KELLY MARTINEZ GOMES

Os lagostins de água doce da América do Sul (Crustacea: Decapoda: Parastacidae): modelagem de distribuição e diversidade genética

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: Biodiversidade

Orientadora: Profa. Dra. Paula Beatriz Araujo

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Dra. Daiana Silva Castiglioni

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Dr. Márcio Borges Martins

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Dr. Leonardo Maltchik Garcia.

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“Words are, in my not-so-humble opinion, our most inexhaustible source of magic. Capable of both inflicting injury, and remedying it. ”

Albus Dumbledore

SUMÁRIO

RESUMO

Os lagostins de água doce são crustáceos encontrados em várias regiões do mundo, com a ocorrência das famílias Astacidae e Cambaridae no hemisfério norte e Parastacidae no hemisfério sul. Os parastacídeos estão distribuídos em quase todos continentes, com a exceção da Antártida e o continente africano. Na América do Sul são conhecidas 16 espécies, pertencentes a três gêneros: *Virilastacus* Hobbs 1991, *Samastacus* Riek 1971 e *Parastacus* Huxley 1879, que podem ocupar ambientes permanentes e temporários. Embora existam informações sobre riqueza de espécies, distribuição e biologia, os dados são insuficientes. Assim, o primeiro capítulo traz a atualização dos dados sobre a distribuição e o reconhecimento de registros de espécies simpatria e sintopia para as espécies sulamericanas. A caracterização dos habitats ocupados pelos lagostins foi também realizada, mas somente para as espécies da porção Atlântica do continente. Os dados de ocorrência foram compilados das coleções zoológicas (CZ), da literatura publicada e das amostragens na região sul do Brasil (Rio Grande do Sul - RS e Santa Catarina - SC). No total, foram obtidos 51 novos registros de ocorrência para América do Sul; incluindo a presença de *Parastacus fluviatilis* Ribeiro & Buckup, 2016 no lugar mais alto do RS e a captura de *P. saffordi* Faxon, 1898 quase quatro décadas após a revisão taxonômica do gênero *Parastacus*. A ocorrência de espécies em simpatria e sintopia foi confirmada, destacando o registro inédito de quatro espécies em um buffer < 5 km no Chile. Os dados obtidos neste estudo representam um avanço sobre o conhecimento da distribuição e a ocupação do habitat, podendo ser usados para melhorar a precisão da avaliação dos riscos de extinção, por exemplo.

As ameaças à sobrevivência dos lagostins de água doce na América do Sul são numerosas e estão relacionadas aos impactos diretos na estrutura do hábitat, como poluição, canalização de cursos d’água e drenagem de áreas úmidas. Entretanto, uma potencial e silenciosa ameaça não tem sido considerada, que são as mudanças climáticas ao longo da distribuição das espécies. O segundo capítulo utilizou o conhecimento atual da ocorrência das espécies para projetar os efeitos das mudanças climáticas sobre a distribuição delas. As principais alterações estimadas foram expansão ou redução do tamanho da área geográfica em dois cenários: atual e 2070. Os dados de ocorrência contabilizaram 216 locais de presença que foram reunidos em quatro grupos: aquáticos, semiaquáticos, semiterrestres Atlântico e semiterrestres Pacífico. O desempenho dos modelos foi avaliado de bom a ótimo e a adequabilidade das áreas no cenário atual foi < 100.000 km² para todos os grupos. A temperatura foi a variável que mais contribuiu para a construção dos modelos (presente e futuro). Potencialmente, esta variável foi responsável pela expansão da área de distribuição das espécies em 2070. Assim, os lagostins apresentaram uma tendência à ocupação de regiões mais altas e menos impactadas pelas ações humanas. Portanto, sugerimos que a atenção seja voltada à conservação das espécies dos grupos semiterrestres da porção Atlântico e do Pacífico. As áreas ocupadas pelas espécies apresentaram alta complexidade ambiental e diferentes níveis de fragmentação. Além disso, as zonas úmidas tendem a desaparecer mais rápido, devido às interferências do clima na dinâmica dos ecossistemas de água doce.

Diante das potenciais modificações do hábitat das espécies e a perda recente de áreas dentro da distribuição dos lagostins, a caracterização genética das populações traz uma nova abordagem sobre a fauna e a conservação dos organismos. Dessa forma, o terceiro capítulo investiga a diversidade genética intraespecífica de *Parastacus brasiliensis* (von Martens 1869) no sul do Brasil. A reconstrução filogenética foi utilizada para identificar a diversidade críptica a partir de marcadores mitocondriais e nucleares. As análises indicaram a existência de cinco unidades evolutivas e somente uma pode ser considerada como *Parastacus brasiliensis* sensu stricto. A distância genética entre *P. brasiliensis* sensu stricto e as demais linhagens "A", "B" e "C" aumenta com distância geográfica, sugerindo que o isolamento por distância pode ser um importante fator de diversificação e eventualmente especiação para os lagostins escavadores. Além disso, uma das quatro linhagens corresponde à subespécie *Parastacus brasiliensis promatensis* Fontoura & Conter 2008, que foi elevada para o nível de espécie. De acordo com esta pesquisa, *P. brasiliensis* ocorre principalmente na Bacia do Lago Guaíba. As populações estudadas no Guaíba I e Guaíba II apresentaram conectividade e fluxo gênico limitado, provavelmente devido à fragmentação do habitat. Com base nos dados de distribuição e genética, o estado de conservação de *P. brasiliensis* foi estabelecido como Quase Ameaçada - "Near Threatened", conforme a IUCN. Estes resultados nos encorajam a propor uma área de preservação para a população isolada do Guaíba II e enfatiza a importância de preservar estes distintos "pools" para a manutenção da diversidade genética desta espécie. O conhecimento limitado sobre os lagostins de água doce e os efeitos das ações antrópicas sobre as populações, reforçam a importância da continuidade de investigações desse cunho.

Outras abordagens podem ser conduzidas a partir dos dados gerados nesta tese como ponto de partida, utilizando os lagostins como uma ferramenta de análise da paisagem, mudanças climáticas e urbanização.

Palavras-chave: Parastacídeos, Habitat de água doce, Crustáceos de água doce.

ABSTRACT

Freshwater crayfishes are crustaceans found in several regions of the world, with the occurrence of the Astacidae and Cambaridae families in the northern hemisphere and Parastacidae in the southern hemisphere. Parastacids are distributed on almost all continents except Antartida and the African continent. In South America 16 species belonging to three genera are known: *Virilastacus* Hobbs 1991, *Samastacus* Riek 1971 e *Parastacus* Huxley 1879, which could occupy permanent and temporary environments. Although there is information on species richness, distribution and biology, the data are insufficient. Thus, the first chapter brings an update on the distribution of crayfishes in South America and the recognition of sympatry and sintopy records. The characterization of the occupied habitat by crayfishes was also carried out, but only for the species that occur in Atlantic portion. Occurrence data were compiled from the records of zoological collections (ZC), literature and samplings performed in southern Brazil (states of Rio Grande do Sul - RS and Santa Catarina – SC). In total, 51 new occurrence records were obtained; including the presence of *Parastacus fluviatilis* Ribeiro & Buckup, 2016 at the highest place on RS and the capture of *P. saffordi* Faxon, 1898 almost four decades after the last taxonomic revision of *Parastacus*. Species occurring in sympatry and sintopy were confirmed, highlighting unpublished record of four species within a buffer < 5 km in Chile. Therefore, the data obtained in this study represent an advance on the knowledge of distribution and habitat occupation, which could be used to improve the precision of extinction risk assessments, for example.

The threats to the survival of freshwater crayfish from South America are numerous and closely related to impacts in habitat structure, such as pollution, watercourse canalization and wetland drainage. Nevertheless, a potential and silent threat has not been considered: the climate changes along the species' distribution. The second chapter used the current knowledge on species occurrence to project the effects of climate changes on distribution. The main alterations estimated were the expansion or reduction in the size of geographical ranges in two scenarios: present and 2070. Occurrence data accounted 216 presence sites that were gathered in four groups: aquatic, semi-aquatic, Atlantic semi-terrestrial and Pacific semi-terrestrial. The model performance was evaluated from good to optimal and suitable areas in the present scenario were < 100, 000 km^2 for all groups. Temperature was the variable that most contributed for the building of models (present and future). It was potentially, responsible for the expansion of the species' distribution area in 2070. Thus, crayfishes show a tendency to occupy higher regions that are less disturbed by human actions. Therefore, we suggest that immediate attention must be paid to the conservation of the Atlantic and Pacific semi-terrestrial groups. Areas occupied by these species presented high environmental complexity and different levels of fragmentation. In addition, wetlands tend to disappear faster due to climate influences on the dynamic of freshwater ecosystems.

In the face of potential modifications to the species' habitats and the recent loss of areas within the current crayfish distribution, the genetic characterization of populations brings a new approach to the study of fauna and the conservation of organisms. Thus, the third chapter investigates the intraspecific genetic diversity of *Parastacus brasiliensis* (von Martens 1869) in South America. Phylogenetic reconstructions based on mitochondrial and nuclear markers were used to identify cryptic diversity. Analyses indicated the existence of five evolutionary significant units and only one can be considered as *P. brasiliensis* sensu stricto. Genetic distances among *P. brasiliensis* sensu stricto and the remaining lineages "A", "B" and "C" increase with geographical distances, suggesting isolation by distance as an important driver of diversification and eventual speciation in these burrowing crayfishes. In addition, one of the other four units corresponds to the subspecies *Parastacus brasiliensis promatensis* Fontoura & Conter 2008, which is here elevated to species level. According to this study, *P. brasiliensis* occurs mainly in the Guaíba Lake basin. The studied populations Guaíba I and II show limited connectivity and gene flow, probably due to habitat fragmentation. Based on genetic and distribution data, the conservation status of *P. brasiliensis* as Near Threatened (NT) has been confirmed, according to IUCN. These findings lead us to encourage the establishment of a preservation area for the isolated Guaíba II population and to highlight the importance of preserving these distinct gene pools in order to maintain species genetic diversity. The restricted knowledge on freshwater crayfishes and the effects of anthropic actions on populations reinforces the importance of these investigations.

