna embedded into mesial margin of endopod in both sexes. Uropod (Fig. 4a) with protopod dorsally divided into four irregular lobes, posterolateral lobe ending in spinous process projected distally; endopod strap-shaped, much longer than wide, exceeding posterior margin of telson, base with rounded lobe ending in a spine protruded distally; exopod triangular with anterodorsal plate shorter than posterodorsal plate, both with distal margin densely setose.

Distribution – Known from Praia do Crispim, Pará to Balneário Camboriú, Santa Catarina, Brazil (Fig. 1c).

Habitat – *Callichirus meridionalis* sp. nov. builds simple burrows with one opening (Fig. 1b), normally inhabited by only one individual, in the intertidal zone of fine-grain size sandy beaches. This species often is the only axiidean species present in the intertidal zone of many beaches. The pinnotherid crabs *Austinia aida* (Righi, 1967) and *A. patagoniensis* (Rathbun, 1918), are normally found living within of the galleries of *C. meridionalis* sp. nov. (Hernández 2018).

Color – Carapace transparent and hyaline; chelipeds white; first and second pleomeres transparent; deep yellow hepatopancreas and reddish-orange ovary visible in pleonal region of mature females; posterior region of the gonads one has an orange coloration in mature males, denoting an ovarian part of testis and consequently the hermaphroditism of individuals belonging to this sex; telson white and hyaline. Pattern of dorsal abdominal grooves more whitish in adult males and females (Fig. 1a).

Etymology – From the Latin, *meridionalis* referring to the southern distribution of the new species.

Discussion

***Taxonomic background of Callichirus major* (Say, 1818)**

The identity of *Callichirus major* (Say, 1818) has been shrouded in uncertainty for many years, despite that larval and molecular evidence have been accumulating to

suggest that it is in fact a species complex (e.g., Staton and Felder 1995; Strasser and Felder 1998, 1999a; Felder and Robles 2009; Peiró 2012; Rio 2018). Long before molecular data was available to suggest splitting the southern Atlantic population previously assigned to *C. major* into a new species, comparative morphological studies by Rodrigues (1985) (see also Rodrigues 1971) have shown that southern (Santos, Brazil) and northern specimens (North Carolina, USA) differed from each other. The differences concerned the presence of a subterminal tooth on the cutting edge of the dactylus of the male major cheliped (absent in northern specimens) and the absence of an acute tooth at the proximal third of the propodus of the female cheliped (present in northern specimens). Rodrigues (1985) suggested that the Brazilian population of *Callichirus* would ultimately prove to be a different species from *C. major*. Rodrigues felt that more specimens from both northern and southern localities were required before a new species was described. Many years later, Strasser and Felder (1999a) commented on several morphological differences between larvae from northern and southern (Brazilian) localities and referred to the Brazilian material as *Callichirus* sp.

Ortmann (1893: 87) described *Anomalocaris macrotelsonis* from northern Brazil based on a fourth larval stage and commented on the resemblance of *A. macrotelsonis* to the mysis of *Callianassa*. However, Ortmann's species has a distinct projecting dorsal carina on the first abdominal segment (Ortmann 1893: 87, pl. VI, fig. 5), a characteristic present neither in *Callianassa* and *Callichirus* nor in other callichirid or callianassid larvae known to date (see also Pohle et al. 2011 and Pohle and Santana 2014 for a compilation of available larval information).

Peiró (2012) corroborated the distinctness of the Brazilian *Callichirus* with molecular data (16S), however, mistakenly assigning the Brazilian specimens to Ortmann's species in combination with the genus *Callichirus*. Surprisingly, he

designated [sic] several males and females from Santos (Brazil) as syntypes [sic] for what he considered to be “*Callichirus macrotelsonis* Ortmann, 1893”. Clearly enough, the fourth larval stage described by Ortmann (1893) is not a *Callichirus* and probably not even a callichirid or callianassid larvae, therefore, Peiró’s action has no taxonomic nor nomenclatural value.

