



## A new species of *Parastacus* Huxley, 1879 (Crustacea, Decapoda, Parastacidae) from a swamp forest in southern Brazil

Felipe Bezerra Ribeiro<sup>1</sup>, Augusto Frederico Huber<sup>1</sup>,  
Christoph D. Schubart<sup>2</sup> and Paula Beatriz Araujo<sup>1</sup>

<sup>1</sup> Programa de Pós-Graduação em Biologia Animal, Departamento de Zoologia  
(Laboratório de Carcinologia), Instituto de Biociências, Universidade Federal do Rio  
Grande do Sul. Porto Alegre, Rio Grande do Sul, Brazil

<sup>2</sup> Zoology and Evolutionary Biology, Universität Regensburg. Regensburg, Germany

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### ABSTRACT

In this contribution we describe a new species of burrowing crayfish of the genus *Parastacus* Huxley, 1879 from a swamp forest in southern Brazil and determine its conservation status. The distinction of the new species is based on morphology and the mitochondrial DNA marker 16S rRNA. The extinction risk was assessed according to the sub-criterion B1 of IUCN that estimates the Extent of Occurrence (EOO). *Parastacus tuerkayi* sp. nov. is morphologically distinguishable from all species of *Parastacus* by having three lines of verrucous tubercles on the dorsomesial margin of the cheliped propodus and a suborbital angle exceeding 90°. The EOO comprises 647,674 km<sup>2</sup>, and the species is classified as “endangered”. Phylogenetic relationships indicate the distinct position of this new species in relation to the already described species.

### KEY WORDS

16S, mtDNA sequence, burrowing crayfish, Neotropical region, taxonomy.

#### CORRESPONDING AUTHOR

Felipe Bezerra Ribeiro  
fbribeiro.ufc@gmail.com

SUBMITTED 17 October 2016  
ACCEPTED 28 november 2016  
PUBLISHED 27 Abril 2017

Guest Editor  
Célio Magalhães

DOI 10.1590/2358-2936e2017008

## INTRODUCTION

The freshwater crayfish of the genus *Parastacus* Huxley, 1879 are currently represented by ten species, distributed in the southern regions of South America, specifically in Chile, Argentina, Uruguay and Brazil (for the latter in the states of Rio Grande do Sul and Santa Catarina) (Buckup and Rossi, 1980; 1993; Ribeiro *et al.*, 2016). According to previous phylogenetic studies, *Parastacus* forms a well supported monophyletic clade and is closely related to *Samastacus* Riek, 1971 and *Viralastacus* Hobbs, 1991 (Crandall *et al.*, 2000; Toon *et al.*, 2010).

Burrowing crayfish differ in both behaviour and type of burrows. Hobbs (1942) classified crayfish burrowing behaviour into three categories, taking into account the complexity of burrows, the connection or not to open waters, seasonality and reproductive period, and time individuals spend inside the burrows. Horwitz and Richardson (1986) classified crayfish burrows based on the relationship to the water availability: (1) located in permanent water bodies, (2) connected to the water-table, water from underground or surface run-off and (3) no connection to water-table, the water supply being the surface run-off. Specifically for *Parastacus*, Riek (1972) classified all species as strong burrowers, but Buckup and Rossi (1980) noted differences in burrowing abilities, depending on habitat.

Molecular tools to complement species descriptions in parastacids were increasingly adopted in the last years (Rudolph and Crandall, 2005; 2007; 2012), especially in the recognition of new species, when cryptic species are involved. The use of DNA sequencing can be very useful in uncovering genetic variation and increasing the speed of species description, thus acting as a stimulus to further conservation efforts (Burnham and Dawkins, 2013).

In this contribution, we describe a new burrowing species of the crayfish genus *Parastacus*, discovered in a small fragment of a swamp forest located inside a theme park in southern Brazil. In addition, the distinctive position of this new species is discussed in a phylogenetic context. Habitat characterization and conservation status of the species based on the IUCN Red List criteria are also discussed.

## MATERIAL AND METHODS

### *Sampling*

Specimens were collected in one small section

of a swamp forest, located inside the Beto Carreiro World Park, in the municipality of Penha, state of Santa Catarina, Brazil (26°48'10"S 48°37'2"W). The type material was deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo, Brazil, and in the Carcinological Collection of the Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio do Grande do Sul (UFRGS), Porto Alegre, Brazil. For sampling, burrows were excavated manually in order to obtain crayfish specimens and to provide some information about the burrow system. In addition, a vacuum pump (7cm x 72 cm) was used to capture the individuals.

### *Morphological analysis*

Drawings were prepared under a stereomicroscope fitted with a *camara lucida* and measurements were performed with vernier calipers with 0.1 mm accuracy and a millimetric ocular on a stereomicroscope. Morphological parameters used were defined by Buckup and Rossi (1980), Hopkins (1970), Morgan (1997) and Ribeiro *et al.* (2016). Measurements of all type specimens can be found in [Tab. 1](#). Size and shape of the S2 pleura were defined according to Ribeiro *et al.* (2016). Sex was determined based on the morphology of the genital apertures, according to Rudolph (1997). Morphological descriptions follow Riek (1971), Buckup and Rossi (1980), Hobbs (1987), Morgan (1997), Holdich (2002) and Ribeiro *et al.* (2016). The taxonomic classification follows De Grave *et al.* (2009). Branchial count follows Huxley (1879).

### *Molecular analysis*

Total genomic DNA was extracted from muscle tissue from walking legs from two fresh specimens collected in the type locality, using the Puregene kit (Qiagen). A fragment of approximately 550 base pairs (bp) of mitochondrial DNA encoding the 16S rRNA was amplified using published primers sets: 16L2 (5'-TGC CTG TTT ATC AAA AAC AT-3') (Schubart *et al.*, 2002) and 1472 (5'-AGA TAG AAA CCA ACC TGG-3') (Crandall and Fitzpatrick 1996; Schubart *et al.*, 2000 as 16H2).

Conditions for the polymerase chain reactions (PCR) were: initial denaturation at 94°C for 4 min, followed by 40 cycles of 95°C for 45 s, annealing at 48 or 50°C for 1 min, elongation at 72°C for 1 min,

and a final extension step at 72°C for 5 min. PCR products were outsourced for sequencing to Macrogen Europe (Amsterdam, The Netherlands). The obtained chromatograms were proofread using Chromas Lite version 2.23 (Technelysium Pty Ltd., 2005). Resulting sequences were blasted in GenBank and compared with the available *Parastacus* assemble. The new sequences were deposited at GenBank under accession numbers KY192525 and KY192526.