Other approaches could be conducted with the data generated in this thesis as a starting point, using crayfish distribution as a tool for landscape, climate change and urbanization analysis.

Key words: Parastacids, Freshwater habitat, Freshwater crustacean.

1. Introdução Geral

Os lagostins de água doce reúnem uma diversidade significativa de espécies, considerando a fauna de crustáceos decápodes ao redor do mundo. São conhecidas ~ 640 espécies, as quais estão divididas em três famílias: Astacidae Latreille 1802, Cambaridae Hobbs 1942 e Parastacidae Huxley 1879 (Fig. 1). Os astacídeos são representados por apenas três gêneros e 10 espécies, com distribuição restrita para a Europa e oeste da América do Norte (Kawai & Crandall 2016). Já Cambaridae reúne a maior riqueza de espécies e diversidade de habitats ocupados dentre as famílias, com 429 espécies conhecidas considerando a América do Norte, México e leste da Ásia (Kawai & Crandall 2016). No Hemisfério Sul, apenas Parastacidae é registrada com 15 gêneros (170 espécies) que ocorrem no sul da América do Sul, Madagascar, Austrália e Nova Zelândia (Crandall & Buhay 2008, Rudolph 2010, 2015, Kawai & Crandall 2016, Ribeiro et al. 2016, 2017). A distribuição descontínua observada para essa família pode ser considerada um padrão, uma vez que também é compartilhada por outros organismos que radiaram pós-quebra da Gondwana (Toon et al. 2010).

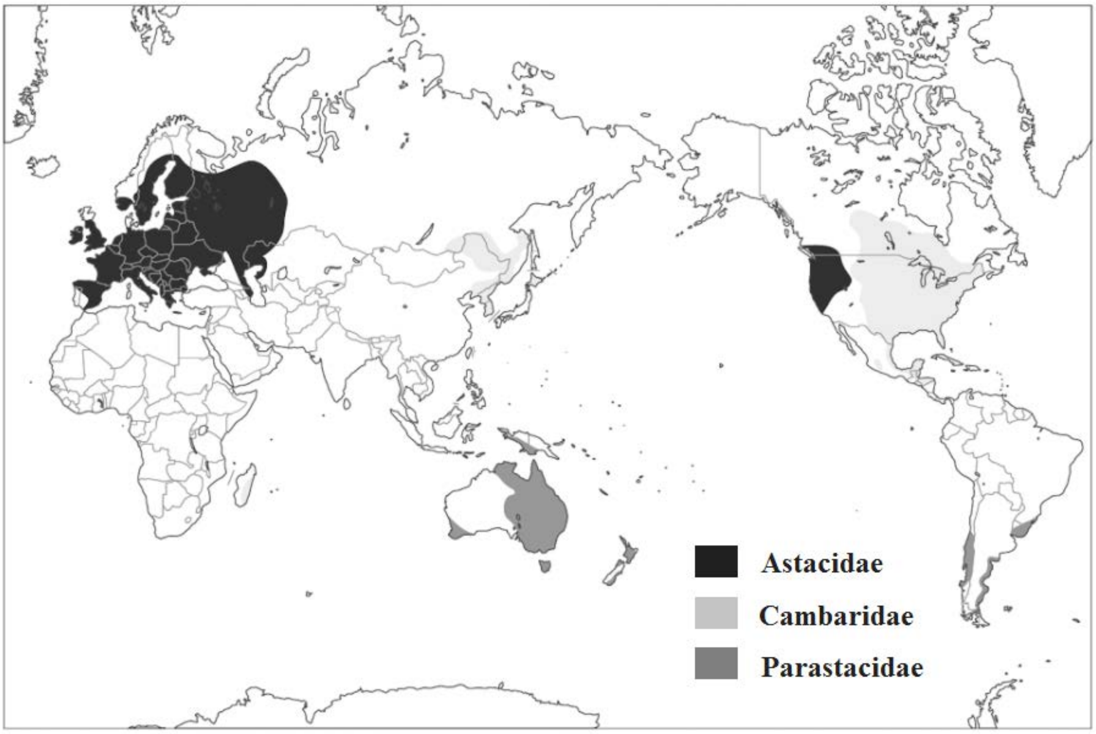

Figura 1. Distribuição das famílias de lagostins de água doce. Fonte: Kawai & Crandall (2016).

Na América do Sul, a distribuição dos lagostins se concentra na porção meridional do continente (Buckup & Rossi 1980, 1993, Rudolph et al. 2010) (Fig. 2). A diversidade conhecida para essa região compreende três gêneros, sendo um deles endêmico do Chile, *Virilastacus* Hobbs 1991. O segundo gênero, *Samastacus* Riek 1971, é o único gênero monotípico de parastacídeo sulamericano, sendo distribuído na Argentina e Chile. Já o terceiro gênero, *Parastacus* Huxley 1879, apresenta uma distribuição disjunta potencialmente relacionada a um processo vicariante e a barreira geográfica formada pela cordilheira dos Andes (Toon et al. 2010), conforme também visto para os caranguejos de água doce do gênero *Aegla* Leach 1820 (Pérez-Losada et al. 2004). Assim, as espécies situadas no lado Pacífico dos Andes, ou seja, na porção centro-sul do Chile, são os lagostins endêmicos *Parastacus nicoleti* (Philippi 1882) e *P. pugnax* (Poepigg 1835). As demais espécies estão situadas no lado no Atlântico dos Andes, compreendendo osul do Brasil (estados do Rio Grande do Sul - RS e Santa Catarina - SC), Uruguai e leste da Argentina (províncias de Entre Ríos, Corrientes e Missiones). No Brasil, também são registrados endemismos, como: *P. brasiliensis* (von Martens 1869), *P. caeruleodactylus* Ribeiro & Araujo 2016, *P. fluviatilis* Ribeiro & Araujo 2016, presentes no RS, *P. laevigatus* Buckup & Rossi 1980 e *P. tuerkayi* Ribeiro, Huber & Araujo 2017 em SC (Buckup & Rossi 1980, Ribeiro et al. 2016, 2017).

A distribuição da maioria dos parastacídeos é restrita, podendo existir regiões de sobreposição e compartilhamento de hábitat por espécies diferentes. Esse tipo de ocorrência é chamada de simpátrica e pode ser verificada para as espécies chilenas (Rudolph 2010, 2015). As espécies do lado Atlântico também podem ocorrer em simpatria (Faxon 1898, Holthuis 1952), porém os registros existentes são limitados e pouco detalhados, necessitando de uma confirmação. Em menor escala, a distribuição dos lagostins ocorre em mosaico na paisagem, conforme a disponibilidade de habitats adequados a sua existência e, assim aumentando as chances de coexistência de espécies com habitats semelhantes (Jones & Bergey 2007, Johnston & Robson 2009).

Os lagostins podem ocupar diferentes ambientes de água doce, dentro e na margem de lagos, riachos, arroios, rios e áreas úmidas (banhados, mata paludosa e turfeiras) (Buckup & Rossi 1980, Rudolph 2005, 2007, 2015, Noro & Buckup 2010). De acordo com o ambiente, algumas características podem influenciar a ocorrência das espécies, como: tipo de solo, distância do recurso d'água e ângulo da margem (Welch & Eversole 2006, Noro & Buckup 2010, Loughman et al. 2012, Helms et al. 2013). O nível do lençol freático também afeta a distribuição dos lagostins na paisagem (Cook et al. 2014). Assim, considerando uma paisagem heterogênea e o deslocamento dos lagostins através dela, podemos compreender a importância da sua composição. Nos habitats temporários, o deslocamento ocorre no sentido de locais mais altos para os mais baixos, conforme a oscilação do lençol freático (Cook et al. 2014). Para habitar esse tipo de ambiente, buscar abrigo, alimento e reproduzir, os lagostins contam com adaptações, como o comportamento escavador.

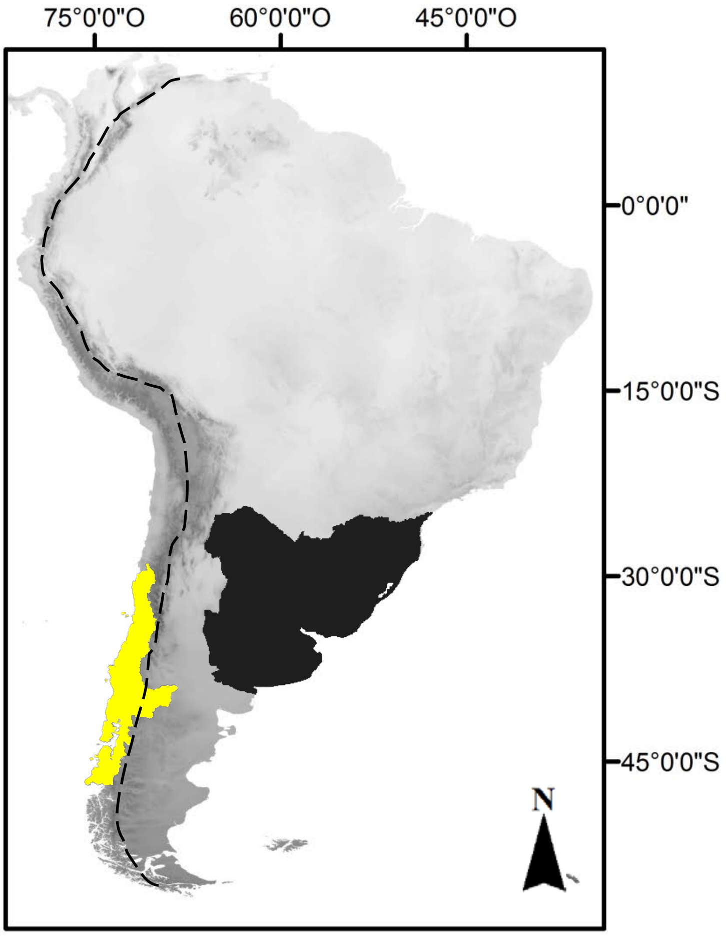

Figura 2. Distribuição de Parastacidae na América do Sul. A linha pontilhada representa a localização da cordilheira dos Andes na América do Sul; a área cinza escuro do mapa corresponde às espécies situadas no lado Atlântico dos Andes; e a área amarela às espécies do lado Pacífico dos Andes.