Rio (2018), based on detailed morphological studies, placed the Brazilian *Callichirus* in a new species, *Callichirus brasiliensis* Rio, 2018, but her nomenclatural action in a master’s dissertation is invalid as it does not fulfil the criteria of the ICZN (1999: Articles 8 and 9). This has been corrected in the present paper.

Neotype designation for Callichirus major (Say, 1818)

Say (1818: 238) described *Callianassa major* (subsequently transferred to *Callichirus* by Stimpson, 1866) from the bay shore of the river St. John, east Florida upon on a single specimen originally housed at the Academy of Sciences of Philadelphia. The holotype has not been found in the collections of the Academy (see Spamer and Bogan 1992: 162). This information has been confirmed by a recent inquiry at the Academy (P. Callomon, personal communication 2020). White (1847: 70) listed 2 “arms” of “*Callianassa major*” presented by Th. Say to the Natural History Museum in London (see Spamer and Bogan 1992: 162). However, no evidence exists to prove that these two “arms” belong to the holotype of *C. major*. Here, the male tl 77 mm (USNM 228086) from Indian River, Flat Just Inside Saint Lucie Inlet, Florida (Manning and Felder 1986) is selected as the neotype of *C. major* (Say, 1818) (Fig. 1a–e) in order to settle the defining characters of *C. major* s. str. and, therefore, ensure the correct use of this name.

Molecular analysis

The divergence rates between *C. major* s. str. (northern localities) and *C. meridionalis* sp. nov. (southern localities) is 5%, the same value as for the well established *C. islagrande* and *C. garthi* and *Filholianassa filholi* (A. Milne-Edwards, 1879) and *F. ceramica* (Fulton & Grant, 1906) (Table 2). Thus, morphology (see below) and molecular support *C. major* and *C. meridionalis* sp. nov. as distinct species.

Callichirus sp. 1 and *Callichirus* sp. 2 (Fig. 5), from the eastern Pacific and Caribbean Colombia, respectively, have a divergence rates of 3% from *C. meridionalis* sp. nov., and 5% from *C. major* (Table 2) These divergence rates also support the separation of *Callichirus* sp. 1 and *Callichirus* sp. 2 into new species. However, additional material of both is needed for morphological studies before conclusions are reached.

The *Callichirus* representatives were found to be nested within a well-supported clade (Fig. 5). The terminals previously assigned to *C. major* s. l. were recovered into a well-supported clade and the sistergroup relationship between *C. meridionalis* sp. nov. and *Callichirus* sp. 2 is also well-supported (bootstrap values of 94 and 77, respectively). *Callichirus* sp. 1 representatives grouped together (bootstrap value 55) but their relationship with *C. major* s. str. and the clade (*C. meridionalis* sp. nov. + *Callichirus* sp. 2) is still unresolved. Resolution of this polytomy will require using additional characters.

Morphological analysis

Morphologically, the new species differs from *C. major* s. str. by a combination of characters including (Figs. 2b, 3a, 4d *versus* Figs. 6a–f): (i) ocular peduncle reaching to about 2/3 of the length of the first article of the antennular peduncle (Fig. 2b) (*versus*