In addition, the following sequences with their respective accession numbers from NCBI database were included in the analysis: *Parastacus defossus* Faxon, 1898 (AF175243.1 and AF175242.1), *Parastacus varicosus* Faxon, 1898 (EU920933.1), *Parastacus nicoleti* (Philippi, 1835) (AF175231.1, AF175232.1, AF175233.1 and AF175234.1), *Parastacus pugnax* (Poepigg, 1882) (AF175238.1, AF175328.1 and AF175239.1) and *Samastacus spinifrons* (Philippi, 1882) (EF199542.1). All sequences were aligned with BioEdit version 7.2.5 (Hall, 1999) using the ClustalW algorithm (Thompson *et al.*, 1994) and adjusted manually, if required.

The best nucleotide substitution model was selected using jMODELTEST 2.1.10 with the Akaike Information Criterion (AIC) (95% confidence) (Darriba *et al.*, 2012), suggesting HKI + G as evolutionary model. Phylogenetic relationships were estimated using Bayesian Inference implemented in BEAST 1.8.3 (Drummond *et al.*, 2012). The gene tree search was run on computational resources provided by CIPRES portal (Miller *et al.*, 2015) using the tool BEAST on XSEDE (Drummond and Rambaut, 2007; Suchard and Rambaut, 2009). We used 10 million generations with Markov Chain Monte Carlo (MCMC) sampling, saving trees every 1,000 steps. The efficiency of the chain was assessed in Tracer 1.6 (Rambaut *et al.*, 2007), and the software TreeAnnotator (BEAST package) was used to summarize the trees, with 10% of initial trees discarded as burn-in. Genetic distances were also calculated by pairwise comparisons using uncorrected p-distances with the software Mega 7.0 (Kumar *et al.*, 2013).

#### *Conservation analysis.*

The extinction risk of the new species was defined according to the B1 sub-criterion of the International Union for Conservation of Nature - IUCN (IUCN, 2012). This sub-criterion takes into consideration

the estimated Extent of Occurrence (EOO) that was calculated in the Arcview 9.3 program (ESRI, 2009). The definition of the hydrographic basins follows the Otto Bacias shape method (level 4) (ANA, 2006).

#### *Abbreviations*

SLP = Thoracic Sternite Lateral Processes

S1 = Abdominal Somite 1

S2 = Abdominal Somite 2

TL = Total Length

CL = Carapace Length

CW = Carapace Width

CD = Carapace Depth

CeL = Cephalon Length

RL = Rostral Length

RW = Rostral Width

RCL = Rostral Carina Length

CMW = Cornea Maximum Width

OW = Orbital Width

POCL = Post Orbital Carina Length

FW = Frontal Width

ASL = Antennal Scale Length

ASW = Antennal Scale Width

AreL = Areola Length

AreW = Areola Width

RPrT/LPrT = Right/Left Propodus Thickness

RPrL/LPrL = Right/Left Propodus Length

RPrW/LPrW = Right/Left Propodus Width

RDL/LDL = Right/Left Dactylus Length

RML/LML = Right/Left Merus Length

AL = Abdomen Length

AW = Abdomen Width

TeL = Telson Length

TeW = Telson Width

The definition of each measurement can be found in Ribeiro *et al.* (2016).

## SYSTEMATICS

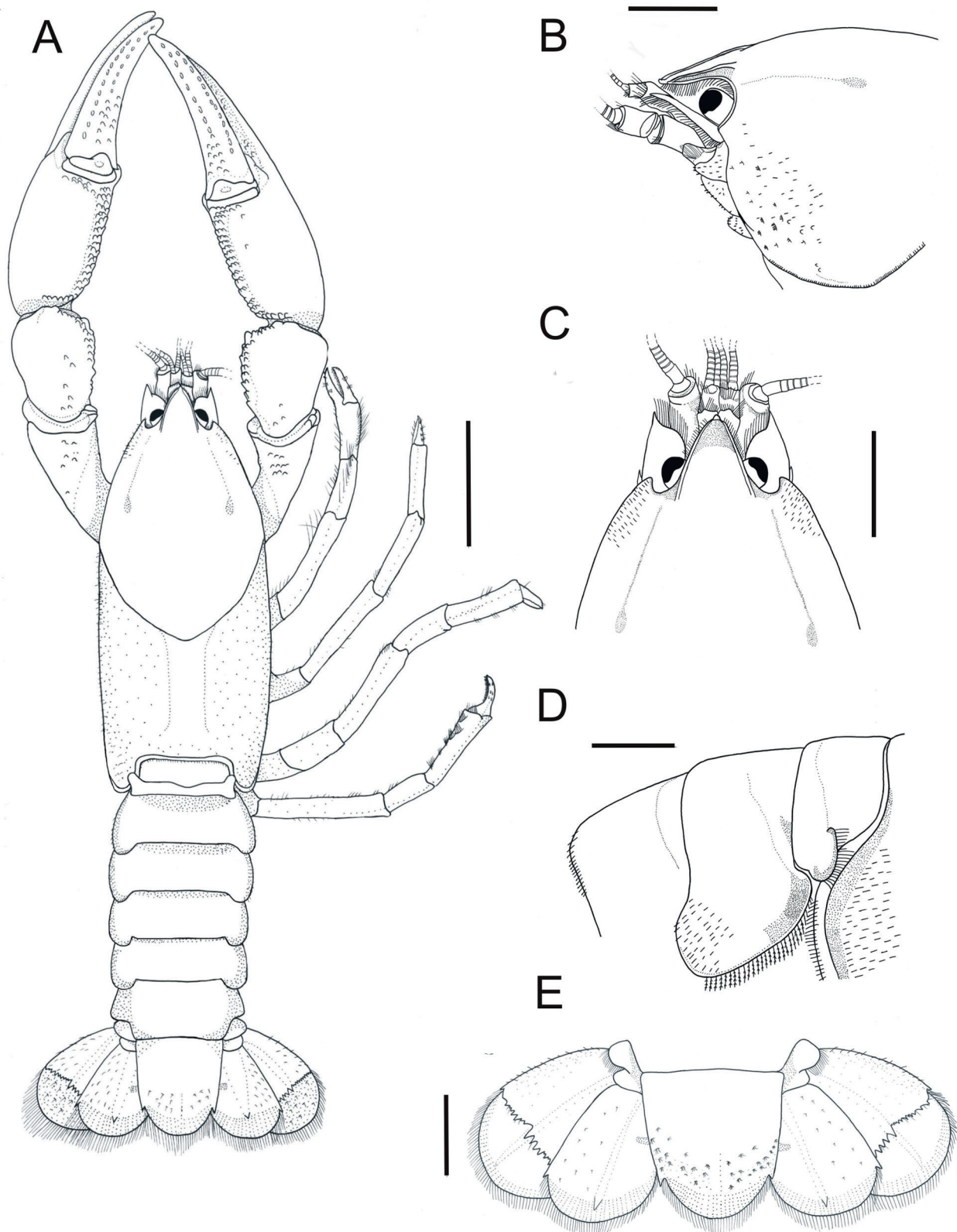
### **Infraorder Astacidea Latreille, 1802**

### **Superfamily Parastacoidea Huxley, 1879**

### **Genus *Parastacus* Huxley, 1879**

### ***Parastacus tuerkayi* sp. nov. Ribeiro, Huber and Araujo (Figs. 1–5)**





**Figure 1.** *Parastacus tuerkayi* sp. nov., holotype (MZUSP 34940). A, habitus, dorsal view; B, cephalon, lateral view; C, cephalon, dorsal view; D, first and second abdominal pleura; E, telson and uropods, dorsal view. Scale bars: A = 1 cm; B – E = 5 mm.