A capacidade escavadora é particular de cada espécie (Berril & Chenoweth 1982) e pode ser categorizada sob alguns aspectos, como morfologia do corpo do indivíduo, tipo de hábitat ocupado, configuração dos túneis escavados (tocas) e o ciclo de vida (Hobbs 1942, Horwitz & Richardson 1986). Assim, uma espécie considerada escavadora primária ou fortemente escavadora, pode construir túneis profundos e complexos (compostos por várias ramificações e aberturas na superfície do solo) e os indivíduos devem passar a maior parte do seu ciclo de vida no interior destas construções; enquanto uma escavadora secundária habita o interior dos cursos d'água nas estações chuvosas e ocupa as tocas durante os períodos secos; e a terciária permanece dentro do curso d'água (Hobbs 1942). Outra forma de classificação é considerar o ambiente e os tipos de tocas construídas. As tocas do tipo 1 são encontradas nas margens dos cursos d'água e suas aberturas ficam normalmente abaixo do nível da água, mas algumas delas podem ficar acima. Quando essas tocas não estão associadas a um curso d'água, porém os túneis seguem até alcançar o lençol freático, elas são chamadas de tipo 2. As tocas tipo 3 estão situadas longe de cursos d'água e do lençol freático, sendo sua umidade mantida pela água da chuva que infiltra no solo (Horwitz & Richardson 1986) (Fig. 3). Na América do Sul, temos representantes que se enquadram em todos essas categorizações, com a exceção dos indivíduos que fazem tocas do tipo 3.

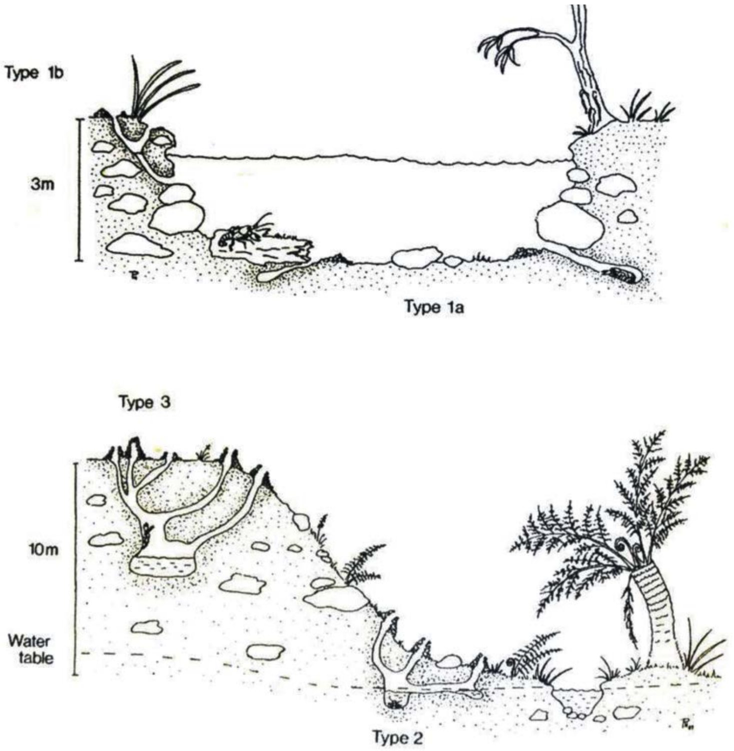

Figura 3. Classificação dos tipos de tocas proposto por Horwitz & Richardson (1986).

A construção dos túneis promove a aeração do solo e auxilia na germinação de plântulas, por esta razão os lagostins são considerados engenheiros do ecossistema (Statzner et al. 2000). Além disso, o desaparecimento desses organismos no ambiente pode acarretar em desequilíbrios na cadeia trófica; pois, são responsáveis pela ciclagem de nutrientes entre os diferentes níveis alimentar, auxiliando na fragmentação da matéria orgânica e na predação de outros invertebrados além de amplo espectro de forrageio devido à dieta onívora, eles (Nyström & Perez 1998, USIO 2002, Reynolds & O'Keeffe 2005, Reynolds et al. 2013). Os predadores desses crustáceos são vertebrados de pequeno e grande porte, como anfíbios, aves, répteis e mamíferos, incluindo o homem (Reynolds 2011, Reynolds et al 2013, Boyle et al. 2014).

O ecossistema de água doce está sendo fortemente impactado pelos diferentes usos e degradações que o homem tem lhe submetido, portanto, os organismos da biota aquática também são afetados. Os lagostins apresentam como agravante a sua distribuição naturalmente restrita e o forte endemismo (Harvey 2002). Além disso, para as espécies sulamericanas a escassez de informações básicas à cerca da distribuição e biologia são insuficientes, dificultando a pesquisa com esses organismos (Almerão et al. 2015).

Na última atualização sobre o estado de conservação mundial dos lagostins de água doce, 53% das espécies de Parastacidae encontravam-se listados em alguma das categorias de ameaça (Vulnerável, Em Perigo e Criticamente em Perigo) propostas pela “International Union for Conservation of Nature” – IUCN (Richman et al. 2015). Na América do Sul as perspectivas sobre o estado de conservação das espécies seguem a mesma tendência (Almerão et al. 2015, Ribeiro et al. 2016, 2017). No entanto, o alto índice de espécies categorizadas como Data Deficient (DD) chama atenção, uma vez que oito das 13 espécies conhecidas estão avaliadas nesta categoria (Almerão et al. 2015). O fato das espécies não estarem inseridas em uma categoria de risco de extinção não excluí a existência de ameaças sobre elas (Almerão et al. 2015).

As espécies recentemente descritas foram avaliadas como ameaçadas de extinção, a partir da sua distribuição restrita e endêmica, além das ameaças como degradação do ambiente em que estão inseridas (Ribeiro et al. 2016). As principais ameaças para a conservação dos crustáceos e tantas outras espécies de água doce têm sido a perda e a degradação do hábitat (Dudgeon 2006, Cumberlidge et al. 2009, Magris et al. 2010). Os ambientes ocupados pelos lagostins podem variar de cursos d’água perenes até áreas úmidas e são fortemente impactados pela urbanização, agricultura e pecuária (Ribeiro et al. 2016, 2017). Desta forma, é possível listar técnicas utilizadas para otimizar a vida humana, mas que são extremamente danosas para a sobrevivências das espécies, como: canalização de curso d'água, drenagem de áreas úmidas, desvio do curso do rio para lavoura, utilização de agroquímicos, despejo de esgoto domésticos e industrial sem tratamento, desmatamento de zonas ripárias (Buckup 2010 a,b,c,d,e,f,g). Ameaças silenciosas como os efeitos climáticos, que aos poucos se fazem presentes no cotidiano, afetam a distribuição de chuvas, intensificam períodos de estiagem também devem ser consideradas (IPCC 2014, Trenberth 2013). Na Austrália, onde as investigações à cerca desse assunto estão mais adiantadas, é possível relacionar a baixa conectividade dos habitats, e consequentemente das populações de lagostins, às mudanças no clima (Richman et al. 2015).

O efeito das mudanças climáticas pode afetar positivamente a expansão de espécies exóticas para outros locais, assim como a disseminação de doenças (Rahel & Olden 2008), principalmente quando se trata do cambarídeo *Procambarus clarkii* (Girard 1852). Predições sobre o potencial invasor dessa espécie foi realizada e os habitats do sul da América do Sul são altamente prováveis de serem colonizados pela espécie (Palaoro et al. 2013). A preocupação à cerca do *P. clarkii* está relacionada à descoberta de populações estabelecidas na cidade de São Paulo e comercialização ilegal no Rio Grande do Sul, Brasil, como espécie ornamental para aquarismo (Loureiro et al. 2015a, b). O Rio Grande do Sul apresenta outra ameaça desse tipo e que coloca em risco a sobrevivência dos lagostins, devido à bioincrustação pelo mexilhão dourado *Limnoperna fortunei* (Dunker 1857) (Darrigran 2002, Mansur et al. 2003, Lopes et al. 2009). A ocorrência deste bivalve já foi registrada na região costeira do estado, na bacia hidrográfica próxima a Reserva ecológica do Taim, onde ocorrem pelo menos duas espécies de lagostins (Lopes et al. 2009).

Diante dessas ameaças e a falta de conhecimento sobre as espécies de lagostins de água doce, novas abordagens e percepções sobre a diversidade desse grupo têm surgido. Atualmente, investigações genéticas que consideram a diversidade críptica dos lagostins como um potencial a ser explorado revelou que esses crustáceos configuram pequenas populações isoladas e com variações morfológicas, que precisam ser conhecidas e consideradas em planos de conservação (Filipová et al. 2010, Larson et al. 2012). Portanto, estudos mais aprofundados englobando aspectos sobre a distribuição, hábitat, taxonomia e genética de populações precisam ser realizados com a fauna de lagostins sulamericanos.

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3. Objetivos

3.1. Objetivo Geral

Conhecer a distribuição das espécies de lagostins de água doce na América do Sul e, a partir desses dados, projetar as áreas potenciais a sua ocorrência em diferentes cenários (presente e futuro); além de investigar a diversidade genética para *Parastacus brasiliensis.*

3.2. Objetivos específicos

Capítulo I

- Atualizar as informações sobre a distribuição dos lagostins de água doce na América do Sul;
- Mapear os registros de simpatia e sintopia das espécies;
- Caracterizar o hábitat dos lagostins da porção atlântica;

Capítulo II

- Estimar a distribuição atual dos lagostins de água doce sulamericanos;
- Projetar os efeitos das mudanças climáticas sobre a distribuição das espécies;
- Avaliar a expansão ou redução da distribuição das espécies no cenário futuro (2070);

Capítulo III

- Investigar as relações filogenéticas entre as populações identificadas como *Parastacus brasiliensis* (von Martens, 1869) ;
- Avaliar a conectividade e as possíveis modificações demográficas a partir de marcadores nuclear e mitocondrial;
- Avaliar o estado de conservação da espécie de acordo com IUCN RedList.

