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

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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 km², 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.
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 Zoolgia da Universidade de Sao Paulo (MZUSP), Sao Paulo, Brazil, and in the Carcinological Collection of the Departamento de Zoolgia, Instituto de Biociencias, 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 Table 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 (Qiaegen). 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, 19/1/1977 (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. *Paratascus nicoleti* – 1 male, Mehuim (next to Valdivia), VIII/1997, leg. niños del Pueblo.


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. |
|----------------------|----------------|----------------|----------------|----------------|
| Sex                  | 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                   | 18.92 | 13.98 | 13.58 | 14.52 |
| Cel                  | 22.58 | 18.09 | 18.51 | 18.78 |
| RL                   | 5.09  | 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                   | 26.26 | 22.58 | 24.42 | 22.28 |
| RP T                 | 7.20  | 3.57  | 6.75  | 5.42  |
| RP L                 | 28.14 | 15.87 | 23.49 | 29.30 |
| RP W                 | 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 |
| LP T                 | 7.36  | 4.82  | 3.92  | 5.46  |
| LP L                 | 29.31 | 18.35 | 15.94 | 20.63 |
| LP W                 | 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  |

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°, unarm (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).

Tailsfan: 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 unarm 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 unarm (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 pereiopod, lateral view (holotype); I, first pereiopod, dorsal view (holotype); J, second pereiopod, 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.
New species of Parastacus (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 pereiopods (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 pereiopods: 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 pereiopods. 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 pereiopods. Male cuticle partition present (Fig. 4B).

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

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Gonopores: presence of both genital apertures on coxae of third and fifth pairs of pereiopods. 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 pereiopods. 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 pleura high and long (Fig. 3C). Transverse suture (diuresis) 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 antenule 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 ± 6.16 mm). FW/CW: 0.4 ± 0.02 (min: 0.38; max: 0.43). RL/RW: 1.14 ± 0.05 (min: 1.08; max: 1.19). MCW/OW: 0.6 ± 0.1 (min: 0.51; max: 0.72). Postorbital carina longer than rostral carina in all specimens analyzed. CW/AW: 1.16 ± 0.09 (min: 1.08; max: 1.29). AreW/RW: 0.93 ± 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 pereiopods 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 antenulle 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 m²) 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 pterydophyta 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 km² based on the

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**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*.

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<tr>
<td>1. <em>P. tuerkayi</em> sp. nov.</td>
<td>0.062</td>
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<td>2. <em>P. defossus</em></td>
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<td>3. <em>P. nicoleti</em></td>
<td>0.131</td>
<td>0.119</td>
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<td>4. <em>P. pugnax</em></td>
<td>0.088</td>
<td>0.064</td>
<td>0.119</td>
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<tr>
<td>5. <em>P. varicosus</em></td>
<td>0.087</td>
<td>0.080</td>
<td>0.104</td>
<td>0.063</td>
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<tr>
<td>6. <em>S. spinifrons</em></td>
<td>0.243</td>
<td>0.231</td>
<td>0.221</td>
<td>0.236</td>
<td>0.231</td>
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</table>

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 km² (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 m²). 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).

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REFERENCES