*Type material.* Holotype: male, Brazil, Santa Catarina, Penha, Beto Carreiro World (26°48'10"S 48°37'02"W), 04/IX/2013, leg. K.M. Gomes and F.B. Ribeiro (MZUSP 34940). Paratypes: 1 ovigerous female, Brazil, Santa Catarina, Penha, Beto Carreiro World (26°48'11"S 48°37'01"W), I/2001, leg. H. Boos Jr. (UFRGS 6376); 1 male, Brazil, Santa Catarina, Penha, Beto Carreiro World, 2001, leg. K. Schaat (UFRGS 3167); 1 male, same data as holotype (UFRGS 6438).

*Comparative material analyzed.* Chile: *P. pugnax* – 1 male and 2 females, La Florida, Concepción, 19/I/1977 (UFRGS 2407); 5 females, Rengo (cordillera), II/1984, leg. A.F. Neto (UFRGS 726); 2 males and 3 females, Laguna San Pedro, Concepción, 18/VII/1970. *Parastacus nicoleti* – 1 male, Mehuim (next to Valdivia), VIII/1997, leg. niños del Pueblo

(UFRGS 2405). Brazil, Rio Grande do Sul: *P. defossus* – 1 male, Costa do Cerro, Lami, Porto Alegre, 19/VII/2005, leg. L.C.E. Daut and J.F. Amato; 1 female, Sítio do Mato, Zona Sul, Porto Alegre (30°4'10.27"S 51°5'10.46"W), 22/III/2014, leg. K.M. Gomes and F.B. Ribeiro. *Parastacus caeruleodactylus* Ribeiro and Araujo in Ribeiro *et al.*, 2016 – 1 female, Morrinhos do Sul (29°17'13.7"S; 49°54'53.42"W), 12/XII/2013, leg. F.B. Ribeiro and K.M. Gomes (UFRGS 5931).

*Etymology.* Named to honor Dr. Michael Türkay from Seckenberg Museum, Frankfurt am Main, Germany, who passed away in 2015. He dedicated several years of his life to the research of freshwater crustaceans, especially freshwater crabs from the Neotropical region, describing several new species and providing invaluable contributions to the taxonomy of freshwater decapods.

**Table 1.** Measurements (mm) of the type series of *Parastacus tuerkayi* sp. nov. For abbreviations, see Material and Methods.

	Holotype (MZUSP 34940)	Paratype (UFRGS 6376)	Paratype (UFRGS 3167)	Paratype (UFRGS 6438)
Sex	M	F	M	M
TL	66.81	54.93	59.37	57.04
CL	33.52	26.45	18.72	28.64
CW	15.23	12.34	14.34	12.55
CD	16.92	13.98	13.58	14.52
CeL	22.58	18.09	18.51	18.78
RL	4.09	3.56	4.01	3.54
RW	3.41	3.13	3.49	3.27
CMW	1.30	1.10	1.40	1.62
OW	2.52	2.15	2.33	2.23
FW	6.27	4.90	5.45	5.47
RCL	4.19	3.80	4.52	4.03
POCL	8.21	5.77	6.39	6.13
ASL	4.51	3.40	3.65	3.85
ASW	2.02	1.30	1.52	1.75
AreL	8.68	7.15	8.59	8.18
AreW	3.08	2.95	3.11	3.32
AW	12.74	11.41	11.10	11.36
AL	26.23	22.58	24.42	22.28
RPrT	7.20	3.57	6.75	5.42
RPrL	28.14	15.87	23.49	29.30
RPrW	12.94	7.00	12.00	10.10
RML	15.05	11.01	12.96	12.09
RDL	17.65	10.45	13.59	12.56
LPrT	7.36	4.82	3.92	5.46
LPrL	29.31	18.35	15.94	20.63
LPrW	12.85	9.44	7.04	10.00
LML	14.91	11.48	11.31	12.19
LDL	17.65	11.49	10.47	12.54
TeL	9.98	8.37	8.75	7.78
TeW	7.65	6.84	6.55	6.74

*Diagnosis.* Narrow front with short triangular rostrum. Rostral apex shaped as inverted “U”, with an upward blunt spine. Suborbital angle  $>90^\circ$ . Postorbital carinae weakly prominent. Cervical groove V-shaped. Areola narrow and barely discernible. Telson subrectangular with sharp spines on lateral margins. Mandible with caudal molar process bicuspidate with one cephalodistal cusp and one small distoproximal cusp. S2 pleurae high and long with deep groove parallel to margin. Internal ventral border of basal article of antennule without sharp spine in males.

*Description of the holotype.* Rostrum: triangular, longer than wide (RW 83.4% of RL), short (10.2% of CL), reaching proximal portion of the second article of the antennular peduncle (Fig. 1A–C). Dorsum straight, apex inverted “U”-shaped, ending in upward blunt spine (Fig. 1B, C). Few plumose setae on lateral margins. Rostral sides slightly convergent and rostral basis parallel. Carinae almost straight, prominent and narrow, extending back to carapace, slightly surpassing rostral basis (Fig. 1B, C).

Cephalon: carapace lacking spines or tubercles. CeL 67.4% of CL. Eyes small (CMW 51.6% of OW); suborbital angle  $>90^\circ$ , unarmed (Fig. 3C). Front narrow (FW 41.2% of CW). Postorbital carinae longer than rostral carinae (RCL 51% of POCL) and weakly prominent. Lateral cephalic edge with moderate setation (Fig. 1A–C).

Thorax: carapace laterally compressed, deep and narrow (CD 50.5% of CL; CW 45.4% of CL). Cervical groove V-shaped. Branchiocardiac grooves inconspicuous (Fig. 1A). Areola narrow, 2.8x as long as wide (25.9% of CL) (Fig. 1A).