4. Estrutura da Tese

Esta tese está estruturada em capítulos que correspondem a artigos desenvolvidos independentemente. Cada um deles usa métodos ou técnicas necessárias, bem como a base prática e teórica para execução dos estudos. Assim, os capítulos abordam respectivamente: a distribuição e caracterização do habitat das espécies de lagostins de água doce na América do sul; modelagem preditiva de distribuição nos cenário presente e futuro (2070) dos lagostins sulamericanos; e diversidade genética de *Parastacus brasiliensis* voltada à conservação da espécie.

5. Capítulos (ARTIGOS)

5.1. Capítulo I

Artigo formatado conforme a revista:

Studies on Neotropical Fauna and Environment

Gomes et al. (2017)

Distribution, sympatry and habitat characterization of South America crayfishes (Crustacea: Decapoda: Parastacidae)

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ABSTRACT

Freshwater crayfishes in South America occupy permanent and temporary environments. Data on distribution and habitats are scarce, mainly for the species localized in the Atlantic portion of the continent. The aims of this study are (1) to update the information on the South American parastacids distribution; (2) to map the records of sympatric and syntopic species; and (3) to characterize the habitats used by the Atlantic species. We compiled the occurrence data from the zoological collections (ZC) and field data carried out in southern Brazil (states of RS and SC) between 2012 and 2015.

Different methods were used to capture the crayfishes: manual excavation, vacuum pump, baited traps, and dip nets. The records obtained from the ZC and samplings were classified as: allopatric, sympatric or syntopic. In total, 51 new occurrence records were obtained; including the presence of *Parastacus fluviatilis* for the highest altitude in RS and the capture of *P. saffordi* almost three decades after its taxonomic revision. For the Atlantic species, the sympatric or syntopic records were numerous for *Parastacus defossus* and *P. brasiliensis* or *P. defossus* and *Parastacus* sp. For the Pacific species, four species were recorded in a buffer zone < 5 km of extension. *Samastacus spinifrons* occurred in sympatry with *P. pugnax* along coastal region of Chile. Six new types of wetlands inhabited by Atlantic crayfishes were recorded. Southern America may be considered as a hotspot of unknown diversity for the freshwater crayfish due to gaps observed in the distribution of species and the scarce data on species habitat and biology. In the future, the data provide in this study can be used to increase the accuracy of the evaluation of the extinction risk of parastacids.

Keywords: South American Parastacids, Geographical range, Syntopy, Freshwater environment

Introduction

Freshwater crayfishes are worldwide distributed and currently represented by three families: Astacidae Latreille 1802 and Cambaridae distributed in the Northern Hemisphere, and Parastacidae Huxley 1879 in the Southern Hemisphere (Crandall & Buhay 2008, Kawai & Crandall 2016). Parastacid crayfishes have a Gondwanan pattern of distribution, being found in South America, Madagascar, New Zealand, New Guinea, and Australia (Toon et al. 2010). Parastacidae comprises 15 genera and 170 species, which corresponds approximately to 22% of total global crayfish diversity (Richman et al. 2015, Ribeiro et al. 2016, 2017). South American parastacids are represented by three genera: *Virilastacus* Hobbs 1991 (four species) is endemic to Chile, *Samastacus* Riek

1971 (one species) occur in Chile and Argentina (Argentinean Patagonia); and *Parastacus* Huxley 1879 (eleven species), is distributed in south-central Chile, southern Brazil, Argentina, and Uruguay (Buckup & Rossi 1980, 1993, Rudolph 2015, Ribeiro et al. 2016, 2017). The restricted distribution of almost all South American crayfishes and the endemism of some species are characteristics shared by members of Parastacidae (Harvey 2002, Richman et al. 2015, Almerão et al. 2015). The high endemism rates seen in this family have been related to the discontinued pattern of habitat occupation and individual features (low dispersal, long cycle-life, and slow maturation) (Harvey 2002).

South American species inhabit springs, perennial or intermittent streams, lakes, and wetlands (swamp forests, marshlands, peatlands, and lowland forests) (Buckup & Rossi 1980, 1993, Rudolph 2010, 2013, 2015, Richman et al. 2015, Ribeiro et al. 2016, 2017). The diversity of environments occupied by crayfishes can be related to their burrower capacity, since the dependence on the burrows was suggested as an important feature that has been present throughout Parastacidae evolution (Crandall et al. 1999). The burrows offer refuge against extreme conditions of the habitat, besides providing access to the water table (Noro & Buckup 2010). In streams, burrows are shallow and built on the margin; in wetlands, the burrows have several openings on the soil surface, with deep and complex tunnels (Buckup & Rossi 1980, Horwitz & Richardson 1986, Noro & Buckup 2010). Thus, in the landscape, it is possible to find different species coexisting within the same stream or wetland (Jones & Bergey 2007, Coughran et al. 2008, Jonhston & Robson 2009).

An interesting feature of crayfish species is their mosaic distribution across the landscape, with up to five species occurring in a same geographical range (Jonhston & Robson 2009). The regional richness of these animals can be quite high (e.g. in regions of Australia) and many species can occur in sympatry, i.e. occurring in the same geographical area. At a local scale, this richness is low and it is possible to verify the occurrence of species sharing the same habitat, i.e. syntopic species (Jones & Bergey 2007, Coughran et al. 2008, Jonhston & Robson 2009).

Records of South American crayfishes occurring in sympatry or syntopy have not yet been clearly addressed in a specific study, and the current information is limited to a few comments in taxonomic or ecological studies (Faxon 1898, Rudolph & Rivas 1988, Holthuis 1952). The insufficient data about species distribution, habitat, and population features of South American crayfishes is worrisome. Eigth species have been categorized at extinction risk (Vulnerable, Endangered, or Critically Endangered categories) (Almerão et al. 2015, Rudolph 2015, Ribeiro et al. 2016, 2017) according to criteria established by the International Union Conservation of Nature — IUCN (IUCN 2014). For those eight species, habitat changes were the main reasons for including them in the Endangered category. Other threats include urbanization, canalization of watercourses, siltation, drainage of wetlands, harvesting for human consumption, agriculture, extraction of *Sphagnum*, and collection of specimens to use as bait (Buckup 2010 a,b,c,d,e,f,g,h). Urbanization is also an important threat since several species occur near or within urban centers (Buckup & Rossi 1980).

Considering the scarcity of studies addressing the distribution of South American parastacids, mainly for Atlantic species, the increasing rates of suitable habitat loss, and the high number of threatened species, the aims of this study are: (1) to update the information on South American parastacids distribution based on zoological collections and field data; (2) to map the records of sympatric and syntopic species; and (3) to characterize the habitats used by Atlantic species. In the future, these data can be used to increase the accuracy of the evaluation of the extinction risk of South American parastacids.

Material and Methods

Occurrence records available in published studies and museums and scientific collections visited from 2013 to 2015 were included, Crustacean Collection of Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil; Laboratorio de Astacología, Universidad de Los Lagos (LAULA), Osorno, Chile. Nevertheless, only georrefered records or those that were possible to attribute their geographic coordinates were used.

Additionally, 177 sites were sampled in southern Brazil (states of Rio Grande do Sul - RS and Santa Catarina - SC) between 2012 and 2015. In order to capture the crayfishes in different environments, different methods were combined: manual excavation, vacuum pump, baited traps, and dip nets. The individuals were fixed in ethanol 70% or 95% and deposited in the UFRGS. Identifications were based on Buckup & Rossi (1980) and Ribeiro et al. (2016), for Atlantic species, and on Buckup & Rossi (1993) and Rudolph & Crandall (2005, 2007, 2012), for Pacific species.

Species were considered sympatric when they occurred in a buffer zone of up to 5 km of extension in the same hydrographic basin. The buffer size was based on sympatric distribution of the Australian species in the catchment (Johnston & Robson 2009). The syntopy was established when two or more species occupied close habitats of up to 100 m of extension in same stream stretch, for example.

The classification of crayfish burrows was made for all South American species based on habitat features, according to Horwitz & Richardson (1986): (1a) burrows within permanent waters; (1b) burrows connected to permanent water bodies; (2) burrows connected to water table from the groundwater table and surface table runoff; and (3) burrows independent of water table, only in the surface runoff.

The habitat occupied by Atlantic species was described according to the observations made in the field and considering definitions of wetlands according to Junk et al. (2014), and also including data from Buckup & Rossi (1980).

Results

The field samplings and the analyses of scientific collections revealed 51 new occurrences of the freshwater crayfishes for Atlantic and Pacific drainages. In total, three new records from scientific collections and 27 referent to field samplings were evidenced for the Atlantic drainage; thus widened and filled some gaps in the geographic range for *Parastacus* sp., *P. defossus* Faxon 1898, and *P. pilimanus* (von Martens 1869) (Tab. I, Fig. 1). For the species of the Pacific drainage, 21 records were considered new, all from scientific collections (Tab. II, Fig. 2). Nine out of 16 known species in South America had a restricted distribution, *V. rucapihuelensis* Rudolph & Crandall 2005, *V. retamali* Rudolph & Crandall 2007, *V. jarai* Rudolph & Crandall 2012 and *P. nicoleti* (Philippi 1882) in Pacific drainage; *P. brasiliensis* (von Martens 1869), *P. laevigatus* Faxon 1898, *P. caeruleodactylus* Ribeiro & Araujo 2016, *P. fluviatilis* Ribeiro & Buckup 2016 and *P. tuerkayi* Ribeiro, Huber & Araujo 2017 in Atlantic drainage.