ocular peduncle reaching to the limit between the first and second articles of antennular peduncle, or a little beyond, in *C. major*, Fig. 6a) (see also Manning and Felder 1986: fig. 1a); (ii) second article of the antennular peduncle about two times longer than the distal one (*versus* second article 2.4 times longer than distal one in *C. major*) (see also Manning and Felder 1986: fig. 1a); (iii) male major cheliped dactylus bifid-tipped (Fig. 3a) (*versus* single-tipped male major cheliped dactylus in *C. major*, Fig. 6b) (see also Manning and Felder 1986: fig. 1c); (iv) male major cheliped with fixed finger armed with only one triangular tooth at the midlength of the cutting edge and the dactylus with a large rounded tooth close to articulation with propodus (Fig. 3a) (*versus* fixed finger armed with two teeth, one proximal small triangular tooth and a large rounded tooth at the midlength of the cutting edge, and the dactylus devoid of tooth and, instead, with a large dentate ridge extending from proximal half of the finger to its distal third, being the remaining surface unarmed to the curved tip in *C. major*, Fig. 6b); (v) male major cheliped fixed finger blunt and slightly curved upward (Fig. 3a) (*versus* male major cheliped fixed finger acute and strongly curved upward in *C. major*, Fig. 6e); (vi) male pleopod 2 exopod well developed (Fig. 4d) (*versus* male pleopod 2 exopod reduced in *C. major*, Figs. 6c, d); (vii) first pair of female pereopods, unequal in size and dissimilar in shape (Figs. 3b, d) (*versus* first female pereopods subequal and similar in *C. major*); and (viii) the female major cheliped, with a large meral hook on flexor margin (Fig. 3b) (*versus* that meral hook absent in *C. major*, Fig. 6f).

Callichirus meridionalis sp. nov. can be easily distinguished from the four other American congeners by the following combination of characters: (i) ocular peduncle elongate with obtuse tip (postcorneal projection absent), not exceeding the second article of the antennular peduncle (*versus* ocular peduncle elongate with postcorneal projections exceeding the second article of the antennular peduncle in *C. garthi*, *C.*

islagrande and *C. seilacheri*); (ii) second article of antennular peduncle slightly exceeding the fourth article of antennal peduncle (*versus* second article of antennular peduncle noteworthily exceeding the fourth article of antennal peduncle in *C. garthi* and *C. islagrande*); (iii) maxilliped 3 merus with distal margin strongly oblique (*versus* distal margin slightly oblique in *C. garthi*, *C. islagrande* and *C. seilacheri*); (iv) male major cheliped having the ventral margin of ischium unarmed (*versus* ventral margin of ischium armed with a hook in *C. islagrande* and *C. seilacheri* and a lobe in *C. garthi*); and (v) male pleopod 2 with the endopod well developed and exopod unsegmented (*versus* male pleopod 2 with the endopod reduced and exopod bisegmented in *C. santarosaensis*) (cf. Manning and Felder 1986, figs. 2a-c; Hernáez et al. 2015, figs. 2a,b, 3a,b; Sakai and Türkay 2012, fig. 10h; Hernáez et al. 2018, figs. 2b,c; see also Figs. 2b, h, 3a, 4d).

Sakai and Türkay (2012) claimed that *C. santarosaensis* stands apart from *C. major* in that (i) the antennular peduncle is about as long as the antennal peduncle and (ii) the telson is bisectioned, slightly converging toward the truncate end. However, Felder and Dworschak (2015) have convincingly showed that misinterpretations of the morphology of antennula, antenna, and telson led Sakai and Türkay (2012) astray, so that no morphological characters remain that justify *C. santarosaensis*. Yet, Felder and Dworschak (2015) argued that the name *C. santarosaensis* can still be applied “on the basis of geographical origins of materials, underpinned by genetic analyses (*sensu* Staton and Felder 1995) when possible”. However, Sakai and Türkay (2012), but also Felder and Dworschak (2015), overlooked that the male second pleopod endopod is greatly reduced in *C. santarosaensis* (Sakai and Türkay 2012: 746, fig. 10h) while well developed in *C. major* s. str. (Figs. 6c, d). This character can be used with confidence in

separating *C. santarosaensis* from *C. major* s. str., unless Sakai and Türkay (2012) have confused the endopod with the exopod in *C. santarosaensis*.

Callichirus meridionalis sp. nov. stands apart from *C. adamas*, the only non-American species in the genus, in the presence of ocular peduncles with obtuse tips without elongated postcorneal projections (*versus* ocular peduncles with elongated postcorneal projections in *C. adamas*) and by the male second pleopod with well develop exopod (*versus* male second pleopod exopod vestigial or strongly reduced in *C. adamas*) (Kensley 1974, figs. 1a, 2h; see also Figs. 2b, 4d).