Abdomen: lacking spines or tubercles, long and narrow (AL 78.2% of CL; AW 83.6% of CW), smooth, covered with small setae on pleural margins (Fig. 1A). Pleural somites with rounded posterior margins. S1 pleurae with a large distal lobe not overlapped by S2 pleurae. S2 pleurae high and short with deep groove parallel to margin (Fig. 1D).

Tailfan: telson uniformly calcified, subrectangular, longer than wide (TeW 76.6% of TeL), with sharp spines on lateral margins; rounded distal margin with abundant long plumose setae and short simple setae. Dorsal surface with tufts of short setae and inconspicuous dorsomedian longitudinal groove (Fig. 1E).

Uropod protopod bilobed, with rounded and unarmed margins; proximal lobe largest. Exopod lateral margin bears a small and sharp spine, mid-dorsal carina weakly prominent, ending in a very sharp spine. Transverse suture (diaeresis) straight, with ten dorsolateral spines (outer) and nine dorsolateral spines (inner) on right exopod and ten dorsolateral spines (outer) and eight dorsolateral spines (inner) on the left exopod. Endopod with mid-dorsal carina weakly prominent, ending in a very sharp spine; lateral margin with one sharp spine at level of exopod transverse suture (Fig. 1E).

Epistome: anterolateral section with conical projection. Posterolateral section smooth and with deep lateral grooves converging to the basis of the anteromedian lobe and reduced median circular concavity. Anteromedian lobe pentagonal, 1.2x longer than wide, apex acute and straight with some serrated setae, reaching median part of antepenultimate article of antennal peduncle; dorsal surface straight, and basis with a shallow groove (Fig. 2A).

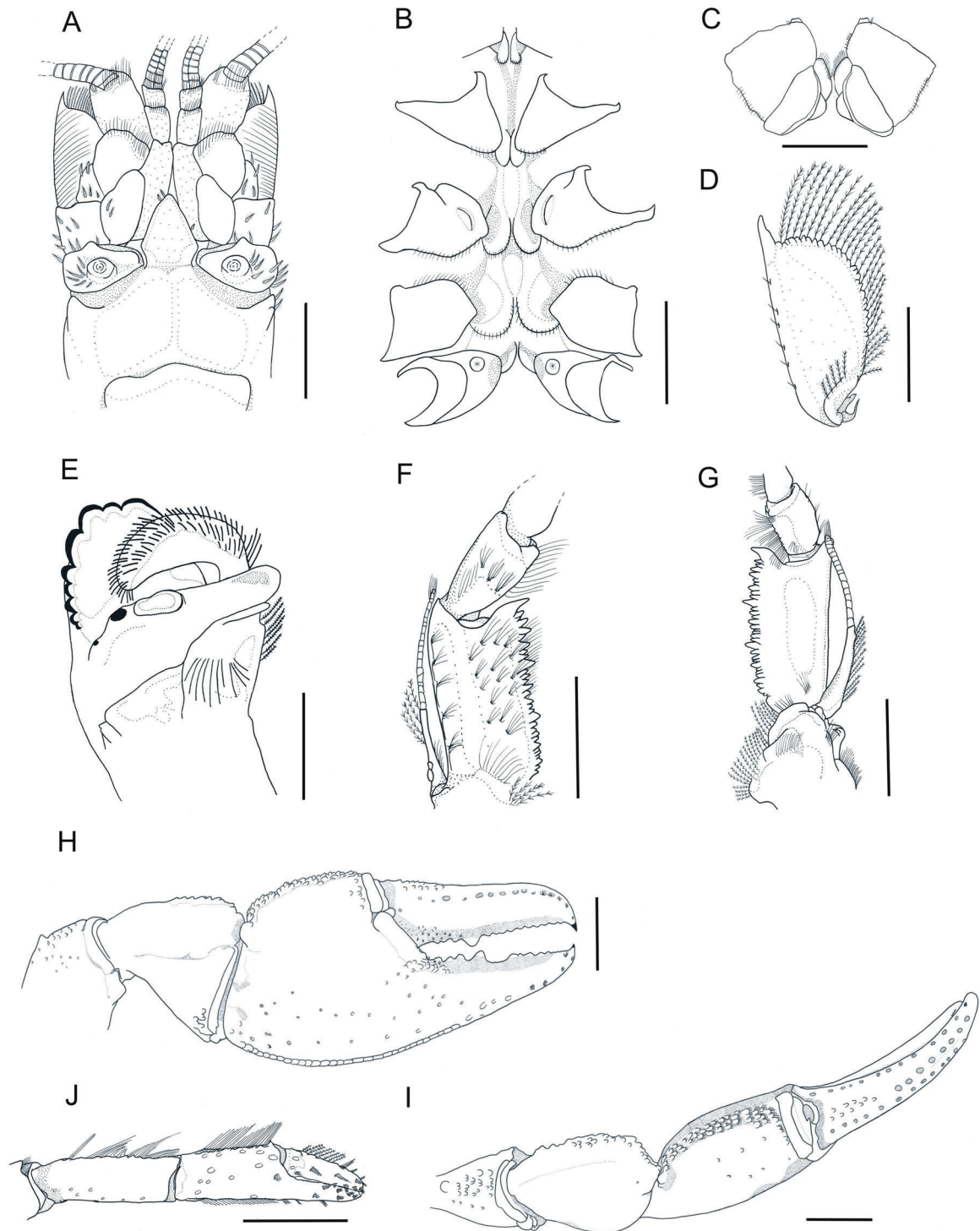
Thoracic sternites: SLP4 smallest and close to each other, median keel present and not inflated; SLP5 small and very close to each other, median keel present and not inflated; SLP6 larger than SLP4, SLP5 and SLP8 and with a slightly concave surface, median keel inflated; SLP7 largest and with surface slightly concave, median keel inflated, bullar lobes absent; SLP8 small and slightly concave, median keel absent, vertical arms of paired sternopleural bridges close to each other, bullar lobes separated and clearly visible (Fig. 2B, C).

Antennule: internal ventral border of basal article without sharp spine (Fig. 2A).

Antenna: when extended back reaching S1. Antennal scale widest at midlength, reaching midlength of third antennal article, ASW 44.8% of ASL (Fig. 2A, D), lateral margin straight, spine strong and distal margin straight. Coxa with prominent carina above nephropore and blunt spine laterally displaced. Basis unarmed (Fig. 2A).

Mandible: cephalic molar process molariform, caudal molar process bicuspidate with one cephalodistal cusp and one distoproximal cusp. Incisive lobe with nine teeth. Third tooth from the anterior margin largest (Fig. 2E).