The new records of Atlantic species widened their distribution area and some gaps in the geographic range were also filled for *Parastacus* sp., *P. defossus* Faxon 1898, and *P. pilimanus* (von Martens 1869). The syntopy records were verified for seven sites in Brazil, four of them came from new samplings in the state of Rio Grande do Sul (Tab. III). These new samplings recorded the presence of *Parastacus* sp. and *P. defossus* in floodplains; and *P. defossus* and *P. brasiliensis* (von Martens 1869) shared stream springs. Analyses of material from the UFRGS evidenced a previous record of syntopy of

P. varicosus Faxon 1898 and *P. pilimanus* in the Taim Ecological Station (protected area), Rio Grande do Sul, and of *P. saffordi* Faxon 1898 and *P. varicosus* from state of Santa Catarina (SC), Brazil.

In order to verify a previous record, we revisited the Agronomic Experimental Station of UFRGS in which, according to UFRGS, *P. defossus* and *P. brasiliensis* would occur in sympatry. However, we could not confirm this information and we only found *P. defossus* and *Parastacus* sp. occurring in syntopy in wetlands of that locality. *Parastacus defossus* and *P. brasiliensis* were found very close to each other and occupying distinct habitats (wetland and stream, respectively) across landscapes of Porto Alegre city; thus, we considered these findings as sympatry records.

A historical record published by Faxon (1898) indicated syntopy between *P. defossus* and *P. saffordi;* the two species co-inhabited the same burrows in their type-locality in Uruguay. We considered this occurrence as inaccurate due to incompatibility of biological data (Tab. III, Fig. 1).

The sympatry and syntopy of species were verified for Pacific parastacids in 12 and four sites, respectively, according to the scientific collections data (Tab. IV, Fig. 2). Syntopy records of different genera in the same habitat, such as *Virilastacus* and *Parastacus* and between *Virilastacus araucanius* (Faxon 1914) and *Virilastacus* sp., were herein confirmed (Tab. IV; Fig. 2). An unpublished record of sympatry among four species, *V. retamali* Rudolph & Crandall 2007, *V. rucapihuelensis* Rudolph & Crandall 2005, *P. nicoleti* (Philippi 1882), and *S. spinifrons* (Philippi 1882), was documented within a range of 5 km of extension. *Samastacus spinifrons* occurred in streams, *V. retamali* in peatlands, and *V. rucapihuelensis* and *P. nicoleti* shared the same habitat in semi-marshands. Similarly, *S. spinifrons* was recorded in sympatry with *P. nicoleti* and

Virilastacus sp. occupying semi-marshlands. The last two records were considered sympatric, but the species that occupied the same habitat can also be considered syntopic.

The habitat of *P. brasiliensis* includes springs and permanent streams of some hydrographic basins of the state of Rio Grande do Sul (RS), Brazil. The streams had different substrates and depths ranging from 3 to 60 cm. This species was found under boulders, rocks and litter, sheltered in crevices of rocks, roots of marginal vegetation, or in burrows built on the margin of streams. Most of the burrows consisted of two simple openings, usually above the water level, or two to three simple openings and one chimney, with approximately 5 cm of height. The excavated tunnels were often shallow due to the presence of roots and rocks on the margins. Thus, the individuals were captured inside tunnels of depths between 10 and 30 cm located in a maximum depth of 60 cm below the water level. Within each burrow, there was only one adult specimen. Some adults were also captured in the stream using dip nets and traps, and at the banks by digging the burrows. Juveniles were collected only within the stream with the aid of dip nets.

The distribution of *P. defossus* is known for the Brazil (RS) and Uruguay (Montevideo). The species occur in swamp forests, flooded fields, floodplains, and wetlands near streams. In urban areas, the individuals are found in roadside ditches. The soil in these localities is poorly drained and remains wet most of the year. The burrows are composed of up to eight openings and chimneys are predominant (average height of 8 cm and maximum of 38 cm). The tunnels are deep and interconnected, they can reach up to 1.5 m of depth. In summer, the tunnels are deeper and openings are mostly sealed. The main methods for capturing these animals are the vacuum pump and manual catch. In addition, it was possible to find a male, a female, and juveniles within the same burrow.

Parastacus fluviatilis is endemic of highland grasslands (Campos de Cima da Serra) in

RS, Brazil, and inhabits low-order streams, lentic habitats formed on the stream side, and burrows built in the banks. In addition, the species can also occur in highland flooded grasslands up to ~1400 m of altitude, where there is a predominance of peat-bog soil. In places with greater and deeper water accumulation (more than 50 cm of depth), it was possible to catch the juveniles together with the vegetation with the aid of sieves. Inside the streams, the traps are efficient, as well as the margin excavation and the use of dip net in the pools. The burrows found had usually simple openings; in one excavated burrow, only one adult individual was seen.

Parastacus sp. occurs in Brazil (RS) and Argentina, it can be found in permanent low-order streams sheltering under pebbles, but mainly in the burrows built on the river banks. In this habitat, the burrows were shallow, and the individuals could be captured 20 cm below the soil surface. This species also inhabits floodplains, wetlands associated with streams and rivers, small pools in forests fed by rain water, and temporary pools. In the regions with greater water accumulation in the wetland, the juveniles are easily captured with sieves and dip net, and the adults build deep burrows in less wet areas. The burrows can reach up to ~80 cm of depth and within each burrow; only one adult individual was collected. The burrows of *Parastacus* sp. are always associated with water bodies or water tables.

The distribution of *P. saffordi* is known for Brazil (RS and SC), Uruguay, and Argentina. This species occupies low-order streams and shelters under pebbles and burrows built on the margin. It can inhabit wetlands fed by rain water which are depressions with water accumulation inside the forest. In this habitat, there is a large quantity of litter which the crayfishes use as a shelter, reason why the capture is more easily made with dip net. However, this species build burrows with simple openings and shallow tunnels reaching up to 45 cm of depth. Ecological features of *P. saffordi* are poorly known.

Parastacus varicosus is widely distributed in Brazil (RS and SC), Uruguay, and Argentina, occurring in water courses under pebbles and litter. The species is also found in floodplains and flooded grasslands associated with the vegetation; thus, the specimens can be catched with dip net. In flooded grasslands of RS coast, the vacuum pump is the main capture method for this species. In this habitat, it is possible to observe burrows with simple or ornamented openings, and the chimneys, when present, can reach up to ~5cm of height. The ecological data about this species is scarce and more studies are necessary.

Parastacus pilimanus occurs in Brazil (RS), Uruguay, and Argentina, in streams, sheltered under pebbles, litter, and burrows; the burrows usually have simple openings built on the edges of the water course. The species also inhabits flooded fields and floodplains of large rivers. In these habitats, the burrows are observed in riparian vegetation and associated with smaller tributaries. The openings of the burrows can be simple or with chimneys that reach 5 to 8 cm of height. In the Pampa biome, the species was collected in streams and the burrows can reach up to more than 1 m of depth. The ecological knowledge of this species is scarce and more studies are necessary.

Interestingly, Atlantic species often build their burrows with two openings that communicate with each other a few centimeters below the soil surface; frequently, only one of the openings has chimney or both have simple openings (i.e. without ornamentation).

Considering the distribution and habitat data available in the literature, as well as the knowledge acquired in the field work, it was possible to classify the species according to the general groups proposed by Horwitz & Richardson (1986): Type 1a — Aquatic, species that occupy exclusively water courses (e.g. *Samastacus spinifrons*) (Table V); Types 1a, b, and 2a— Semi-Aquatic, species that inhabit water bodies and some types of wetlands, such as *P. brasiliensis, P. fluviatilis, P. pilimanus, P. saffordi, P. varicosus*, and *Parastacus sp.*; and Type 2 — Semi-Terrestrial, species that occur only in wetlands (e.g. *P. nicoleti*) and rarely in streams (e.g. *P. pugnax*) such the genus *Virilastacus*, and species *P. caeruleodactylus*, *P. defossus*, *P. nicoleti*, and *P. pugnax* (Table V). *Parastacus laevigatus* was not considered in this categorization due to the lack of information on its biology.

Discussion

The narrow range distribution of crayfishes verified for most species in this study, it is shared with other representatives that radiated post-Gondwanan break-up dispersing from east to west (Toon et al. 2010). In South America, the disjoint range of the species is related to the formation of the Andes which influenced drainages (Pérez-Losada et al. 2004, Bedatou et al. 2008) and, consequently, the colonization of different environments. There are measures to define the area size of a short-range endemic (SRE) to separate species with restricted occurrence in Parastacidae. Harvey (2002), used a maximum range of < 10,000km² for Australian species, but Eberhard et al. (2009), decreased this scale and utilized < 1,000 km² for some subterranean species. Knowledge about crayfishes in South America is still scarce. Several species could be classified as endemic if we consider the limited range associated with specific habitats (hydrographic basin or wetland) within the same country. Nevertheless, for an accurate assessment of

SRE, it is necessary to perform standardized samplings, especially for species categorized as data deficient in conservation studies (i.e. Almerão et al. 2015).

The lack of data is linked to insufficient investments in studies concerning these animals, since the last publication on distribution update and taxonomy review was performed by Buckup & Rossi in 1980. Currently, three new species were described for the genus *Parastacus* (Ribeiro et al. 2016, 2017). Therefore, analyses of scientific collections and samplings brought new data mainly for the Atlantic species. The occurrence of *Parastacus* at ~1400 m of altitude was recorded for the first time; the higher altitude ever documented for this genus. This report corresponds to the occurrence of *P. fluviatilis* in the Pico Monte Negro, the highest place of the state of Rio Grande do Sul, Brazil. To date, crayfish record in altitude streams was known for *P. promatensis* at 800 m of altitude (Fontoura & Conter 2008). The similarity between these species is related to the habitat occupied; both crayfishes occur in streams situated in the in highlands fields — Campos de Cima da Serra; however, *P. fluviatilis* can also be found in the flooded grassland.

