FELIPE BEZERRA RIBEIRO

Taxonomia e relações filogenéticas dos lagostins de água doce do gênero *Parastacus* Huxley, 1879 (Crustacea, Decapoda, Parastacidae)

Tese apresentada ao Programa de Pós-Graduação em Biologia Animal, Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Doutor em Biologia Animal.

Área de Concentração: Biologia Comparada

Orientadora: Profa. Dra. Paula Beatriz de Araujo

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Taxonomia e relações filogenéticas dos lagostins de água doce do gênero *Parastacus* Huxley, 1879 (Crustacea, Decapoda, Párastacidae)

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Aprovada em _____ de __________________de 2017.

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Professora Dra. Georgina Bond-Buckup – Universidade Federal do Rio Grande do Sul

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Professor Dr. Fernando Mantelatto – Universidade de São Paulo

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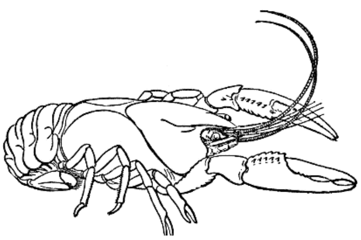
Professora Dra. Jocélia Grazia – Universidade Federal do Rio Grande do Sul

Aos lagostins de água doce, minha família, minha orientadora e meus amigos queridos ...



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“There is grandeur in this view of life, with its several powers, having been originally breathed into a few forms or into one; and that, whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved.”

Charles Darwin

“(…) Once there was a child`s dream
One night the clock struck twelve
The window open wide
Once there was a child`s heart
The age I learned to fly
And took a step outside

Fly to a dream far across the sea

All the burdens gone (…)”

Tuomas Holopainen (Dark Chest of Wonders
- Nightwish

Sumário

Prefácio

Os lagostins de água doce constituem um grupo impar entre os crustáceos decápodos, sendo amplamente distribuídos pelo mundo, em especial no sul da América do Sul. O gênero *Parastacus*, até então com apenas oito espécies, sempre foi um grupo subestimado no quesito de riqueza específica. O aumento do conhecimento acerca da diversidade e evolução do grupo é o grande objetivo desta tese. Ao longo deste trabalho foi possível explorar ferramentas morfológicas e moleculares, assim como a análise de diversos espécimes depositados em várias coleções do mundo e também oriundos de expedições de campo.

A tese inicia com uma introdução geral, onde são abordadas aspectos gerais de classificação diversidade e bioecologia dos lagostins de água doce, além de diversidade críptica, filogenia e taxonomia molecular. Posteriormente, esta tese está estruturada em quatro capítulos que correspondem a artigos desenvolvidos de forma relacionada, abordando taxonomia e sistemática filogenética usando dados moleculares e morfológicos.

O capítulo I - **Two new species of South American crayfish genus *Parastacus* Huxley, 1879 (Crustacea, Decapoda. Parastacidae) [Duas novas espécies de lagostins sulamericanos do gênero *Parastacus* Huxley, 1879 (Crustacea, Decapoda. Parastacidae)]** aborda a descrição de duas novas espécies para o gênero *Parastacus*, incluindo novos caracteres morfológicos e morfométricos nas descrições, além de uma descrição do habitat, análise de distribuição e avaliação do estado de conservação.

O capítulo II - **A new species of *Parastacus* Huxley, 1879 (Crustacea, Decapoda, Parastacidae) from a swamp forest in southern Brazil [Uma nova espécie de *Parastacus* Huxley, 1879 (Crustacea, Decapoda, Parastacidae) de uma mata paludosa no sul do Brasil]** é referente à descrição de uma nova espécie para o gênero *Parastacus*, incluindo uma análise molecular com um gene mitocondrial, além de uma descrição do habitat, análise de distribuição e avaliação do estado de conservação.

No capítulo III - **A molecular phylogenetic investigation of South American freshwater crayfish (Crustacea: Decapoda: Parastacidae) with emphasis on**

***Parastacus* [Uma investigação filogenética molecular dos lagotins de água doce da América do Sul (Crustacea: Decapoda: Parastacidae) com ênfase em *Parastacus*],** são investigadas a monofilia e as relações filogenéticas do gênero *Parastacus* e dos dois outros gêneros sulamericanos (*Samastacus* e *Virilastacus*). Além disso, novas espécies são identificadas sob o ponto de vista molecular e um novo gênero também é proposto.

Por fim, no capítulo IV - **Taxonomic review of the genus *Parastacus* Huxley, 1879 (Crustacea: Decapoda: Astacidea: Parastacidae) [Revisão Taxonômica do gênero *Parastacus* Huxley, 1879 (Crustacea: Decapoda: Astacidea: Parastacidae)]**, a taxonomia das espécies já descrita para o gênero *Parastacus* é revisada, sendo todas as espécies redescritas. Além disso, um gênero novo é proposto e dez novas espécies são descritas. Mapas de distribuição são atualizados e uma nova chave de identificação é proposta para os lagostins sulamericanos.

Resumo

Os lagostins de água doce sulamericanos (Família Parastacidae) são representados por três gêneros: *Parastacus* Huxley, 1879, *Samastacus* Riek, 1971 and *Virilastacus* Hobbs, 1991. Esse grupo é distribuído no Sul do Brasil (Rio Grande do Sul and Santa Catarina), Uruguai, Argentina e Chile. Os objetivos dessa tese foram revisar a taxonomia do gênero *Parastacus* e investigar a sua monofilia e relações filogenéticas entre suas espécies e entre os outros gêneros sulamericanos. Para esse propósito, várias coleções e museus ao redor do mundo foram analisados (Brasil, Argentina, Uruguai, Chile, Estados Unidos, Alemanha, Holanda, Inglaterra e França) em adição a coletas realizadas entre Março de 2013 a Setembro de 2016. Os espécimes foram analisados em estereomicroscópio e os desenhos foram preparados com auxílio de *camara lucida*. Para as análises genéticas, uma abordagem multigênica foi utilizada com dois marcadores mitocondriais (Cox1 e 16S) e um nuclear (28S). A análise filogenética foi realizada por Inferência Bayesiana e a distância genética p também foi calculada. Adicionalmente, o risco de extinção foi assessado para algumas espécies de acordo com o sub-critério B1 da IUCN que leva em consideração a estimativa da Área de Extenção de Ocorrência. Oito espécies foram redescritas: *Parastacus brasiliensis* (von Martens, 1869), *P. defossus* Faxon, 1898, *P. laevigatus* Buckup & Rossi, 1980, *P. pilimanus* (Von Martens, 1869), *P. pugnax* (Poepigg, 1835), *P. promatensis* Fontoura & Conter, 2008 e *P. varicosus* Faxon, 1898. Um novo gênero foi proposto para alocar a espécie *P. nicoleti* (Philippi, 1882) que também foi redescrita. Treze novas espécies foram descritas. Assim como, chaves de identificação, descrições, diagnoses, sinonímias e mapas de distribuição foram providos. As árvores filogenéticas resultantes corroboraram com a monofilia de *Parastacus* e do clado sulamericano, além de dar suporte para o estabelecimento de um novo gênero e novas espécies. Com essa tese, o aumento da riqueza específica para *Parastacus* é de mais de 150% e as novas informações sobre habitat e distribuição trarão suporte para futuros estudos de conservação e manejo.

Palavras-chave: lagostins escavadores, Parastacídeos, Taxonomia, Filogenia Molecular

Abstract

South American freshwater crayfish (Family Parastacidae) are represented by three genera: *Parastacus* Huxley, 1879, *Samastacus* Riek, 1971 and *Virilastacus* Hobbs, 1991. This group is distributed in Southern Brazil (Rio Grande do Sul and Santa Catarina), Uruguay, Argentina and Chile. The goals of this thesis is to review the taxonomy of the genus *Parastacus* and to investigate the monophyly and phylogenetic relationships within *Parastacus* and among South American crayfish genera. For this purpose, several collections and museums around the world were analyzed (Brazil, Argentina, Uruguay, Chile, United States of America, Germany, Netherlands, England, and France) in addition to collectings carried out from March 2013 to September 2016. Specimens were analyzed under a stereomicroscope and drawings were prepared with the aid of a *camara lucida*. For genetic analysis, a multigenic approach was used with two mitochondrial (Cox1 and 16S) and one nuclear (28S) markers. Phylogenetic analysis were performed with Bayesian Inference and genetic *p*-distances were also calculated. In addition, the extinction risk was assessed according to the sub-criterion B1 of IUCN that estimates the Extent of Occurrence (EOO) for some species. Eight species are redescribed: *Parastacus brasiliensis* (von Martens, 1869), *P. defossus* Faxon, 1898, *P. laevigatus* Buckup & Rossi, 1980, *P. pilimanus* (Von Martens, 1869), *P. pugnax* (Poepigg, 1835), *P. promatensis* Fontoura & Conter, 2008 and *P. varicosus* Faxon, 1898. A new genus is proposed to encompass the species *P. nicoleti* (Philippi, 1882) and this species is also redescribed. In addition, 13 new species are described. Identification keys, descriptions, diagnoses, synonymies and distribution maps of the genera and species are provided. Phylogenetic trees corroborated the monophyly of *Parastacus* and the South American crayfish clade, and give support for the establishment of a new genus and new species. With this thesis, the increase in specific richness for *Parastacus* is more than 150% and new information about habitat and distribution will bring support for future conservation and management studies.

Palavras-chave: burrowing crayfish, Parastacids, Taxonomy, Molecular Phylogeny

Introdução geral

Classificação e diversidade

Os lagostins de água doce ("Astacida") são um grupo diverso de crustáceos decápodos (~640 espécies), pertencentes à infraordem Astacidea Latreille, 1802, constituindo um grande clado monofilético (SCHOLTZ & RICHTER, 1995; RODE & BABCOCK, 2003; BRACKEN et al., 2009). Atualmente, os lagostins são divididos em duas superfamílias. A superfamília Astacoidea Latreille, 1802, distribuída no hemisfério norte, é constituída pelas famílias Cambaridae Hobbs, 1842 e Astacidae Latreille, 1802. A superfamília Parastacoidea Huxley, 1879 é distribuída no hemisfério sul, sendo constituída apenas pela família Parastacidae Huxley, 1879 (HOLDICH, 2002; SINCLAIR et al., 2004; CRANDALL & BUHAY, 2008; BRACKEN *et al.*, 2009) (Figura 1). A família Parastacidae inclui 15 gêneros (~170 espécies), das quais 11 são encontradas na Austrália, Nova Guiné e Nova Zelândia, três na América do Sul e um em Madagascar (CRANDALL & BUHAY, 2008; TOON *et al.*, 2010; RIBEIRO *et al.*, 2016; 2017).

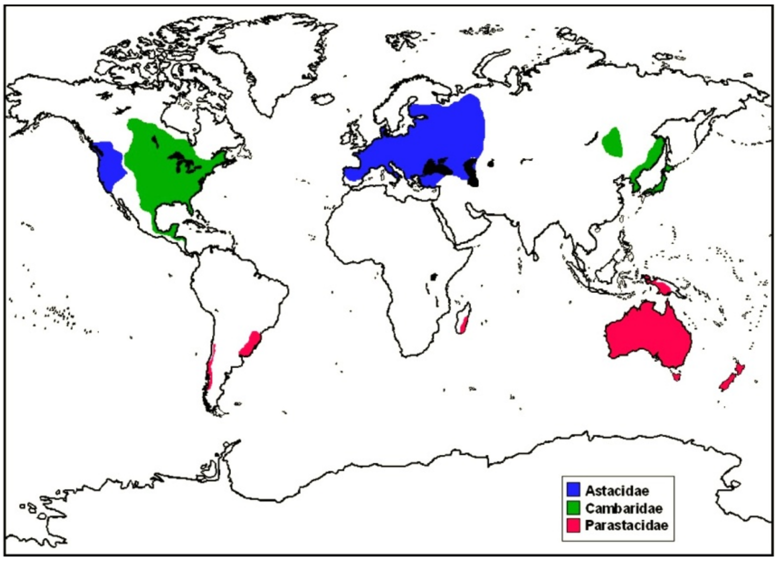

Figura 1. Distribuição mundial das famílias de lagostins de água doce. Modificado de HOBBS (1988).

As espécies nativas da América do Sul pertencem a três gêneros: *Parastacus* Huxley, 1879 (12 espécies); *Samastacus* Riek, 1971 (uma espécie); e *Virilastacus* Hobbs, 1991 (quatro espécies) (BUCKUP & ROSSI, 1980; 1993; RUDOLPH & CRANDALL, 2005; 2007; 2012; RIBEIRO *et al*., 2016; 2017). Essas espécies são distribuídas no sul do Brasil, Uruguai, centro-sul do Chile e sul e nordeste da Argentina formam um grupo monofilético estabelecido a ~85 milhões de anos (CRANDALL et al., 2000b; TOON *et al.*, 2010) (Figura 2). Fósseis oriundos da Patagônia central foram identificados como astacídeos que ocorreram do Jurássico Superior ao Cretáceo Superior, sugerindo que durante este período as espécies se distribuíram ao longo do sul da América do Sul (TOON *et al.*, 2010). Supostamente, este padrão de distribuição tem sido modelado por transgressões que ocorreram do período Cretáceo até a metade do período Paleógeno (COLLINS *et al.*, 2011).

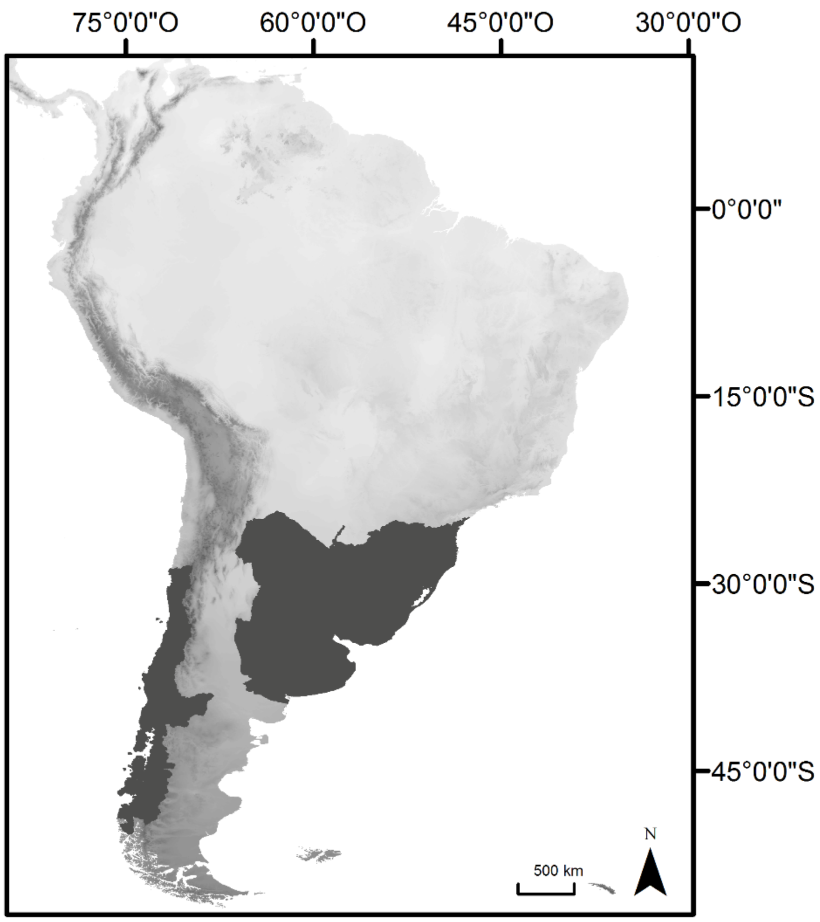

Figura 2. Distribuição geográfica dos gêneros de lagostins sulamericanos representada pela área em negrito.

Taxonomia molecular e diversidade críptica

A conservação dos ecossistemas aquáticos depende primariamente do reconhecimento da biodiversidade. A taxonomia molecular surgiu nas últimas décadas como uma ferramenta importante para aumentar a rapidez da descrição das espécies. Os métodos empregados permitem identificar espécies crípticas que podem não ser totalmente evidentes com a utilização apenas de caracteres morfológicos (HEBERT *et al.*, 2003; BICKFORD *et al.*, 2007; BURNHAM & DAWKINS, 2013).

As espécies crípticas ou complexo de espécies são definidas quando duas ou mais espécies distintas são classificadas como uma única espécie nominal devido ao fato de serem superficialmente morfologicamente pouco distinguíveis (BICKFORD *et al.*, 2007). A descoberta de espécies crípticas é provável que não seja aleatória em relação a taxon e bioma e, portanto, poderia ter profundas implicações para a evolução, biogeografia e planejamento de conservação (BICKFORD *et al.*, 2007). A presença de espécies crípticas já foi documentada em diversos gêneros de lagostins de água doce (MATHEWS *et al.*, 2008; SINCLAIR *et al.*, 2011; HELMS *et al.*, 2015; LARSON *et al.*, 2016). Para a parastacídeos da América do sul, o uso de ferramentas moleculares na descrição de espécies vem sendo adotado frequentemente nos últimos anos (RUDOLPH & CRANDALL, 2005; 2007; 2012; RIBEIRO *et al.* 2017) e a presença de espécies crípticas já vem sendo identificada (I. Miranda *comunicação pessoal*).

Filogenia

Os primeiros estudos que abordaram a história evolutiva dos lagostins de água doce foram desenvolvidos por Huxley (1880) que, através de morfologia comparativa, estabeleceu uma classificação dos gêneros em categorias supragenéricas baseadas na estrutura das brânquias e na ornamentação da carapaça.

Posteriormente ao trabalho de Huxley, os estudos evolutivos dos lagostins receberam pouca atenção até o século posterior com os trabalhos de HOBBS (1942; 1974; 1988) e RIEK (1969, 1971, 1972). Esses trabalhos foram baseados em inferências das relações taxonômicas e a morfologia. Hobbs deteve-se aos lagostins do hemisfério Norte (Cambaridae e Astacidae), enquanto Riek ao hemisfério Sul (Parastacidae).

HOBBS (1988) classificou os lagostins com base principalmente na morfologia dos apêndices e no arranjo branquial. Outros caracteres externos como o formato do rostro e a morfologia dos quelípodos também foram considerados, mas com um menor valor taxonômico. RIEK (1972), com base em apenas 13 caracteres morfológicos, entre eles os atributos referentes aos níveis de desenvolvimento dos sulcos cefalotoráxicos, à orientação da movimentação dos dáctilos do 1° par de pereiópodos (quelípodos) e as modificações sexuais secundários dos machos, inferiu as relações filogenéticas entre os gêneros da família Parastacidae.

Outras análises filogenéticas morfológicas mais recentes realizadas por ALBRECHT (1982), SCHOLTZ (1995, 1998, 1999, 2002) e SCHOLTZ & RICHTER (1995) foram baseadas em caracteres embrionários, de juvenis e de adultos para tentar estabelecer as afinidades filogenéticas dos lagostins com outros táxons e estabelecer a possível monofilia do grupo. A análise cladística de SCHOLTZ & RICHTER (1995) da ordem Decapoda proveu um suporte para a separação das lagostas queladas da superfamília Nephropoidea (“Homarida”) dos lagostins de água doce (Astacoidea e Parastacoidea/ “Astacida”) e determinou que os lagostins formariam um clado junto com as infraordens Brachyura, Anomura e Thalassinida ou que seriam um ramo que emergiria dos Thalassinida.

Outros trabalhos adicionais e relevantes para a compreensão da filogenia dos lagostins inclui estudos de teloblastos (SCHOLTZ, 1993) e ultra-estrutura do esperma (JAMIESON, 1991); e RNAr 18S (KIM & ABELE, 1990) forneceram evidências para a origem monofilética dos lagostins de água doce. Os estudos com marcadores moleculares se tornaram comuns a partir das décadas de 1980 e 1990 e então muitos estudos com vários marcadores foram publicados, abordando variações cromossômicas, de alozimas e de nucleotídeos (ALBRECT & VON HAGEN, 1981; PATAK *et al.*, 1989; AUSTIN, 1995a,b; CRANDALL & FITZPATRICK, 1996; CRANDALL & CRONIN, 1997; LAWLER & CRANDALL, 1998; CRANDALL *et al.*, 2000a; CRANDALL *et al.*, 2000b; FETZNER & CRANDALL, 2002).

Dentro da família Parastacidae, uma série de filogenias baseadas em evidências a partir de métodos moleculares têm sido formuladas. PATAK & BALDWIN (1984) e PATAK *et al*. (1989) estudaram marcadores eletroforéticos e imunoquímicos entre gêneros dessa família. AUSTIN (1995a, b), usou alozimas para reconstruir a filogenia de vários parastacídeos. Além disso, uma série de estudos (LAWLER & CRANDALL, 1998; PONNIAH & HUGHES, 1998; CRANDALL *et al*, 1999; CRANDALL *et al.*, 2000b) utilizaram DNA mitocondrial 16S para reconstruir a filogenia dos parastacídeos. CRANDALL *et al*. (2000a) ratifica a monofilia da família Parastacidae e também mostra que os gêneros sulamericanos formam um grupo monofilético, estreitamente relacionado com os gêneros *Paranephrops* White, 1842 e *Parastacoides* Clark, 1936 na Austrália. De acordo com TOON *et al*., (2010), a família Parastacidae se originou no início do Jurássico (183 Ma) e o clado sulamericano divergiu dos outros parastacídeos em torno 158 milhões de anos atrás, constituindo um grupo irmão para todos os outros lagostins do hemisfério sul.

A monofilia dos lagostins de água doce foi também avaliada por RODE & BABCOCK (2003), baseando-se em caracteres morfológicos externos da carapaça e dos apêndices. Em sua análise, foram incluídos grupos fósseis e a família de lagostas marinhas Nephropidae. As sinapomorfias de Astacida foram definidas como os padrões distintos de sulcos na região dorsal do cefalotórax e a mobilidade do último segmento toráxico. Em relação à família Parastacidae, esta foi considerada monofilética, com exceção do gênero *Gramastacus* Riek, 1972 e suas sinapomorfias compreendem a ausência total do primeiro par de pleópodos e pelo padrão diferencial de calcificação da porção distal do télson.

No entanto, não existem estudos mais aprofundados tanto do ponto de vista morfológico quanto molecular para lagostins sulamericanos, sendo necessário ainda estabelecer as relações filogenéticas entre as espécies do gênero *Parastacus* e deste gênero com os demais da família.

Habitat e hábitos escavadores

Uma característica conspícua na evolução dos lagostins de água doce é sua diversidade ecológica, sendo diretamente relacionada à irradiação nos ambientes dulcícolas. As espécies desenvolveram diferentes hábitos de vida, em grande parte subterrâneo de acordo com o habitat ocupado (HORWITZ & RICHARDSON, 1986).

As espécies do gênero *Parastacus* foram classificadas por RIEK (1972) na categoria ecológica de hábitos escavadores pronunciados, juntamente com os gêneros australianos *Engaeus* Erichson, 1846, *Engaewa* Riek, 1967 e *Tenuibranchiurus* Riek, 1951. Essa classificação foi baseada em caracteres morfológicos como os dáctilos dos quelípodos com movimentação vertical, tamanho corporal pequeno ou moderado, abdomen reduzido em largura e comprimento e sulco cervical em formato de V. Essas espécies podem ser encontradas em riachos, banhados, planícies de inundação, matas paludosas com solo permanentemente ou temporariamente inundado com grande quantidade de matéria orgânica (BUCKUP & ROSSI, 1980; RIBEIRO *et al.*, 2016; 2017). Mesmo que todas as espécies do gênero *Parastacus* tenham o potencial de desenvolver seus hábitos escavadores, elas podem ser classificadas de acordo com a maior ou menor extensão desses hábitos que estão diretamente relacionados com o habitat e refletidos na morfologia corporal (BUCKUP & ROSSI, 1980). No gênero *Parastacus*, as espécies podem construir sistemas complexos de galerias (~1m de profundidade) em áreas alagadas (p. ex., *P. defossus*) ou matas paludosas (p. ex. *P. caeruleodactylus*); outras constroem galerias similares ao longo de corpos d'água (p. ex. *P. brasiliensis* e *P. fluviatilis*) (BUCKUP & ROSSI, 1980; FONTOURA & BUCKUP, 1989; BUCKUP, 1999; NORO & BUCKUP, 2010; RIBEIRO *et al*., 2016). Além disso, são espécies de hábitos notívagos, podendo sair de suas habitações em busca de alimento no interior da água ou ambientes próximos, alimentando-se de matéria orgânica de origem animal e vegetal (FRIES, 1980). Mas mesmo assim, essas espécies são caracterizadas por uma baixa capacidade de dispersão (DALOSTO, 2012).

Existem duas principais classificações propostas para o nível escavador dos lagostins de água doce. A classificação de HOBBS (1942) leva em consideração a complexidade das tocas, a conexão com corpos d’água, estratégia reprodutiva e tempo que o lagostim permanece no nível subterrâneo. Dessa forma, os lagostins foram classificados em três níveis: (1) escavadores primários: passam a maior parte de sua vida no interior das tocas, as quais são profundas e complexas, podendo apresentar vários túneis; (2) escavadores secundários: passam a maior parte de sua vida no interior das tocas, as quais podem ser profundas e complexas; os adultos podendo permanecer na superfície durante as estações chuvosas; (3) escavadores terciários vivem nos corpos d’água durante a maior parte da sua vida, usando as tocas apenas como abrigo em épocas reprodutivas, proteção contra predadores e dissecação, construindo túneis simples. Já a classificação de HORWITZ & RICHARDSON (1986), leva em consideração a relação entre as tocas e a conexão com os corpos d’água e lençol freático (Figura 3). As tocas do **Tipo 1 (a e b)** ocorrem diretamente no interior dos corpos d'água ou estão diretamente conectados aos mesmos; as tocas do **Tipo 2** são conectados ao lençol freático; e as tocas do **Tipo 3** são independentes do lençol freático (Figura 3).

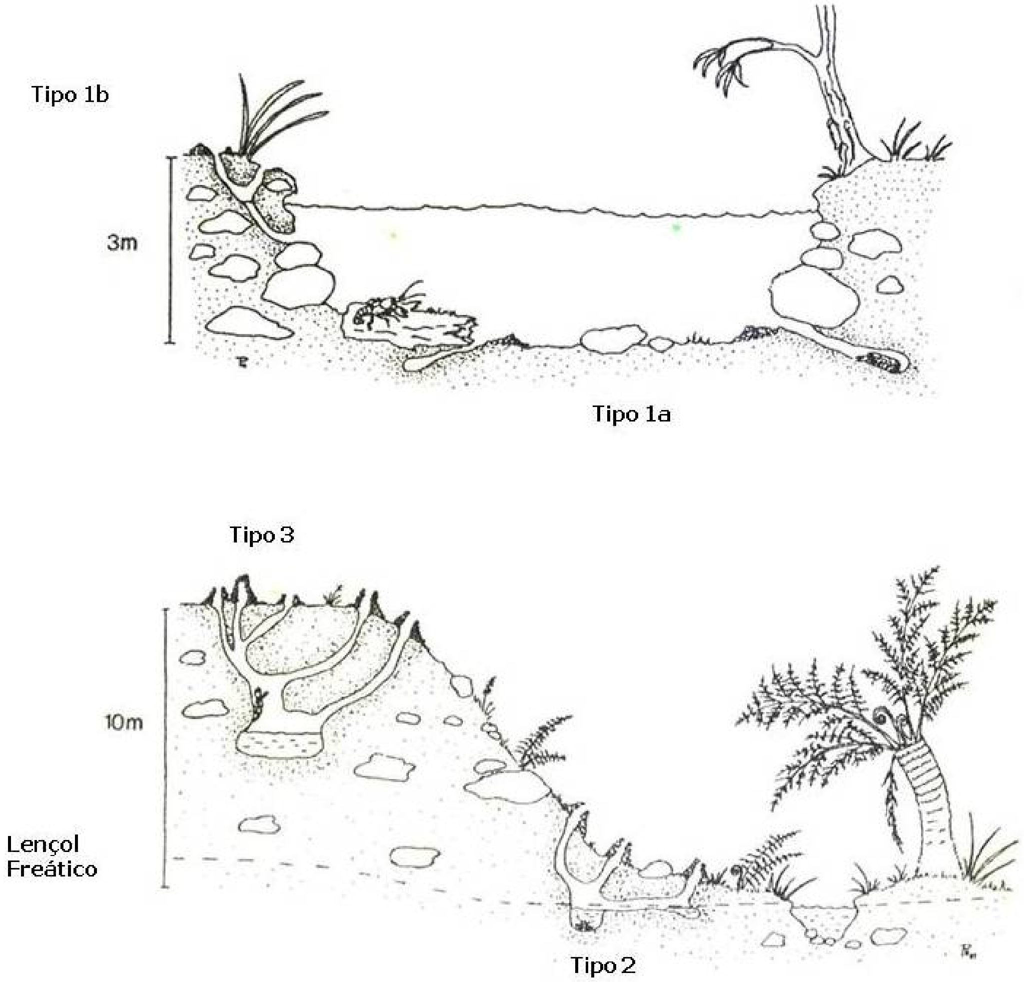

Figura 3. Classificação dos lagostins escavadores de acordo com HORWITZ & RICHARDSON (1988). Modificado de RICHARDSON (2007).

Biologia reprodutiva

As características reprodutivas do gênero *Parastacus* ainda são pobremente conhecidas e constituem um dos pontos mais controversos na biologia das espécies (RUDOLPH & ALMEIDA, 2000). Todas as espécies deste gênero apresentam a intersexualidade, ou seja, a presença de características sexuais primárias e/ou secundárias no mesmo indivíduo (SAGI *et al.*, 1996). Esse fenômeno também pode ser encontrado em outros parastacídeos, como os gêneros australianos *Cherax* Erichson, 1846, *Engaeus* Erichson, *Engaewa* (HORWITZ, 1988; VASQUÉZ & LÓPEZ-GRECO, 2007) e os sulamericanos *Samastacus* e *Virilastacus* Hobbs, 1991 (RUDOLPH, 1999; RUDOLPH & ALMEIDA, 2000, RETAMAL AND RUDOLPH, 2005). No gênero *Parastacus*, a intersexualidade é caracterizada pela presença de gonóporos supernumerários e dutos genitais no mesmo indivíduo (RUDOLPH & ALMEIDA, 2000). Tal fato é conhecido desde a metade do século XIX (VON MARTENS, 1869; FAXON, 1898) e pode estar relacionado com um hermafroditismo rudimentar (LÖNNBERG, 1898).

O sistema sexual dos lagostins sulamericanos pode ser classificado como de três tipos: (1) gonocorismo, encontrado nas espécies dos gêneros *Samastacus* e *Virilastacus*; (2) intersexualidade permanente, encontrada em *P. pugnax*, *P. varicosus*, *P. saffordi*, *P. pilimanus*; (3) hermafroditismo protândrico parcial, encontrado em *P. nicoleti* e *P. brasiliensis* (RUDOLPH & ALMEIDA, 2000). Esse tipo de hermafroditismo é caracterizado pela presença mútua das gônadas masculina e feminina em um mesmo indivíduo, mas em diferentes estágios da vida, sendo o masculino o primeiro sexo (RUDOLPH, 1997; RUDOLPH & ALMEIDA, 2000; RUDOLPH *et al.*, 2001; RUDOLPH & VERDI, 2010).

Conservação

Os lagostins de água doce constituem um grupo de crustáceos sujeito a ameaças de extinção. A categorização das espécies segundo seu risco de extinção é definida de acordo com os critérios da União Internacional para a Conservação da Natureza - IUCN (IUCN 2012). Essa classificação leva em consideração vários aspectos de uma espécie, como o tamanho populacional, a distribuição, fragmentação do habitat, entre outros. Segundo RICHMAN *et al.*, (2015), cerca de 32% de todas as espécies de lagostim estão sob algum nível de risco de extinção. As principais ameaças à conservação dos lagostins de água doce incluem a urbanização, poluição, mudanças climáticas, canalização de rios, agricultura e a presença de espécies invasoras (RICHMAN *al.*, 2015). Na América do Sul, as espécies de lagostins estão ameaçadas por causas similares, sendo sujeitas principalmente aos impactos diretos da urbanização e de atividades de pesca, como no caso da espécie *P. pugnax*, na qual as populações vêm sofrendo uma redução acentuada devido ao regime de sobrepesca (RUDOLPH, 2010; ALMEIRÃO *et al*., 2015). Além disso, a presença da espécie exótica invasora *Procambarus clarkii* Girard, 1852 foi detectada no estado de São Paulo (LOUREIRO *et al*., 2015a,b). Tal fato se torna bastante preocupante, uma vez que foi detectada a presença do fungo de carapaça *Aphanomyces astaci* Schikora, 1906 nas populações brasileiras de *Procambarus* (PEIRÓ *et al*., 2016). Esse fungo causa uma doença conhecida como “praga do lagostim” que pode dizimar populações inteiras de espécies nativas de lagostins

(GUTIÉRREZ-YURRITA *et al*., 1999; SOUTY-GROSSET *et al*., 2006). Felizmente, indivíduos de *P. clarkii* ainda não foram detectados em ambientes naturais no sul do Brasil, onde as espécies nativas de lagostins ocorrem.

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Objetivos

Objetivo geral

- Revisar a taxonomia de *Parastacus* e investigar a hipótese de monofilia e relações filogenéticas do gênero.

Objetivos específicos

- Estabelecer os limites do gênero *Parastacus*;
- Revisar as espécies existentes e descrever novas espécies;
- Elaborar uma nova chave de identificação para o grupo;
- Através de uma análise filogenética molecular, investigar a monofilia do gênero *Parastacus* e do clado sulamericano (*Parastacus* + *Samastacus* + *Virilastacus*);

CAPÍTULO I

Two new species of South American freshwater crayfish genus *Parastacus* Huxley, 1879 (Crustacea: Decapoda: Parastacidae)

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Two new species of South American freshwater crayfish genus *Parastacus* Huxley, 1879 (Crustacea: Decapoda: Parastacidae)

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Abstract

Two new species of *Parastacus* Huxley, 1879 are described from material collected in the state of Rio Grande do Sul, southern Brazil: *Parastacus fluviatilis* **sp. nov.** from highland streams and *Parastacus caeruleodactylus* **sp. nov.** from wetlands. *Parastacus fluviatilis* **sp. nov.** is distinguished mainly by large chelipeds with dense setae cover on the cutting edge of fingers, telson subtriangular with two lateral blunt spines and strongly concave ventral surface of lateral process of thoracic sternites 6 and 7. *Parastacus caeruleodactylus* **sp. nov.** is distinguished mainly by blue cheliped fingers and a large gap between them, reduced abdomen, dorsal and ventral margins of dactylus, propodus and carpus of second pair of pereiopods with tufts of long setae and mid-dorsal carina of exopod of uropods unarmed. According to IUCN Red List criteria both species are considered endangered. Habitat characterization and a method for defining the shape of second abdominal pleura are also provided.

Key words: burrowing crayfish, freshwater decapods, neotropical crustaceans, parastacid, taxonomy

Introduction

The freshwater crayfishes of the family Parastacidae Huxley, 1879 currently comprise about 178 species in 15 genera (Crandall & Buhay 2008; Toon *et al*. 2010). In South America, the family is represented by only 13 species, grouped in three genera: *Parastacus* Huxley, 1879, *Samastacus* Riek, 1971 and *Virilastacus* Hobbs, 1991 (Buckup & Rossi 1980; 1993; Riek 1971; Rudolph & Crandall 2012). The genus *Parastacus* has a disjunct distribution, with two species occurring on the plains of the southern Andean region (Philippi 1882; Poepigg 1835; Rudolph 2010), *Parastacus nicoleti* (Philippi, 1882) and *P. pugnax* (Poepigg, 1835); and six species in northeastern Argentina, Uruguay and southern Brazil: *P. brasiliensis* (von Martens, 1869), *P. defossus* Faxon, 1898, *P. laevigatus* Buckup & Rossi, 1980, *P. pilimanus* (von Martens, 1869), *P. saffordi* Faxon, 1898 and *P. varicosus* Faxon, 1898 (Buckup & Rossi 1980). All non-Andean species of *Parastacus*, except for *P. laevigatus*, are recorded in Brazil in the state of Rio Grande do Sul (RS); *P. brasiliensis* is endemic to RS, occurring in the Guaiba hydrographic region (Buckup 2003).

These crayfishes are found in wetlands, lotic and lentic environments, where they burrow to a greater or lesser extent (Buckup & Rossi 1980; 1993). This behavior is associated with the water-table level and with ecological, morphological and reproductive traits, as in other crayfish species (Horwitz & Richardson 1986). According to Riek (1972), species of *Parastacus* are of moderate size and are strong burrowers.

The limited distributions and specific habitat requirements of these crustaceans make them highly vulnerable to human impacts. Moreover, the lack of accurate data concerning the distribution range, population size and reproductive features impedes evaluation of the status of and threats to their populations, specifically the IUCN risk categories (Almerão *et al.* 2015; Richman *et al.* 2015). Neotropical crayfishes have been little studied, especially with regard to taxonomy. Compared to the species richness of other crayfish genera, the number of species described from South America is very low, although important taxonomic contributions have been made for the

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endemic Chilean genus *Virilastacus* (Rudolph & Crandall 2005; 2007; 2012). The most recent description of a species of *Parastacus* was published in 1980 by Buckup & Rossi, who described *P. laevigatus*, endemic to the state of Santa Catarina, Brazil. This contribution describes two new species within the genus. The habitats and conservation statuses of these species based on the IUCN Red List criteria are also discussed.

Material and methods

The descriptions are based on material deposited in scientific collections and additional specimens collected in new localities. In streams, specimens were captured with a PVC trap 50 mm in diameter and 20 cm long (see Fontoura & Buckup 1989), closed with a wire screen and baited with chicken liver. The traps were installed in late afternoon, immersed in flowing water to attract the crayfish. A vacuum pump 7 cm in diameter and 72 cm long was used to capture wetland species. In addition, some burrows were also slowly and carefully excavated manually in order to extract the crayfish and provide information on the structure of the burrow system. At each site, geographical coordinates and altitude were recorded, using a Garmin eTrex Legend® H GPS. Field observations included landforms, vegetation, soil type, and associated fauna.

Specimens were taken to the laboratory, photographed to record color pattern of the fresh material, and then killed by cryoanesthesia and preserved in 96% ethanol. Specimens from scientific collections (see below) were also examined. Drawings were prepared by means of a stereomicroscope fitted with a *camera lucida*. Vernier calipers with 0.01 cm accuracy, and a millimetric ocular on a stereomicroscope were used for the measurements. For species of *Parastacus*, few measurements have been used in taxonomic studies, according to Buckup & Rossi (1980). We decided to employ other morphological parameters that have been used for other genera of the family Parastacidae, following Hopkins (1970) and Morgan (1997) and create new ones to improve the taxonomic descriptions (Fig. 2; Table 1). Measurements of all type series specimens can be found in supplementary data (Appendix 1 and 2).

In order to define the size and shape of the S2 pleura, The following measurements for S2 size (x) were taken: y = pleura maximum height and z = maximum anteroposterior distance (Fig. 1). Based on these measurements, the size of the pleura was defined: z:x ratio $\leq$ 1.2 short pleura; 1.2 < z:x ratio $\leq$ 1.3 moderate; and z:x ratio > 1.3 long; for height, we used the ratios: y:x ratio < 1.7 low pleura and y:x ratio > 1.7 high pleura.

Considering that all species of *Parastacus* have supernumerary gonopores (intersexuality), which makes sex identification difficult (von Martens 1869; Faxon 1898; Lönnberg 1898; Riek 1971; Rudolph & Almeida 2000), the sex was determined based on the morphology of the genital apertures: intersex males have female gonopores that are semi-ellipsoidal without setae on or near the borders, and are covered with a calcified cuticle; intersex females have ellipsoidal female gonopores with setae on the surface of the coxa close to its borders, and are covered with a non-calcified membrane; male gonopores are similar in males and females, which open on the apical end of a small, fixed, calcified and truncated phallic papilla, close to the inner border of the ventral surface of the coxae of the fifth pereiopods (Buckup & Rossi 1980; Almeida & Buckup 2000; Rudolph & Verdi 2010).

The terminology used in the morphological descriptions follows Riek (1972), Buckup & Rossi (1980), Hobbs (1987), Morgan (1997) and Holdich (2002). The taxonomic classification is according to De Grave *et al.* (2009). Branchial count follows Huxley (1879).

The type material was deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP), state of São Paulo, Brazil. Paratypes were deposited in the Carcinological Collection of the Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio do Grande do Sul (UFRGS), and in the Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul (MCP), both in Porto Alegre, state of Rio Grande do Sul, Brazil. Comparative material is also deposited in UFRGS and MCP.

The extinction risk of the species was assessed according to the criteria of the International Union for Conservation of Nature - IUCN (IUCN 2012), using B1 subcriterion that takes into consideration the estimated Extent of Occurrence (EOO). To calculate the EOO [performed in the Arcview 9.3 program (ESRI 2009)] the definition of the hydrographic basins according to the Otto Bacias shape method (levels 5 and 6) (ANA 2006) was used. This method considers the contribution area of the stretches from the hydrographic system for the basin classification (Pfafstetter 1989; ANA 2006). Ottobacia 1 corresponds the broadest level and Ottobacia 6 the most restricted, being that the main watercourse has a contribution area greater than its tributary. EOO was obtained from the sum of the Ottobacias areas that presented the occurrence records of the species.

TABLE 1. Morphological measurements with respective abbreviations and definitions according with authors. *First time used for the genus *Parastacus*.

Measurement abbreviations	Name	Definition	Reference
TL	Total Length	The distance from tip of rostrum to posterior margin of telson	Buckup & Rossi (1980); Morgan (1997)
CL	Carapace Length	The distance from tip of rostrum to mid-dorsal posterior margin of carapace	Buckup & Rossi (1980); Morgan (1997)
CW*	Carapace Width	Maximum width at the widest points of carapace	Morgan (1997)
CD*	Carapace Depth	Maximum depth at the deepest part, from dorsal carapace to ventral margin between pereiopods	Morgan (1997)
CeL*	Cephalon Length	Distance from tip of rostrum to cervical groove	Present contribution
RL	Rostral Length	The distance from tip of rostrum to orbital level	Buckup & Rossi (1980)
RW	Rostral Width	Maximum width of rostrum at orbital level	Buckup & Rossi (1980); Morgan (1997)
RCL*	Rostral Carina Length	Total length of rostral carina	Morgan (1997)
CMW*	Cornea Maximum Width	The distance from apex to basis of cornea	Present contribution
OW*	Orbital Width	The distance from the suborbital angle to rostral rim	Present contribution
POCL*	Post Orbital Carina Length	Total length of post orbital carina length when present	Present contribution
FW*	Frontal Width	The distance between both suborbital angles	Present contribution
ASL*	Antennal Scale Length	The distance from tip to the basis of antennal scale	Hopkins (1970)
ASW*	Antennal Scale Width	Maximum width at the widest points of antennal scale	Hopkins (1970)
AreL*	Areola Length	The distance along the midline from the cervical groove to dorsal posterior region of carapace.	Morgan (1997)
AreW	Areola Width	Maximum distance between branchiocardiac grooves	Buckup & Rossi (1980)
RPrT/LPrT*	Right/Left Propodus Thickness	First cheliped propodus thickness (depth), maximum distance measured between dorsal and ventral palm surfaces	Morgan (1997)
RPrL/LPrL	Right/Left Propodus Length	First cheliped propodus length, the distance from propodal basis to apex of fixed finger	Hopkins (1970); Buckup & Rossi (1980); Morgan (1997)
RPrW/LPrW	Right/Left Propodus Width	First cheliped propodus width, the maximum height between proximal and distal edges in the mesial margin	Hopkins (1970); Buckup & Rossi (1980); Morgan (1997)
RDL/RDL	Right/Left Dactylus Length	Dactylus length of the first cheliped	Buckup & Rossi (1980), Morgan (1997)
RML/LML*	Right/Left Merus Length	Merus length of the first cheliped	Present contribution
AL*	Abdomen Length	The distance between the beginning of the first abdominal segment to the end of the last abdominal segment	Present contribution
AW*	Abdomen Width	Maximum width at level of third abdominal somite	Morgan (1997)
TeL*	Telson Length	The distance from porterion margin of abdominal somite 6 to the tip of telson	Morgan (1997)
TeW*	Telson Width	The width measured at level of telson basis	Present contribution

Other Abbreviations:

SLP	Thoracic Sternite Lateral Processes
S1	Abdominal Somite 1
S2	Abdominal Somite 2
m	males
f	females

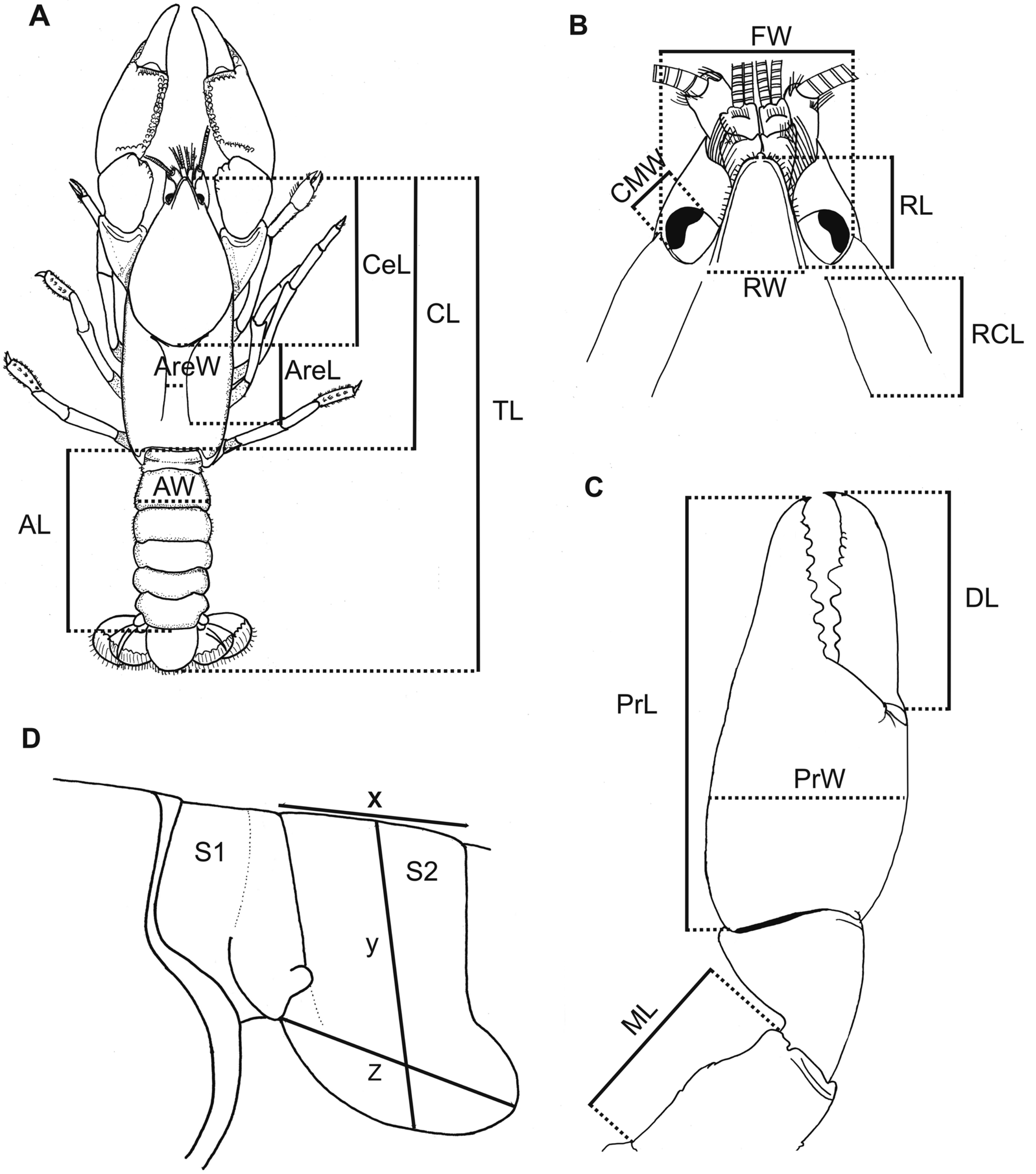

FIGURE 1. Main measurements used in the descriptions of new species of genus *Parastacus* Huxley, 1879 in present contribution. For abbreviations see Material and Methods section and Table I.

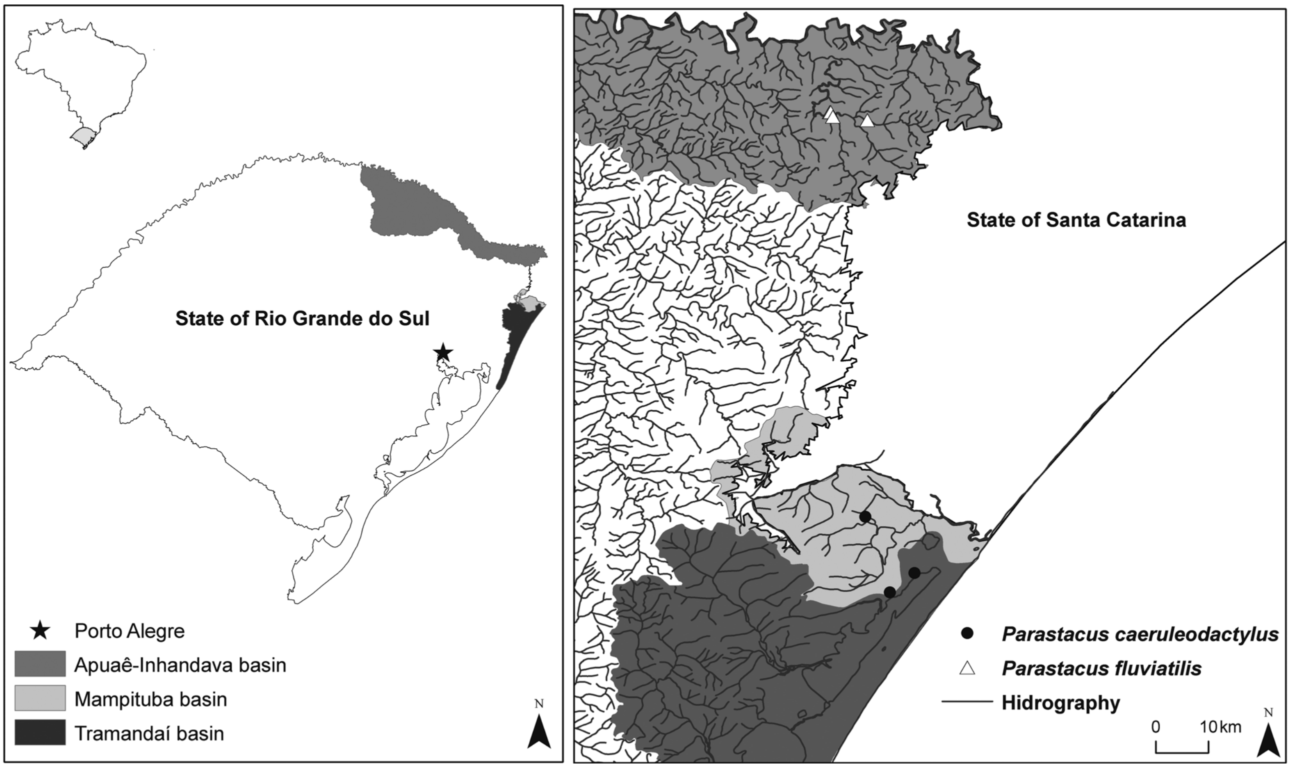

FIGURE 2. Distribution of ***Parastacus fluviatilis*** Ribeiro & Buckup **sp. nov.** and ***Parastacus caeruleodactylus*** Ribeiro & Araujo **sp. nov** in state of Rio Grande do Sul, southern Brazil.

Results

Systematics

Infraorder Astacidea Latreille, 1802

Superfamily Parastacoidea Huxley, 1879

Family Parastacidae Huxley, 1879

Genus *Parastacus* Huxley, 1879

Parastacus Huxley, 1879: 759, 771 [Type species, by subsequent designation (Faxon, 1898: 683): *Astacus pilimanus* von Martens, 1859: 15. Gender: masculine.]

Diagnosis. See Hobbs (1991), p. 801.

Parastacus fluviatilis Ribeiro & Buckup sp. nov.
(Figs. 2–5)

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Holotype. ♂, Brazil, Rio Grande do Sul, São José dos Ausentes, Apuaê-Inhandava Basin, Silveira river (28°35'54.45"S; 49°59'1.36"W), 07/IX/1999, col. L. & T. Buckup (MZUSP 34288).

Paratypes. 1–3: Brazil, Rio Grande do Sul—two ♂ and one ♀, same data as holotype (UFRGS 2704); 4–5: two ♂, São José dos Ausentes, affluent of Silveira river, Fazenda Potreirinhos (28°35'53.40"S; 49°58'55.99"W), 28/IV/1996, col. P.B. Araujo (UFRGS 2294); 6–7: two ♂, São José dos Ausentes, Fazenda Potreirinhos, 02/V/1997, col. O.R. Naches (UFRGS 2295); 8–9: two ♂, São José dos Ausentes, affluent of Silveira river, Fazenda Potreirinhos (28°35'53.40"S; 49°58'55.99"W), 14/V/2014, col. K.M. Gomes, F.B. Ribeiro & D.C. Kenne (UFRGS 6195); 10–11: two ♂, São José dos Ausentes, margin of Silveira River, near the gap of Silveira and Divisa rivers, Fazenda Potreirinhos (28°36'21.29"S; 49°58'41.74"W) 15/V/2014, col. K.M. Gomes, F.B. Ribeiro & D.C. Kenne (UFRGS 6107); 12: one ♀, São José dos Ausentes, Marco river (28°36'42.99"S; 49°55'11") (UFRGS 2236); 13–15: two ♂ and one ♀, Bom Jesus, Fazenda Sr. Argemiro P. Borges, 28/I/1979, col. O. Camargo (UFRGS 1363).

Comparative material analyzed. Brazil, Rio Grande do Sul: *Parastacus pilimanus*—one ♂ and one ♀, Rio Grande, Taim, 09/VI/1975, col. L. Buckup (UFRGS 277); one ♀, Dom Pedrito, 05/III/1957, col. C.P. Coreto (UFRGS 1374); one ♂ and one ♀, São Gabriel (30°34'16.86"S; 54°29'42.22"W), 21/IX/2012, col. K.M. Gomes (UFRGS 5756); one ♂, Ibirapuitã river, Alegrete, 25/II/1982, col. B. Irgang (UFRGS 542); *Parastacus brasiliensis*—three ♂, Mariana Pimentel (30°20'41"S; 51°33'55"W), 12/IV/2010, col. W. Beduchaud, K.M. Gomes & S. Santos (UFRGS 4890); one ♂ and one ♀, Porto Alegre, Praça da Vila Jardim Renascença, Zona Sul (30°5'79"S; 51°11'30.62"W), 10/VII/2013, col. K.M. Gomes, F.B. Ribeiro & G.C. Dalló (UFRGS 5860); Santa Catarina: *P. laevigatus*—one ♀ and five juveniles, Estrada da Cidra, Chacara dos Ipês, Joinville, 05/08/1961, col. Rosenberg (UFRGS 1369).

Etymology. The Latin epithet *fluviatilis* alludes to the riverine habitats where this species was found. We suggest the common name "the highland streams crayfish" for this new species.

Diagnosis. Narrow front with short triangular rostrum. Rostral apex shaped as inverted "U", with blunt spine. Postorbital ridges present and easily distinguished only in anterior portion. Cervical groove strongly V-shaped. Areola narrow and with no lateral elevation on branchiostegite grooves. Cutting-edge surface of fixed finger and dactylus with dense cover of simple and pappose setae. Telson subtriangular with small blunt lateral spines. Mandible with caudal molar process unicuspidate with one big cephalodistal cusp. Ventral surface of lateral process of thoracic sternites 6 and 7 strongly concave.

Description. Rostrum: triangular, wider than long (RL 98.7% of RW), short (12.4% of CL), reaching middle of second article of antennular peduncle (Fig. 3A, B, C). Dorsum slightly concave, apex inverted "U"-shaped, ending in straight blunt spine. Few plumose setae on lateral margins. Rostral sides slightly convergent and rostral basis slightly divergent. Carinae almost straight, prominent and wide, extending back to carapace, surpassing rostral basis (Fig. 3B, C).

Cephalon: Carapace lacking spines or tubercles. CeL 63.9% of CL. Eyes small (CMW 64.4% of OW); suborbital angle 90°, unarmed (Fig. 3C). Front narrow (FW 40% of CW). Postorbital carinae longer than rostral carinae (RCL 62.6% of POCL), conspicuous anteriorly and barely discernible posteriorly. Lateral cephalic edge with sparse setation.

Thorax: carapace laterally compressed, deep and narrow (CD 50.8% of CL; CW 44.1% of CL). Cervical groove strongly V-shaped. Branchiocardiac grooves conspicuous and without carina (Fig. 3A). Areola narrow, 3.1x as long as wide (29.3% of CL) (Fig. 3A).

Abdomen: lacking spines or tubercles, long and wide (AL 79% of CL; AW 80.3% of CW), smooth, with few small setae on pleural margins (Fig. 3A, D). Pleural somites with rounded posterior margins. S1 pleurae with small distal lobe not overlapped by S2 pleurae. S2 pleurae with shallow groove parallel to margin, high and moderately long (Fig. 3E, F).

Tailfan: telson calcified in the proximal portion and weakly calcified in the distal margin, subtriangular, longer than wide (TeW 81.6% of TeL), with small blunt spines on lateral margins; rounded distal margin with abundant long plumose setae and short simple setae. Dorsal surface with tufts of short setae and a dorsomedian longitudinal sulcus (Fig. 3G). Uropod protopod bilobed, with rounded and unarmed margins; proximal lobe largest. Exopod lateral margin unarmed, mid-dorsal carina weakly prominent, ending in small spine. Transverse suture (diaeresis) straight, with two dorsolateral spines (outer) and one dorsolateral spine (inner) on each of right and left exopodites. Endopod, mid-dorsal carina weakly prominent, ending in spine, lateral margin with small spine at level of exopod transverse suture (Fig. 3G).

Epistome: anterolateral section with three marginal tubercles, posteriormost tubercle smallest; also with single keel and small circular median concavity. Posterolateral section with cluster of squamose setiferous tubercles. Anteromedian lobe irregularly pentagonal, 1.03x longer than wide, reaching midlength of antepenultimate article of antennal peduncle; lateral margins keeled; ventral surface slightly concave and basis deeply grooved (Fig. 4A).

Thoracic sternites: SLP4 small and very close to each other, median keel present and not inflated; SLP5 smallest and close to each other, median keel present and not inflated; SLP6 larger than SLP4 and SLP5 and with concave surface, median keel inflated; SLP7 largest and with surface strongly concave, median keel inflated and rhomboidal, bullar lobes absent; SLP8 smaller than SLP6 and concave, median keel absent, vertical arms of paired sternopleural bridges widely separated, bullar lobes separated and clearly visible (Fig. 4B, C).

Antennule: internal ventral border of basal article with sharp spine (Fig. 4A).

Antenna: when extended back reaching second somite of pleon. Antennal scale widest at midlength, reaching midlength of third antennal article, ASW 40.8% of ASL (Fig. 4A, D), lateral margin slightly curved, spine strong and distal margin emarginate. Coxa with weakly prominent carina above nephropore, with strong blunt dorsolateral spine. Basis unarmed (Fig. 4A).

Mandible: cephalic molar process molariform, caudal molar process unicuspidate with one big cephalodistal cusp. Incisive lobe with nine teeth. The third tooth from the anterior is the largest. (Fig. 4E).

Third maxilliped: ischium bearing several setiferous puctuations but with numerous short smooth simple setae on outer margin and on ventrolateral surface, but some tufts presents longer setae (Fig. 4F) and the dorsal surface presents few setiferous punctuations (Fig. 4G). Merum ventral surface entirely covered by long smooth simple setae (Fig. 4F). *Crista dentata* of right and left ischium each with 28 teeth. Merus, entire ventral surface densely covered with simple setae. Exopod longer than ischium, with flagellum reaching proximal margin of merus (Fig. 4F, G).

First pair of pereiopods (chelipeds): large and subequal, laterally flattened (RPrT 23.2% of RPrL; LPrT 22.6% of LPrL) (Fig. 3A). Ischium ventral surface with 11 tubercles. Merus: right merus (RML) 48.2% of propodus length (RPrL); left merus (LML) 49.5% of propodus length (LPrL); ventral surface with two longitudinal series of tubercles: inner series with 15 tubercles, outer 12 and mesial 16, arranged irregularly on right merus; inner series bearing 11 tubercles, outer 13 and mesial 15, arranged irregularly on left merus. Dorsal and midventral spines absent. Carpus with dorsomedial surface divided longitudinally by shallow groove (Fig. 3A; Fig. 4I). Internal dorsolateral margin with row of tubercles, increasing in size distally; inner surface with 1–2 small mesial tubercles. Carpal spine absent (Fig. 4I). Propodus width (RPrW and LPrW) 39.61% of length in right cheliped and 41.75% in left cheliped. Dorsal surface of palm with two rows of tubercles (Fig. 4H, I). Inner margin bearing few small tubercles. Ventral surface bearing two rows of squamose tubercles, reaching beginning of fixed finger (Fig. 4H). Dactylus: moving subvertically, right dactylus (RDL) 54.6% of propodus length (RPrL), left dactylus (LDL) 58.4% of left propodus (LPrL); dorsal surface without tubercles, but with rows of bristle tufts (Fig. 4I). Cutting edge of fingers covered with tufts of pappose setae; fixed finger with eight teeth and dactylus with nine teeth, uniformly distributed, decreasing in size distally (Fig. 4H, I).

Second pair of pereiopods: ventral and dorsal surface of carpus, propodus and dactylus with sparse covering of simple setae (Fig. 4J).

Gonopores: Presence of both genital apertures on coxae of third and fifth pairs of pereiopods. Female gonopores semi-ellipsoidal (maximum diameter 1.5 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 follows the same described by Huxley (1879) and Hobbs (1991) with the epipodite of the first maxiliped with rudimentary podobranchia filaments.

Measurements. Holotype male, CL 38.66 mm and TL 76.66 mm. In type series, CL ranging from 18.25 to 40.88 mm (27.96 ± 7.04 mm). Female paratypes larger than males. FW/CW: 0.44 ± 0.04 (min: 0.39; max: 0.51). RL/RW: 1.00 ± 0.19 (min: 0.74; max: 1.35). CMW/OW: 0.67 ± 0.11 (min: 0.5; max: 0.83). Postorbital carina longer than rostral carina in all specimens analyzed. CW/AW: 1.16 ± 0.12 (min: 1.04; max: 1.51). AuW/RW: 1.18 ± 0.35 (min: 0.71; max: 1.72).

Color of live specimens. Rostrum greenish brown. Cephalothorax anterior region brown, lateral region light brown. First pair of pereiopods brown with greenish-brown fingers and whitish setae coverture on cutting edge. Pereiopod pairs 2–5 light brown. Dorsal pleon brown to greenish with light-brown to greenish-brown marks on lateral margins. Tailfan brown with shades of light brown to greenish brown on dorsal surface of telson (Fig. 5E, F).

Remarks. Female paratypes differ from holotype in their larger sizes, narrower rostrum, larger pleurae of abdominal somites (Fig. 3 D, E, F) and shorter chelipeds. Allometric differences between males and females, as found in other crayfishes, are possible (Reynolds 2002), but were not analyzed here. All paratypes presents both masculine and feminine gonopores in the same individual. Male paratypes also present female gonopores semi-ellipsoidal (average maximum diameter 1.24 ± 0.37 mm) covered by a calcified membrane. Female paratypes presents female gonopores ellipsoidal (average maximum diameter 1.88 ± 0.31 mm) covered by a thin and less calcified membrane. Male gonopores are very similar in males and females.

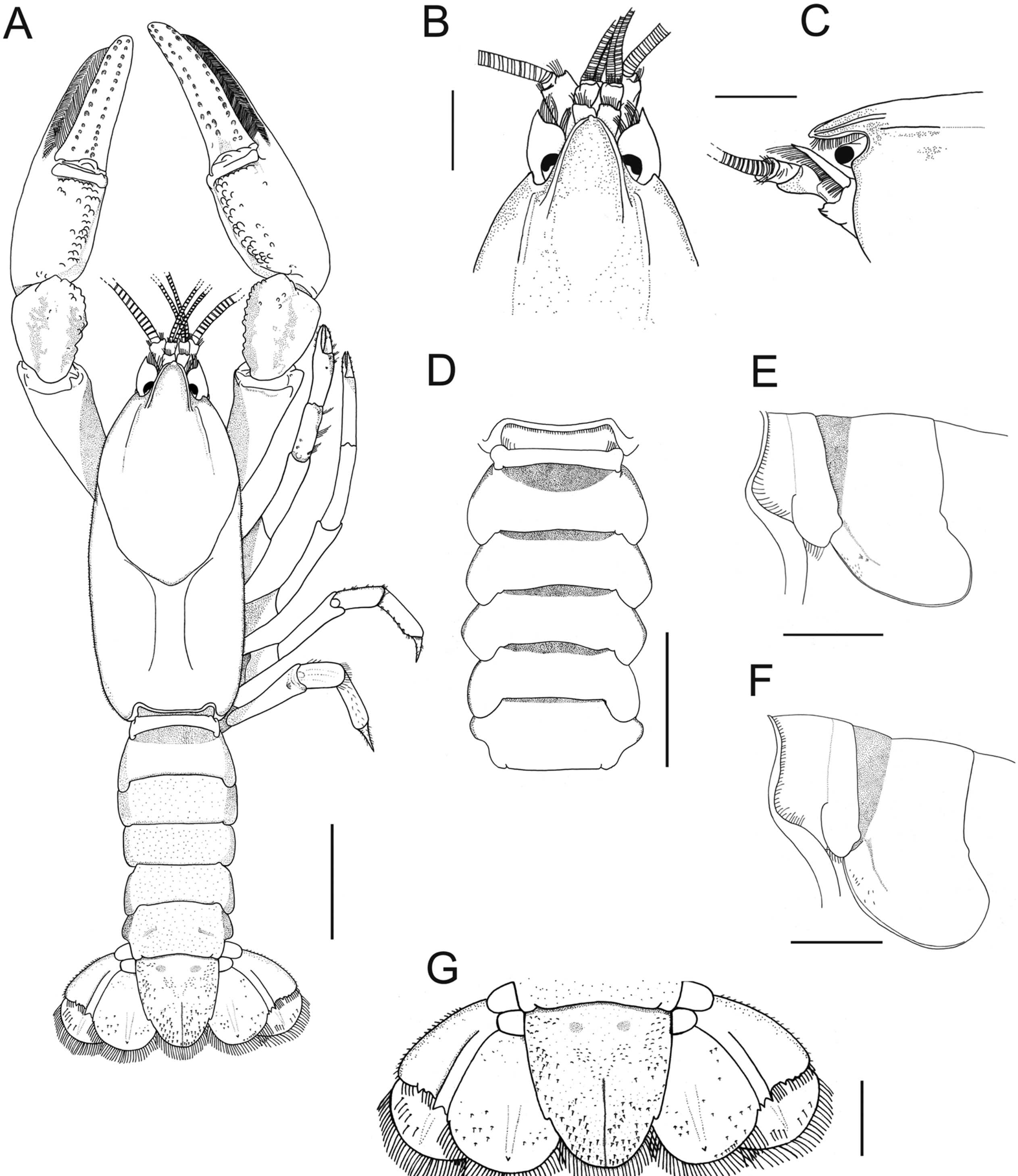

FIGURE 3. ***Parastacus fluviatilis*** Ribeiro & Buckup **sp. nov.**, holotype and paratypes: A, habitus dorsal view (holotype); B, cephalon dorsal view (holotype); C, cephalon lateral view (holotype); D, female abdominal somites dorsal view (paratype 1); E, first and second abdominal pleura (holotype); F, first and second abdominal pleura (paratype 1); G, tailfan (holotype). Scale bars: A—1.5 cm; B, C, E, F, G—5 mm; 5mm; D—1.3 cm.

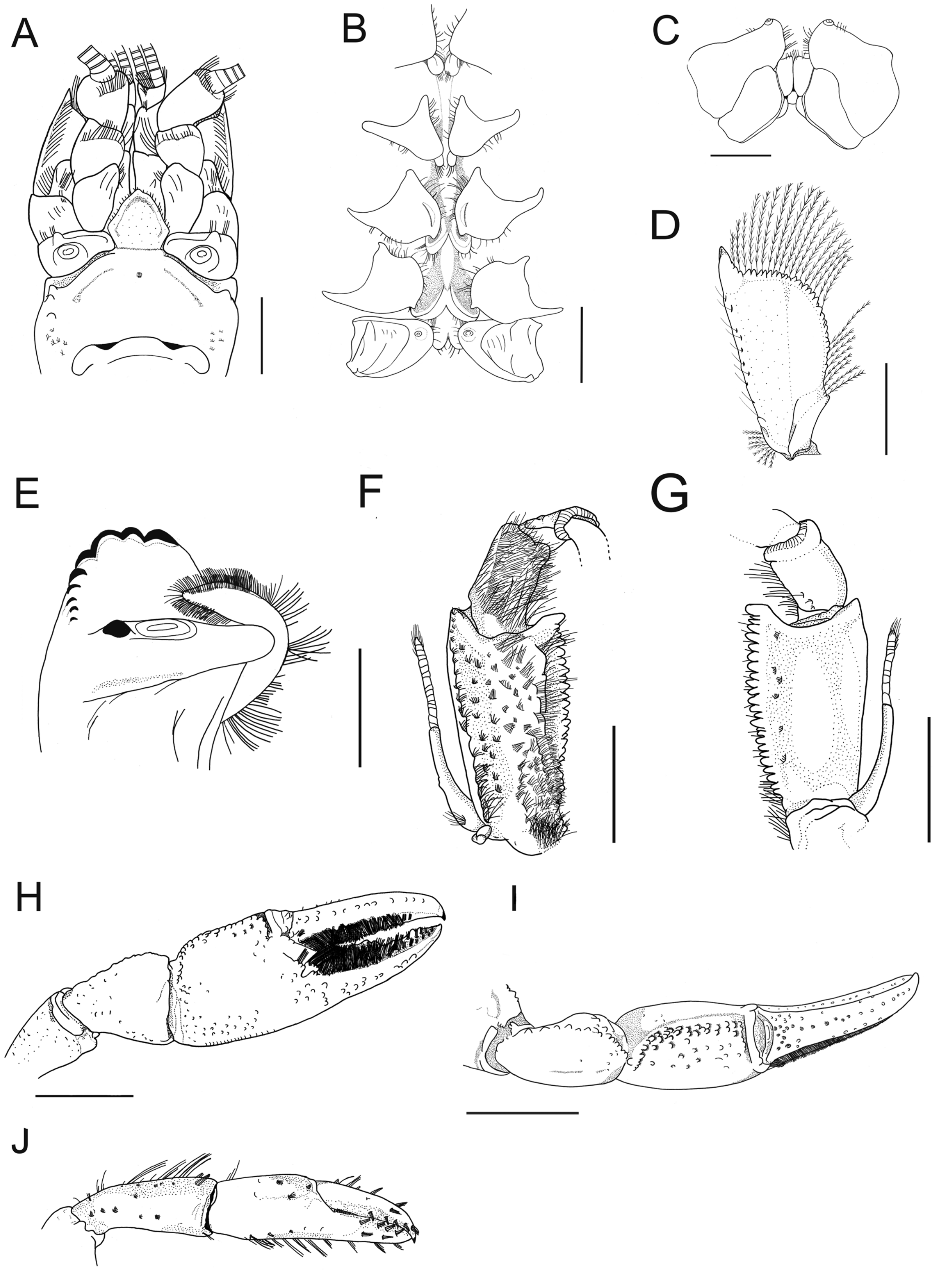

FIGURE 4. ***Parastacus fluviatilis*** Ribeiro & Buckup **sp. nov.**, holotype and paratypes: A, epistome (holotype); B, thoracic sternites and gonopores (holotype); C, thoracomere 8, caudal view (holotype); D, antennal scale lateral view (paratype 1); E—mandible (paratype 1); F—third maxilliped ventral view (paratype 1); G—third maxilliped dorsal view (paratype 1); H—first pereiopod lateral view (holotype); F, first pereiopod dorsal view (holotype); G, second pereiopod lateral view (holotype). Scale bars: A, C—2.5 mm; B, J—5 mm; D, E—2 mm; F, G—5 mm; H, I—1 cm.

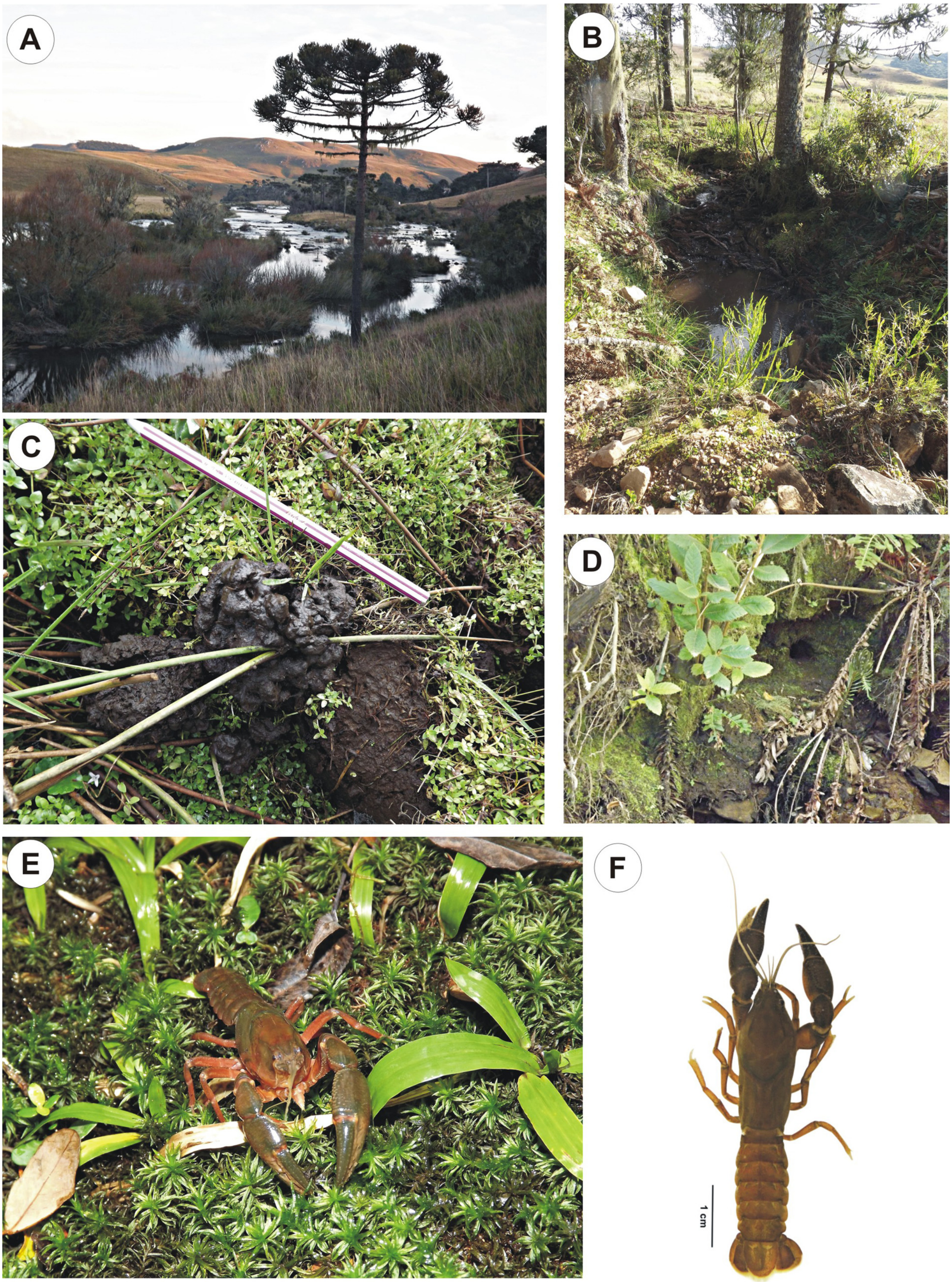

FIGURE 5. ***Parastacus fluviatilis*** Ribeiro & Buckup **sp. nov.** habitat and live specimens. A, Silveira river; B, Tipic habitat, an affluent of Silveira river; C, sealed chimney at margin of Silveira river; D, tipical oppening of burrows; E, living specimen walking among vegetation at margin of Silveira river; F, living specimen, showing colour pattern. Photo A by Kelly M. Gomes. All other photos by Felipe B. Ribeiro.