Third maxilliped: ischium bearing few setiferous punctuations, but with some long smooth simple setae on outer margin (Fig. 2F); dorsal surface without setae



**Figure 2.** *Parastacus tuerkayi* sp. nov., holotype (MZUSP 34940) and paratypes (UFRGS 3167, UFRGS 6438). A, epistome (holotype); B, thoracic sternites and gonopores (holotype); C, thoracomere 8, caudal view (holotype); D, antennal scale, lateral view (UFRGS 3167); E, mandible (UFRGS 6438); F, third maxilliped, ventral view (UFRGS 6438); G, third maxilliped, dorsal view (UFRGS 6438); H, first pereopod, lateral view (holotype); I, first pereopod, dorsal view (holotype); J, second pereopod, lateral view (holotype). Scale bars: A = 3.3 mm; B, C, H, I, J = 5 mm; D, E = 1.6 mm; F, G = 3.33 mm.



(Fig. 2G). Merus ventral surface sparsely covered by long smooth simple setae in the median-proximal region (Fig. 2F). *Crista dentata* bearing 29 and 26 teeth on right and left ischium respectively. Merus, dorsal surface sparsely covered with simple setae. Exopod longer than ischium, with flagellum reaching proximal margin of merus (Fig. 2F, G).

First pair of pereopods (chelipeds): large and subequal, laterally flattened (RPrT 25.6% of RPrL; LPrT 25.1% of LPrL) (Fig. 1A). Ischium ventral surface with 14 tubercles. Merus: right merus (RML) 53.5% of propodus length (RPrL); left merus (LML) 50.9% of propodus length (LPrL); ventral surface with two longitudinal series of tubercles: inner series with 17 tubercles, outer 16 and mesial 26, arranged irregularly on right merus; inner series bearing 17 tubercles, outer 16 and mesial 30, arranged irregularly on left merus. Dorsal and midventral spines present. Carpus with dorsomedial surface divided longitudinally by shallow groove (Fig. 1A; Fig. 2I). Internal dorsolateral margin with row of tubercles, increasing in size distally; inner surface with 20 small mesial tubercles. Carpal spine absent (Fig. 2I). Propodus width (RPrW and LPrW) 46% of length in right cheliped and 43.8% in left cheliped. Dorsal surface of palm with three rows of verrucous tubercles (Fig. 2H, I). Inner margin without tubercles. Ventral surface bearing two rows of squamose tubercles, trespassing the beginning of the fixed finger (Fig. 2H). Dactylus: moving subvertically, right dactylus (RDL) 62.8% of propodus length (RPrL), left dactylus (LDL) 60.2% of left propodus (LPrL); dorsal surface with squamose tubercles in the proximal portion (Fig. 4I). Cutting edge of fingers visible. Fixed finger with eleven teeth, third and fourth teeth largest. Dactylus with 14 teeth, third tooth largest (Fig. 2H, I).

Second pair of pereopods: ventral and dorsal surface of carpus, propodus and dactylus with sparse cover of simple long setae (Fig. 2J).

Gonopores: presence of both genital apertures on coxae of third and fifth pairs of pereopods. Female gonopores semi-ellipsoidal (maximum diameter 1.56 mm) with well-calcified membrane. Male gonopores rounded, opening onto apical end of a small, fixed, calcified and truncated phallic papilla, close to inner border of ventral surface of coxae of fifth pair of pereopods. Male cuticle partition present (Fig. 4B).

Branchial count: 20 + epr + r. Branchial arrangement

as described by Huxley (1879) and Hobbs (1991), with the epipod of the first maxilliped with rudimentary podobranchial filaments.

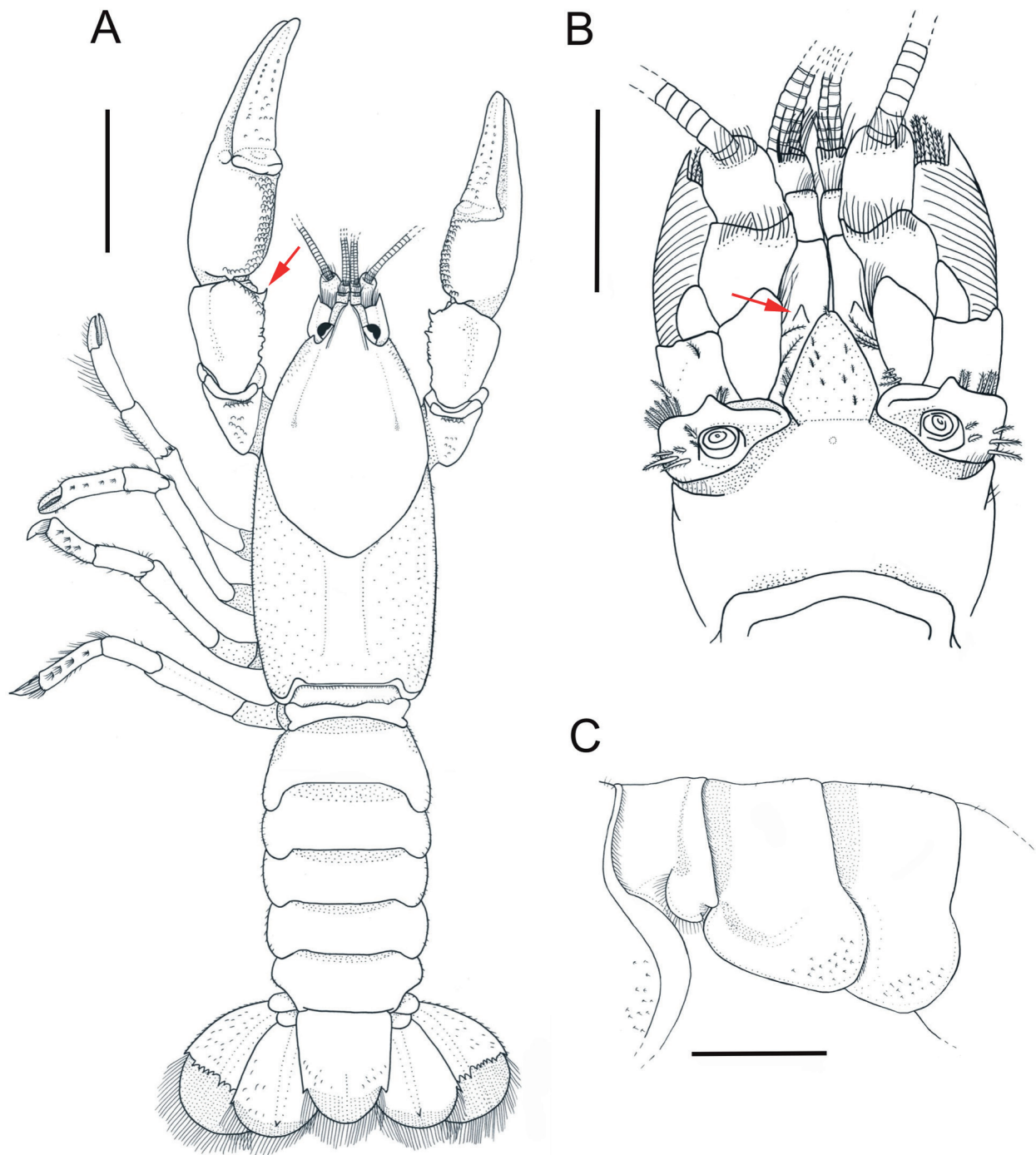
*Description of the female paratype:* Differs from the holotype in the following morphological characters: rostrum less sharp at apex, RW 81.9% of RL (Fig. 3A). Post orbital carinae shorter (RCL 65.8% of POCL) (Fig. 3A). Areola 2.4x as long as wide, constituting 27% of CL (Fig. 3A). S2 pleurae high and long (Fig. 3C). Transverse suture (diaresis) with seven dorsolateral spines (outer) and five dorsolateral spines (inner) on right exopod and five dorsolateral spines (outer) and six dorsolateral spines (inner) on left exopod. Anteromedian lobe of epistome 1.1x longer than wide. Internal ventral border of basal article of antennule with a sharp spine (Fig. 3B). Antennal flagellum reaching S2. *Crista dentata* bearing 24 and 28 teeth on the right and left ischium, respectively. Chelipeds shorter than in male. Merus of chelipeds with up to two spines in the midventral region. Carpal spine present in both chelipeds, right cheliped bears two spines (Fig. 3A). Female gonopores ellipsoidal (maximum diameter 1.21 mm) covered by a thin and non-calcified membrane.