Callichirus now consists of 7 species, namely: *C. adamas* (known from Senegal to Orange River mouth, South Africa), *C. garthi* (known from Huanchaco, Peru to Tubul, Chile), *C. islagrande* (currently known from the Gulf of Mexico), *C. meridionalis* sp. nov. (from Pará to Santa Catarina, Brazil), *C. major* (from North Caroline, USA to the Caribbean coast of Colombia), *C. santarosaensis* (known only from the northern coast of the Gulf of Mexico, Florida, USA) and *C. seilacheri* (From Jalisco, Mexico to Puntarenas, Costa Rica).

Callichirus meridionalis sp. nov. is the fourth western Atlantic species in *Callichirus*. However, the specimens from the Caribbean Colombia probably belong to an undescribed species sister to *C. meridionalis* sp. nov. Both differ from *C. major* s. str. in having shorter ocular peduncles that do not reach to the second article of antennular peduncle, and male pleopod 2 with well-developed exopod. However, the Caribbean Colombia population and *C. meridionalis* sp. nov. can be easily separated from each other in that the Colombian specimens have single-tipped male major cheliped dactylus, whereas *C. meridionalis* sp. nov. has bifid-tipped male major cheliped dactylus, and in the lack of a hook on the ventral margin of merus of the female major cheliped, whereas the ventral margin of the female major cheliped merus is armed with

a hook in *C. meridionalis* sp. nov. The following key facilitates the identification of the species of *Callichirus*.

Key to the species of the genus *Callichirus* Stimpson, 1866.

- 1.- Ocular peduncle elongate with obtuse tip, not exceeding or slightly exceeding to the junction between the first and second articles of the antennular peduncle..... 2
- Ocular peduncle elongate with postcorneal projection noteworthy exceeding to the junction between the first and second articles of the antennular peduncle..... 5
- 2.- Ocular peduncle reaching to about 2/3 of the length of the first article of the antennular peduncle. Male pleopod 2 with endopod and exopod well developed..... 3
- Ocular peduncle reaching to the limit between the first and second articles of antennular peduncle, or a little beyond. Male pleopod 2 with endopod or exopod reduced..... 4
- 3.- Male major cheliped dactylus with bifid-tipped. Female major cheliped merus armed with ventral hook..... *C. meridionalis* sp. nov.
- Male major cheliped dactylus with single-tipped. Female major cheliped merus unarmed.....*Callichirus* sp. 2 (Caribbean Colombia)
- 4.- Male pleopod 2 with endopod underdeveloped. Antennular peduncle about as long as antennal peduncle..... *C. santarosaensis*
- Male pleopod 2 with exopod reduced. Antennular peduncle longer than antennal peduncle..... *C. major* s. str.
- 5.- A1 peduncle article 2 reaching as far as A2 peduncle article 4. Maxilliped 3 propodus with V-shaped depression distally..... *C. seilacheri*

563	- A1 peduncle article 2 exceeding A2 peduncle article 4. Maxilliped 3 propodus rounded	
564	or straight distally.....	6
565	6.- Male major cheliped ischium unarmed ventrally. Telson emarginate posteriorly, with	
566	rounded tooth on distomedial depression.....	<i>C. garthi</i>
567	- Male major cheliped ischium armed with a ventral hook. Telson emarginate	
568	posteriorly, unarmed distomedial depression.....	7
569	7.- Male pleopod 2 with exopod reduced. Male major cheliped merus unarmed	
570	ventrally.....	<i>C. adamas</i>
571	- Male pleopod 2 with exopod well developed. Male major cheliped merus armed with	
572	ventral hook.....	<i>C. islagrande</i>

573

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876 **Table 1.** Taxa included in the molecular phylogenetic analyses to place *Callichirus meridionalis* sp. nov. within the context of the genus

877 *Callichirus* Stimpson, 1866.