Parastacus fluviatilis **sp. nov.** is morphologically similar to *P. pilimanus* (von Martens, 1869) and *P. laevigatus* in having the cutting edge of the cheliped fingers covered with long setae (Buckup & Rossi, 1980). It differs from *P. pilimanus* in that the latter has a longer rostrum, rows of verrucous tubercles on the dorsal surface of the dactylus of the first pereiopods/chelipeds (*vs*. dorsal surface without tubercles, but with rows of bristle tufts in *P. fluviatilis* **sp. nov.**), and two sharp spines on the right side of the coxa of the antennal peduncle above the nephropore (please, follow the example of previous comparison). And *P. fluviatilis* differs from *P. laevigatus* mainly in the absence of a carpal spine in chelipeds, the post orbital ridges well prominent, cervical groove strongly V-shaped and larger body sizes. *Parastacus fluviatilis* **sp. nov.** differs from all other *Parastacus* species in having a subtriangular telson, postorbital ridges well distinguished only in anterior portion and a strongly concave ventral surface of the lateral process of thoracic sternites 6 and 7.

Habitat and ecology. *Parastacus fluviatilis* **sp. nov.** was collected at altitudes above 1,300 m in streams of the Serra Geral plateau. This physiographic region is called Campos de Cima da Serra (Fig. 5), i.e., the Brazilian Subtropical Highland Grasslands (Iganci *et al.* 2011). The streams and rivers in this region characteristically have stone and pebble bottoms. They are bordered by few riparian forests, composed mainly of undergrowth and shrubby vegetation (Bond-Buckup 2008).

The specimens were collected with traps in first- and third-order streams, and in backwaters, with dip nets. Shallow burrows were found on the streambanks, usually with one simple opening; when present, the chimney was short (4–5 cm high) (Fig. 5C). *P. fluviatilis* **sp. nov.** is ecologically similar to *P. brasiliensis* in that both inhabit streams and build shallow burrows in the streambanks (Buckup & Rossi 1980).

Distribution. *Parastacus fluviatilis* **sp. nov.** appears to have an extremely limited distribution, being found only in mountain streams of northeastern Rio Grande do Sul, in São José dos Ausentes and Bom Jesus municipalities (Fig. 2). The main streams where the species occurs are Silveira and Marco, tributaries in the Apuaê-Inhandava hydrographic basin, Uruguay Hydrographic Region (Justus 1990).

Conservation status. The EOO was estimated at approximately 1,140 km² based on the Otto Bacia shape level 6 (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). The species is categorized as EN under subitem "a": it is known to occur at no more than 5 locations, as established from its presence in the sub-basins (Silveira and Marco rivers), and local threats; and subitem "b" (iii): continuing decline in quality of habitat by the threats observed in the field, such as deforestation of riparian vegetation for cattle ranching, planting of exotic species (e.g., *Pinus* sp.), agriculture (e.g., sweet potato, *Ipomoea*) and the presence of the exotic rainbow trout *Oncorhynchus mykiss* Walbaum, 1972. We suggest that the conservation status of this species be classified as ENDANGERED B1 ab(iii).

Parastacus caeruleodactylus Ribeiro & Araujo sp. nov.

(Figs. 2, 6–9)

Zoobank: urn:lsid:zoobank.org:act:1BD9ED38-24DA-4C6B-9981-5497EC81BF3A

Holotype. ♂, Brazil, Rio Grande do Sul, Morrinhos do Sul (29°17'13.7"S; 49°54'53.42"W), 12/XII/2013, col. F.B. Ribeiro & K.M. Gomes (MZUSP 34287)

Paratypes. 1: Brazil, Rio Grande do Sul—one ♀, same data as holotype (UFRGS 5931); 2: one ♀, same data as holotype (UFRGS 5932); 3: one ♂, Dom Pedro de Alcântara, RPPN Mata do Professor Baptista (29°23'06"S; 49°50'20"W), 16/IV/2014, col. D.C. Kenne & K.M. Gomes (UFRGS 5934); 3: one ♂, Dom Pedro de Alcântara, RPPN Mata do Professor Baptista (29°23'06"S; 49°50'20"W), 16/IV/2014, col. D.C. Kenne & K.M. Gomes (UFRGS 5935); 5–6: one ♂ Dom Pedro de Alcântara, and one juvenile, RPPN Mata do Professor Baptista (29°23'06"S; 49°50'20"W), 16/IV/2014, col. D.C. Kenne & K.M. Gomes (UFRGS 5936); 7: one ♀, Dom Pedro de Alcântara, RPPN Mata do Professor Baptista (29°23'06"S; 49°50'20"W), 16/IV/2014, col. D.C. Kenne & K.M. Gomes (UFRGS 5950); 8: one ♂, Morro Azul, 12/X/1998, col. L. Buckup & G. Bond-Buckup (UFRGS 2706); 9: one ♂, Torres, Colônia de São Pedro, 13/X/1985 (MCP 1067).

Comparative material analyzed. Brazil, Rio Grande do Sul: *Parastacus defossus*—one ♂, Porto Alegre, Lami, Costa do Cerro, 19/VII/2005, col. L.C.E. Daut & J.F. Amato (UFRGS 4199); one ♂, Porto Alegre, Lami, Costa do Cerro, 19/VII/2005, col. L.C.E. Daut & J.F. Amato (UFRGS 4200); one ♂, Porto Alegre, Morro do Coco (30°15'40.82" S; 51°2'8.27"W), 15/X/2013, col. K.M. Gomes & C.T. Wood (UFRGS 5867); one ♂ and one ♀, Porto Alegre, Lami, 08/VI/2002, col. L. Buckup & G. Bond-Buckup (UFRGS 3360); five ♀, Porto Alegre, Lami (30°11’41”S; 50°06’00”W), 2005, col. C. Noro (UFRGS 4021); Chile: *Parastacus nicoleti*—one ♂, Mehuim (next to Valdivia), VIII/1997, col. niños del Pueblo (UFRGS 2405); *Parastacus pugnax*—one ♂ and one ♀, La Florida, Concepción, 19/I/1977 (UFRGS 2407).

Diagnosis. Rostrum triangular and short. Rostral apex inverted V-shaped, ending in inconspicuous blunt spine. Postorbital carinae obsolete. Cervical groove U-shaped. Areola very narrow and barely discernible. Cheliped propodus globose with large gap between dactylus and fixed finger. Fingers of chelipeds blue. Dorsal margin of dactylus and dorsal and ventral margins of propodus and carpus of second pair of pereiopods with tufts of long simple setae. Mandible caudal molar process unicuspidate with one big cephalodistal cusp. Abdomen shorter and narrower than cephalothorax. Telson subrectangular with small sharp lateral spines. Mid-dorsal carina of exopod of uropods unarmed.

Description. Rostrum: Triangular, wider than long (RL 97.3% of RW), short (11.33% of CL), reaching distal end of second antennular article (Fig. 6A, B, C). Dorsum straight, apex inverted V-shaped, ending in inconspicuous straight blunt spine. Dense plumose setae on lateral margins (Fig. 6C). Rostral sides convergent and rostral basis divergent. Carinae long, prominent and narrow, extending back to carapace, surpassing rostral basis (Fig. 6B, C).
Cephalon: Carapace lacking spines or tubercles. CeL 62.7% of CL. Eyes small (CMW 60% of OW), suborbital angle 90° and unarmed (Fig. 6C). Front narrow (FW 34.4% of CW). Postorbital carinae longer than rostral carinae (RCL 54.3% of POCL) and weakly prominent (obsolete). Lateral cephalic edge with conspicuous setation (Fig. 6C).

Thorax: carapace laterally expanded, deep and wide (CD 54.7% of CL; CW 44.7% of CL; CW 81.7% of CD). Cervical groove U-shaped. Branchiocardiac groove barely visible. Areola narrow and barely discernible, 3.3x as long as wide (26.5% of CL). (Fig. 6A).

Abdomen: lacking spines or tubercles, short and narrow (AL 30.7% of CL; AW 68.6% of CW), smooth and with conspicuous setation on pleural margins (Fig. 6A, D). Pleural somites with rounded distal margins. S1 pleura with small distal lobe not overlapped by S2 pleura. S2 pleura with deep groove parallel to margin, moderately elongated (Fig. 6E, F). AW 48.1% of AL.

Tailfan: telson well calcified in the proximal portion, weakly calcified in the distal margin, subrectangular, longer than wide (TelW 77% of TelL) with small sharp spines on lateral margins; rounded distal margin with abundant long and short simple setae. Dorsal surface with tufts of short setae and a rudimentary dorsomedian longitudinal sulcus (Fig. 6G). Uropod protopod bilobed with rounded and unarmed distal margins, proximal lobe largest. Exopod lateral margin unarmed, mid-dorsal carina weakly prominent, ending in small spine. Transverse suture (diaeresis) straight with 6 dorsolateral spines (outer) and 11 dorsolateral spines (inner) on right exopod and with 6 (outer) and 9 (inner) spines on left exopod. Endopod mid-dorsal carina weakly prominent and unarmed, outer lateral margin with small spine at level of exopod transverse sulture (Fig. 6G).

Epistome: anterolateral section with conical projection. Posterolateral section with cluster of squamose setiferous tubercles and lateral grooves converging to basis of anteromedian lobe, and reduced median circular concavity. Anteromedian lobe irregularly pentagonal, 1.3x longer than wide, apex rounded and slightly concave, reaching median part of antepenultimate article of antennal peduncle; dorsal surface straight, and basis deeply grooved (Fig. 7A).

Thoracic sternites: SLP4 smallest and very close to each other, median keel present and not inflated; SLP5 smaller than SLP6,7,8 and very close to each other, median keel present and not inflated; SLP6 smaller than SLP7,8 and separated from each other, median keel present and inflated; SLP7 largest and separated from each other with dorsal surface slightly concave, median keel present and inflated, bullar lobes absent; SLP8 smaller than SLP7, median keel absent (Fig. 7B), vertical arms of paired sternopleural bridges widely separated, bullar lobes very close to each other (Fig. 7C).

Antennule: inner ventral border of basal article with blunt spine (Fig. 7A).

Antenna: when extended back reaching posterior edge of carapace. Antennal scale widest at distal to midlength and reaching basis of third antennal article, ASW 44.4% of ASL (Fig. 6A, D), lateral margin straight, spine strong and distal margin emarginate. Coxa with weakly prominent carina above nephropore, with strong blunt mesial spine. Basis unarmed (Fig. 6A).

Mandible: cephalic molar process molariform, caudal molar process unicuspidate with one big cephalodistal cusp and. Incisor lobe with nine teeth. The third tooth from the anterior is the largest (Fig. 7E).

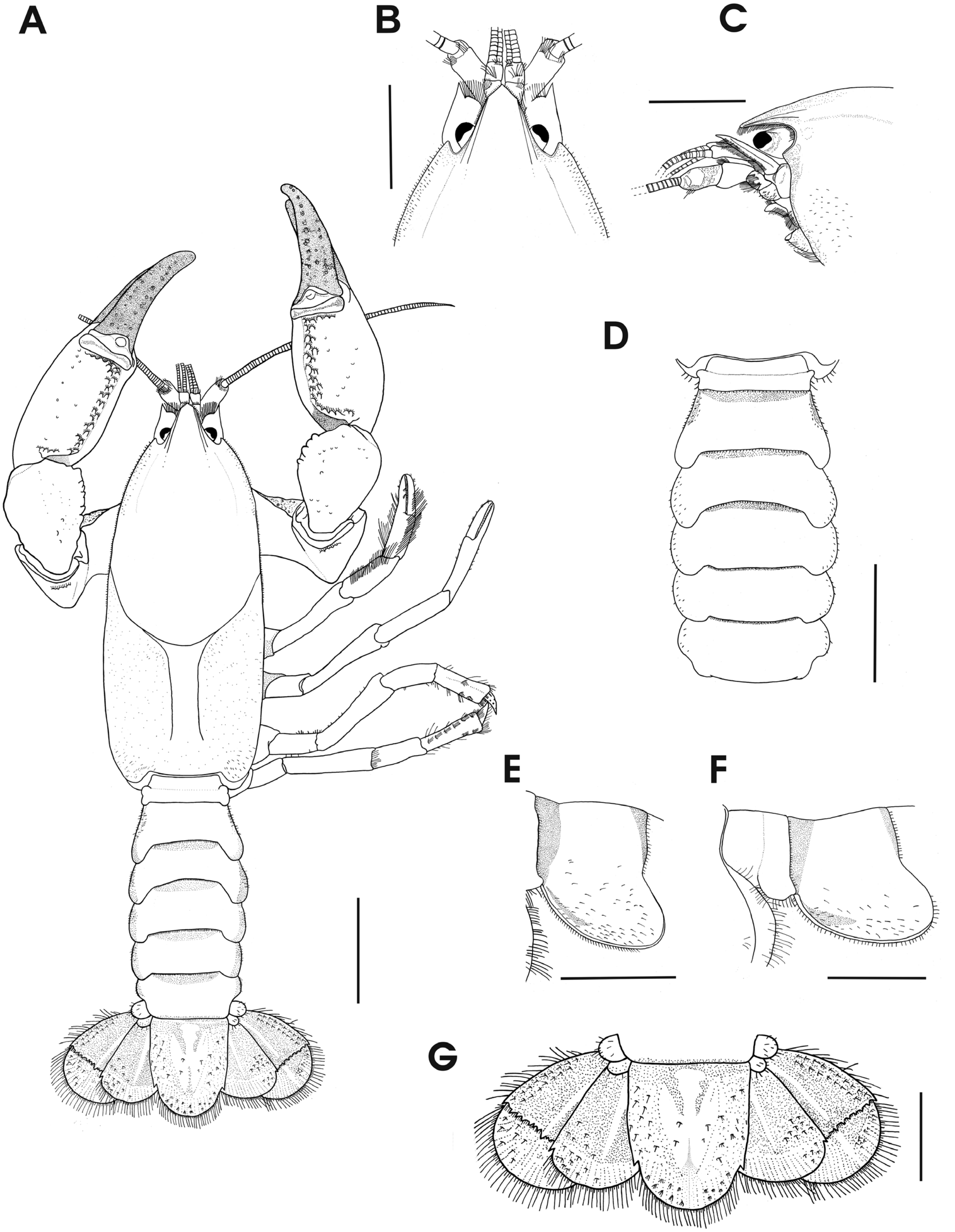

FIGURE 6. ***Parastacus caeruleodactylus*** Ribeiro & Araujo **sp. nov.**, holotype and paratypes: A, habitus dorsal (holotype); B, cephalon dorsal view (holotype); C, cephalon lateral view (holotype); D, female abdominal somites dorsal view (paratype 1); E, first and second abdominal pleura (holotype); F, first and second abdominal pleura (paratype 1); G, tailfan (holotype). Scale bars: A, D—1 cm; B, C, E, F, G—5 mm.

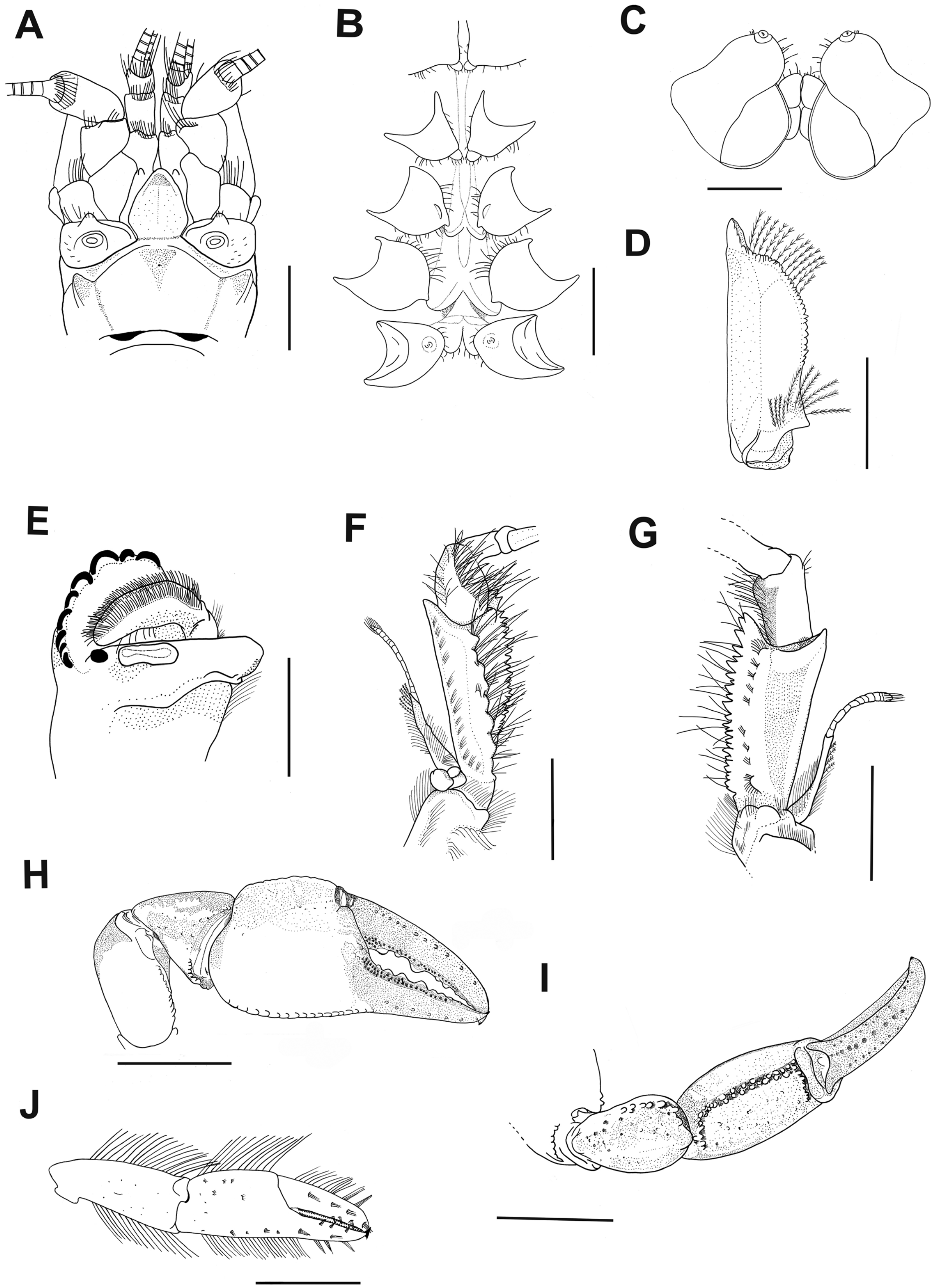

FIGURE 7. ***Parastacus caeruleodactylus*** Ribeiro & Araujo **sp. nov.**, holotype and paratypes: A, epistome (holotype); B, thoracic sternites and gonopores (holotype); C, thoracomere 8 caudal view (holotype); D—antennal scale lateral view (paratype 1); E, mandible (paratype 8); F, third maxilliped ventral view (paratype 1); G, third maxilliped dorsal view (paratype 1); H, first pereiopod lateral view (holotype); I, first pereiopod dorsal view (holotype); J, second pereiopod lateral view (holotype). Scale bars: A, C—2.5 mm; B, J—5 mm; D, E—2 mm; F, G—5 mm; H, I—1 cm.

FIGURE 8. ***Parastacus caeruleodactylus*** Ribeiro & Araujo **sp. nov.** Habitat and live specimen. A, Swamp forest; B, open chimney in moist soil; C, closed chimney; D, living specimen in moist soil.

Third maxilliped: ischium bearing few setiferous puctuations with few long simple setae on outer margin and on ventrolateral surface and dorsal surface with one row of setiferous punctuations (Fig. 7F, G). Merum ventral surface partially covered by long smooth simple setae (Fig. 7F). *Crista dentata* of right and left ischium each bearing 25 teeth. Merus entire ventral surface sparsely covered with simple setae. Exopod longer than ischium, flagellum reaching proximal margin of merus (Fig. 7F, G).

First pair of pereiopods (chelipeds): short, subequal and globose (RPrT 26.8% of RPrL; LPrT 26.1% LPrL) (Fig. 6A). Ischium ventral surface with 11 tubercles. Merus: right merus (RML) 53.8% of propodus length (RPrL), left merus (LML) 52.7% of propodus length (LPrL); ventral surface with two laterolongitudinal series of tubercles: right merus, inner and outer series bearing 13 tubercles and mesial with several small and medium-sized tubercles, irregularly arranged; left merus, inner series bearing 13 tubercles, external 12, and mesial with same pattern as right merus; dorsal and ventromedial spines absent. Carpus medial dorsal surface straight, not divided by a groove (Fig. 6A, 7I). Inner dorsolateral margin with row of tubercles, increasing in size distally. Inner surface bearing some small squamose tubercles. Carpal spine absent (Fig. 7I) Propodus width (RPrW and LPrW) 53.1% of length in right cheliped and 48.5% in left cheliped. Dorsal line of palm with two rows of verrucose tubercles, with tufts of short simple and pappose setae on base of tubercles. Ventral surface bearing two rows of squamose tubercles, reaching beginning of fixed finger (Fig. 7H). Dactylus: right dactylus (RDL) 66.1% of propodus length (RPrL), left dactylus (LDL) 62.3% of propodus length (LPrL); dorsal surface without tubercles, but with rows of bristle tufts (Fig. 4I). Cutting edge of fingers visible. Fixed finger with eight teeth, third and fourth teeth largest. Dactylus with eight teeth, first and third teeth largest. Wide gap between dactylus and fixed finger (Fig. 7H).

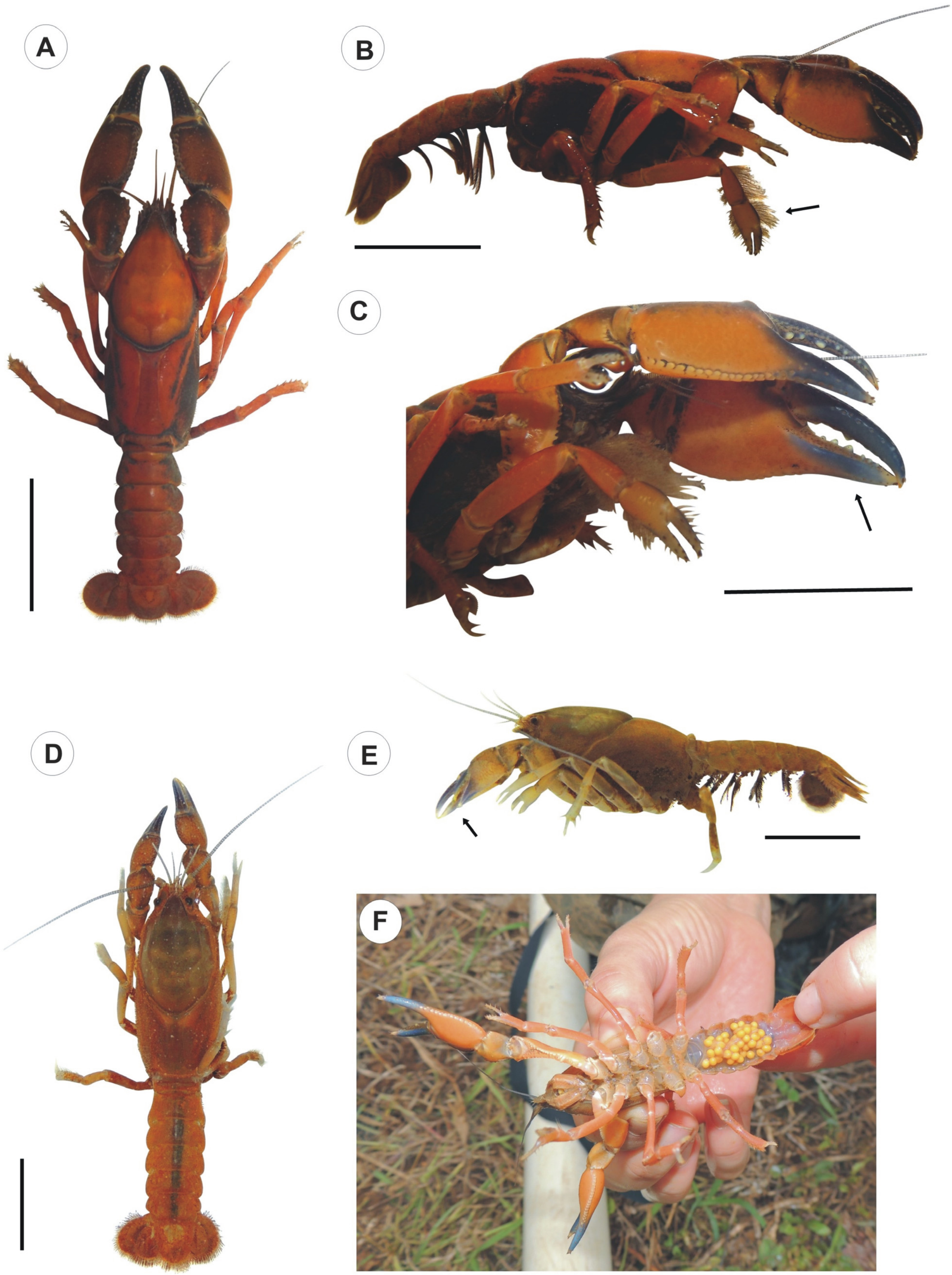

FIGURE 9. ***Parastacus caeruleodactylus*** Ribeiro & Araujo **sp. nov.** Living specimens. A, adult male dorsal view; B, adult male lateral view, arrow indicates tufts of long setae coverture in the dorsal and ventral regions of dactylus, propodus and carpus of second pereiopod; C, adult male chelipeds, arrow indicates blue coloration of fingers; D, juvenile dorsal view; E, juvenile lateral view; F, ovigerous female ventral view. Scale bars: A, B, C—2.5 cm; D, E—1cm.

Second pair of pereiopods: ventral and dorsal margins of dactylus and dorsal and ventral margins of propodus and carpus with tufts of long simple setae (Fig. 7J).

Gonopores: presence of both genital apertures on coxae of third and fifth pairs of pereiopods. Female gonopores semi-ellipsoidal (maximum diameter 1.2 mm) with a well-calcified membrane. Male gonopores rounded, opening onto apical end of 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. 7B).

Branchial count: 20 + epr + r. Branchial arrangement follows the same described by Huxley (1879) and Hobbs (1991) with the epipodite of the first maxiliped with rudimentary podobranchia filaments.

Etymology. A combination of the Latin epithets *caeruleus,* which alludes to the blue color + *dactylus,* which alludes to fingers. We recommend the common name "the blue-fingered burrowing crayfish" for this new species.

Measurements. Holotype male, CL 35.4 mm and TL 68 mm. In the type series, CL ranging from 8.18 to 39.95 mm (27.71 ± 10.6 mm). Female paratypes larger than males. FW/CW: 0.37 ± 0.05 (min: 0.31; max: 0.46). RL/RW: 0.83 ± 0.16 (min: 0.49; max: 0.97). CMW/OW: 0.40 ± 0.18 (min: 0.23; max: 0.82). Postorbital carina longer than rostral carina in all specimens analyzed. CW/AW: 1.39 ± 0.26 (min: 0.7; max: 1.72). AuW/RW: 1.05 ± 0.29 (min: 0.68; max: 1.76).

Color of living specimens. Rostrum orange-brown. Cephalothorax anterior and lateral regions orange-brown in adults and brown with shades of greenish brown in juveniles. First pair of pereiopods orange-brown with cerulean-blue to dark-blue fingers. Pereiopods 2–5 light orange to orange. Dorsal pleon orange-brown. Tailfan orange-brown with shades of orange to light orange on dorsal surface of telson (Fig. 8D, 9).

Remarks. Female paratypes differ from the holotype in the larger body size and larger pleurae of the abdominal somites. *Parastacus caeruleodactylus* **sp. nov.** is morphologically closely related to strong burrowing species of the genus *Parastacus*, as *P. defossus*, *P. nicoleti* and *P. pugnax* in having chelipeds with a globose propodus and a narrow abdomen in relation to CW. However, it differs from all other *Parastacus* species in the large gap between the dactylus and the fixed finger of the first pair of pereiopods; the blue coloration of these fingers and in having the dense setation of the dactylus, propodus and carpus of the second pair of pereiopods.

All paratypes presents both masculine and feminine gonopores in the same individual. Male paratypes also present female gonopores semi-ellipsoidal (average maximum diameter 0.97 ± 0.47 mm) covered by a calcified membrane. Female paratypes presents female gonopores ellipsoidal (average maximum diameter 1.04 ± 0.07 mm) covered by a thin and less calcified membrane. Male gonopores are very similar in males and females.

Habitat and ecology. *Parastacus caeruleodactylus* **sp. nov.** was collected in flat wetlands near the foothills of the Serra Geral and in the coastal region forests in northeastern Rio Grande do Sul. This physiographic region belongs to the Atlantic Forest Biome and it is characterized by swamp forests (Fig. 8A) with permanently or temporarily flooded soils with large amounts of organic matter (Dorneles & Waechter 2004; Rambo 2005). Burrows of *P. caeruleodactylus* had chimneys averaging 10 cm in height and width; some individuals were captured in burrows 1 m deep, with only one opening. We found ovigerous females bearing different numbers of eggs, with a maximum of 40 eggs in the initial stage of development attached to the pleopods (see Fig. 9F). Ecologically, *P. caeruleodactylus* **sp. nov.** resembles the strong burrowers species of the genus *Parastacus*, e.g. *P. defossus*, *P. nicoleti* and *P. pugnax*; and some other parastacid species in South America, as in the genus *Virilastacus* and in Australian genera *Engaeus* Erichson, 1846, *Engaewa* Riek, 1967 and *Tenuibranchiurus* Riek, 1951, and even in the cambarid genus *Fallicambarus* Hobbs, 1969. Ecological features shared by these species include deep burrow system with complex chimneys and particular morphological adaptations to the burrowing lifestyle, as reduced abdomen and reduced eyes; globose cheliped propodus with dactylus moving subvertically or obliquely; branchial chamber extended (narrow areola); and the abundance of setae along the carapace, abdomen and appendages (Horwitz & Richardson 1986).

Distribution. *Parastacus caeruleodactylus* **sp. nov.** appears to have a limited distribution, since it has been found only in swamp forests in northeastern Rio Grande do Sul, in the municipalities of Morrinhos do Sul, Morro Azul, and Dom Pedro de Alcântara. Colônia de São Pedro is an older name for the Dom Pedro de Alcântara municipality (Fig. 2). The main drainages where the species occurs are the Tramandaí and Mampituba hydrographic basins, Coastal Hydrographic Region (Justus 1990).

Conservation status. The EOO was estimated at 1,152 km² based on the Otto Bacias shape level 5, indicating that this species can be categorized as Endangered (EOO less than 5,000 km², according to the IUCN). Subitem "a" for an EOO that is severely fragmented, and subitem "b" (iii) Continuing decline in quality of habitat, are appropriate due to the threats existing in the occurrence areas of *P. caeruleodactylus*. Rice cultivation may be one cause of habitat loss and fragmentation, because the swamp areas are deforested for cultivation or drained for cattle ranching and construction of human dwellings. The coastal region is largely agricultural, and the use of agrochemicals has been reported (Cabrera *et al.* 2008). We therefore suggest that this species be classified as "ENDANGERED B1 ab (iii)".

Discussion

Conservation status. The global fauna of crustaceans, especially freshwater crayfishes, is under threat. For the world crayfish fauna, 36% of the species are under some level of risk of extinction (Almerão *et al.* 2015; Richman *et al.* 2015). The threats observed for crayfishes include urban development, pollution, damming and water management, climate change, harvesting, agriculture, and invasive species (Richman *et al*. 2015). In Brazil, freshwater crustacean species in general are threatened by similar causes, but more specifically by the removal of riparian forest, which causes siltation and discharge of domestic, urban, industrial, and agricultural effluents, which degrade water quality (Magris *et al.* 2010).

The Campos de Cima da Serra region harbors endemic and threatened species of crustaceans such as anomuran crabs of the genus *Aegla* Leach, 1820 (Bond-Buckup 2008). The Upper Uruguay river region, which includes the Silveira and Marco rivers, is recognized as an important freshwater ecoregion for biodiversity conservation (Abell *et al.* 2008). For aeglids, this region has both high species richness and several endemic species (Pérez-Losada *et al.* 2009). Nevertheless, this diverse fauna is under threat, including *P. fluviatilis* **sp. nov.** and aeglids, which may be subject to the same pressures. Removal of riparian vegetation can cause a host of problems. Riparian vegetation is responsible for the regulation of hydrological dynamics, transfer of energy and material between terrestrial and aquatic systems, and buffering against silting and contamination of watercourses (Klapproth & Tjaden 1996; Pusey & Arthington 2003). The importance of riparian vegetation and the negative effects produced by its absence were reviewed for fish faunas by Pusey & Arthington (2003).

The presence of the rainbow trout *Oncorhynchus mykiss* is another potential threat to crayfish, because this trout is a voracious predator of invertebrates (Billy & Usseglio-Polatera 2002; Winckler-Sosinski 2008) and can potentially reduce the number of juvenile crayfish in local populations. Trout are reintroduced periodically in streams in this region, mainly in the Silveira River, for sport fishing (Wincler-Sosinski *et al.* 2005). The consequences of the presence of rainbow trout in the state of Rio Grande do Sul have been analyzed only for the ichthyofauna, which has undergone reductions in species richness, abundance and biomass at sites stocked with trout (Wincler-Sosinski *et al.* 2009). In other countries, introduction of this species caused declines of frog populations (Finley & Vredenburg, 2007).

The Tramandaí–Mampituba ecoregion, which encompasses the distribution of *P. caeruleodactylus* **sp. nov.**, is an important area for species diversity and ecosystem conservation (Abell *et al.* 2008). Nevertheless, the coastal region as a whole is predominantly used for agriculture, silviculture and cattle ranching (Becker *et al*. 2007). Rice farms cover much of the range of *P. caeruleodactylus* **sp. nov.**, which was collected on the edges of rice fields. The use of agrochemicals in rice cultivation is common, and involves a high risk of contamination of surface and groundwaters (Cabrera *et al.* 2008).

In addition, the presence of the alien crayfish species *Procambarus clarkii* Girard, 1852 in Brazil can be an important threat for native crayfish populations. Fortunately, established populations of this species were found only in southeast region, which has no native crayfish species, but a possible range expansion of *P. clarkii* toward the South of Brazil, where there are endemic species of the genus *Parastacus*, is worrying (Loureiro *et al*. 2015a,b)

The distribution, ecology, behavior and habitat requirements of both species of crayfishes should be studied more extensively in order to better understand and assess threats to their populations. Currently, most freshwater crustacean species are evaluated as Data Deficient (DD), due to a scarcity of needed information (Richman *et al.* 2015; Cumberlidge *et al.* 2009). In Brazil, 75% of freshwater crustaceans are assessed as DD (Magris *et al.* 2010).

The classification of *P. fluviatilis* **sp. nov.** and *P. caeruleodactylus* **sp. nov.** as Endangered by criterion B1 is based on knowledge of their distributions and current threats; this criterion is considered appropriate for assessment of invertebrate species (Cardoso *et al*. 2011). Both species have particular features such as restricted distributions and possible narrow habitat specificity that may exacerbate the risk of their extinction. Furthermore, burrowing species have a limited ability to disperse, because they spend very little time above ground, have small and disconnected populations, and the individuals need to reach the water table (Reynolds *et al.* 2013).

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APPENDIX 1. Measurements (mm) of type series of ***Parastacus fluviatilis*** Ribeiro & Buckup **sp. nov**. – Broken appendage. For abbreviations see Material and Methods section.

	CRAYFISHES						
	Holotype (MZUSP 34288)	Paratype 1 (UFRGS 2704)	Paratype 2 (UFRGS 2704)	Paratype 3 (UFRGS 2704)	Paratype 4 (UFRGS 2294)	Paratype 5 (UFRGS 2294)	Paratype 6 (UFRGS 2295)
Sex	M	F	M	M	M	M	M
TL	76.66	84.15	66.37	53.02	54.44	54.75	46.61
CL	38.66	40.71	33.85	26.71	26.67	27.09	23.59
CW	17.06	17.48	14.69	10.18	10.43	10.54	9.15
CD	19.63	22.61	22.03	10.23	14.47	14.13	13.25
CeL	24.71	22.54	21.6	17.15	18.55	17.77	15.09
RL	4.81	4.18	4.17	3.44	2.76	2.55	3.16
RW	4.87	5.40	4.84	3.07	2.9	3.15	3.23
CMW	1.61	1.59	2	1.31	1.45	1.38	1.19
OW	2.5	2.89	2.4	2.27	1.96	1.85	1.7
FW	6.84	7.44	6.38	4.76	4.04	4.82	4.44
RCL	6.06	5.29	5.74	3.43	3.37	4.47	3.72
POCL	9.68	9.86	7.28	5.25	7.7	7.35	6.99
ASL	4.38	4.64	9.66	3.34	3.17	3.47	3.09
ASW	1.79	2.45	3.61	1.77	1.63	2.05	1.41
AreL	11.35	12.89	9.6	9.85	8.99	9.84	8.95
AreW	3.68	3.86	3.61	4.75	4.02	4.72	3.54
AW	13.7	16.22	13.02	8.25	9.3	9.24	7.96
AL	30.56	33.83	26.94	21.3	27.51	27.24	17.59
RPrT	8.12	7.46	7.9	5.33	5.55	-	4.41
RPrL	34.99	29.17	28.65	18.74	21.03	-	16.05
RPrW	13.86	12.26	13	9	9.29	-	7.65
RML	16.86	16.72	14.32	11.16	11.69	-	8.42
RDL	19.1	18.23	15.38	10.9	11.89	-	9.4
LPrT	7.93	7.36	7.67	5.38	5.82	5.7	4.53
LPrL	34.11	28.22	27.14	18.67	20.85	19.9	16.02
LPrW	14.24	12.32	13.01	9.1	9.03	9.4	7.49
LML	16.9	17.22	14.55	10.24	11.86	11	8.91
LDL	19.91	17.30	15.68	11.06	11.17	12.01	9.37
TeL	11.32	12.56	10.08	7.91	8.65	7.96	6.44
TeW	9.24	10.66	8.12	6.75	6.87	6.73	5.59

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APPENDIX 1. (Continued)

	CRAYFISHES								
	Paratype 7 (UFRGS 2295)	Paratype 8 (UFRGS 6195)	Paratype 9 (UFRGS 6195)	Paratype 10 (UFRGS 6107)	Paratype 11 (UFRGS 6107)	Paratype 12 (UFRGS 2236)	Paratype 13 (UFRGS 1363)	Paratype 14 (UFRGS 1363)	Paratype 15 (UFRGS 1363)
Sex	M	M	M	M	M	F	M	F	M
TL	34.7	31.73	46.09	62.85	48.25	78.2	54.55	57.8	43.33
CL	18.25	18.29	22.44	30.01	23.42	40.88	27.26	28.54	21.1
CW	8.6	7.34	9.54	13.69	9.56	17.01	11.36	11.97	8.63
CD	11.87	8.26	10.52	13.98	11.49	18.76	16.89	12.94	13.37
CeL	12.22	12.26	14.75	19.53	15.07	26.74	18.03	18.53	14.17
RL	1.83	2.75	3.47	4.05	3.73	4.9	3.38	2.72	2.11
RW	2.04	2.22	2.65	4.18	2.76	4.42	3.05	3.69	2.57
CMW	0.91	0.92	1.11	1.14	1.03	1.42	1.14	1.25	1.14
OW	1.81	1.21	1.32	2.02	1.75	2.64	1.68	1.99	1.37
FW	4.15	3.12	4.04	5.37	4.81	6.91	4.9	5.64	4.42
RCL	3.23	2.39	2.93	4.53	3.68	5.43	3.48	3.97	4.1
POCL	5.73	4.68	5.94	7.51	5.13	9.76	8.03	5.83	4.12
ASL	2.39	2.96	3.17	4.67	3.27	4.85	3.20	3.73	2.41
ASW	1.10	1.23	1.39	1.53	1.2	1.93	1.65	1.70	1.13
AreL	6.35	4.28	5.55	7.7	6.05	10.57	9.29	10.22	7.64
AreW	3.73	1.8	2.4	3.86	3.67	5.46	5.24	4.34	3.19
AW	5.68	6.84	8.53	12.07	9.03	16.08	9.66	9.28	8.23
AL	14.29	13.7	17.76	21	19.43	25.88	21.59	23.72	17.35
RPrT	2.85	5.42	-	9.84	7.74	12.85	4.38	4.23	-
RPrL	10.54	11.54	-	20.99	15.99	27.75	17.46	16.45	-
RPrW	5.28	3.4	-	5.74	5.07	7.62	7.69	7.04	-
RML	7.39	6.9	-	11.39	9.1	15.56	10.96	9.65	-
RDL	6.37	6.23	-	12.58	8.83	-	10.7	10.4	-
LPrT	3.13	4.23	7.14	13.43	7.67	-	6.03	4.39	4.47
LPrL	10.02	9.69	13.68	25.19	15.43	-	19.95	17.21	13.96
LPrW	5.31	2.48	4.11	8.12	5.02	-	9.74	7.29	7.22
LML	7.06	6.16	8.21	13.07	8.94	-	10.48	10.51	8.1
LDL	5.92	5.75	7.89	14.36	8.66	-	11.5	10.28	7.95
TeL	5.13	5.28	6.6	8.86	6.72	13.01	8.11	7.92	6.05
TeW	4.22	4.62	5.72	7.52	6.28	10.28	6.46	7.07	5.32

APPENDIX 2. Measurements (mm) of type series of ***Parastacus caeruleodactylus*** Ribeiro & Araujo **sp. nov.** – Broken appendage. For abbreviations see Material and Methods section.

	CRAYFISHES									
	Holotype (MZUSP 34287)	Paratype 1 (UFRGS 5931)	Paratype 2 (UFRGS 5932)	Paratype 3 (UFRGS 5934)	Paratype 6 (UFRGS 5935)	Paratype 7 (UFRGS 5936)	Paratype 8 (UFRGS 5936)	Paratype 4 (UFRGS 5950)	Paratype 9 (UFRGS 2706)	Paratype 10 (MCP 1067)
Sex	M	F	F	M	M	M	Juvenile	F	M	M
TL	68	77.43	54.72	58.66	30.23	30.47	15.89	57.12	66.64	62.38
CL	35.4	39.95	32.99	30.6	15.98	15.82	8.18	28.54	36.22	33.43
CW	15.83	17.73	14.68	13.69	7.23	7.12	3.71	12.25	15.47	15.13
CD	19.36	20.74	17.65	18.47	8.53	5.82	4.66	16.94	15.59	17.93
CeL	22.2	25.47	21.54	19.39	10.78	10	5.09	18.46	22.25	21.42
RL	4.01	3.7	3.43	2.49	1.71	1.64	1.11	2.73	3.62	3.52
RW	4.12	4.14	3.45	3.52	2.47	3.33	1.24	3.22	4.46	3.62
CMW	2.2	1.84	1.72	2.3	1.18	0.66	0.48	1.88	1.13	2.03
OW	5.59	7.9	6.18	7.38	4.01	1.49	0.58	6.31	1.83	6.44
FW	5.45	5.9	5.04	4.34	2.44	3.07	1.74	4.11	5.87	6.23
RCL	7.38	7.5	6.76	6.1	3.34	2.09	1.47	5.29	5.46	7.06
POCL	7.38	9.28	8.14	7.83	3.51	3.59	1.64	6.73	7.98	7.77
ASL	3.58	4.51	3.42	3.99	1.81	2.08	1.20	3.68	4.86	4.28
ASW	1.59	1.76	1.46	1.56	0.66	0.59	0.49	1.29	1.62	1.59
AreL	9.39	13.98	11.5	11.02	5.65	4.89	2.29	10.3	9.68	11.45
AreW	2.81	3.91	4.85	4.64	3.72	2.39	0.95	3.37	4.43	4.08
AW	22.57	12.74	10.13	7.94	4.8	4.75	2.48	9.05	10.89	11.02
AL	10.87	27.36	24.64	22.61	11.95	16.17	6.6	21.06	24.82	25.01
RPrT	7.38	4.38	8.63	7.1	2.19	2	0.72	2.93	7.59	7.68
RPrL	27.5	19.3	29.44	23.66	7.77	6.86	3.59	12.29	25.11	31.08
RPrW	14.16	8.24	15.94	12.9	3.81	3.74	1.31	5.52	13.16	14.37
RML	14.79	12.6	13.61	12.66	5.59	5.57	2.59	9.35	15.03	15.65
RDL	18.17	13.01	17.49	14.04	4.92	3.97	2.09	7.96	14.28	18.08
LPrT	7.28	8.59	4.41	-	2.29	2.01	0.91	5.29	7.45	3.93
LPrL	27.89	28.72	16.71	-	7.8	7.26	3.14	17.29	24.43	17.79
LPrW	13.53	15.29	7.62	-	4.05	3.7	1.39	10.14	13	8.31
LML	14.7	15.49	10.78	-	5.76	4.96	2.80	11.26	14.37	11.74
LDL	17.39	17.47	10.9	-	4.53	4.30	1.89	8.91	13.48	12.01
TeL	9.2	10.81	8.23	7.77	4.33	4.21	2.43	7.61	9.10	8.84
TeW	7.09	8.22	6.38	6.06	3.8	3.75	1.70	5.64	6.90	6.66

CAPÍTULO II

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

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ORIGINAL ARTICLE

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

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ZOOBANK http://zoobank.org/urn:lsid:zoobank.org:pub:E302E7F9-0594-40EE-B179-D7F8A68A19A9

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 turkayi* 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^2, 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 .

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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)

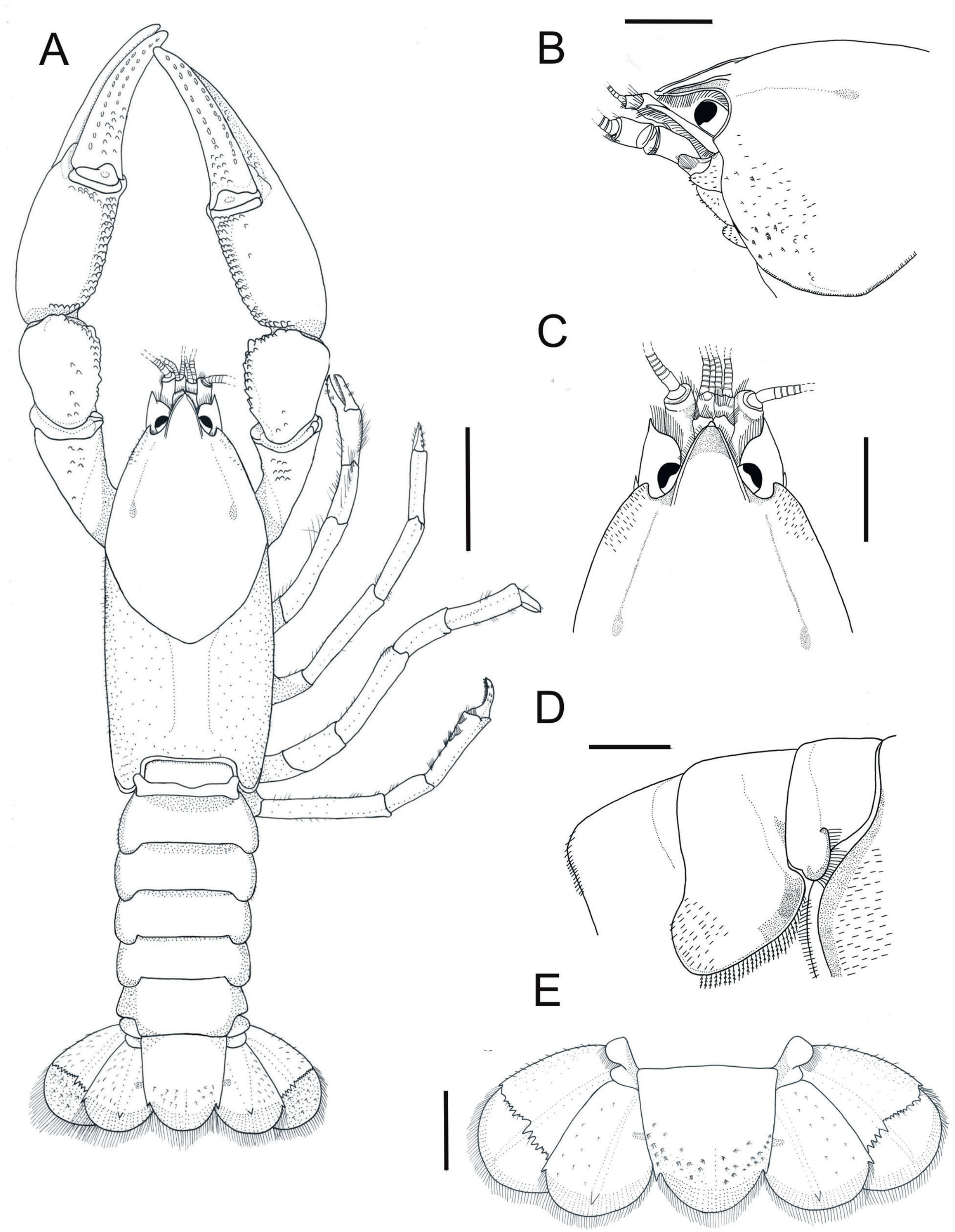

Figure 1. *Parastacus tuerkayi* sp. nov., holotype (MZUSP 34940). A, habitus, dorsal view; B, cephalon, lateral view; C, cephalon, dorsal view; D, first and second abdominal pleura; E, telson and uropods, dorsal view. Scale bars: A = 1 cm; B – E = 5 mm.

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. *Paratascus 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°. 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°, 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

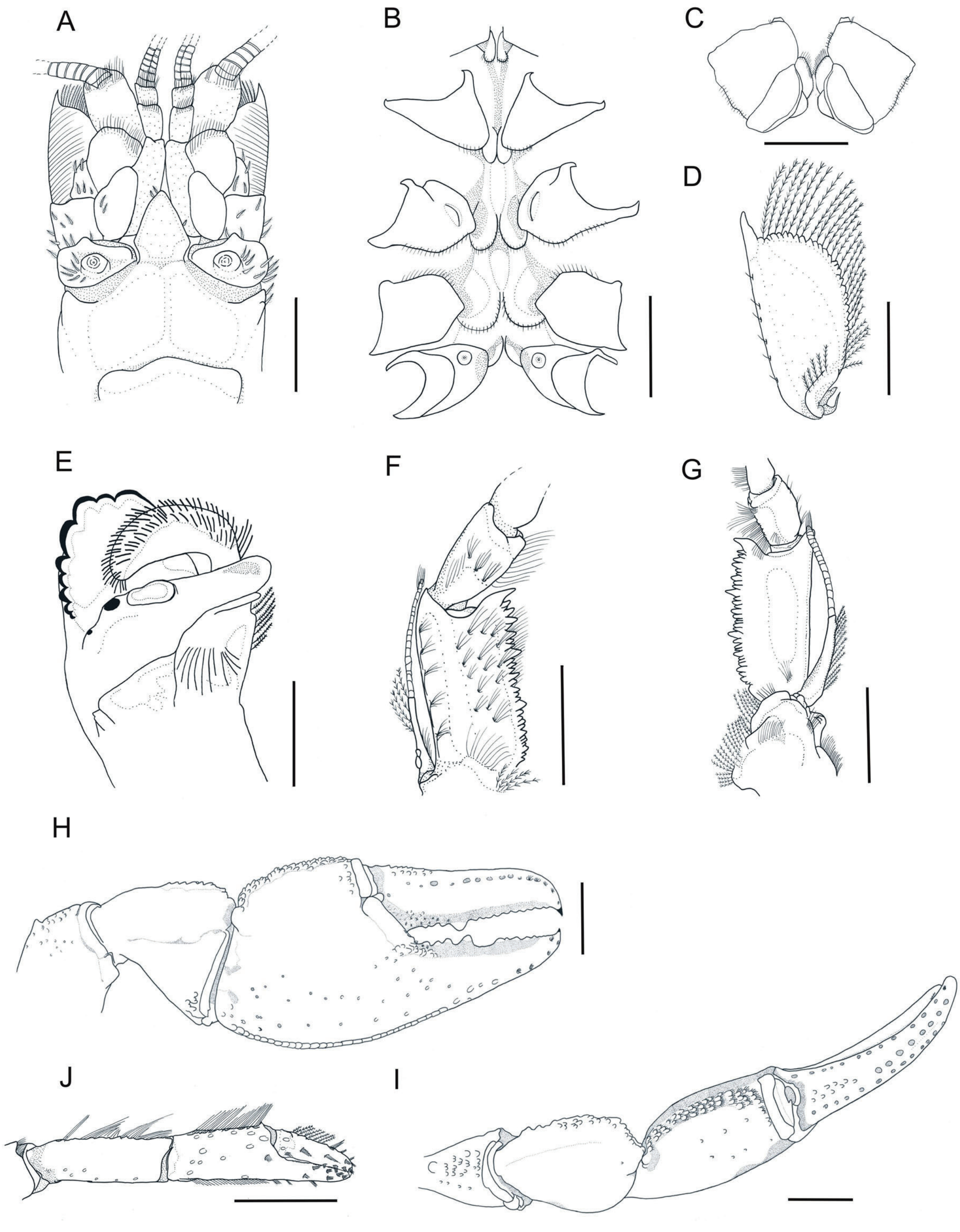

Figure 2. *Parastacus tuerkayi* sp. nov., holotype (MZUSP 34940) and paratypes (UFRGS 3167, UFRGS 6438). A, epistome (holotype); B, thoracic sternites and gonopores (holotype); C, thoracomere 8, caudal view (holotype); D, antennal scale, lateral view (UFRGS 3167); E, mandible (UFRGS 6438); F, third maxilliped, ventral view (UFRGS 6438); G, third maxilliped, dorsal view (UFRGS 6438); H, first 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.

(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).

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 antenulle 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-

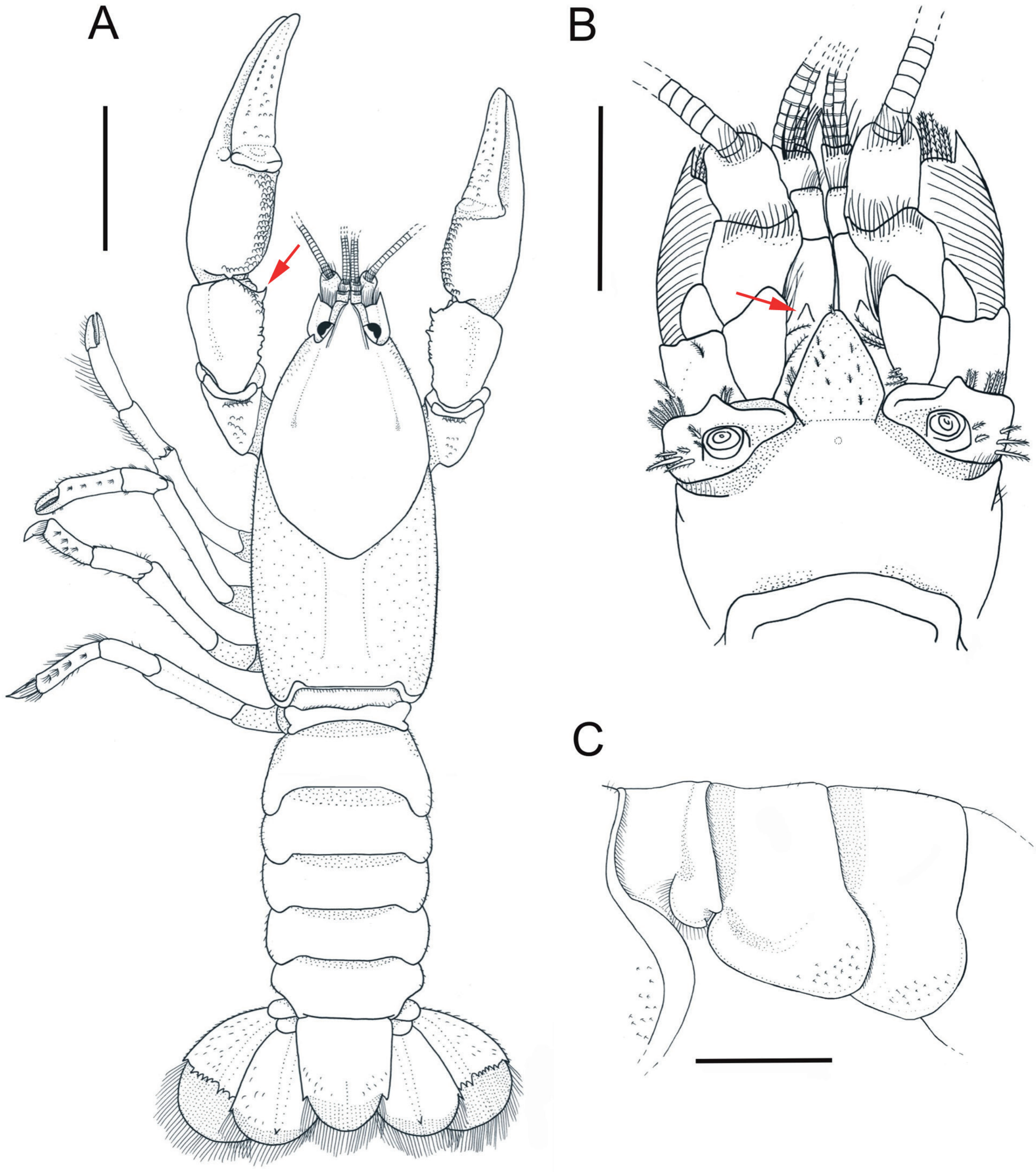

Figure 3. *Parastacus tuerkayi* sp. nov., female paratype (UFRGS 6376). A, habitus, dorsal view; B, epistome; C, first and second abdominal pleura. Scale bars: A = 1 cm; B, C = 5 mm. Red arrows indicate the spine on the carpus and on the internal ventral border of basal article of antenulle respectively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

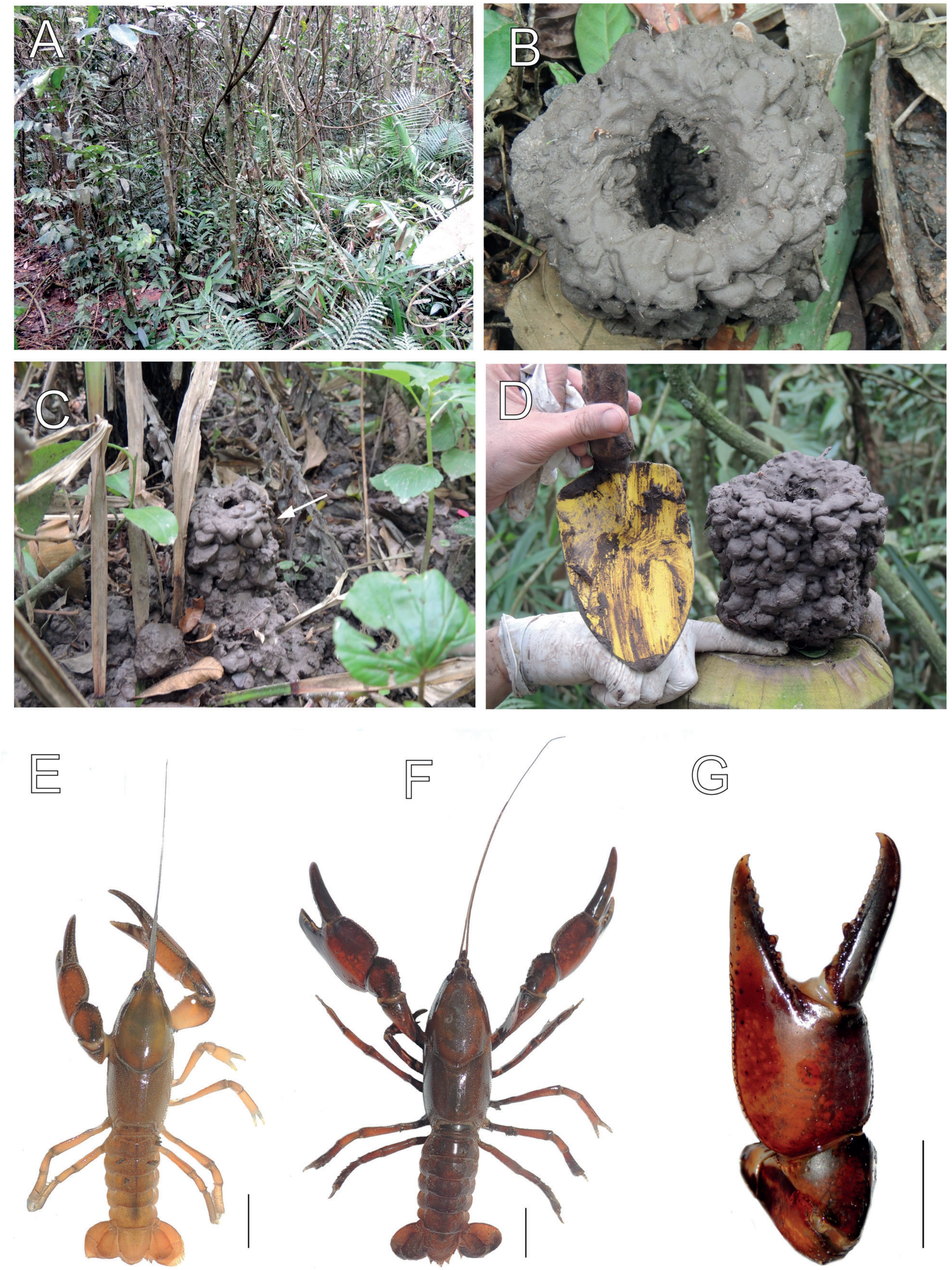

Figure 4. *Parastacus tuerkayi* sp. nov., habitat and living specimens. A, Typical habitat, a swamp forest; B, Opened chimney superior view; C and D, opened chimney lateral view, white arrow indicates the chimney; E and F, living specimens, habitus dorsal view UFRGS 6438 and holotype, respectively); 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 the16S 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^2) 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 km^2 based on the

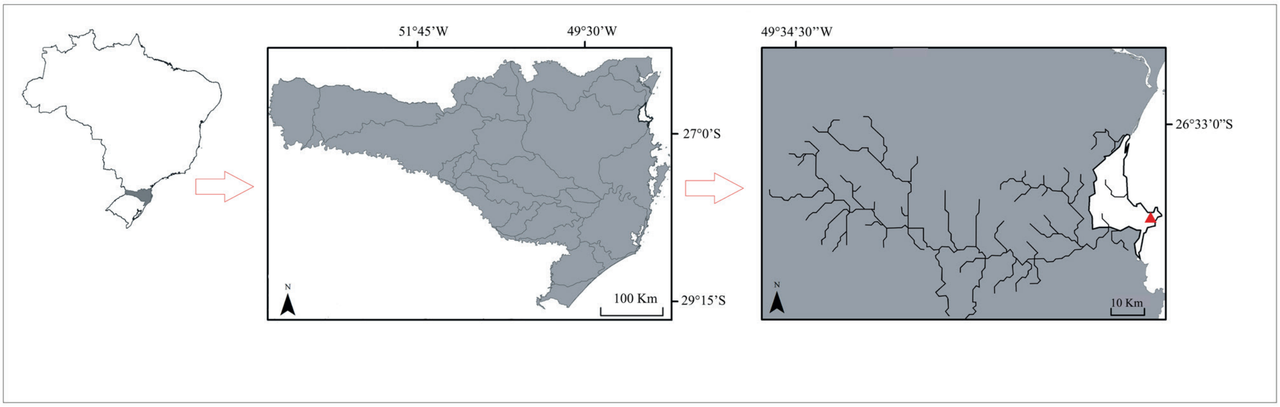

Figure 5. Distribution of *Parastacus tuerkayi* sp. nov. in the state of Santa Catarina, southern Brazil. The type locality is represented by a red triangle.

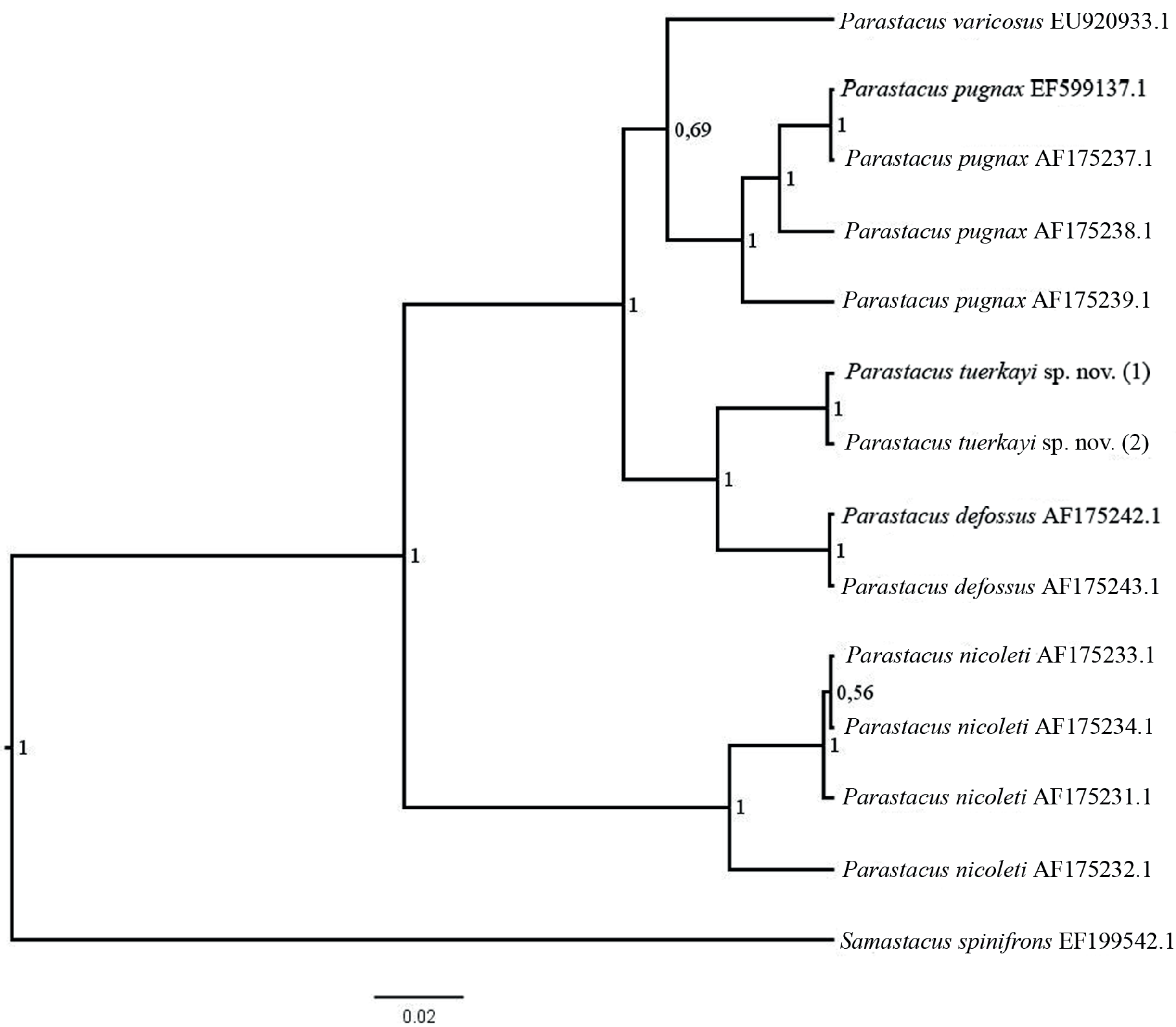

Figure 6. Bayesian inference estimate of phylogenetic relationships among selected species of South American freshwater crayfish based on 16S rRNA gene data from the mitochondrial genome. *Parastacus tuerkayi* sp. nov. (1) and (2) are respectively paratype (UFRGS 6438) and holotype.

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. *Parastacus tuerkayi* sp. nov.					
2. *Parastacus defossus*	0.062				
3. *Parastacus nicoleti*	0.131	0.119			
4. *Parastacus pugnax*	0.088	0.064	0.119		
5. *Parastacus varicosus*	0.087	0.080	0.104	0.063	
6. *Samastacus spinifrons*	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^2 (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^2). 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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CAPÍTULO III

A molecular phylogenetic investigation of the South American freshwater crayfish (Crustacea, Decapoda, Parastacidae) with emphasis on *Parastacus*

Manuscript prepared to be submitted to the Journal: Invertebrate Systematics

A molecular phylogenetic investigation of South American freshwater crayfish (Crustacea: Decapoda: Parastacidae) with emphasis on *Parastacus*

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Running head: Phylogeny of the South American Parastacidae.

Abstract.

South American freshwater crayfish from the genera *Parastacus*, *Samastacus* and *Virilastacus* constitutes the most basal clade with respect to all other parastacids. The evolutionary relationships among the species of these genera have been poorly studied until now. The inclusion of a multigenic approach with a large dataset may help to solve taxonomic questions and to identify potential cryptic species. Using phylogenetic analysis based on three genes (Cox1, 16S and 28S), the relationships among South American genera were evaluated. We corroborated the monophyly of *Parastacus*, and propose a new genus to encompass *Parastacus nicoleti* (Philippi, 1882). Additionally, we verified the presence of ten new species along the genus *Parastacus*.

Additional keywords: Systematics, freshwater crayfish, neotropical crustaceans, parastacids.

Introduction

Freshwater crayfishes comprise about 640 species worldwide distributed (except continental Africa and Antartica) (Crandall and Buhay 2008) and are currently represented by the two super-families Astacoidea Latreille, 1802 and Parastacoidea Huxley, 1879 (Crandall et al. 2000; Crandall and Buhay 2008). The monophyly of freshwater crayfish was already hypothesized by morphological (Dixon et al. 2003; Rode and Babcock 2003; Karasawa et al. 2013; Stern and Crandall 2016) and molecular studies (Crandall *et al*. 2000a,b; Toon *et al*. 2010; Stern and Crandall 2016). The superfamily Parastacoidea is represented only by the family Parastacidae Huxley, 1879. The distribution of the extant species is restricted to the Southern Hemisphere, being found in Australia, New Zealand, New Guinea, Madagascar and South America (Crandall and Buhay 2008; Toon et al. 2010). South American parastacids are composed by three genera: *Parastacus* Huxley, 1879 (eleven species); *Samastacus* Riek, 1971 (one species); and *Virilastacus* Hobbs, 1991 (four species) (Buckup and Rossi 1980; 1993; Riek, 1971; Hobbs 1991; Rudolph and Crandall 2005; 2007; 2012; Ribeiro *et al*.,2016; 2017).

The phylogenetic relationships of the South American freshwater crayfish have been poorly studied until now. Riek (1972) defined two ecological divisions within Parastacidae: the strong burrowers and the moderate burrowers. This classification was based mainly on the orientation of the cheliped dactylus movement and the development of the cephalothoracic grooves. Strong burrowers, a group that includes the South American *Parastacus* and the Australian genera *Engaeus* Erichson, 1846, *Engaewa* Riek, 1967 and *Tenuibranchiurus* Riek, 1951, also known as *Parastacus* species-group, are represented by individuals whose dactylus of chelipeds move in a vertical plane. In moderate burrowers, also known as *Euastacus* species-group, the dactylus of chelipeds move in a horizontal plane. This group includes the South American genus *Samastacus*, the Australian genera *Euastacus* Clark, 1939, *Euastacoides* Riek, 1956, *Astacopsis* Huxley, 1879, *Cherax* Erichson, 1846, *Parastacoides* Clark, 1936, *Geocharax* Clark, 1936 and *Gramastacus* Riek, 1972; the Madagascarian genus *Astacoides* Guérrin-Méneville, 1839; and the New Zealander genus *Paranephrops* White, 1842. Starobogatov (1995) suggested a close affinity of the South American crayfishes to one another and *Paranephrops.* Later, Crandall *et al*. (2000b) conducted a subsequent analysis in which they refuted Riek's and corroborated

Starobogatov's hypothesis. The authors used mitochondrial DNA to investigate these phylogenetic relationships and hypothesized that the South American crayfish genera form a well-supported monophyletic clade closely related to *Paranephrops* and *Parastacoides*. In the same analysis, the genus *Parastacus* constitutes a monophyletic clade with the sister clade composed by the monophyletic group *Samastacus* + *Virilastacus*. Nevertheless, there is a clear distinction of two subclades within *Parastacus*. The Chilean species, *P. pugnax* (Poepigg, 1835) and *P. nicoleti* (Philippi, 1882), split in these two supported clades: one formed solely of *P. nicoleti*, and the other clade represented by *P. pugnax* and the other species from Brazil, Uruguay and Argentina (Crandall *et al*. 2000b).

Rode and Babcock (2003) conducted a cladistic analysis of the freshwater crayfish and some related lobster groups, using morphology of extant and fossil taxa. They recovered Astacidae and Cambaridae as a monophyletic group with Parastacidae as a sister group. The work supported the South American crayfish as a paraphyletic group. However, the exact tree search methods used by these authors were not well explained, raising doubts about their results (see Stern and Crandall 2016).

Toon *et al.* (2010) provided a phylogenetic and biogeographic background of Parastacidae using a multilocus analysis of all genera with a large subset of species. According to the authors, the South American crayfish are a monophyletic group and the most basal clade with respect to all other parastacids. They also stated that the South American group was established around 85 Mya. The trace fossil records from central Patagonia (Argentina) dated from the Late Jurassic to the Late Cretaceous corroborates that the freshwater crayfish were previously spread throughout the southern South America (Bedatou *et al*. 2008).

Stern and Crandall (2016) used morphological data set produced by Rode and Babcock (2003), plus mitochondrial and nuclear DNA, to estimate phylogenies and obtained discrepant topologies. However, the topologies differ between morphological and molecular data showing that the South American crayfish could be paraphyletic and monophyletic respectively. The characters used in this morphological analysis were clearly insufficient and highlighted the strong need to provide more specific characters in order to better understand the evolution of crayfish in a generic level.

Thus, it is evident that several aspects of the phylogeny of the South American Parastacidae remain unclear. Then, the aim of this contribution is to test the phylogenetic relationships of this group of genera (*Parastacus*, *Samastacus* and *Virilastacus*) using a multigenic approach.

Material and Methods

Sampling

Samplings were carried out from March 2013 to June 2016 in several localities of the states of Rio Grande do Sul (RS) and Santa Catarina (SC), Brazil. We used a combination of sampling methods for catch crayfish in different habitats: manual excavation, vacuum pump (7cm x 72 cm), PVC trap closed with a wire screen and baited with cattle liver and cat food and a dip net. Additional samples were obtained from the following institutions: in Brazil – Crustacean Collection of the Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre (RS), ; Museu de Ciência e Tecnologia da Pontificia Universidade Católica do Rio Grande do Sul (MCP), Porto Alegre (SP); and Museu de Zoologia da Universidade de São Paulo (SP); and in Netherlands – Rijskmuseum van Naturlijke Historie, Naturalis, Leiden, (RMNH) (Table 1).

DNA extraction, amplification and sequencing

Muscle tissue was dissected from the walking legs or abdomen from fresh and museum specimens. Total genomic DNA then was extracted using the Puregene kit (Qiagen). For the DNA amplification, we chose the mitochondrial genes cytochrome *c* oxidase subunit 1 (Cox1) and 16 rRNA (16S), and the nuclear 28S rRNA gene (28S). Corresponding primer sets are summarized in Table 2.

The mitochondrial markers 16S and Cox1 were already used in phylogenetic studies for over a decade and are a common choice in decapods (Schubart *et al*., 2000; Mantelatto *et al*., 2007; Vergamini *et al*., 2011; Anker and Baeza, 2012; Carvalho *et al*., 2016). Nuclear markers are more conserved than mitochondrial ones and have different evolution rates, allowing us to infer phylogenetic relationships in a broader range (Hwang and Kim, 1999). The combined use of both kind of markers (mitochondrial and nuclear) is very helpful for the reconstruction of robust phylogenies and thus the evolutionary history of species relationships (Crandall *et al.* 2000; Schubart 2009; Rossi & Mantelatto 2013; Tsang *et al.* 2014; Baeza, 2016).

Polymerase chain reactions (PCR) conditions 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 45s to 1 min, elongation at 72°C for 1 min, and a final extension step at 72°C for 5 min. In cases of unspecific amplification in standard PCR, a touchdown PCR was performed as described by Thiercelin and Schubart (2014). This procedure was employed mainly for 28S PCRs. Annealing temperatures were calculated from primer melting temperatures (16S and 28S: 48°C, Cox1: 50°C). 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 XXXXX and XXXXX. In addition, some sequences obtained from GenBank were included in the analysis. These sequences correspond to the following species of south American crayfishes: *Parastacus brasiliensis* (von Martens, 1869), *P. pilimanus* (von Martens, 1869), *P. saffordi* Faxon, 1898, P. *tuerkayi* Ribeiro, Huber & Araujo, 2017, *P. varicosus* Faxon, 1898, *Samastacus spinifrons* (Philippi, 1882), *Virilastacus araucanius* (Faxon, 1914), *V. rucapihuelensis* Rudolph & Crandall, 2005, *V. retamali* Rudolph & Crandall, 2007, *V. jarai* Rudolph & Crandall, 2012. We also included the following parastacid species as outgroups: *Engaeus fossor* (Erichson, 1846) (Australia), *Paranephrops planifrons* White, 1842 and *P. zealandicus* (White, 1847). Their respective accession numbers from NCBI database were included in the analysis in the Table 1.