*Measurements.* Holotype male, CL 33.52 mm and TL 66.81 mm. Paratype female, CL 26.45 mm and TL 54.93 mm. In type series, CL ranging from 18.72 to 33.52 mm ( $26.83 \pm 6.16$  mm). FW/CW:  $0.4 \pm 0.02$  (min: 0.38; max: 0.43). RL/RW:  $1.14 \pm 0.05$  (min: 1.08; max: 1.19). MCW/OW:  $0.6 \pm 0.1$  (min: 0.51; max: 0.72). Postorbital carina longer than rostral carina in all specimens analyzed. CW/AW:  $1.16 \pm 0.09$  (min: 1.08; max: 1.29). AreW/RW:  $0.93 \pm 0.05$  (min: 0.89; max: 1.01).

*Color of living specimens.* Rostrum reddish brown. Cephalothorax anterior and lateral regions greenish brown to reddish brown. First pair of pereopods reddish brown with dark reddish brown fingers. Pereiopod pairs 2–5 light brown to reddish brown. Dorsal abdomen light brown to dark reddish brown. Tailfan light brown to reddish brown (Fig. 4E–G).

*Remarks.* All paratypes present both masculine and feminine gonopores in the same individual. Male paratypes also present female gonopores semi-





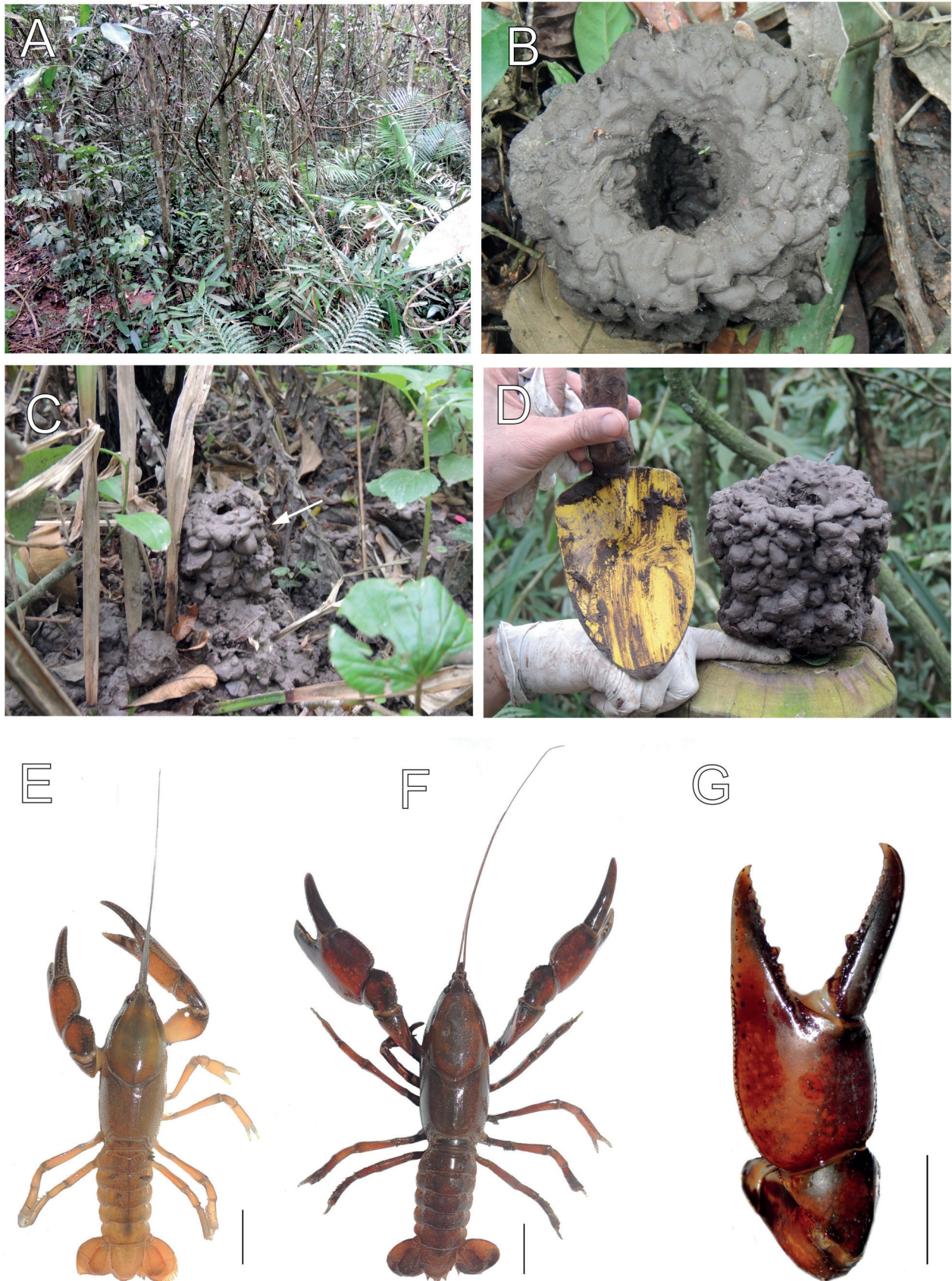
**Figure 3.** *Parastacus tuerkayi* sp. nov., female paratype (UFRGS 6376). A, habitus, dorsal view; B, epistome; C, first and second abdominal pleura. Scale bars: A = 1 cm; B, C = 5 mm. Red arrows indicate the spine on the carpus and on the internal ventral border of basal article of antennule respectively in A and B.

ellipsoidal (average maximum diameter 1.18 mm) covered by a calcified membrane. Male gonopores are very similar in male and female paratypes.