Genbank taxon name	Taxon name	Sampling locality	Country	Ocean	Catalog n°	GenBank accession n°	Reference
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Praia do Perequê-açu, São Paulo	Brazil	WA	CCDB 2938-3	JX878478.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Praia do Perequê-açu, São Paulo	Brazil	WA	CCDB 2938-2	JX878477.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Ilha comprida, São Paulo	Brazil	WA	CCDB 3184	JX878476.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Ilhéus, Bahia	Brazil	WA	CCDB 3102	JX878475.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Maxaranguape, Rio Grande do Norte	Brazil	WA	CCDB 429	JX878474.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Maxaranguape, Rio Grande do Norte	Brazil	WA	CCDB 429	JX878473.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Barra do Sai, Sao Paulo	Brazil	WA	MZUSP 14557	JX878470.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Jaboatão dos Guararapes, Pernambuco	Brazil	WA	MZUESC n.n.	JX878469.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Acaú, Paraíba	Brazil	WA	MOUFPE 8391	JX878468.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Itapoá, Santa Catarina	Brazil	WA	CCDB 2184	JX878467.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Itapoá, Santa Catarina	Brazil	WA	CCDB 2184	JX878466.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Luis Correia, Piauí	Brazil	WA	CCDB 2973	JX878465.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Luis Correia, Piauí	Brazil	WA	CCDB 2973	JX878464.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Balneário Camboriú, Santa Catarina	Brazil	WA	CCDB 2868	JX878463.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Balneário Camboriú, Santa Catarina	Brazil	WA	CCDB 2868	JX878462.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Bragança, Pará	Brazil	WA	CCDB 2864	JX878461.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Bragança, Pará	Brazil	WA	CCDB 2864	JX878460.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Passo do Camaragibe, Alagoas	Brazil	WA	CCDB 2678	JX878454.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Passo do Camaragibe, Alagoas	Brazil	WA	CCDB 2678	JX878453.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	São Sebastião, São Paulo	Brazil	WA	CCDB 2677	JX878452.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	São Sebastião, São Paulo	Brazil	WA	CCDB 2677	JX878451.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Praia do Lázaro, São Paulo	Brazil	WA	CCDB 2661	JX878450.1	Peiró (2012)
<i>Callichirus</i> sp. 3	<i>Callichirus meridionalis</i> sp. nov.	Praia do Lázaro, São Paulo	Brazil	WA	CCDB 2661	JX878449.1	Peiró (2012)
<i>Callichirus major</i>	<i>Callichirus meridionalis</i> sp. nov.	São Paulo	Brazil	WA	ULLZ 6056	EU882918.1	Robles et al. (2020)
<i>Callichirus major</i>	<i>Callichirus meridionalis</i> sp. nov.	São Paulo	Brazil	WA	ULLZ 6056	EU882917.1	Robles et al. (2020)
<i>Callichirus</i> aff. <i>major</i>	<i>Callichirus meridionalis</i> sp. nov.	São Paulo	Brazil	WA	ULLZ 6056	MN238261.1	Robles et al. (2020)