Alignment and phylogenetic analysis

All sequences were aligned with BioEdit version 7.2.5 (Hall, 1999) using the ClustalW algorithm (Thompsom *et al*., 1994) and adjusted manually, when required. Poorly aligned blocks of sequence data and large indels were removed with aid of Gblocks (Castresana 2000) allowing less strict flanking positions. The best nucleotide substitution model for each gene was selected using JMODELTEST 2.1.10 with the Akaike Information Criterion (AIC) (95% of confidence) (Darriba *et al*. 2012), suggesting HKI + G as evolutionary model for 16S, GTR+I+G for Cox1 and Tr+N +G for 28S. However, for the latter, we used the corresponding model in BEAST 1.8.3 TN93+G. Phylogenetic relationships were estimated using Bayesian Inference implemented in BEAST 1.8.3 (Drummond *et al*. 2012) for individual and concatenated dataset. We used MESQUITE 3.2 (Maddison and Maddison 2017) to concatenate gene datasets. The gene tree searches were run on computational resources provided by CIPRES gateway (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.* 2014), and the software TreeAnnotator (BEAST package) was used to summarize the trees, with 25% of initial trees discarded as burn-in. The index of nucleotide substitution saturation (Iss) of the sequences was evaluated using the software DAMBE v. 5.3.109 (Xia 2013).

Genetic distances

Genetic distances were calculated for each gene by pairwise comparisons using uncorrected p-distances with the software Mega 7.0 with pairwise deletion and 1000 bootstrap replications (Kumar *et al.* 2016). The average values of the intraspecific and interspecific distances were also calculated.

RESULTS

Phylogenetic Analysis

From the total of 57 individuals used (including outgroups), we obtained 42 16S sequences for 42 specimens, 27 Cox1 sequences for 27 specimens and 25 28S sequences for 25 specimens. The full alignment of three genes was ~2100 bp long. After alignment and removal of difficult to align regions, the dataset was reduced to 1464 bp.

For 16S, the final alignment consisted of 56 sequences of 441 bp (ΠA = 35.1%; ΠC = 11.7%; ΠT = 33.9%; ΠG = 19.3%). These sequences contained 174 variable sites (39.45%), of which 136 (30.84%) were parsimony informative. The sequences were not saturated, based on an Iss that was significantly lower than the Iss_c (Iss = 0.221, Iss_c = 0.996, p < 0.001). For Cox1, the final alignment consisted of 40 sequences of 573 bp (ΠA = 25.1%; ΠC = 20.5%; ΠT = 36.6%; ΠG = 17.8%). These sequences contained 235 variable sites (41.01%), of which 220 (38.39%) were parsimony informative. The sequences were not saturated, based on an Iss that was significantly lower than the Iss_c (Iss = 0.197, Iss_c = 0.709, p < 0.001). For 28S, the final alignment consisted of 33 sequences of 451 bp (ΠA = 17.8%; ΠC = 31.3%; ΠT = 15%; ΠG = 35.9%). These sequences contained 98 variable sites (21.72%), of which 64 (14.19%) were parsimony informative. Overall, the sequences were not saturated, based on an Iss that was significantly lower than the Iss_c (Iss = 0.064 Iss_c = 0.703, $p < 0.001$).

South American freshwater crayfish were recovered as a monophyletic group with high support values (posterior probability 1.0), with a basal clade formed by the species of the genus *Samastacus* + *Virilastacus* (Fig. 1). This group is sister of a group formed by two clades strongly supported: one formed solely by *Parastacus nicoleti* and other including all Atlantic species of *Parastacus* + *P. pugnax* that we called *Parastacus strict sensu*. *Parastacus nicoleti* is the more basal clade within the *Parastacus lato sensu* group. Within *Parastacus strict sensu* group, a more basal clade is formed by *P. pugnax* + *P. saffordi* and *P. varicosus.* Remaining species are joined in a big clade. All nominal species of the genus *Parastacus* included in this analysis had high support values including ten new species identified in present study. According to Bayesian tree (Fig. 1).

Genetic distances

Genetic distances within species of *Parastacus* ranged from 0 to 2.8% for 16S (Table 3), 0 to 5.6% for Cox1 (Table 5) and 0 to 2.8% for 28S (Table 7). Genetic distances amongst congeneric species ranged from 2.5 to 11.6% for 16S (Table 4), 7% to 14% for Cox1 and and from 0.1 % to 3.4% for 28S. For 16S, genetic distances among *Parastacus* species and *S. spinifrons* ranged from 13.6% to 15.8%; and among *Parastacus* species and *Virilastacus* species ranged from 13.8% to 16.7%.

Discussion

Our multilocus phylogenetic analysis based on 16S, Cox1 and 28S markers, corroborated the monophyly of the South American freshwater crayfish genera. The separation of genera *Samastacus* and *Virilastacus* and their sister group relationships are supported by high posterior probabilities values. The monophyly of South American clade was first proposed by Crandall *et al.* (2000b). This monophyletic group was corroborated by the subsequent study of Toon *et al.* (2010). The authors also noticed the early divergence of South American group within Parastacidae. This radiation of lineages started 116Ma and the three South American genera were established around 85 Ma (Toon *et al*. 2010). The close affinity among these genera corroborated the Starobogatov’s (1995) hypothesis that South American clade form a group to the exclusion of Australian parastacids.

Regarding the phylogenetic relationships within *Parastacus*, Crandall *et al*. (2000) noticed a clear distinction of two subclades well supported by high bootstrap values for both maximum likelihood and parsimony analysis. The authors also commented that it should be interesting to obtain data from more *Parastacus* species to better explore the relationships among species within this genus. It has been accepted until now that the present *Parastacus* species belong to the same genus. However, there has been no extensive work in terms of establishing phylogenetic relationships among the species of *Parastacus*. Our phylogenetic tree showed the clear distinction of the two clades within *Parastacus* (*Parastacus stricto sensu* + *P. nicoleti*) with high support values. This result added to some morphological differences among *P. nicoleti* and remaining *Parastacus* species, suggest the creation of a new genus to encompass *P. nicoleti*. Morphological characters shared only by *P. nicoleti* are the protandric hermaphroditism, the intersexuality not present in all individuals; the lack of lateral spines and median longitudinal suture in telson, the conspicuous tubercles in the proximal portion of dactylus and the third maxilliped flagellum reaching the median portion of merus (Buckup and Rossi 1993; Rudolph and Almeida 2000). These morphological characters are usually used to separate genera within Parastacidae (Hobbs 1974).

Our study showed that the used markers are reliable tools for phylogenetic studies and in the distinguishment amongst *Parastacus* species and it can be used when traditional taxonomy is not robust enough to do so and to avoid the problems of morphologically based identification. However, molecular phylogenetics is not a substitute for conventional taxonomy, but should be used as a complement when traditional taxonomy does not recognize discrete morphological variation.

Genetic distances has been widely categorized as an important component of biodiversity evaluations (Ehrlich and Wilson 1991; Humphries *et al.* 1995). In the present contribution, the results obtained in the analyses of genetic *p*-distance allowed us to document the hidden diversity within *Parastacus* with the identification of ten new species well supported. Genetic distances also showed high divergence among *P. nicoleti* and other *Parastacus* species with similar values to divergence among *Parastacus* species and *Samastacus* and *Virilastacu*s. The most important implication of these findings is the recognition and conservation of the involved species. The unrecognized diversity within *Parastacus* presented here is not entirely surprising as it was already reported (I. Miranda, *comunicação pessoal*) and it seems to be recurrent in other crayfish groups (Schultz *et al*. 2007; Dawkins *et al*. 2010; Larson 2016).

This is the first phylogenetic approach for inferring relationships among South American crayfish genera including a large data set (multigenic concatenated analysis of several species). One of our most significant findings is the extent of diversity within *Parastacus*, suggesting that *Parastacus* might be better represented by two different genera, one including *P. nicoleti* and other representing the remaining *Parastacus* samples used in this study (9 of the currently recognized 11 species). This information could help in future phylogenetic and biogeographic studies in order to better understanding the evolutionary history and distribution of the freshwater crayfish species in South America continent.

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Figure captions.

Figure 1. Phylogram based on Bayesian Inference analysis of concatenated sequences 16s, Cox1 and 28S sequences.

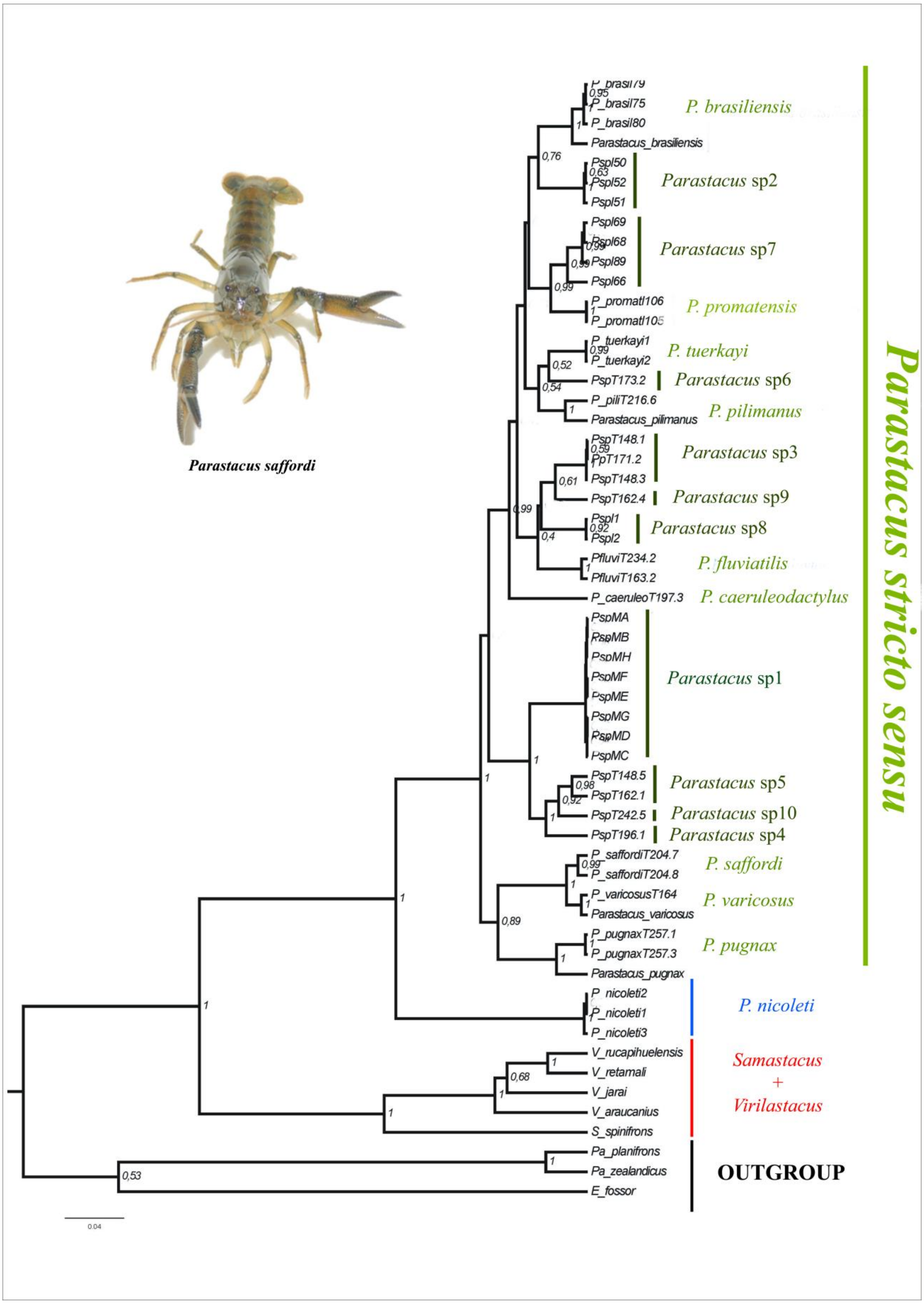


Table 1. Parastacid crayfish species used for the molecular phylogenetic reconstruction (Cox1, 16S and 28S) with respective site of collection and GenBank accession numbers.

Species	Collection Site	Cox1	16S	28S
Parastacus brasiliensis I75	Porto Alegre (RS) – Brazil	XXXXX	–	–
Parastacus brasiliensis I79	Porto Alegre (RS) – Brazil	XXXXX	XXXXX	XXXXX
Parastacus brasiliensis I80	Porto Alegre (RS) – Brazil	XXXXX	XXXXX	XXXXX
Parastacus brasiliensis	Porto Alegre (RS) – Brazil	EF599158	EU175244	EU921138
Parastacus caeruleodactylus	Morrinhos do Sul (RS) – Brazil	XXXXX	XXXXX	XXXXX
Parastacus fluviatilis T234.2	São José dos Ausentes (RS) – Brazil	–	XXXXX	XXXXX
Parastacus fluviatilis T163.2	Morro Montenegro (RS) – Brazil	–	XXXXX	XXXXX
Parastacus pilimanus T216.6	Manoel Viana (RS) – Brazil	–	XXXXX	XXXXX
Parastacus pilimanus	Brazil	FJ965967	AF175246	FJ965967
Parastacus pugnax T253.1	Chile	XXXXX	XXXXX	XXXXX
Parastacus pugnax	Chile	XXXXX	XXXXX	XXXXX
Parastacus pugnax	Chile	EF599157	AF175237	FJ966040
Parastacus promatensis	Chile			
Parastacus nicoleti A	Chile	–	AF175234.1	–
Parastacus nicoleti B	Chile	–	AF175233.1	–
Parastacus nicoleti C	Chile	–	AF175232.1	–
Parastacus saffordi T2014.7	Siderópolis (SC) – Brazil	XXXXX	XXXXX	XXXXX
Parastacus saffordi T2014.8	Siderópolis (SC) – Brazil	XXXXX	XXXXX	XXXXX
Parastacus varicosus T164	Porto Alegre (RS) – Brazil	XXXXX	XXXXX	XXXXX
Parastacus varicosus	Brazil	FJ965969	EU920933	EU920990
Parastacus sp. 1 MA	Mariana Pimentel (RS) – Brazil	XXXXX	XXXXX	–
Parastacus sp. 1 MB	Mariana Pimentel (RS) – Brazil	XXXXX	XXXXX	–
Parastacus sp. 1 MC	Mariana Pimentel (RS) – Brazil	XXXXX	XXXXX	–
Parastacus sp. 1 MD	Mariana Pimentel (RS) – Brazil	XXXXX	XXXXX	–
Parastacus sp. 1 ME	Mariana Pimentel (RS) – Brazil	XXXXX	XXXXX	–
Parastacus sp. 1 MF	Mariana Pimentel (RS) – Brazil	XXXXX	XXXXX	–
Parastacus sp. 1 MG	Mariana Pimentel (RS) – Brazil	XXXXX	XXXXX	–
Parastacus sp. 1 MH	Mariana Pimentel (RS) – Brazil	XXXXX	XXXXX	–
Parastacus sp. 2 I50	Dom Feliciano (RS) - Brazil	XXXXX	XXXXX	XXXXX
Parastacus sp. 2 I51	Dom Feliciano (RS) - Brazil	–	XXXXX	XXXXX
Parastacus sp. 2 I52	Dom Feliciano (RS) - Brazil	XXXXX	XXXXX	–
Parastacus sp. 3 T148.1	Porto Alegre (RS) - Brazil	XXXXX	XXXXX	XXXXX
Parastacus sp. 3 T148.3	Porto Alegre (RS) - Brazil	XXXXX	XXXXX	XXXXX
Parastacus sp. 3 T171.2	Porto Alegre (RS) - Brazil	XXXXX	XXXXX	XXXXX
Parastacus sp. 4 T193.4	Maracajá (SC) – Brazil	–	XXXXX	–
Parastacus sp. 5 T173.2	Florianópolis			
Parastacus sp. 6 T148.5	Banhado dos Pachecos (RS) - Brazil	XXXXX	XXXXX	XXXXX
Parastacus sp. 6 T162.1	Morro da Borússia (RS) - Brazil	XXXXX	XXXXX	–
Parastacus sp. 7 I66	Silveira Martins (RS) – Brazil	XXXXX	XXXXX	–
Parastacus sp. 7 I68	Silveira Martins (RS) – Brazil	XXXXX	XXXXX	–
Parastacus sp. 7 I69	Silveira Martins (RS) – Brazil	XXXXX	XXXXX	XXXXX
Parastacus sp. 7 I89	Silveira Martins (RS) – Brazil	–	XXXXX	XXXXX
Parastacus sp. 8 I1	Caraá (RS) – Brazil	–	XXXXX	XXXXX
Parastacus sp. 8 I2	Caraá (RS) – Brazil	XXXXX	XXXXX	XXXXX
Parastacus sp. 9 T162.4	Morro da Borússia (RS) – Brazil	–	XXXXX	XXXXX
Parastacus sp. 10 T242.5	Eldorado do Sul (RS) – Brazil	–	XXXXX	XXXXX
Samastacus spinifrons	Chile	EF599159	AF175241	EU921137
Virilastacus araucanius	Chile	EF599156	AF175236	FJ966042
Virilastacus jarai	Chile	JQ844468	JQ8444651	–
Virilastacus retamali	Chile	FF599154	–	–
Virilastacus rucapihuelensis	Chile	EF599150	–	–
Engaeus fossor	Australia	EU921144	EU921121	EU921134
Paranephrops planifrons	New Zealand	DQ006415	AF135995	EU921141
Paranephrops zealandicus	New Zealand	DQ006416	DQ006670	FJ006416

Table 2. Primers used for PCR amplification and direct DNA sequencing.

Gene/Primer name		Sequence	Reference
Cox1			
-	COL1b	CCWGCTGGDGGWGGDGAYCC	Schubart (2009)
-	COL6	TYTCHACAAAYCATAAAGAYATYGG	Schubart (2009)
-	COL6a	CWACAAATCATAAAGAYATTGG	Schubart (2009)
-	COL6b	ACAAATCATAAAGATATYGG	Schubart and Huber (2006)
-	COH16	CATYWTTCTGCCATTTTAGA	Schubart (2009)
-	COH6	TADACTTCDGGRTGDCCAAARAAYCA	Schubart and Huber (2006)
-	Coat880	AAG TAA AAT ATG CTC GAG	Zank (2014)
16S			
-	16L2	TGCCTGTTTATCAAAAACAT	Schubart *et al*. (2002)
-	16H2	AGATAGAAACCAACCTGG	Schubart *et al.* (2000)
28S			
-	28D2L	TACCGTGAGGGAAAGYTGAAA	Thiercelin & Schubart (2014)
-	28H2	CGATTTGCACGTCAGAATTGCT'	Thiercelin & Schubart (2014)

Table 3. Genetic divergence matrix (p-distances) of the16S gene within *Parastacus* species (bold) and other South American genera and selected outgroups (*Engaeus* and *Paranephrops*). d – genetic p-distance; S.E. – Standardized Error; n/c – not calculated.

Species	d	S.E.
P. brasiliensis	0.010	0.004
P. caeruleodactylus	**n/c**	**n/c**
P. fluviatilis	0.005	0.003
Parastacus nicoleti	0	0
P. pilimanus	0.028	0.008
P. promatensis	0	0
P. pugnax	0.019	0.005
P. saffordi	0.009	0.004
P. tuerkayi	0	0
P. varicosus	0	0
***Parastacus* sp1**	0	0
***Parastacus* sp2**	0.001	0.001
***Parastacus* sp3**	0	0
***Parastacus* sp4**	**n/c**	**n/c**
***Parastacus* sp5**	0.005	0.003
***Parastacus* sp6**	**n/c**	**n/c**
***Parastacus* sp7**	0.007	0.002
***Parastacus* sp8**	0	0
***Parastacus* sp9**	**n/c**	**n/c**
***Parastacus* sp10**	**n/c**	**n/c**
S. spinifrons	**n/c**	**n/c**
V. araucanius	**n/c**	**n/c**
V. jarai	**n/c**	**n/c**
V. retamali	**n/c**	**n/c**
V. rucapihuelensis	**n/c**	**n/c**
E. fossor	**n/c**	**n/c**
P. planifrons	**n/c**	**n/c**
P. zealandicus	**n/c**	**n/c**

Table 4. Genetic divergence matrix (p-distances) of the 16S gene among *Parastacus* species (bold) and other South American genera and selected outgroups (*Engaeus* and *Paranephrops*). Genetic distances are represented below and standardized errors above.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1.	***P. brasiliensis***		0.012	0.009	0.014	0.010	0.009	0.010	0.012	0.010	0.011	0.013	0.009	0.010	0.013	0.013	0.010	0.009	0.008	0.011	0.013	0.017	0.017	0.018	0.018	0.017	0.020	0.018	0.017
2.	***P. caeruleodactylus***	0.071		0.012	0.015	0.012	0.013	0.011	0.012	0.012	0.012	0.013	0.012	0.012	0.013	0.013	0.012	0.012	0.012	0.014	0.013	0.016	0.017	0.018	0.017	0.017	0.021	0.017	0.017
3.	***P. fluviatilis***	0.042	0.064		0.014	0.010	0.009	0.009	0.011	0.010	0.010	0.012	0.009	0.009	0.013	0.012	0.009	0.010	0.009	0.010	0.013	0.017	0.018	0.018	0.018	0.018	0.021	0.018	0.018
4.	***Parastacus nicoleti***	0.106	0.100	0.097		0.013	0.012	0.013	0.013	0.014	0.012	0.015	0.014	0.014	0.015	0.014	0.014	0.013	0.013	0.014	0.015	0.016	0.017	0.017	0.017	0.017	0.019	0.016	0.016
5.	***P. pilimanus***	0.062	0.072	0.052	0.097		0.010	0.010	0.011	0.009	0.011	0.012	0.010	0.011	0.013	0.013	0.010	0.010	0.009	0.010	0.014	0.017	0.017	0.017	0.017	0.017	0.020	0.017	0.017
6.	***P. promatensis***	0.044	0.077	0.041	0.084	0.056		0.009	0.011	0.010	0.010	0.012	0.009	0.010	0.012	0.012	0.009	0.007	0.008	0.010	0.013	0.016	0.017	0.017	0.017	0.017	0.019	0.016	0.016
7.	***P. pugnax***	0.061	0.064	0.044	0.089	0.055	0.050		0.008	0.010	0.008	0.011	0.010	0.009	0.012	0.012	0.009	0.009	0.009	0.009	0.013	0.016	0.016	0.017	0.017	0.016	0.020	0.016	0.016
8.	***P. saffordi***	0.071	0.066	0.059	0.085	0.069	0.056	0.043		0.010	0.004	0.012	0.011	0.011	0.012	0.012	0.011	0.011	0.010	0.011	0.013	0.017	0.017	0.017	0.018	0.018	0.020	0.017	0.017
9.	***P. tuerkayi***	0.049	0.066	0.041	0.100	0.045	0.050	0.062	0.057		0.010	0.012	0.010	0.010	0.012	0.013	0.009	0.010	0.009	0.011	0.014	0.017	0.017	0.018	0.018	0.017	0.020	0.017	0.017
10.	***P. varicosus***	0.062	0.061	0.050	0.074	0.064	0.051	0.037	0.010	0.052		0.011	0.011	0.010	0.012	0.012	0.011	0.010	0.010	0.010	0.013	0.016	0.017	0.017	0.017	0.017	0.020	0.017	0.017
11.	***Parastacus* sp1**	0.083	0.071	0.068	0.109	0.080	0.075	0.075	0.064	0.068	0.054		0.012	0.012	0.012	0.010	0.012	0.011	0.012	0.013	0.012	0.018	0.018	0.018	0.018	0.018	0.020	0.018	0.018
12.	***Parastacus* sp2**	0.041	0.064	0.039	0.104	0.052	0.037	0.060	0.063	0.051	0.056	0.078		0.009	0.013	0.012	0.010	0.008	0.008	0.010	0.013	0.016	0.016	0.017	0.017	0.016	0.020	0.017	0.016
13.	***Parastacus* sp3**	0.046	0.057	0.036	0.099	0.053	0.043	0.044	0.054	0.039	0.045	0.055	0.034		0.013	0.012	0.010	0.009	0.009	0.009	0.013	0.017	0.018	0.018	0.018	0.018	0.021	0.018	0.017
14.	***Parastacus* sp4**	0.089	0.079	0.079	0.116	0.092	0.075	0.085	0.063	0.079	0.063	0.060	0.078	0.077		0.010	0.012	0.012	0.012	0.014	0.010	0.017	0.017	0.018	0.017	0.017	0.020	0.016	0.016
15.	***Parastacus* sp5**	0.079	0.076	0.068	0.096	0.083	0.063	0.072	0.063	0.072	0.057	0.043	0.067	0.065	0.044		0.012	0.011	0.012	0.013	0.008	0.017	0.018	0.019	0.018	0.019	0.022	0.017	0.017
16.	***Parastacus* sp6**	0.047	0.066	0.038	0.099	0.051	0.040	0.049	0.058	0.040	0.055	0.073	0.050	0.044	0.075	0.067		0.009	0.008	0.011	0.013	0.017	0.017	0.018	0.018	0.018	0.020	0.018	0.018
17.	***Parastacus* sp7**	0.048	0.068	0.043	0.098	0.059	0.029	0.050	0.059	0.053	0.049	0.064	0.039	0.041	0.067	0.051	0.039		0.007	0.010	0.012	0.016	0.016	0.017	0.017	0.016	0.019	0.016	0.015
18.	***Parastacus* sp8**	0.029	0.064	0.036	0.095	0.047	0.029	0.052	0.057	0.039	0.050	0.065	0.030	0.036	0.073	0.059	0.028	0.027		0.009	0.013	0.017	0.017	0.017	0.018	0.017	0.019	0.017	0.016
19.	***Parastacus* sp9**	0.054	0.074	0.044	0.094	0.053	0.042	0.046	0.057	0.047	0.047	0.068	0.043	0.033	0.086	0.069	0.052	0.046	0.039		0.014	0.018	0.018	0.018	0.019	0.018	0.020	0.017	0.017
20.	***Parastacus* sp10**	0.085	0.079	0.074	0.115	0.095	0.067	0.081	0.074	0.085	0.069	0.061	0.073	0.073	0.046	0.025	0.079	0.060	0.069	0.077		0.017	0.018	0.019	0.018	0.018	0.022	0.017	0.017
21.	*S. spinifrons*	0.146	0.136	0.151	0.145	0.157	0.143	0.151	0.149	0.159	0.144	0.168	0.148	0.138	0.152	0.147	0.155	0.136	0.145	0.148	0.144		0.014	0.015	0.015	0.016	0.020	0.016	0.016
22.	*V. araucanius*	0.154	0.146	0.156	0.148	0.157	0.141	0.150	0.151	0.152	0.148	0.153	0.142	0.155	0.145	0.140	0.148	0.136	0.145	0.158	0.146	0.110		0.011	0.011	0.010	0.020	0.016	0.016
23.	*V. jarai*	0.152	0.143	0.156	0.140	0.164	0.138	0.154	0.156	0.155	0.148	0.155	0.146	0.156	0.157	0.152	0.161	0.145	0.152	0.156	0.157	0.109	0.062		0.011	0.011	0.018	0.017	0.016
24.	*V. retamali*	0.151	0.144	0.154	0.155	0.167	0.146	0.157	0.167	0.160	0.159	0.162	0.140	0.157	0.155	0.156	0.171	0.145	0.155	0.164	0.153	0.118	0.065	0.057		0.007	0.020	0.016	0.017
25.	*V. rucapihuelensis*	0.149	0.147	0.147	0.155	0.165	0.139	0.149	0.159	0.158	0.152	0.162	0.138	0.149	0.151	0.163	0.163	0.142	0.148	0.161	0.155	0.118	0.060	0.066	0.028		0.019	0.017	0.016
26.	*E. fossor*	0.212	0.221	0.222	0.201	0.215	0.201	0.216	0.222	0.229	0.213	0.229	0.211	0.226	0.224	0.227	0.219	0.200	0.206	0.214	0.228	0.221	0.204	0.189	0.214	0.209		0.019	0.019
27.	*P. planifrons*	0.157	0.161	0.161	0.143	0.161	0.138	0.148	0.157	0.159	0.149	0.169	0.151	0.150	0.145	0.138	0.157	0.134	0.150	0.143	0.146	0.155	0.139	0.155	0.153	0.151	0.167		0.007
28.	*P. zealandicus*	0.147	0.157	0.149	0.137	0.154	0.128	0.143	0.153	0.151	0.146	0.162	0.145	0.139	0.137	0.131	0.149	0.126	0.142	0.140	0.139	0.149	0.137	0.147	0.148	0.144	0.174	0.023	

Table 5. Genetic divergence matrix (p-distances) of the Cox1 gene within *Parastacus* species (bold) and other South American genera and selected outgroups (*Engaeus* and *Paranephrops*). d – genetic p-distance; S.E. – Standardized Error; n/c – not calculated.

Species	**d**	**S.E.**
P. brasiliensis	0.017	0.004
P. caeruleodactylus	**n/c**	**n/c**
P. pugnax	0.056	0.009
P. pilimanus	**n/c**	**n/c**
P. saffordi	0.002	0.002
P. varicosus	0.019	0.005
***Parastacus* sp1**	0.014	0.004
***Parastacus* sp2**	0.002	0.001
***Parastacus* sp3**	**n/c**	**n/c**
***Parastacus* sp5**	**n/c**	**n/c**
***Parastacus* sp6**	0.005	0.003
***Parastacus* sp7**	0.034	0.006
***Parastacus* sp8**	0	0
S. spinifrons	**n/c**	**n/c**
V. araucanius	**n/c**	**n/c**
V. jarai	**n/c**	**n/c**
V. retamali	**n/c**	**n/c**
V. rucapihuelensis	**n/c**	**n/c**
E. fossor	**n/c**	**n/c**
Paranephrops planifrons	**n/c**	**n/c**
Paranephrops zealandicus	**n/c**	**n/c**

Table 6. Genetic divergence matrix (p-distances) of the Cox1 gene among *Parastacus* species (bold) and other South American genera and selected outgroups (*Engaeus* and *Paranephrops*). Genetic distances are represented below and standardized errors above.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1. P. brasiliensis		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
2. P. caeruleodactylus	0.10		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
3. P. pugnax	0.12	0.13		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
4. P. pilimanus	0.10	0.13	0.14		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
5. P. saffordi	0.13	0.14	0.13	0.16		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
6. P. varicosus	0.12	0.14	0.14	0.16	0.05		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
***7. Parastacus* sp1**	0.11	0.12	0.10	0.13	0.13	0.12		0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
***8. Parastacus* sp2**	0.08	0.12	0.13	0.12	0.14	0.13	0.10		0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
***9. Parastacus* sp3**	0.10	0.11	0.13	0.13	0.15	0.14	0.12	0.12		0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
***10. Parastacus* sp5**	0.10	0.12	0.10	0.12	0.12	0.11	0.06	0.12	0.10		0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
***11. Parastacus* sp6**	0.08	0.09	0.12	0.12	0.13	0.13	0.09	0.10	0.08	0.08		0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
***12. Parastacus* sp7**	0.08	0.11	0.12	0.11	0.13	0.12	0.10	0.10	0.11	0.11	0.10		0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
***13. Parastacus* sp8**	0.07	0.08	0.12	0.11	0.13	0.13	0.09	0.09	0.07	0.09	0.05	0.08		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
14. S. spinifrons	0.25	0.23	0.24	0.24	0.24	0.24	0.25	0.25	0.25	0.25	0.24	0.24	0.24		0.02	0.02	0.02	0.02	0.02	0.02	0.02
15. V. araucanius	0.22	0.21	0.22	0.21	0.22	0.22	0.22	0.21	0.22	0.22	0.22	0.22	0.22	0.20		0.01	0.01	0.01	0.02	0.02	0.02
16. V. jarai	0.23	0.24	0.23	0.22	0.22	0.23	0.24	0.23	0.25	0.24	0.24	0.24	0.25	0.21	0.13		0.01	0.01	0.02	0.02	0.02
17. V. retamali	0.23	0.23	0.23	0.23	0.22	0.22	0.23	0.24	0.24	0.23	0.23	0.24	0.24	0.21	0.12	0.12		0.01	0.02	0.02	0.02
18. V. rucapihuelensis	0.23	0.24	0.23	0.23	0.23	0.23	0.23	0.25	0.23	0.22	0.23	0.22	0.24	0.21	0.14	0.13	0.09		0.02	0.02	0.02
19. E. fossor	0.22	0.22	0.24	0.22	0.24	0.23	0.22	0.22	0.23	0.22	0.21	0.23	0.22	0.24	0.20	0.20	0.21	0.21		0.02	0.02
20. Paranephrops planifrons	0.22	0.22	0.21	0.23	0.22	0.22	0.23	0.22	0.22	0.23	0.22	0.22	0.22	0.24	0.23	0.22	0.22	0.24	0.20		0.01
21. Paranephrops zealandicus	0.22	0.22	0.21	0.23	0.20	0.21	0.22	0.21	0.21	0.23	0.21	0.22	0.21	0.24	0.23	0.21	0.22	0.22	0.19	0.09	

Table 7. Genetic divergence matrix (p-distances) of the 28S gene within *Parastacus* species (bold) and other South American genera and selected outgroups (*Engaeus* and *Paranephrops*). d – genetic p-distance; S.E. – Standardized Error; n/c – not calculated.

Species	**D**	**S.E**
1. P. brasiliensis	0.003	0.002
2. P. caeruleodactylus	**n/c**	**n/c**
3. P. fluviatilis	0	0
4. P. promatensis	0	0
5. P. pugnax	0.002	0.002
6. P. saffordi	0	0
7. P. tuerkayi	**n/c**	**n/c**
8. P. varicosus	**n/c**	**n/c**
9. Parastacus sp2	0	0
10. Parastacus sp3	0.004	0.002
11. Parastacus sp5	0.028	0.007
12. Parastacus sp6	**n/c**	**n/c**
13. Parastacus sp7	0.007	0.004
14. Parastacus sp8	0	0
15. Parastacus sp9	**n/c**	**n/c**
16. Parastacus sp10	**n/c**	**n/c**
17. S. spinifrons	**n/c**	**n/c**
18. V. araucanius	**n/c**	**n/c**
19. E. fossor	**n/c**	**n/c**
20. P. planifrons	**n/c**	**n/c**
21. P. zealandicus	**n/c**	**n/c**

Table 8. Genetic divergence matrix (p-distances) of the 28S gene among *Parastacus* species (bold) and other South American genera and selected outgroups (*Engaeus* and *Paranephrops*). Genetic distances are represented below and standardized errors ab

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1.	***P. brasiliensis***		0.001	0.001	0.006	0.001	0.001	0.003	0.001	0.001	0.003	0.007	0.004	0.006	0.001	0.001	0.008	0.009	0.008	0.014	0.013	0.013
2.	***P. caeruleodactylus***	0.001		0.000	0.006	0.001	0.000	0.003	0.000	0.000	0.002	0.007	0.004	0.006	0.000	0.000	0.008	0.008	0.008	0.014	0.013	0.013
3.	***P. fluviatilis***	0.001	0.000		0.006	0.001	0.000	0.003	0.000	0.000	0.002	0.007	0.004	0.006	0.000	0.000	0.008	0.008	0.008	0.014	0.013	0.013
4.	***P. promatensis***	0.021	0.020	0.020		0.006	0.006	0.007	0.006	0.006	0.007	0.009	0.007	0.006	0.006	0.006	0.009	0.009	0.009	0.014	0.013	0.013
5.	***P. pugnax***	0.003	0.001	0.001	0.021		0.001	0.003	0.001	0.001	0.003	0.007	0.004	0.006	0.001	0.001	0.008	0.008	0.008	0.014	0.013	0.013
6.	***P. saffordi***	0.001	0.000	0.000	0.020	0.001		0.003	0.000	0.000	0.002	0.007	0.004	0.006	0.000	0.000	0.008	0.008	0.008	0.014	0.013	0.013
7.	***P. tuerkayi***	0.006	0.004	0.004	0.022	0.006	0.004		0.003	0.003	0.004	0.008	0.004	0.006	0.003	0.003	0.008	0.009	0.008	0.014	0.013	0.013
8.	***P. varicosus***	0.001	0.000	0.000	0.020	0.001	0.000	0.004		0.000	0.002	0.007	0.004	0.006	0.000	0.000	0.008	0.008	0.008	0.014	0.013	0.013
9.	***Parastacus* sp2**	0.001	0.000	0.000	0.020	0.001	0.000	0.004	0.000		0.002	0.007	0.004	0.006	0.000	0.000	0.008	0.008	0.008	0.014	0.013	0.013
10.	***Parastacus sp3***	0.006	0.004	0.004	0.024	0.006	0.004	0.009	0.004	0.004		0.008	0.004	0.006	0.002	0.002	0.008	0.009	0.008	0.014	0.013	0.013
11.	***Parastacus_sp5***	0.036	0.034	0.034	0.052	0.035	0.034	0.039	0.034	0.034	0.037		0.008	0.009	0.007	0.007	0.006	0.010	0.010	0.014	0.014	0.014
12.	***Parastacus_sp6***	0.008	0.007	0.007	0.024	0.008	0.007	0.007	0.007	0.007	0.011	0.041		0.007	0.004	0.004	0.009	0.009	0.009	0.014	0.013	0.013
13.	***Parastacus_sp7***	0.019	0.018	0.018	0.018	0.019	0.018	0.022	0.018	0.018	0.022	0.051	0.022		0.006	0.006	0.009	0.009	0.009	0.014	0.013	0.013
14.	***Parastacus_sp8***	0,001	0,000	0,000	0,020	0,001	0,000	0,004	0.000	0.000	0.004	0.034	0.007	0.018		0.000	0.008	0.008	0.008	0.014	0.013	0.013
15.	***Parastacus_sp9***	0.001	0.000	0.000	0.020	0.001	0.000	0.004	0.000	0.000	0.004	0.034	0.007	0.018	0.000		0.008	0.008	0.008	0.014	0.013	0.013
16.	***Parastacus_sp10***	0.033	0.031	0.031	0.044	0.031	0.031	0.033	0.031	0.031	0.035	0.025	0.038	0.047	0.031	0.031		0.010	0.010	0.014	0.013	0.013
17.	*S._spinifrons*	0.039	0.038	0.038	0.044	0.039	0.038	0.042	0.038	0.038	0.042	0.063	0.042	0.045	0.038	0.038	0.053		0.003	0.014	0.014	0.014
18.	*V._araucanius*	0.037	0.035	0.035	0.042	0.037	0.035	0.040	0.035	0.035	0.040	0.061	0.040	0.043	0.035	0.035	0.051	0.004		0.014	0.014	0.014
19.	*E._fossor*	0.099	0.098	0.098	0.106	0.099	0.098	0.100	0.098	0.098	0.098	0.114	0.104	0.109	0.098	0.098	0.109	0.109	0.109		0.015	0.015
20.	*Paranephrops_planifrons*	0.090	0.089	0.089	0.100	0.090	0.089	0.093	0.089	0.089	0.093	0.109	0.091	0.099	0.089	0.089	0.100	0.104	0.104	0.120		0.000
21.	*Paranehprops_zealandicus*	0.090	0.089	0.089	0.100	0.090	0.089	0.093	0.089	0.089	0.093	0.109	0.091	0.099	0.089	0.089	0.100	0.104	0.104	0.120	0.000	

Supplementary data

Figure 1. Phylogram based on Bayesian Inference analysis of 16S rRNA gene sequences.

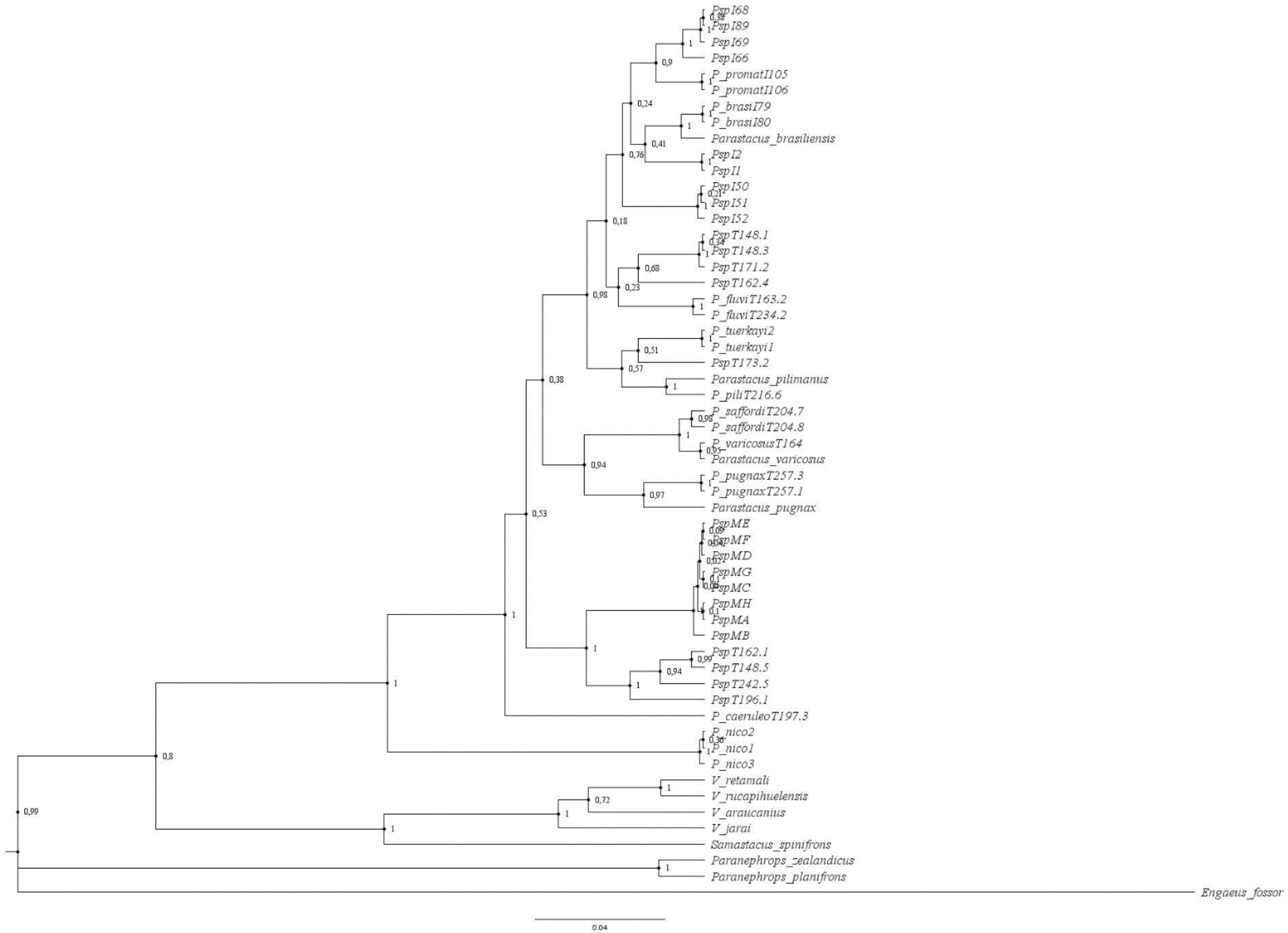


Figure 2 Phylogram based on Bayesian Inference analysis of Cox1 gene sequences.

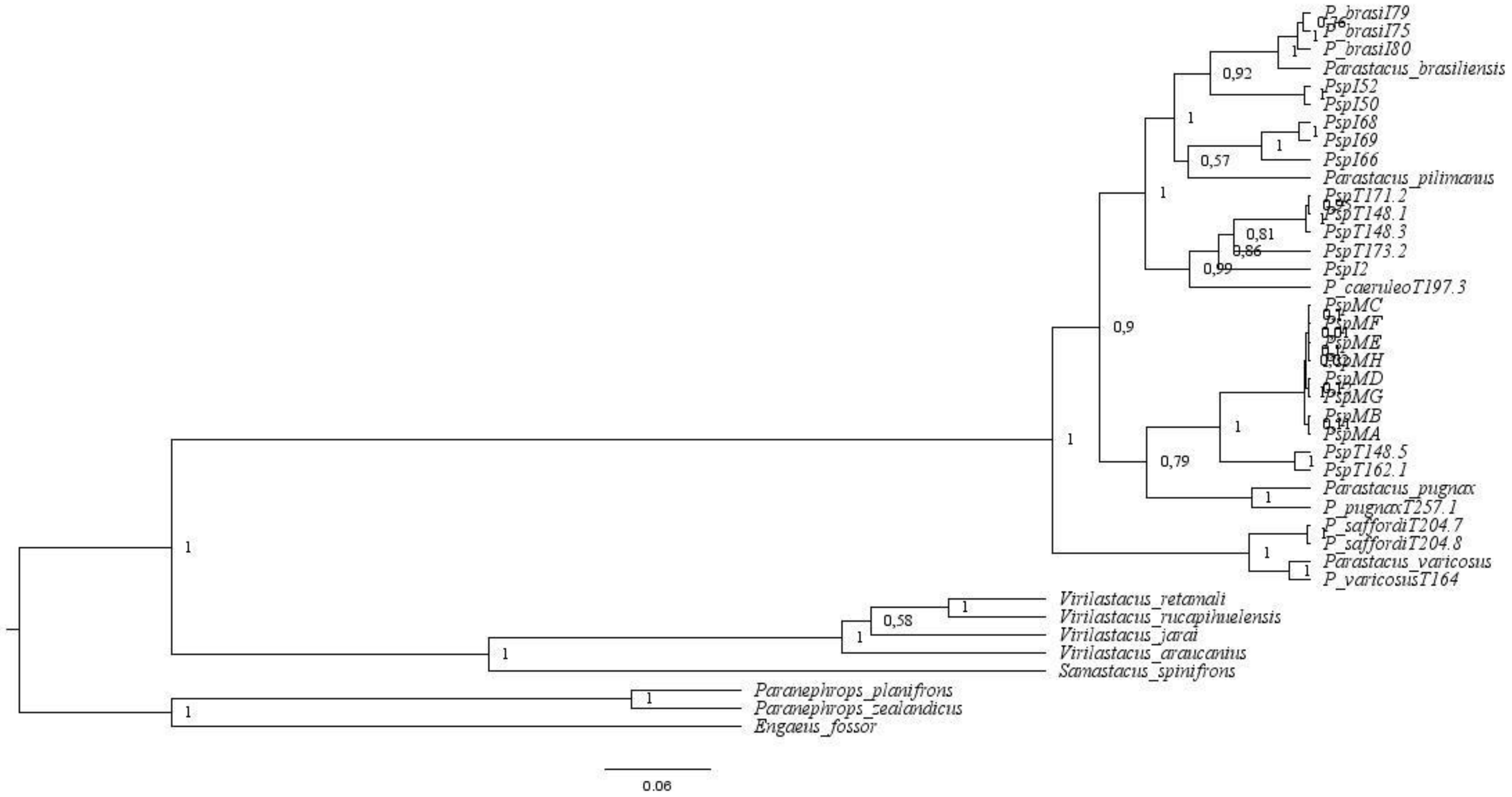
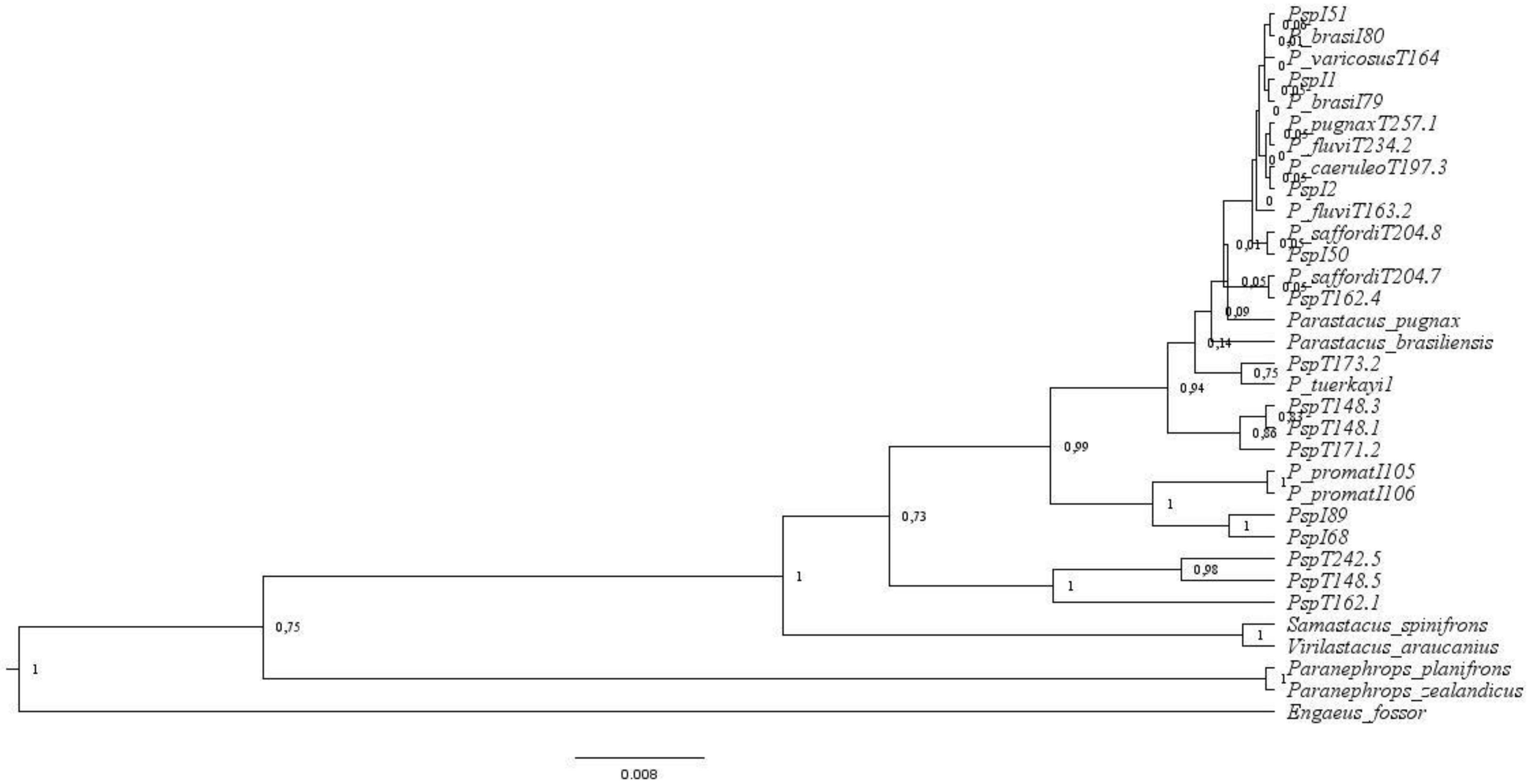


Figure 3 Phylogram based on Bayesian Inference analysis of 28S gene sequences.



ANEXO 1

Invertebrate Systematics - Author Instructions

All manuscripts should be submitted via ScholarOne Manuscripts.

Invertebrate Systematics is an international journal for publication of original and significant contributions on the systematics and biodiversity of invertebrates worldwide.

Submission of a paper implies that the results reported have not been published and are not being considered for publication elsewhere. The journal assumes that all authors of a multi-authored paper agree to its submission. The journal will use its best endeavours to ensure that work published is that of the named authors except where acknowledged and, through its reviewing procedures, that any published results and conclusions are consistent with the primary data. It takes no responsibility for fraud or inaccuracy on the part of the authors. All papers are refereed. Authors may suggest the names of suitable referees.

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Scope

Invertebrate Systematics publishes original and significant contributions on the systematics and evolution of invertebrates worldwide. Morphological and molecular studies are welcomed. Systematic revisions should provide comprehensive treatment of a clearly defined group, and contain information on the phylogeny, biogeography and/or other aspects of biodiversity and general biology of the group. The aim of the work must be clear and all papers should include a discussion indicating the significance of the work, and its broader implications. Contributions on the systematics of selected species that are of economic, medical or veterinary importance may also be considered if these aspects are substantially highlighted in the work. Review or discussion papers on methodology, theoretical systematics, cladistics, phylogeny, molecular biology and biogeography pertinent to invertebrate systematic biology are also encouraged. Pivotal reviews of general invertebrate systematics, containing innovative data or overviews of current theories, are also actively sought.

Submission of manuscripts

To submit your paper, please use our online journal management system ScholarOne Manuscripts, which can be reached directly through this link or from the link on the journal´s homepage. If a first-time user, register via the ´Register here´ link, or use your existing username and password to log in. Then click on the ´Author Centre´ link and proceed.

A covering letter must accompany the submission and should include the name, address, fax and telephone numbers, and email address of the corresponding author. The letter should also contain a statement justifying why the work should be considered for publication in the journal, and that the manuscript has not been published or simultaneously submitted for publication elsewhere. Suggestions of possible referees are welcome.

If you encounter any difficulties, or you have any queries, please contact:
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Authors are advised to read recent issues of the journal to note details of the scope of papers, headings, tables, illustrations, style, and general form. Observance of these and the following details will shorten the time between submission and publication. Poorly prepared and unnecessarily lengthy manuscripts have less chance of being accepted.

For manuscripts involving phylogenetic analyses, electronic copies of the data sets in Nexus or Nona/WinClada format should be supplied with the submitted manuscript (e.g. morphological data sets, aligned nucleotide sequence data).

There are no page charges for publishing in Invertebrate Systematics.

Format of manuscripts
Papers must be typed with double- or 1.5-line spacing throughout and with a margin of at least 3 cm on the left-hand side. All pages of the manuscript must be numbered consecutively, including those carrying references, tables and figure captions, all of which are to be placed after the text. Illustrations, both line drawings and photographs, are to be numbered as figures in a common sequence, and each must be referred to in the text. Figures that are of the same quality as those to be reproduced in the published paper must be included at the end of the electronic file and must be clearly numbered. Colour figures are accepted but will be printed at the author´s expense; cost is dependent upon the number of pages involved and the editor may be consulted for an estimate.

Authors are advised to note the layout of headings, tables and illustrations exemplified in the latest issues of the Journal. Strict observance of these and the requirements listed under ´Preparation of manuscripts´ will shorten the interval between submission and publication.

Rapid communications
The Journal publishes preliminary communications of results that are of special significance or of current and extreme interest. Such papers should yield no more than ten pages when printed, including illustrations, tables and references, and should conform with every aspect of the Notice to Authors. Illustrations must be submitted in a

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Review articles

The Journal welcomes review articles and they should be submitted in the same way as research papers. They should be formatted as simply as possible, using no more than three levels of heading and normal or body text style for the main text. Summary diagrams should be used where possible to reduce the amount of description required to introduce a topic. Authors should remember the wide readership of the Journal when preparing their article, and are advised to discuss the review with the Editor-in-Chief or a member of the Editorial Board before submission.

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Perspective articles are similar to reviews in that they critically assess specific topics of broad interest, explore significant questions, examine the validity of current views in the field, and recommend directions for future research. However, they also give authors the freedom to present thought-provoking ideas, develop novel hypotheses, and speculate on controversial topics. In the interests of provoking discussion among researchers, Perspectives will be made freely available online.

Perspective articles will be commissioned by members of the Editorial Board but prospective authors are welcome to submit proposals to the Editor-in-Chief, who will assess their suitability for publication. Like all content in Invertebrate Systematics, Perspective articles are subject to peer review.

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The Journal welcomes submission of suitably eye-catching, high-quality images for consideration for the cover after the paper has been accepted. The image will reflect the content of one of the papers in the issue and must be suitable for reproduction at very high resolution as the final image will be large (approx. 180 × 165 mm). Submission of an image does not guarantee publication. The choice will be based on several factors, including image quality, interest and appeal, suitability for the Journal, and relevance to the content of the issue.

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General presentation. The work should be presented clearly and concisely in English. The title should reflect the key points of interest in the paper, and should include the order and family (or higher categories if necessary). The names and addresses of all authors should be presented on the first page, together with the full postal address and email address (or facsimile number) of the corresponding author. The introduction

should indicate the reason for the work and include essential background references. Authors must observe the International Code of Zoological Nomenclature and decisions of the International Commission on Zoological Nomenclature. Authors are expected to register all new taxonomic names with ZooBank, the Official Registry of Zoological Nomenclature, after the manuscript has been accepted for publication. All nucleotide sequence data (aligned and unaligned) should be submitted
to Genbank, EMBL or, DDBJ. Morphology data matrices should also be made available online through a permanent site, such as the journal´s website or TreeBASE.

Title. Readers see your title first, and the quality of your title often determines whether they keep reading. Ensure that your title is informative and interesting, and provides information on your key findings. Include higher classification categories. The title should contain all keywords necessary to facilitate retrieval by modern searching techniques.

Running title. Provide an abridged title not exceeding 50 letter spaces, for use at the top of the typeset page.

Abstract. The abstract should comprise the following 4 components: an opening sentence that outlines the context of the study or the problem being examined; the methods used; a summary of the key results; a discussion of the implications of the principal findings. The abstract should not exceed 200 words, but should contain enough information to facilitate retrieval by modern searching techniques.

Short summary. Authors of accepted papers will be asked to provide a short summary for use in the journal's Table of Contents. The short summary should contain the same 4 components as the Abstract, but should be shorter and free from scientific jargon. The first sentence should engage the reader, explaining why your work is important. The second sentence should introduce the problem addressed in the paper; the third the main discovery. The final sentence should describe how the results fit into the bigger picture.

Introduction. The opening paragraph(s) of the Introduction should be written for the general reader of the journal rather than the experts on the group concerned. Taxonomic history should be kept to a minimum and should include only essential information, as much of the relevant history is better placed later in the paper. Although the biology of a particular group might be obvious to the author and other experts and so is often not mentioned, this information is likely to be of interest to the general reader and can also provide useful context for the paper – the ecological importance of a group as predators, scavengers, etc. Finally, the last part of the Introduction should include a clear statement of the aims of the study.

Phylogenetic methods. Analyses must be repeatable and therefore the programs used and the choice of models and program settings should be clearly explained. Measures of support should be shown (e.g. bootstrap, decay index or jacknife values).

Headings. Headings for all taxonomic categories from subspecies upwards should be centred. The name of a genus should be preceded by the word ´Genus´ and followed by the unabbreviated name of the author. Similarly the author of a species should follow the species name. The date should not be given in headings. The abbreviations ´gen. nov.´, ´sp. nov.´, ´subsp. nov.´ must be used for indicating a new genus, species, or subspecies and should be separated from the new name by a comma. Genera and species should be treated in alphabetical order, unless another logical order is preferred, in which case the reason for the order should be given in the Methods section, so that a species of interest can be found easily.

Synonymies. If adequate synonymies and references are reasonably accessible in the literature, these need not be repeated in full, but a reference to that source must be given. The reference to the original description should always appear immediately below the centred headings. References given, whether to the accepted name or synonyms, should include the author, date, page number and any figure numbers, but should exclude the name of the publication, as this is given under author and date in a list of references at the end of the paper. Synonymies should not be further annotated. Multiple synonyms should be arranged in order of date of first application to the unit in question, and, under each name the separate references (if more than one is given) should be in chronological order.

Citation of type species of genera and location of primary types of known species. The type species, with author and date, should be cited immediately beneath the synonymy for each genus treated. The author and date of publication of a taxonomic name should be separated by a comma. The names of two or more authors should be linked with an ampersand (&). For each known species treated, the museum in which the primary type (holotype, lectotype or neotype) is preserved should be similarly stated, or an account given of the steps taken to ascertain the whereabouts of the type in the event that it could not be located.

Type designation and lodgment. Authors are required to follow the requirements of the International Code of Zoological Nomenclature (Fourth Edition, effective from 1 January 2000) with respect to designation of types and their lodgment. Types should be lodged in publicly accessible formal repositories, such as a museum or other public institution.

It is expected that all material has been collected under appropriate collection permits and approved ethics guidelines, and a statement to this effect should be included in the Acknowledgments. Authors should be aware of the provisions of the regulations that govern the import and export of all specimens of wildlife to and from the countries in which they have worked. Among other things the regulations often require that any specimen exported from the country that is subsequently designated a primary type must be lodged in an appropriate instituition of the source country, e.g. The Australian Wildlife Protection (Regulation of Exports and Imports) Act 1982 and associated Regulations 1984, requires that any specimen exported from Australia after 1 May 1984 and that is subsequently designated a primary type of an Australian native animal must be lodged in an Australian institution.

Material examined. Concise lists of specimens examined should be presented for each species.
Type specimens: full details should be provided for type material and information on specimen labels should be replicated with supplementary details (e.g. current country names, altitudes, etc.) provided in square brackets. If the day of the month is included, the month is to be given in lower-case roman numerals. The year is never abbreviated. Authors should consult recent issues of the journal to ensure lists are consistent with journal style with respect to punctuation, use of bold headings for country and state names, etc. Non-type specimens: lists should be reduced to a bare minimum, and at most confined to the number and sex of specimens, locality name and repository (with the registration or accession number of specimens). Lists should be arranged in alphabetical or other appropriate order of localities within States or similar major regions. Where the sex of specimens is given, the symbol ´#´ should be used for males, and ´@´ for females to enable easy replacement during typesetting. Significant information regarding distribution, habitat, host association, seasonality, behaviour, or biology should be summarized in the body of the paper, e.g. in the Remarks section. Authors are encouraged to provide distribution maps where appropriate. If authors request, a full list of all material examined, including complete specimen information, can be submitted as an additional file to be placed on the journal´s website as Supplementary Material.

Descriptions. The ´telegraphic´ style is required for descriptions and diagnoses. Diagnoses should contain only the distinguishing characters or combination of characters for that taxon. Comparative comments are to be placed under ´Remarks´. The use of figures to illustrate descriptions is encouraged and should permit some reduction in the length of the verbal description of the parts figured. Authors should subdivide long descriptions by using appropriate subordinate headings.

Keys. Keys should use clear-cut characters that can be interpreted unambiguously. The judicious use of triplets, instead of couplets, is permissible to improve the efficiency of the key. Headings to keys should be self-explanatory. Tabular (i.e. synoptic or special purpose) keys are permitted where appropriate.

Footnotes. Footnotes are discouraged and should be used only when essential. They should be placed within horizontal rules immediately under the lines to which they refer.

References. In the text, references are cited chronologically by the author and date and are not numbered. Names of two coauthors are linked by ´and´; for three or more, the first author´s name is followed by ´et al.´. Citation of authorities (name and date) should be given when a taxon name is first mentioned. Two or more coauthors of a name are linked by ´&´. All references cited must be listed alphabetically at the end of the paper; all entries in this list must correspond to references in the text. No editorial responsibility can be taken for the accuracy of the references and authors are requested to check these with special care. Titles must be included for all references. Papers that have not been accepted for publication may not be included in the list of references and must be cited either as ´unpublished data´ or as ´personal communication´; the use of such citations is discouraged. Authors are referred to the latest issues of the Journal for the style to be used in citing references to books and other literature. Titles of periodicals must not be abbreviated.

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- Haswell, W. A. (1882). ´Catalogue of the Australian Stalk- and Sessile-eyed Crustacea.´ (Australian Museum: Sydney, Australia.)
- Sluys, R., and Ball, I. R. (1988). A synopsis of the marine triclads of Australia and New Zealand (Platyhelminthes : Tricladida : Maricola). Invertebrate Taxonomy 2, 915-959.
- Voss, G. L. (1988). Evolution and phylogenetic relationships of deep-sea octopods (Cirrata and Incirrata). In ´The Mollusca. Vol. 12. Palaeontology and Neontology of Cephalopods´. (Eds M. R. Clarke and E. R. Trueman.) pp. 253-276. (Academic Press: London, UK.)
- Erzinçlioglu, Y. Z. (1984). ´Studies on the Morphology and Taxonomy of the Immature stages of Calliphoridae, with Analysis of Phylogenetic Relationships within the Family, and Between It and Other Groups in the Cyclorrhapha (Diptera).´ PhD thesis., (University of Durham:, UK.)
- Huelsenbeck, J. P., and Ronquist, F. (2001). ´MrBayes 2.01: Bayesian Inference of Phylogeny.´ Available at http://morphbank.ebc.uu.se/mrbayes/.

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Statistical evaluation of results. The tests should be described briefly and, if necessary, supported by references. Numbers of individuals, mean values, ranges and measures of variability should be stated. It should be made clear whether the standard deviation or the standard error of the mean has been given.

Tables

Each table (including data matrices and character lists, where appropriate) must be numbered with arabic numerals and must be accompanied by a title. A headnote containing material relevant to the whole table should start on a new line, as it will be set in a different font. Tables should be arranged with regard to the dimensions of the printed page (17.5 by 22.5 cm in two 8.5-cm columns) and the number of table columns kept to a minimum. Excessive subdivision of column headings is undesirable and long headings should be avoided by the use of explanatory notes, which should be incorporated into the headnote. Footnotes should be kept to a minimum and reserved for specific items in columns. Horizontal rules should be inserted only above and below the column headings and at the foot of the table. Vertical rules must not be used. Each table must be referred to in the text. Only in exceptional circumstances will the presentation of essentially the same data in both tabular and graphical form be permitted; where adequate, the graphical form should be used. Short tables can frequently be incorporated into the text as a sentence or as a brief untitled tabulation.

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Authors should submit their illustrations in electronic format (see ´Electronic files´). All illustrations should conform to the general instructions for layout as follows.

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Line drawings should be scanned at high resolution, at least 800 dpi at final (printed) size, and saved in black and white bitmap format as TIFF files. Fine line drawings with a lot of variable grey shading should be saved in greyscale format as TIFF files. Photographs should be scanned at a resolution of at least 300 dpi at final size and saved in greyscale format as TIFF or Photoshop files. It is preferable for labels to be applied electronically to the scanned images, rather than scanning manually labeled figures. Electronic files of colour figures or photographs should be saved in CMYK colour not in RGB colour, because the CMYK format is required for printing. Authors should note that colours change when converted to CMYK from RGB and when printed from different types of printer; hence it is important to provide a hard copy in which the colours are correct and match the CMYK file version.

Computer-generated figures, including cladograms, prepared using either a draw or chart/graph program must be saved in one of the following formats: Adobe Illustrator (.ai) (preferred format), encapsulated postscript (.eps), encapsulated metafile (.emf), Windows metafile (.wmf) or Excel; cladograms should be saved as EMF or WMF files (from PAUP*, trees can be exported as PICT files or opened in TreeView and saved in WMF format; from WinClada, trees can be saved in EMF format); illustrations created using PowerPoint should be saved in PowerPoint; CorelDraw files should be saved as EPS or .AI files; charts created on a Macintosh computer should be saved as EPS, PS or PICT files. In all cases they should be editable vector graphic files. Avoid using 3D surface area charts because print quality is often poor. Remove colours from all charts and graphs. Figures embedded in Word are often difficult to import successfully into typesetting programs; thus, if you can only provide Word files for your figures, please also make sure that you give us high-quality, hardcopy originals, not larger than A4 size, for scanning if necessary.

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CAPÍTULO IV

Taxonomic review of the genus *Parastacus* Huxley, 1879 (Crustacea: Decapoda: Astacidea: Parastacidae)

Manuscript prepared to be submitted to the Journal: Zootaxa

Manuscript to be submited to Zootaxa

Monograph

ZOOTAXA

Taxonomic review of the genus *Parastacus* Huxley, 1879 (Crustacea: Decapoda: Astacidea: Parastacidae)

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Short running title: REVIEW OF *PARASTACUS*

Abstract

The freshwater crayfish genus *Parastacus* Huxley, 1879 (Parastacidae) is reviewed. Eight species are redescribed: *Parastacus brasiliensis* (von Martens, 1869), *P. defossus* Faxon, 1898, *P. laevigatus* Buckup & Rossi, 1980, *P. pilimanus* (Von Martens, 1869), *P. pugnax* (Poepigg, 1835), *P. promatensis* Fontoura & Conter, 2008, *P. saffordi* and *P. varicosus* Faxon, 1898. A new genus is proposed to encompass *P. nicoleti* (Philippi, 1882) from Chile, and this species is also redescribed. In addition, 10 new species are described. Identification keys, descriptions, diagnoses, synonymies and distribution maps of the genera and species are provided.

Key words: burrowing crayfish, freshwater decapods, neotropical crustaceans, parastacid, taxonomy

Introduction

The freshwater crayfishes are a conspicuous and diverse group of decapod crustaceans, worldwide distributed, except in continental Africa and Antarctica (Crandall and Buhay 2008). They are divided in two superfamilies: Astacoidea Latreille, 1882, which occurs in the northern hemisphere and is composed by the families Astacidae Latreille, 1802 and Cambaridae Hobbs, 1842; and Parastacoidea Huxley, 1879, which is composed only by the family Parastacidae Huxley, 1879 and is distributed in the southern hemisphere (Crandall and Buhay, 2008).

Parastacidae includes 15 genera and about 170 species distributed in Australia, New Zealand, New Guinea, Madagascar and South America (Crandall & Buhay, 2008; Toon *et al.* 2010). The South American parastacids are constituted by three genera, *Parastacus* Huxley, 1879 including 11 species, *Samastacus* Riek, 1971, including a single species, and *Virilastacus*, including four species (Ribeiro *et al*. 2016; 2017; Rudolph 2010; Rudolph and Crandall 2005; 2007; 2012).

A review of the taxonomic history of Parastacus

The first authors who described crayfish from South America used the generic name *Astacus* Fabricius, 1775 for all species (Poeppig 1835; H. Milne Edwards 1837; Nicolet 1849; von Martens 1869; and Philippi 1882; 1894). Poeppig (1835) mentioned a crayfish with a native name “Camaron de tierra” that possess burrowing habits and was found in the neighborhood of Talcahuano, Chile. For this species, he gave the name *A. pugnax* (Poepigg, 1835), but did not provide any morphological description. Milne Edwards (1837) provided a brief description of *A. chilensis* Milne-Edwards, 1837. Erichson (1846) reviewed the genus *Astacus* and proposed five subgenera. He placed all the American crayfish in the subgenus *Cambarus*, including *Astacus (C.) Chilensis*.

Philipp’s contributions concerning the Chilean crayfish, provided the description of the species *A. nicoleti* Philippi, 1882, *A. spinifrons* Philippi, 1882 and *A. bimaculatus* Phillipi, 1894 without mentioning the subgenus *Cambarus*. Nicolet (1849) briefly described and illustrated a crayfish species from Chile without name it. The description of *A. nicoleti* by Philippi (1882) was based on the species described by Nicolet (1849). Bahamonde (1958) believes that Philippi had the opportunity to analyze Nicolet’s material, which no longer exists in the collection of the “Museo Nacional de Historia Natural”, Chile.

Von Martens (1869) described *A. pilimanus* von Martens, 1869 and *A. Brasiliensis* von Martens, 1869 based on material collected by Hensel in southern Brazil (Buckup & Bond-Buckup 1994). Huxley (1879), in an extensive analysis of several crayfish genera, recognized that the South American ones were morphologically closely related to the crayfish from Madagascar and Australia. He analyzed two well-preserved males of *A. brasiliensis* and *A. pilimanus* and noticed that their branchial structure were so much alike and resembled those of the genus *Cherax* Erichson, 1846. Huxley (1879) created the family Parastacidae and the genus *Parastacus* based on the branchial traits, the lack of the first pair of pleopods, and the telson incompletely divided by a transverse suture.

However, the generic name *Parastacus* was not widely applied to the South American crayfish until the work of Faxon (1898), *e.g.* Philippi (1882). Faxon (1898) analyzed the collections of American museums, expanding the Huxley’s diagnosis and described five new species, *P. defossus* Faxon, 1898, *P. saffordi* Faxon, 1898, *P. varicosus* Faxon, 1898, *P. hassleri* Faxon, 1898 and *P. agassizii* Faxon, 1898. However, he did not realize that *P. hassleri* and *P. agassizii* were previously described under the names *A. pugnax* and *A. spinifrons* respectively. He also proposed to call *A. chilensis* as *Parastacus nicoletii*.

Porter (1904) provided some data about the Chilean *Parastacus* species *P. hassleri* and *P. agassizi*. Ortmann (1902) recorded the occurrence of *P. saffordi* and *P.*

defossus in Brazil, based on material from Rio Grande do Sul collected by Hermann von Ihering and also suggested possible occurrence of *Parastacus* for the state of Santa Catarina. He also considered *P. chilensis*, *P. spinifrons* and *P. bimaculatus* as doubtful species. Lenz (1902) classified some sympatric specimens from Tumbes, Chile as *P. nicoletii* and *P. hassleri*. Rathbun (1910), in a checklist of crustaceans from Peru and adjacent countries, placed *Parastacus* species in the family Astacidae and, cited *P. chilensis* and *P. hassleri* and added *P. nicoletii* and *P. agassizii*. She said that Professor E. L. Bouvier of the "Muséum National d'Histoire Naturelle", France examined the type of *A. chilensis* and confirmed it to belong to *Parastacus*.

In 1914, Faxon described *P. araucanius* Faxon, 1914 from Chile, improved the description of *P. spinifrons* (Philippi, 1882) and commented on the validity of *P. bimaculatus* (Philippi, 1894). Porter (1917) provided a new record of *P. nicoleti* from Chile, considering Faxon as the author of the species.

Holthuis (1952) commented that the taxonomic status of *P. chilensis* remained still uncertain and that the type should be reexamined to establish the specific characters. He also included *P. bimaculatus* as a synonym of *P. spinifrons*, discussed about the burrowing capabilities of *P. araucanius* and confirmed *P. pugnax* as a valid species.

Bahamonde (1958) redescribed and validated *P. nicoleti* based on the analysis of several specimens, but stated that the type specimen was lost. He provided drawings, pictures, measurements and comments on the species' distribution and biological aspects.

Riek (1971), in a review of the South American crayfish, proposed that the two species previously assigned to *Parastacus* should belong to a new genus, *Samastacus* Riek, 1971, establishing the new combinations *S. araucanius* (Faxon, 1914) and *S. spinifrons* (Philippi, 1882). According to this author, *Parastacus* was represented by burrowing species with the chelae moving vertically and *Samastacus* by the stream and lake dwelling species with chelae moving horizontally. Riek (1971) also included *P. brasiliensis* in the synonymy of *P. pilimanus* without a convincing morphological explanation and proposed some diagnostic characters for both genera and keys for the already described species.

Buckup & Rossi (1980) reviewed all Brazilian species of *Parastacus*, providing several new records and describing *Parastacus laevigatus* Buckup & Rossi, 1980 from the state of Santa Catarina. In that review, the authors also considered *P. brasiliensis* as a valid species based on morphological and morphometric analysis.

The American astacologist Dr. Horton H. Hobbs Jr. published several papers about freshwater crayfish. In 1974, Hobbs provided a synopsis of the families and genera of crayfish, giving a new diagnosis for *Parastacus* and in 1989, provided an illustrated checklist for all species and genera found in the Americas. In 1991, he proposed a new genus, *Virilastacus* Hobbs, 1991, for *S. araucanius*, redescribing the species based on material from the Museum of Comparative Zoology, Cambridge, Massachusetts, United States of America. In that contribution, the author also included modifications of the diagnosis for the genera *Parastacus* and *Samastacus* and commented about the parastacid mandibular features as a character to take into consideration.

Buckup & Rossi (1993) redescribed all parastacid species from the South-Andean region and provided new illustrations and an identification key. However, the authors did not mention *Virilastacus* proposed by Hobbs (1991) because their paper was in press when the contribution of Hobbs was published.

Fontoura & Conter (2008) proposed the division of *P. brasiliensis* in two subspecies, *P. brasiliensis brasiliensis* and *P. brasiliensis promatensis* based mainly in a morphometric analysis. However, there is evidence that the latter should be elevated to a species level, *P. promatensis* Fontoura & Conter, 2008, based in genetic and distribution analysis (I. Miranda, *personal communication*).

Virilastacus remained a monotypic genus until 2005, when *V. rucapihuelensis* Rudolph & Crandall, 2005 was described. Later on two more species were added to the genus, *V. retamali* Rudolph & Crandall, 2007 and *V. jarai* Rudolph & Crandall, 2012 (Rudolph and Crandall 2005; 2007; 2012). The genus *Samastacus* remains monotypic, but *S. spinifrons* has two morphotypes (Rudolph *et al*. 2016). After a gap of 36 years, three new species of *Parastacus* were described: *P. fluviatilis* Ribeiro & Buckup, 2016; *P. caeruleodactylus* Ribeiro & Araujo, 2016; and *P. tuerkayi* Ribeiro, Huber & Araujo, 2017 (Ribeiro *et al*. 2016; 2017).