Parastacus saffordi was previously searched without success in habitats known for its potential occurrence (Buckup, L. *pers. comm.*). During the samplings, we collected the species in the state of Santa Catarina, almost three decades after the record of Buckup & Rossi (1980). The reason for this long-time gap between records may be related to alterations observed in the water bodies since the southern region of the state have been suffering intense activity of coal mining (Brandelero et al. 2016, Pompêo et al. 2004, Cesar et al. 2013). Thus, records obtained for *P. saffordi* are concentrated in highland streams and small depressions in woods fed by rainfall. These habitats are commonly less disturbed and one of the records is located in a protected area, Ecological Sanctuary Aguaí (SC). In general, the presence of crayfishes is under estimated in protected areas due to neglection of freshwater fauna in surveys during the creation of areas for species conservation (Richman et al. 2015). An example is the occurrence of *Parastacus* sp. in the Lami Biological Reserve (RS), unknown until our on-site survey.

The knowledge on habitats occupied by Atlantic species was previously limited to streams, riverbanks and, flooded grasslands (Buckup & Rossi 1980); recently, the occurrence was amplified to lowland forests (Ribeiro et al. 2016, 2017). In our study, we amplified the knowledge about habitats occupied. Six new types of wetlands inhabited by crayfishes were recorded: inland wetlands that encompass the flooded grasslands of altitude, floodplains, riparian wetlands along small rivers (1 - 5 order), rain water fed wetlands in small depressions, swampy forests, and coastal wetlands with temporary ponds (Fig. 3). The wetlands are essential for the control of hydrological regime and maintenance of aquatic biota (Junk et al. 2014). Wetlands also contribute for the complexity of the landscape, creating a mosaic of seasonal habitat with perennial environments (Johnston & Robson 2009, Bennett & Sauders 2010).

The diversity of habitats occupied by species is partially related to environmental characteristics such as moisture and soil texture, proximity with the stream or floodplain, and water table depth (Welch & Eversole 2006, Noro & Buckup 2010, Loughman et al. 2012; Helms et al. 2013). The interaction between the physical factors of the habitat and crayfish biology influences the behavior of the species, including the burrows distribution in the environment (Helms et al. 2013). Therefore, the burrowing behavior is an adaptation of several crayfishes to survive in intermittent streams (Jones & Bergey 2007), to search for shelter, feeding, reproduction, and exploration of different habitats (Helms et al. 2013). The characteristic and extension (stronger or weaker) of this behavior is particular of each species (Berril & Chenoweth 1982).

There are many classifications for the burrower potential of the crayfishes. Hobbs (1942), considered life-history features and burrows occupation in North American species for classifying burrows. Horwitz & Richardson (1986), proposed categories for the environment, burrows types and their connection with water table in Australian species. Both categorizations can be applied in South American crayfishes, but Hobbs's classification is less wide. So, we used the classification proposed by Horwitz & Richardson (1986). *Samatacus spinifrons* was classified as Type 1a because it inhabits permanent water courses and lakes, shelters under rocks, edges crevices, and non-ramified burrows. *Parastacus brasiliensis* and other Atlantic species can be classified as Type 1a and 1b, the burrows can be found in edge streams under or above water surface; however, this species can also inhabit burrows of Type 2 that are connected with the water table in flood beds surrounding small creeks. Only *P. defossus* was considered exclusively of type 2 among Atlantic species. This crayfish occupies distinct habitats and has adaptation to live within a burrow such as tolerance to low oxygen levels (Silva-Castiglioni et al. 2010). All others Pacific species were classified in the category of the Type 2, which corresponds to its ecological attributes.

The co-occurrence of four Pacific species can be explained by the habitat specificity showed by them. *Samastacus spinifrons* inhabits streams (Type 1a), *V. retamali* lives in geogenous peatland, *V. rucapihuelens* and *P. nicoleti* inhabit semi-marshlands (all of the Type 2) (Tab. IV). Similarly, the Atlantic species occupied different habitats, *P. brasiliensis* occurred in streams (Type 1 a, b, and Type 2a), and *P. defossus* in wetlands (Type 2). In water sources, however, these species shared the same environment. The syntopic record in the same stream of *P. varicosus* and *P. saffordi,* based on data of the UFRGS, was unique among occurrences of South American crayfishes. For others parastacids, this type of occurrence is already known (Jones &

Bergey 2007, Johnston et al. 2009). In the wetlands, the segregation of habitat was also evident in most of the occurrence sites of *P. defossus* and *Parastacus* sp. (Type 1 a, b and Type 2). The representatives classified as Type 1 a, b and Type 2 for a single species, are not present in Chile; but in the Atlantic portion these types are numerous and commonly recorded. A particularity is seen in the burrows built in wetlands of Atlantic species, they are always connected to permanent resources (streams or water tables).

We considered as improbable the record of *P. defossus* and *P saffordi* cohabiting the same burrow made by Faxon (1898) due to the current knowledge about South American crayfishes. Crayfishes present different social behavior that co-evolved along with the burrowing behavior. Species which have more terrestrial habitats, such as *P. defossus*, present extended parental care and higher tolerance to cohabitation with others co-specifics; differently from organisms most related to water courses (Noro & Buckup 2010, Richardson 2007).

Some requirements are identified as key factors for the establishment of the sympatry (Cothran et al 2015) such as the divergence of ecological traits associated with the habitat use, foraging, activity patterns, and shelter preferences (Bennett & Sauders 2010, Baumart et al. 2015, Jones & Bergey 2007). Neverthless, in the co-occurrence of species ecologically similar such as *P saffordi* and *P. varicosus*, or *P. pugnax* and *V. araucanius*, the separation may be evident in the level of microhabitat use (Johnston & Robson 2009). The degree of divergence depends on the fitness of each species, on the environment occupied, and on the interaction between species. This happens because small differences may be sufficient for species with similar fitness to coexist (Cothran et al 2015). However, traits related to resource competition and ability to avoid predators cannot be enough for explaining the occurrence of sympatric species (Cothran et al

2015). Thus, environmental heterogeneity may be other factor considered in this complex interaction (Bennett & Sauders 2010).

Availability of suitable habitat in the landscape determines the occurrence pattern of the species (Johnston & Robson 2009). Therefore, changes promoted in the landscape or within the stream that modify the abiotic conditions may affect the crayfish fauna, especially in endemic and specialist species (Jones et al. 2007). Those changes consequently reduce populations and alter the burrower activity due to soil compaction, for example (March & Robinson 2006). In streams, small impoundments locally affect the crayfish assemblage, but its effects can be extended over 2 km downstream (Adam 2013). The siltation of the water body resulted from tree-harvesting, road construction, or changes in large scale due to land use, are potential threat to stream organisms (Jones et al. 2007). The landscape may act as refuges for individuals during environments disturbances such as riparian forest in agricultural areas near to water bodies (March & Robinson 2006). In wetlands, crayfishes may move from higher-to lower-elevated habitats according to the water level (Cook et al. 2014); therefore, alterations in the hydric dynamic can compromise the displacement of animals across in environment.

Habitat degradation is one of the main threats for crayfishes and for other freshwater species around the world (Richman et al. 2015, Magris et al. 2010, Cumberlidge et al. 2009). Wetlands harbor most species of South American crayfish (Buckup & Rossi 1980, Rudolph 2015, Ribeiro et al. 2016, 2017) and those ecosystems are globally declining in area and quality (Ramsar 2015). The area loss has been more pronounced in inlands than in coastal regions (Davidson 2014). The Brazilian wetlands have been frequently considered as wastelands by politicians and urban planners; thus, this ecosystem is quickly becoming a solid-waste dumping, housing areas, or roads (Junk et al. 2014). The conversion of wetland into croplands is extensively noticed in south

Brazil, and deforestation of the natural vegetation cover alters the hydrological dynamic, drainage, and flooding (Junk et al. 2014). In rice croplands of the state of Rio Grande do Sul, the population of *P. caeruleodactylus* uses swampy forests as refuge for ovigerous females (Ribeiro et al. 2016).

Southern America may be considered as a hotspot of unknown diversity for the freshwater crayfish due to gaps observed in the distribution of species and the scarce data. Knowledge about species distribution and habitat classification is the first step for future ecological studies.

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Tables, Figures and Captions

Table I. New occurrence records of freshwater crayfishes (Atlantic species) from new samplings and scientific collections in southern Brazil. UFRGS: Crustacean Collection of Universidade Federal do Rio Grande do Sul, Rio Grande do Sul, Brazil.

Table II. New occurrence records of freshwater crayfishes (Pacific species) from the scientific collections. LAULA: Laboratorio de Astacología, Universidad de Los Lagos, Osorno, Chile.

Table III. Occurrence records of Atlantic freshwater crayfish in sympatry or syntopy, and habitat type, from the scientific collections and new samplings. **UFRGS**: Crustacean Collection of Universidade Federal do Rio Grande do Sul, Rio Grande do Sul, Brazil. **Faxon W. 1898**. Observations on the Astacidae in the United States National Museum and in the Museum of Comparative Zoology, with descriptions of new species. Proceedings of the United States National Museum, 20: 642–694.

Table IV. Occurrence records of Pacific freshwater crayfish in sympatry or syntopy, and habitat type, from the scientific collections. **LAULA**: Laboratorio de Astacología, Universidad de Los Lagos, Osorno, Chile. **Rudolph EH. 2010**. Sobre la distribución geográfica de las especies chilenas de Parastacidae (Crustacea: Decapoda: Astacidea). Boletín de Biodiversidad de Chile, 3: 32–46.