<i>Callichirus aff. major</i>	<i>Callichirus meridionalis</i> sp. nov.	São Paulo	Brazil	WA	ULLZ 6055	MN238260.1	Robles et al. (2020)
<i>Callichirus</i> sp. 2	<i>Callichirus</i> sp. 2	Cartagena	Colombia	WA	CCDB 3426	JX878472.1	Peiró (2012)
<i>Callichirus</i> sp. 2	<i>Callichirus</i> sp. 2	Cartagena	Colombia	WA	CCDB 3425	JX878471.1	Peiró (2012)
<i>Callichirus</i> sp. 1	<i>Callichirus</i> sp. 1	Playa Curu, Puntarenas	Costa Rica	EP	CCDB 2869	JX878459.1	Peiró (2012)
<i>Callichirus</i> sp. 1	<i>Callichirus</i> sp. 1	Playa Curu, Puntarenas	Costa Rica	EP	CCDB 2869	JX878458.1	Peiró (2012)
<i>Callichirus</i> sp. 1	<i>Callichirus</i> sp. 1	Playa Curu, Puntarenas	Costa Rica	EP	CCDB 2869	JX878457.1	Peiró (2012)
<i>Callichirus</i> sp. 1	<i>Callichirus</i> sp. 1	Playa Curu, Puntarenas	Costa Rica	EP	CCDB 2869	JX878456.1	Peiró (2012)
<i>Callichirus</i> sp.	<i>Callichirus</i> sp. 1	Baja California	Mexico	EP	ULLZ 4163	EU882922.1	Felder and Robles (2009)
<i>Callichirus seilacheri</i>	<i>Callichirus garthi</i>	E Tongoy, Coquimbo	Chile	EP	ULLZ 9002	KR003900.1	Robles and Felder (2015)
<i>Callichirus</i> sp.	<i>Callichirus islagrande</i>	Gulf of Mexico, Tabasco	Mexico	WA	ULLZ 5773	KR003899.1	Robles and Felder (2015)
<i>Callichirus</i> sp.	<i>Callichirus islagrande</i>	Gulf of Mexico, Tabasco	Mexico	WA	ULLZ 5773	KR003898.1	Robles and Felder (2015)
<i>Callichirus islagrande</i>	<i>Callichirus islagrande</i>	Gulf of Mexico, Misissipi	USA	WA	ULLZ 6052	EU882916.1	Robles et al. (2020)
<i>Callichirus major</i>	<i>Callichirus major</i>	Isles Dernieres, Louisiana	USA	WA	unknown	AF436041.1	Morrison et al. (2002)
<i>Callichirus major</i>	<i>Callichirus major</i>	Unknown	unknown	WA	KC1864	DQ079707.1	Porter et al. (2005)
<i>Callichirus seilacheri</i>	<i>Callichirus seilacheri</i>	Playa Curu, Puntarenas	Costa Rica	EP	CCDB 561	JX878455.1	Peiró (2012)
<i>Callichirus seilacheri</i>	<i>Callichirus seilacheri</i>	Unknown	Nicaragua	EP	ULLZ 6053	EU882921.1	Robles et al. (2020)
<i>Callichirus seilacheri</i>	<i>Callichirus seilacheri</i>	Baja California	Mexico	EP	ULLZ 6054	EU882920.1	Robles et al. (2020)
<i>Callianassa aqabaensis</i>	<i>Aqaballianassa aqabaensis</i>	Unknown	Jordan	Red Sea	ULLZ 7924	EU874925.1	Robles et al. (2020)
<i>Sergio mirim</i>	<i>Audacallichirus mirim</i>	São Paulo	Brazil	WA	CCDB 2975	MF490166.1	Mantelatto et al. (2018)
<i>Biffarius delicatulus</i>	<i>Biffarius biformis</i>	Indian River, Florida	USA	WA	USNM 309754	EU874953.1	Robles et al. (2020)
<i>Callianassa ceramica</i>	<i>Filholianassa ceramica</i>	South of Port Authority Pier, Queenscliff	Australia	WP	NMV J40715	KU350630.1	Tan et al. (2017)
<i>Callianassa ceramica</i>	<i>Filholianassa ceramica</i>	Anglesea beach, Victoria	Australia	WP	unknown	KU362925.1	Gan et al. (2016)
<i>Callianassa filholi</i>	<i>Filholianassa filholi</i>	Unknown	New Zealand	WP	NMV J44818	EU874949.1	Robles et al. (2020)
<i>Lepidophthalmus siriboia</i>	<i>Lepidophthalmus siriboia</i>	Estuario do Roteiro	Brazil	WA	ULLZ 5611	KR003930.1	Robles and Felder (2015)
<i>Lepidophthalmus siriboia</i>	<i>Lepidophthalmus siriboia</i>	Estuario do Roteiro	Brazil	WA	ULLZ 5611	KR003929.1	Robles and Felder (2015)
<i>Sergio guassutunga</i>	<i>Neocallichirus guassutunga</i>	Bocas Del Toro, Bocas del Toro	Panamá	WA	BOC-035b	MK971568.1	Venera-Pontón et al. (2020)
<i>Sergio guassutunga</i>	<i>Neocallichirus guassutunga</i>	Isla Colón, Bocas Del Toro	Panamá	WA	ULLZ13324	MK971507.1	Venera-Pontón et al. (2020)
<i>Sergio guassutunga</i>	<i>Neocallichirus guassutunga</i>	Isla Colón, Bocas Del Toro	Panamá	WA	ULLZ13323	MK971363.1	Venera-Pontón et al. (2020)
<i>Sergio guassutunga</i>	<i>Neocallichirus guassutunga</i>	Isla Colón, Bocas Del Toro	Panamá	WA	ULLZ13322	MK971307.1	Venera-Pontón et al. (2020)
<i>Callianassa</i> sp.	<i>Pugnatrypaea</i> sp.	Gulf of Mexico, Louisiana	USA	WA	ULLZ 6058	EU882915.1	Robles et al. (2020)