The aim of this contribution is to review the taxonomy of the species of *Parastacus.* This review is based on material deposited in museums from Brazil, Uruguay, Argentina, Chile, Germany, Netherlands, France, England and the United States of America. All the species of *Parastacus* are revisited, one genus and ten new species are described. In addition, information on the geographical distribution and an identification key are also provided.

Material and Methods

All descriptions are based on material deposited in scientific collections and additional specimens collected in field expeditions. During fieldwork, we observed that the species can occupy different habitats. For this reason, we employed different techniques: (1) for stream and pond dwelling species, we used a dip net to sample along the margins and a PVC trap (50 mm x 20 cm) closed with a wire screen and baited with chicken liver. We installed the traps in late afternoon immersing it in the running water and removing it in the next morning (for more details see Fontoura & Buckup 1989); (2) for burrowing species, we used a vacuum pump (7cm x 72 cm) and manual excavation. The latter provided information on the burrow systems.

In laboratory, fresh specimens were photographed to record color pattern in life. After, they were crioanesthetized and preserved in 96% ethanol. Drawings were prepared under a stereomicroscope fitted with a *camara lucida*. All measurements were performed with vernier calipers with 0.1 mm accuracy and a millimetric ocular on a stereomicroscope. Measurements of the type material of the new species can be found in the supplementary data (Appendix 1). The definition of each measurement can be found in Ribeiro *et al*. (2016).

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). Size and width of abdomen were defined in relation to cephalothorax length and width. We used the relation between AL and CL: AL ≥ 79% of CL long, AL < 79% of CL short; and AW and CW: AW ≥ 80% of CW wide, AW < 80% of CW narrow. In order to define size of the eyes, we employed the relation between CMW and OW: CMW ≥ 65% of OW large (macrophtalm), CMW < 65% of OW small (microphtalm). In order to define the front width in relation to the cephalothorax width, we used the relation between FW and CW: FW ≥ 47% of CW wide, FW < 47% of CW narrow.

Morphological descriptions and terminologies follow Riek (1971), Buckup & Rossi (1980), Hobbs (1987), Morgan (1997), Holdich (2002) and Ribeiro *et al*. (2016; 2017). The taxonomic classification follows De Grave *et al*. (2009). Branchial count follows

Huxley (1879). For some descriptions, we included additional characters not mentioned in the original descriptions or past redescriptions.

Burrowing behavior and burrows were classified according to Hobbs (1942) and Horwitz and Richardson (1986) when data was available. Hobbs's classification categorizes crayfish burrowing behaviour in three groups based on the complexity of burrow structure, the conection to open waters, seasonality and reproductive period and time that the crayfish spends inside the burrow: primary burrowers spend most of their lives inside the burrow and built complex burrows; secondary burrowers spend much of their life inside the burrow, but can stay in the surface during wet seasons and build less complex burrows; and tertiary burrowers live in open water during most of their lives, using burrows as a shelter to brood eggs, to avoid dissecation or freezing or protect against predators, and build simple burrows. Horwitz and Richardson's classification take into account the relation of the burrow to surface water and the water table: type 1 burrows occur in or are directly connected to water bodies; type 2 burrows are connected to the water table; and type 3 burrows are independent to the water table.

Two keys are provided: (1) a key to identify specimens to generic level; and (2) a key to identify *Parastacus* species. All keys have been prepared based on preserved material. Then, color descriptions were not included in the keys. Characters have been chosen that can be examined without damage the specimens. Brief locality notes are also given (in italics in brackets) for all species to assist in the confirming identification.

The type material was deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP), state of São Paulo, Brazil. Paratypes were deposited in the MZUSP, Museu Nacional, Rio de Janeiro (MNRJ), Carcinological Collection of the Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio do Grande do Sul (UFRGS), and in the Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul (MCP).

Abbreviations:

Measurements

SLP = Thoracic Sternite Lateral Processes

S1 = Abdominal Somite 1

S2 = Abdominal Somite 2

S3 = Abdominal Somite 3

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

Museums and collections

FCEN–UBA = Facultad de Ciencias Exatas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina;

FC–UDELAR = Facultad de Ciencias de la Universidad de la Republica, Montevideu, Uruguay;

MCP = Museu de Ciência e Tecnologia da Pontificia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil;

MNRJ = Museu Nacional, Rio de Janeiro, Brazil;

MACN–In = Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, Argentina;

MFA-ZI = Museo Provincial de Ciencias Naturales Florentio Ameghino, Santa Fé, Argentina;

MLP = Facultad de Ciencias Naturales y Museo de La Plata, La Plata, Argentina;

MNHNCL = Museo Nacional de Historia Natural de Chile, Santiago, Chile;

MNB = Museum für Naturkunde, Berlin, Germany;

MZUSP = Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil;

MZ UNISINOS = Museu de Zoologia da Universidade do Vale do Rio dos Sinos, São Leopoldo, Rio Grande do Sul, Brazil;

BMNH= Britsh Museum of Natural History, London, England;

UFC = Universidade Federal do Ceará, Fortaleza, Ceará, Brazil;

UFRGS = Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil;

USNM = National Museum of Natural History, Smithsonian Institute, Washington, DC, United States of America;

RMNH = Rijskmuseum van Naturlijke Historie, Naturalis, Leiden, Netherlands;

SMF = Senckenberg Museum, Frankfurt, Germany;

ZCUMCN = Museu de Ciências Naturais do Centro Universitário, Universidade Integrada Vale do Taquari, Lajeado, Rio Grande do Sul, Brazil;

ZSM = Zoologische Staatssammlung München, Munich, Germany.

Other abbreviations

snd – sex not determined

w/n – without number

Systematic account

Infraorder Astacidea Latreille, 1802

Superfamily Parastacoidea Huxley, 1879

Family Parastacidae Huxley, 1879

Genus *Parastacus* Huxley, 1879

Astacus.—Poeppig, 1835:314.

Parastacus Huxley, 1879: 759, 771.

Type species. *Astacus pilimanus* by subsequent designation (Faxon, 1898: 683): *Astacus pilimanus* von Martens, 1859: 15. Gender: masculine.]

Diagnosis.

Moderate body size. Carapace lacking spines and tubercles; rostrum mainly triangular, but spatulate in *P. brasiliensis* and quadratic in *P. brevirostris* **sp. nov.**; postorbital carinae ranging from obsolete to well developed; cervical groove V or U–shaped, deeply impressed; branchiocardic grooves inconspicuous or conspicuous with anterolateral part often hardly dinguinshable and very close to cervical groove, two usually merging dorsolaterally. Abdomen lacking tubercles or spines; first abdominal pleurae partly overlapped by that of second; posterior margin of second abdominal pleura rounded, except in *P. varicosus*. Telson subtriangular or subrectangular, entirely or weakly calcified with dorsomedian longitudinal groove (sometimes inconspiscuous) and with blunt or sharp lateral spines. Epistome with anteromedian lobe pentagonal or hexagonal shape. Antennal scale lateral margin straight to curved with a terminal strong spine. Third maxilliped with mesial half of ventral surface of ischium bearing setiferous punctuations, but *P. varicosus* and *P. saffordi* presents a line of sharp and blunt spines in the middle part, respectively; setae coverture sparse or dense; flagellum of exopodite reaching, or slightly overreaching, basal part of merus. Caudal molar process of mandible unicuspidate, bicuspidate or tricuspidate.; incisive lobe with eight to ten teeth, usually the third is the largest. Chelipeds carpus can be divided by a groove impression; propodus dorsal region with squamose or verrucous tubercles that can form well defined rows, ventral region with two rows of tubercles that can reach or surpass the beginning of the fixed finger; dactylus moving subvertically. Male genitalia consisting of a fixed and slightly elevated ventromesial ridge bearing a small noncalcified papilla; male cuticle partition (*sensu* Morgan, 1986) present; all member with supernumerary gonopores in both sexes (permanent intersexuality). Branchial count 20 + epr + r, or 20 + ep + r (podobranchs on segments VIII–XIII; anterior arthrobranchs on VIII–XIII; posterior arthrobranchs on IX–XIII, that on 13 rudimentary pleurobranchs on XI–XIV; and epipodite on VII bearing few branchial filaments). SLP8 with a deep median suture or widely separated.

Remarks. The first diagnosis for *Parastacus* was proposed by Huxley (1879). He created this genus and the family Parastacidae to group Southern hemisphere crayfish based mainly on branchial structure. The author informed that the podobranchiae are devoid of more than a rudiment of a lamina and that the stem may be alate. In addition, podobranchiae of the first maxilliped has the form of an epipodite, but it can bears branchial filaments. The subsequent diagnosis for *Parastacus* were provided by Faxon (1898) and Riek (1971). Hobbs (1991) improved the diagnosis for *Parastacus* and discussed about the mandible morphology of South American Parastacidae and included mandible as a character in the genera diagnosis. However, the drawing that describes the mandible of *P. varicosus* is apparentely wrong, since both species present the caudal molar process bicuspidate and not quadricuspidate as pointed by Hobbs' illustration. *Parastacus* is morphologically similar to the Australian genera *Engaeus* Erichson, 1846 and *Engaewa* Riek, 1967 in having the dactylus of chelipeds moving subvertically and male genitalia consisting of a small papilla. *Parastacus* is the unique genus with all individuals of the species with supernumerary gonopores (intersexuality). This phenomenon was already recorded in some populations of the South American species *S. spinifrons* (Rudolph 2002), *V. araucanius* (Rudolph & Rivas 1988; Martinez *et al*. 1994) and the Australian genera *Engaewa, Engaeus, Euastacus* Clark, 1936 and *Cherax* Erichson, 1846 (Horwitz 1988; Sokol 1988; Medley 1993; Sagi et al. 1995). For a review of crayfish reproductive traits see Yazicioglu et al. (2016).

Distribution. Brazil (states of Rio Grande do Sul and Santa Catarina), Uruguay, Argentina and Chile (Buckup and Rossi, 1980; 1991; Rudolph, 2010).

Parastacus pilimanus **(von Martens, 1869)**

(Fig. 1–4, 50)

Astacus pilimanus von Martens, 1869: 15, pl. 2, fig. 1.

Parastacus pilimanus.—Huxley 1879:771 [by implication].—Faxon 1898: 683, 684.—Moreira 1901: 16, 80.—Ortmann 1902: 292.—Faxon 1914: 405.—Riek 1971:133.—Buckup and Rossi 1980:665, figs. 2-4, 8, 21.—Hobbs 1989: 80, fig. 366; 1991: 801, fig. 3e.—Buckup & Bond-Buckup, 1994: 19.—Collins *et al*. 2004: 254, fig. 1c.

Type materal examined. *Lectotype*. One snd, Brazil, Rio Grande do Sul, Porto Alegre (30°2'S; 51°12'W), col. Hensel (MNB 3323). *Paralectotypes*. Brazil, Rio Grande do Sul – one snd, same data as lectotype (MNB 3323); four m#, Santa Cruz, Pardo River (29°42'S; 52°25'W) (MNB 3447) (Fig. 1).

Material examined. Brazil, Rio Grande do Sul – one m#, col. Ihering (BMNH 1886.1); one m#, Alvorada, 04/X/1999 (MCP 2150); one f#, Torres, 1944, col. A. Ceroni (UFRGS 327); one m# and one f#, Maquiné, Estrada do Ligeiro, 02/VIII/1999, col. F. G. Becker, T. Finker & P. Colombo (UFRGS 6233); one f#, São Sebastião do Caí, IV/1997, col. M. Verdade (UFRGS 2191); two f#, Taim, 09/VI/1975, col. L. Buckup (UFRGS 276); one m#, Taim, 09/VI/1975, col. L. Buckup (UFRGS 277H); one m# and one f#, Taim, 09/VI/1975, col. L. Buckup (UFRGS 277P); 12 m#, one f# and 28 juveniles, Rio Grande, Taim, 07/VIII/1976 (UFRGS 205); six m#, Rio Grande, Estação Ecológica do Taim, 19/IX/1998, col. G. Bond-Buckup & C. Jara (UFRGS 2413); one f# and one juvenile, Rio Grande, Povo Novo, Estrada do Brete, col. A. L. F. Santos (UFRGS 3169); one m#, Povo Novo, Estrada do Brete, 23/VII/1982, col. A.LF. Santos (UFRGS 3170); two m# and one juvenile, Faxinal do Soturno, 28/VII/1995 (MCP 1653); two m#, Faxinal do Soturno, col. N. Fontoura, G. F. Rey & M. P. Barros (MCP 1915); four m# and 18 juveniles, Restinga Seca, 28/III/1995 (MCP 1650); one f#, Vale Vêneto (29°38'33"S; 53°33'02"W), 2012, col. M.M. Dalosto (UFRGS 5779); three m# and one f#, Vale Vêneto, XI/2012, col. M. M. Dalosto (UFRGS 6073); one m#, Santa Maria, CISM (29°45'12.58"S; 53°51'13.6°W); one f#, Santa Maria, Base Aérea (29° 49'27.33"S; 53°37'19.506"W), 2012, col. M. M. Dalosto (UFRGS 5784); one m#, São Gabriel (30°34'16.86"S; 54°29'42.22"W), 21/IX/2012, col. K. M. Gomes (UFRGS 5785); two f#, São Gabriel (30°34'16.86"S; 54°29'42.22"W), 21/IX/2012, col. K. M. Gomes (UFRGS 5786); one m#, Santiago, Rota 1 (UFRGS 2310); one m#, Alegrete, Rio Ibirapuitã, 25/II/1982, col. B. Irgang (UFRGS 542); one m#, Manoel Viana, 28/VII/1998, col. J. Ferzola & P. Ferzola (UFRGS 2697); three m#, Porto Xavier, Ijuí river, 22/VI/1989 (MCP 1378); one m#, Pirapó, Ijuí river, 22/VI/1989 (MCP 1348); two m#, Garruchos (28°7'43.93"S; 55°31'10.85"W), 17/IX/2013, col. F. B. Ribeiro & K. M. Gomes (UFRGS 5787); one f#, São Borja, Arroio do Barreiro, 28/II/1988 (MCP 1308); one m#, São Borja, Arroio do Barreiro, 21/IV/1989 (MCP 1350); three m#, São Borja, Arroio Barreiro, 23/VI/1989 (MCP 1370); one f#, Uruguaiana, Barragem e Rio Touro Passo, 14–18/X/1985, col. P. Lucena & Marchini (UFRGS 1376); one f#, Uruguayana, Barragem e Rio Touro Passo, 14–18/X/1985, col. P. Lucena & Marchini (UFRGS 1376); one f#, Uruguayana, 1914, col. E. Garbe (USNM 50671); one m#, Itaqui, 1914, col. E. Garbe (MZUSP 949); one m#, Montenegro, affluent of Maratá stream, 15/V/2001, col. D. Pereira (UFRGS 3204); one f#, Dom Pedrito, 05/III/1957, col. C. P. Coreto (UFRGS 1374); one m#, Quaraí, affluent of the river Garupá, BR – 293, 12/XI/1987, col. G. Bond-Buckup (UFRGS 2339); one m#, Quaraí, affluent of the river Garupá, 12/XI/1987; col. G. Bond, N. Fontoura & F. Bento (UFRGS 2345); one m# and one f#, Bagé, 22/VIII/1987 (UFRGS 2350); one m#, Bagé, Estância Santa Odessa, IV/2004, col. Filho.

Uruguay – four m# and one juvenile (MNHN–As 361); one m#, Río Negro, Estância Morgan, 20/XII/1968, col. C. S. Carbonell (FC–UDELAR 165); one snd, Lavallega, 20/VI/1981, col. F. Amestoy (USNM 177853); one snd, Rocha, 20/VIII/1981, F. Amestoy (USNM 177854); two snd, 31/XII/1986, leg. (USNM 219130); one snd, 1921, F. Felipponi (USNM 62318).

Argentina – one f#, Catamarca, col. E. Boman (MACN–In 30838); two m#, Marte Caseros, Corrientes (MACN–In 19817); one snd, Santo Tomé, Corrientes, col. A. Nani VII/1947 (MACN–In 6592); one snd, Mercedes, Corrientes, col. V. C. Pedera (FCEN–UBA w/n).

Diagnosis and description. von Martens (1869) and Buckup and Rossi (1980).

Remarks. Additional characters not mentioned in the original description by von Martens (1869) or in the redescription by Buckup and Rossi (1980) are: eyes small (Fig. 2A, B, C); front narrow (Fig. 2A, B); anterolateral section with a conical projection (Fig. 3A); antenna when extended back reaching S2; antennal scale lateral margin curved (Fig. 3D); antennule internal ventral border of basal article unarmed (Fig. 3A); mandible with cephalic molar process molariform and caudal molar process bicuspidate, incisive lobe with nine teeth; the third tooth from the anterior is the largest (Fig. 3E); SLP4 and SLP5 equal in size and separated to each other with median keel not inflated; SLP6 conical and with two concavities on surface and larger than SLP4 and SLP5, median keel present and inflated; SLP7 largest and with a concave surface, median keel present and inflated; SLP8 smaller than SLP 7, median keel absent, vertical arms of paired sternopleural bridges close to each other, bullar lobes not visible (Fig. 3B, C); male cuticle partition present. Taking into account that von Martens (1869) did not designate the holotype, Buckup & Bond-Buckup (1994) concluded that the type series consisted of a set of syntypes and designated the largest specimen of lot MNB 3323 as lectotype and the other specimens as paralectotypes, with Porto Alegre as the type-locality. This species is morphologically similar to *P. fluviatilis* and *P. laevigatus* in having the cutting edge of cheliped fingers covered by dense tufts of long setae and triangular rostrum, but it differs from both in having irregular lines of verrucous tubercles in the dorsal margin of cheliped dactylus and longer rostrum with apex V-shaped.

Distribution. Brazil: State of Rio Grande do Sul; Argentina: Provinces of Catamarca, Entre Ríos, Corrientes and Santa Fé; Uruguay: Departments of Rivera, Rocha, Serro Largo, Federación and Flores (Fig. 50).

Color of live specimens. Rostrum, cephalothorax anterior and lateral regions, dorsal pleon and tailfan greenish brown. Pereiopod pairs 2 – 5 light brown (Fig. 4D).

Habitat. Streams, floodplain rivers, flooded grasslands (Buckup and Rossi 1980) (Fig. 4A). Burrows can reach a depth of up to one meter and with several branches and small chimneys (Fig. 4B, C) (Buckup and Rossi 1980). Burrows of *P. pilimanus* can be identified as and type 1 and type 2 according to Horwitz and Richardson's (1986) classification and the species can be considered a primary burrower based on Hobbs' (1942) classification.

Parastacus brasiliensis (von Martens, 1869)

(Fig. 5–8, 50)

Astacus Brasiliensis von Martens 1869: 16, 17, pl. 2, fig. 2 and 2b.

Astacus brasiliensis.—Huxley 1879: 771.

Parastacus brasiliensis.—Huxley 1879: 771, fig. 2D; 1880: 250, fig. 64.—Ortmann 1902: 293.—Faxon 1914: 405.—Riek 1971: 133.—Buckup and Rossi 1980: 667, figs. 5–7, 8, 21.—Hobbs 1989: 79, fig. 365; 1991: 801, fig. 3k.—Buckup & Bond-Buckup, 1994: 19.

Parastacus braziliensis.—Moreira 1901: 80 [erroneous spelling]

Astacus braziliensis.—Moreira 1901: 80 [erroneous spelling]

Parastacus brasiliensis brasiliensis.—Fontoura & Conter 2008: 29.

Type locality. Porto Alegre, Rio Grande do Sul, Brazil (Buckup and Bond-Buckup, 1994).

Type material re-examined. *Lectotype.* one m#, Brazil, Rio Grande do Sul, Porto Alegre, col. Hensel (MNB 3322). *Paralectotypes*. Brazil, Rio Grande do Sul – same data as lectotype (MNB 3323); two m#, one f# and 11 juveniles, Porto Alegre (30°2'S; 51°13'W), col. Hensel (MNB 3448) (Fig. 5).

Material examined. Brazil, Rio Grande do Sul: one f#, Nova Petrópolis, Arroio Isabela, Picada Café, 20/VII/1980, col. P. A. Buckup, L. Malabarba & R. E. Reis (UFRGS 1360); one m#, km 42, estrada Porto Alegre – Taquara, 06/II/1983; one m# ando ne f#, Porto Alegre, Vila Jardim, 25/V/1963, col. N. Klevaco (UFRGS 282); one snd, Viamão, Parque Saint'Hillaire, 10/XII/1976, col. Edna (UFRGS 275) two m#, Parque Saint'Hilaire, col. Edna (UFRGS 279); one f#, Parque Saint'Hilaire, Viamão, col. E. Ferronato (UFRGS 329); one f#, Parque Saint'Hilaire, Viamão, col. E. Ferronato (UFRGS 330); three snd, Viamão, Parque Saint'Hilaire (UFRGS 583); one f#, Viamão, Parque Saint'Hilaire, VII/1981 (UFRGS 584); one m#, Viamão, Parque Saint'Hilaire, V/1987, col. Turma de Carcinologia (UFRGS 1354); one juvenile, Viamão, Parque Saint'Hilaire, 14/III/1985, col. L. Buckup, G. Bond-Buckup & O. Facchini (UFRGS 1361); one m#, Viamão, Parque Saint'Hilaire, 13/XII/1976, col. L. Buckup (UFRGS 2351); one m#, Viamão, Parque Saint'Hilaire 12/VIII/1994 (UFRGS 2701); one m#, Viamão, Condomínio Cantegril (30°4'19.9" S; 51°4'15.88"W); one snd, Praça da Vila Renascença, Porto Alegre (30°5'51.79"S; 51°11'30.26"W), 10/II/2013, col. K.M. Gomes, F.B. Ribeiro & G.C. Dalló (UFRGS 5861); one f#, Porto Alegre, Parque Nacional Morro do Osso (30°07'19"S; 51°14'01"W), 2014, col. K. M. Gomes & A. Kessler (UFRGS 5338); one f#, Porto Alegre, Morro Santana, 06/IV/2013, col. K. M. Gomes (UFRGS 5757);one f#, Morro da Agronomia, Porto Alegre, 26/XI/1975, col. Bruno (UFRGS 278); one snd (dry specimen), riacho em Porto Alegre, 26/V/1958, col. Odilo & Artur (UFRGS 1353); four juveniles, Arroio do Campus do Vale, Porto Alegre, 5/X/1977, col. G. Bond-Buckup (UFRGS 274); two juveniles, Guaíba, Fazenda São Maximiliano, 28/IV/1984, col. N. F. Fontoura (UFRGS 1667); two m#, Gravataí, Morungava, 1/XIII/1978, col. P. A. Buckup (UFRGS 326); one m#, Vila Nova, 20/XI/1979, col. V. Bitencourt (UFRGS 177); one snd, Itacolomi Mountain, 6/IV/1980, col. K. Kleebank (UFRGS 1358); five juveniles, Estação Experimental Agronômica UFRGS, Eldorado do Sul, 23/IX/1979, col. L. Buckup (UFRGS 324); one f#, Estação Experimental Agronômica UFRGS, Eldorado do Sul, 18/XII/1978, col. Flamonion (UFRGS 331); one ovigerous f# (with only three eggs), Taquara, col. V. Ihering (MNB 6494); one m# and two juveniles, Jardim Botânico de Lajeado, Lajeado, 26/VIII/2004, col. L. Johann (ZCUMCN 0055); two m#, Jardim Botânico de Lajeado, Lajeado, 26/VIII/2004, col. L. Johann & C. Birkheuer (ZCUMCN 0057); one m#, Jardim Botânico de Lajeado, Lajeado, 30/V/2005, col. L. Johann & C. Birkheuer (ZCUMCN 0088), one m#, Jardim Botânico de Lajeado, Lajeado, 30/V/2005, col. L. Johann & C. Birkheuer (ZCUMCN 0092); one m#, Roca Sales, 29/04/2006, col. L. K. Ruckert (ZCUMCN 0096); one m#, Colinas, 24/V/2004, col. D. Winter (ZCUMCN 0020); one m# and two f#, Arroio do Meio, 22/V/2005, col. S. Henz (ZCUMCN 0021); one m#, Arroio do Meio, /V/2005, col. S. Henz (ZCUMCN 0024); one m#, Ilópolis, 10/VI/2004, col. D. Bona (ZCUMCN 0022); one m#, Lajeado, 26/V/2004, col. E. Ost (ZCUMCN 0023); one f#, Venâncio Aires, 01/III/2004; one snd, Venâncio Aires (29°36’7.2”S; 52°16’4.8”W) XII/2014, col. N. N. Uhlman (UFRGS 6003); three m#, Mariana Pimentel (30°20’41”S; 51°33’55”W), 12/IV/2010, col. W. Beduchaud, K.M. Gomes & S. Santos (UFRGS 4890); four m# and one f#, Mariana Pimentel, 9/X/1986 (UFRGS 2337); three f#, Mariana Pimentel (30°20’00’S; 51°22’39”W), col. N. F. Fontoura (UFRGS 2338); three m# and one f#, Mariana Pimentel, 09/V/1988 (UFRGS 2352); one m#, Mariana Pimentel, 03/II/1998 (UFRGS 2736); one f#, Mariana Pimentel, 14/X/1997 (UFRGS 2709); one m#, Mariana Pimentel, 18/III/1998 (UFRGS 2752); one snd, Mariana Pimentel, 31/III/1998 (UFRGS 2722); one snd, Mariana Pimentel, 31/III/1998 (UFRGS 2742); one snd, Mariana Pimentel, 23/I/1999 (UFRGS 2753); one m#, Mariana Pimentel, 14/II/1994 (UFRGS 3356); one m#, Mariana Pimentel, Estrada do Boqueirão, Cerro da Cavalhada, 20/VIII/1982, col. R. V. Sá Filho (UFRGS 1357); one m#, Mariana Pimentel, affluent of river Ribeirão Pequeno (30°21’13”S; 51°32’03”W), 24/vi/2010, col. K. M. Gomes & Sandro Santos (UFRGS 4927); six m# and one juvenile, Horto Florestal Mariana, 03/III/1997, col. W. Bruschi Jr (UFRGS 2233); two m#, Mariana Pimentel, Horto Florestal Mariana,11/XI/1997), col. W. Bruschi Jr & G. Veisciprova (UFRGS 2265); two m# and three juveniles, Horto Florestal Mariana, Arroio Menor, Jacuí Basin, 23/V/1987, col. W. Bruschi Jr & G. Veisciprova (UFRGS 2221); two juveniles, Horto Florestal Mariana, Arroio Menor, 13/IX/1997 (UFRGS 2250); one m#, Horto Florestal Mariana, Arroio da Cascata, 11/XI/1993, col. W. Bruschi Jr & G. Veisciprova (UFRGS 2258) two m#, Horto Florestal Mariana, Arroio jusante (UFRGS 2265); two m# and three f#, Horto Florestal Ramos, Mariana Pimentel, Arroio dos Ratos Basin, 02/IX/1997, W. Bruschi Jr. (UFRGS 2215); five m# and one juvenile, Horto Florestal Mariana, Mariana Pimentel, 14/VIII/1997, col. W. Bruschi Jr. (UFRGS 2218); one m#, Horto Florestal Santo Amaro, Arroio Mosquito, 26/XI/1997, col. W. Bruschi Jr & G. Veisciprova (UFRGS 2274); one m#, Horto Florestal Ramos, Arroio 3, col. col. W. Bruschi Jr & Cristiano (2260); two juveniles, Horto Florestal Ramos, Arroio dos Ratos, 09/XII/1997 (UFRGS 2256); four snd, Arroio Tolotti, Horto Florestal Ramos, Mariana Pimentel, 29/X/2001, col. W. Bruschi Jr (UFRGS 3197); one m#, Afluente Arroio Ribeiro Pequeno, Mariana Pimentel (30°21’13”S; 51°32’03”W), 24/VI/2010, col. K.M. Gomes & S. Santos (UFRGS 4927); one m#, Sertão Santana (30°27’10”S; 51°35’36”W), 23/VI/2010, col. K.M. Gomes & S. Santos (UFRGS 4924); one m# and one f#, Taquara, 1981 (UFRGS 2342); one m#, Taquara, Fazenda Fialho, 07/IX/1998, col. P. Buckup four m#, Dois Irmãos, 1981 (UFRGS 2346); one snd, Gravataí, Morro Agudo, 13/IV/2002, col. A. Zimmer (UFRGS 3499).

Diagnosis and description. Von Martens (1869) and Buckup and Rossi (1980).

Remarks. Additional characters not mentioned in the original description by von Martens (1869) or in the redescription by Buckup and Rossi (1980) are: eyes small (Fig. 6A, B, C); front narrow (Fig. 6A, B); anterolateral section with a small conical projection (Fig. 7A); antenna when extended back reaching telson; antennal scale lateral margin straight (Fig. 7D); antennule internal ventral border of basal article with sharp spine (Fig. 7A); mandible with cephalic molar process molariform and caudal molar process bicuspidate, incisive lobe with nine teeth; the third tooth from the anterior is the largest (Fig. 7E); SLP4 smallest and very close to each other, medial keel present and not inflated; SLP5 larger than SLP4 and close to each other, median keel present and not inflated; SLP6 larger than SLP4 and SLP5 with concave dorsal surface , medial keel present and inflated; SLP7 largest and with concave surface, median keel present and inflated; SLP8 smaller than SLP 7, median keel absent, vertical arms of paired sternopleural bridges close to each other, bullar lobes visible and well separated to each other (Fig. 7B, C); male cuticle partition present. Taking into account that von Martens

(1969) did not designate the holotype, Buckup & Bond-Buckup (1980) concluded that the type series consisted of a set of syntypes and designated the largest specimen of lot MNB 3322 as lectotype and the other specimens as paralectotypes, with Porto Alegre as the type-locality. This species is morphological similar to *P. fluviatilis*, *P. promatensis* and *P. tuerkayi* in having large and laterally flattened chelipeds, triangular rostrum and lateral margin of antennal scale straight, but it can be distinguished by the concavity of the rostrum dorsal surface.

Distribution. Brazil: State of Rio Grande do Sul (Buckup & Rossi, 1980; I. Miranda, *personal communication*) (Fig. 50).

Color of living specimens. Rostrum, cephalothorax anterior and lateral regions, dorsal pleon and tailfan brown or black. Pereiopod pairs 2 – 5 brown or black (Fig. 8E).

Habitat and Ecology. Streams and springs (Fig. 8A, C) (Buckup & Rossi 1980; Miranda *et al.* submitted). Burrows are built in stream margins and are not complex and usually shallow (less than 50 cm), consisting in up to two single openings with chimneys with average height of 5 cm (Fig. 8B, D) (Buckup & Rossi 1980; Miranda *et al.* submitted). Burrows of *P. brasiliensis* can be identified as type 1a and 2 according to Horwitz and Richardson's (1986) classification and the species can be considered a secondary burrower based on Hobbs' (1942) classification.

Parastacus pugnax (Poeppig, 1835)

(Fig. 9–10, 52)

Astacus pugnax Poepigg, 1835: 314.

Astacus chilensis H. Milne Edwards, 1837:333.

Astacus chiliensis.—Gray 1845:411 [erroneous spelling].

Astacus (Cambarus) chilensis.—Erichson 1846:100.

Astacus (Astacus) Chilensis.—Herklots 1861:144.

Parastacus Chilensis.—Von Ihering, 1893:46.

Parastacus hassleri Faxon, 1898:687, pl 70, figs. 1-3.

Parastacus Hassleri.—Lonnberg, 1898: 349, figs. 1-3.

Parastacus chilensis.—Rathbun, 1910:602.—Holthuis, 1952:81.

Astacus chilensis auct.—Bahamonde, 1951:92.

Parastacus pugnax.—Holthuis 1952:84.—Riek 1971:133, fig. lb.—Buckup & Rossi 1993: 168, fig. 1.—Hobbs 1989: 80, fig. 371.—Rudolph 2010: 37, fig. 1B.— Rudolph 2013: 1481, fig. 8.

Type locality. Talcahuano, Chile

Material examined. Chile: *Parastacus pugnax* – one m# and two #f, La Florida, Concepción, 19/I/1977 (UFRGS 2407); 5 females, Rengo (cordillera), II/1984, leg. A.F. Neto (UFRGS 726); two m# and three f#, Laguna San Pedro, Concepción, 18/VII/1970; five m# and three f#, Nuble (between Parral and San Carlos, near Panamerican Highway), 03/VII/1981, col. J.C. Miguel (RMNH.CRUS.D. 34684); three m# and three f#, Concepción, Los Bastros (along the road towards Coronel), 24/V/1980, col. Lekalovic (RMNH.CRUS.D. 32955); one m#, Carahue, 28/08/1995, leg. E. Rudolph (FC–UDELAR 28); five m# and one f#, Chili (presented by the Chilean envoy from International Exhibition of Fisheries) (BMNH 1883.20); three m# and one f#, Tumbes, VI/1994 (MNB 10465–1); two m# and one f#, Tumbes (MNB 10465–2); one m#, Concepción, col. Pöpigg (MNB 26983); one f#, Quebrada de Cordova, El Tabo, 24/VII/1958, col. J. Reys (MNHCL/CRU 2318); two f#, Hacienda Bucalemu, 6/VIII/1959, col. N. Bahamonde (MNHCL/CRU 2384); seven m# and two f#, Linares, 23–24/VIII/1986, col. M. Rebolledo (MNHCL/CRU D –11093–A); one m# and one f#, Estero, Las Toscas, Quinahue, 10,20/I/1959, col. Lopéz (MNHCL/CRU 2284); five m# and five f#, Arredores de Puerto Alto, col. M. Cadoceu (MNHCL/CRU DA2193); one f#, San Carlos, V/1955 (MNHCL/CRU 2194); one f#, Quebrada de Córdoba, El Tabo, 17/IV/1958, col. S. Spinoza & M. Riquelme (MNHCL/CRU 2301); one m# and two f#, San Carlos de Buli, San Carlos, 28/VII/1964, col. A. Avilla (MNHCL/CRU 2422); two f# and four juveniles, Parral, 04/V/1959, col. Montero (MNHCL/CRU 2372); two m# and two f#, Linares, VIII/1986, col. M. Rebolledo (MNHCL/CRU 11.093-A); one m# (dry specimen), San Carlos (8ª Región, Bío-Bío, 26/IX/2000, col. E. Del Valle Leina (MNHCL/CRU 11374); one m# (dry specimen), Quinahue, cerca de Santa Cruz, VIII/1952, col. M. T. Lopéz (MNHCL/CRU 2058); one m#, Quebrada de Córdova, El Tabo, 1/I/1956, col. E. Hermosilla (MNHCL/CRU 2069); two m# (dry specimen), Quinahue, Santa Cruz, col. M. T. Lopéz (MNHCL/CRU 2057); one juvenile (dry specimen), Río Mapocho, El Monte, 30/XI/1957(MNHCL/CRU 2118); one m# (dry specimen), Bucalemu, 22/VIII/1957, col. N. Bahamonde (MNHCL/CRU 2084); two m#

and two f#, Guaraculén, XII/1952, col. M. Espinoza (MNHCL/CRU 2063); one m# (dry specimen, Río Aconquagua, II/1956, col. H. Etchevery (MNHCL/CRU 2068); three snd, Estación Piscicultura Curicó, 3/VIII/1955 (MNHCL/CRU 2182); two m# and five f# (one ovigerous) (dry specimens), Fundo "La Torina", 19/I/1959, col. Serrano (MNHCL/CRU 2364), one m#, Cauquenes, 1952, col. Alvarado (MNHCL/CRU 2184); two f#, Entre Dichato y Quinehue, 10,20/II/1959, col. L. Peña (MNHCL/CRU 2147); four m# and one f#, Estero Villa Alegre, IV/1953, col. P. Sepúlveda (MNHCL/CRU 2048); one m#, Laguna San Pedro, Concepción, IX/1955, col. J. Concha (MNHCL/CRU 2050); four m# and one f#, Talco, II/1957, col. R. Henriquez (MNHCL/CRU 2059); two m#, Chillán, 20/IX/1957, col. N. Bahamonde (MNHCL/CRU 2091); seven m# and one f#, Villa Alegre, IV/1953, col. P. Sepúlveda (MNHCL/CRU 2051); 34 m#, Carahue, 1/IV/1959, col. N. Bahamonde (MNHCL/CRU 2021); 43 m#, Andalién, Concepción, 13/VI/1958, col. P. Cassel (MNHCL/CRU 2293); one m# (BMNH 1883.16); five m# and one f# (BMNH #1883.20); one m#, Manganal, IX/1894, col. Lakaste (MNHN-As 358); ten m# and one f#, Puga Borne, Manganal (Itata), IX/1894, col. M. Lakaste (MNHN-As 359); one snd (dry specimen), col. M. Fontanier (MNHN-As 357); one snd (dry specimen) (Type of *Astacus chilensis*) (MNHN-As 356).

Diagnosis and Description. Poeppig (1835), Buckup & Rossi (1993), Rudolph (2010)

Remarks. Specimens from MNB 10465 were erroneously identified as *P. nicoleti* by Buckup and Bond-Buckup (1994). Several lots were previously identified as *P. chilensis* in MNB. Poeppig (1835) did not provide a morphological description for *Astacus pugnax*. There was not a designated type for this species until the contribution of Hobbs (1989), who said that the holotype of *P. hassleri* (MCZ 3401), a synonym of *P. pugnax* should be the neotype of *P. pugnax*. The designated holotype of *P. chilensis* by H. Milne-Edwards (1837), also a synonym of *P. pugnax*, is deposited in the Museum d'Histoire Naturelle in Paris, and was examined. Additional morphological characters not mentioned in previous descriptions were included: eyes small (Fig. 9A, B, C); front narrow (Fig. 9A, B); anterolateral section with a small conical projection (Fig. 10A); antenna when extended back reaching the posterior edge of carapace; antennal scale lateral margin straight (Fig. 10D); antennule internal ventral border of basal article unarmed (Fig. 10A); mandible with cephalic molar process molariform and caudal molar process bicuspidate, incisive lobe with ten teeth; the third tooth from the anterior is the largest (Fig. 10E); SLP4 and SLP5 subequal in size and very close to each other, medial keel present and not inflated; SLP6 larger than SLP4 and SLP5 with slightly concave dorsal surface , medial keel present and inflated; SLP7 largest and with slightly concave surface, median keel present and slightly inflated; SLP8 smaller than SLP 7, median keel absent, vertical arms of paired sternopleural bridges close to each other, bullar lobes not visible (Fig. 7B, C); male cuticle partition present. This species is morphological similar to *P. tuerkayi* in having the post orbital carinae weakly prominent, the areola narrow and barely discernible and the abdomen narrower than the cephalothorax, but it can be distinguished by the rostral carinae strongly convergent and by the number of teeth in the mandible incisive lobe.

Distribution. Chile (Rudolph, 2010) (Fig. 52).

Color of live specimens. Rudolph, 2010.

Habitat and Ecology. Vegas or Hualves, occurring in underground waters in small valleys or depressions between mountains or topographic depressions, usually associated with perennial forests (Rudolph, 2013). *Parastacus pugnax* build burrows with few ramifications and variable depths (Rudolph, 2010; 2013). *Parastacus pugnax* can also be found in lotic systems, where it builds burrows on the margins (Rudolph, 2012). Burrows of *P. pugnax* can be identified as type 2 according to Horwitz and Richardson's (1986) classification and the species can be considered a primary burrower based on Hobbs' (1942) classification.

***Parastacus defossus* Faxon, 1898**

(Fig. 11–12, 50)

Parastacus defossus Faxon, 1898: 686, pl. 67: figs. 3, 4.—Ortmann 1902: 293.—Riek 1971: 134.—Buckup and Rossi 1980: 677.—Hobbs 1989: 79, fig. 370; 1991: 801, fig. 3f.

Type Locality. Montevideu (Uruguay).

Type material re-examined. Holotype, #m, Uruguay, Montevideo, mouth of La Plata, col. W. E. Safford, U. S S. "Vandalia" (USNM 19647).

Material examined. Uruguay – one m#, 1897, col. Rodrigues (MACN–In 4970–2); two m#, Maldonado, Ruta 13, km 180, col. I. Larossa (FC–UDELAR XXXX).

Diagnosis and description. Faxon (1898).

Remarks. The occurrence of this species in Brazil was first proposed by Ortmann (1902). In the review of Buckup & Rossi (1980), they consider that this species ocurrs in the state of Rio Grande do Sul, Brazil. The authors redescribed the species based on Brazilian specimens. However, the analysis of photos of the type material of *P. defossus* and additional material from FC-UDELAR and all Brazilian material previously identified as *P. defossus*, allowed us to confirm that this species does not occur in Brazil. Additional morphological characters not mentioned in previous descriptions include: eyes small (Fig. 11A, B, C); front narrow (Fig. 11A, B); anterolateral section with an inconspicuous conical projection (Fig. 12A); antennal scale lateral margin straight (Fig. 10D); antennule internal ventral border of basal article unarmed (Fig. 12A); mandible with cephalic molar process molariform and caudal molar process bicuspidate, incisive lobe with nine teeth; the second and third teeth from the anterior are the largest (Fig. 12E); SLP4 very close to each other, medial keel present and not inflated; SLP5 smallest and close to each other, median keel present and not inflated; SLP6 larger than SLP4 and SLP5 with slightly concave dorsal surface , medial keel present and inflated; SLP7 largest and with slightly concave surface, median keel present and slightly inflated; SLP8 smaller than SLP 7, median keel absent, vertical arms of paired sternopleural bridges close to each other, bullar lobes visible and close to each other (Fig. 12B, C); male cuticle partition present. This species is morphological similar to *P. pugnax* and *P. caeruleodactylus* in having globose chelipeds, post orbital carinae weakly prominent, telson subrectangular and triangular rostrum, but it is distinguishable in having shorter dactylus and carpus with one blunt spine near the distal end.

Distribution. Uruguay: provinces of Montevideo and Maldonado (Faxon, 1898) (Fig. 50).

Color of live specimens. Not available data.

Habitat and Ecology. Burrows. More data not available.

Parastacus saffordi Faxon, 1898

(Fig. 13–15, 50)

Parastacus saffordi Faxon, 1898: 683, pl. 68.—Ortmann 1902: 293.—Riek, 1971:134.—Buckup and Rossi, 1980:673, figs. 9-11.21.—Hobbs 1989: 80, fig. 368; 1991: 801, fig. 3b.

Type locality. Montevideu (Uruguay).

Type material re-examined. Holotype, #m, Uruguay, Montevideo, mouth of La Plata, col. W. E. Safford, U. S S. "Vandalia" (USNM 12581) [Photos examined].

Material examined. Brazil, Santa Catarina: one m# and one f#, Cubatão river, 1960, col. Rosenberger (UFRGS 2705); one m#, Siderópolis, Reserva do Aguaí, 2014, col. C. Feltrin (UFRGS 5862); two m# and one snd, Siderópolis, Reserva do Aguaí, 2014, col. C. Feltrin (UFRGS 5863); two m#, Siderópolis, Reserva do Aguaí, 2014, col. C. Feltrin (UFRGS 5864); one m#, Siderópolis, Estrada para Jordão Baixo (28°35'31.18"S; 49°30'10.15"W), 10/XII/2013, col. K.M. Gomes & F.B. Ribeiro (UFRGS 5875); one m#, Siderópolis, Estrada para Jordão Baixo (28°35'31.18"S; 49°30'10.15"W), 10/XII/2013, col. K.M. Gomes & F.B. Ribeiro (UFRGS 5876); one snd, Siderópolis, Estrada para Jordão Baixo (28°35'31.18"S; 49°30'10.15"W), 10/XII/2013, col. K.M. Gomes & F.B. Ribeiro (UFRGS 5877);

Brazil, Rio Grande do Sul: one m#, Novo Hamburgo, Lomba Grande, Estrada da Integração, 26/X1985, col. Guacira & Jackson (UFRGS 1356),

Uruguay: one m#, Montevideu (MZUSP 251); one snd, Maldonado, Barra do Maldonado, II/1975 (FCEN–UBA s/n)

Diagnosis and description. Faxon (1898) and Buckup & Rossi (1980).

Remarks. Additional morphological characters not mentioned in previous descriptions include: eyes large (Fig. 13A, B, C); front wide (Fig. 13A, B); anterolateral section with a sharp conical projection (Fig. 14A); antennal scale lateral margin straight (Fig. 10D); antennule internal ventral border of basal article with one sharp spine (Fig. 14A); mandible with cephalic molar process molariform and caudal molar process bicuspidate or tricuspidate, incisive lobe with ten teeth; the third tooth from the anterior are the largest (Fig. 14E); SLP4 smallest and separeted to each other, medial keel present and not inflated; SLP5 separeted to each other, median keel present and not inflated; SLP6 larger than SLP4 and SLP5 with slightly concave dorsal surface , medial keel present and inflated; SLP7 largest and with slightly concave surface, median keel present and slightly inflated; SLP8 smaller than SLP 7, median keel absent, vertical arms of paired sternopleural bridges close to each other in the superior portion, bullar lobes visible and separated to each other (Fig. 14B, C); male cuticle partition present. This species ressembles *P. varicosus* in having a long and triangular rostrum, cheliped propodus densely covered by verrucous tubercles, post orbital carinae and largest lobe of protopod of exopod with one spine and branchiocardic grooves with elevation, but it is distinguishable by the shorter merus and internal margin of cutting edge of finger covered by tufts of setae on the proximal portion.

Distribution. Brazil: States of Rio Grande do Sul and Santa Catarina (Buckup and Rossi, 1980; 2003); Uruguay: departments of Montevideu and Maldonado.

Color of living specimens. Rostrum, cephalothorax anterior and lateral regions, dorsal pleon and tailfan light green, dark green or greenish brown. Pereiopod pairs 2 – 5 light green (Fig. 15, C, D).

Habitat and Ecology. Swamps and wet lands away from temporary or permanent water bodies and sometimes in streams, where they can build shallow burrows in the margins (Buckup & Rossi, 1980). Burrows of *P. saffordi* can be identified as type 2 according to Horwitz and Richardson's (1986) classification and the species can be considered a secondary or tertiary burrower based on Hobbs' (1942) classification.

Parastacus varicosus Faxon, 1898

(Fig. 16–17, 50)

Parastacus varicosus Faxon, 1898: 685, pl. 69.—Ortmann 1902: 293.—Riek, 1971:134,fig. 1a, d, e.—Buckup & Rossi, 1980:675, figs. 12-14,21.—Hobbs 1989: 80, fig. 367; 1991: 801, fig. 3d.—Buckup & Bond-Buckup 1994: 19.
Parastacus pilimanus.—Correa *et al*. 2013: 156, fig. 1, 2.

Type locality. Colima (Mexico) [obviously an erroneous locality data]

Type material re-examined. Holotype, #m, Colima (USNM 4133) [Photos examined].

Material examined. Brazil, Santa Catarina: one m#, Joinville, Estrada Timbé (direção Chaparral) (26°12'30"S; 48°50'47"W), 19/II/2001, col. L. Buckup & G. Bond-Buckup (UFRGS 3067); one m# and one f#, Joinville, Estrada Timbé (direção Chaparral) (26°12'30"S; 48°50'47"W), 20/II/2001, col. L. Buckup & G. Bond-Buckup (UFRGS 3066); one m#, Joinville, Estrada Timbé (direção Chaparral) (26°12'30"S; 48°50'47"W), 08/X/2003, col. L. Buckup & G. Bond-Buckup (UFRGS 2663); two m#, Siderópolis, Jordão Baixo, 01/XII/19777, col. L. Buckup (UFRGS 271).
Brazil, Rio Grande do Sul: one m#, Novo Hamburgo, Lomba Grande, Estrada da Integração, 27/X/1985, col. G. Oliveira & J. Muller (UFRGS 1354); six m#, Canoas, floodplain of Sinus river, Tabaí-Canoas, 16/IX/1983 (MCP 981); five m#, one m#, Novo Hamburgo, Lomba Grande, Estrada da Integração, 28/IX/1985, col. J. Muller (UFRGS 1355); seven m#, Novo Hamburgo, Lomba Grande, Estrada da Integração, 25/X/1985, col. Guacira & Jackson (UFRGS 2343); one snd, Novo Hamburgo, Lomba Grande, Estrada da Integração, col. G. Oliveira & J. Muller (UFRGS 879); one m#, Porto Alegre, várzea do Rio Gravataí, 08/XI/2000, col. Malabarba, Berg, Anza & Azevedo (UFRGS 4790); one m#, Taim, 21/XI/1979, col. Helena (UFRGS 2347); one f#, Taim, 6–10/VII/1979, col. P. Buckup, C. Souto & K. Leyser (UFRGS 1370); six m#, Pelotas, 22/VII1965 (MZUSP 6934); one f#, Rio Grande do Sul, col. Hering (BMNH 1983:5); one m#, Mostardas, 21/I/1977 (UFRGS 280); one m#, Gravataí, Fazenda Alencastro, 29/X/1976 (UFRGS 280).
Uruguay: one m# and three juveniles, Rocha, Ruta 9 Pte Arroyo Valizas, Charco al Norte de La Carretera, 09/V/1981, col. R. V. Ferreira, F. Achaval & J. Bergan (FC–UDELAR 276); one snd, Bocas del Sarandi, III/1995 (FC–UDELAR 313); two m#, Departamento de Rocha, Camino a laguna de Rocha (Frente al Velódromo), IV/1996

(FC–UDELAR 314); one m# and one f#, Departamento de Rocha (FC–UDELAR 355); one snd, Departamento de Maldonado, Gruta de Salamanca, Ruta 13 entre Aigua y Velazquez (FC–UDELAR 384); one snd, Montevideu, Parque Centenário, XII/1938, col. Carcelles, Alberto & M. Rosa (MACN–In 23568); three f# and 19 m#, Maldonado, IX/1927, col. C. R. Mazzoni (MACN–In 17514); one snd, Republica Oriental do Uruguay, 1897, col. F. M. Rodriguez (MACN–In 4570–1)
Argentina: one ovigerous f# with juveniles, Entre Ríos (MACN–In 30723);

Diagnosis and description. Faxon (1898) and Buckup & Rossi (1980).

Remarks. This species also occurs in the municipality of Siderópolis – SC, being erroneously identified as *P. saffordi* by Buckup and Rossi (1980). Regarding the type locality, it is obviously erroneous as pointed out by Hobbs (1987) and Buckup & Rossi (1980), because the genus *Parastacus* only occurs in South America. Additional morphological characters not mentioned in previous descriptions include: eyes large (Fig. 16A, B, C); front wide (Fig. 16A, B); anterolateral section with a very sharp conical projection (Fig. 17A); antennal scale lateral margin straight (Fig. 17D); antennule internal ventral border of basal article with one sharp spine (Fig. 17A); mandible with cephalic molar process molariform and caudal molar process bicuspidate or tricuspidate, incisive lobe with nine teeth; the third tooth from the anterior are the largest (Fig. 14E); SLP4 smallest and separeted to each other, medial keel present and not inflated; SLP5 widely separeted to each other, median keel present and not inflated; SLP6 larger than SLP4 and SLP5 with slightly concave dorsal surface , medial keel present and inflated; SLP7 largest and with slightly concave surface, median keel present and slightly inflated; SLP8 smaller than SLP 7, median keel absent, vertical arms of paired sternopleural bridges close to each other in the superior portion, bullar lobes not visible (Fig. 17B, C); male cuticle partition present. This species is morphologically similar to *P. saffordi* as mentioned before, but it can be distinguishable by the serrated externally branchiocardic grooves, longer merus propodus and dactylus of chelipeds and anteriomedian lobe of epistome with acute apex.

Distribution. Brazil: States of Rio Grande do Sul and Santa Catarina (Buckup and Rossi, 1980; 2003); Uruguay: departments of Montevideu, Maldonado and Rocha; Argentina: Province of Entre Ríos).

Color of living specimens. Not available.

Habitat and Ecology. Swamps and wet lands away from temporary or permanent water bodies and sometimes in streams, where they can build shallow burrows in the margins (Buckup & Rossi, 1980). Burrows of *P. varicosus* can be identified as type 2 according to Horwitz and Richardson's (1986) classification and the species can be considered a secondary or tertiary burrower based on Hobbs' (1942) classification.

***Parastacus laevigatus* Buckup & Rossi, 1980**

(Fig. 18–19, 50)

Parastacus laevigatus Buckup and Rossi, 1980: 677, figs. 18, 21.—Hobbs 1989: 79, fig. 369; 1991: 801.—Boos Jr. *et al* 200

Type locality. Joinville, state of Santa Catarina, Brazil.

Type Material examined. *Holotype.* m#, Brazil, Santa Catarina, Joinville, Estrada da Cidra, Chacara dos Ipês. 05/VIII/1961, col. Rosenberg (MN UFRJ w/n). *Paratypes*. one f# and five juveniles, same data as holotype (MN UFRJ w/n); one f#, Cubatão Grande, São Francisco do Sul, Santa Catarina, 25/IX/1959, col. H. Stick (MN UFRJ w/n); one f#, Brazil (UFRGS 2039).

Material examined. Brazil: Santa Catarina – one ovigerous f# (with three eggs), São Francisco do Sul, E. Gounelle, 1914 (MNHN – IU 2013 – 14858).

Diagnosis and description. Buckup & Rossi (1980).

Remarks. The type material was apparentely lost, but it was found at UFRGS collection during the review of the material. This species was never found again in nature since 1962 and it is probably extint. Additional morphological characters not mentioned in previous descriptions include: eyes small (Fig. 16A, B, C); front narrow (Fig. 16A, B); anterolateral section with a blunt conical projection (Fig. 17A); antennal scale lateral margin straight (Fig. 17D); antennule internal ventral border of basal article with one sharp spine (Fig. 17A); mandible with cephalic molar process molariform and caudal molar process unicuspidate, incisive lobe with eight teeth; the second tooth from the anterior are the largest (Fig. 14E); SLP4 close to each other, medial keel present and not inflated; SLP5 smallest separeted to each other, median keel present and not inflated; SLP6 larger than SLP4 and SLP5 with slightly concave dorsal surface , medial keel present and inflated; SLP7 largest and with slightly concave surface, median keel present and slightly inflated; SLP8 smaller than SLP 7, median keel absent, vertical arms of paired sternopleural bridges close to each, bullar lobes not visible (Fig. 17B, C); male cuticle partition present. This species is morphological similar to *P. pilimanus* and *P. fluviatilis* in having the chelipeds laterally flattened and with the cutting edge of the finger covered by tufts of long setae, but it can be distinguishable by the post orbital carinae obsolete and the carpal spine present.

Distribution. Brazil: State of Santa Catarina (Buckup and Rossi, 1980) (Fig. 50).

Color of live specimens. Data not available.

Habitat and Ecology. Data not available

Parastacus promatensis Fontoura & Conter, 2008

(Fig. 20–21, 50)

Parastacus brasiliensis promatensis Fontoura & Conter, 2008: 29, fig. 1.

Type locality. São Francisco de Paula, state of Rio Grande do Sul, Brazil.

Type material re-examined. *Holotype*: m#, Brazil, Rio Grande do Sul, São Francisco de Paula, Riacho Garapiá, CPCN Pró-Mata (PUCRS), 25/II/1997 (MCP 2085); *Paratypes*: six m# and two f#, same data as holotype (MCP 2086).

Material examined. Brazil, Rio Grande do Sul: one f#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 15/I/2006, col. L. C. C. Daudt (UFRGS 4160); two f# and 1 m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS) (29°29’21.99”S; 50°13’3.972”), 2014 (UFRGS 5949); two f# and two m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 05/IV/1995, col. N. Fontoura (UFRGS 2110); one f#, São Francisco de Paula, Riacho Garapiá, CPCN Pró –

Mata (PUCRS), 08/VII/2005, col. L.C.C. Daudt (UFRGS 4156); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 09/VII/2005, col. L. C. C. Daudt (UFRGS 4148); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 09/VII/2005, col. L.C.C. Daudt (UFRGS 4159); one f#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 15/I/2006, col. L.C.C. Daudt (UFRGS 4157); one f#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 09/VII/2005, col. L.C.C. Daudt (UFRGS 4155); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 09/VII/2005, col. L. C. C. Daudt (UFRGS 4153); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 09/VII/2005, col. L.C.C. Daudt (UFRGS 4150); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 09/VII/2005, col. L.C.C. Daudt (UFRGS 4152); one f#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 09/VII/2005, col. L.C.C. Daudt (UFRGS 4149); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 09/VII/2005, col. L.C.C. Daudt (UFRGS 4151); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 09/VII/2005, col. L.C.C. Daudt (UFRGS 4154); one f#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 09/VII/2005, col. L.C.C. Daudt (UFRGS 4158); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 15/I/2006, col. L.C.C. Daudt (UFRGS 4161); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 15/I/2006, col. L. C. C. Daudt (UFRGS 4162); one f#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 15/I/2006, col. L.C.C. Daudt (UFRGS 4163); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 15/I/2006, col. L.C.C. Daudt (UFRGS 4164); five f# and four m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), X/2012, col. G.T. Oliveira (UFRGS 6151); one f#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 09/VII/2005 (MCP 2639); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 09/VII/2005 (MCP 2640); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 09/VII/2005 (MCP 2641); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 09/VII/2005 (MCP 2642); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 09/ VII/2005 (MCP 2643); one f#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 09/VII/2005 (MCP 2644); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 15/I/2006 (MCP 2645); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 15/I/2006 (MCP 2646); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 15/I/2006 (MCP 2647); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 15/I/2006 (MCP 2648); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 15/I/2006 (MCP 2649); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 15/I/2006 (MCP 2650); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 15/I/2006 (MCP 2651) one f#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 15/I/2006 (MCP 2652); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 15/I/2006 (MCP 2653); one m#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 22/V/2006 (MCP 2654); four m# and one f#, São Francisco de Paula, Riacho Garapiá, CPCN Pró – Mata (PUCRS), 21/IX/2009 (MCP 2981).

Diagnosis and Description. Fontoura & Conter (2008).

Remarks. This species was first described as a subspecies of *P. brasiliensis* based mainly on a morphometric analysis of individuals previously identified as *P. brasiliensis* from the locality of São Francisco de Paula, Rio Grande do Sul, Brazil. However, there is genetic and morphological evidences to elevate this taxon to species level (I. Miranda, *personal communication*). Additional morphological characters not mentioned in previous descriptions include: eyes small (Fig. 20A, B, C); front narrow (Fig. 20A, B); anterolateral section with a blunt conical projection and tubercles (Fig. 21A); antennal scale lateral margin straight (Fig. 21D); antennule internal ventral border of basal article without one sharp spine (Fig. 21A); mandible with cephalic molar process molariform and caudal molar process bicuspidate, incisive lobe with nine teeth; the third tooth from the anterior are the largest (Fig. 21E); SLP4 smallest and close to each other, medial keel present and not inflated; SLP5 close to each other, median keel present and not inflated; SLP6 larger than SLP4 and SLP5 with slightly concave dorsal surface , medial keel present and slightly inflated; SLP7 largest and with slightly concave surface, median keel present and inflated; SLP8 smaller than SLP 7, median keel absent, vertical arms of paired sternopleural bridges close to each, bullar lobes visible and widely separated (Fig. 21B, C); male cuticle partition present. This species is morphological similar to *P. brasiliensis* and *P. fluviatilis* in having the chelipeds long and laterally flattened, triangular rostrum and post orbital carinae prominent, but it can be distinguishable by the longer dactylus of chelipeds and the entire lateral margin of chelipeds covered by verrucous tubercles irregularly distributed.

Distribution. Brazil, state of Rio Grande do Sul (Fig. 50).

Color of live specimens. Data not available.

Habitat and Ecology. Streams at high altitudes (~850 m) (Fontoura & Conter, 2008). No data is available concerning burrow structure.

Parastacus fluviatilis Ribeiro & Buckup, 2016

(Fig. 50)

Type locality. São José dos Ausentes, state of Rio Grande do Sul, Brazil

Additional material examined. Brazil, Rio Grande do Sul: one f#, São José dos Ausentes, Divisa river, 3/X/2002, col. G. Bond-Buckup (UFRGS 2059); one m#, São José dos Ausentes, Morro Monte Negro, 03/X/2002, col. G. Bond-Buckup (UFRGS 2293); one juvenile, São José dos Ausentes, Pico Monte Negro (28°36'59.81"S; 49°47'48.48"W),14/V/2013, col. F.B. Ribeiro, K.M. Gomes & D.C. Kenne (UFRGS 6435); one m#, Silveira/São José dos Ausentes, Divisa river (28°38'43.91"S; 49°56'27.67"W), 15/V/2013, col. F.B. Ribeiro & K.M. Gomes (UFRGS 6436).

Diagnosis and Description. Ribeiro *et al.* (2016).

Remarks. The record of this species at about 1400 m of altitude in Pico Monte Negro, São José dos Ausentes (RS) constitutes the higher record of freshwater crayfish in South America.

Distribution. Brazil: State of Rio Grande do Sul (Ribeiro *et al.* 2016).

Color of live specimens. Ribeiro et al. (2016).

Habitat and Ecology. Streams at high altitudes (~1,400m) (Ribeiro *et al.* 2016). This species builds shallow burrows with small chimneys in the river margins. (Ribeiro *et al.* 2016). Burrows of *P. fluviatilis* can be identified as type 2 according to Horwitz and

Richardson's (1986) classification and the species can be considered a secondary or burrower based on Hobbs' (1942) classification.

Parastacus caeruleodactylus Ribeiro & Araujo, 2016

(Fig. 50)

Type locality. Morrinhos do Sul, state of Rio Grande do Sul, Brazil (Ribeiro *et al.* 2016).

Diagnosis and Description. Ribeiro et al. (2016).

Distribution. Brazil: State of Rio Grande do Sul (Ribeiro *et al.*, 2016).

Color of live specimens. Ribeiro et al. (2016).

Habitat and Ecology. Swamp forests (Ribeiro *et al.* 2016). *Parastacus caeruleodactylus* builds deep burrows with chimneys averaging 10 cm in height and width (Ribeiro *et al.* 2016). Burrows of *P. caeruleodactylus* can be identified as type 2 according to Horwitz and Richardson's (1986) classification and the species can be considered a primary burrower based on Hobbs' (1942) classification.

Parastacus tuerkayi Ribeiro, Huber & Araujo, 2017

(Fig. 50)

Type locality. Penha, State of Santa Catarina, Brazil (Ribeiro *et al. in press*).

Diagnosis and Description. Ribeiro et al. (2017).

Distribution. Brazil: State of Santa Catarina, Brazil (Ribeiro *et al*., 2017) (Fig. 50).

Color of live specimens. Ribeiro et al. (2017).

Habitat. Swamp forests (Ribeiro *et al*., 2017). *Parastacus tuerkayi* build burrows that 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 (Ribeiro *et al*., 2017). Burrows of *P. tuerkayi* can be identified as type 2 according to Horwitz and Richardson's (1986) classification and the species can be considered a primary burrower based on Hobbs' (1942) classification.

***Parastacus* sp. nov. 1**

(Figs. 22–23, 51)

Parastacus defossus.—Almeida and Buckup, 1999: 116, figs. 1–9.

Holotype. m#, Brazil, Rio Grande do Sul, Mariana Pimentel, Lago Guaíba Basin (30°21'11.72"S; 51°35'2.59"W), 25/VI/2016, col. F. B. Ribeiro, K. M. Gomes & A. Hirschmann (MZUSP XXXXX).

Paratypes. Brazil, Rio Grande do SuL – 1–3: one f# and two m#, Mariana Pimentel (30°21'11.72"S; 51°35'2.59"W), 08/VII/2016, col. F. B. Ribeiro, K. M. Gomes, A. Hirschmann & A. F. Huber (UFRGS 6414); 4–5: two f#, same data as holotype (UFRGS 6410).

Additional material examined. Brazil, Rio Grande do Sul: one m#, Mariana Pimentel, 24/IX/1997 (UFRGS 2708); one snd, Mariana Pimentel, 01/XII/1997 (UFRGS 2710); one f#, Mariana Pimentel, 20/VII/1998 (UFRGS 2711); one f#, Mariana Pimentel, 11/III/1998 (UFRGS 2712); one f#, Mariana Pimentel, 28/V/1998 (UFRGS 2713); one snd, Mariana Pimentel, 01/XII/1997 (UFRGS 2714); one m#, Mariana Pimentel, 28/V/1998 (UFRGS 2715); one m#, Mariana Pimentel, 08/VI/1998 (UFRGS 2716); one f#, Mariana Pimentel, 01/IX/1997 (UFRGS 2717); one m#, Mariana Pimentel, 23/IX/1997 (UFRGS 2718); one m#, Mariana Pimentel, 20/I/1998 (UFRGS 2720); one m#, Mariana Pimentel, 08/IV/1998 (UFRGS 2723); one m#, Mariana Pimentel, 25/IV/1998 (UFRGS 2725); one snd, Mariana Pimentel, 01/VI/1998 (UFRGS 2726); one f#, Mariana Pimentel, 09/XII/ 1997 (UFRGS 2724); one m#, Mariana Pimentel, 12/VI/1998 (UFRGS 2727); one f#, Mariana Pimentel, 19/I/1998 (UFRGS 2728); one f#, Mariana Pimentel, 20/I/1998 (UFRGS 2729); one m#, Mariana Pimentel, 02/IX/1997 (UFRGS 2730); one f#, Mariana Pimentel, 12/III/1998 (UFRGS 2731); one f#, Mariana Pimentel, 29/IX/1997 (UFRGS 2732); one m#, Mariana Pimentel, 01/VI/1998 (UFRGS 2733); one m#, Mariana Pimentel, 19/I/1997 (UFRGS 2734); one snd, Mariana Pimentel, 02/XI/1997 (UFRGS 2737); one m#, Mariana Pimentel, 29/IX/1997 (UFRGS 2738); one m#, Mariana Pimentel, 22/IX/1997 (UFRGS 2739); one f#, Mariana Pimentel, 08/IX/1997 (UFRGS 2740); one m#, Mariana Pimentel, 13/III/1998 (UFRGS 2741); one m#, Mariana Pimentel, 29/IX/1997 (UFRGS 2743); one m#, Mariana Pimentel, 30/III/1998 (UFRGS 2744); one f#, Mariana Pimentel, 03/IV/1998 (UFRGS 2745); one f#, Mariana Pimentel, 19/IV/1998 (UFRGS 2746); one snd, Mariana Pimentel, 28/IV/1997 (UFRGS 2747); one m#, Mariana Pimentel, 13/I/ 1997 (UFRGS 2748); one snd, Mariana Pimentel, 20/I/1998 (UFRGS 2749); one m#, Mariana Pimentel, 19/I/1998 (UFRGS 2750); one m#, Mariana Pimentel, 08/IV/1998 (UFRGS 2751); five m#, Mariana Pimentel, several dates (6, 10, 11/VIII/1998) (UFRGS 2756); ten f#, Mariana Pimentel, several dates (24/VI/1998; 17, 20/VII/1998; 17, 18, 25/VIII/1998; 25/XI/1998; 09/XII/1998;) eight m#, Mariana Pimentel, several dates (26/VI/1998; 25/VIII/1998; 04, 05, 20/XII/1998; 20, 25/XI/1998) (UFRGS 2758); eight f#, Mariana Pimentel, several dates (29/VII/1998; 06, 10, 11, 12, 17/VIII/1998) (UFRGS 2759); one m#, Mariana Pimentel, 14/I/1997 (UFRGS 2760); INCLUIR PUC

Diagnosis. Narrow front with short triangular rostrum with apex inverted "U"-shaped, not ending in a spine. Epistome anteromedian lobe pentagonal as long as wide. Post orbital carinae obsolete. Cervical groove strongly V-shaped. Telson subtriangular. Globose chelipeds.

Description. Rostrum: triangular, wider than long (RL 83.2% of RW), short (11.1% of CL), reaching the half of the second article of the antennular peduncle (Fig. 1A–C). Dorsum straight, apex inverted "U"-shaped, not ending in a spine (Fig. 1B, C). Few plumose setae on lateral margins. Rostral sides slightly convergent and rostral basis divergent. Carinae straight, prominent and narrow, extending back to carapace, slightly surpassing rostral basis (Fig. 22B, C).

Cephalon: CeL 66% of CL. Eyes small (CMW 70% of OW); suborbital angle 90°, unarmed (Fig. 3C). Front narrow (FW 39.5% of CW). Postorbital carinae longer than rostral carinae (RCL 65.9% of POCL) and weakly prominent. Lateral cephalic edge with dense setation (Fig. 22A–C).

Thorax: carapace laterally compressed, deep and narrow (CD 57.3% of CL; CW 46.2% of CL). Cervical groove strongly V-shaped. Branchiocardiac grooves inconspicuous (Fig. 1A). Areola narrow, 4.3x as long as wide (33.6% of CL) (Fig. 22A).

Abdomen: long and narrow (AL 70.8% of CL; AW 64.6% of CW), smooth, covered with small setae on pleural margins (Fig. 22A). Pleural somites with rounded posterior margins. S2 pleurae low and short with shallow groove parallel to margin (Fig. 22D, E, F).

Tailfan: telson uniformly calcified, subtriangular, longer than wide (TeW 79.1% of TeL), with small 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. 22E). Uropod protopod bilobed, with rounded and unarmed margins; proximal lobe largest. Exopod lateral margin bears a small and sharp spine, mid-dorsal carina few prominent, ending in a very sharp spine. Transverse suture (diaeresis) straight, with seven dorsolateral spines (outer) and five dorsolateral spines (inner) on right exopod and six dorsolateral spines (outer) and six dorsolateral spine (inner) on the left exopod. Endopod, mid-dorsal carina few prominent, not ending in a spine; lateral margin with one sharp spine at level of exopod transverse suture (Fig. 22E).

Epistome: anterolateral section with blunt conical projection. Posterolateral section smooth and with deep lateral grooves converging to the basis of the anteromedian lobe, and without median circular concavity. Anteromedian lobe pentagonal, as long as 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 deep groove (Fig. 23A).

Thoracic sternites: SLP4 and SLP5 subequal in size 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 straight, median keel absent, vertical arms of paired sternopleural bridges close to each other, bullar lobes close to each other and clearly visible (Fig. 23B, C).

Antennule: internal ventral border of basal article with a small sharp spine (Fig. 23A).

Antenna: when extended back reaching the posterior margin of carapace. Antennal scale widest at midlength, reaching the end of third antennal article, ASW 51.14% of ASL (Fig. 23A, D), lateral margin straight, spine strong and distal margin emarginated. Coxa with a weak prominent carina above nephropore and blunt spine laterally displaced. Basis unarmed (Fig. 23A).

Mandible: cephalic molar process molariform, caudal molar process bicuspidate with one cephalodistal cusp and one small distoproximal cusp. Incisive lobe with eight teeth. The second tooth from the anterior margin is the largest (Fig. 23E).

Third maxilliped: ischium bearing few setiferous puctuations in the middle, and simple long setae on inner margin and small simple setae on outer margin (Fig. 23F); dorsal surface with few setae (Fig. 23G). Merus ventral surface densely covered by long and short simple setae in the median region (Fig. 23F). *Crista dentata* bearing 25 and 26 teeth in right and left ischium respectively. Merus, dorsal surface with a setose midline. Exopod longer than ischium, with flagellum reaching proximal margin of merus (Fig. 2F, G).

First pair of pereiopods (chelipeds): small and subequal, globose (RPrT 33.29% of RPrL; LPrT 31.96% of LPrL) (Fig. 22A). Ischium ventral surface with setiferous punctuations. Merus: right merus (RML) 62.27% of propodus length (RPrL); left merus (LML) 55.80% of propodus length (LPrL); ventral surface with two longitudinal series of tubercles: inner series with 12 tubercles, outer 13 and mesial 8, arranged irregularly on right merus; inner series bearing 13 tubercles, outer 13 and mesial 9, arranged irregularly on left merus. Dorsal and midventral spines absent. Carpus with dorsomedian surface not divided by a transversal groove (Fig. 22A; Fig. 23I). Internal dorsolateral margin with row of tubercles, increasing in size distally; inner surface with few small tubercles. Carpal spine absent (Fig. 23I). Propodus width (RPrW and LPrW) 57.8% of length in right cheliped and 57.62% in left cheliped. Dorsal surface of palm with irregularly distributed verrucous tubercles (Fig. 23H, I). Inner margin without tubercles. Ventral surface bearing two rows of squamose tubercles, trespassing the beginning of the fixed finger (Fig. 23H). Dactylus: right dactylus (RDL) 58.7% of propodus length (RPrL), left dactylus (LDL) 60.2% of left propodus (LPrL); dorsal surface with rows of setiferous punctuations (Fig. 4I). Cutting edge of fingers visible. Fixed finger with six teeth, third and fourth teeth largest. Dactylus with seven teeth, second tooth largest (Fig. 23H, I).

Second pair of pereiopods: ventral and dorsal surface of carpus, propodus and dactylus with moderate covering of simple long setae (Fig. XJ).

Gonopores: Presence of both genital apertures on coxae of third and fifth pairs of pereiopods. Female gonopores semi-ellipsoidal (maximum diameter 0.97 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. 23B).

Remarks. Number of teeth in *Crista dentata* of third maxilliped ranges to 23 to 28 teeth in type series. *Parastacus* **sp. nov. 1** is morphologically similar to *P. defossus*, *P. caeruleodactylus* and *P. pugnax* in having globose chelipeds, short and triangular rostrum and subtriangular telson, narrow abdome and post orbital carinae weakly prominent, but it is distinguishable by the rostrum apex “U”-shaped and without spine and epistome anteromedian lobe pentagonal as long as wide.

Distribution. Brazil: State of Rio Grande do Sul.

Color of live specimens. Rostrum, cephalothorax anterior and lateral regions, dorsal pleon and tailfan brown brown to dark brown Pereiopod pairs 2 – 5 brown.

Habitat and Ecology. Flooded grasslands. Burrows are complex and usually deep (up to 1m), consisting in several openings with chimneys with average height of 5 cm. Burrows of *Parastacus* **sp. nov. 1** can be identified as type 2 according to Horwitz and Richardson’s (1986) classification and the species can be considered a primary burrower based on Hobbs’ (1942) classification.

***Parastacus* sp. nov. 2**

(Figs. 24–25, 51)

Holotype. m#, Brazil, Rio Grande do Sul, Dom Feliciano, Camaquã Basin, Horto Herval (30°29 2.616"S; 52° 9'36.75"W), 05/X/2012, col. K.M. Gomes (MZUSP XXXXX).

Paratypes. Brazil, Rio Grande do Sul – 1: one m#, Dom Feliciano, Horto Florestal Lageado (30°32' 6"S; 52°5 4.99"W), 05/X/2012, col. K.M. Gomes (UFRGS 5497); 2: one juvenile, Dom Feliciano, Horto Florestal Xavier (30°32'54.6"S; 52°7'30.72"W), 05/X/2012, col. K.M. Gomes (UFRGS 5493).

Diagnosis. Wide front with short trapezoid rostrum wider than long with apex inverted “U”-shaped, ending in a straight blunt and small spine. Epistome anteromedian lobe hexagonal. Post orbital carinae obsolete. Cervical groove weakly V-shaped. Telson subrectangular. Globose chelipeds.

Description. Rostrum: trapezoid, wider than long (RL 75.1% of RW), short (12.1% of CL), reaching the middle of the second article of the antennular peduncle (Fig. 24A–C). Dorsum straight, apex inverted "U"-shaped, ending in straight blunt and small spine (Fig. 24B, C). Few plumose setae on lateral margins. Rostral sides slightly convergent and rostral basis slightly divergent. Carinae angled, prominent and narrow, extending back to carapace not surpassing rostral basis (Fig. 24B, C).

Cephalon: CeL 69.2% of CL. Eyes large (CMW 74.7% of OW); suborbital angle >90°, unarmed (Fig. 24C). Front wide (FW 41.8% of CW). Postorbital carinae longer than rostral carinae (RCL 51% of POCL) and weakly prominent. Lateral cephalic edge with very sparse setation (Fig. 24A–C).

Thorax: carapace laterally compressed, deep and wide (CD 55.7% of CL; CW 48.7% of CL). Cervical groove weakly V-shaped. Branchiocardiac grooves inconspicuous (Fig. 24A). Areola wide, 2.2x as long as wide (31.9% of CL) (Fig. 24A).

Abdomen: long and wide (AL 90.4% of CL; AW 80.3% of CW), smooth, not covered 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. 24D).

Tailfan: telson uniformly calcified, subrectangular, longer than wide (TeW 89.2% 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. 24E). Uropod protopod bilobed, with rounded and unarmed margins; proximal lobe largest. Exopod lateral margin bears a small and sharp spine, mid-dorsal carina few prominent, ending in a very sharp spine. Transverse suture (diaeresis) straight, with nine dorsolateral spines (outer) and seven dorsolateral spines (inner) on right exopod and eight dorsolateral spines (outer) and seven dorsolateral spine (inner) on the left exopod. Endopod, mid-dorsal carina few prominent, ending in a small and sharp spine; lateral margin with one small and sharp spine at level of exopod transverse suture (Fig. 24E).

Epistome: anterolateral section with sharp conical projection. Posterolateral section smooth and with deep and setose lateral grooves converging to the basis of the anteromedian lobe, and reduced median circular concavity. Anteromedian lobe hexagonal, 1.2x wider than long, apex straight with some serrated setae, reaching median part of antepenultimate article of antennal peduncle; dorsal surface concave at apex, and basis with a shallow groove (Fig. 25A).