Table V. Distribution of freshwater crayfish species in South America and burrow type, according to Horwitz & Richardson (1986). **Buckup & Rossi 1980:** Buckup L, Rossi A. 1980. O Gênero *Parastacus* no Brasil (Crustacea, Decapoda, Parastacidade). Rev. Bras. Biol., 40: 663–681. **Gomes et al.:** Gomes KM, Ribeiro FB, Rudolph EH, Araujo PB. 2017. Distribution, sympatry and habitat characterization of South America crayfishes (Crustacea: Decapoda: Parastacidae). Unpublished. **Ribeiro et al. 2016:** Ribeiro FB, Buckup L, Gomes KM, Araujo PB. 2016. Two new species of South American freshwater crayfish genus *Parastacus* Huxley, 1879 (Crustacea: Decapoda: Parastacidae). Zootaxa, 30, 4158(3): 301–24. DOI 10.11646/zootaxa.4158.3.1. **Ribeiro et al. 2017:** Ribeiro FB, Huber AF, Schubart CD, Araujo PB. 2017. A new species of *Parastacus* Huxley, 1879 (Crustacea, Decapoda, Parastacidae) from a swamp forest in southern Brazil. Nauplius, 25: 1–14. DOI 10.1590/2358-2936e2017008. **Rudolph 2002:** Rudolph EH. 2002. Sobre la biología del camarón de río *Samastacus spinifrons* (Philippi, 1882) (Decapoda, Parastacidae). Gayana, 66(2): 147–159. **Rudolph 2010:** Rudolph EH. 2010. Sobre la distribución geográfica de las especies chilenas de Parastacidae (Crustacea: Decapoda: Astacidea). Boletín de Biodiversidad de Chile, 3: 32–46. **Rudolph 2013:** Rudolph EH. 2013. *Parastacus pugnax* (Poeppig, 1835) (Crustacea, Decapoda, Parastacidae): conocimiento biológico, presión extractiva y perspectivas de cultivo. Lat. Am. J. Aquat. Res., 41(4): 611–632. **Rudolph 2015:** Rudolph EH. 2015. Current state of knowledge on *Virilastacus* species (Crustacea, Decapoda, Parastacidae). Lat. Am. J. Aquat. Res., 43(5): 807–818.

Figure 1. Distribution of species of Atlantic freshwater crayfish based on previous and new records, from scientific collections and new samplings. Black arrow indicates the syntopy mentioned by Faxon (1898).

Figure 2. Distribution of species of Pacific freshwater crayfish based on previous and new records, from scientific collections and bibliography. S. a: Sympatry zone "a"; S. b:

Sympatry zone "b"; the black arrows are highlighting the sympatry of three or four species in a range of up to 5 km.

Figure 3. New occurrences records of Atlantic crayfishes in wetlands situated in Brazil according to Junk et al. (2014). **A**. Rain water fed wetlands in small depressions; **B**. Burrow with simple openings (type 2); **C**. *Parastacus saffordi* Faxon 1898; **D**. River floodplain; **E**. Burrow (type 2) with ornamented opening (chimney), height of 5 cm. **F**. *Parastacus* sp. **G**. Flooded grassland of altitude; **H**. *Parastacus fluviatilis* Ribeiro & Buckup 2016.

Table I. New occurrence records of freshwater crayfishes (Atlantic species) from new samplings and scientific collections in southern Brazil. UFRGS: Crustacean Collection of Universidade Federal do Rio Grande do Sul, Rio Grande do Sul, Brazil.

Species	**Habitat**	**Decimal Coordinates**		**Database**
P. pilimanus	Stream	-29.920650’S	-55.76724’W	Sampling
P. pilimanus	Stream	-29.66068’S	-50.21387’W	Sampling
Parastacus sp	Stream	-30.350390’S	-51.02902’W	Sampling
Parastacus sp	Stream	-30.354490’S	-51.03354’W	Sampling
Parastacus sp	Temporary pool	-30.355980’S	-51.04880’W	Sampling
P. brasiliensis	Stream	-30.134718’S	-51.10329’W	Sampling
P. brasiliensis	Stream	-30.184127’S	-51.23080’W	Sampling
P. brasiliensis	Stream	-30.344720’S	-51.59333’W	UFRGS
P. brasiliensis	Stream	-30.091516’S	-51.09739’W	UFRGS
P. brasiliensis	Stream	-30.333330’S	-51.37750’W	UFRGS
P. brasiliensis	Stream	-30.154330’S	-51.10165’W	Sampling
P. brasiliensis	Stream	-29.80252’S	-50.9682’W	Sampling
P. fluviatilis	Flooded grassland	-28.616578’S	49.79681’W	Sampling
P. fluviatilis	Stream	-28.64553’S	-49.9410’W	Sampling
P. saffordi	Stream	-28.388322’S	-49.44943’W	Sampling
P. saffordi	rain water fed wetlands in small depressions	-28.521381’S	-49.52413’W	Sampling
P. saffordi	Stream	-29.5375’S	-49.91444’W	Sampling

Table II. New occurrence records of freshwater crayfishes (Pacific species) from the scientific collections. LAULA: Laboratorio de Astacología, Universidad de Los Lagos, Osorno, Chile.

Pacific species	**Decimal Coordinates**		**Database**
V. araucanius	-39.8916666'S	-73.4397222'W	LAU-LA
V. araucanius	-39.9488888'S	-73.1716666'W	LAU-LA
P. nicoleti	-40.5980555'S	-73.4666666'W	LAU-LA
P. nicoleti	-39.9988888'S	-73.6513888'W	LAU-LA
P. nicoleti	-40.1677777'S	-73.4494444'W	LAU-LA
P. nicoleti	-40.7211111'S	-73.4897222'W	LAU-LA
P. nicoleti	-40.7916666'S	-73.425'W	LAU-LA
P. nicoleti	-39.8708333'S	-73.4725'W	LAU-LA
P. pugnax	-37.6108333'S	-73.5313888'W	LAU-LA
P. pugnax	-37.6108333'S	-73.5313888'W	LAU-LA
V. rucapihuelensis	-40.5857916'S	-73.6447861'W	LAU-LA
Virilastacus sp	-39.3444444'S	-73.1716666'W	LAU-LA
Virilastacus sp	-39.2316666'S	-72.8786111'W	LAU-LA
Virilastacus sp	-39.1788888'S	-73.0027777'W	LAU-LA
Virilastacus sp	-39.2069444'S	-72.9361111'W	LAU-LA
Virilastacus sp	-39.2080555'S	-72.9331944'W	LAU-LA
S. spinifrons	-39.94889'S	-73.1716666'W	LAU-LA
S. spinifrons	-40.0833333'S	-73.08333'W	LAU-LA

Table III. Occurrence records of Atlantic freshwater crayfish in sympatry or syntopy, and habitat type, from the scientific collections and new samplings. **UFRGS**: Crustacean Collection of Universidade Federal do Rio Grande do Sul, Rio Grande do Sul, Brazil. **Faxon W. 1898**. Observations on the Astacidae in the United States National Museum and in the Museum of Comparative Zoology, with descriptions of new species. Proceedings of the United States National Museum, 20: 642–694.

Atlantic species	**Decimal Coordinates**		**Sympatry or Syntopy**	**Habitat**	**Database**
Parastacus sp. *P. defossus* *P. brasiliensis*	-30.10159'S	-51.69422’W	Syntopy	Floodplains	Sampling and UFRGS
Parastacus sp *P. defossus*	-29.8936’S	-51.16000’W	Syntopy	Swamp forest	Sampling
Parastacus sp. *P. defossus*	-29.6885’S	-51.1089’W	Syntopy	Swamp forest	Sampling
Parastacus sp. *P. defossus*	-30.35325’S -30.34564’S	-51.58405’W -51.56899’W	Sympatry	Wetlands near to streams Swamp forest	Sampling
P. defossus *P. brasiliensis*	-30.19442’S	-51.09206’W	Sympatry	Wetlands near to streams Stream	Sampling
P. defossus *P. brasiliensis*	-30.11417’S	-51.14280’W	Sympatry	Wetlands near to streams Stream	Sampling
P. defossus *P. brasiliensis*	-30.16395’S	-51.07972’W	Sympatry	Wetlands near to streams Stream	Sampling
P. defossus *P. brasiliensis*	-30.1232’S	-51.11949’W	Syntopy	Spring	Sampling
P. defossus *P. brasiliensis*	-30.22011’S	-51.07339’W	Sympatry	Wetlands near to streams Stream	Sampling
P. defossus *P. brasiliensis*	-29.71846’S	-53.72892’W	Sympatry	Wetlands near to streams Stream	Sampling
P. pilimanus *P. varicosus*	-32.57361’S	-52.5661’W	Syntopy	Flooded grassland	UFRGS
P. saffordi *P. varicosus*	-28.5685’S	-49.4352’W	Syntopy	Stream	UFRGS
P. defossus *P. saffordi*	-34.92173’S	-56.1642’W	Syntopy	Unknown	Faxon 1898

Table IV. Occurrence records of Pacific freshwater crayfish in sympatry or syntopy, and habitat type, from the scientific collections. **LAULA**: Laboratorio de Astacología, Universidad de Los Lagos, Osorno, Chile. **Rudolph EH. 2010**. Sobre la distribución geográfica de las especies chilenas de Parastacidae (Crustacea: Decapoda: Astacidea). Boletín de Biodiversidad de Chile, 3: 32–46.