878	<i>Callianassa</i> sp.	<i>Pugnatrypaea</i> sp.	Gulf of Mexico, Louisiana	USA	WA	ULLZ 8279	EU882903.1	Robles et al. (2020)
	Catalog number abbreviations: CCDB, Crustacean Collection of the Department of Biology, FFCLRP, University of São Paulo; MOUFPE, Museu de Oceanografia Prof. Petrônio Alves Coelho; MZUESC, Museu de Zoologia da Universidade Estadual de Santa Cruz; MZUSP, Museum of Zoology of University of São Paulo; NHMW, Naturhistorisches Museum Wien; NMV, Museums Victoria, Melbourne, Australia; ULLZ, University of Louisiana at Lafayette Zoological Collection; USNM, United States National Museum, Smithsonian Institution. N. n., not numbered; EP, eastern Pacific; WP, western Pacific; EA, eastern Atlantic; WA, western Atlantic.							
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884 **Table 2.** Estimation of evolutionary divergence over sequence pairs from the portion of the mitochondrial 16S rDNA with ~510bp.

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	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 <i>Callichirus meridionalis</i> sp. nov.															
2 <i>Callichirus</i> sp. 2	0,03														
3 <i>Callichirus</i> sp. 1	0,05	0,05													
4 <i>Callichirus major</i>	0,05	0,06	0,06												
5 <i>Callichirus seilacheri</i> ?	0,08	0,09	0,09	0,09											
6 <i>Callichirus garthi</i>	0,09	0,09	0,10	0,10	0,06										
7 <i>Callichirus islagrande</i>	0,09	0,08	0,09	0,09	0,05	0,05									
8 <i>Callichirus seilacheri</i>	0,12	0,11	0,12	0,11	0,07	0,06	0,06								
9 <i>Audacallichirus mirim</i>	0,19	0,17	0,19	0,19	0,20	0,19	0,19	0,21							
10 <i>Filhollianassa ceramica</i>	0,19	0,19	0,18	0,20	0,21	0,19	0,19	0,22	0,22						
11 <i>Filhollianassa filholi</i>	0,20	0,18	0,18	0,20	0,21	0,19	0,19	0,22	0,22	0,05					
12 <i>Lepidophthalmus siriboia</i>	0,21	0,19	0,20	0,20	0,21	0,20	0,21	0,23	0,16	0,22	0,22				
13 <i>Biffarius biformis</i>	0,23	0,21	0,22	0,23	0,23	0,22	0,22	0,24	0,22	0,19	0,18	0,24			
14 <i>Pugnatrypaea</i> sp.	0,24	0,23	0,22	0,23	0,22	0,25	0,23	0,23	0,25	0,20	0,22	0,26	0,19		
15 <i>Neocallichirus guassutinga</i>	0,25	0,24	0,23	0,27	0,26	0,26	0,26	0,28	0,25	0,27	0,28	0,29	0,31	0,32	
16 <i>Aqaballianassa aqbaensis</i>	0,26	0,25	0,26	0,27	0,29	0,25	0,26	0,28	0,28	0,24	0,22	0,29	0,23	0,23	0,37