Thoracic sternites: SLP4 small and very close to each other, median keel present and not inflated; SLP5 smallest and separated to each other, median keel present and not inflated; SLP6 larger than SLP4 and SLP5 a concave surface, median keel not inflated; SLP7 largest and with surface slightly concave, median keel inflated, bullar lobes absent; SLP8 straight, median keel absent, vertical arms of paired sternopleural bridges close to each other, bullar lobes not visible (Fig. 25B, C).

Antennule: internal ventral border of basal article with small sharp spine (Fig. 25A).

Antenna: when extended back reaching the posterior margin of carapace. Antennal scale widest at midlength, reaching the end of third antennal article, ASW 57.1% of ASL (Fig. 25A, D), lateral margin straight, spine strong and distal margin emarginate. Coxa with prominent carina above nephropore and blunt spine above the nephropore. Basis unarmed (Fig. 25A).

Mandible: cephalic molar process molariform, caudal molar process bicuspidate with one cephalodistal cusp and one small distoproximal cusp. Incisive lobe with eight teeth. The third tooth from the anterior margin is the largest (Fig. 25E).

Third maxilliped: ischium glabrous in the middle, setose inner margin and sparse small and simple setae on outter margin (Fig. 25F); dorsal surface without setae (Fig. 25G). *Crista dentata* bearing 22 and 23 teeth in right and left ischium respectively. Merus ventral surface bearing few setae in the middle and densely setose inner margin. Exopod longer than ischium, with flagellum reaching proximal margin of merus (Fig. 25F, G).

First pair of pereiopods (chelipeds): small and subequal, laterally flattened (RPrT 28.1% of RPrL; LPrT 25.68% of LPrL) (Fig. 24A). Ischium ventral surface with 7 tubercles. Merus: right merus (RML) 68.8% of propodus length (RPrL); left merus (LML) 62.8% of propodus length (LPrL); ventral surface with two longitudinal series of tubercles: inner series with 12 tubercles, outer 16 and mesial 10, arranged irregularly on right merus; inner series bearing 13 tubercles, outer 16 and mesial 12, arranged irregularly on left merus. Dorsal and midventral spines present. Carpus dorsomedial surface not divided longitudinally by groove (Fig. 24A; Fig. 25I). Internal dorsolateral margin with row of tubercles, increasing in size distally; inner surface with few mesial tubercles. Carpal spine absent (Fig. 25I). Propodus width (RPrW and LPrW) 47.32% of length in right cheliped and 47.1% in left cheliped. Dorsal surface of palm squamose tubercles irregularly distributed (Fig. 25H, I). Inner margin without tubercles. Ventral surface bearing two rows of squamose tubercles, trespassing the beginning of the fixed finger (Fig. 25H). Dactylus: right dactylus (RDL) 63.8% of propodus length (RPrL), left dactylus (LDL) 61.9% of left propodus (LPrL); dorsal surface with squamose tubercles in the proximal portion (Fig. 4I). Cutting edge of fingers visible. Fixed finger with three subequal teeth. Dactylus with four teeth subequal in size (Fig. 25H, I).

Second pair of pereiopods: ventral and dorsal surface of carpus, propodus and dactylus with sparse covering of simple long setae (Fig. 25J).

Gonopores: Presence of both genital apertures on coxae of third and fifth pairs of pereiopods. Female gonopores semi-ellipsoidal (maximum diameter 0.74 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. 25B).

Remarks. *Parastacus* **sp. nov. 2** was previously identified as *P. brasiliensis* in UFRGS collection. *Parastacus* **sp. nov. 2** ressembles juveniles of *P. brasiliensis* in having dorsal surface of palm squamose tubercles irregularly distributed. It differs from all other *Parastacus* species by the trapezoid rostrum.

Distribution. Brazil: State of Rio Grande do Sul.

Color of live specimens. Not available data.

Habitat and Ecology. Stream (Fig. 4A). Burrows are simple and shallow and found in the stream margins. Burrows of *Parastacus* **sp. nov. 2** can be identified as type 2 according to Horwitz and Richardson's (1986) classification and the species can be considered a secondary burrower based on Hobbs' (1942) classification.

Distribution. Brazil: State of Rio Grande do Sul.

Parastacus sp. nov. 3

(Figs. 26–28, 51)

Parastacus defossus.—Buckup and Rossi, 1980: 677, figs. 15-17, 21.

Holotype. m#, Brazil, Rio Grande do Sul, Porto Alegre, Sítio do Mato (Zona Sul), Lago Guaíba Basin (30°6'51.03"S; 51°8'34.08"W), 22/III/2014, col. K. M. Gomes & F. B. Ribeiro (MZUSP XXXXX).

Paratypes. Brazil, Rio Grande do Sul – 1: one f#, same data as holotype (UFRGS 5870); one m#, same data as holotype (UFRGS 5857); one f#, same data as holotype (UFRGS 5859); one m#, Porto Alegre, Morro da Extrema, VII/1999, col. A. O. Almeida (UFC 419); 5 – 7: two m# and one f#, Porto Alegre, Lami (30°11'28.07"S; 51° 9'1.44"W), 12/XII/2014, col. K. M. Gomes, D. Kenne & A. C. L. Oliveira (UFRGS 6351); 8 – 9: two m#, Porto Alegre, Haras Cambará (30°12'47.26"S; 51°7'25.91"W) 19/XI/2014, col. K. M. Gomes & F. B. Ribeiro (UFRGS 6341).

Diagnosis. Narrow front with short triangular rostrum wider than long with apex inverted "U"-shaped, ending in a straight blunt and small spine. Epistome anteromedian lobe pentagonal with apex very acute. Post orbital carinae obsolete. Cervical groove weakly V-shaped. Telson subrectangular. Globose chelipeds with fixed finger bearing only three teeth.

Description. Rostrum: triangular, wider than long (RL 74.5% of RW), short (9% of CL), reaching the end of the second article of the antennular peduncle (Fig. 26A–C). Dorsum concave, apex inverted "U"-shaped, ending in straight blunt and small spine (Fig. 26B, C). Few plumose setae on lateral margins. Rostral sides slightly convergent and rostral basis divergent. Carinae straight, prominent and narrow, extending back to carapace surpassing rostral basis (Fig. 26B, C).

Cephalon: CeL 64.1% of CL. Eyes small (CMW 57.4% of OW); suborbital angle 90°, unarmed (Fig. 26C). Front narrow (FW 41.9% of CW). Postorbital carinae longer than rostral carinae (RCL 63.9% of POCL) and weakly prominent. Lateral cephalic edge with dense setation (Fig. 1A–C).

Thorax: carapace laterally compressed, deep and wide (CD 52.4% of CL; CW 46.6% of CL). Cervical groove weakly V-shaped. Branchiocardiac grooves conspicuous (Fig. 26A). Areola narrow, 3.7x as long as wide (31.3% of CL) (Fig. 26A).

Abdomen: short and narrow (AL 78.2% of CL; AW 75.9% of CW), smooth, covered with setae on pleural margins (Fig. 26A). Pleural somites with rounded posterior margins. S1 pleurae with a large distal lobe not overlapped by S2 pleurae. S2 pleurae high and short with shallow groove parallel to margin (Fig. 26D).

Tailfan: telson uniformly calcified, subrectangular, longer than wide (TeW 77.6% 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. 26E). Uropod protopod bilobed, with rounded and unarmed margins; proximal lobe largest. Exopod lateral margin bears a small and sharp spine, mid-dorsal carina few prominent, ending in a sharp spine. Transverse suture (diaeresis) straight, with five dorsolateral spines (outer) and five dorsolateral spines (inner) on both exopods. Endopod, mid-dorsal carina few prominent, ending in a minuscle and sharp spine; lateral margin with one small and sharp spine at level of exopod transverse suture (Fig. 26E).

Epistome: anterolateral section with sharp conical projection. Posterolateral section smooth and with deep lateral grooves converging to the basis of the anteromedian lobe, and median circular concavity well developed. Anteromedian lobe pentagonal, 1.2x wider than long, apex acute, reaching median part of antepenultimate article of antennal peduncle; dorsal surface straight at apex, and basis with a shallow groove (Fig. 27A).

Thoracic sternites: SLP4 smallest and very close to each other, median keel present and not inflated; SLP5 small and separated to each other, median keel present and not inflated; SLP6 larger than SLP4 and SLP5 with concave surface, median keel not inflated; SLP7 largest and with surface slightly concave, median keel inflated, bullar lobes absent; SLP8 straight, median keel absent, vertical arms of paired sternopleural bridges close to each other, bullar lobes not visible (Fig. 27B, C).

Antennule: internal ventral border of basal article with small blunt spine (Fig. 2A).

Antenna: when extended back reaching the posterior margin of carapace. Antennal scale widest at midlength, reaching the end of third antennal article, ASW 41.9% of ASL (Fig. 27A, D), lateral margin straight, spine strong and distal margin emarginate. Coxa with prominent carina and one blunt spine above nephropore. Basis unarmed (Fig. 27A).

Mandible: cephalic molar process molariform, caudal molar process bicuspidate with one cephalodistal cusp and one small distoproximal cusp. Incisive lobe with nine teeth. The third tooth from the anterior margin is the largest (Fig. 27E).

Third maxilliped: ischium, ventral surface with ventral longitudinal parallel rows of long and simple setae along the entire surface (Fig. 27F); dorsal surface with few small and simple setae (Fig. 27G). *Crista dentata* bearing 21 and 22 teeth in right and left ischium respectively. Merus ventral surface follows the same pattern of ischium. Exopod longer than ischium, with flagellum reaching proximal margin of merus (Fig. 27F, G).

First pair of pereiopods (chelipeds): small and subequal, globose (RPrT 30.53% of RPrL; LPrT 28.2% of LPrL) (Fig. 27A). Ischium ventral surface with seven tubercles. Merus: right merus (RML) 72.6% of propodus length (RPrL); left merus (LML) 68.6% of propodus length (LPrL); ventral surface with two longitudinal series of tubercles: inner series with 13 tubercles, outer eight and mesial 15, arranged irregularly on right merus; inner series bearing 10 tubercles, outer 12 and mesial 18, arranged irregularly on left merus. Dorsal and midventral spines absent. Carpus with dorsomedial surface divided longitudinally by shallow groove (Fig. 27A; Fig. 27I). Internal dorsolateral margin with row of tubercles, increasing in size distally; inner surface with few small mesial tubercles. Carpal spine absent (Fig. 27I). Propodus width (RPrW and LPrW) 53.1% of length in right cheliped and 52.5% in left cheliped. Dorsal surface of palm with rows of verrucous tubercles irregularly distributed (Fig. 27H, I). Inner margin without tubercles. Ventral surface bearing two rows of squamose tubercles, reaching the beginning of the fixed finger (Fig. 27H). Dactylus: moving subvertically, right dactylus (RDL) 60.9% of propodus length (RPrL), left dactylus (LDL) 58% of left propodus (LPrL); dorsal surface with setiferous puctuations (Fig. 27I). Cutting edge of fingers visible. Fixed finger with three teeth, second tooth largest. Dactylus with six teeth, second tooth largest (Fig. 27H, I).

Second pair of pereiopods: ventral and dorsal surface of carpus, propodus and dactylus with dense cover of simple long setae (Fig. 27J).

Gonopores: Presence of both genital apertures on coxae of third and fifth pairs of pereiopods. Female gonopores semi-ellipsoidal (maximum diameter 1.62 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. 27B).

Remarks. *Parastacus* **sp. nov. 3** was previously identified as *P. defossus* in UFRGS collection. *Parastacus* **sp. nov. 3** ressembles *P. defossus* and *P. caeruleodactylus* in having short globose chelipeds, abdomen narrow than cephalothorax and telson subrectangular. It is distinguishable from all *Parastacus* species in having the following combination of characters: anteromedian lobe of epistome apex very acute, lateral margin of antennal scale curved and fixed finger with only three teeth.

Distribution. Brazil: State of Rio Grande do Sul.

Color of live specimens. Rostrum, cephalothorax anterior and lateral regions, dorsal pleon and tailfan light brown to dark brown Pereiopod pairs 2 – 5 brown (Fig. 28D).

Habitat and Ecology. Flooded grasslands and swamp forests (Fig. 28A). Burrows are complex and deep (up to 1m deep) with chimneys up to 30 cm. (Fig. 28B, C). Burrows of *Parastacus* **sp. nov. 3** can be identified as type 2 according to Horwitz and Richardson's (1986) classification and the species can be considered a primary burrower based on Hobbs' (1942) classification.

Parastacus sp. nov. 4

(Figs. 29–31, 51)

Holotype. m#, Brazil, Santa Catarina, Maracajá, Parque Ecológico Maracajá (28°52'29.98" S; 49°28' 9.15"W), 11/XII/2013, col. F.B. Ribeiro (MZUSP XXXXX).

Paratypes. Brazil, Santa Catarina, m#, same data as holotype (UFRGS 5856).

Diagnosis. : Anteromedian lobe of epistome apex very acute, lateral margin of antennal scale curved and fixed finger with only three teeth.

Description. Rostrum: triangular, longer than wide (RW 89.5% of RL), short (12.9% of CL), reaching the half of the second article of the antennular peduncle (Fig. 29A–C). Dorsum straight, apex inverted "U"-shaped, ending in a straight spine (Fig. 29B, C). Few plumose setae on lateral margins. Rostral sides slightly convergent and rostral basis divergent. Carinae angled, prominent and narrow, extending back to carapace, slightly surpassing rostral basis (Fig. 29B, C).

Cephalon: CeL 67.4% of CL. Eyes large (CMW 65.82% of OW); suborbital angle 90°, unarmed (Fig. 29C). Front narrow (FW 38.1% of CW). Postorbital carinae longer than rostral carinae (RCL 65% of POCL) and weakly prominent. Lateral cephalic edge with dense setation (Fig. 29A–C).

Thorax: carapace laterally compressed, deep and wide (CD 59.4% of CL; CW 44.9% of CL). Cervical groove weakly V-shaped. Branchiocardiac grooves inconspicuous (Fig. 29A). Areola narrow, 2.6x as long as wide (27.2% of CL) (Fig. 29A).

Abdomen: short and wide (AL 77.6% of CL; AW 82.9% of CW), smooth, covered with small setae on pleural margins (Fig. 29A). Pleural somites with rounded posterior margins. S2 pleurae low and short with shallow groove parallel to margin (Fig. 29D, E, F).

Tailfan: telson uniformly calcified, subtriangular, width and length subequal (TeL 94% of TeW), with small 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. 29E). Uropod protopod bilobed, with rounded and unarmed margins; proximal lobe largest. Exopod lateral margin bears a small and sharp spine, mid-dorsal carina few prominent, ending in a sharp spine. Transverse suture (diaeresis) straight, with five dorsolateral spines (outer) and seven dorsolateral spines (inner) on right exopod and six dorsolateral spines (outer) and five dorsolateral spine (inner) on the left exopod. Endopod, mid-dorsal carina few prominent, not ending in a spine; lateral margin with one sharp spine at level of exopod transverse suture (Fig. 29E).

Epistome: anterolateral section with blunt conical projection. Posterolateral section smooth and with deep lateral grooves converging to the basis of the anteromedian lobe, and with a median groove. Anteromedian lobe pentagonal, 1.2x longer than wide, apex rounded with surpassing median part of antepenultimate article of antennal peduncle; dorsal surface straight, and basis with a deep groove (Fig. 30A).

Thoracic sternites: SLP4 and SLP5 subequal in size and very close to each other, median keel present and not inflated; SLP6 larger than SLP4 and SLP5 with a slightly concave surface, median keel inflated; SLP7 largest and with surface slightly concave, median keel inflated, bullar lobes absent; SLP8 straight, median keel absent, vertical arms of paired sternopleural bridges close to each other, bullar lobes separated to each other and clearly visible (Fig. 30B, C).

Antennule: internal ventral border of basal article without spine (Fig. 30A).

Antenna: when extended back reaching S1. Antennal scale widest at midlength, reaching the end of third antennal article, ASW 45.2% of ASL (Fig. 30A, D), lateral margin curved, spine strong and distal margin emarginate. Coxa with prominent carina and one blunt spine above nephropore. Basis unarmed (Fig. 30A).

Mandible: cephalic molar process molariform, caudal molar process bicuspidate with one cephalodistal cusp and one small distoproximal cusp. Incisive lobe with eight teeth. The second tooth from the anterior margin is the largest (Fig. 30E).

Third maxilliped: ischium, ventral surface with tufts of setae in the middle and inner margin (Fig. 30F); dorsal surface with few small and simple setae (Fig. 30G). *Crista dentata* bearing 22 and 21teeth in right and left ischium respectively. Merus ventral surface follows the same pattern of ischium. Exopod longer than ischium, with flagellum reaching proximal margin of merus (Fig. 30F, G).

First pair of pereiopods (chelipeds): small and subequal, globose (RPrT 31.4% of RPrL; LPrT 28.9% of LPrL) (Fig. 29A). Ischium ventral surface with 14 and 15 tubercles in the right and left ischium respectively. Merus: right merus (RML) 67.95% of propodus length (RPrL); left merus (LML) 97.6% of propodus length (LPrL); ventral surface with two longitudinal series of tubercles: inner series with 13 tubercles, outer 10 and mesial eight, arranged irregularly on right merus; inner series bearing 11 tubercles, outer six and mesial nine, arranged irregularly on left merus. Dorsal spine absent and and midventral spine present. Carpus not divided by a transversal groove (Fig. 29A; Fig. 30I). Internal dorsolateral margin with row of tubercles, increasing in size distally; inner surface with few small median tubercles. Carpal spine absent (Fig. 2I). Propodus width (RPrW and LPrW) 53.7% of length in right cheliped and 48.1% in left cheliped. Dorsal surface of palm with irregularly distributed squamose tubercles (Fig. 30H, I). Inner margin without tubercles. Ventral surface bearing two rows of squamose tubercles, reaching the beginning of the fixed finger (Fig. 30H). Dactylus: moving subvertically, right dactylus (RDL) 60.1% of propodus length (RPrL), left dactylus (LDL) 47.61% of left propodus (LPrL); dorsal surface with rows of setiferous punctuations (Fig. 30I). Cutting edge of fingers visible. Fixed finger with six teeth, third and teeth largest. Dactylus with eight teeth, second tooth largest (Fig. 30H, I).

Second pair of pereiopods: ventral and dorsal surface of carpus, propodus and dactylus with dense covering of simple long setae (Fig. 30J).

Gonopores: Presence of both genital apertures on coxae of third and fifth pairs of pereiopods. Female gonopores semi-ellipsoidal (maximum diameter 1.16 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. 30B).

Remarks. *Parastacus* **sp. nov. 4** ressembles *P. defossus* and *P. caeruleodactylus* in having short globose chelipeds. It is distinguishable from all *Parastacus* species in having the following combination of characters: anteromedian lobe of epistome apex very acute, lateral margin of antennal scale curved and fixed finger with only three teeth.

Distribution. Brazil: State of Santa Catarina.

Color of live specimens. Rostrum, cephalothorax anterior and lateral regions, dorsal pleon and tailfan brown to dark brown. Chelipeds reddish brown. Pereiopod pairs 2 – 5 brown (Fig. 31C).

Habitat and Ecology. Swamp forests (Fig. 31A). Burrows are complex and deep (up to 1m deep) with chimneys up to 15 cm. (Fig. 31B). Burrows of *Parastacus* **sp. nov. 4** can be identified as type 2 according to Horwitz and Richardson's (1986) classification and the species can be considered a primary burrower based on Hobbs' (1942) classification.

Parastacus sp. nov. 5

(Figs. 32–34, 51)

Holotype. m#, Brazil, Rio Grande do Sul, Viamão, Banhado dos Pachecos (30°5'40.95"S; 50°51'25.99"W), 20/VIII/2013, col. F. B. Ribeiro & K. M. Gomes (MZUSP XXXX).

Paratypes. Brazil, Rio Grande do Sul – two m#, Viamão, Banhado dos Pachecos (30°5'49.99"S; 50°51'7.02"W) (UFRGS 5865); one m#, Osório, XI/1981, col. L. Schirmer (UFRGS 1359); one f# and one juvenile, Osório, Morro da Borússia (29°53.63'S; 50°16.89'W), 22/IV/2015 (UFRGS 6439).

Diagnosis. Rostrum wider than long. Rostral carinae not surpassing rostral basis when extending back to carapace. Globose chelipeds with different sizes. Anteromedian lobe of epistome with apex very acute.

Description. Rostrum: triangular, wider than long (RL 90% of RW), short (11.1% of CL), reaching the middle of the second article of the antennular peduncle (Fig. 32A–C). Dorsum straight, apex inverted "V"-shaped, ending in upward blunt and small spine (Fig. 32B, C). Few plumose setae on lateral margins. Rostral sides slightly convergent and rostral basis divergent. Carinae straight, prominent and narrow, extending back to carapace not surpassing rostral basis (Fig. 32B, C).

Cephalon: Carapace lacking spines or tubercles. CeL 68% of CL. Eyes small (CMW 77.4% of OW); suborbital angle > 90°, unarmed (Fig. 32C). Front narrow (FW 33.5% of CW). Postorbital carinae longer than rostral carinae (RCL 47.7% of POCL) and weakly prominent. Lateral cephalic edge with dense setation (Fig. 32A–C).

Thorax: carapace laterally compressed, deep and wide (CD 57.8% of CL; CW 45.8% of CL). Cervical groove weakly V-shaped. Branchiocardiac grooves conspicuous (Fig. 32A). Areola narrow, 2x as long as wide (25% of CL) (Fig. 32A).

Abdomen: lacking spines or tubercles, short and wide (AL 79.7% of CL; AW 80.9% of CW), smooth, covered with setae on pleural margins (Fig. 32A). Pleural somites with rounded posterior margins. S1 pleurae with a large distal lobe not overlapped by S2 pleurae. S2 pleurae low and short with deep groove parallel to margin (Fig. 32D).

Tailfan: telson uniformly calcified, subrectangular, longer than wide (TeW 82.4% 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. 32E). Uropod protopod bilobed, with rounded and unarmed margins; proximal lobe largest. Exopod lateral margin bears a small and sharp spine, mid-dorsal carina few prominent, ending in a sharp spine. Transverse suture (diaeresis) straight, with six dorsolateral spines (outer) and four dorsolateral spines (inner) on right exopod left exopod. Endopod, mid-dorsal carina few prominent, ending in a minuscle and sharp spine; lateral margin with one sharp spine at level of exopod transverse suture (Fig. 32E).

Epistome: anterolateral section with blunt conical projection with tubercles. Posterolateral section smooth and with deep lateral grooves converging to the basis of the anteromedian lobe, and median concavity well developed. Anteromedian lobe pentagonal, 1.06x wider than long, apex very acute, reaching median part of antepenultimate article of antennal peduncle; dorsal surface straight in the middle and concave margins; basis with a shallow groove (Fig. 33A).

Thoracic sternites: SLP4 small and very close to each other, median keel present and not inflated; SLP5 smallest and separated to each other, median keel present and not inflated; SLP6 larger than SLP4 and SLP5, subequal to SLP8, with concave surface, median keel inflated; SLP7 largest and with surface slightly concave, median keel inflated, bullar lobes absent; SLP8 straight, median keel absent, vertical arms of paired sternopleural bridges close to each other, bullar lobes visible (Fig. 33B, C).

Antennule: internal ventral border of basal article with sharp spine (Fig. 33A).

Antenna: when extended back reaching S3. Antennal scale widest at midlength, reaching the end of third antennal article, ASW 42% of ASL (Fig. 33A, D), lateral margin straight, spine strong and distal margin emarginate. Coxa with prominent carina and one blunt spine above nephropore. Basis unarmed (Fig. 33A).

Mandible: cephalic molar process molariform, caudal molar process bicuspidate with one cephalodistal cusp and one small distoproximal cusp. Incisive lobe with eight teeth. The second tooth from the anterior margin is the largest (Fig. 33E).

Third maxilliped: ischium, ventral surface with tufts of small setae in the middle and inner margin and few single setae in the outer margin (Fig. 33F); dorsal surface with few small and simple setae (Fig. 33G). *Crista dentata* bearing 22 and 23 teeth in right and left ischium respectively. Merus ventral surface with tufts of long and small. Exopod reaches the end of ischium (Fig. 33F, G).

First pair of pereiopods (chelipeds): large and with different sizes, globose (RPrT 23.9% of RPrL; LPrT 31.2% of LPrL) (Fig. 32A). Ischium ventral surface with nine and 11 tubercles in the right and left ischium respectively. Merus: right merus (RML) 71.1% of propodus length (RPrL); left merus (LML) 48.3% of propodus length (LPrL); ventral surface with two longitudinal series of tubercles: inner series with 15 tubercles, outer 12 and mesial nine, arranged irregularly on right merus; inner series bearing 17 tubercles, outer eight and mesial 15, arranged irregularly on left merus. Dorsal and midventral spines present. Carpus groove impression absent (Fig. 32A; Fig. 33I). Internal dorsolateral margin with row of tubercles, increasing in size distally; inner surface with up to 14 small mesial tubercles. Carpal spine absent (Fig. 33I). Propodus width (RPrW and LPrW) 48.5% of length in right cheliped and 54.1% in left cheliped. Dorsal surface of palm with two rows of verrucous tubercles (Fig. 33H, I). Inner margin without tubercles. Ventral surface bearing two rows of squamose tubercles, reaching the beginning of the fixed finger (Fig. 33H). Right dactylus (RDL) 64.7% of propodus length (RPrL), left dactylus (LDL) 56.1% of left propodus (LPrL); dorsal surface with squamose tubercles in the proximal portion (Fig. 33I). Cutting edge of fingers visible. Fixed finger with nune teeth, third tooth largest. Dactylus with eleven teeth, second tooth largest (Fig. 33H, I).

Second pair of pereiopods: ventral and dorsal surface of carpus, propodus and dactylus with moderate cover of simple long setae (Fig. 33J).

Gonopores: Presence of both genital apertures on coxae of third and fifth pairs of pereiopods. Female gonopores semi-ellipsoidal (maximum diameter 1.06 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. 33B).

Remarks. This species ressembles *P. defossus* in small sizes, globose chelipeds and post orbital carinae obsolete. *Parastacus* **sp. nov. 5** is distinguishable from all congeneric species by heterochely (chelipeds with diffent sizes) with left cheliped larger and by the anteromedian lobe of epistome with apex very acute.

Distribution. Brazil: State of Rio Grande do Sul (Fig. 51).

Color of living specimens. Rostrum, cephalothorax anterior and lateral regions, dorsal pleon and tailfan brown to dark brown Pereiopod pairs 2 – 5 brown (Fig. 34D).

Habitat and Ecology. Peat bogs (Fig. 34A). Burrows are shallow with one or two tunnels and can reach up to 35 cm deep (Fig. 34B) Chimneys can reach up to 5 cm height and within the burrows can be found two adults individuals, including the female in the ovigerous stage. According to Horwitz and Richardson's (1986) the burrows of *Parastacus* **sp. nov. 5** are of type 2 and it can be considered a primary burrower based on Hobbs' (1942) classification.

Parastacus sp. nov. 6

(Figs. 35–37, 51)

Holotype. m#, Brazil, Santa Catarina, Florianópolis, Fazenda Experimental da Ressacada – UFSC, 23/IX/2013, col. F. B. Ribeiro, M. P. Almerão & P. B. Araujo (MZUSP XXXXX).

Paratypes. Brazil, Santa Catarina – one f#, same data as holotype (UFRGS 6489); one f#, same data as holotype (UFRGS 6490); one f#, same data as holotype (UFRGS 6491); one f#, same data as holotype (UFRGS 6492); one m#, same data as holotype (UFRGS 6493).

Diagnosis. Rostrum triangular and with apex U-shaped. Carpal spine present in chelipeds. Dorsal surface of chelipeds palm with three rows of verrucous tubercles.

Description.

Rostrum: triangular, longer than wide (RL 82.9% of RW), short (11.1% of CL), reaching the half of the second article of the antennular peduncle (Fig. 35A–C). Dorsum deflexed, apex inverted "U"-shaped, ending in a downward spine (Fig. 35B, C). Few plumose setae on lateral margins. Rostral sides slightly convergent and rostral basis divergent. Carinae straight, prominent and narrow, extending back to carapace, slightly surpassing rostral basis (Fig. 35B, C).

Cephalon: Carapace lacking spines or tubercles. CeL 63.2% of CL. Eyes large (CMW 71.27% of OW); suborbital angle > 90°, unarmed (Fig. 35C). Front narrow (FW 44.5% of CW). Postorbital carinae longer than rostral carinae (RCL 70.6% of POCL) and prominent. Lateral cephalic edge with sparse setation (Fig. 35A–C).

Thorax: carapace laterally compressed, deep and narrow (CD 54.8% of CL; CW 43.3% of CL). Cervical groove strongly V-shaped. Branchiocardiac grooves conspicuous (Fig. 35A). Areola narrow, 2.6 as long as wide (30.21% of CL) (Fig. 35A).

Abdomen: lacking spines or tubercles, long and wide (AL 81.7% of CL; AW 94.9% of CW), smooth, covered with small setae on pleural margins (Fig. 35A). Pleural somites with rounded posterior margins. S2 pleurae low and short with deep groove parallel to margin (Fig. 35D, E, F).

Tailfan: telson uniformly calcified, subtriangular, longer than wide (TeW 84.3% of TeL), with small 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. 35E). Uropod protopod bilobed, with rounded and unarmed margins; proximal lobe largest. Exopod lateral margin bears a small and sharp spine, mid-dorsal carina few prominent, ending in a sharp spine. Transverse suture (diaeresis) straight, with eleven dorsolateral spines (outer) and nine dorsolateral spines (inner) on right exopod and ten dorsolateral spines (outer) and seven dorsolateral spine (inner) on the left exopod. Endopod, mid-dorsal carina few prominent, ending in a small spine; lateral margin with one sharp spine at level of exopod transverse suture (Fig. 35E).

Epistome: anterolateral section with blunt conical projection. Posterolateral section smooth and with deep lateral grooves converging to the basis of the anteromedian lobe, and with a small median concavity. Anteromedian lobe pentagonal, 1.2x longer than wide, apex rounded with surpassing median part of antepenultimate article of antennal peduncle; dorsal surface concave at apex with serrated setae, and basis with a shallow groove (Fig. 36A).

Thoracic sternites: SLP4 very close to each other, median keel present and not inflated; SLP5 smallest, median keel present and not inflated; SLP6 larger than SLP4 and SLP5 with a slightly concave surface, median keel inflated; SLP7 largest and with surface slightly concave, median keel inflated, bullar lobes absent; SLP8 straight, median keel absent, vertical arms of paired sternopleural bridges close to each other, bullar lobes separated to each other and clearly visible (Fig. 36B, C).

Antennule: internal ventral border of basal article without spine (Fig. 36A).

Antenna: when extended back reaching S2. Antennal scale widest at midlength, reaching the end of third antennal article, ASW 45.9% of ASL (Fig. 36A, D), lateral margin straight, spine strong and distal margin emarginate. Coxa with prominent carina and one blunt spine above nephropore. Basis unarmed (Fig. 36A).

Mandible: cephalic molar process molariform, caudal molar process unicuspidate with one cephalodistal cusp. Incisive lobe with nine teeth. The third tooth from the anterior margin is the largest (Fig. 36E).

Third maxilliped: ischium, ventral surface with tufts of setae (Fig. 36F); dorsal surface with few sparse small and simple setae (Fig. 36G). *Crista dentata* bearing 25 and 26 teeth in right and left ischium respectively. Merus ventral surface with longitudinal rows of long and simple setae. (Fig. 36F, G).

First pair of pereiopods (chelipeds): large and subequal, laterally flatenned (RPrT 22.1% of RPrL; LPrT 22.41% of LPrL) (Fig. 36A). Ischium ventral surface with 17 tubercles. Merus: right merus (RML) 53.7% of propodus length (RPrL); left merus (LML) 54.8% of propodus length (LPrL); ventral surface with two longitudinal series of tubercles: inner series with 16 tubercles, outer 14 and mesial 28, arranged irregularly on right merus; inner series bearing 17 tubercles, outer 14 and mesial 29, arranged irregularly on left merus. Dorsal and midventral spines present. Carpus divided by a shallow transversal groove (Fig. 35A; Fig. 36I). Internal dorsolateral margin with row of tubercles, increasing in size distally; inner surface with few small mesial tubercles. Carpal spine present (Fig. 36I). Propodus width (RPrW and LPrW) 45.6% of length in right cheliped and 46% in left cheliped. Dorsal surface of palm with three rows of verrucous tubercles (Fig. 36H, I). Inner margin without tubercles. Ventral surface bearing two rows of squamose tubercles, reaching the beginning of the fixed finger (Fig. 36H), right dactylus (RDL) 58.7% of propodus length (RPrL), left dactylus (LDL) 60.2% of left propodus (LPrL); dorsal surface with rows of setiferous punctuations (Fig. 4I). Cutting edge of fingers visible. Fixed finger with nine teeth, third teeth largest. Dactylus with nine teeth, third tooth largest (Fig. 36H, I).

Second pair of pereiopods: ventral and dorsal surface of carpus, propodus and dactylus with sparse covering of simple long setae (Fig. 36J).

Gonopores: Presence of both genital apertures on coxae of third and fifth pairs of pereiopods. Female gonopores semi-ellipsoidal (maximum diameter 1.26 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. 36B).

Remarks. *Parastacus* **sp. nov. 6** ressembles *P. tuerkayi* in having large chelipeds with dorsal surface of palm with three rows of verrucous tubercles, but it is distinguishable in the shape of epistome anteromedian lobe and number of teeth in mandible cephalic molar process.

Distribution. Brazil: State of Santa Catarina (Fig. 51).

Color of living specimens. Rostrum, cephalothorax anterior and lateral regions, dorsal pleon and tailfan reddish brown. Pereiopod pairs 2 – 5 reddish brown (Fig. 37D).

Habitat and Ecology. Peat bogs (37A). Burrows are shallow due to the soil permanently flooded. Simple burrows without ornamentation and with chimney can reach up to 5 cm height (37B, C). Burrows of *Parastacus* **sp. nov. 6** can be are of type 2 according to Horwitz and Richardson's (1986) and considered a primary burrower species based on Hobbs' (1942) classification.

Parastacus sp. nov. 7

(Figs. 38–39, 51)

Holotype. m#, Brazil, Rio Grande do Sul, Silveira Martins (29°39'25.13"S; 53°37'33.6" W), 23/IX/2013, col. M. M. Dalosto

Paratypes. Brazil, Rio Grande do Sul – two f#, same data as holotype (UFRGS 6484); one m#, same data as holotype (UFRGS 6489); one m#, same data as holotype (UFRGS 5793); one m#, same data as holotype (UFRGS 5778); one m#, same data as holotype (UFRGS 5795); one f#, same data as holotype (UFRGS 5794).

Diagnosis. Inner margin of chelipeds palm with tubercles irregularly distributed. Ventral surface bearing two rows of squamose tubercles, surpassing the beginning of the fixed finger. Incisive lobe of mandible with seven teeth, second is the largest.

Description.

Rostrum: spatulated, longer than wide (RL 85.9% of RW), short (11.71% of CL), reaching the half of the second article of the antennular peduncle (Fig. 38A–C). Dorsum deflexed, apex inverted "U"-shaped, ending in a downward spine (Fig. 38B, C). Few plumose setae on lateral margins. Rostral sides slightly convergent and rostral basis slightly divergent. Carinae straight, prominent and narrow, extending back to carapace, surpassing rostral basis (Fig. 38B, C).

Cephalon: CeL 69.8% of CL. Eyes large (CMW 73.7% of OW); suborbital angle > 90°, unarmed (Fig. 38C). Front narrow (FW 43.3% of CW). Postorbital carinae longer than rostral carinae (RCL 76.8% of POCL) and weakly prominent. Lateral cephalic edge with sparse setation (Fig. 38A–C).

Thorax: carapace laterally compressed, deep and wide (CD 47.29% of CL; CW 40.7% of CL). Cervical groove weakly V-shaped. Branchiocardiac grooves inconspicuous (Fig. 38A). Areola narrow, 2x as long as wide (23.23% of CL) (Fig. 38A).

Abdomen: long and wide (AL 80.36% of CL; AW 96.6% of CW), smooth, not covered by setae on pleural margins (Fig. 38A). Pleural somites with rounded posterior margins. S2 pleurae low and short with deep groove parallel to margin (Fig. 38D, E, F).

Tailfan: telson uniformly calcified, subrectangular, longer than wide (TeW 76.6% of TeL), with small 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. 38E). Uropod protopod bilobed, with rounded and unarmed margins; proximal lobe largest. Exopod lateral without spine, mid-dorsal carina few prominent, ending in a sharp spine. Transverse suture (diaeresis) straight, with eight dorsolateral spines (outer) andfive dorsolateral spines (inner) on right exopod and six dorsolateral spines (outer) and seven dorsolateral spine (inner) on the left exopod. Endopod, mid-dorsal carina few prominent, ending in a small spine; lateral margin with one sharp spine at level of exopod transverse suture (Fig. 38E).

Epistome: anterolateral section with blunt conical projection. Posterolateral section smooth and with deep lateral grooves converging to the basis of the anteromedian lobe, and with a small median concavity. Anteromedian lobe pentagonal, 1.1x longer than wide, apex rounded with serrated setae, surpassing median part of antepenultimate article of antennal peduncle; dorsal surface straight, and basis with a shallow groove (Fig. 39A).

Thoracic sternites: SLP4 and SLP5 subequal and separated to each other, median keel present and not inflated; SLP6 larger than SLP4 and SLP5 with a slightly concave surface, median keel inflated; SLP7 largest and with surface slightly concave, median keel inflated, bullar lobes absent; SLP8 straight, median keel absent, vertical arms of paired sternopleural bridges close to each other, bullar not visible (Fig. 39B, C).

Antennule: internal ventral border of basal article with a sharp spine (Fig. 39A).

Antenna: when extended back reaching S3. Antennal scale widest at midlength, reaching the end of third antennal article, ASW 80.3% of ASL (Fig. 39A, D), lateral margin straight, spine strong and distal margin emarginate. Coxa with prominent carina and one blunt laterally displaced in relation to the nephropore. Basis unarmed (Fig. 39A).

Mandible: cephalic molar process molariform, caudal molar process bicuspidate with one cephalodistal cusp and one small distoproximal cusp. Incisive lobe with seven teeth. The second tooth from the anterior margin is the largest (Fig. 39E).

Third maxilliped: ischium, ventral surface entirely covered by tufts of long and simple (Fig. 39F); dorsal surface glabrous (Fig. 39G). *Crista dentata* bearing 22 and 23 teeth in right and left ischium respectively. Merus ventral surface follows the same pattern than that of ischium (Fig. 39F, G).

First pair of pereiopods (chelipeds): large and subequal, laterally flatenned (RPrT 17.3% of RPrL; LPrT 24.6% of LPrL) (Fig. 39A). Ischium ventral surface with 13 tubercles. Merus: right merus (RML) 66.6% of propodus length (RPrL); left merus (LML) 56.29% of propodus length (LPrL); ventral surface with two longitudinal series of tubercles: inner series with 14 tubercles, outer 18 and mesial 35, arranged irregularly on right merus; inner series bearing 14 tubercles, outer 18 and mesial 26, arranged irregularly on left merus. Dorsal and midventral spines present. Carpus divided by a shallow transversal groove (Fig. 38A; Fig. 39I). Internal dorsolateral margin with row of tubercles, increasing in size distally; inner surface with several small median tubercles. Carpal spine absent (Fig. 39I). Propodus width (RPrW and LPrW) 34.2% of length in right cheliped and 40.5% in left cheliped. Dorsal surface of palm with verrucous tubercles irregularly distributed (Fig. 39H, I). Inner margin with tubercles irregularly distributed. Ventral surface bearing two rows of squamose tubercles, surpassing the beginning of the fixed finger (Fig. 39H), right dactylus (RDL) 58.7% of propodus length (RPrL), left dactylus (LDL) 57.1% of left propodus (LPrL); dorsal surface with rows of verrucous rtubercles (Fig. 39I). Cutting edge of fingers visible. Fixed finger with 12 teeth, third teeth largest. Dactylus with 16 teeth, third tooth largest (Fig. 39H, I).

Second pair of pereiopods: ventral and dorsal surface of carpus, propodus and dactylus with sparse covering of simple long setae (Fig. 39J).

Gonopores: Presence of both genital apertures on coxae of third and fifth pairs of pereiopods. Female gonopores semi-ellipsoidal (maximum diameter 1.44 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. 39B).

Remarks. *Parastacus* **sp. nov. 7** ressembles *P. brasiliensis* in having large and laterally flattened chelipeds, telson subrectangular and triangular or spatulated rostrum, but it differs in the nner margin of chelipeds palm with tubercles irregularly distributed and in the number of teeth in the mandible incisive lobe.

Distribution. Brazil: State of Rio Grande do Sul (Fig. 51).

Color of living specimens. Data not available.

Habitat and Ecology. Data not available.

Parastacus sp. nov. 8

(Figs. 40–41, 51)

Holotype. m#, Brazil, Rio Grande do Sul, Caraá (29°42'26.39"S; 50°17'27.41"W), 04/XII/2012, col. M.P. Almerão (MZUSP XXXXX);

Paratypes. Brazil, Rio Grande do Sul – five m#, same data as holotype (UFRGS 6440).

Diagnosis. Rostrum wider than long, apex ending ina upwards spine. S3 longer than S2 with an elevation ressembling a humpback.

Description.

Rostrum: triangular, wider than long (RL 83.2% of RW), short (11.1% of CL), not reaching the half of the second article of the antennular peduncle (Fig. 40A–C). Dorsum upwards, apex inverted "U"-shaped, ending in a upwards spine (Fig. 40B, C). Few plumose setae on lateral margins. Rostral sides slightly convergent and rostral basis slightly divergent. Carinae straight, prominent and narrow, extending back to carapace, slightly surpassing rostral basis (Fig. 40B, C).

Cephalon: CeL 66% of CL. Eyes small (CMW 51.6% of OW); suborbital angle > 90°, unarmed (Fig. 40C). Front narrow (FW 39.5% of CW). Postorbital carinae longer than rostral carinae (RCL 65.9% of POCL) and weakly prominent. Lateral cephalic edge with some tubercles and sparse setation (Fig. 40A–C).

Thorax: carapace laterally compressed, deep and narrow (CD 57.3% of CL; CW 46.2% of CL). Cervical groove weakly U-shaped. Branchiocardiac grooves conspicuous (Fig. 40A). Areola narrow, 4.3x as long as wide (33.6% of CL) (Fig. 40A).

Abdomen: long and narrow (AL 70.8% of CL; AW 64.6% of CW), smooth, not covered by setae on pleural margins (Fig. 40A). Pleural somites with rounded posterior margins. S2 pleurae high and long without groove parallel to margin (Fig. 40D, E, F). S3 longer than S2 with an elevation ressembling a humpback.

Tailfan: telson uniformly calcified, subtriangular, longer than wide (TeW 76.6% of TeL), with small 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. 40E). Uropod protopod bilobed, with rounded and unarmed margins; proximal lobe largest. Exopod lateral with a small spine, mid-dorsal carina few prominent, ending in a 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 spine (inner) on the left exopod. Endopod, mid-dorsal carina few prominent, ending in a small spine; lateral margin with one sharp spine at level of exopod transverse suture (Fig. 40E).

Epistome: anterolateral section with inconspicuous blunt conical projection. Posterolateral section smooth and with deep lateral grooves converging to the basis of the anteromedian lobe, and with longitudinal median groove. Anteromedian lobe pentagonal, 1.2x longer than wide, apex acute with serrated setae, reaching median part of antepenultimate article of antennal peduncle; dorsal surface straight, and basis with a shallow groove (Fig. 41A).

Thoracic sternites: SLP4 close to each other, median keel present and not inflated; SLP5 smallest and close to each other, median keel inflated; SLP7 largest and with surface slightly concave, median keel inflated, bullar lobes absent; SLP8 straight, median keel absent, vertical arms of paired sternopleural bridges close to each other, bullar not visible (Fig. 41B, C).

Antennule: internal ventral border of basal article with a sharp spine (Fig. 41A).

Antenna: when extended back reaching telson. Antennal scale widest at midlength, reaching the end of third antennal article, ASW 44.8% of ASL (Fig. 41A, D), lateral margin straight, spine strong and distal margin emarginate. Coxa with prominent carina and one blunt above the nephropore. Basis unarmed (Fig. 41A).

Mandible: cephalic molar process molariform, caudal molar process bicuspidate with one cephalodistal cusp and one small distoproximal cusp. Incisive lobe with nine teeth. The third tooth from the anterior margin is the largest (Fig. 41E).

Third maxilliped: ischium, ventral surface entirely covered by tufts of short and simple (Fig. 41F); dorsal surface glabrous (Fig. 41G). *Crista dentata* bearing 27 and 20 teeth in right and left ischium respectively. Merus ventral surface entirely covered by tufts of long and simple setae (Fig. 41F, G).

First pair of pereiopods (chelipeds): large and subequal, laterally flatenned (RPrT 25.6% of RPrL; LPrT 25.1% of LPrL) (Fig. 41A). Ischium ventral surface with 10 and seven tubercles in the right and left ischium respectively. 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 12 tubercles, outer eight and mesial 17, arranged irregularly on right merus; inner series bearing 13 tubercles, outer 10 and mesial 15, arranged irregularly on left merus. Dorsal and midventral spines absent. Carpus not divided by a shallow transversal groove (Fig. 40A; Fig. 41I). Internal dorsolateral margin with row of tubercles, increasing in size distally; inner surface without mesial tubercles. Carpal spine absent (Fig. 41I). Propodus width (RPrW and LPrW) 41.8% of length in right cheliped and 41.8% in left cheliped. Dorsal surface of palm with verrucous tubercles irregularly distributed (Fig. 41H, I). Inner margin without tubercles. Ventral surface bearing two rows of squamose tubercles, reaching the beginning of the fixed finger (Fig. 41H), right dactylus (RDL) 59.6% of propodus length (RPrL), left dactylus (LDL) 59.6% of left propodus (LPrL); dorsal surface with rows of setiferous punctuations (Fig. 41I). Cutting edge of fingers visible. Fixed finger with seven teeth with four large. Dactylus with eight teeth, the last two smallest (Fig. 41H, I).

Second pair of pereiopods: ventral and dorsal surface of carpus, propodus and dactylus with sparse covering of simple long setae (Fig. 41J).

Gonopores: Presence of both genital apertures on coxae of third and fifth pairs of pereiopods. Female gonopores semi-ellipsoidal (maximum diameter 1.05 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. 41B).

Remarks. This species ressembles *P. brasiliensis* in having large and laterally flattened chelipeds, but it is distinguished from other congeneric species by the S3 longer than S2 with an elevation ressembling a humpback.

Distribution. Brazil: State of Rio Grande do Sul (Fig. 51).

Color of living specimens. Data not available.

Habitat and Ecology. Data not available.

Parastacus sp. nov. 9

(Figs. 42–44, 51)

Holotype. m#, Brazil, Rio Grande do Sul, Osório, Morro da Borússia, 05/XI/2013, col. K. M. Gomes (MZUSP XXXXX);

Paratypes. Brazil, Rio Grande do Sul – two m#, same data as holotype (UFRGS 6441)

Diagnosis. Carapace laterally compressed, deep and narrow. Telson subtriangular with very small lateral spines.

Description.

Rostrum: triangular, wider than long (RL 78.5% of RW), long (11.7% of CL), reaching the proximal portion of the third antennular peduncle (Fig. 42A–C). Dorsum straight, apex inverted "V"-shaped, ending in a straight spine (Fig. 42B, C). Few plumose setae on lateral margins. Rostral sides slightly convergent and rostral basis slightly divergent. Carinae angled, prominent and narrow, extending back to carapace, slightly surpassing rostral basis (Fig. 42B, C).

Cephalon: CeL 64.1% of CL. Eyes large (CMW 72.8% of OW); suborbital angle 90°, unarmed (Fig. 42C). Front narrow (FW 48.6% of CW). Postorbital carinae longer than rostral carinae (RCL 98.5% of POCL) and weakly prominent. Lateral cephalic edge with sparse setation (Fig. 42A–C).

Thorax: carapace laterally compressed, deep and narrow (CD 53.8% of CL; CW 36.5% of CL). Cervical groove U-shaped. Branchiocardiac grooves inconspicuous (Fig. 42A). Areola narrow, 2.5x as long as wide (25.04% of CL) (Fig.42A).

Abdomen: long and narrow (AL 68.3% of CL; AW 97.1% of CW), smooth, not covered by setae on pleural margins (Fig. 42A). Pleural somites with rounded posterior margins. S2 pleurae low and short with shallow groove parallel to margin (Fig. 42D, E, F).

Tailfan: telson uniformly calcified, subtriangular, longer than wide (TeW 79.9% of TeL), with very small 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. 42E). Uropod protopod bilobed, with rounded and unarmed margins; proximal lobe largest. Exopod lateral with a very small spine, mid-dorsal carina few prominent, ending in a sharp spine. Transverse suture (diaeresis) straight, with five dorsolateral spines (outer) and four dorsolateral spines (inner) on right exopod and six dorsolateral spines (outer) and five dorsolateral spine (inner) on the left exopod. Endopod, mid-dorsal carina few prominent, ending in a very small spine; lateral margin with one sharp spine at level of exopod transverse suture (Fig. 42E).

Epistome: anterolateral section with a blunt conical projection. Posterolateral section smooth and with deep lateral grooves converging to the basis of the anteromedian lobe, and with small median concavity. Anteromedian lobe pentagonal, 1.2x longer than wide, apex acute with serrated setae, surpassing median part of antepenultimate article of antennal peduncle; dorsal surface straight, and basis with a shallow groove (Fig. 43A).

Thoracic sternites: SLP4 and SLP5 subequal in size, close to each other, median keel present and not inflated; SLP6 larger than SLP4 and SLP5 and close to each other, median keel inflated; SLP7 largest and with surface slightly concave, median keel inflated, bullar lobes absent; SLP8 straight, median keel absent, vertical arms of paired sternopleural bridges close to each other, bullar not visible (Fig. 43B, C).

Antennule: internal ventral border of basal article with a blunt spine (Fig. 43A).

Antenna: when extended back reaching S3. Antennal scale widest at midlength, reaching the end of third antennal article, ASW 39.75% of ASL (Fig. 43A, D), lateral margin straight, spine strong and distal margin straight. Coxa with prominent carina and one blunt above the nephropore. Basis unarmed (Fig. 43A).

Mandible: cephalic molar process molariform, caudal molar process bicuspidate with one cephalodistal cusp and one small distoproximal cusp. Incisive lobe with ten teeth. The third tooth from the anterior margin is the largest (Fig. 43E).

Third maxilliped: ischium, ventral surface partially covered by tufts of short and simple setae (Fig. 43F); dorsal surface glabrous (Fig. 43G). *Crista dentata* bearing 22 and 25 teeth in right and left ischium respectively. Merus ventral surface covered by tufts of long and simple setae in the inner margin (Fig. 43F, G).

First pair of pereiopods (chelipeds): large and subequal, laterally flatenned (RPrT 25.8% of RPrL; LPrT 25.8% of LPrL) (Fig. XA). Ischium ventral surface with nine tubercles. Merus: right merus (RML) 62.38% of propodus length (RPrL); left merus (LML) 60.1% of propodus length (LPrL); ventral surface with two longitudinal series of tubercles: inner series with 15 tubercles, outer 13 and mesial six, arranged irregularly on right merus; inner series bearing 16 tubercles, outer 14 and mesial fou, arranged irregularly on left merus. Dorsal and midventral spines present. Carpus not divided by a shallow transversal groove (Fig. 42A; Fig. 43I). Internal dorsolateral margin with row of tubercles, increasing in size distally; inner surface with few small mesial tubercles. Carpal spine present (Fig. 43I). Propodus width (RPrW and LPrW) 20.1% of length in right cheliped and 42.2% in left cheliped. Dorsal surface of palm with two lines of verrucous tubercles (Fig. 43H, I). Inner margin without tubercles. Ventral surface bearing two rows of squamose tubercles, reaching the beginning of the fixed finger (Fig. 43H), right dactylus (RDL) 59% of propodus length (RPrL), left dactylus (LDL) 66.9% of left propodus (LPrL); dorsal surface with rows of setiferous punctuations (Fig. 43I). Cutting edge of fingers visible. Fixed finger with five teeth, third tooth is slightly larger. Dactylus with six teeth, third tooth largest (Fig. 43H, I).

Second pair of pereiopods: ventral and dorsal surface of carpus, propodus and dactylus with sparse covering of simple long setae (Fig. 43J).

Gonopores: Presence of both genital apertures on coxae of third and fifth pairs of pereiopods. Female gonopores semi-ellipsoidal (maximum diameter 1.24 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. 43B).

Remarks. This species ressembles *P. pilimanus* in the shape of chelipeds and rostrum. It can be distinguishable from all congeneric species by the very small lateral spines of telson.

Distribution. Brazil: State of Rio Grande do Sul.

Color of living species. Rostrum, cephalothorax anterior and lateral regions, dorsal pleon and tailfan brown to dark brown. Pereiopod pairs 2 – 5 brown (Fig. 44D).

Habitat and Ecology. Stream and wetlands associated with low order stream (1-5 order) (44A). *Parastacus* **sp. nov. 9** also can be found under litter and entrances of tree roots or simple burrows in the banks, with up to 40 cm deep. The accumulated sediment within and at margin are also inhabited by adult individuals. Burrow with chimney was not observed during the samplings. Species burrows can be identified as type 1 a and b, in wetlands of type 2 (Horwitz and Richardson, 1986). *Parastacus* **sp. nov. 9** is considered secundary burrower according to Hobbs (1942) classification.

Parastacus sp. nov. 10

(Figs. 45–47, 51)

Holotype. m#, Brazil, Rio Grande do Sul, Eldorado do Sul, Estação Agronômica da UFRGS, 19/III/2013, col. K. M. Gomes (MZUSP XXXXX);

Paratypes. Brazil, Rio Grande do Sul – two m# ando one f#, same data as holotype (UFRGS 5755)

Diagnosis. Rostrum very short with sort and convergent carinae. Post orbital carinae prominent. Mandible caudal molar process unicuspidate, lobe incisive with eight teeth. Globose chelipeds and carpal spine present.

Description.

Rostrum: triangular, wider than long (RL 83% of RW), short (10.9% of CL), surpassing the half of the second antennular peduncle (Fig. 45A–C). Dorsum straight, apex inverted "V"-shaped, ending in a small blunt spine (Fig. 45B, C). Few plumose setae on lateral margins. Rostral sides slightly convergent and rostral basis slightly divergent. Carinae straight, prominent and narrow, extending back to carapace, slightly surpassing rostral basis (Fig. 45B, C).

Cephalon: CeL 62.5% of CL. Eyes small (CMW 71.33% of OW); suborbital angle > 90°, unarmed (Fig. 3C). Front narrow (FW 37.5% of CW). Postorbital carinae longer than rostral carinae (RCL 88.3% of POCL) and weakly prominent. Lateral cephalic edge with dense setation (Fig. 45A–C).

Thorax: carapace laterally compressed, deep and narrow (CD 56.7% of CL; CW 45.6% of CL). Cervical strongly V-shaped. Branchiocardiac grooves inconspicuous (Fig. 1A). Areola narrow, 2.5x as long as wide (26.4% of CL) (Fig. 45A).

Abdomen: long and narrow (AL 70.1% of CL; AW 72.3% of CW), smooth, covered by small setae on pleural margins (Fig. 45A). Pleural somites with rounded posterior margins. S2 pleurae low and short with shallow groove parallel to margin (Fig. 45D, E, F).

Tailfan: telson uniformly calcified, surectangular, longer than wide (TeW 97.2% 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. 45E). Uropod protopod bilobed, with rounded and unarmed margins; proximal lobe largest. Exopod lateral not ending in a spine, mid-dorsal carina few prominent, ending in a 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 spine (inner) on the left exopod. Endopod, mid-dorsal carina few prominent, not ending in a spine; lateral margin with one sharp spine at level of exopod transverse suture (Fig. 45E).

Epistome: anterolateral section with a blunt conical projection. Posterolateral section smooth and with deep lateral grooves converging to the basis of the anteromedian lobe, and with small median concavity. Anteromedian lobe pentagonal, 1.2x longer than wide, apex acute, surpassing median part of antepenultimate article of antennal peduncle; dorsal surface straight, laterally keeled, and basis with a shallow groove (Fig. 46A).

Thoracic sternites: SLP4 and SLP5 subequal in size, SLP4 close to each other and SLP5 separeted to each other, median keel present and not inflated; SLP6 larger than SLP4 and SLP5 and close to each other, median keel not inflated; SLP7 largest and with surface slightly concave, median keel inflated, bullar lobes absent; SLP8 straight, median keel absent, vertical arms of paired sternopleural bridges close to each other, bullar not visible (Fig. 46B, C).

Antennule: internal ventral border of basal article with a sharp spine (Fig. 46A).

Antenna: when extended back reaching S3. Antennal scale widest at midlength, reaching the end of third antennal article, ASW 33.2% of ASL (Fig. 46.A, D), lateral margin straight, spine strong and distal margin emarginate. Coxa with prominent carina and one blunt above the nephropore. Basis unarmed (Fig. 46A).

Mandible: cephalic molar process molariform, caudal molar process unicuspidate with one cephalodistal cusp. Incisive lobe with eight teeth. The third tooth from the anterior margin is the largest (Fig. 46E).

Third maxilliped: ischium, ventral surface partially covered by tufts of short and long simple setae (Fig. 2F); dorsal surface glabrous (Fig. 46G). *Crista dentata* bearing 24 and 22 teeth in right and left ischium respectively. Merus ventral surface covered by tufts of long and simple setae in the inner margin (Fig. 46F, G).

First pair of pereiopods (chelipeds): small and subequal, globose (RPrT 32.9% of RPrL; LPrT 27.9% of LPrL) (Fig. 45A). Ischium ventral surface with six and 12 tubercles in the right and left ischium respectively. Merus: right merus (RML) 58.8% of propodus length (RPrL); left merus (LML) 79.2% of propodus length (LPrL); ventral surface with two longitudinal series of tubercles: inner series with 12 tubercles, outer nine and mesial 19, arranged irregularly on right merus; inner series bearing 10 tubercles, outer 10 and mesial 17, arranged irregularly on left merus. Dorsal and midventral spines present. Carpus not divided by a shallow transversal groove (Fig.

45A; Fig. 46). Internal dorsolateral margin with row of tubercles, increasing in size distally; inner surface with 20 small mesial tubercles. Carpal spine present (Fig. 46I). Propodus width (RPrW and LPrW) 56.4% of length in right cheliped and 49.1% in left cheliped. Dorsal surface of palm with verrucous tubercles irregularly distributed (Fig. 46H, I). Inner margin without tubercles. Ventral surface bearing two rows of squamose tubercles, reaching the beginning of the fixed finger (Fig. 46H), right dactylus (RDL) 61.3% of propodus length (RPrL), left dactylus (LDL) larger than of left propodus (LPrL); dorsal surface with rows of setiferous punctuations (Fig. 46I). Cutting edge of fingers visible. Fixed finger with five teeth, second tooth largest. Dactylus with six teeth, second tooth largest (Fig. 46H, I).

Second pair of pereiopods: ventral and dorsal surface of carpus, propodus and dactylus densely covered of simple long setae (Fig. 46J).

Gonopores: Presence of both genital apertures on coxae of third and fifth pairs of pereiopods. Female gonopores semi-ellipsoidal (maximum diameter 1.27 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. 46B).

Remarks. *Parastacus* sp. 10 ressembles *P. defossus* in having globose chelipeds, narrow abdomen, subrectangular telson and rostrum triangular, but differs in the post orbital carinae more prominent, cephalic molar process of mandible bicuspidate and bullar lobes not visible.

Distribution. Brazil: state of Rio Grande do Sul.

Color of living specimens. Rostrum, cephalothorax anterior and lateral regions, dorsal pleon and tailfan light brown to brown. Pereiopod pairs 2 – 5 light brown (Fig. 47C).

Habitat and Ecology. Floodplains (Fig. 47A). Presence of complex burrows (with several ramifications) and depth can exceed 1m. deep The chimneys reach up to 15 cm, but simple burrows are also found mainly in bank stream. Inside the burrow is possible to find adults and juveniles (Fig. 47B). *Parastacus* **sp. nov. 10** can be identified as type

2 according to Horwitz and Richardson's (1986) classification and the species can be considered a primary burrower based on Hobbs' (1942) classification.

Genus *xxxxxxxxx* gen. nov.

Type species. *Astacus nicoleti* by subsequent designation (Ortmann, 1902: 293): *Astacus nicoleti* Philippi, 1882: 624. Gender: masculine.]

***Gen. nov. nicoleti* (Philippi, 1882) new combination**

(Fig. 48–49, 52)

Astacus chilensis.—Nicolet, 1849: 211 [not H. Milne-Edwards, 1837]

Astacus Nicoleti Philippi, 1882: 624.

Astacus nicoleti.—Faxon, 1914: 354.

Astacus Ricoleti.—Philippi, 1882:626 [erroneous spelling].

Parastacus nicoletii Faxon, 1898: 689 [as a new species].

Parastacus nicoleti.—Ortmann, 1902: 293.—Porter, 1917: 98, fig. 61.—Bahamonde, 1958: 183, figs. 1–17.—Riek, 1971: 133.—Hobbs 1989: 80, fig. 372.—Buckup and Rossi, 1993: 168, fig. 4.—Rudolph, 2010: 37 , fig. 1C, 2C.—Rudolph 2013: 1488, fig. 14.—Ríos-Escalante, 2016: 121, fig. 3(4).

Parastacus Nicoleti.—Porter, 1904: 255.

Parastacus nicoletti.—Bahamonde, 1951: 91 [erroneus spelling]

Neotype: Chile: one m# Miraflores, Río Valdivia, XI/1957, col. Killian (MNHNCL/CRU XXXX)

Material examined. Chile: one m#, Mehuim (next to Valdivia), VIII/1997, col. niños del Pueblo (UFRGS 2405); one snd, Valdivia (UFRGS 1372); 2 m# and 4 f#, Miraflores, Río Valdivia, XI/1957, col. Killian (MNHNCL/CRU 2211); one m#, Río Chaquigua, 18/III/1985, col. L. Peña (MNHNCL/CRU 2026); one snd, Valdivia, Fundo "Palos Altos", 1954, col. M.T. Lopéz (MNHNCL/CRU 2072); 12 m#, 4 f# and one juvenile, Valdivia, Jardim Botánico, 15/X/1950, col. E. Killian (MNHNCL/CRU 2137); 1 m# (dry specimen), Fondo Bellavista (cerca La Unión), 13/II/1958, col. B.G. López (MNHNCL/CRU 2151); one snd (dry specimen), Valdivia, Catripuye, II/1954, col. G. Lopéz (MNHNCL/CRU 2116); one m#, Valdivia, VIII/1959, col. López (MNHNCL/CRU 2181); two m#, one f#, nine juveniles and one snd, Valdivia, Fundo "Palos Altos", 08/II/1959, col. T. López (MNHNCL/CRU 2373); one m#, Fundo

Bellavista (cerca de La Union), 13/II/1958 (MNHNCL/CRU 2254); one m# and two f#, Valdivia, Miraflores, XI/1957, col. E. Killian (MNHNCL/CRU 2257); three m#, five f# and two juveniles, Fundo Encantrao (25km north of Valdivia), 24/II/1958, col. G. López (MNHNCL/CRU 2260); Fundo Palos Altos, VII/1959, col. López (MNHNCL/CRU 2374); one m# and one ovigerous f# (bearing 37 eggs), Valdivia, 09/IX/1967, col. M.T. López (BMNH 1972.101); one m# and one f#, Valdivia, 1925, col. Ohde (ZSM 67/01); one f#, Máfil, Valdivia, 26/08/1994, col. E. Rudolph (FC–UDELAR 246); one m# and one ovigerous f# (with approximately 37 eggs), Valdivia, Provincia Valdivia, 09/IX/1967, col. M. T. Lopéz (BMNH 1972.101);

Diagnosis. Carapace lacking spines and tubercles; rostrum quadratic and truncated**;** postorbital carinae obsolete; cervical groove weakly V-shaped, deeply impressed; branchiocardiac grooves inconspicuous with anterolateral part often hardly distinguishable and very close to cervical groove, two usually merging dorsolaterally. Abdomen lacking tubercles or spines; first abdominal pleurae not overlapped by that of second; posterior margin of second abdominal pleura rounded. Telson subtriangular weakly calcified not divided by a dorsomedian longitudinal groove and without lateral spines. Epistome with anteromedian lobe pentagonal. Antennal scale lateral margin straight with terminal strong spine. Third maxilliped with mesial half of ventral surface of ischium bearing setiferous punctuations; setae coverture moderate; flagellum of exopodite reaches mesial part of merus. Caudal molar process of mandible bicuspidate; incisive lobe with eight teeth, the second is the largest. Chelipeds carpus not divided by a groove impression; propodus dorsal region with two lines of well-developed verrucous tubercles, ventral region with two rows of tubercles that reach the beginning of the fixed finger; dactylus moving obliquely. Male genitalia consisting of a fixed and slightly elevated ventromesial ridge bearing a small noncalcified papilla; male cuticle partition (*sensu* Morgan, 1986) present; supernumerary gonopores in males; protandric hermaphroditism. Branchial count 20 + epr + r, or 20 + ep + r (podobranchs on segments VIII–XIII; anterior arthrobranchs on VIII–XIII; posterior arthrobranchs on IX–XIII, 13 rudimentary pleurobranchs on XI–XIV; and epipodite on VII bearing few branchial filaments). SLP8 with a deep median suture.

Description and Diagnosis. Phillipi (1882), Bahamonde (1868) and Buckup and Rossi (1993).

Remarks. Type material of this species was apparentely lost as pointed by Bahamonde (1958). Buckup and Rossi (1993) redescribed this species and indicated that the lateral spines in telson are present in all species of *Parastacus*, except *P. nicoleti*. Telson without lateral spines is also found in the Australian genus *Engaeus*. Additional morphological characters not mentioned in previous descriptions include: eyes small (Fig. 48A, B, C); front narrow (Fig. 48A, B); anterolateral section with inconspicuous projection (Fig. 49A); antennal scale lateral margin curved (Fig. 49D); antennule internal ventral border of basal article without spine (Fig. 49A); mandible with cephalic molar process molariform and caudal molar process bicuspidate, incisive lobe with eight teeth; the second tooth from the anterior are the largest (Fig. 49E); SLP4 and SLP5 subequal in size and separated to each other, medial keel present and not inflated; SLP6 larger than SLP4 and SLP5 with slightly concave dorsal surface , medial keel present and slightly inflated; SLP7 largest and with slightly concave surface, median keel present and inflated; SLP8 smaller than SLP 7, median keel absent, vertical arms of paired sternopleural bridges close to each, bullar lobes visible and closed (Fig. 49B, C); male cuticle partition present. This is the only species of the genus.

Distribution. Chile (Rudolph, 2010) (Fig. 52).

Color of live specimens. Rostrum, cephalothorax anterior and lateral regions, dorsal pleon and tailfan reddish brown. Pereiopod pairs 2 – 5 and chelipeds reddish brown (Rudolph, 2010).

Habitat and Ecology. Vegas or hualves. This species occurs in underground waters in small valleys or depressions between mountains or topographic depressions, usually associated with perennial forests (Rudolph, 2013). **Gen. nov.** *nicoleti* build deep burrows with multiple entrance tunnels (Rudolph, 2013). **Gen. nov.** *nicoleti* can be identified as type 2 according to Horwitz and Richardson's (1986) classification and the species can be considered a primary burrower based on Hobbs' (1942) classification.

Key to the South American freshwater crayfish genera

1. Telson without lateral spines ………………………………………… **Gen. nov.**
   (This genus is endemic of Chile and it is monotypic)

Telson with lateral spines …………………………………………………. 2

2. Coxa of fifth pair of pereiopods without male cuticle partition……………………………………………………….. *Virilastacus*
   (This genus is endemic of Chile and it has four species)
   Coxa of fifth pair of pereiopods with male cuticle partition present ……………………………………………………………..…………3
3. Dactylus of chelipeds moving sub-horizontally ……………………*Samastacus*
   (This genus can be found in northern Patagonia between the provinces of Río Negro and Neuquén, in Argentina, and widely distributed in central portion of Chile)
   Dactylus of chelipes moving sub-vertically ………………………..*Parastacus*
   (This genus is distributed in central portion of Chile, north of Argentina, Uruguay and southern Brazil, in the states of Rio Grande do Sul and Santa Catarina).

Key to species of *Parastacus*

1. Rostrum trapezoid…………………………………...…… *Parastacus* **sp. nov. 2**
   (This species has an extremily limited distribution, being found only in the municipality of Dom Feliciano, state of Rio Grande do Sul, Brazil)
   Rostrum triangular or spatulated ……………………………………………… 2
2. Cutting edge of cheliped fingers external face hardly visible, covered by tufts of long setae ……………………………………………………….………… 3
   Cutting edge of cheliped fingers visible, not covered by tufts of long setae ...……………………………………………………………….……….… 4
3. Post orbital carinae obsolete ……………………………….………… *P. laevigatus*
   (This species has a limited distribution, being found only in the north of the state of Santa Catarina, north limit of Parastacus *distribution. It is probably extinct in the nature.)*
   Post orbital carinae prominent ………………………………..……………... 5
4. Proximal lobe of exopod protopod with spine …………………………………. 6
   Proximal lobe of exopod protopod unarmed …….……………………………. 7
5. Telson subtriangular ………………………………………………..…. *P. fluviatilis*
   (This species is found only in streams in the region of Campus de Cima da –RS up to 1.400 m of altitude)
   Telson surectangular ……………………………………………….. *P. pilimanus*
   (Widely distributed in the state of Rio Grande do Sul – Brazil, Uruguay and Argentina)
6. Internal surface of merus, carpus and propodus covered by tufts of long setae; Ventral margin of ischium of maxilliped bears a longitudinal line of strong spines ……………………………………………………….……… *P. varicosus*

(This species is distributed in the states of Santa Catarina and Rio Grande do Sul – Brazil and Uruguay and Argentina)

Internal surface of merus, carpus and propodus not covered by tufts of long setae. Tufts of setae present in the internal cutting edge of chelipeds. Ventral margin of ischium of maxilliped bears a longitudinal line of tubercles ….. *P. saffordi*

(This species is distributed in the state of Rio Grande do Sul – Brazil and in Uruguay)

7. Chelipeds short and globose ………………………………………………… 8

Chelipeds long and laterally flattened ………………………………………. 9

8. Cervical groove U – shaped …………………………...……*P. caeruleodactylus*

(This species has a limited distribution being found only in swamp forests in the northeast region of the State of Rio Grande do Sul, Brazil)

Cervical groove V – shaped ……………………………………………….. 10

9. S3 longer than S2 and curved in lateral view……….….… *Parastacus* **sp. nov. 8**

(This species has an extremely limited distribution, being found only in streams in the Municipality of Caraá, southwest portion of the state of Santa Catarina, Brazil).

S3 and S2 with similar sizes and not curved in lateral view …………………. 11

10. Ventral region of the first segment of antennular peduncle without a spine ………………………………………………………….. *Parastacus* **sp. nov. 4**

(This species has an extremely limited distribution, being found only in swamp forests in the Municipality of Maracajá, southwest portion of the state of Santa Catarina, Brazil).

Ventral region of the first segment of antennular peduncle bears a spine ……. 12

11. Apex of anteromedian lobe of epistome acute ………..;;;………………...... 13

Apex of anteromedian lobe of epistome straight ..………………. *P. promatensis*

(This species has an extremely limited distribution, being found only in streams in the Municipality of São Francisco de Paula, state of Rio Grande do Sul, Brazil, up to 1.400 m of altitude).

12. Chelipeds subequal in size …………………………………………………. 14

Chelipeds with different sizes …………………….……...…. *Parastacus* **sp. nov. 5**

(*This species is found in wetlands from the central east to the northeast portion of the state of Rio Grande do Sul, Brazil, in the municipalities of Viamão and Osório.)*

13. Lateral sides of cheliped propodus with irrelugar lines of tubercles …..…………………………………………….………… *Parastacus* **sp. nov.** 7

(This species is found only in the central portion of the state of Rio Grande do Sul, Brazil, in the Pampas Biome)

Lateral sides of cheliped propodus smooth …………………………………... 15

14. Lateral margin of antennal scale curved ……………..….. *Parastacus* **sp. nov. 3**

(This species is only found in the central east portion of the state of Rio Grande do Sul, Brazil, in the municipality of Porto Alegre)

Lateral margin of antennal scale straight …………………………………... 17

15. Rostrum apex V – shaped ………………………………. *Parastacus* **sp. nov. 9**

(This species has an extremely limited distribution, being found only in the municipality of Osório, state of Rio Grande do Sul, Brazil, in the Borússia Mountain).

Rostrum apex U – shaped ………………………………………………...…16

16. Post orbital carinae prominent …………………..………………………… 18

Post orbital carinae obsolete ………………………………………… *P. tuerkayi*

(This species is found in swamp forests in the municipality of Penha, state of Santa Catarina, Brazil).

17. Rostum apex not ending in a tubercle or spine ……..…… *Parastacus* **sp. nov. 1**

(This species has an extremely limited distribution, being found only in the municipality of Mariana Pimentel, state of Rio Grande do Sul, Brazil).

Rostrum apex ending in a tubercle or spine …………………………………. 19

18. Mandible caudal molar process unicuspidate …………… *Parastacus* **sp. nov. 6**

(This species has an extremely limited distribution, being found only in the island of Florianópolis, state of Santa Catarina, Brazil).

Mandible caudal molar process bicuspidate ……………………... *P. brasiliensis*

(This species is found only in the central portion of the state of Rio Grande do Sul, Brazil).

19. Branchiocardiac grooves inconspicuous….………..…… *Parastacus* **sp. nov. 10**

(*This species has an extremely limited distribution, being found only in the municipality of Eldorado do Sul, state of Rio Grande do Sul, Brazil).*

Branchiocardiac grooves conspicuous …………………………………….. 20

20. Dactylus of chelipeds larger than dorsal surface of palm and incisive lobe of mandible with ten teeth ………………………………………………. *P. pugnax*

(This ispecies is found only in the central portion of Chile).

Datylus of chelipeds equal or shorter than dorsal surface of palm and incisive lobe of mandible with nine teeth ………………………………..…..*P. defosssus*

(This species is found only in the south portion of Uruguay).

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Figure captions

Figure 1. ***Parastacus pilimanus*** (von Martens, 1869), lectotype: A – cephalothorax dorsal view (MNB 3323); B – cephalothorax lateral view (MNB 3323); C – abdome (MNB 3323); D – cheliped lateral view (MNB 3323). Scale bars: 1 cm.

Figure 2. ***Parastacus pilimanus*** (von Martens, 1869): A – habitus dorsal view (UFRGS 277); B – cephalon dorsal view (UFRGS 277); C – cephalon lateral view (UFRGS 277); D – female abdominal somites dorsal view (UFRGS 277); E – first and second male abdominal pleura (UFRGS 277); F – first and second female abdominal pleura (UFRGS 277); G – tailfan (UFRGS 277). Scale bars: A, C, D, E – 1 cm; B, F, G – 5 mm;

Figure 3. ***Parastacus pilimanus*** (von Martens, 1869): A – epistome (UFRGS 277); B – thoracic sternites and gonopores (UFRGS 277); C – thoracomere 8, caudal view (UFRGS 277); D – antennal scale lateral view (UFRGS 277); E – mandible (UFRGS 277); F – third maxilliped ventral view (UFRGS 277); G – third maxilliped dorsal view (paratype 4); H – first pereiopod lateral view (UFRGS 277); I – first pereiopod dorsal view (UFRGS 277); J – second pereiopod lateral view (UFRGS 277).Scale bars: A, C, F, G, I, J – 5 mm; B, H – 1 cm; D, E – 2.5 mm.

Figure 4. ***Parastacus pilimanus*** (von Martens, 1869), habitat and living specimen: A – habitat; B – burrow with chimney; C – burrow opening; D – living specimen. White arrows indicate chimney and burrow opening.

Figure 5. ***Parastacus brasiliensis*** (von Martens, 1869), lectotype: A – habitus dorsal dorsal view (MNB 3322); B – habitus lateral view (MNB 3322); C – habitus dorsal view (MNB 3322); D – habitus dorsal view (MNB 3323). Scale bars: 1 cm.