*Parastacus tuerkayi* sp. nov. is morphologically similar to *P. caeruleodactylus*, *P. defossus*, *P. nicoleti* and *P. pugnax* in having the post orbital carinae weakly prominent, the areola narrow and barely discernible

and the abdomen narrower than the cephalothorax. *Parastacus tuerkayi* sp. nov. is also similar to *P. nicoleti* in having the dorsal surface of dactylus with tubercles in the proximal portion. *Parastacus tuerkayi* sp. nov. differs from all other *Parastacus* species in having three well defined lines of verrucous tubercles in the dorsomesial margin of the palm of chelipeds and the





**Figure 4.** *Parastacus tuerkayi* sp. nov., habitat and living specimens. A, Typical habitat, a swamp forest; B, Opened chimney superior view; C and D, opened chimney lateral view, white arrow indicates the chimney; E and F, living specimens, habitus dorsal view UFRGS 6438 and holotype, respectively); G, living specimen (holotype), cheliped lateral view. Scale bars: E, F, G = 10 mm.

internal ventral border of basal article of antennules without a sharp spine.

**Phylogenetic position.** The phylogenetic relationships based on 512bp of the 16S rRNA gene provide clear evidence for the separation of *P. tuerkayi* sp. nov. from other species of the genus *Parastacus* with high values of posterior probability (Fig. 6). Genetic distances estimated between *P. tuerkayi* sp. nov. and other *Parastacus* species range from 6.2% (*P. defossus*) to 13.1% (*P. nicoleti*) for the 16S gene (Tab. 2). Intraspecific genetic distance was not more than 0.03%.

**Habitat and ecology.** *Parastacus tuerkayi* sp. nov. was collected in a small fragment (approximately 500 m<sup>2</sup>) of a swamp forest located inside the theme park “Beto Carreiro World” in the coastal region of the state of Santa Catarina. This physiographic region belongs to the Atlantic Forest Biome and the vegetation is composed predominantly by Myrtaceae, Poaceae, Piperaceae (genus *Piper*) and some pteridophyta of the family Blechnaceae (genus *Blechnum*) (P. Brack pers. comm.). Soil is mainly composed by clay and temporarily flooded with a large amount of organic matter derived from leaf decomposition (F. B. Ribeiro pers. obs.). Found in a flooded area, burrows of *P. tuerkayi* sp. nov. can be identified as type 2 according to Horwitz and Richardson’s (1986) classification.

Based on Hobbs’ (1942) classification, *P. tuerkayi* sp. nov. can be considered a primary burrower, in which the individuals spend almost their entire life underground and build deep and relatively complex burrows.

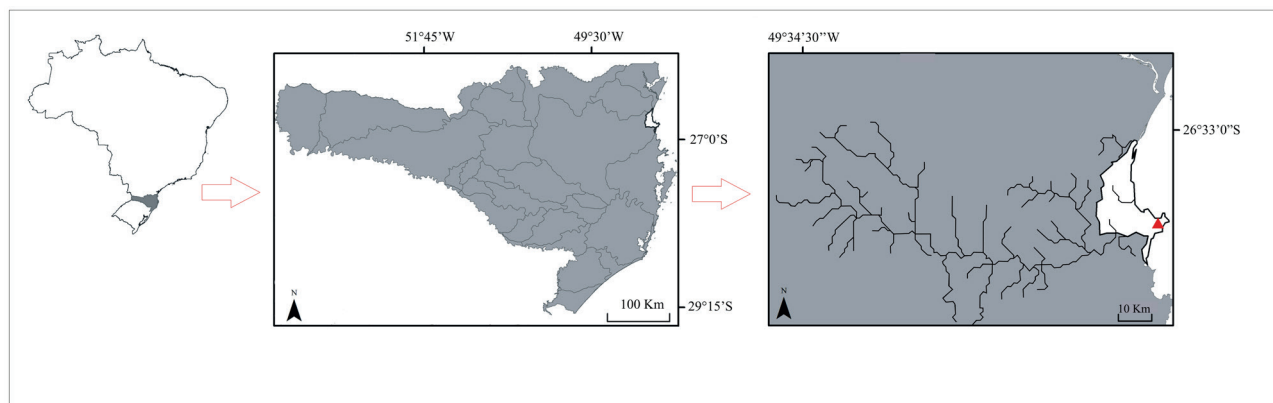
Burrows can reach a depth of up to one meter, but with few branches and with long (up to 15 cm) and large (up to 12 cm) chimneys.

This burrow structure is very similar to the one of *P. caeruleodactylus* that is also found in swamp forests in the state of Rio Grande do Sul, near the foothills of the Serra Geral mountains and in the coastal region, and *P. pugnax*, found in small valleys or depressions between mountains or topographic depressions, usually associated with perennial forests in Chile (Rudolph, 2013; Ribeiro et al., 2016). *Parastacus tuerkayi* sp. nov. is ecologically similar to *P. pugnax*, *P. caeruleodactylus*, *P. defossus* and *P. nicoleti*. These species share some morphological adaptations to the burrowing life style, as the narrow areola, which is indicative of one extended branchial chamber; carapace, abdomen and appendages covered by setae in some regions, reduced eyes and the abdomen narrower than the cephalothorax (Horwitz and Richardson 1986; Richardson, 2007).

Regarding reproductive biology, the ovigerous female (paratype UFRGS 6376) bears approximately 20 eggs (average maximum diameter 2.4 mm) attached to its pleopods. The low fecundity is also a characteristic shared by strong burrowing species (Richardson, 2007).