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Table 3. Branchial formula for *Callichirus meridionalis* **sp. nov.**

	Maxillipeds			Pereopods				
	1	2	3	1	2	3	4	5
Exopods	1	1	-	-	-	-	-	-
Epipods	1	1	-	-	-	-	-	-
Podobranchs	-	-	-	-	-	-	-	-
Arthrobranchs		1	2	2	2	2	2	-
Pleurobranchs	-	-	-	-	-	-	-	-

Legends of figures

Fig. 1. a, male and female specimen of the burrowing shrimp *Callichirus meridionalis* sp. nov. from the Brazilian coast (MZUSP 39027); b, intertidal flat at Gonzaga beach showing a topotypical habitat of the new species, inset indicating a burrow opening of *C. meridionalis* sp. nov.; c, collection sites for *C. meridionalis* sp. nov. along the Brazilian coast. Scale bar: 5mm.

Fig. 2. *Callichirus meridionalis* sp. nov. a, b, holotype, male, cl 18.8 mm, MZUSP 41251; c–h, paratype, male, cl 18.6 mm, MZUSP 41253. a, body, lateral view; b, carapace front, eyestalks, and antennular and antennal peduncles, dorsal view; c, right mandible, external surface; d, right maxillulla, external surface; e, right maxilla, external surface; f, right first maxilliped, external surface; g, right second maxilliped, external surface; h, right third maxilliped, external surface. Scale bars: a = 1 cm; b–h = 2 mm.

Fig. 3. *Callichirus meridionalis* sp. nov., a, c, e–h, holotype, male, cl 18.8 mm, MZUSP 41251; b, d, paratype, female, cl 20.4 mm, MZUSP 41252. a, c, male major and minor chelipeds, respectively, lateral view; b, d, female major and minor chelipeds, respectively, lateral view; e–h, second, third, fourth, and fifth pereopods, respectively, lateral view. Scale bars: a–d = 1 cm; e–h = 2 mm.

Fig. 4. *Callichirus meridionalis* sp. nov., a, b, d, f, holotype, male, cl 18.8 mm, MZUSP 41251; c, e, paratype, female, cl 20.4 mm, MZUSP 41252. a, sixth abdominal somite, uropods, and telson, dorsal view; b, c, male and female first pleopods, respectively, external surface; d, e, male and female second pleopods, respectively, external surface; f, third to fifth pleopods, external surface. Scale bars: a, c, e, f = 2 mm; b, d = 1 mm.

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920 **Fig. 5.** Molecular phylogenetic tree represented as maximum likelihood topology of the
 921 partial mitochondrial DNA sequence of the 16S rDNA gene to place *Callichirus meridionalis*
 922 sp. nov. Nodal support values represent the frequencies observed using 1000 bootstrap
 923 pseudo-replicates. Values below 50% are not represented.

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925 **Fig. 6.** *Callichirus major*, a, b, neotype, male cl 13.2 mm, Indian River, Florida, USA,
 926 USNM 228086. c, e, male cl 20.6 mm, New River, North Caroline, USA, USNM 266227. d,
 927 male cl 24.4 mm, Louisiana, Gulf of Mexico, USA, USNM 79171. f, female cl 16.3 mm,
 928 Indian River, Florida, USA, USNM 228087. a, carapace front, eyestalks, and antennular and
 929 antennal peduncles, dorsal view; b, male major cheliped claw, lateral view; c, d, male second
 930 pleopod, external surface; e, male major cheliped claw, lateral view; f, female major cheliped,
 931 lateral view. Scale bars: a = 2 mm; b, e, f = 1 cm; c, d = 1 mm.

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Figure 1.

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Figure 5.

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