Figure 6. ***Parastacus brasiliensis*** (von Martens, 1869): A – habitus dorsal view (UFRGS 5868); B – cephalon dorsal view (UFRGS 5866); C – cephalon lateral view (UFRGS 5868); D – female abdominal somites dorsal view (UFRGS 278); E – first and second male abdominal pleura (UFRGS 5868); F – first and second female abdominal pleura (UFRGS 278); G – tailfan (UFRGS 5868). Scale bars: A, D, E, G – 10 mm; B, C, F – 5 mm;

Figure 7. ***Parastacus brasiliensis*** (von Martens, 1869): A – epistome (UFRGS 278); B – thoracic sternites and gonopores (UFRGS 5868); C – thoracomere 8, caudal view (UFRGS 278); D – antennal scale lateral view (UFRGS 5868); E – mandible (UFRGS 5868); F – third maxilliped ventral view (UFRGS 278); G – third maxilliped dorsal view (paratype 4 UFRGS 278); H – first pereiopod lateral view (UFRGS 276); I – first pereiopod dorsal view (UFRGS 278); J – second pereiopod lateral view (UFRGS 5868). Scale bars: A, C, F, G, I, J – 5 mm; B, H – 10 mm; D, E – 3.33 mm;

Figure 8. ***Parastacus brasiliensis*** (von Martens, 1869), habitat and living specimens: A, C – Habitat, streams; B – chimney; D – single opening; E – living specimen.

Figure 9. ***Parastacus pugnax*** (Poeppig, 1835): A – habitus dorsal view (UFRGS 726); B – cephalon dorsal view (UFRGS 726); C – cephalon lateral view (UFRGS 726); D – female abdominal somites dorsal view (UFRGS 2407); E – first and second male abdominal pleura (UFRGS 726); F – first and second female abdominal pleura (UFRGS 2407); G – tailfan (UFRGS 726). Scale bars: A, E –10 mm; B, C, D, F, G - 5 mm;

Figure 10. ***Parastacus pugnax*** (Poeppig, 1835): A – epistome (UFRGS 2407); B – thoracic sternites and gonopores (UFRGS 726); C – thoracomere 8, caudal view (UFRGS 726); D – antennal scale lateral view (UFRGS 726); E – mandible (UFRGS 726); F – third maxilliped ventral view (UFRGS 726); G – third maxilliped dorsal view (UFRGS 726); H – first pereiopod lateral view (UFRGS 726); I – first pereiopod dorsal view (UFRGS 726); J – second pereiopod lateral view (UFRGS 726).Scale bars: A, C, D, H, I, – 5 mm; B – 10 mm; F, G, J – 3.33 mm;

Figure 11. ***Parastacus defossus*** Faxon 1898, holotype and paratypes: A – habitus dorsal view (UFRGS 277); B – cephalon dorsal view (UFRGS 277); C – cephalon lateral view (UFRGS 277); D – first and second male abdominal pleura (UFRGS 277); E – tailfan (UFRGS 277). Scale bars: A, C, D, E – 10 mm; B, F, G – 5 mm.

Figure 12. ***Parastacus defossus*** Faxon 1898, holotype and paratypes: A – epistome (FC – UDELAR w/n); B – thoracic sternites and gonopores (FC – UDELAR w/n); C – thoracomere 8, caudal view (FC – UDELAR w/n); D – antennal scale lateral view (FC –

UDELAR w/n); E – mandible (FC – UDELAR w/n); F – third maxilliped ventral view (FC – UDELAR w/n); G – third maxilliped dorsal view (FC – UDELAR w/n); H – first pereiopod lateral view (FC – UDELAR w/n); I – first pereiopod dorsal view (FC – UDELAR w/n); J – second pereiopod lateral view (FC – UDELAR w/n).Scale bars: A, I– 3.33 mm; B, C, F, G, H, J – 5 mm; D, E – 2.5 mm.

Figure 13. ***Parastacus saffordi*** Faxon 1898, holotype and paratypes: A – habitus dorsal view (UFRGS 1356); B – cephalon dorsal view (UFRGS 1356); C – cephalon lateral view (UFRGS 1356); D – first and second male abdominal pleura (UFRGS 1356); E – tailfan (UFRGS 356). Scale bars: A – 10 mm; B, C, D, E – 5 mm.

Figure 14. ***Parastacus saffordi*** Faxon 1898, holotype and paratypes: A – epistome (UFRGS 1356); B – thoracic sternites and gonopores (UFRGS 1356); C – thoracomere 8, caudal view (UFRGS 1366); D – antennal scale lateral view (UFRGS 5875); E – mandible (UFRGS 1351); F – third maxilliped ventral view (UFRGS 1356); G – third maxilliped dorsal view (UFRGS 1356); H – first pereiopod lateral view (UFRGS 1356) I – first pereiopod dorsal view (UFRGS 1356); J – second pereiopod lateral view (UFRGS 1356).Scale bars: A, C, F, G, H, I, J – 5 mm; B – 10mm; D – 1.5 mm

Figure 15. ***Parastacus saffordi*** Faxon 1898, habitat and living specimens: A – Habitat, rain water fed wetlands in small depressions; B – single opening; C, D – living specimen. Scale bars: C, D – 1 cm.

Figure 16. ***Parastacus varicosus*** Faxon 1898, holotype and paratypes: A – habitus dorsal view (UFRGS 281); B – cephalon dorsal view (UFRGS 1354); C – cephalon lateral view (UFRGS 1354); D – female abdominal somites dorsal view (UFRGS 3066); E – first and second male abdominal pleura (UFRGS 1354); F – first and second female abdominal pleura (UFRGS 3066); G – tailfan (UFRGS 281). Scale bars: A, B, C, D– 10 mm; E – 10 mm.

Figure 17. ***Parastacus varicosus*** Faxon 1898, holotype and paratypes: A – epistome (UFRGS 1354); B – thoracic sternites and gonopores (UFRGS 1354); C – thoracomere 8, caudal view (UFRGS 1353); D – antennal scale lateral view (UFRGS 1355); E – mandible (UFRGS 1354); F – third maxilliped ventral view (UFRGS 1354); G – third

maxilliped dorsal view (UFRGS 1354); H – first pereiopod lateral view (UFRGS 1355); I – first pereiopod dorsal view (UFRGS 1355); J – second pereiopod lateral view (UFRGS 1354). Scale bars: A, C, F, G, H, I – 5 mm; B, J – 10 mm; D, E – 2,5 mm;

Figure 18. ***Parastacus laevigatus*** Buckup & Rossi 1980, holotype and paratypes: A – habitus dorsal view (holotype, MN UFRJ w/n); B – cephalon dorsal view (holotype, MN UFRJ w/n); C – cephalon lateral view (holotype, MN UFRJ w/n); D – female abdominal somites dorsal view (paratypr, MN UFRJ w/n); E – first and second male abdominal pleura (holotype, MN UFRJ w/n); F – first and second female abdominal pleura (paratypr, MN UFRJ w/n); G – tailfan (holotype, MN UFRJ w/n). Scale bars: A – 10 mm; B, C – 2,5 mm; D, E – 5 mm.

Figure 19. ***Parastacus laevigatus*** Buckup & Rossi 1980, holotype and paratypes: A – epistome (MN UFRJ w/n); B – thoracic sternites and gonopores (MN UFRJ w/n); C – thoracomere 8, caudal view (MN UFRJ w/n); D – antennal scale lateral view (UFRGS 1366); E – mandible (MN UFRJ w/n); F – third maxilliped ventral view (UFRGS 1366); G – third maxilliped dorsal view (UFRGS 1366); H – first pereiopod lateral view (MN UFRJ w/n); I – first pereiopod dorsal view (MN UFRJ w/n); J – second pereiopod lateral view (MN UFRJ w/n).Scale bars: A, B, I – 5 mm; C – 3.33 mm; D – 1,5 mm; E – 1 mm; F, G – 2,5mm; H, J – 10 mm;

Figure 20. ***Parastacus promatensis*** Fontoura & Conter 2008, holotype and paratypes: A – habitus dorsal view (MCP 2086); B – cephalon dorsal view (MCP 2086); C – cephalon lateral view (MCP 2086); D – female abdominal somites dorsal view (MCP 2086); E – first and second male abdominal pleura (MCP 2086); F – first and second female abdominal pleura (MCP 2086); G – tailfan (MCP 2086). Scale bars: A – 20 mm; B, G – 5 mm; C, D, E, F – 10 mm;

Figure 21. ***Parastacus promatensis*** Fontoura & Conter 2008, holotype and paratypes: A – epistome (MCP 2086); B – thoracic sternites and gonopores (MCP 2086); C – thoracomere 8, caudal view (MCP 2086); D – antennal scale lateral view (UFRGS 4157); E – mandible (UFRGS 4157); F – third maxilliped ventral view (UFRGS 4157); G – third maxilliped dorsal view (UFRGS 4157); H – first pereiopod lateral view (MCP 2086); I – first pereiopod dorsal view (MCP 2086); J – second pereiopod lateral view

(FC – UDELAR w/n).Scale bars: B, H, I – 10 mm; A, C, F, G, J – 5 mm; D, E – 3.33 mm;

Figure 22. ***Parastacus*** **sp. nov. 1**, holotype and paratypes: A – habitus dorsal view (holotype); B – cephalon dorsal view (holotype); C – cephalon lateral view (parátipo); D – female abdominal somites dorsal view (UFRGS 6414); E – first and second abdominal pleura (Holótipo); F – first and second abdominal pleura (UFRGS 6414); G – tailfan (holotype). Scale bars: A, D – 10 mm; B, C, E, F, G – 5 mm;

Figure 23. ***Parastacus*** **sp. nov. 1,** holotype and paratypes: A – epistome (UFRGS 6410); B – thoracic sternites and gonopores (holotype); C – thoracomere 8, caudal view (holotype); D – antennal scale lateral view (paratype 4, UFRGS 6410); E – mandible (paratype 4, UFRGS 6410); F – third maxilliped ventral view (paratype 4, UFRGS 6410); G – third maxilliped dorsal view (paratype 4, UFRGS 6410); H – first pereiopod lateral view (holotype); F – first pereiopod dorsal view (holotype).Scale bars: A, C, F, G, J – 2.5 mm; B, H, I – 5 mm; D, E – 1,5 mm;

Figure 24. ***Parastacus*** **sp nov. 2**, holotype: A – habitus dorsal view (holotype); B – cephalon dorsal view (holotype); C – cephalon lateral view (holotype); D – first and second male abdominal pleura (holotype); E – tailfan (holotype). Scale bars: A, D – 5 mm; B, C, E, 2.5 mm;

Figure 25. ***Parastacus*** **sp. nov. 2**, holotype and paratypes: A – epistome (holotype); B – thoracic sternites and gonopores (holotype); C – thoracomere 8, caudal view (holotype); D – antennal scale lateral view (holotype); E – mandible (paratype 1, UFRGS 5497); F – third maxilliped ventral view (paratype 1, UFRGS 5497); G – third maxilliped dorsal view (paratype 1, UFRGS 5497); H – first pereiopod lateral view (holotype); F – first pereiopod dorsal view (holotype); G – second pereiopod lateral view (holotype). Scale bars: A, C – 3.3 mm; B – 5 mm; D – 1.5 mm; E – 1 mm; H, I, J – 2.5 mm; F, G – 2 mm;

Figure 26. ***Parastacus*** **sp. nov. 3**, holotype and paratypes: A – habitus dorsal view (holotype); B – cephalon dorsal view (holotype); C – cephalon lateral view (holotype D – female abdominal somites dorsal view (paratype 1, UFRGS 5870); E – first and

second male abdominal pleura (holotype); F – first and second female abdominal pleura (paratype 1, UFRGS 5870); G – tailfan (paratype 1, UFRGS 5870). Scale bars: A – 10 mm; B, C, D, E, F, G – 5 mm.

Figure 27. ***Parastacus*** **sp. nov. 3**, holotype and paratypes: A – epistome (holotype); B – thoracic sternites and gonopores (holotype); C – thoracomere 8, caudal view (holotype); D – antennal scale lateral view (UFRGS 5857); E – mandible (paratype 2, UFRGS 5857); F – third maxilliped ventral view (paratype 1, UFRGS 5870); G – third maxilliped dorsal view (paratype 1, UFRGS 5870); H – first pereiopod lateral view (holotype); I – first pereiopod dorsal view (holotype); J – second pereiopod lateral view (holotype). Scale bars: C – 2.5 mm; A, B, C, H, I, J – 5 mm; D - 1 mm; E – 2 mm; F, G – 3.33mm;

Figure 28. ***Parastacus*** **sp. nov. 3,** habitat and living specimens: A, B – Habitat, flooded grassland; C – burrow with chimney (~ 35 cm height); D – living specimen. Scale bars: C, D – 1 cm.

Figure 29. ***Parastacus*** **sp. nov. 4**, holotype and paratypes: A – habitus dorsal view (holotype); B – cephalon dorsal view (holotype); C – cephalon lateral view (holotype); D – first and second male abdominal pleura (holotype); E – tailfan (holotype). Scale bars: A – 10 mm; C, D – 5 mm; B, E – 3.33 mm.

Figure 30. ***Parastacus*** **sp. nov. 4**, holotype and paratypes: A – epistome (holotype); B – thoracic sternites and gonopores (holotype); C – thoracomere 8, caudal view (holotype); D – antennal scale lateral view (paratype, UFRGS 5856); E – mandible (paratype, UFRGS 5856); F – third maxilliped ventral view (paratype, UFRGS 5856); G – third maxilliped dorsal view (paratype, UFRGS 5856); H – first pereiopod lateral view (holotype); I – first pereiopod dorsal view (holotype); J – second pereiopod lateral view (holotype).Scale bars: A, B – 5 mm; C, J, H, I – 3.33 mm; E - 1.5 mm; D – 1 mm; F, G – 2 mm;

Figure 31. *Parastacus* sp. nov. 4, habitat and living specimens: A – Habitat, swamp forest; B – burrow with chimney (~ 5 cm height); C – living specimen. Scale bars: C – 1 cm.

Figure 32. *Parastacus* sp. nov. 5, holotype and paratypes: A – habitus dorsal view (holotype); B – cephalon dorsal view (paratype 5, UFRGS 1359); C – cephalon lateral view (paratype 5, UFRGS 1359); D – female abdominal somites dorsal view (paratype 4, UFRGS 6439); E – first and second male abdominal pleura (holotype); F – first and second female abdominal pleura (paratype 4, UFRGS 6439); G – tailfan (paratype 2, UFRGS 5865). Scale bars:a – 10 mm; B, C, D, E, F, G – 5 mm;

Figure 33. *Parastacus* sp. nov. 5, holotype and paratypes: A – epistome (UFRGS 1359); B – thoracic sternites and gonopores (paratype 5, UFRGS 1359); C – thoracomere 8, caudal view (holotype); D – antennal scale lateral view (paratype 2, UFRGS 5865); E – mandible (paratype 2, UFRGS 5865); F – third maxilliped ventral view (paratype 2, UFRGS 5865); G – third maxilliped dorsal view (paratype 2, UFRGS 5865); H – first pereiopod lateral view (holotype); I – first pereiopod dorsal view (holotype); J – second pereiopod lateral view (holotype); Scale bars: A – 2 mm; B, H, I – 5 mm; D, F, G – 1,5 mm; C, J – 2,5 mm; E – 1 mm;

Figure 34. *Parastacus* sp. nov. 5, habitat and living specimens: A – Habitat, peatland; B – burrow with chimney (~ 5 cm high); C – ovigerous female; D – living specimen. Scale bars: C, D – 1 cm.

Figure 35. *Parastacus* sp. nov. 6, holotype and paratypes: A – habitus dorsal view (holotype); B – cephalon dorsal view (holotype); C – cephalon lateral view (holotype); D – female abdominal somites dorsal view (paratype 1, UFRGS 6489); E – first and second male abdominal pleura (holotype); F – first and second female abdominal pleura (paratype 1, UFRGS 6489); G – tailfan (holotype). Scale bars: A – 10 mm; B, E, F, G – 5 mm; C – 2 mm.

Figure 36. *Parastacus* sp. nov. 6, holotype and paratypes: A – epistome (holotype); B – thoracic sternites and gonopores (holotype); C – thoracomere 8, caudal view (holotype); D – antennal scale lateral view (paratype 2, UFRGS 6490); E – mandible (paratype 2,

UFRGS 6490); F – third maxilliped ventral view (paratype 1, UFRGS 6489); G – third maxilliped dorsal view (paratype 1, UFRGS 6489); H – first pereiopod lateral view (holotype); I – first pereiopod dorsal view (holotype); J – second pereiopod lateral view (holotype).Scale bars: A, J, C – 3,33 mm; B, I – 5 mm; F, G – 2,5 mm; E, D – 1,5 mm; H – 10 mm;

Figure 37. ***Parastacus*** **sp. nov. 6,** habitat and living specimens: A – Habitat, peatland; B – burrow opening; C – burrow with chimney (~ 5 cm high); D – living specimen. Scale bars: D – 1 cm.

Figure 38. ***Parastacus*** **sp. nov. 7,** holotype and paratypes: A – habitus dorsal view (holotype); B – cephalon dorsal view (holotype); C – cephalon lateral view (holotype); D – female abdominal somites dorsal view (parátipo); E – first and second male abdominal pleura (holotype); F – first and second female abdominal pleura (parátipo); G – tailfan (holotype). Scale bars: A, D – 10 mm; B, C, E, F, G – 5 mm;

Figure 39. ***Parastacus*** **sp. nov.7,** holotype and paratypes: A – epistome (holotype); B – thoracic sternites and gonopores (holotype); C – thoracomere 8, caudal view (parátipo); D – antennal scale lateral view (UFRGS 5973); E – mandible (UFRGS 5793; F – third maxilliped ventral view (UFRGS 5795); G – third maxilliped dorsal view (UFRGS 5795); H – first pereiopod lateral view (holotype); I – first pereiopod dorsal view (holotype); J – second pereiopod lateral view (holotype).Scale bars: A, C, F, G, H, I, J – 5 mm; B – 10 mm; D – 2 mm; E – 2,5 mm;

Figure 40. ***Parastacus*** **sp. nov. 8,** holotype: A – habitus dorsal view (holotype); B – cephalon dorsal view (holotype); C – cephalon lateral view (holotype); D – first and second male abdominal pleura (holotype); E – tailfan (holotype). Scale bars: A – 1 cm; B, C, D, E – 5 mm.

Figure 41. ***Parastacus*** **sp. nov.8,** holotype and paratypes: A – epistome (holotype); B – thoracic sternites and gonopores (holotype); C – thoracomere 8, caudal view (holotype); D – antennal scale lateral view (paratype 2, UFRGS 6440); E – mandible (paratype 2, , UFRGS 6440); F – third maxilliped ventral view (paratype 1, UFRGS

6440); G – third maxilliped dorsal view (paratype 1); H – first pereiopod lateral view (holotype); I – first pereiopod dorsal view (holotype); J – second pereiopod lateral view (holotype).Scale bars: A, C – 2.5 mm; B, I – 5 mm; D, E – 2 mm; F, G – 5 mm; H, J– 1 cm.

Figure 42. ***Parastacus*** **sp. nov. 9,** holotype: A – habitus dorsal view (holotype); B – cephalon dorsal view (holotype); C – cephalon lateral view (holotype); D – first and second male abdominal pleura (holotype); E – tailfan (holotype). Scale bars: A – 1 cm; B, C, D, E – 5 mm.

Figure 43. ***Parastacus*** **sp. nov. 9,** holotype and paratypes: A – epistome (holotype); B – thoracic sternites and gonopores (holotype); C – thoracomere 8, caudal view (holotype); D – antennal scale lateral view (paratype 2, UFRGS 6476); E – mandible (paratype 2); F – third maxilliped ventral view (paratype 1, UFRGS 6476); G – third maxilliped dorsal view (paratype 1, UFRGS 6476); H – first pereiopod lateral view (holotype); I – first pereiopod dorsal view (holotype); J – second pereiopod lateral view (holotype).Scale bars: A, C – 2.5 mm; B, I – 5 mm; D, E – 2 mm; F, G – 5 mm; H, J– 1 cm.

Figure 44. ***Parastacus*** **sp. nov. 9,** habitat and living specimens: A – Habitat, stream and banks; B – burrow opening; C – living specimen. Scale bars: C – 1 cm.

Figure 45. ***Parastacus*** **sp. nov. 10,** holotype: A – habitus dorsal view (holotype); B – cephalon dorsal view (holotype); C – cephalon lateral view (holotype); D – female abdominal somites dorsal view (paratype 1, UFRGS 5755); E – first and second male abdominal pleura (holotype); F – first and second female abdominal pleura (paratype 1, UFRGS 5755); G – tailfan (holotype). Scale bars: A – 1 cm; B, C, D, E – 5 mm.

Figure 46. ***Parastacus*** **sp. nov. 10,** holotype and paratypes: A – epistome (holotype); B – thoracic sternites and gonopores (holotype); C – thoracomere 8, caudal view (holotype); D – antennal scale lateral view (paratype 2, UFRGS 5755); E – mandible (paratype 2, UFRGS 5755); F – third maxilliped ventral view (paratype 1, UFRGS 5755); G – third maxilliped dorsal view (paratype 1); H – first pereiopod lateral view (holotype); I – first pereiopod dorsal view (holotype); J – second pereiopod lateral

view (holotype). Scale bars: A, C – 2.5 mm; B, I – 5 mm; D, E – 2 mm; F, G – 5 mm; H, J– 1 cm.

Figure 47. *Parastacus* sp. nov. 10, habitat and living specimens: A – Habitat, floodplain river; B – burrow with chimney (~ 14 cm high); C – living specimen. Scale bars: C – 1 cm.

Figure 48. Gen. nov. *nicoleti*, A – habitus dorsal view (UFRGS 2405); B – cephalon dorsal view (UFRGS 2405); C – cephalon lateral view (UFRGS 2405); D – first and second male abdominal pleura (UFRGS 2405); E – tailfan (UFRGS 2405). Scale bars: A, E – 1 cm; B, C, D – 5 mm.

Figure 49. Gen. nov. *nicoleti*, A – epistome (UFRGS 2405); B – thoracic sternites and gonopores UFRGS 2405); C – thoracomere 8, caudal view (UFRGS 2405); D – antennal scale lateral view (UFRGS 2405); E – mandible (UFRGS 2405); F – third maxilliped ventral view (paratype 1); G – third maxilliped dorsal view (UFRGS 2405); H – first pereiopod lateral view (UFRGS 2405); I – first pereiopod dorsal view (UFRGS 2405); J – second pereiopod lateral view (UFRGS 2405).Scale bars: A, C – 2.5 mm; B, I – 5 mm; D, E – 2 mm; F, G – 5 mm; H, J– 1 cm.

Figure 50. Distribution of the species previously described of *Parastacus* in Brazil (state of Rio Grande do Sul e Santa Catarina), Uruguay and Argentina.

Figure 51. Distribution of the ten new species of *Parastacus* in the state of Rio Grande do Sul e Santa Catarina, Brazil.

Figure 52. Distribution of the species previously described of *Parastacus pugnax* (Poeppig, 1835) and new genus in the south-central of Chile.

FIGURE 1

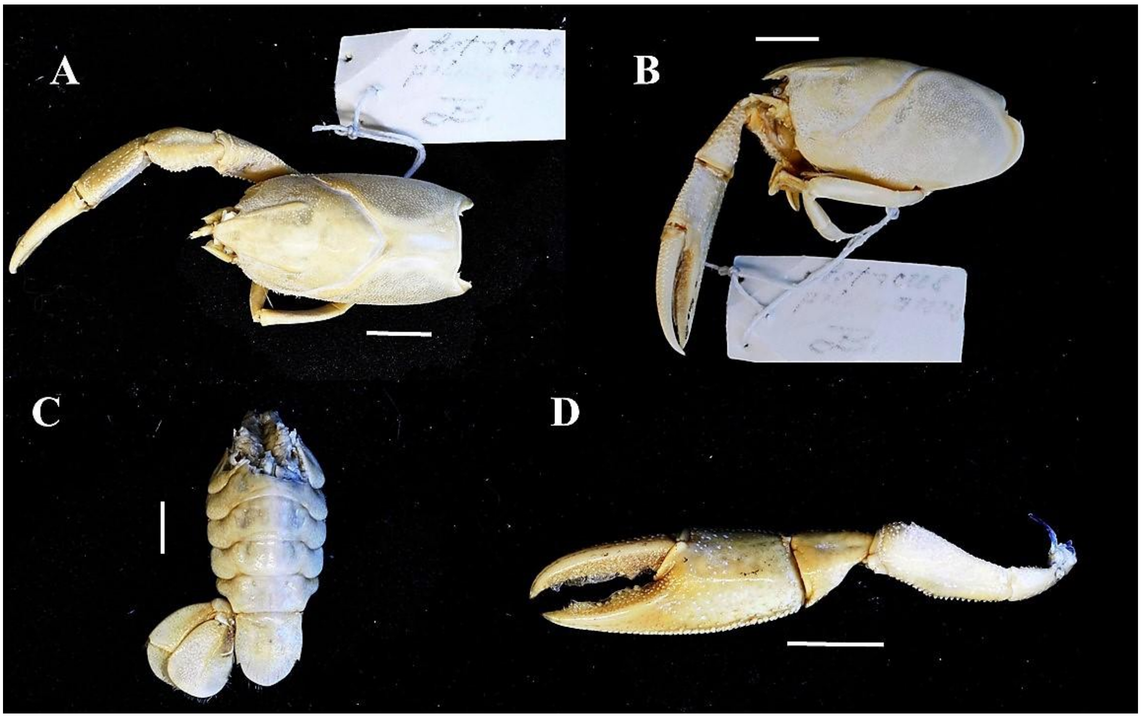
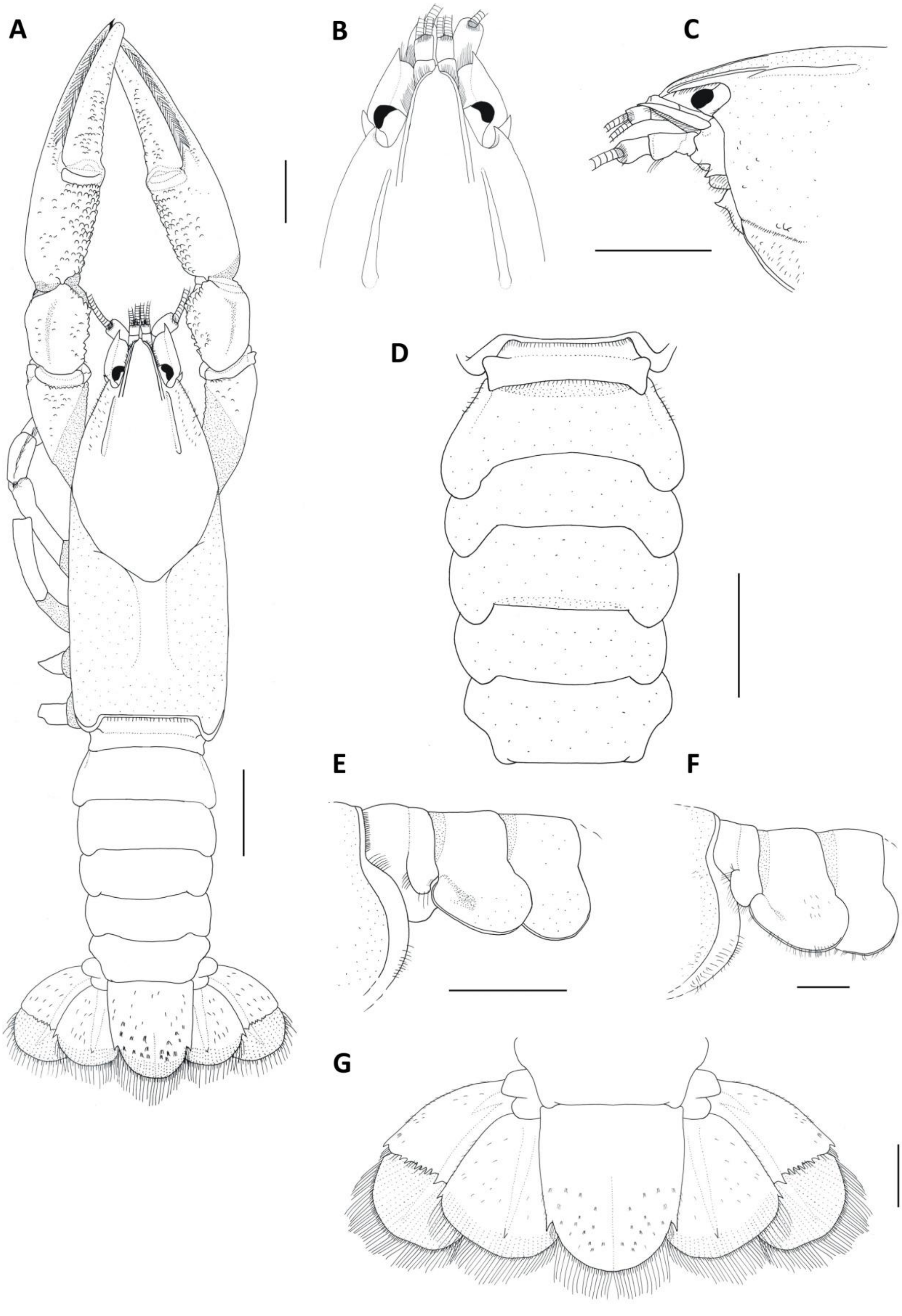


FIGURE 2



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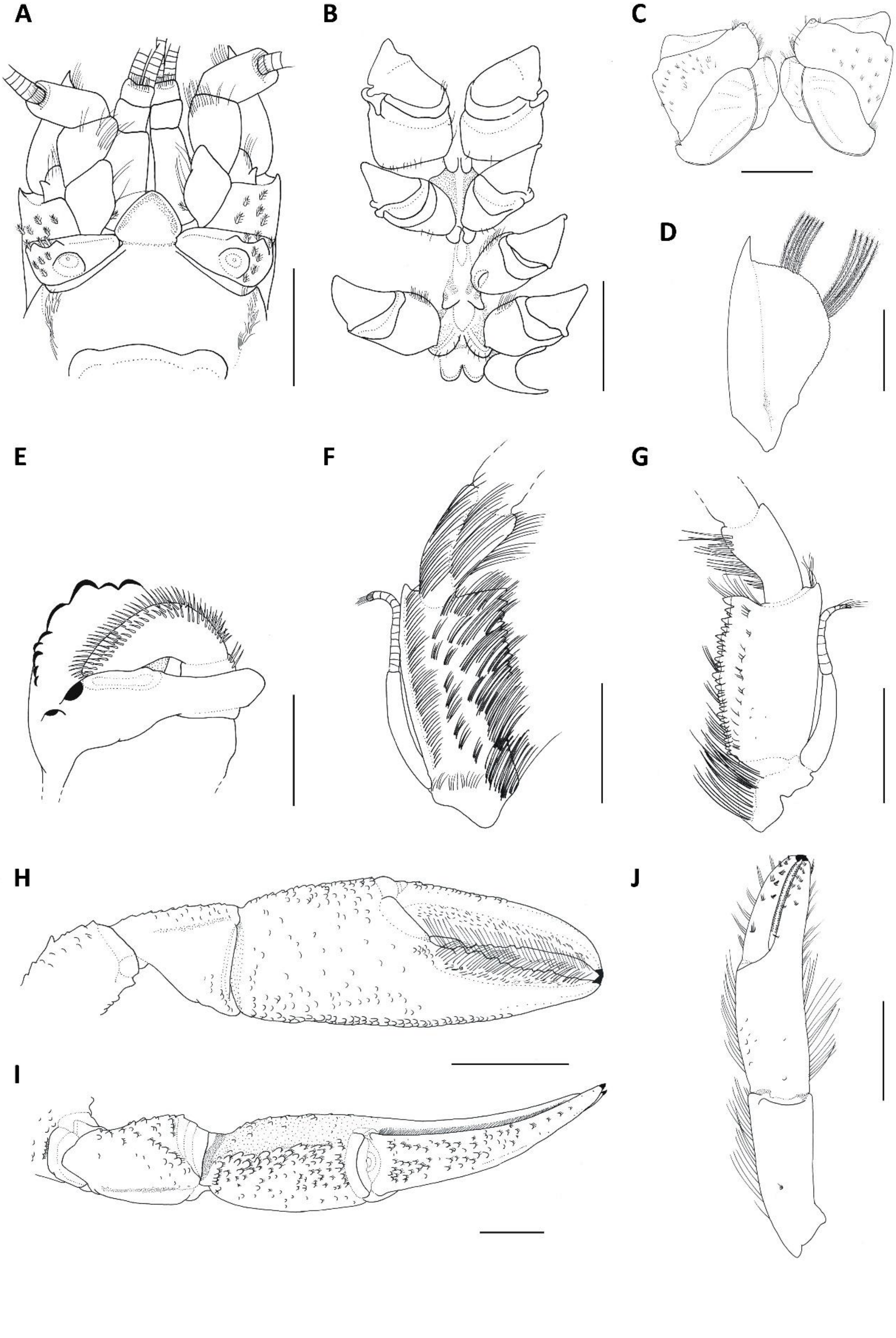
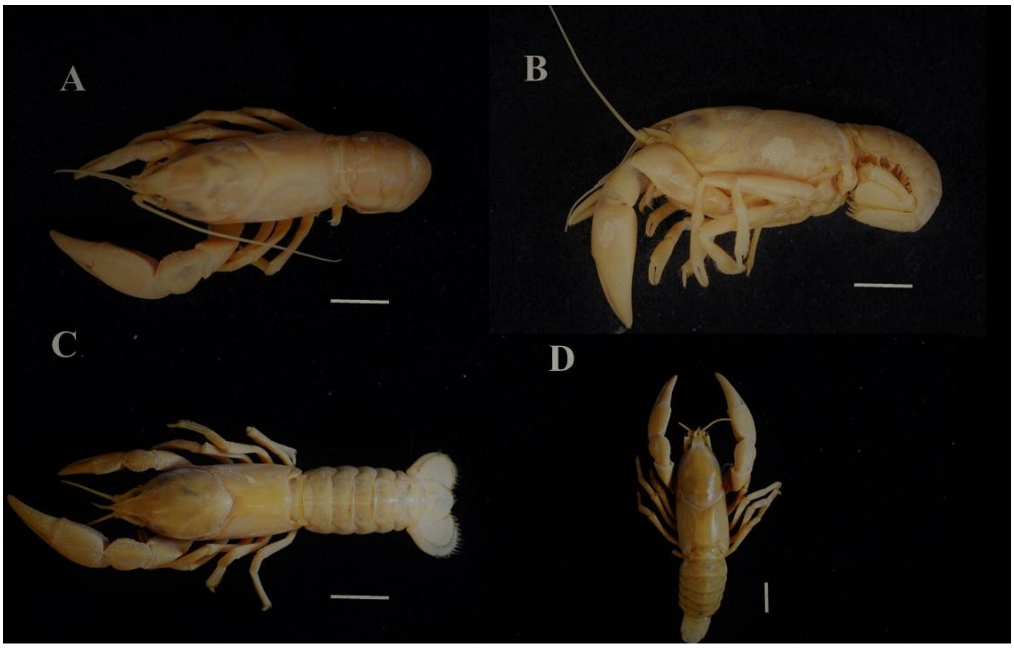


FIGURE 4

FIGURE 5



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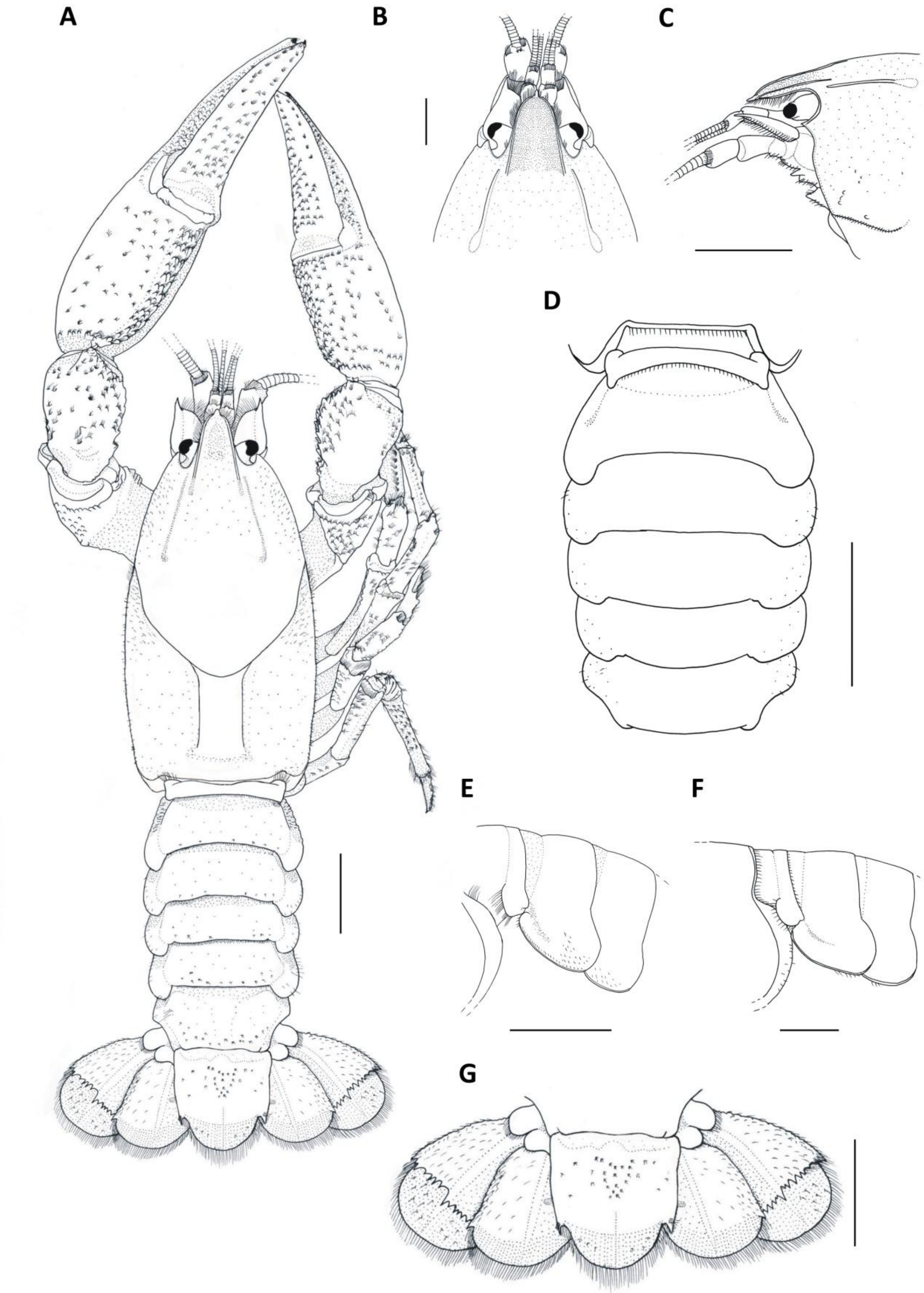

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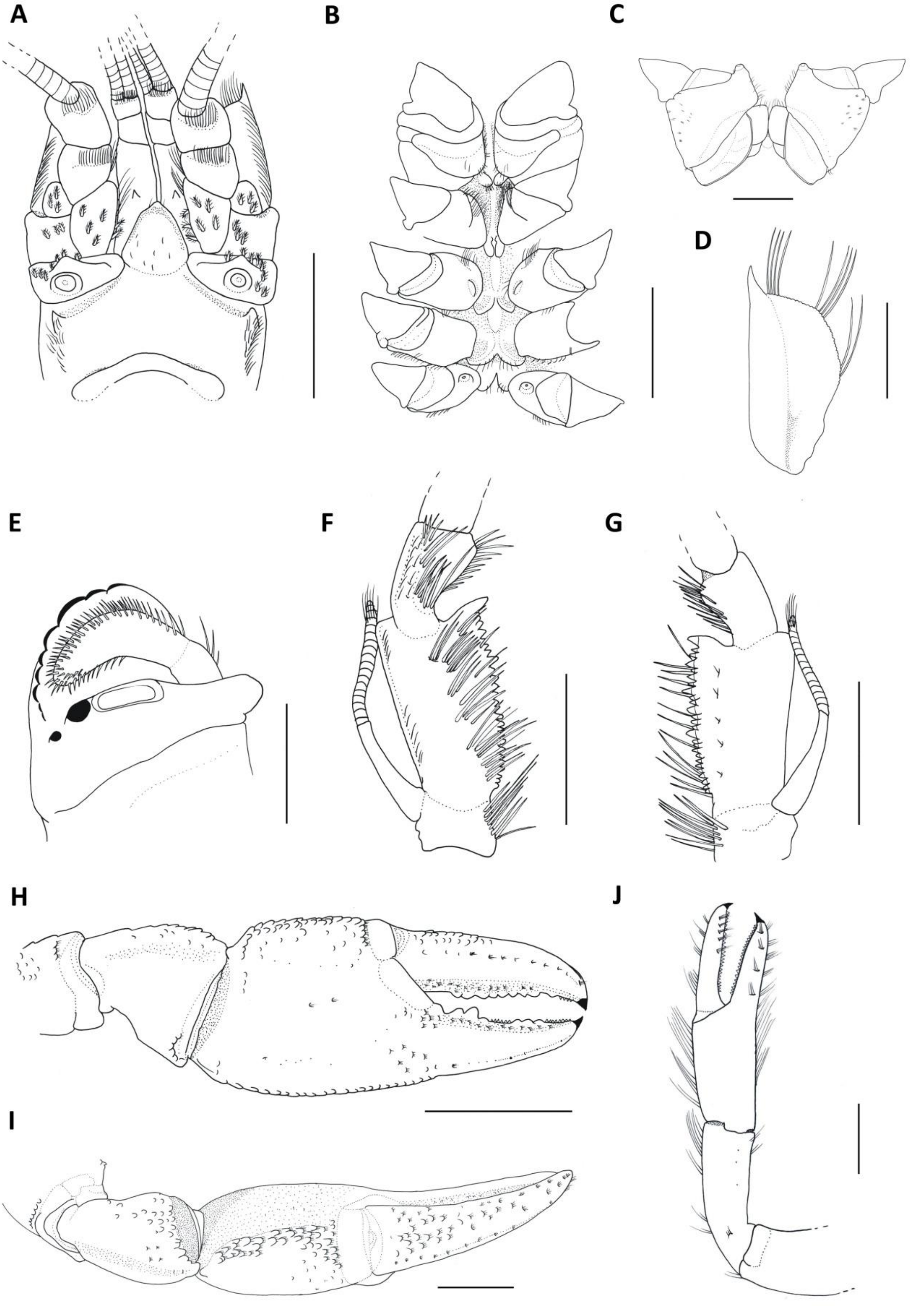

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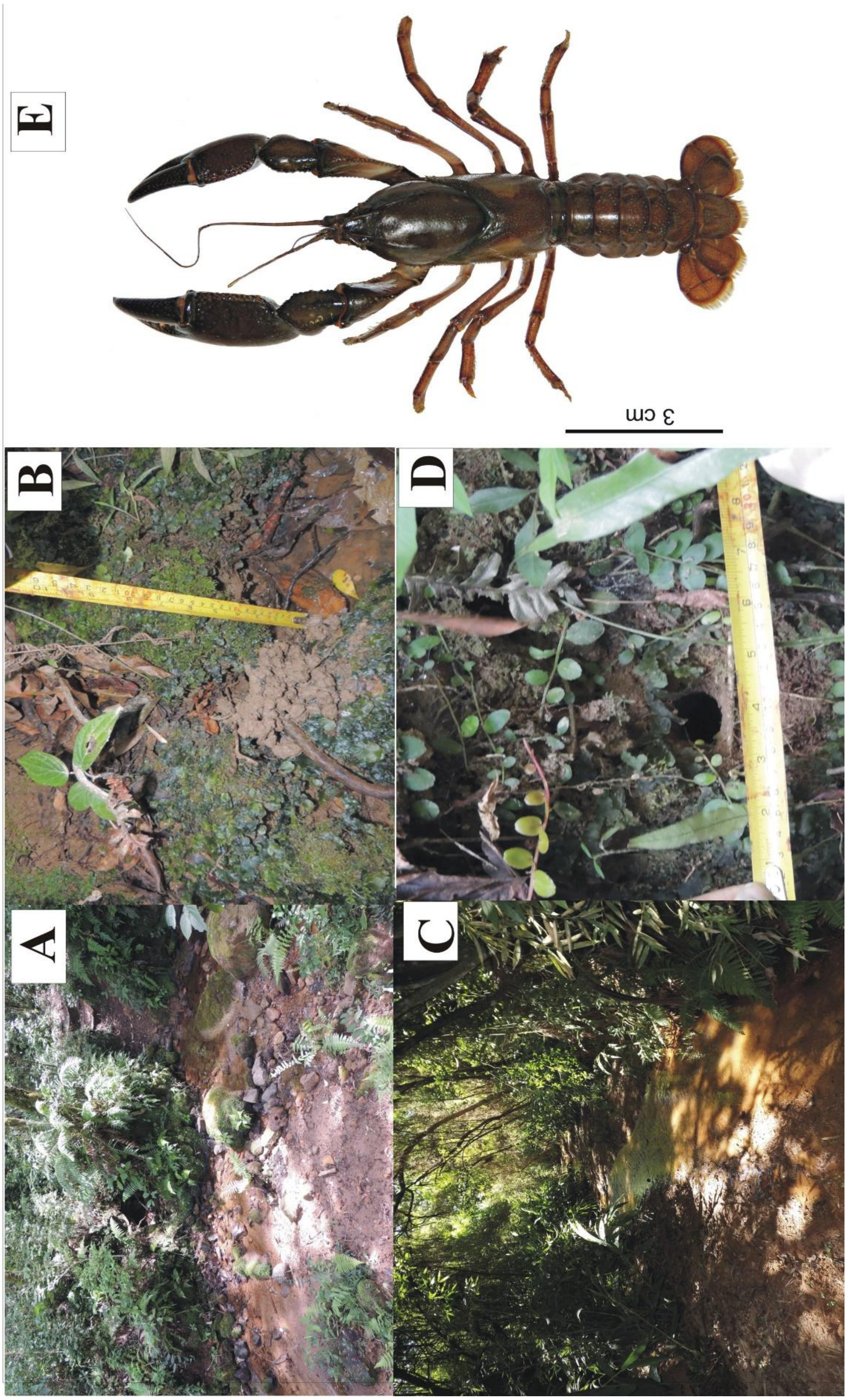

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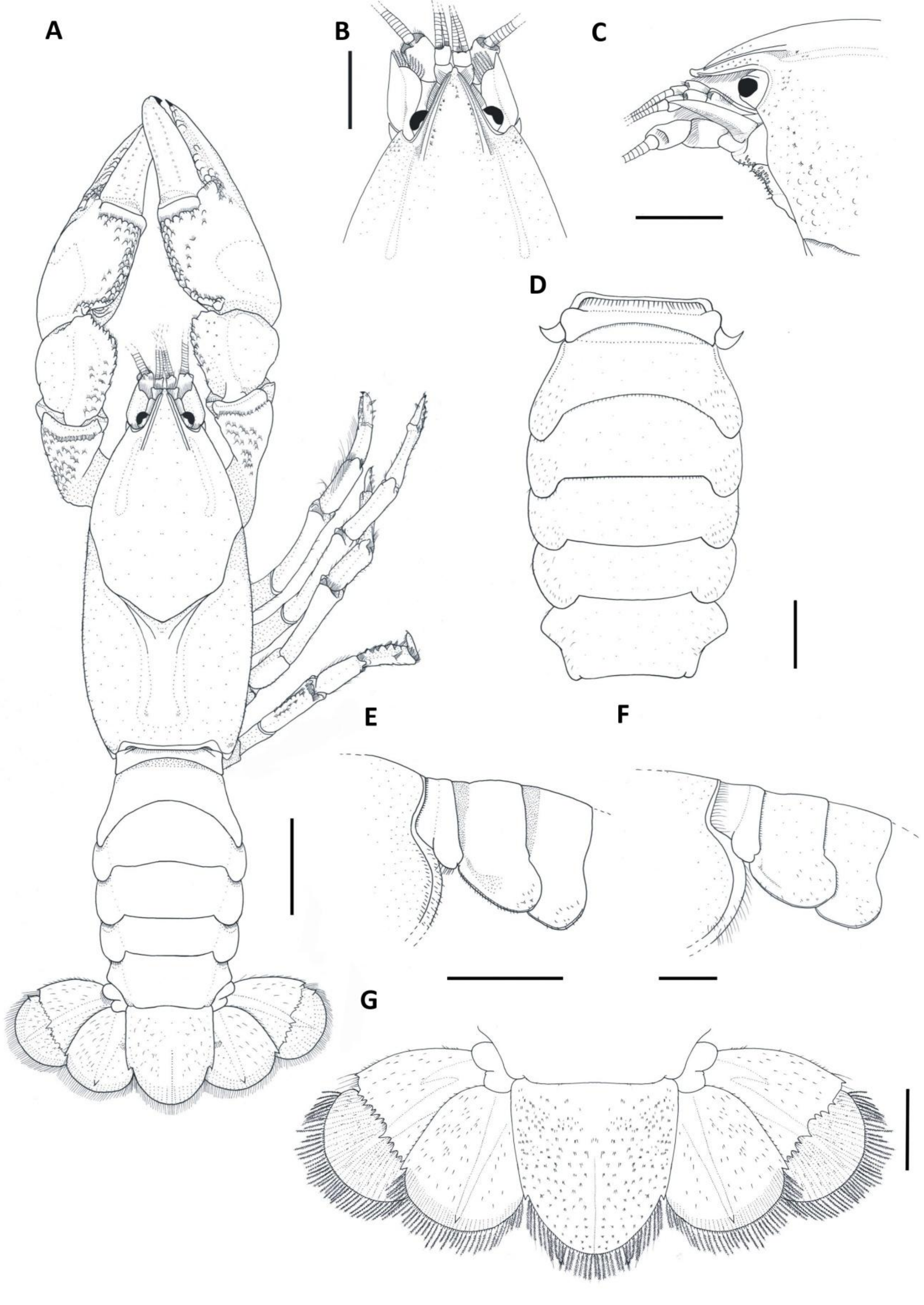
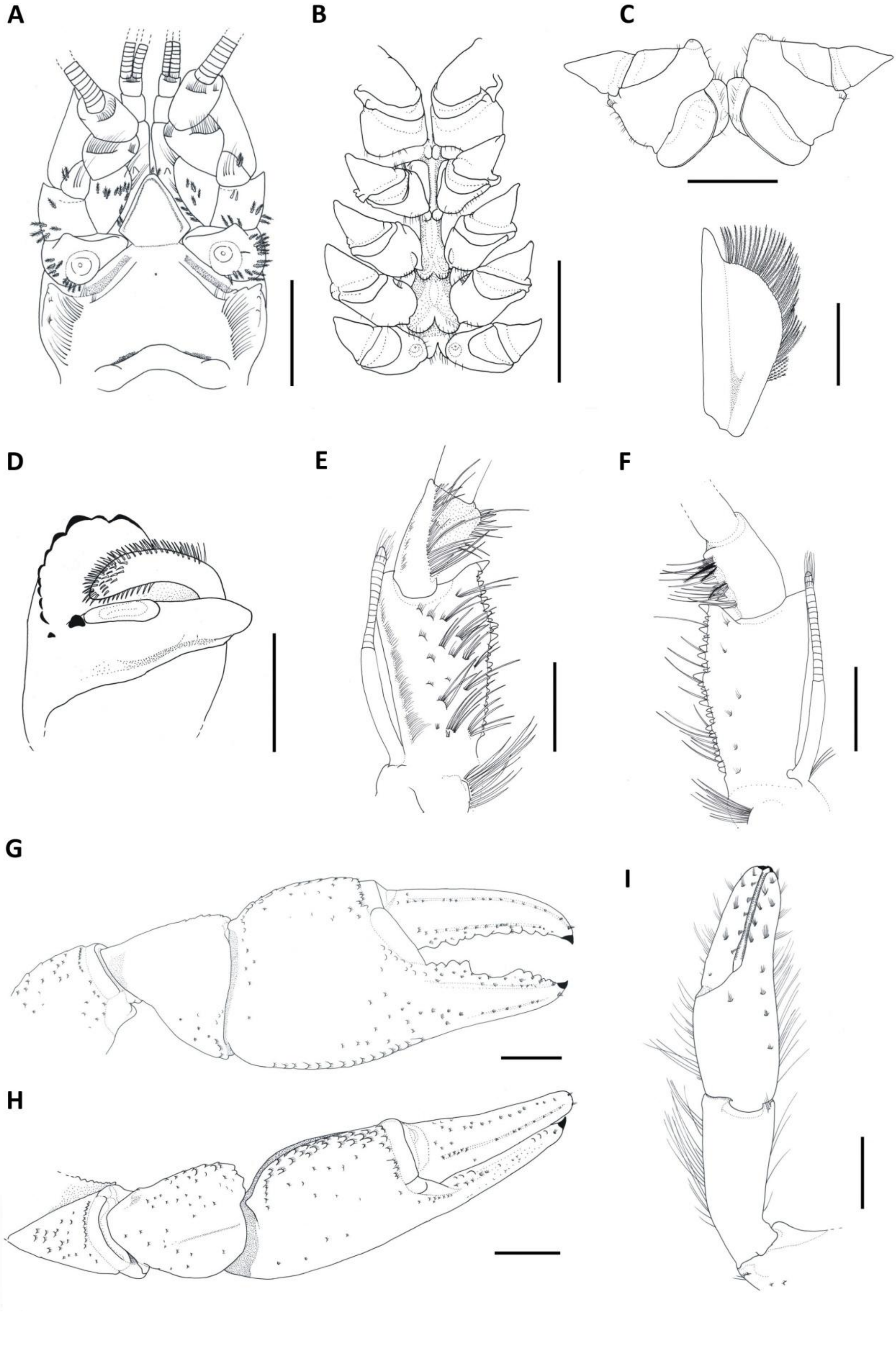


FIGURE 10



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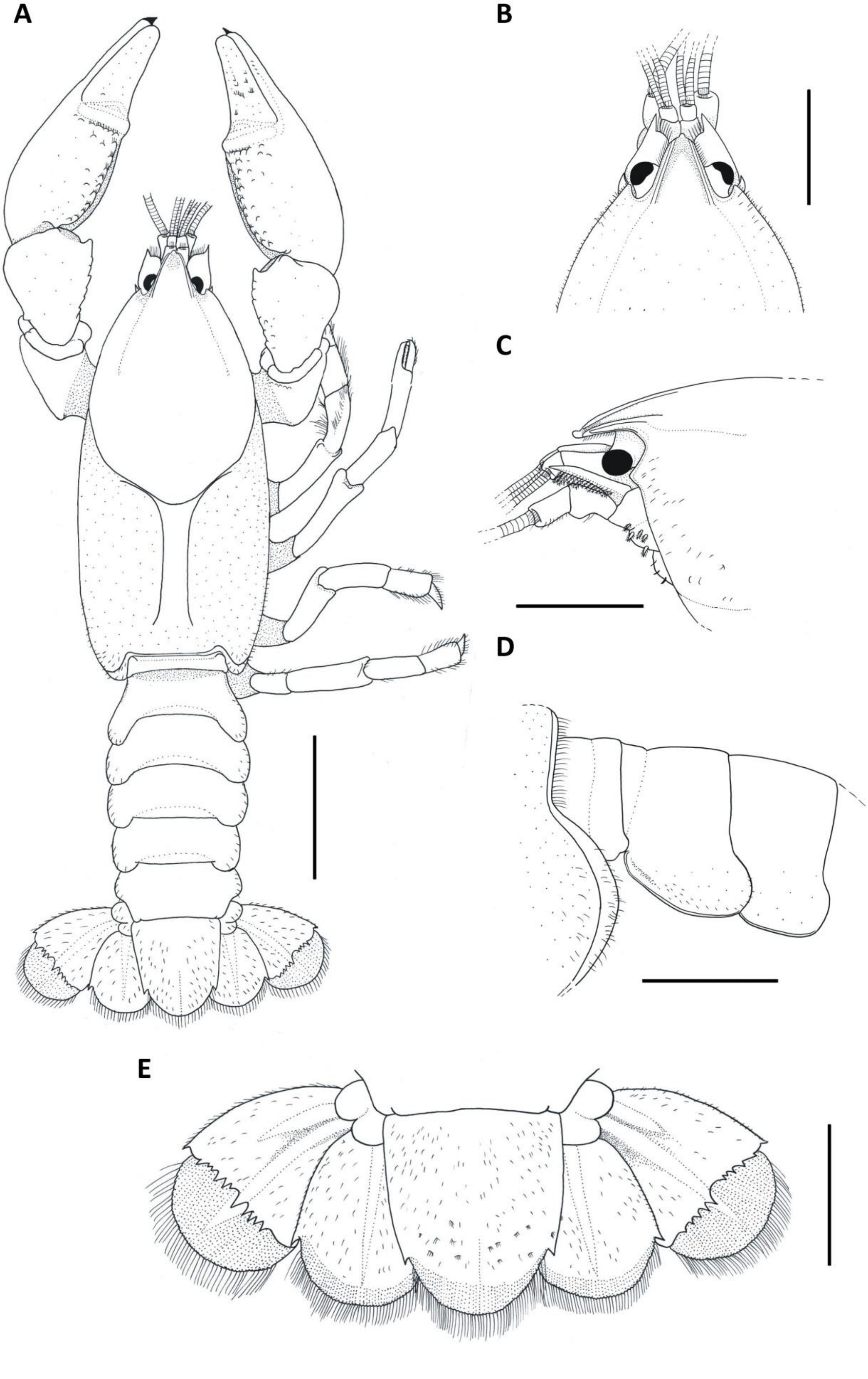

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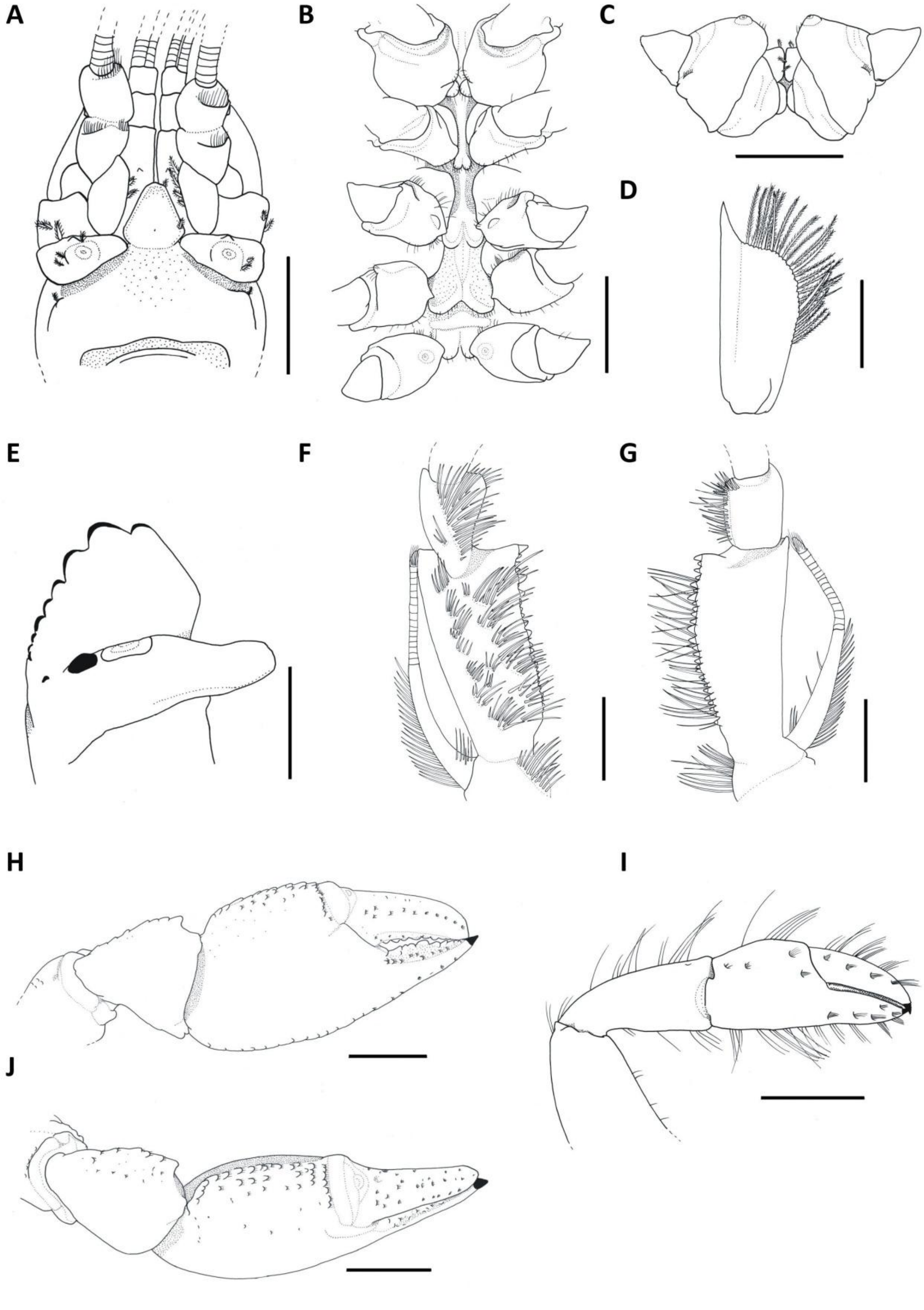

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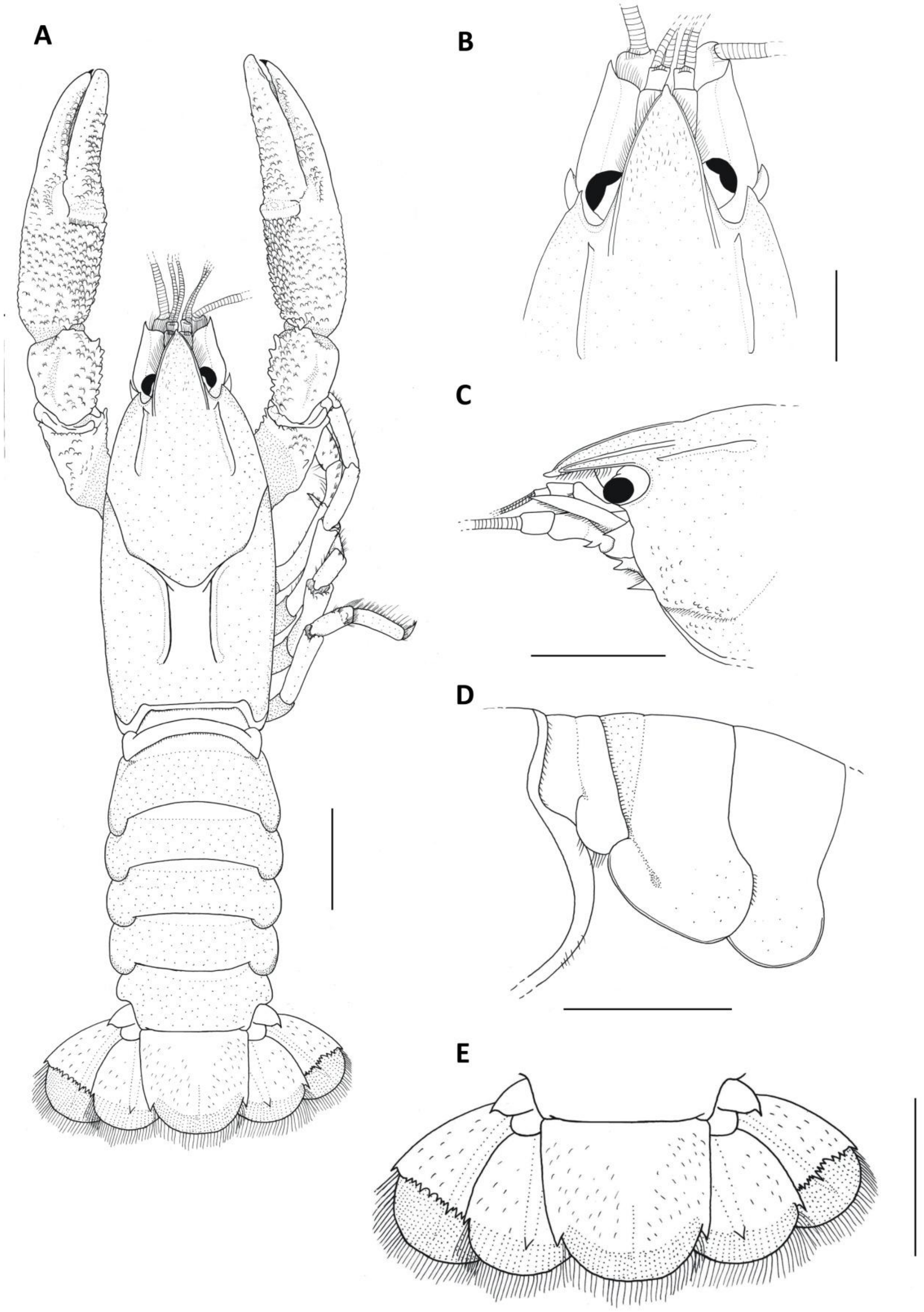

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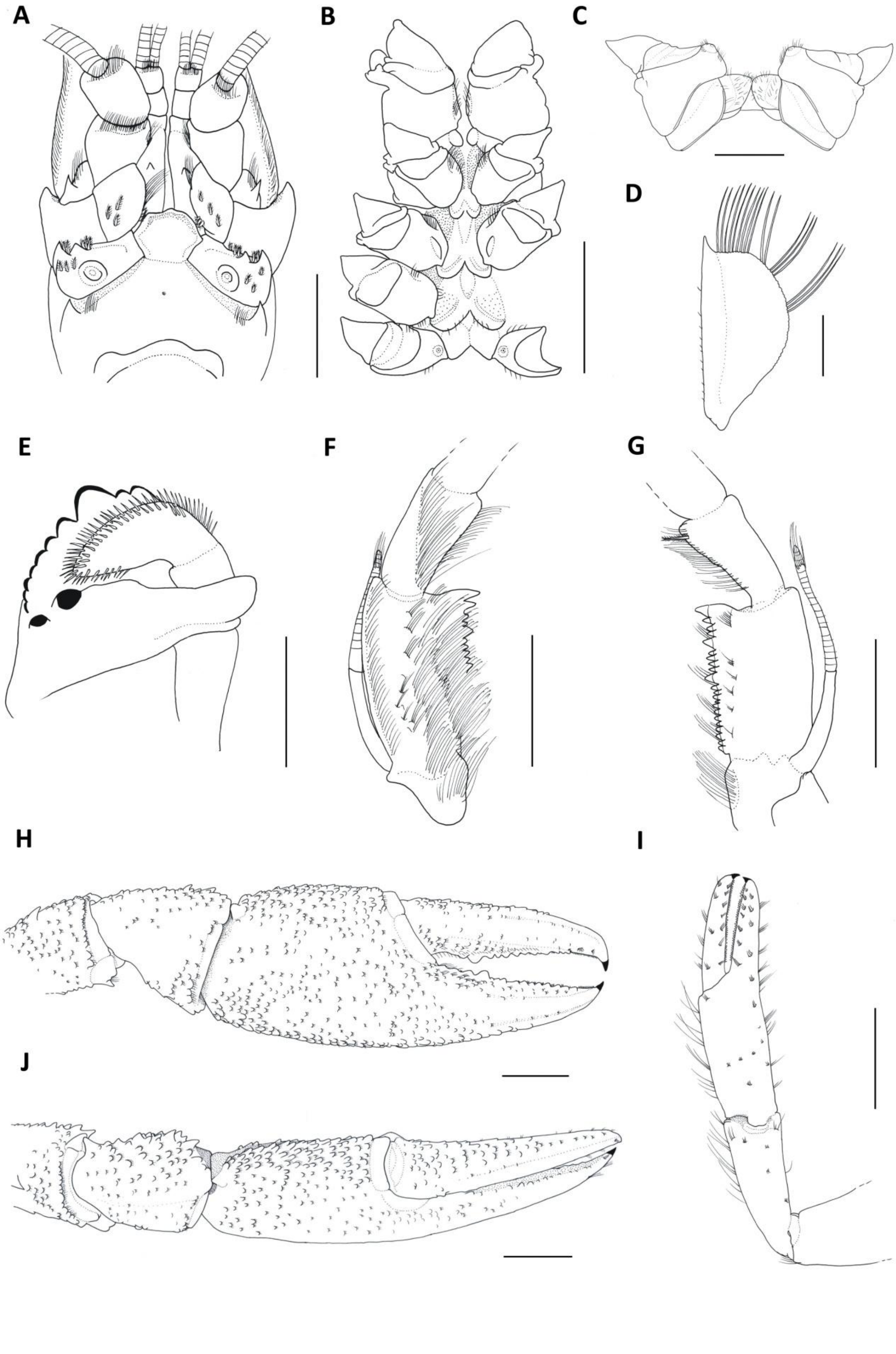

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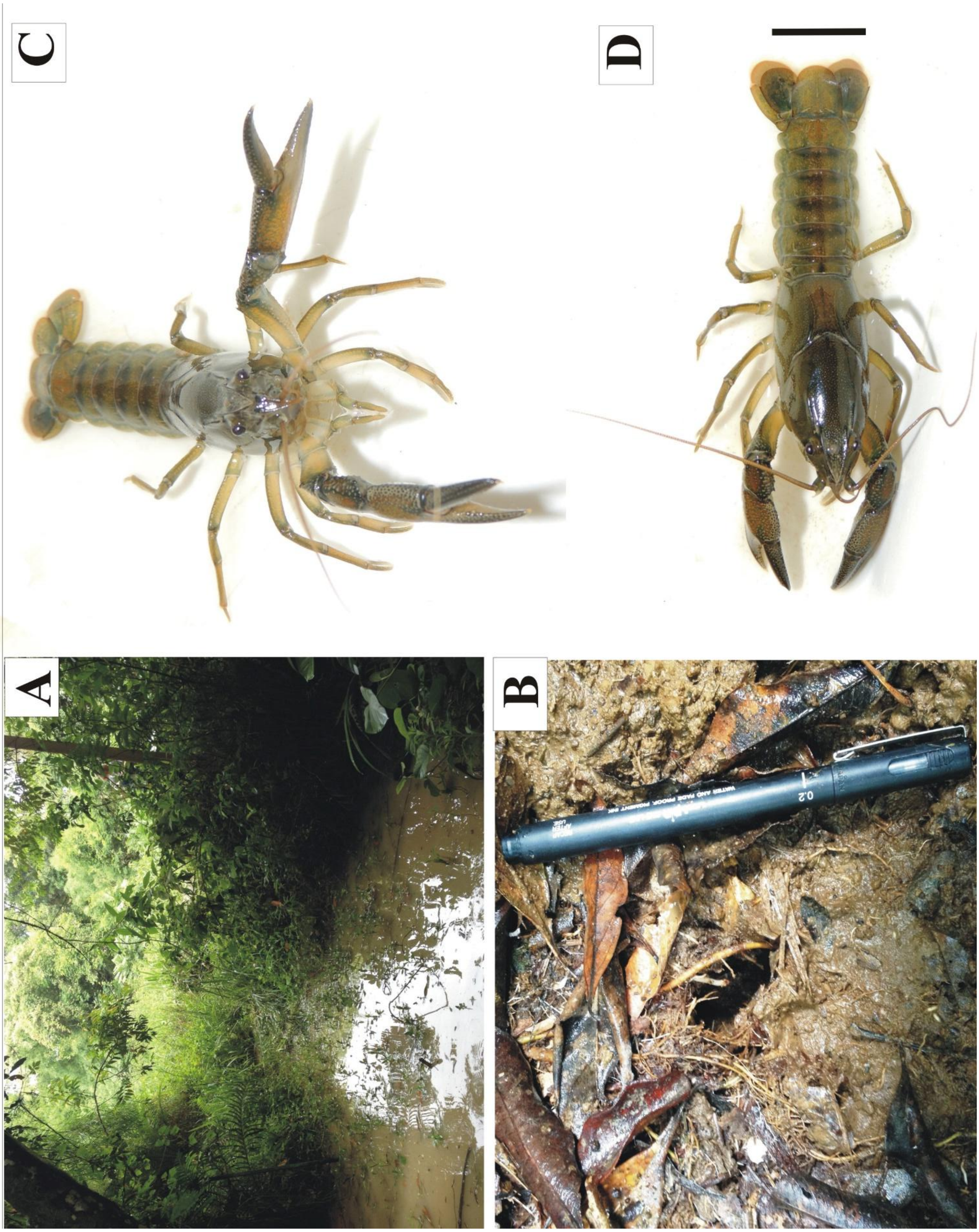

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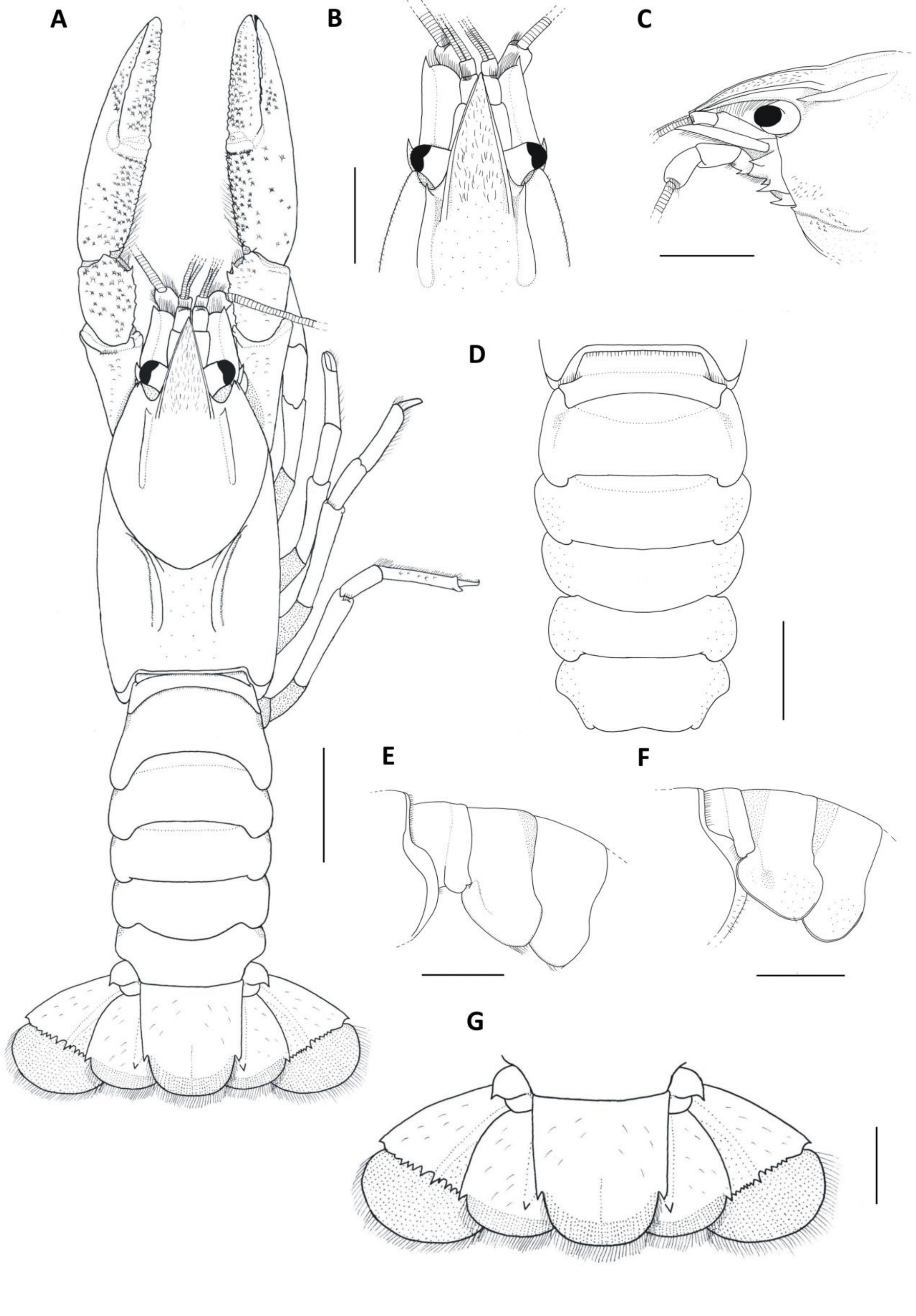