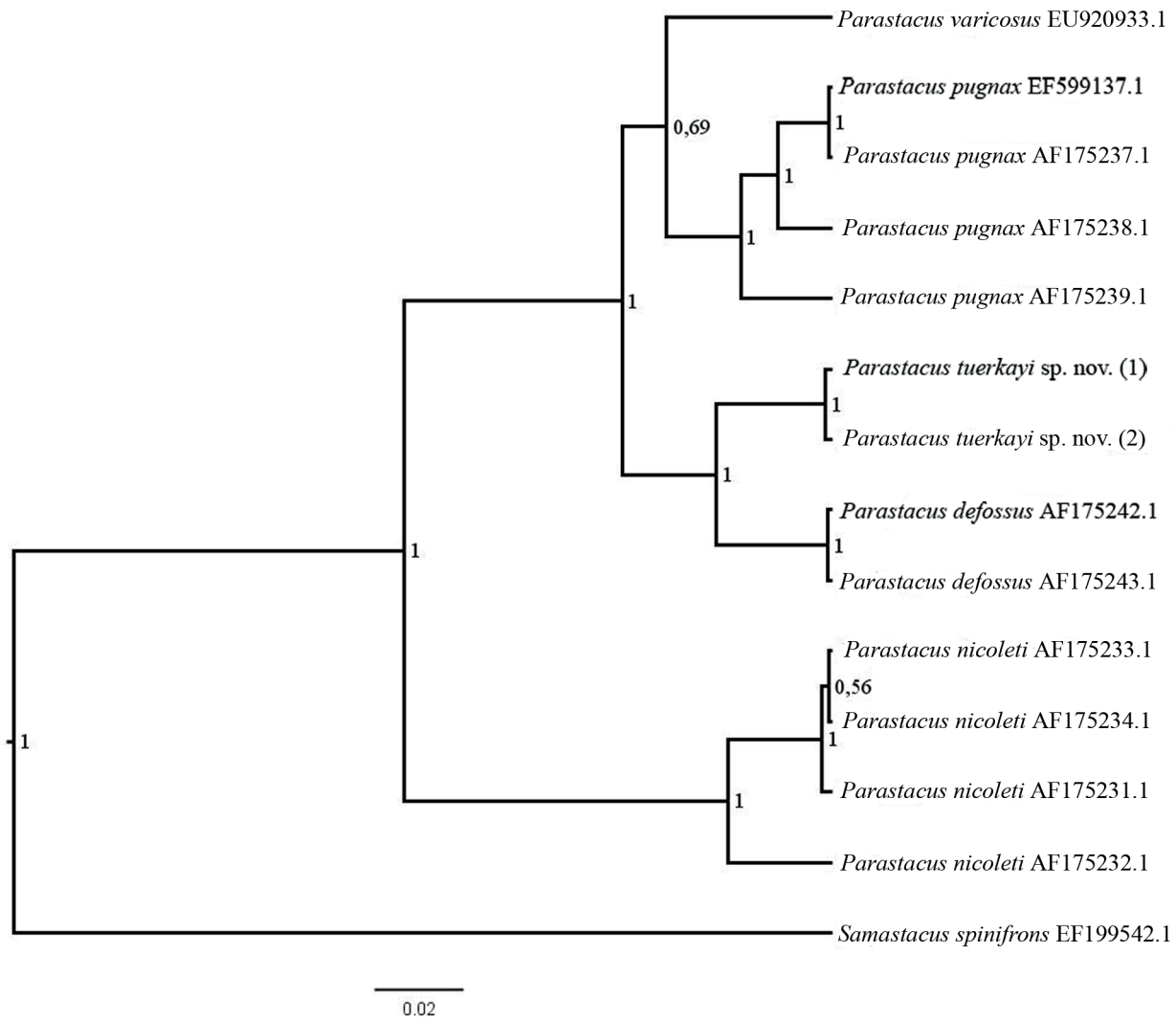
**Distribution.** *Parastacus tuerkayi* sp. nov. appears to have an extremely limited distribution, being found only in the municipality of Penha, state of Santa Catarina, southern Brazil (Fig. 5).

**Conservation status.** The EOO was estimated as comprising approximately 647.674 km<sup>2</sup> based on the



**Figure 5.** Distribution of *Parastacus tuerkayi* sp. nov. in the state of Santa Catarina, southern Brazil. The type locality is represented by a red triangle.





**Figure 6.** Bayesian inference estimate of phylogenetic relationships among selected species of South American freshwater crayfish based on 16S rRNA gene data from the mitochondrial genome. *Parastacus tuerkayi* sp. nov. (1) and (2) are respectively paratype (UFRGS 6438) and holotype.

**Table 2.** Genetic divergence matrix (p-distances) of the 16 S mitochondrial gene among *Parastacus tuerkayi* sp. nov. and other selected species of the genus *Parastacus* (*P. defossus*, *P. nicoleti*, *P. pugnax* and *P. varicosus*) and *Samastacus spinifrons*.

	1	2	3	4	5
1. <i>Parastacus tuerkayi</i> sp. nov.					
2. <i>Parastacus defossus</i>	0.062				
3. <i>Parastacus nicoleti</i>	0.131	0.119			
4. <i>Parastacus pugnax</i>	0.088	0.064	0.119		
5. <i>Parastacus varicosus</i>	0.087	0.080	0.104	0.063	
6. <i>Samastacus spinifrons</i>	0.243	0.231	0.221	0.236	0.231

Otto Bacia shape level 4 (ANA, 2006), indicating that this species can be included in the Endangered – EN category, in which the EOO is less than 5,000 km<sup>2</sup> (IUCN, 2012). The species is categorized as EN under subitem “a”: for an EOO, which is severely

fragmented; and subitem “b” (iii): continuing decline in quality of habitat. Both subitems are appropriate, due to the threats existing in the species occurrence area. Urbanization may be the main cause of habitat loss and fragmentation, since *P. tuerkayi* sp. nov. was

found inside a theme park in a small fragment of a swamp forest (approximately 500 m<sup>2</sup>). In addition, this region of the state of Santa Catarina is a target of intense urban real estate speculation and tourism. We therefore suggest that the conservation status of this species be classified as ENDANGERED B1ab(iii).

## ACKNOWLEDGMENTS

This study is part of the Doctorate thesis of F.B. Ribeiro in the Post-Graduation Program in Animal Biology at the Universidade Federal do Rio Grande do Sul. The authors would like to thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) that provided a Doctorate Scholarship to F.B. Ribeiro; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) that supplied a Productivity Research Scholarship to P. B. Araujo (PQ 305900/2014-5); and Programa de Pós Graduação em Biologia Animal – Universidade Federal do Rio Grande do Sul that provided additional support to sampling. The authors also would like to thank Professor Dr. Paulo Brack (UFRGS) for plant identification, Kelly M. Gomes for the help in sampling and conservation analysis, Dr. Ivana Miranda for the help in laboratory genetic procedures and the anonymous reviewers for their suggestions. All sampled specimens were collected according to the Brazilian laws (SISBIO license number 45759-2).

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