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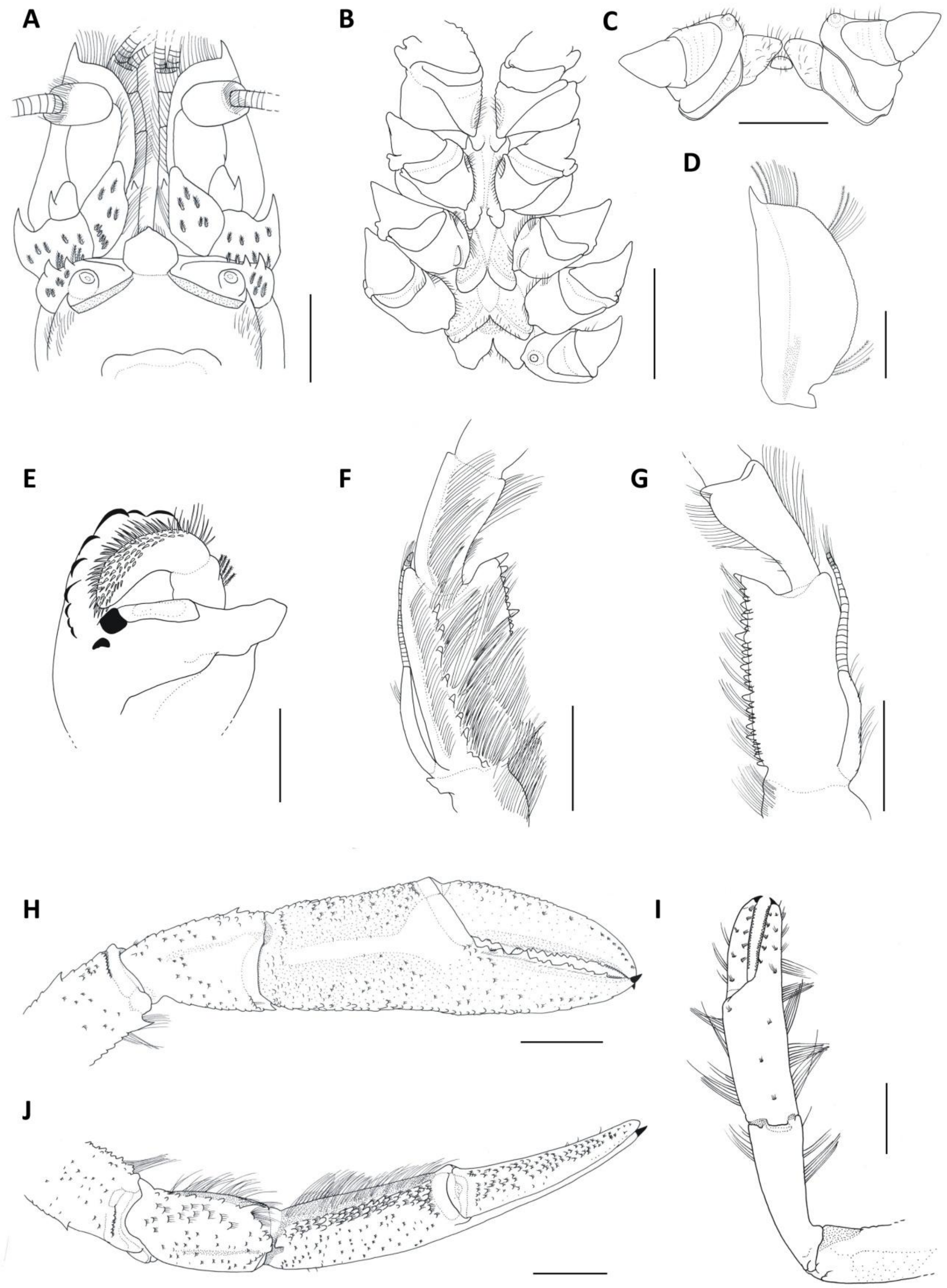

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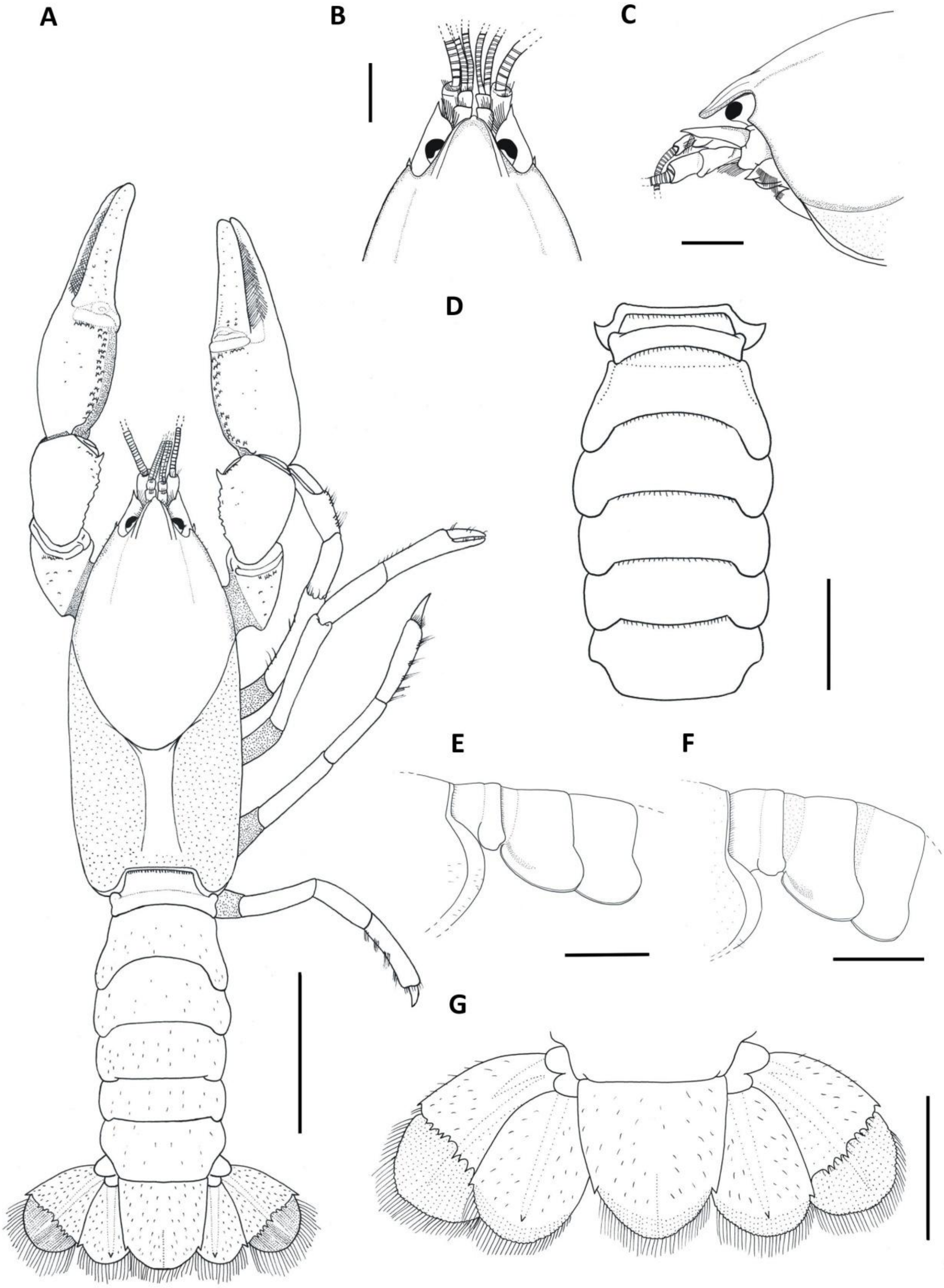

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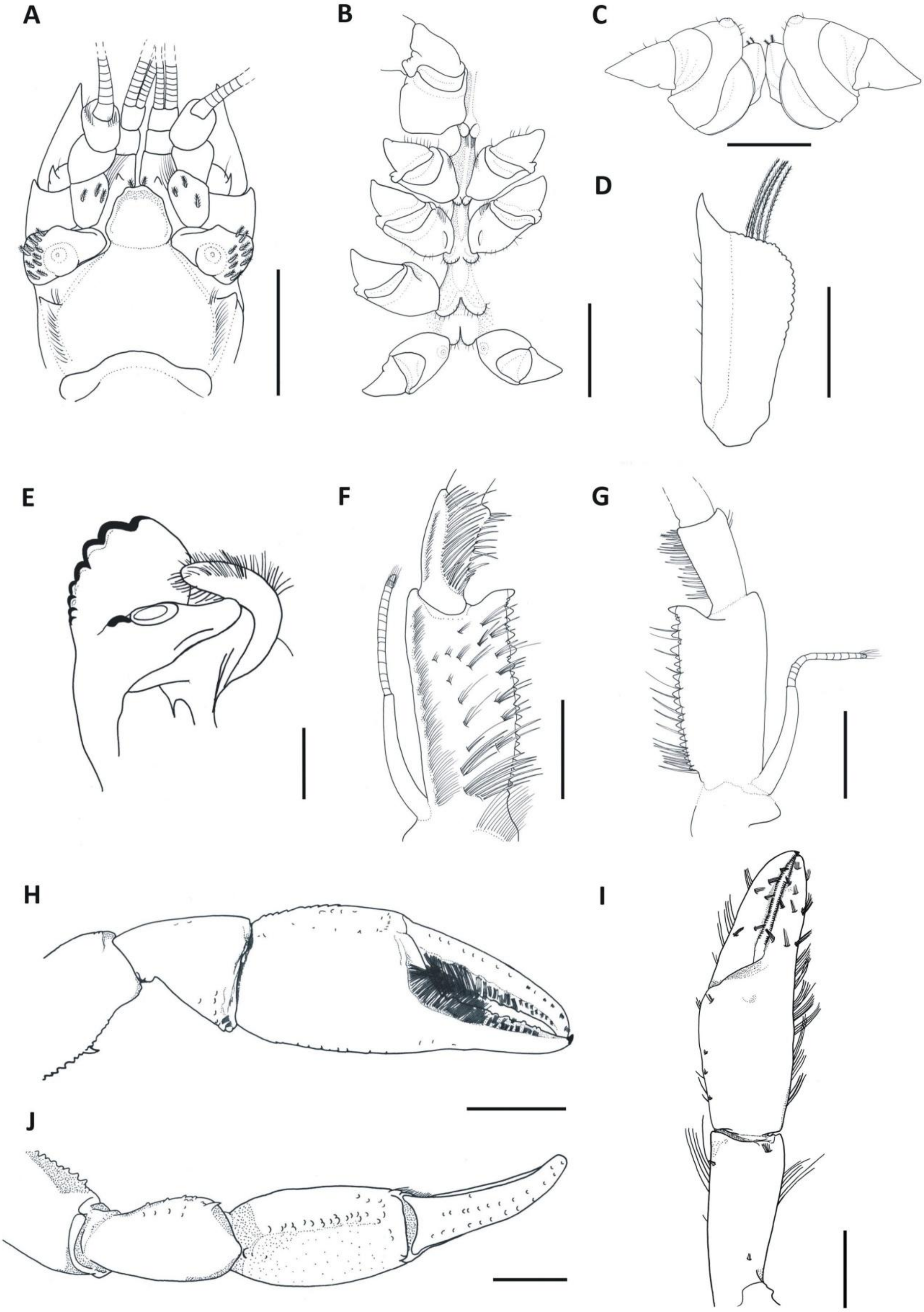

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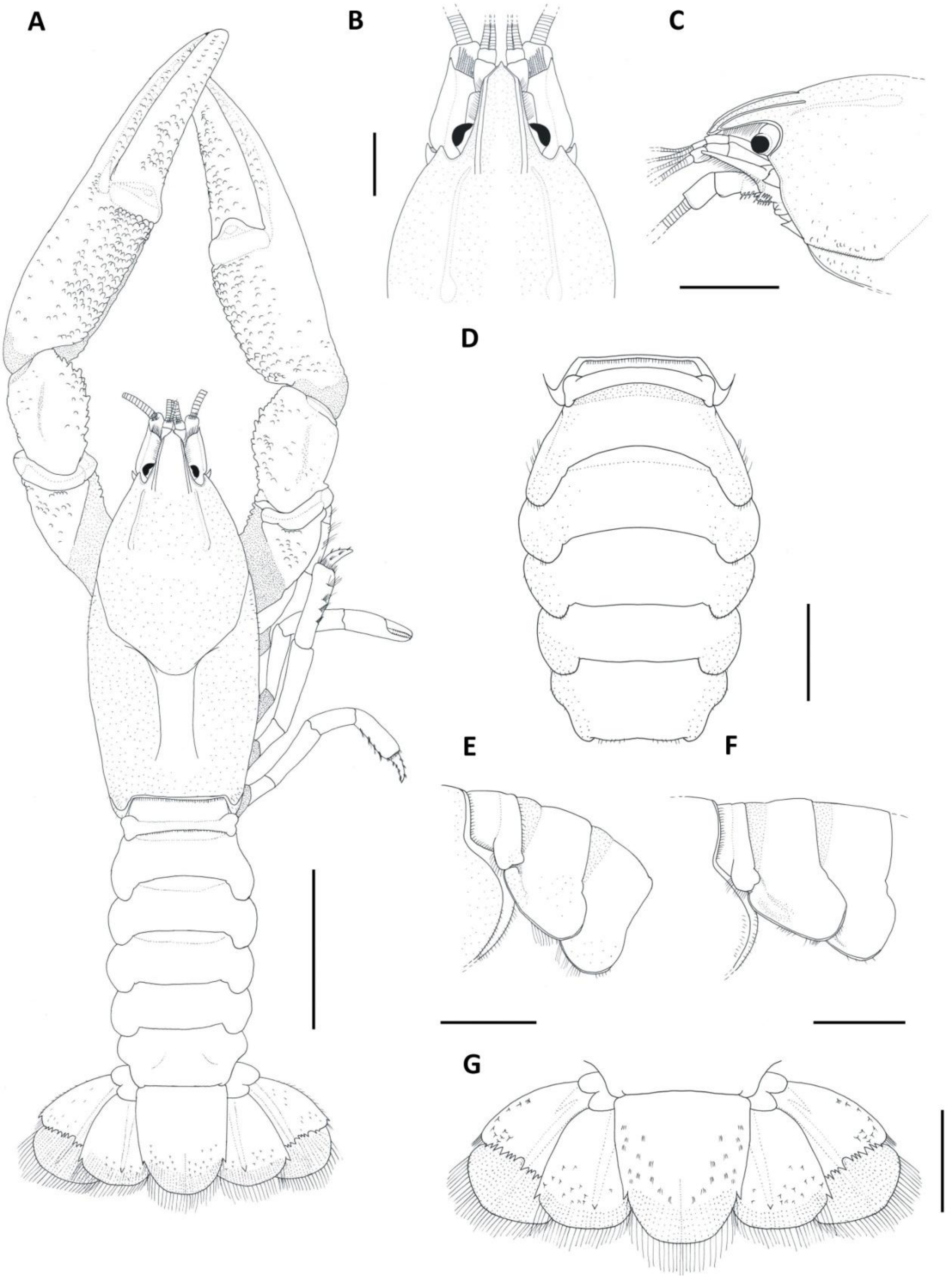

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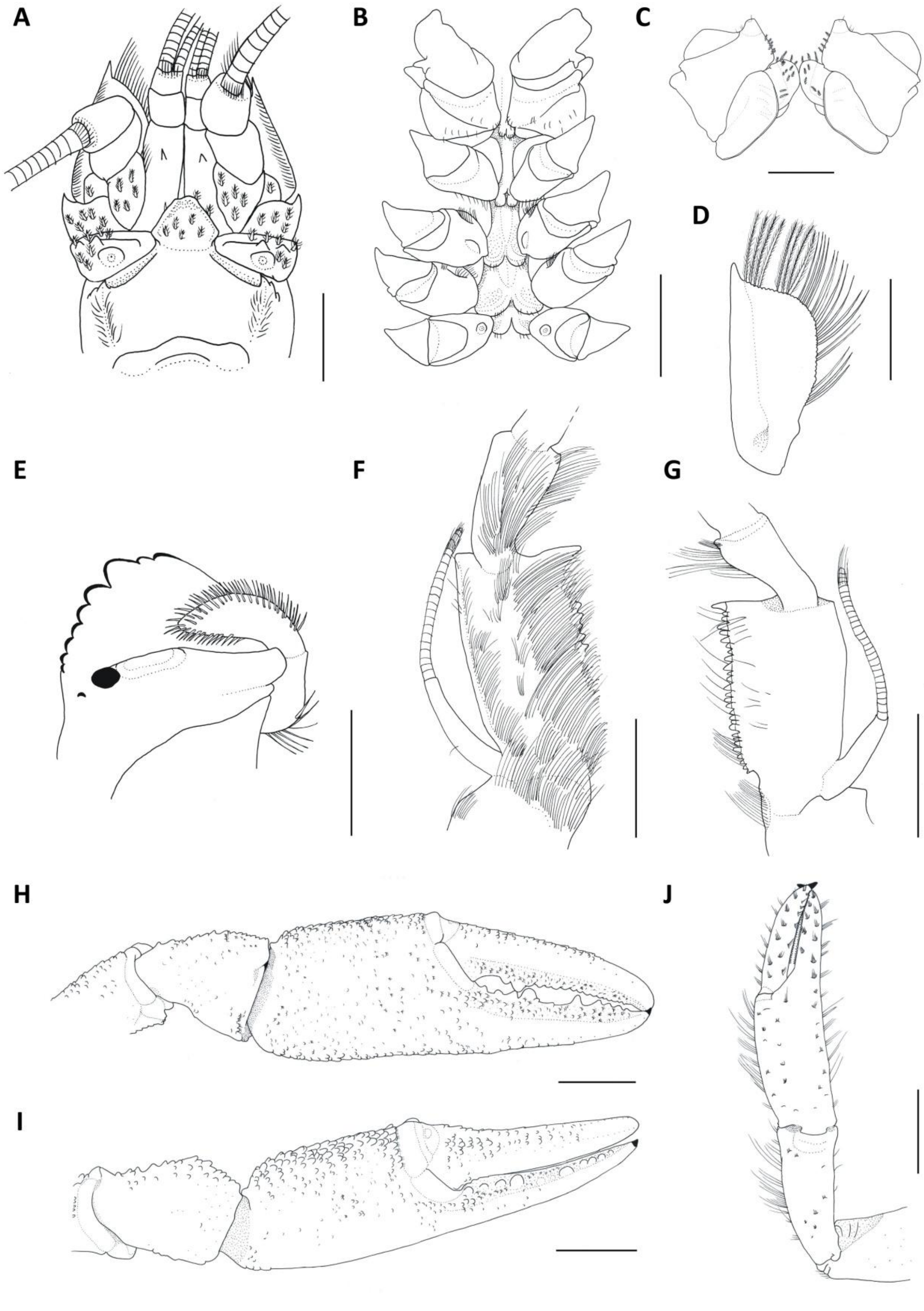

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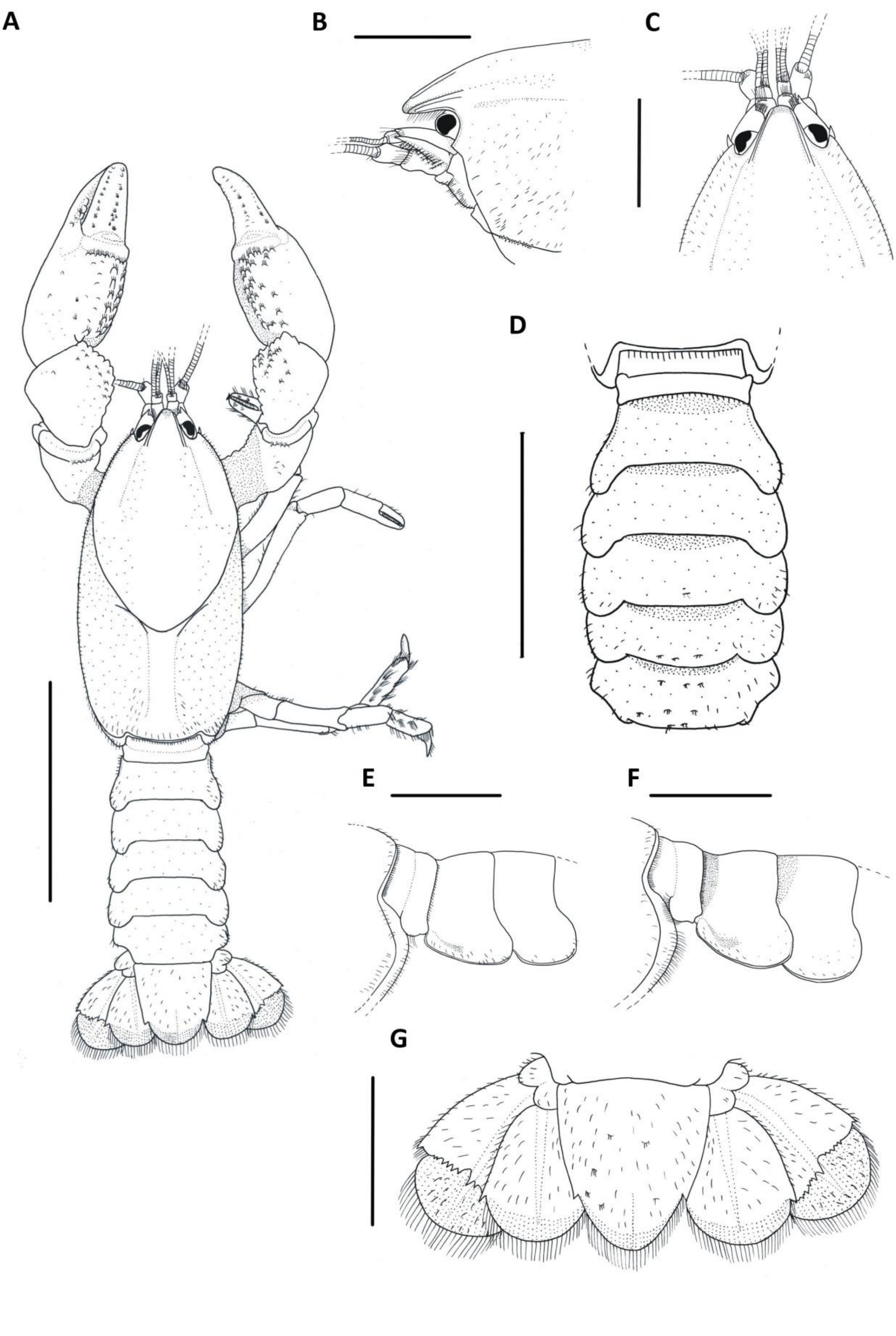

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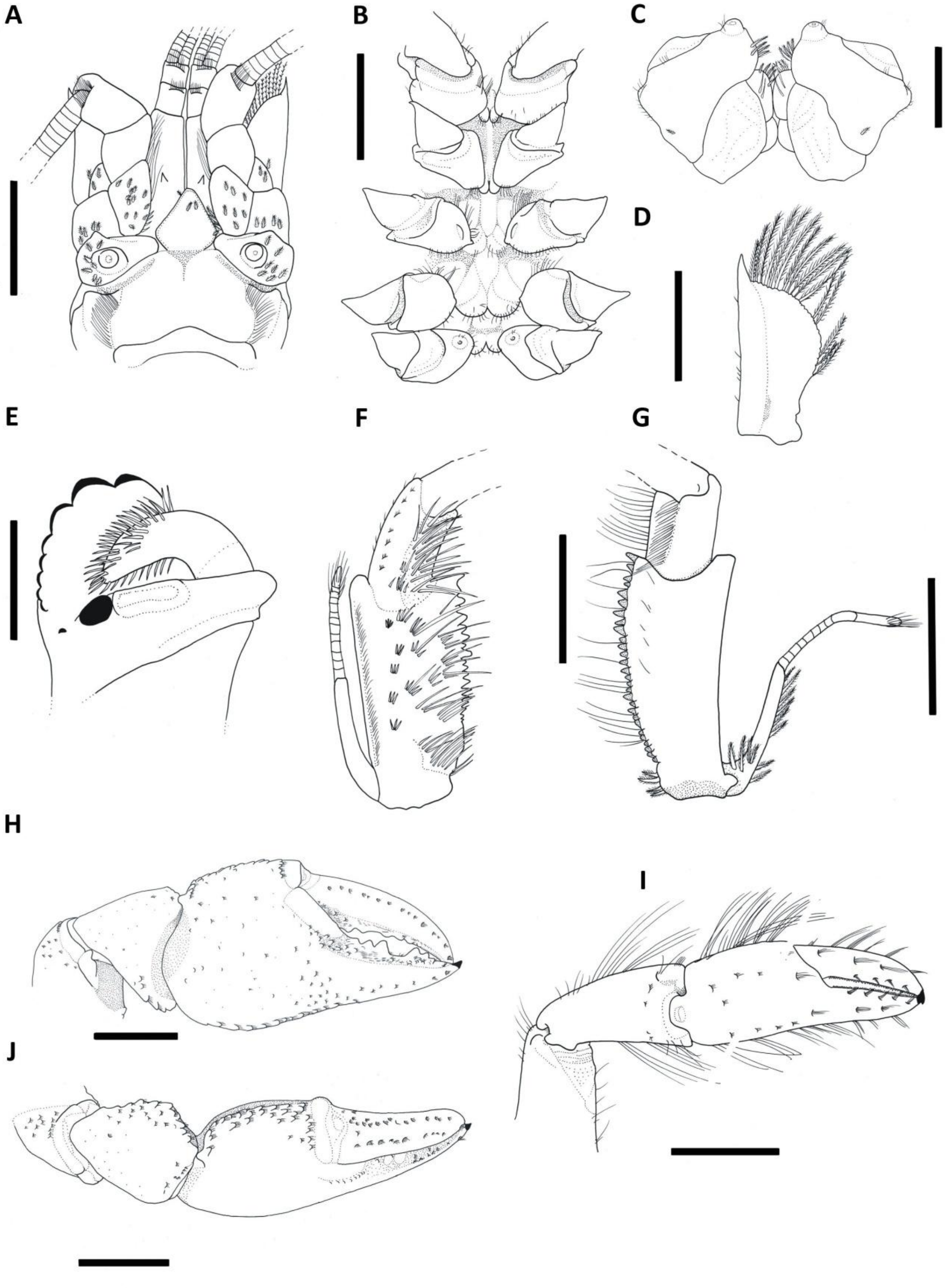

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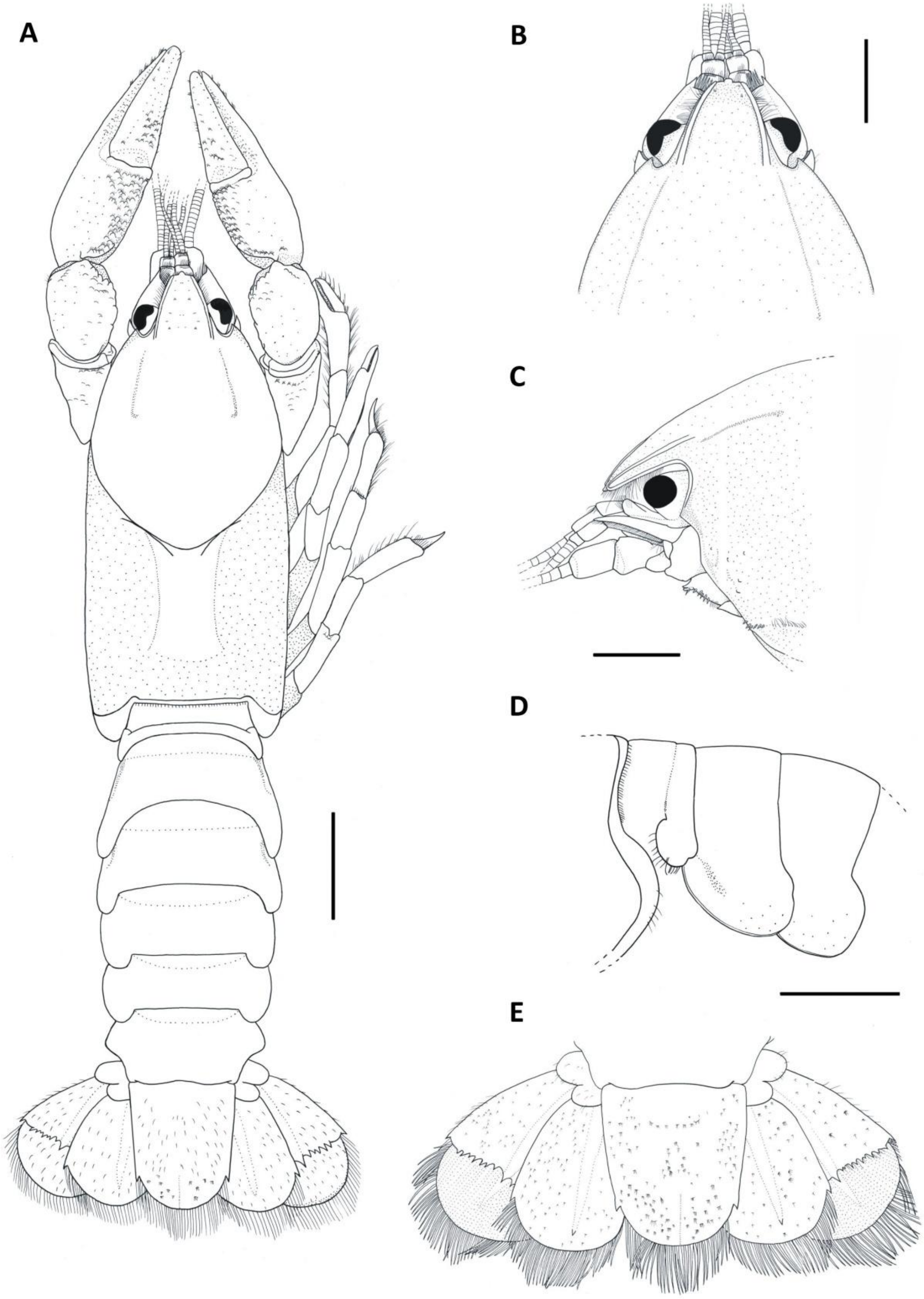

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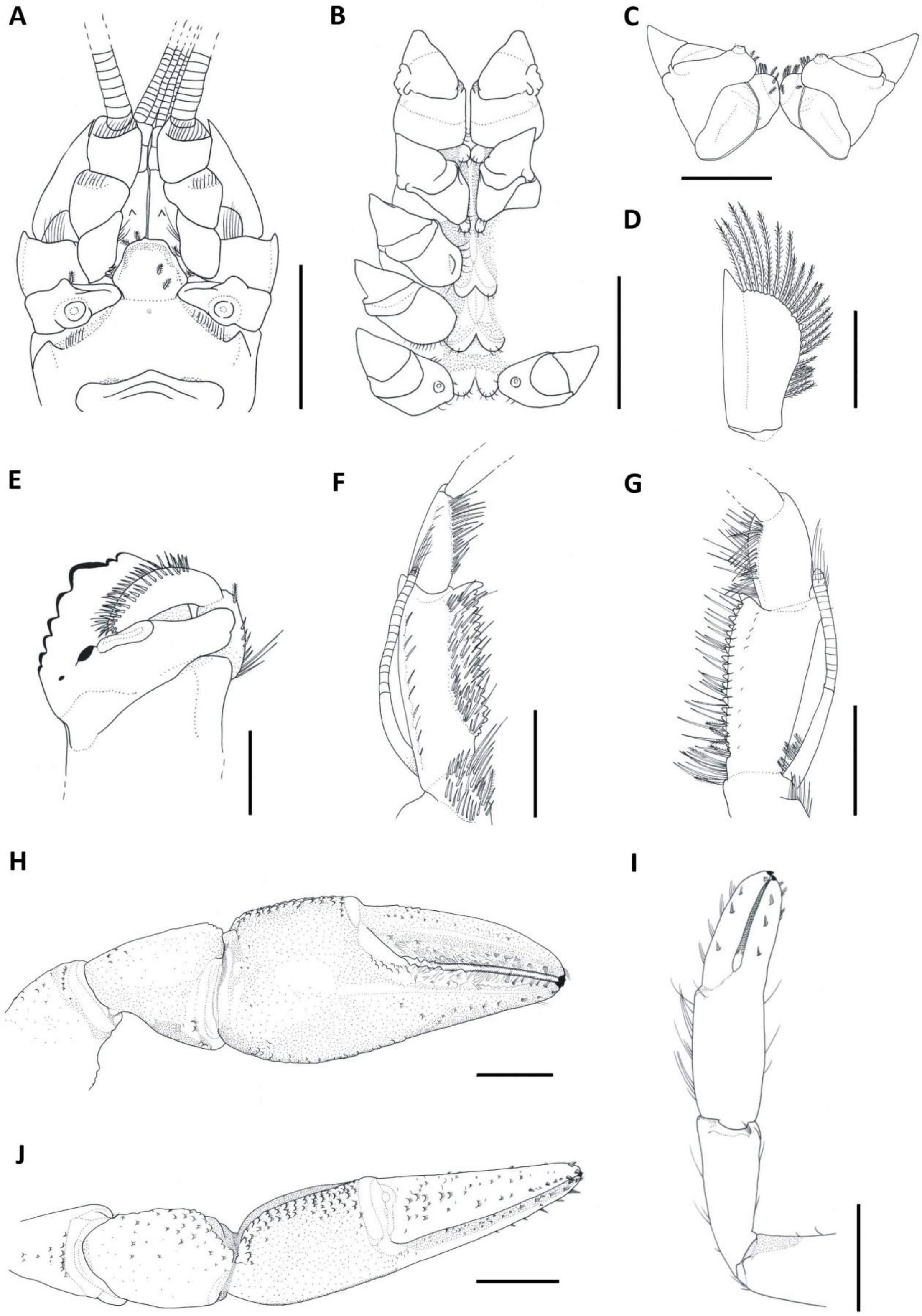

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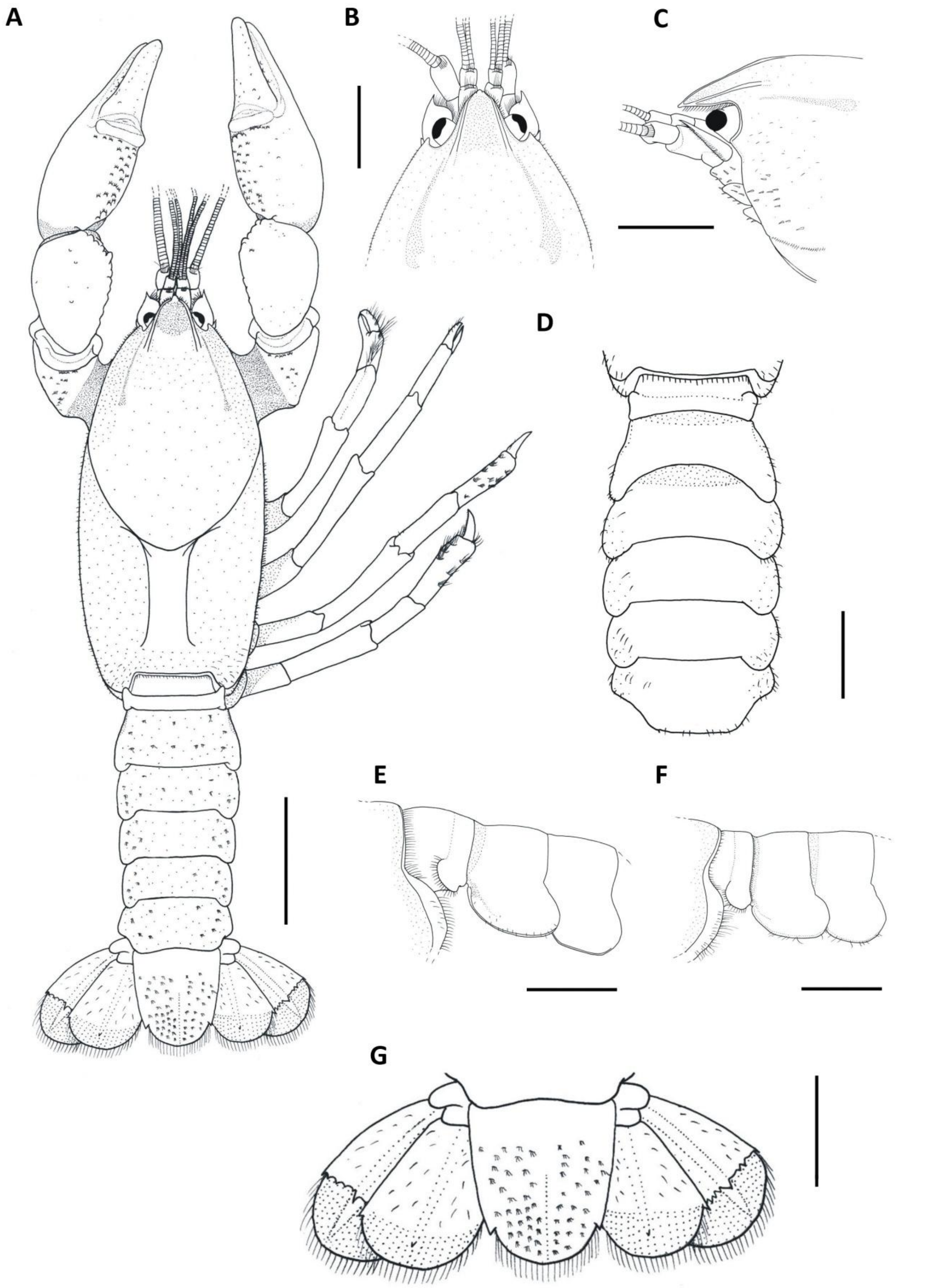

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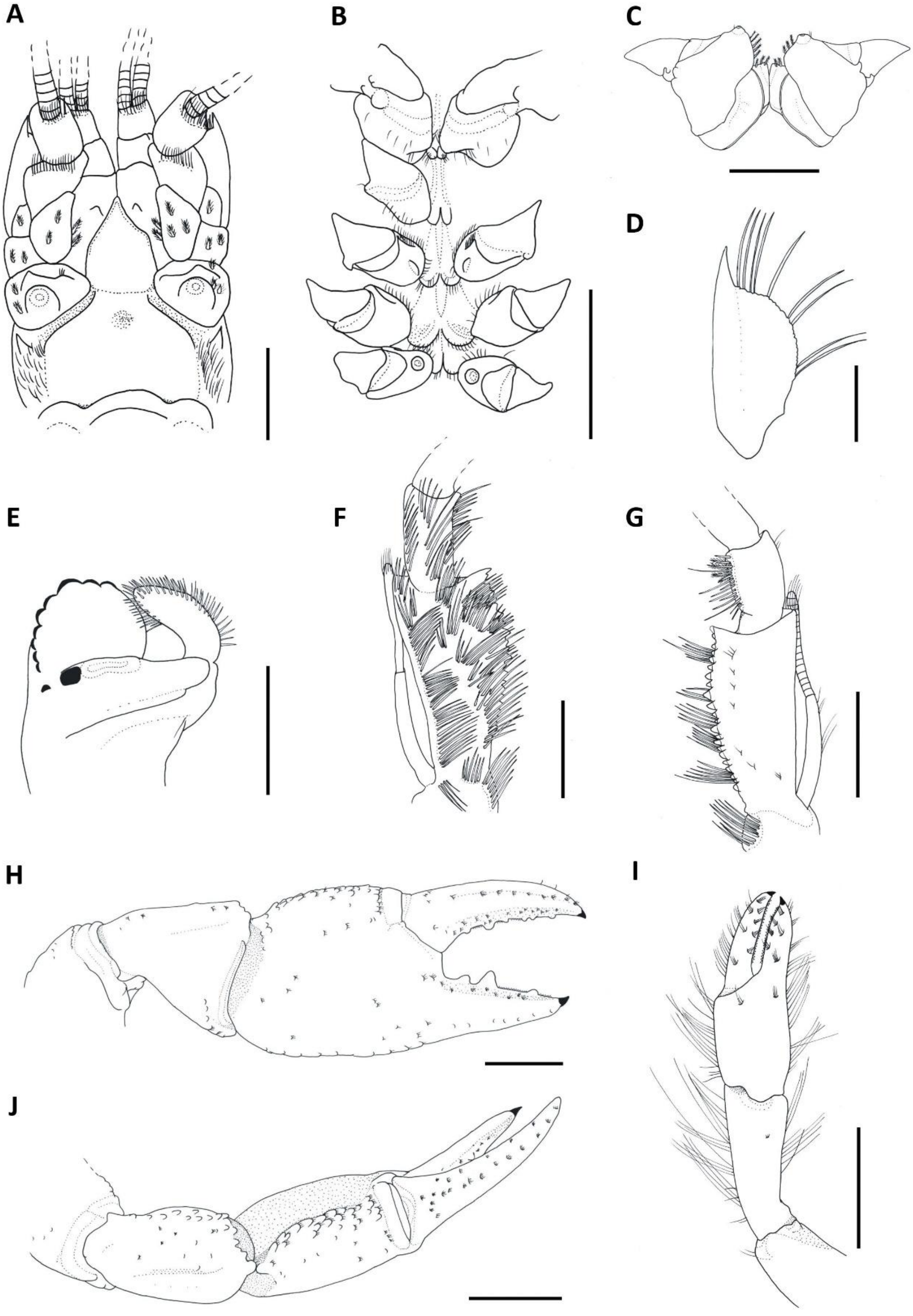

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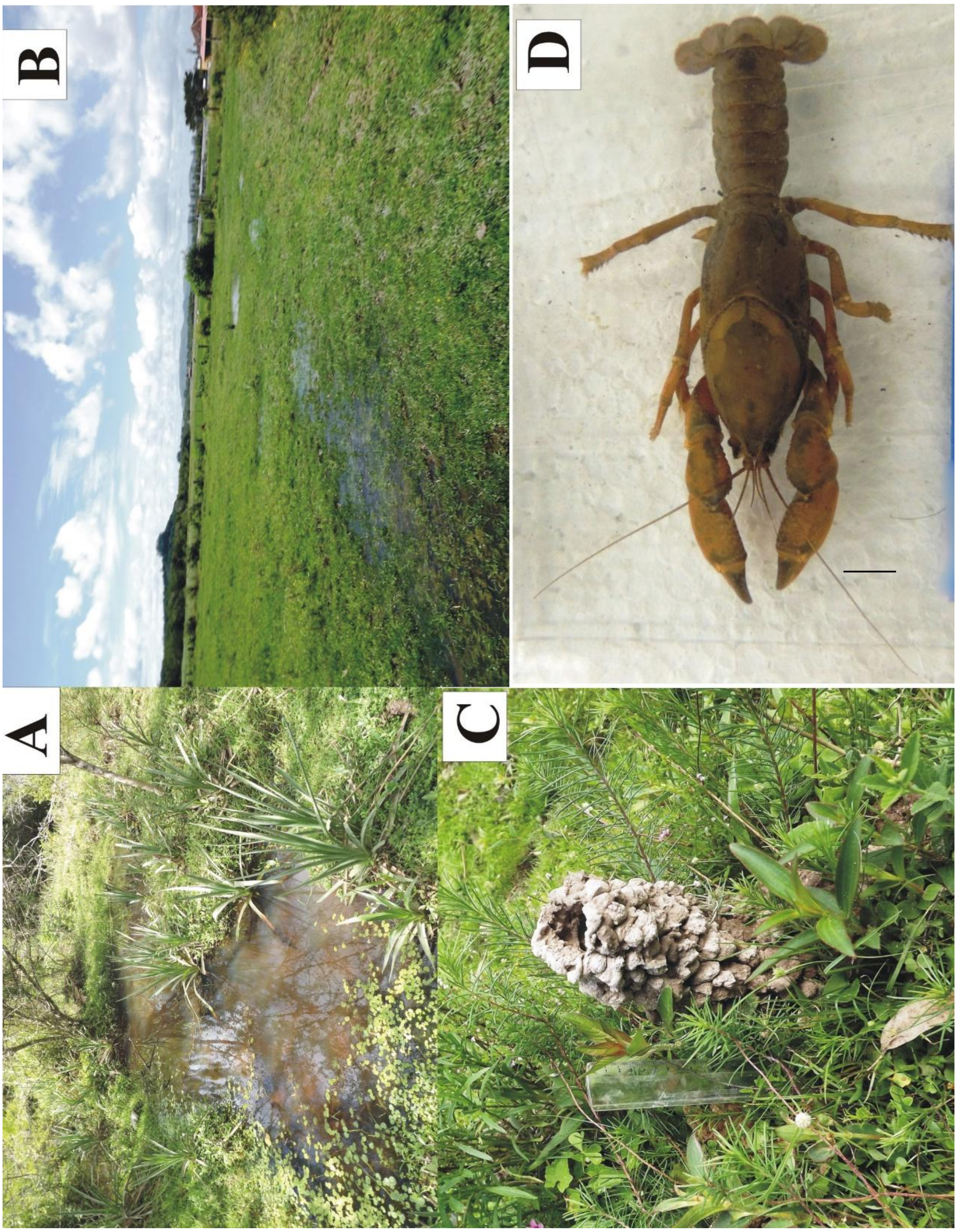

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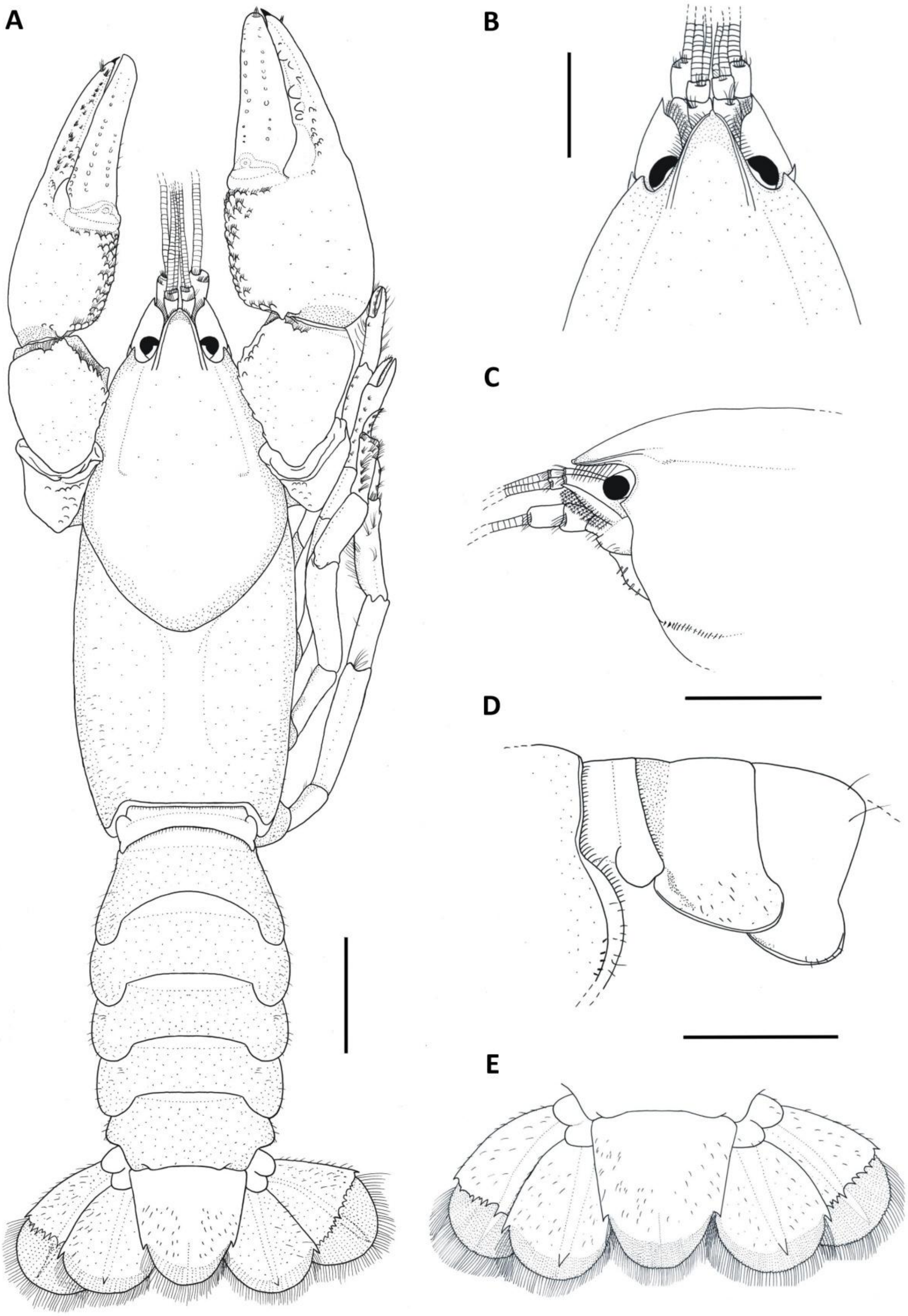

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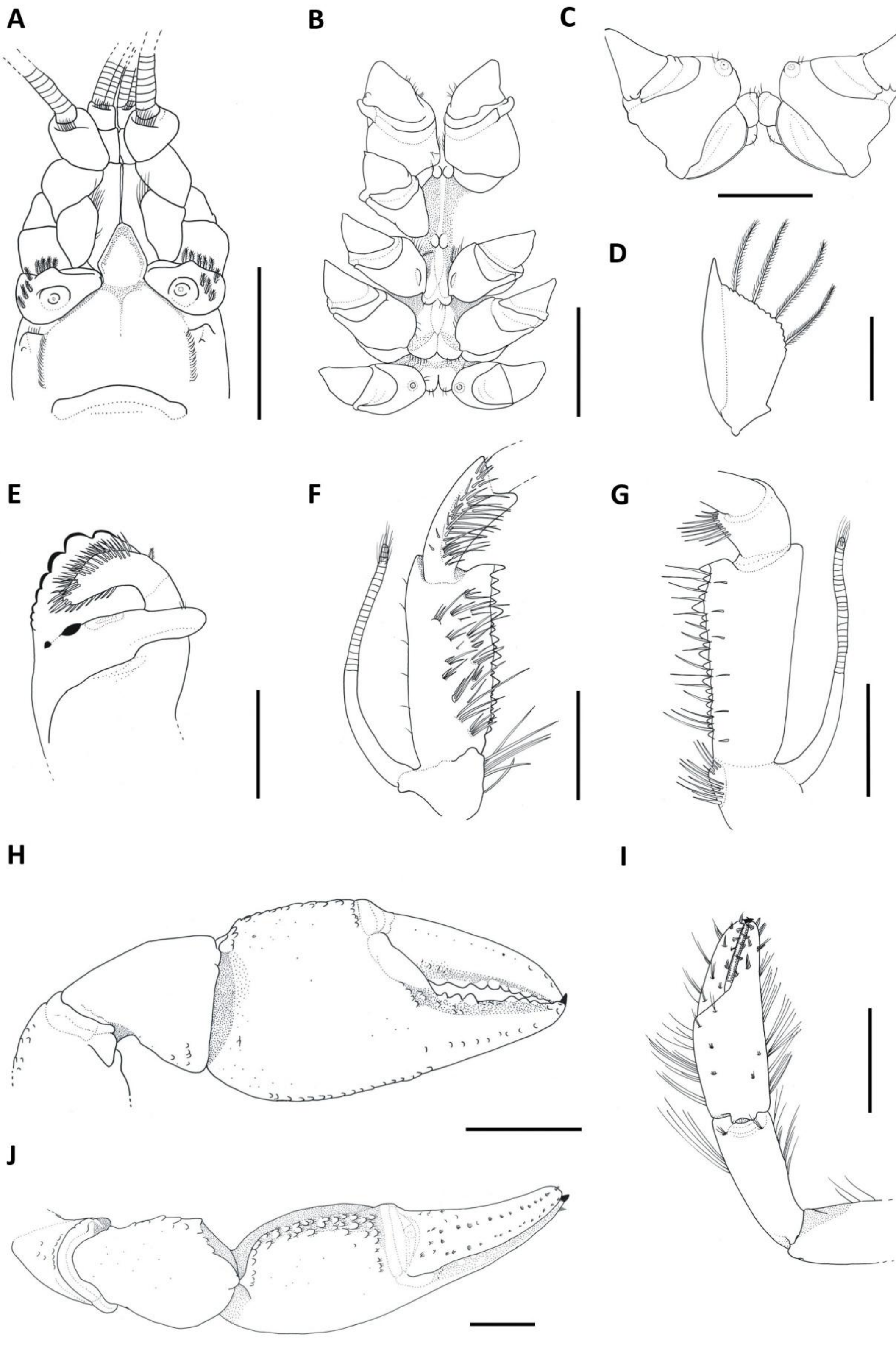

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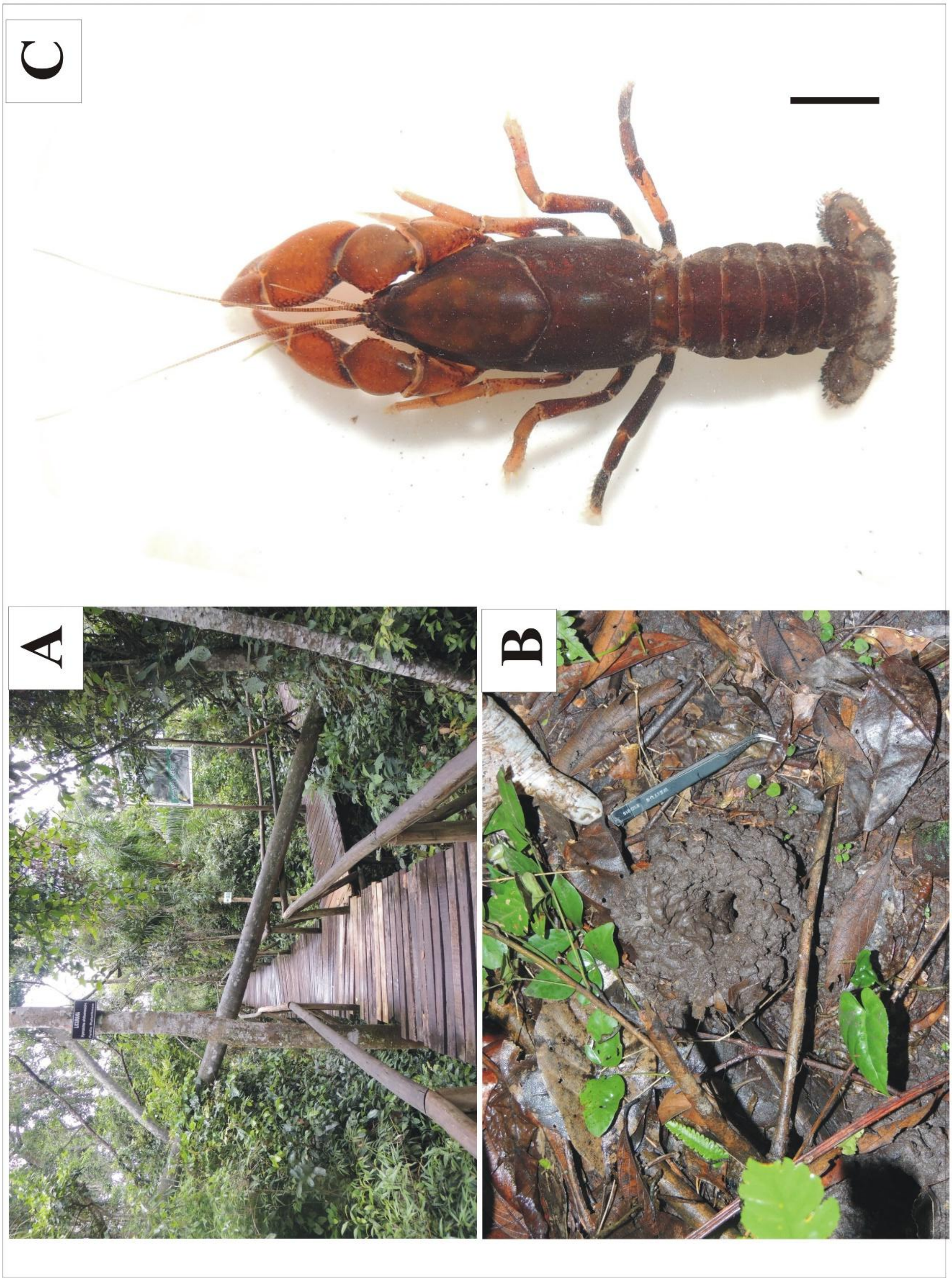

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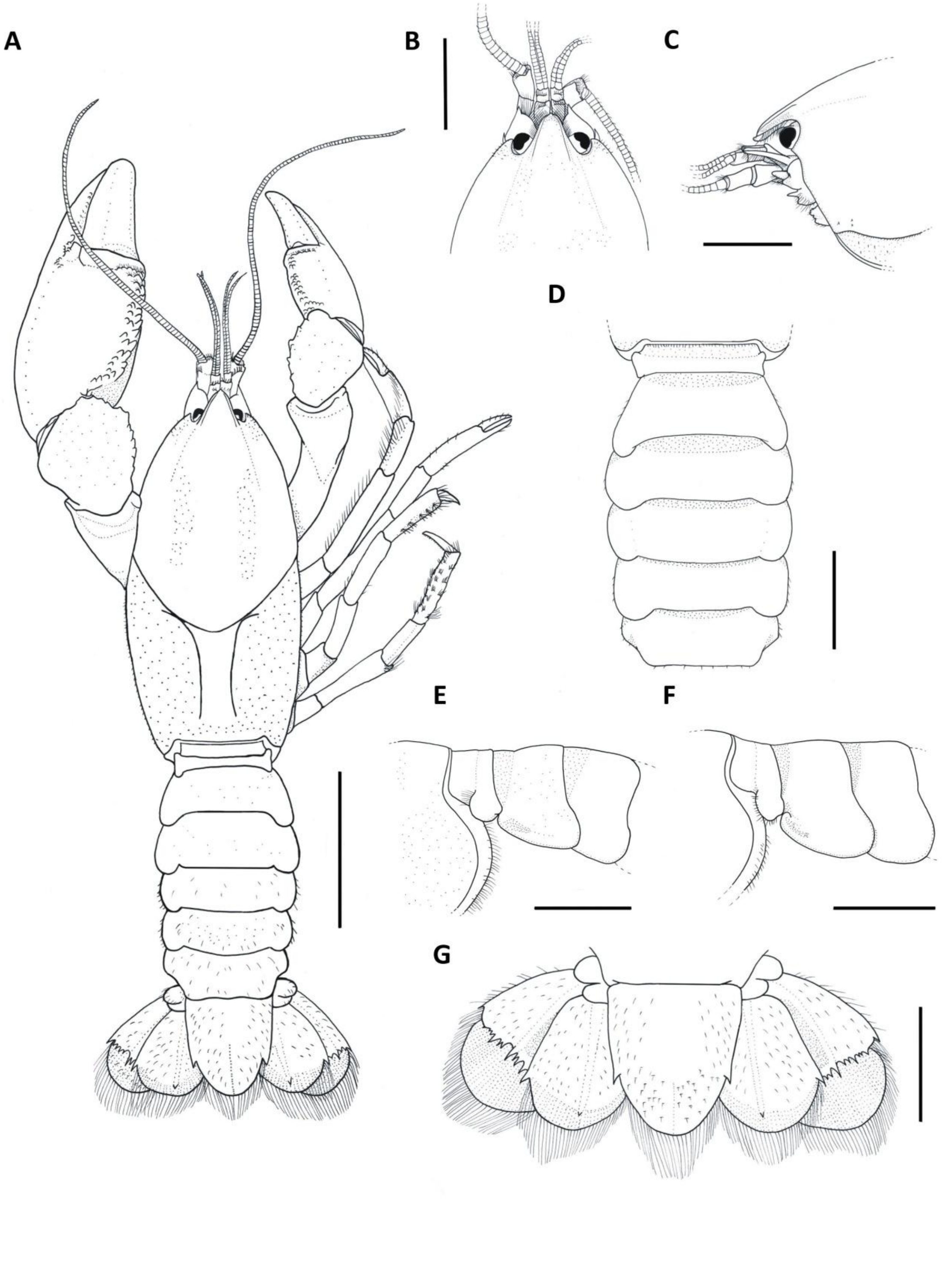

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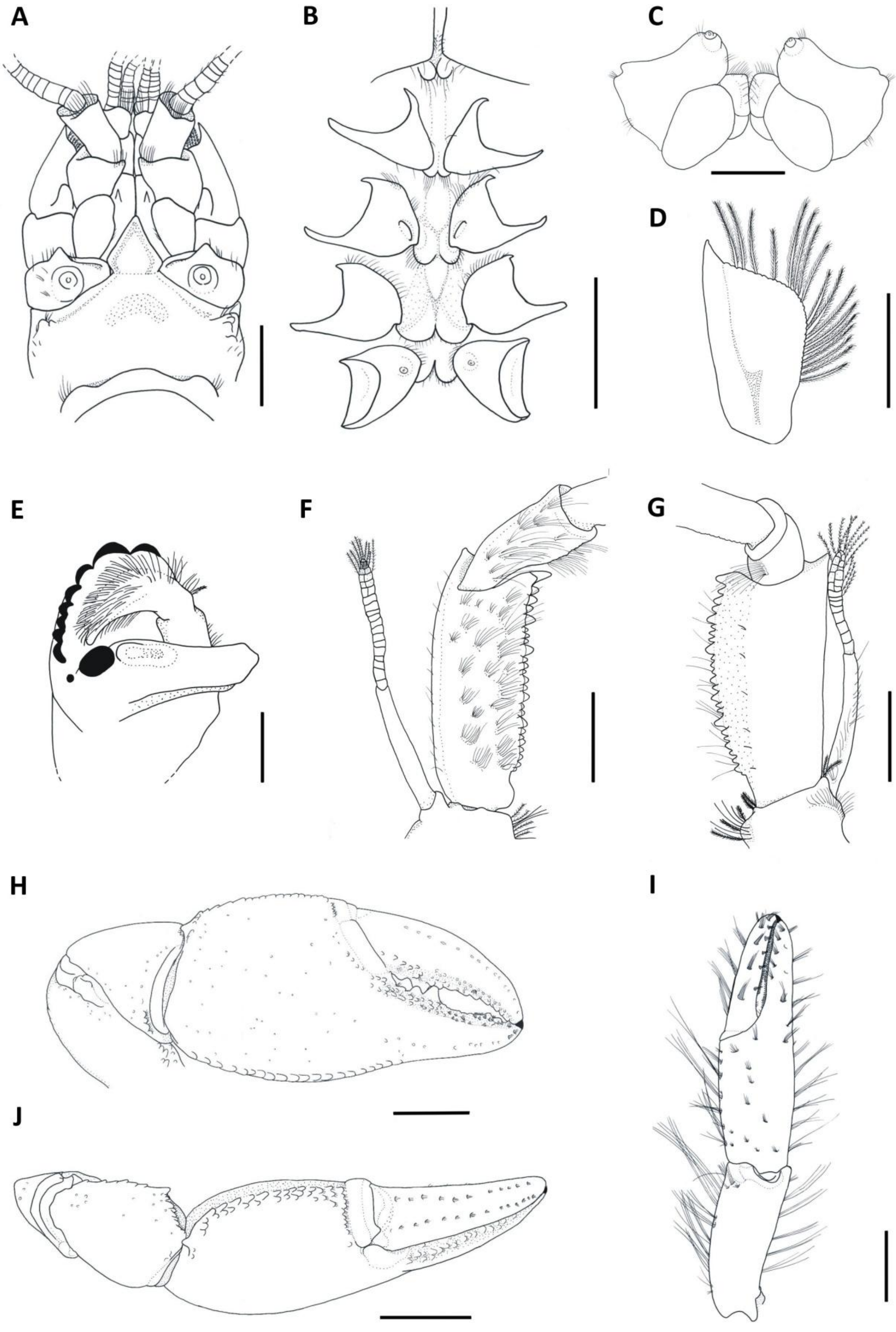

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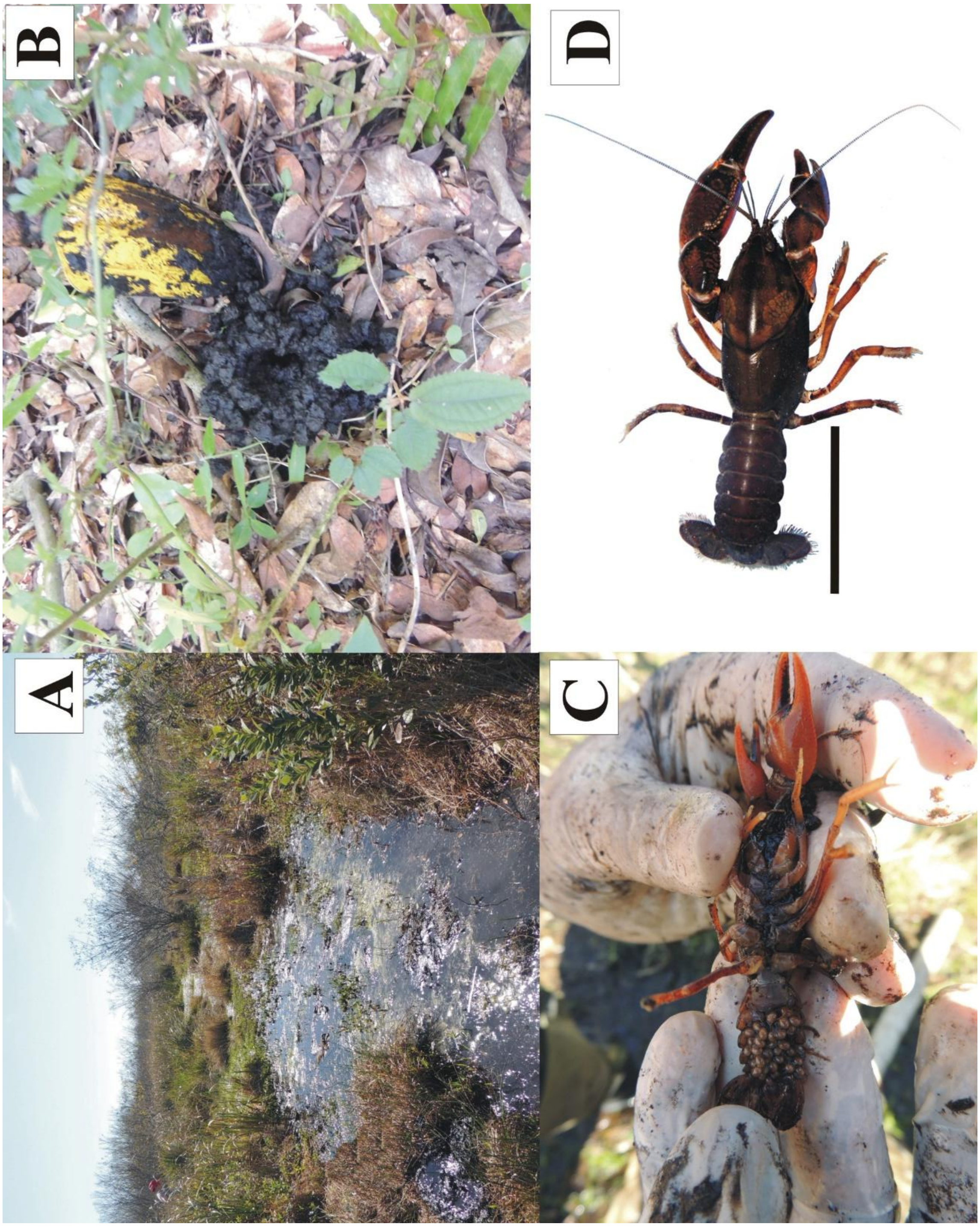

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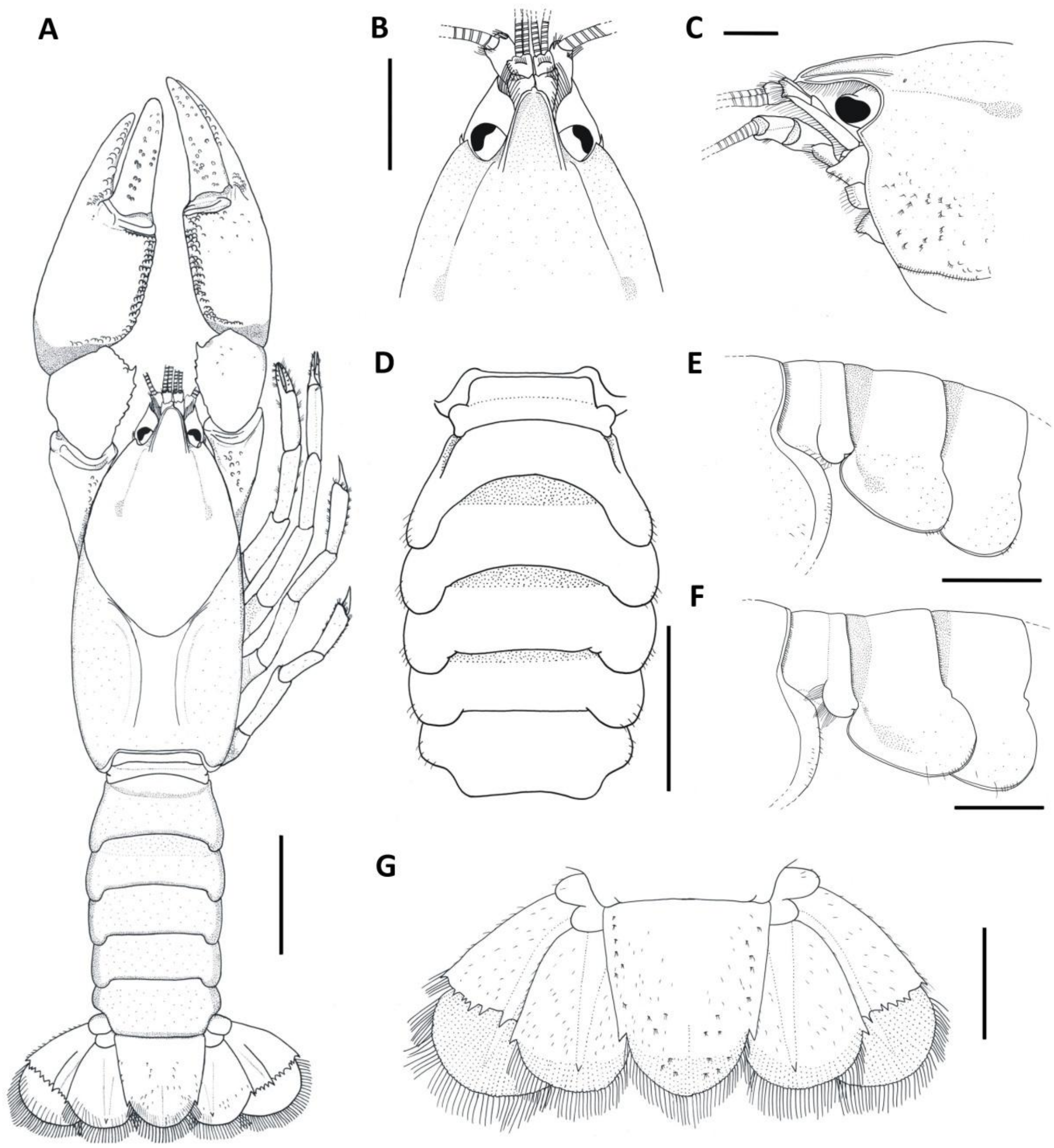

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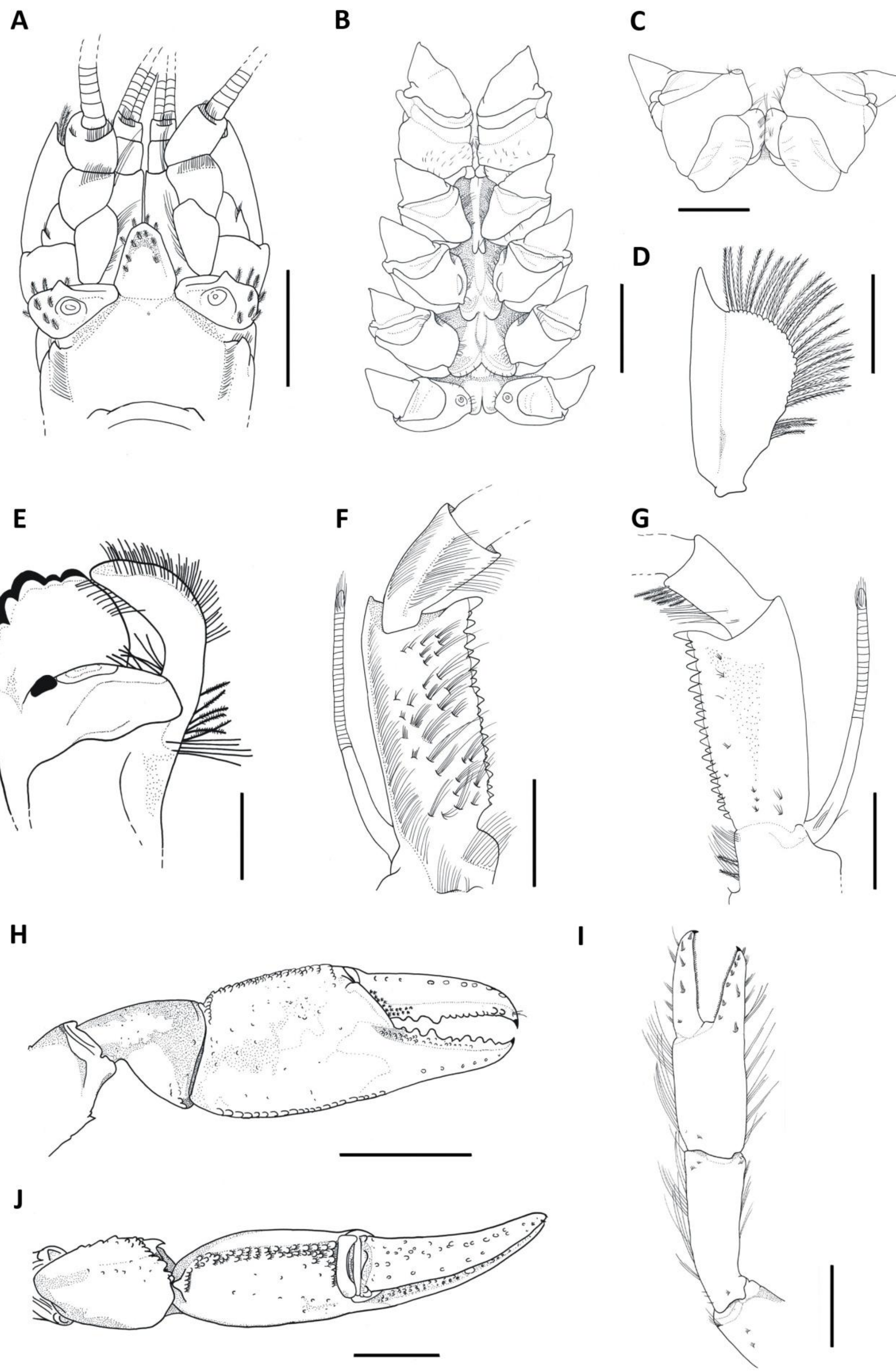
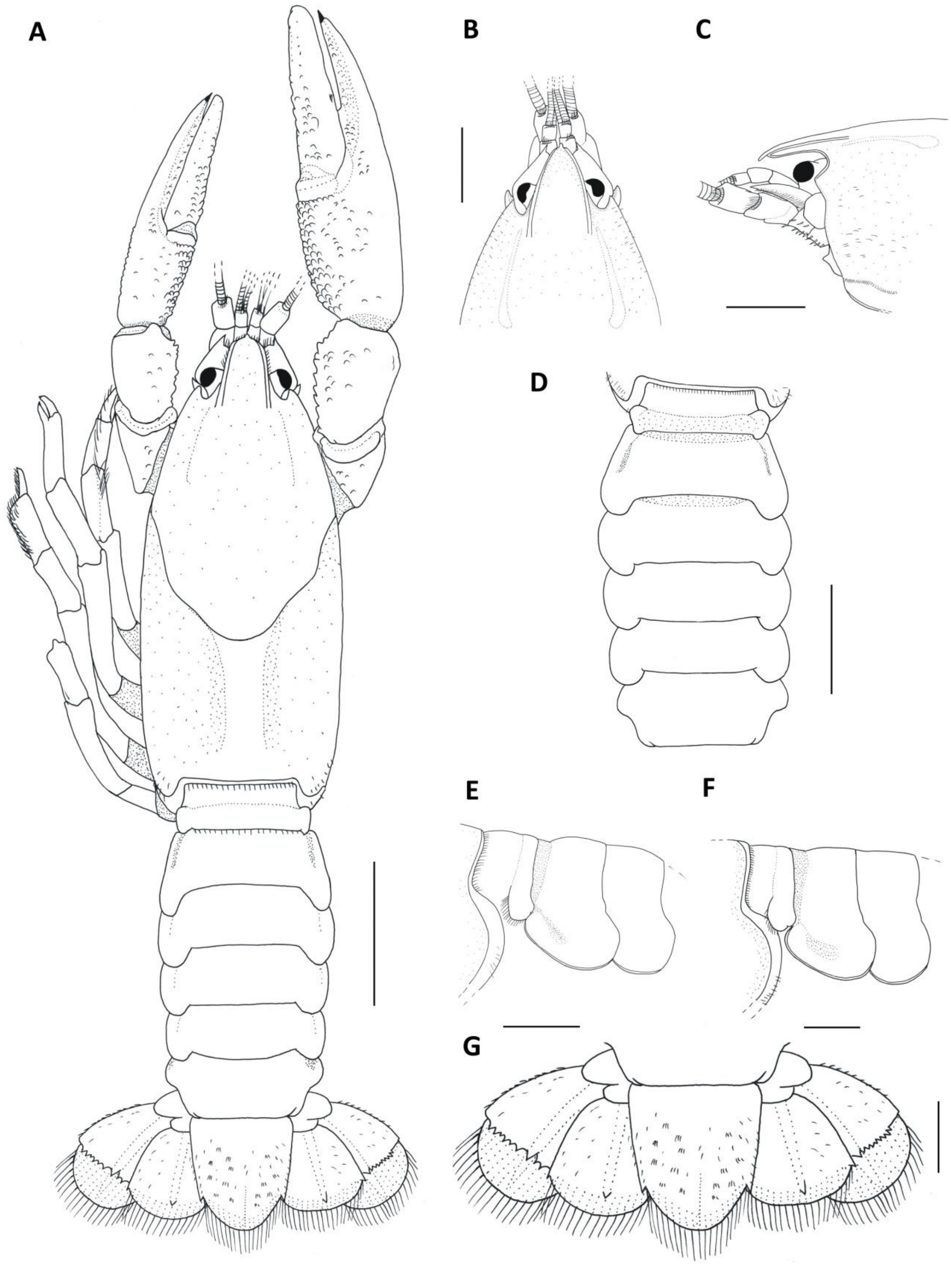


FIGURE 37

FIGURE 38



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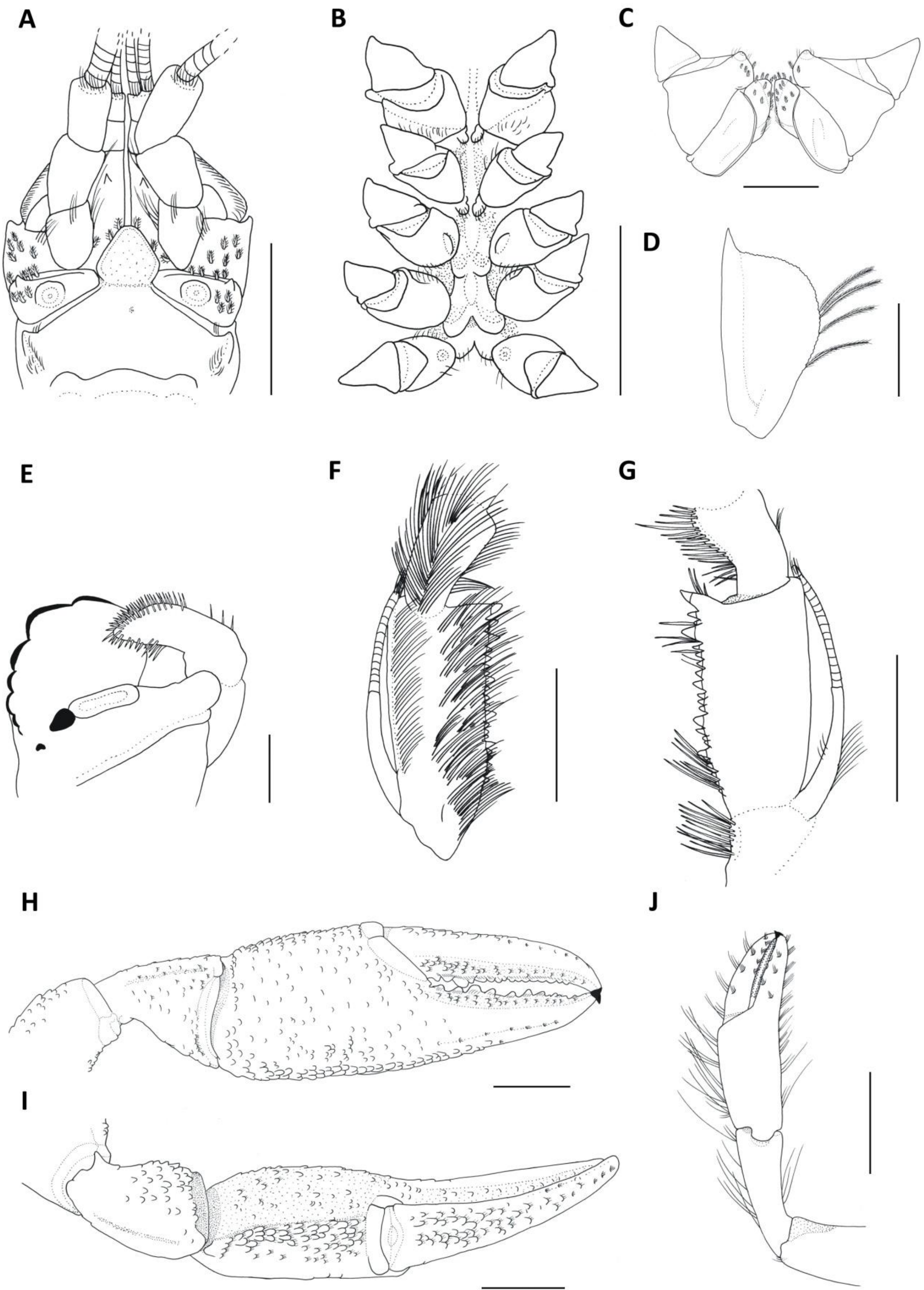

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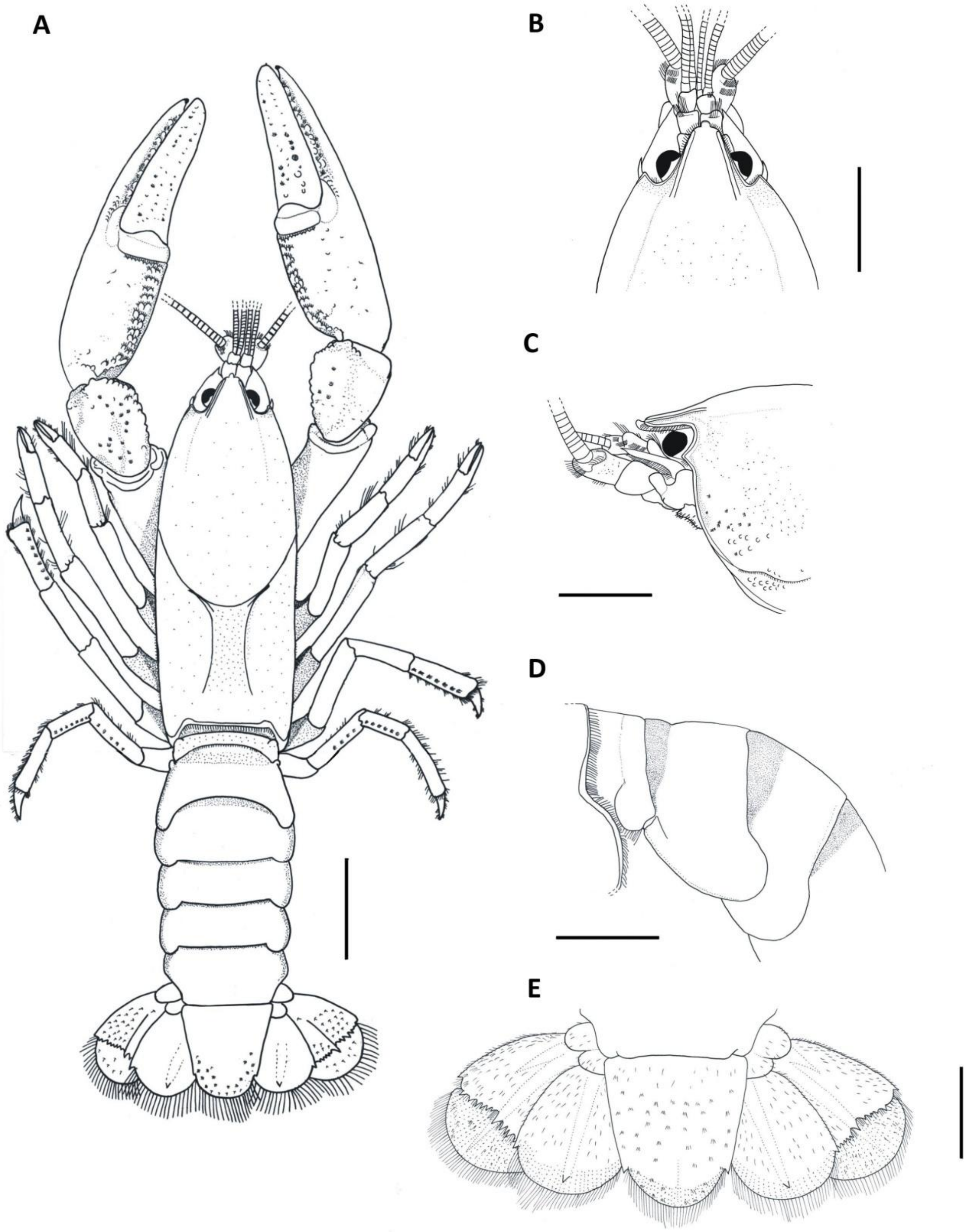


FIGURE 41

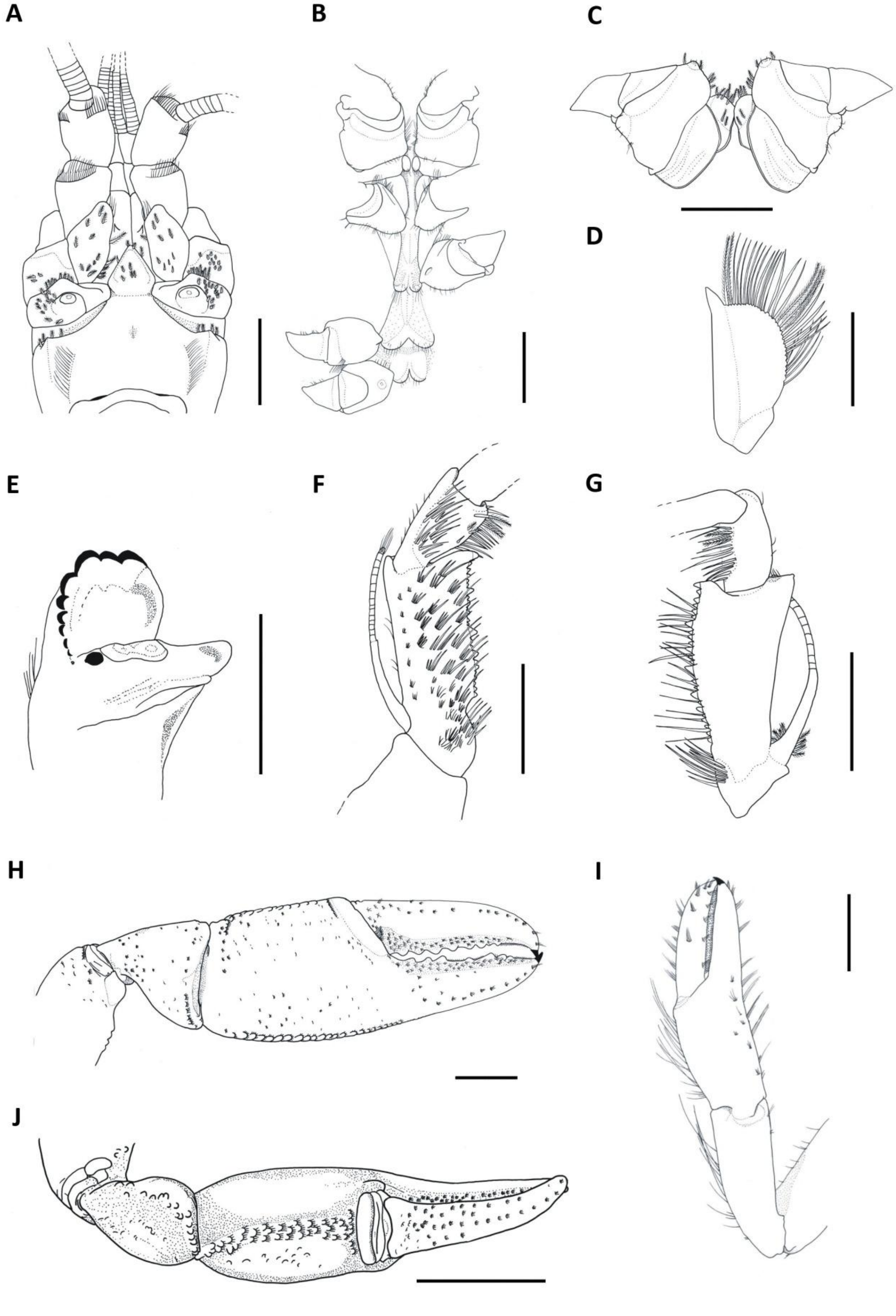


FIGURE 42

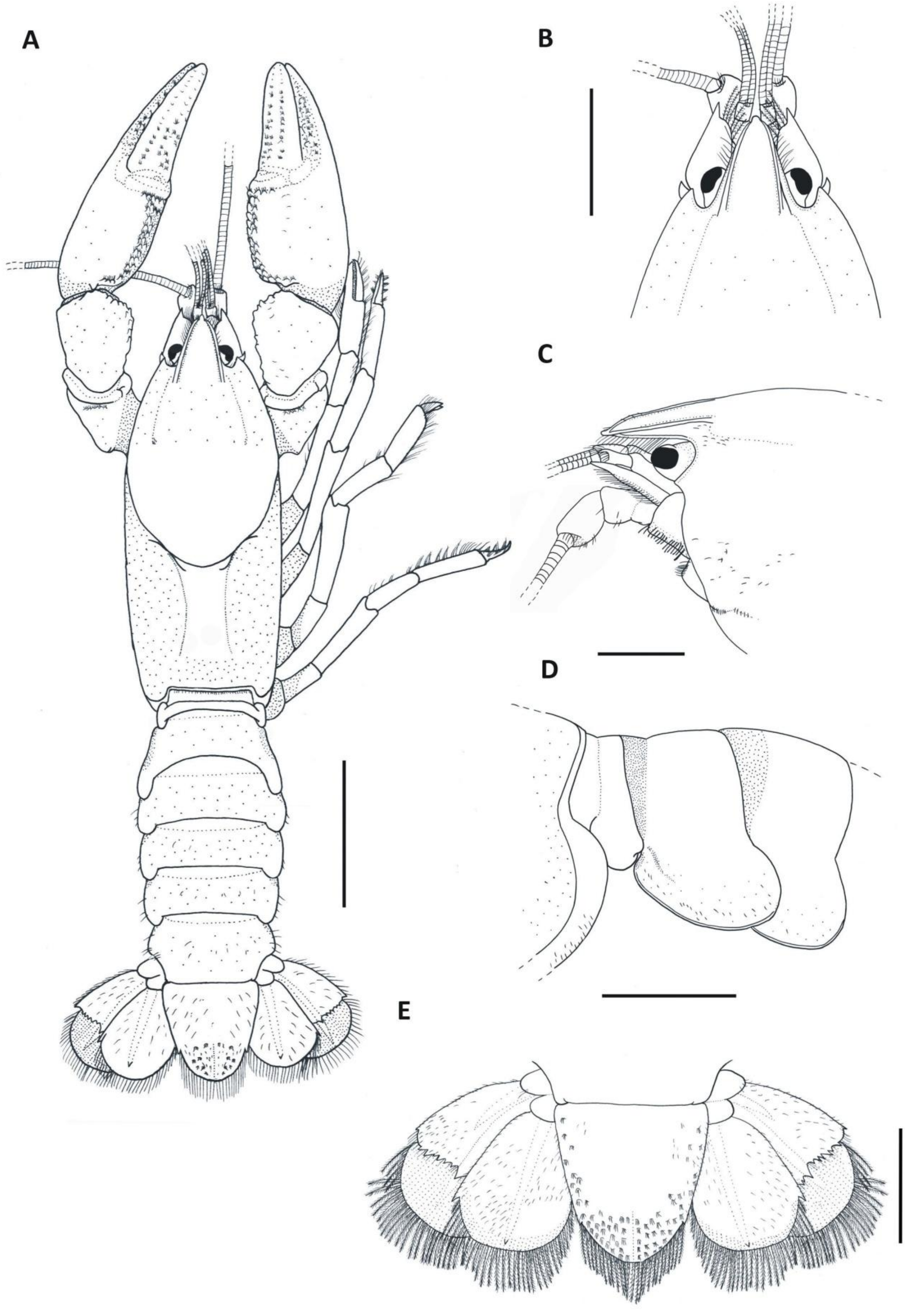


FIGURE 43

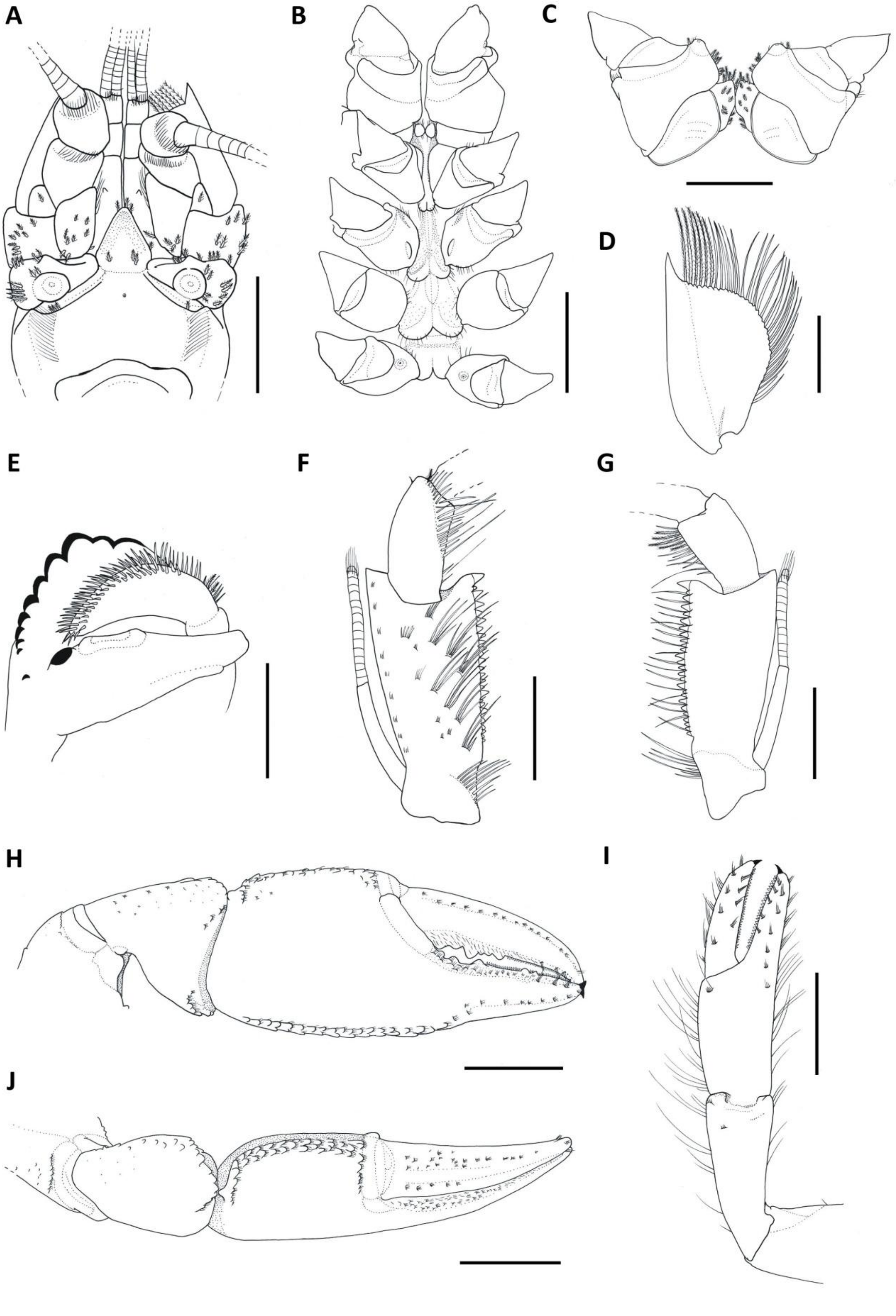


FIGURE 44

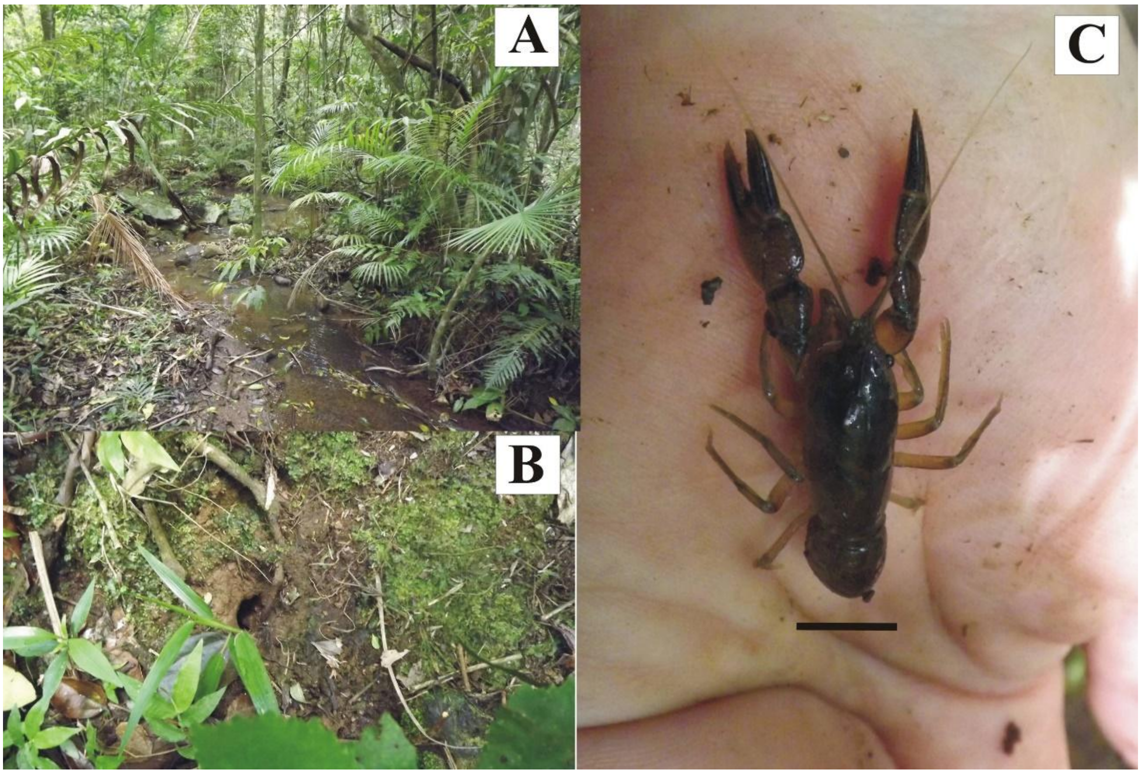


FIGURE 45

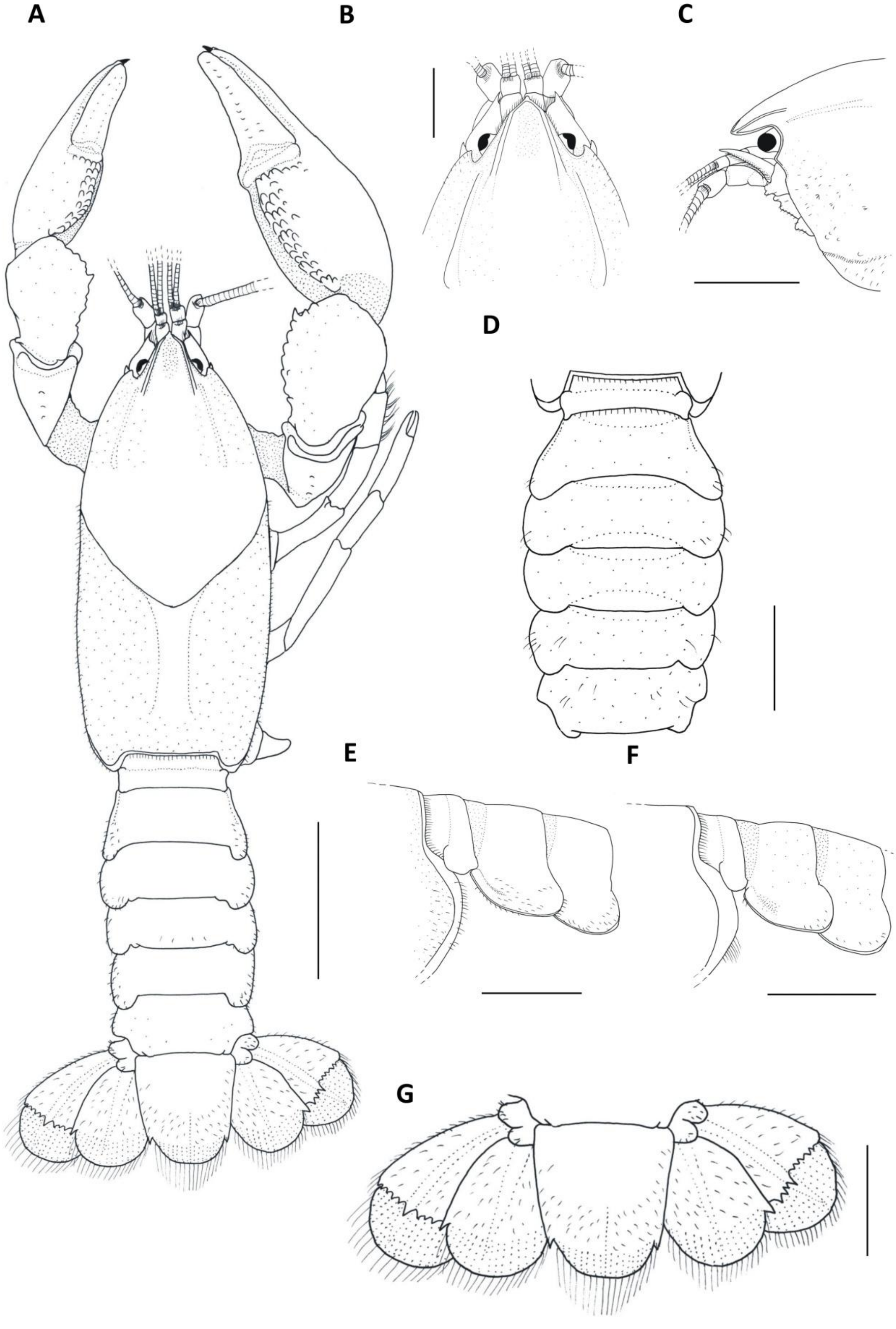


FIGURE 46

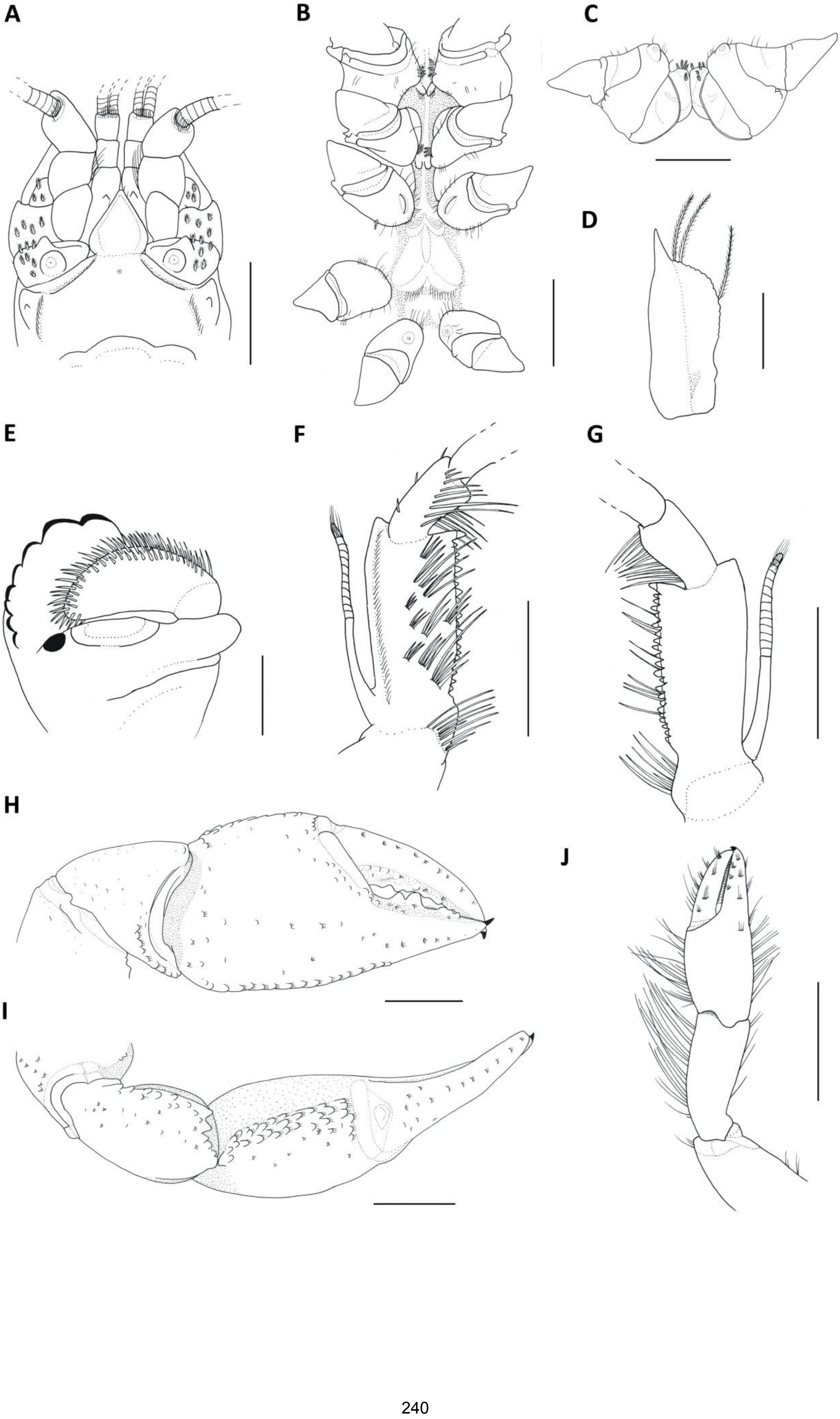


FIGURE 47

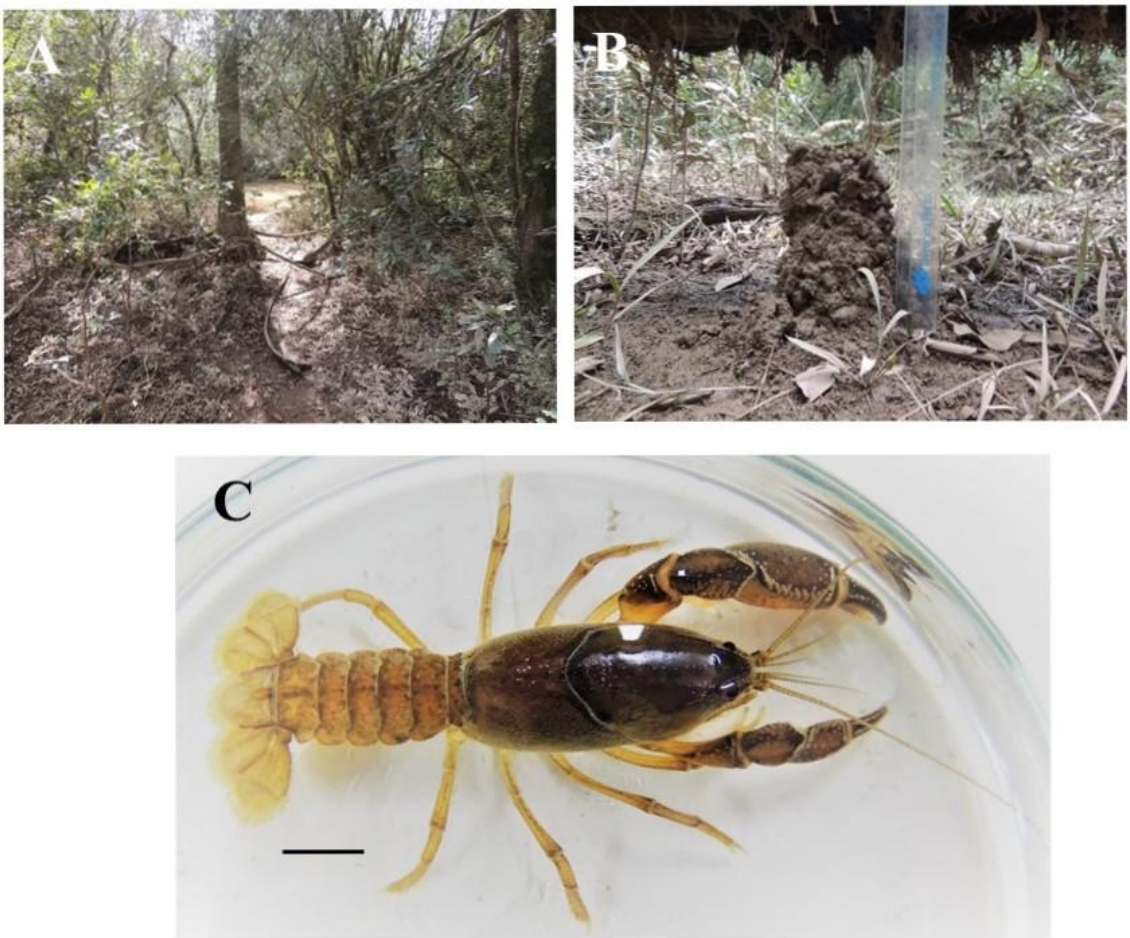


FIGURE 48

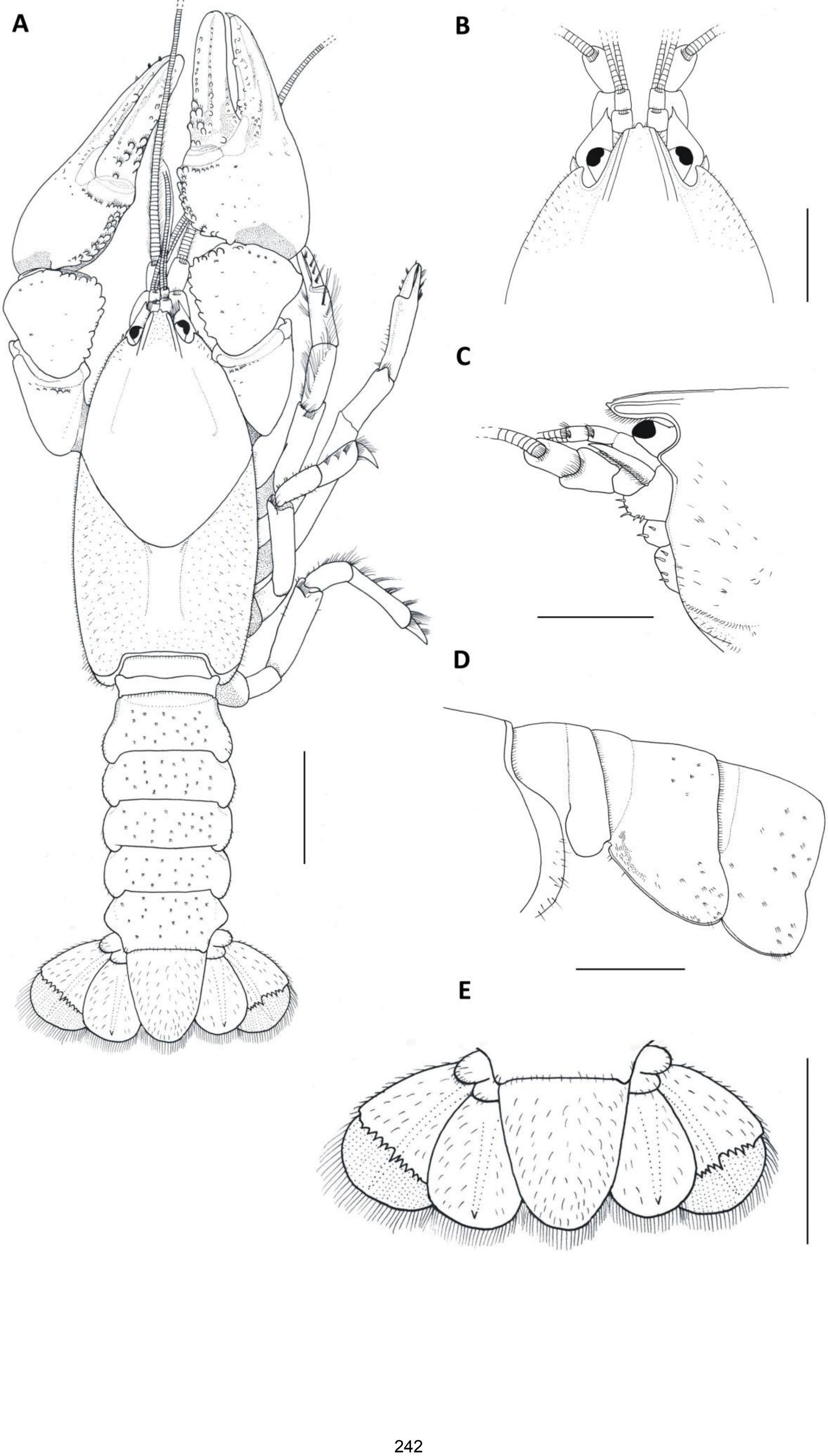


FIGURE 49

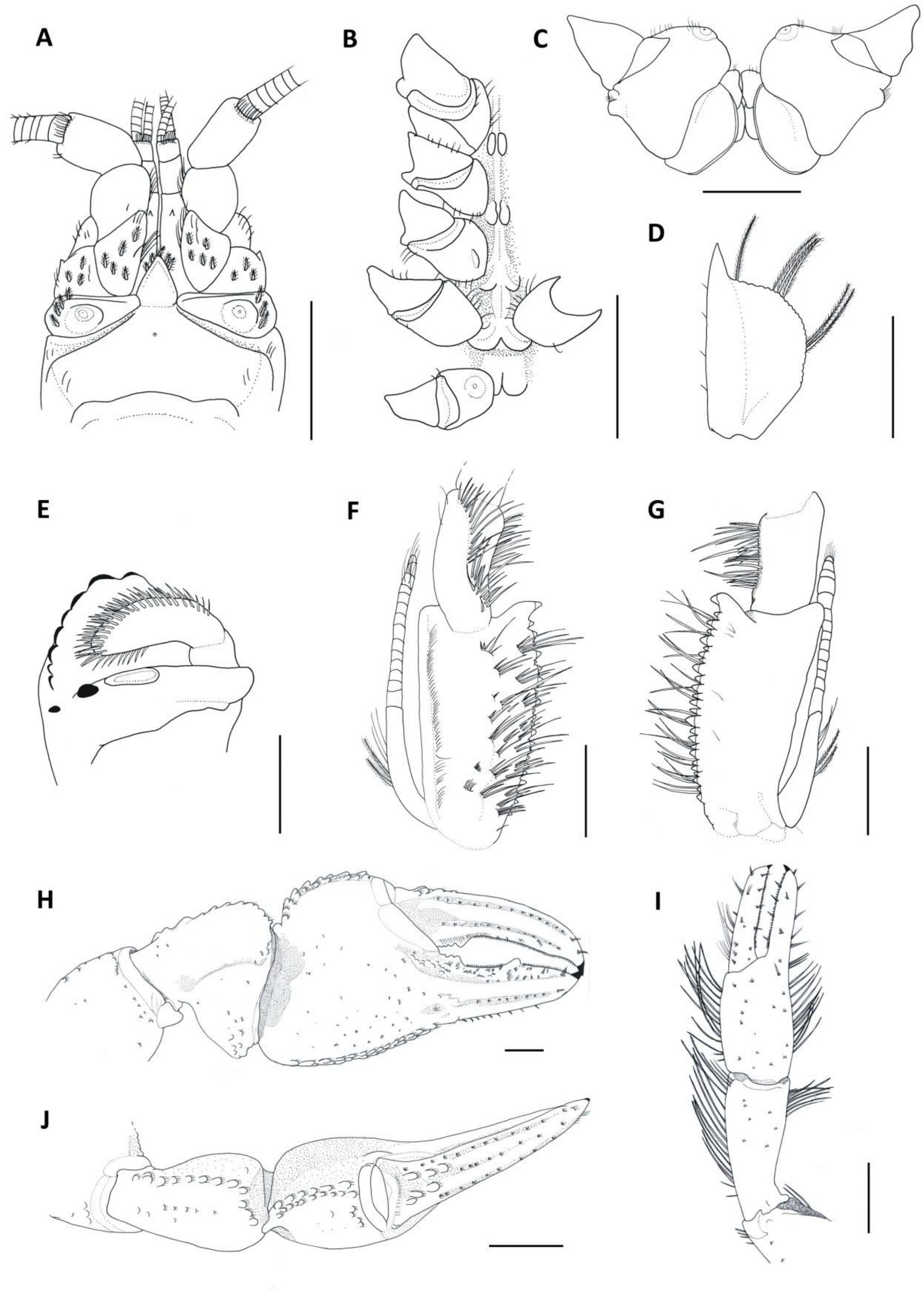


FIGURE 50

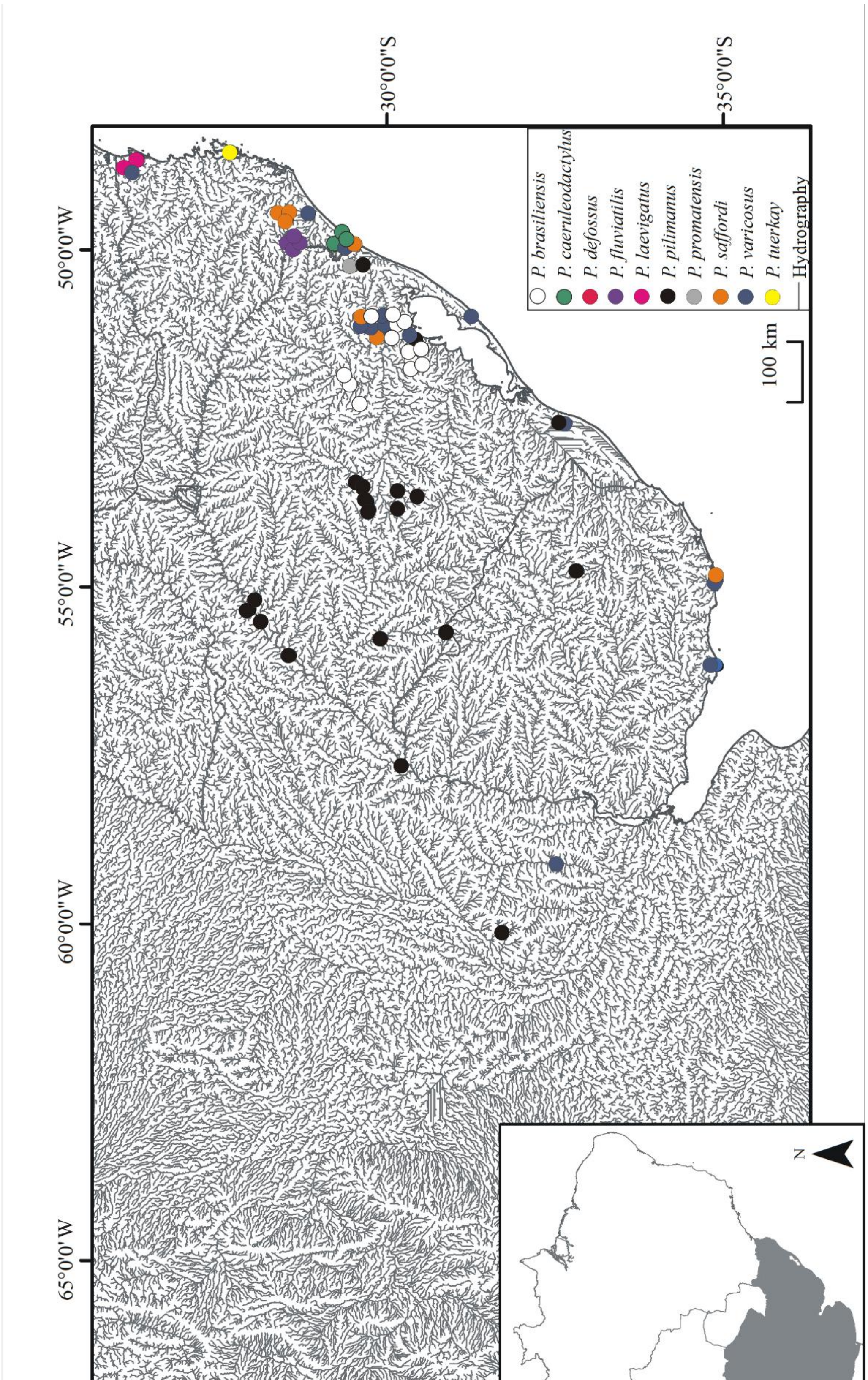
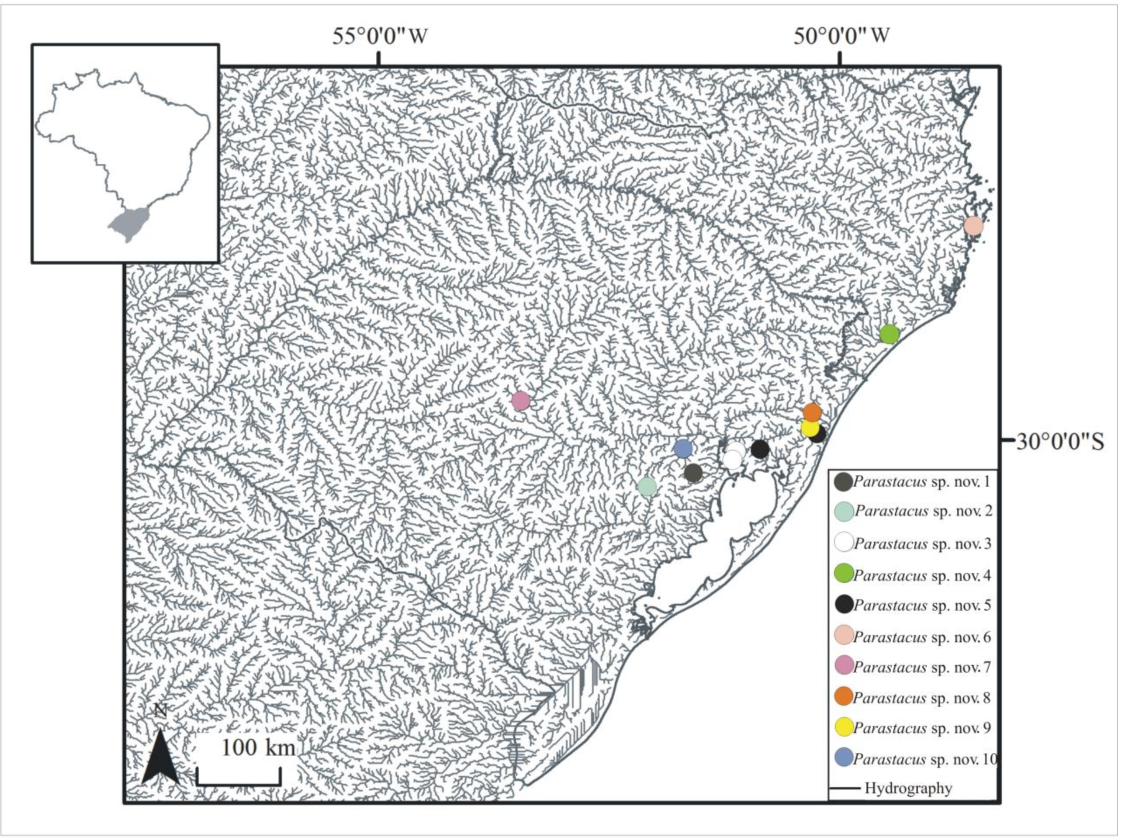
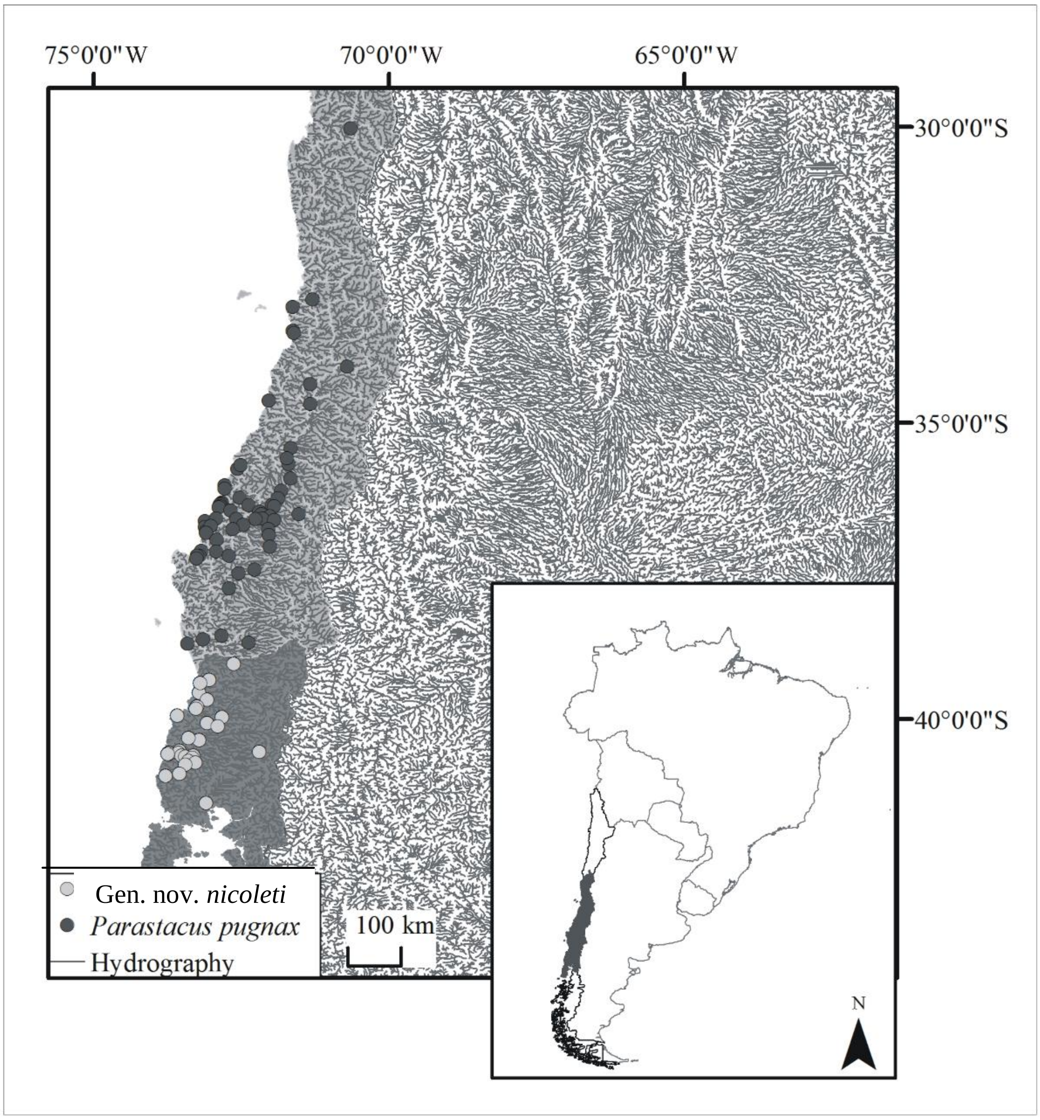

30°0'0"S
35°0'0"S
50°0'0"W
55°0'0"W
60°0'0"W
65°0'0"W
P. brasiliensis
P. caeruleodactylus
P. defossus
P. fluviatilis
P. laevigatus
P. pilimanus
P. promatensis
P. saffordi
P. varicosus
P. tuerkay
Hydrography
100 km
N

FIGURE 51



55°0'0" W
50°0'0" W
30°0'0"S
N
100 km
Parastacus sp. nov. 1
Parastacus sp. nov. 2
Parastacus sp. nov. 3
Parastacus sp. nov. 4
Parastacus sp. nov. 5
Parastacus sp. nov. 6
Parastacus sp. nov. 7
Parastacus sp. nov. 8
Parastacus sp. nov. 9
Parastacus sp. nov. 10
Hydrography

FIGURE 52


75°0'0"W
70°0'0"W
65°0'0"W
30°0'0"S
35°0'0"S
40°0'0"S
Gen. nov. nicoleti
Parastacus pugnax
Hydrography
100 km
N

Supplementary data

Appendix 1. Measurements (mm) of type series of *Parastacus sp. nov. 1* – Broken appendage. For abbreviations see Material and Methods section.

	CRAYFISHES					
	Holotype (MZUSP XXXXX)	Paratype 1 (UFRGS 6414)	Paratype 2 (UFRGS 6414)	Paratype 3 (UFRGS 6414)	Paratype 4 (UFRGS 6410)	Paratype 5 (UFRGS 6410)
Sex	M	F	M	M	F	F
TL	42.71	44.28	44.63	33.93	35.82	42.30
CL	22.22	21.75	22.34	17.37	18.17	21.78
CW	10.27	8.45	9.62	7.81	8.13	9.39
CD	12.74	13.27	13.08	10.06	8.99	11.67
CEL	14.67	14.39	14.99	11.44	1.84	13.86
RL	2.46	1.96	2.29	1.88	1.92	2.60
RW	3.03	2.99	2.26	2.19	2.37	2.92
CMW	1.08	0.90	1.00	1.03	0.94	0.95
OW	1.54	2.46	1.24	1.23	1.21	1.43
FW	4.04	3.55	3.49	3.46	3.07	3.47
RCL	2.96	3.26	3.04	2.32	2.51	2.77
POCL	4.49	4.07	4.37	3.63	3.29	4.35
ASL	2.12	2.18	1.99	1.96	1.80	1.94
ASW	1.09	0.96	1.05	0.86	0.90	0.87
AuL	7.47	6.00	6.11	4.19	5.06	6.18
AuW	1.72	1.80	1.85	1.80	1.70	1.88
AW	6.64	7.89	7.71	5.37	5.78	6.87
AL	15.73	16.22	16.31	16.13	13.68	16.54
RPrT	5.25	2.72	3.92	2.93	3.18	3.45
RPrL	15.77	8.89	12.70	9.93	10.02	11.87
RPrW	9.12	4.74	7.13	5.47	5.79	6.62
RML	9.82	7.61	8.34	7.12	6.89	8.79
RDL	9.26	7.59	8.11	6.09	6.07	7.26
LPrT	5.07	3.96	4.00	3.02	2.323	4.33
LPrL	15.86	13.50	12.79	9.70	8.11	13.47
LPrW	9.14	7.39	7.31	5.58	4.07	8.24
LML	8.85	8.49	8.86	6.52	6.30	8.76
LDL	9.94	8.16	8.14	6.23	5.51	8.42
TeL	6.33	6.53	6.38	4.83	5.28	6.11
TeW	5.01	5.72	5.52	4.16	4.66	5.44

Appendix 2. Measurements (mm) of type series of *Parastacus brevirostris* **sp. nov.** Ribeiro & Araujo **sp. nov.** – Broken appendage. For abbreviations see Material and Methods section.

CRAYFISHES			
	Holotype (MZUSP XXXXX)	Paratype 1 (UFRGS 5497)	Paratype 2 (UFRGS 5493)
Sex	M	M	M
TL	42.33	35.09	21.88
CL	19.69	17.74	11.41
CW	9.59	7.73	4.95
CD	10.74	9.62	6.32
CEL	13.63	12.38	7.95
RL	2.38	2.40	1.81
RW	3.17	2.94	2.00
CMW	1.15	0.95	0.70
OW	1.54	1.43	0.94
FW	4.01	3.62	2.49
RCL	3.71	3.32	2.00
POCL	4.19	4.03	2.12
ASL	2.33	2.27	1.48
ASW	1.33	0.94	0.75
AuL	6.28	4.73	3.32
AuW	1.81	2.01	1.58
AW	7.70	7.34	4.23
AL	17.81	14.34	9.96
RPrT	3.11	2.82	1.62
RPrL	11.03	9.93	5.61
RPrW	5.22	4.53	2.61
RML	7.60	6.99	4.20
RDL	7.05	5.74	3.33
LPrT	3.17	2.73	1.57
LPrL	11.14	10.32	5.79
LPrW	5.25	4.68	2.62
LML	7.00	6.40	4.37
LDL	6.90	5.64	3.55
TeL	6.40	5.08	3.26
TeW	5.71	4.64	2.88

Appendix 3. Measurements (mm) of type series of *Parastacus crandalli* **sp. nov**. – Broken appendage. For abbreviations see Material and Methods section.

	CRAYFISHES									
	Holotype (MZUSP XXXXX)	Paratype 1 (UFRGS 5870)	Paratype 2 (UFRGS 5887)	Paratype 3 (UFRGS 5859)	Paratype 4 (UFRGS #419)	Paratype 5 (UFRGS 6351)	Paratype 6 (UFRGS 6353)	Paratype 7 (UFRGS 6352)	Paratype 8 (UFRGS 6343)	Paratype 9 (UFRGS 6341)
Sex	M	F	M	F	M	M	F	M	M	M
TL	59.83	53.60	43.59	47.27	52.90	57.48	57.07	55.34	56.13	44.31
CL	29.30	27.20	21.48	23,43	27.83	28.59	28.14	28.24	28.42	24.35
CW	13.65	12.30	9.57	10.67	12.52	12.37	12.95	13.05	13.50	11.12
CD	15.37	15.49	11.62	13.45	16.67	16.34	16.78	16.76	16.61	14.27
CEL	18.79	18.42	14.33	15.59	18.66	18.76	18.53	18.53	19.08	16.32
RL	2.65	3.75	2.90	3.00	3.68	3.29	3.42	3.70	3.76	3.17
RW	3.96	4.27	3.72	3.60	4.19	4.53	4.59	4.31	4.29	4.01
CMW	1.32	1.21	1.12	1.19	1.15	1.27	1.29	1.26	1.14	1.06
OW	2.30	1.77	1.48	1.61	1.60	1.71	1.69	1.76	1.48	1.65
FW	5.72	5.06	3.47	4.48	4.23	5.52	5.05	5.37	4.52	4.55
RCL	5.10	4.07	3.15	3.95	4.53	4.80	5.27	4.39	5.19	3.92
POCL	7.98	6.30	4.67	5.05	5.28	6.58	6.14	6.59	5.94	5.02
ASL	2.96	2.63	2.09	2.49	2.83	2.69	2.97	3.01	3.11	2.43
ASW	1.24	1.08	1.03	0.99	1.16	1.30	1.36	1.27	1.25	1.10
AuL	9.19	6.45	5.86	5.72	6.61	6.78	7.41	7.66	8.02	5.40
AuW	2.50	2.11	1.92	1.54	2.67	3.00	2.17	2.52	3.20	2.60
AW	10.36	9.38	7.24	8.28	9.28	10.65	10.72	10.11	9.88	8.61
AL	22.57	21.19	17.48	18.56	19.70	22.23	18.57	20.66	22.20	16.86
RPrT	5.64	4.02	3.12	3.61	5.58	5.84	4.38	5.34	5.77	5.14
RPrL	10.10	14.71	10.99	11.89	17.41	18.77	18.88	17.28	19.02	16.20
RPrW	9.82	7.49	5.40	6.17	9.00	10.46	8.11	9.03	9.55	8.76
RML	13.41	11.03	7.90	9.54	1.96	12.97	11.78	12.19	12.88	10.80
RDL	11.25	8.20	7.15	7.75	9.83	11.46	9.79	10.34	11.21	9.98
LPrT	5.27	4.06	3.07	3.63	5.36	5.83	4.52	5.29	5.64	4.84
LPrL	18.71	14.70	10.69	12.27	17.35	18.22	15.73	17.28	18.73	16.00
LPrW	9.82	7.52	5.45	6.26	9.06	10.32	8.17	8.91	9.36	8.43
LML	12.84	10.91	8.04	9.58	12.56	13.23	11.69	12.37	13.02	10.65
LDL	10.85	9.37	6.57	7.75	9.67	11.27	9.25	10.41	10.84	9.49
TeL	8.86	7.43	6.34	7.18	8.00	7.99	8.44	7.87	7.95	7.43
TeW	6.88	5.58	5.20	5.74	6.29	6.32	6.76	6.33	6.61	5.29

Appendix 4. Measurements (mm) of type series of *Parastacus fossorius* **sp. nov**. – Broken appendage. For abbreviations see Material and Methods section.

	CRAYFISHES	
	Holotype (MZUSP XXXXX)	Paratype 1 (UFRGS 5856)
Sex	M	M
TL	43.03	36.08
CL	21.26	18.18
CW	9.55	7.82
CD	12.64	10.16
CEL	14.33	11.96
RL	2.76	1.91
RW	2.47	2.50
CMW	1.04	0.79
OW	1.58	0.95
FW	3.64	2.85
RCL	3.42	2.39
POCL	5.26	4.81
ASL	2.43	0.79
ASW	1.10	1.65
AuL	5.78	3.58
AuW	2.22	1.52
AW	7.92	6.43
AL	16.51	13.76
RPrT	4.64	2.80
RPrL	14.76	10.26
RPrW	7.93	4.95
RML	10.03	7.11
RDL	8.88	6.49
LPrT	3.71	2.50
LPrL	12.81	10.33
LPrW	6.17	4.96
LML	12.51	6.96
LDL	8.48	6.30
TeL	6.14	5.52
TeW	5.53	4.30

Appendix 5. Measurements (mm) of type series of *Parastacus* **sp. nov**. 5 – Broken appendage. For abbreviations see Material and Methods section.

	CRAYFISHES					
	Holotype (MZUSP XXXXX)	Paratype 1 (UFRGS 5865)	Paratype 2 (UFRGS 5865)	Paratype 3 (UFRGS 6439)	Paratype 4 (UFRGS 6439)	Paratype 5 (UFRGS 1359)
Sex	M	M	M	M	F	M
TL	45.07	40.96	24.78	22.68	45.44	50.78
CL	22.39	20.73	12.29	10.77	22.43	24.98
CW	10.25	9.34	5.29	4.65	9.73	10.84
CD	12.95	12.09	6.60	6.19	11.87	13.45
CEL	15.17	13.04	8.20	7.24	14.97	17.27
RL	2.49	2.17	1.34	1.39	2.36	2.64
RW	2.74	2.61	1.69	1.69	2.99	2.72
CMW	0.89	0.81	0.42	0.46	0.88	1.10
OW	1.15	1.40	0.91	0.68	1.24	1.94
FW	3.43	3.47	1.95	1.79	3.31	4.72
RCL	2.86	3.00	1.58	1.90	2.76	2.74
POCL	4.79	4.84	3.69	2.38	4.66	5.82
ASL	2.19	1.95	1.25	1.36	2.48	2.70
ASW	0.92	0.77	0.64	0.46	0.80	1.14
AuL	5.70	5.54	3.01	2.85	5.56	5.43
AuW	2.82	1.99	1.11	1.25	1.70	2.26
AW	8.30	8.00	4.40	4.23	8.66	9.45
AL	17.64	15.75	9.78	9.20	17.49	20.08
RPrT	3.28	2.11	2.37	-	-	3.19
RPrL	13.74	10.37	7.62	-	-	13.91
RPrW	6.66	3.10	3.46	-	-	5.84
RML	9.77	6.88	4.82	-	-	9.78
RDL	8.89	6.89	4.40	-	-	9.33
LPrT	6.56	4.30	-	1.52	3.05	6.45
LPrL	20.98	14.43	-	6.49	13.23	24.88
LPrW	1.36	8.05	-	2.81	6.14	11.80
LML	10.13	8.67	-	4.56	8.98	11.74
LDL	11.78	7.94	-	3.32	8.47	13.59
TeL	6.81	6.02	4.24	3.26	6.93	8.08
TeW	5.61	4.87	3.10	2.83	5.31	5.77

Appendix 6. Measurements (mm) of type series of *Parastacus* **sp. nov. 6** – Broken appendage. For abbreviations see Material and Methods section.

	CRAYFISHES					
	Holotype (MZUSP XXXXX)	Paratype 1 (UFRGS #6489)	Paratype 2 (UFRGS #6490)	Paratype 3 (UFRGS #6491)	Paratype 4 (UFRGS #6492)	Paratype 5 (UFRGS #6493)
Sex	M	F	F	F	F	M
TL	58.48	65.02	52.89	56.63	60.12	67.13
CL	29.36	31.08	25.04	26.59	28.76	33.35
CW	12.81	14.00	11.02	11.76	11.77	15.23
CD	16.08	16.87	14.13	14.84	14.31	20.17
CEL	18.55	21.03	16.59	17.23	18.02	22.14
RL	3.90	3.65	2.77	3.12	3.82	3.56
RW	3.39	2.72	2.28	2.56	2.71	2.96
CMW	1.34	1.40	1.16	1.18	1.08	1.54
OW	1.88	2.14	1.86	1.96	2.06	2.09
FW	5.70	5.26	3.96	4.10	4.16	5.57
RCL	4.68	4.07	3.01	4.37	3.42	4.36
POCL	6.63	5.26	5.28	5.28	6.23	7.66
ASL	3.27	3.33	2.50	2.95	2.99	3.35
ASW	1.50	1.65	1.30	1.42	1.40	1.71
AuL	8.87	8.78	6.33	7.25	7.42	8.89
AuW	3.38	4.87	3.05	3.17	2.90	3.20
AW	12.16	13.63	10.32	10.73	10.78	13.03
AL	23.99	26.09	20.92	22.98	23.32	25.42
RPrT	5.35	5.69	4.51	5.06	4.41	3.26
RPrL	24.15	23.38	17.40	18.23	18.19	13.44
RPrW	11.01	11.34	8.62	9.59	9.04	4.92
RML	12.98	14.00	10.86	11.09	12.09	10.89
RDL	14.17	14.00	10.38	10.87	11.24	8.70
LPrT	5.35	4.33	4.12	5.10	4.14	8.67
LPrL	23.87	19.60	15.67	18.58	18.28	32.92
LPrW	10.99	8.73	8.28	9.79	8.88	16.03
LML	13.08	13.41	11.09	11.29	11.82	17.75
LDL	13.34	12.60	9.99	11.06	10.80	19.91
TeL	8.31	9.37	7.78	8.40	9.28	9.14
TeW	7.01	8.28	6.52	6.79	6.90	7.75

Appendix 7. Measurements (mm) of type series of *Parastacus* **sp. nov**. 7. – Broken appendage. For abbreviations see Material and Methods section.

CRAYFISHES								
	Holotype (MZUSP XXXXX)	Paratype 1 (UFRGS 6484)	Paratype 2 (UFRGS 6484)	Paratype 3 (UFRGS 6489)	Paratype 4 (UFRGS 5793)	Paratype 5 (UFRGS 5778)	Paratype 6 (UFRGS 5795)	Paratype 7 (UFRGS 5794)
Sex	M	F	F	M	M	M	M	F
TL	61.97	83.77	58.22	51.85	60.63	60.51	69.88	77.86
CL	30.57	40.55	25.04	25.40	30.47	29.28	74.94	39.00
CW	12.17	16.50	11.28	11.37	12.36	12.26	15.39	17.13
CD	15.95	19.18	14.08	12.47	16.34	15.02	18.58	21.50
CEL	20.76	28.32	19.83	17.00	20.24	20.18	24.26	26.58
RL	4.60	5.53	3.97	3.61	4.47	4.40	5.02	5.70
RW	4.33	4.75	3.25	2.99	3.34	3.28	3.82	9.08
CMW	1.41	1.94	1.42	1.19	1.19	1.43	1.62	1.72
OW	2.18	2.63	1.86	1.80	2.03	2.06	2.51	2.45
FW	6.45	7.19	5.34	4.02	4.56	4.88	6.50	8.18
RCL	6.72	7.89	5.74	4.81	6.20	6.57	7.41	7.81
POCL	6.65	10.22	6.81	5.28	6.23	7.36	8.81	8.08
ASL	4.39	5.35	3.89	3.58	3.92	3.90	5.38	5.05
ASW	1.81	6.66	1.93	1.68	2.00	2.02	2.45	2.54
AuL	7.67	9.42	7.49	5.65	7.56	7.36	9.12	8.80
AuW	6.32	4.68	3.83	3.14	3.72	4.31	4.18	4.77
AW	11.68	16.00	1.30	9.75	12.17	1.34	12.84	15.78
AL	24.56	32.99	23.80	15.65	23.51	24.19	26.10	18.94
RPrT	6.45	4.75	5.50	6.21	3.39	3.09	9.10	6.82
RPrL	25.52	27.40	22.67	21.18	19.35	15.53	35.52	29.76
RPrW	9.73	9.37	8.96	9.71	6.70	5.63	13.50	10.63
RML	12.31	18.26	12.42	11.41	12.76	1.56	18.17	18.81
RDL	14.68	16.09	13.57	11.03	11.98	9.24	19.57	17.71
LPrT	4.21	8.18	3.04	4.33	6.03	5.60	5.47	6.33
LPrL	20.74	33.29	16.20	17.76	24.47	22.39	29.66	30.52
LPrW	7.49	13.53	5.86	7.12	9.92	9.07	5.95	10.89
LML	12.99	18.72	11.38	10.99	13.49	12.90	18.31	18.74
LDL	14.58	19.01	10.62	10.45	13.90	11.46	17.53	17.70
TeL	8.98	12.30	8.00	7.06	8.87	8.90	9.73	11.61
TeW	7.67	9.42	6.95	5.88	7.35	7.03	8.09	9.03

Appendix 8. Measurements (mm) of type series of *Parastacus* **sp. nov**. 8. – Broken appendage. For abbreviations see Material and Methods section.

	CRAYFISHES					
	Holotype (MZUSP XXXXX)	Paratype 1 (UFRGS 6440)	Paratype 2 (UFRGS 6440)	Paratype 3 (UFRGS 6440)	Paratype4 (UFRGS 6440)	Paratype5 (UFRGS 6440)
Sex	M	M	M	M	JUV	JUV
TL	59.29	66.29	51.13	51.63	37.92	33.88
CL	28.86	31.80	26.02	26.61	17.98	16.70
CW	10.87	12.97	9.49	9.58	5.83	5.88
CD	15.89	16.89	13.23	13.54	8.92	8.60
CEL	18.94	20.91	16.81	17.37	11.88	11.10
RL	3.44	3.36	3.31	3.57	2.21	2.14
RW	3.75	4.27	3.53	3.79	2.58	2.60
CMW	1.34	1.53	1.25	1.31	0.99	0.89
OW	2.24	2.27	1.62	1.72	1.15	1.18
FW	5.65	5.30	3.87	4.10	2.89	2.82
RCL	3.57	4.40	3.61	3.54	2.73	2.60
POCL	5.50	7.76	6.24	4.92	3.71	3.50
ASL	3.69	3.87	3.24	3.10	2.31	2.24
ASW	1.71	1.81	1.42	1.37	1.02	0.86
AuL	7.22	9.49	7.00	8.01	5.03	4.27
AuW	2.82	3.98	4.12	3.89	2.65	2.67
AW	10.81	11.78	9.94	9.64	6.26	6.01
AL	24.36	27.28	19.62	20.60	15.80	14.00
RPrT	6.15	8.88	5.95	-	-	3.50
RPrL	24.84	31.60	20.29	-	-	12.44
RPrW	10.40	13.81	5.77	-	-	5.40
RML	13.92	15.27	10.88	-	-	7.25
RDL	14.82	16.61	10.48	-	-	6.36
LPrT	6.23	5.36	5.58	-	3.47	-
LPrL	24.89	23.87	20.43	-	12.46	-
LPrW	10.68	9.52	9.80	-	6.00	-
LML	12.71	14.39	10.88	-	7.74	-
LDL	14.39	14.82	11.48	-	7.17	-
TeL	8.66	9.19	7.65	7.47	5.30	5.16
TeW	7.44	7.88	6.16	6.42	4.45	4.22

Appendix 9. Measurements (mm) of type series of *Parastacus* **sp. nov**. 9. – Broken appendage. For abbreviations see Material and Methods section.

CRAYFISHES			
	Holotype (MZUSP XXXXX)	Paratype 1 (UFRGS #6476)	Paratype 2 (UFRGS #6476)
Sex	M	M	M
TL	51.14	53.86	50.89
CL	25.31	26.01	25.07
CW	9.25	10.19	9.84
CD	13.63	14.33	12.81
CEL	16.23	17.63	16.81
RL	3.78	3.70	3.77
RW	2.97	3.70	3.25
CMW	1.34	1.37	1.09
OW	1.84	1.80	1.90
FW	4.50	4.29	4.09
RCL	4.09	3.94	3.91
POCL	4.03	5.73	5.84
ASL	3.27	2.82	3.26
ASW	1.30	1.68	1.70
AuL	6.34	6.74	6.54
AuW	2.54	2.60	3.26
AW	9.53	10.30	9.98
AL	17.30	21.74	19.96
RPrT	4.67	6.40	4.17
RPrL	18.03	20.52	16.67
RPrW	3.65	10.61	7.66
RML	11.28	11.20	9.90
RDL	10.67	11.51	9.86
LPrT	4.63	3.78	5.02
LPrL	17.94	15.90	18.48
LPrW	8.11	6.83	8.88
LML	10.79	10.09	10.01
LDL	12.01	9.58	10.11
TeL	7.81	7.51	7.47
TeW	6.24	6.66	6.46

Appendix 10. Measurements (mm) of type series of *Parastacus* **sp. nov**. 10. – Broken appendage. For abbreviations see Material and Methods section.

CRAYFISHES					
		Holotype (MZUSP XXXXX)	Paratype 1 (UFRGS #5755)	Paratype 2 (UFRGS #5755)	Paratype 3 (UFRGS #5755)
Sex	Sex	M	F	M	F
TL	TL	50.83	45.33	44.97	44.57
CL	CL	27.20	21.92	22.89	22.10
CW	CW	12.41	9.52	9.73	9.98
CD	CD	15.42	11.98	12.47	11.59
CEL	CEL	16.99	14.45	15.54	14.56
RL	RL	2.98	2.44	2.64	2.53
RW	RW	3.59	2.56	2.45	2.56
CMW	CMW	1.12	0.91	0.74	1.05
OW	OW	1.57	1.27	1.16	1.44
FW	FW	4.65	3.27	3.27	3.46
RCL	RCL	5.49	2.73	3.13	2.80
POCL	POCL	6.22	4.57	5.24	5.26
ASL	ASL	3.13	1.90	1.97	1.99
ASW	ASW	1.04	0.88	0.93	2.94
AuL	AuL	7.19	6.61	6.42	7.55
AuW	AuW	2.84	2.06	3.26	2.55
AW	AW	8.97	7.88	7.21	8.55
AL	AL	19.07	17.56	16.51	17.74
RPrT	RPrT	6.47	2.08	4.86	4.07
RPrL	RPrL	19.66	9.37	16.84	13.98
RPrW	RPrW	11.09	3.48	8.21	7.37
RML	RML	11.57	7.08	10.11	9.59
RDL	RDL	12.05	6.20	10.36	8.32
LPrT	LPrT	3.83	3.61	5.00	3.69
LPrL	LPrL	13.73	13.88	17.05	13.94
LPrW	LPrW	6.74	6.67	8.28	7.30
LML	LML	10.88	9.29	10.39	9.40
LDL	LDL	13.96	8.67	10.58	8.92
TeL	TeL	5.99	7.00	6.89	6.24
TeW	TeW	6.16	5.35	5.04	5.20

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- Aim and scope
- Research article
- Correspondence
- Special issues with collected papers (e.g. Festschrift)
- Preparation of manuscripts
- Submission of manuscripts
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Author (2002) Title of website, database or other resources, Publisher name and location (if indicated), number of pages (if known). Available from: http://xxx.xxx.xxx/ (Date of access).

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Considerações Finais

Esta tese contribuiu para a geração de conhecimento acerca da diversidade e evolução dos lagostins de água doce da América do Sul, especialmente o gênero *Parastacus*. Especificamente, foi provida evidência para a remoção de uma espécie (*P. nicoleti*) do gênero *Parastacus*, com a subsequente criação de um novo gênero monotípico para alocar essa espécie, além da descrição de 13 espécies novas para o grupo. Essas novas entidades configuram um aumento de mais de 150% da riqueza específica para um grupo até então bastante subestimado.

A extensiva experiência em campo e no laboratório de biologia molecular, além do estágio sanduíche no exterior, configuraram importantes passos para o amadurecimento de minha carreira como cientista. A utilização de novas ferramentas para a realização de estudos taxonômicos trazem reais ganhos para os trabalhos. Para os estudos taxonômicos com lagostins, a inclusão de uma análise de distribuição, aliada à análise do estado de conservação e análise molecular, foi essencial para o enriquecimento do trabalho.

Os dados taxonômicos e moleculares gerados nesta tese servirão como base para futuros estudos de cunho filogenéticos e biogeográficos, além de contribuir para o conhecimento da diversidade e futuras atividades de manejo e conservação das espécies.