Pacific species	Decimal Coordinates		Sympatry or Syntopy	Habitat	Database
P. pugnax *S. spinifrons*	-33.05'S -33096'S	-71.633'W -71.638'W	Sympatric	Wetland and stream	Rudolph 2010
P. pugnax *S. spinifrons*	-35.433'S	-71.666'W	Sympatric	Wetland and stream	Rudolph 2010
P. pugnax *S. spinifrons*	-35.702'S	-71.7007'W	Sympatric	Wetland and stream	Rudolph 2010
P. pugnax *S. spinifrons*	-36.582108'S -36.55074'S	-72.01632'W -72.05087'W	Sympatric	Wetland and stream	Rudolph 2010
P. pugnax *S. spinifrons*	-36.4'S	-71.9833'W	Sympatric	Wetland and stream	Rudolph 2010
P. pugnax *S. spinifrons*	-36.599'S	-72.10058'W	Sympatric	Wetland and stream	Rudolph 2010
P. pugnax *S. spinifrons*	-36.551674'S -36.5833'S	-72.05093'W -72.0167'W	Sympatric	Wetland and stream	Rudolph 2010
P. pugnax *S. spinifrons*	-36.1167'S	-72.7833'W	Sympatric	Wetland and stream	Rudolph 2010
P. pugnax *S. spinifrons*	-36.766667'S	-73.11666'W	Sympatric	Wetland and stream	Rudolph 2010
P. pugnax *S. spinifrons*	-38.716667'S -38.6667'S	-73.10'W -73.15'W	Sympatry	Wetland and stream	Rudolph 2010
Virilastacus sp *P. nicoleti* *S. spinifrons*	-39.344'S -39.31667'S -39.4'S	-73.17667'W -73.2'W -73.2'W	Sympatry and Syntopy	Semi-marshland	LAU-LA
P. nicoleti *V. araucanius*	-46. 56'S	-72°55'W	Syntopy	Semi-marshland	LAU-LA
V. retamali *V. rucapihuelensis* *P. nicoleti* *S. spinifrons*	-40. 58333'S	-73.56667'W	Sympatry and Syntopy	Peatland Semi-marshland Semi-marshland Stream	LAU-LA
P. pugnax *V. araucanius*	-36.75'S -36.7666'S	-73.0167'W -73.01666'W	Syntopy	Semi-marshland	Rudolph 2010
P. pugnax *V. araucanius*	-36.9333'S -36.9833'S	-72.91666'W -72.9167'W	Syntopy	Semi-marshland	Rudolph 2010
P. pugnax *V. jarai*	-37.4833'S -37.4444'S	-72.2833'W -72.31031'W	Syntopy	Semi-marshland	Rudolph 2010

Table V. Distribution of freshwater crayfish species in South America and burrow type, according to Horwitz & Richardson (1986).

Buckup & Rossi 1980: Buckup L, Rossi A. 1980. O Gênero *Parastacus* no Brasil (Crustacea, Decapoda, Parastacidade). Rev. Bras. Biol., 40: 663–681. **Gomes et al.:** Gomes KM, Ribeiro FB, Rudolph EH, Araujo PB. 2017. Distribution, sympatry and habitat characterization of South America crayfishes (Crustacea: Decapoda: Parastacidae). Unpublished. **Ribeiro et al. 2016:** Ribeiro FB, Buckup L, Gomes KM, Araujo PB. 2016. Two new species of South American freshwater crayfish genus *Parastacus* Huxley, 1879 (Crustacea: Decapoda: Parastacidae). Zootaxa, 30, 4158(3): 301–24. DOI 10.11646/zootaxa.4158.3.1. **Ribeiro et al. 2017:** Ribeiro FB, Huber AF, Schubart CD, Araujo PB. 2017. A new species of *Parastacus* Huxley, 1879 (Crustacea, Decapoda, Parastacidae) from a swamp forest in southern Brazil. Nauplius, 25: 1–14. DOI 10.1590/2358-2936e2017008. **Rudolph 2002:** Rudolph EH. 2002. Sobre la biología del camarón de río *Samastacus spinifrons* (Philippi, 1882) (Decapoda, Parastacidae). Gayana, 66(2): 147–159. **Rudolph 2010:** Rudolph EH. 2010. Sobre la distribución geográfica de las especies chilenas de Parastacidae (Crustacea: Decapoda: Astacidea). Boletín de Biodiversidad de Chile, 3: 32–46. **Rudolph 2013:** Rudolph EH. 2013. *Parastacus pugnax* (Poeppig, 1835) (Crustacea, Decapoda, Parastacidae): conocimiento biológico, presión extractiva y perspectivas de cultivo. Lat. Am. J. Aquat. Res., 41(4): 611–632. **Rudolph 2015:** Rudolph EH. 2015. Current state of knowledge on *Virilastacus* species (Crustacea, Decapoda, Parastacidae). Lat. Am. J. Aquat. Res., 43(5): 807–818.

Species	Country (ies)	Burrow Type	Habitat	Database
***Parastacus* sp**	Brazil (RS) and Argentina	Type 1a, b and Type 2	Streams, floodplains, wetlands associated with streams, small pools within forest and temporary puddle	Gomes et al. 2017 (unpublished data)

P. brasiliensis	Brazil (RS)	Type 1a, b and Type 2	Springs and streams (low order)	Buckup & Rossi 1980, Gomes et al. 2017 (unpublished data)
P. pilimanus	Brazil (RS) and Argentina	Type 1a, b and Type 2	Streams, floodplain river and flooded field	Buckup & Rossi 1980, Gomes et al. 2017 (unpublished data)
P. saffordi	Brazil (RS and SC), Uruguay and Argentina	Type 1a, b and Type 2	Streams, wetlands associated with streams and small pools within forest	Buckup & Rossi 1980, Gomes et al. 2017 (unpublished data)
P. defossus	Brazil (RS) and Uruguay	Type 2	Swamp forest, flooded field, springs, floodplain and wetlands near to streams.	Buckup & Rossi 1980, Gomes et al. 2017 (unpublished data)
P. caeruleodactylus	Brazil (RS)	Type 2	Swamp forest	Ribeiro et al. 2016
P. laevigatus	Brazil (SC)	Unknown	Unknown	Unknown
P. tuerkayi	Brazil (SC)	Type 2	Flooded grassland	Ribeiro et al. 2017

P. nicoleti	Chile (south-central)	Type 2	Semi-marshland, perennially green areas	Rudolph 2010
P. pugnax	Chile (south-central)	Type 2	Semi-marshland, perennially green areas	Rudolph 2013
Virilastacus araucanius	Chile (south-central)	Type 2	Semi-marshland, perennially green areas	Rudolph 2015
V. jarai	Chile	Type 2	Semi-marshland fragment, perennially green areas	Rudolph 2015
V. retamali	Chile	Type 2	Geogenous peatlands	Rudolph 2015
Samastacus spinifrons	Argentina and Chile	Type 1a	River and Lakes	Rudolph 2002

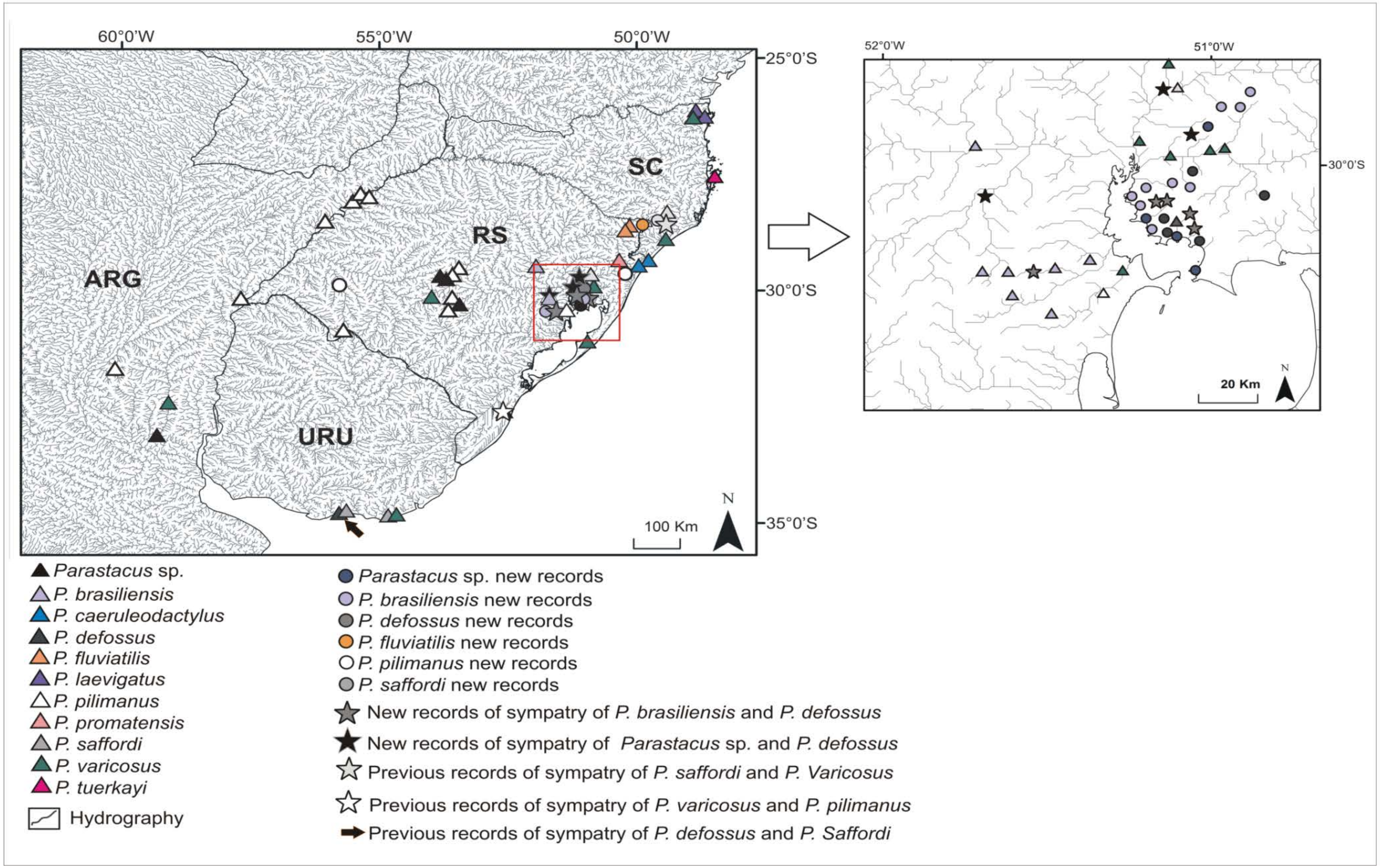

Figure 1. Distribution of species of Atlantic freshwater crayfish based on previous and new records, from scientific collections and new samplings. Black arrow indicates the syntopy mentioned by Faxon (1898).

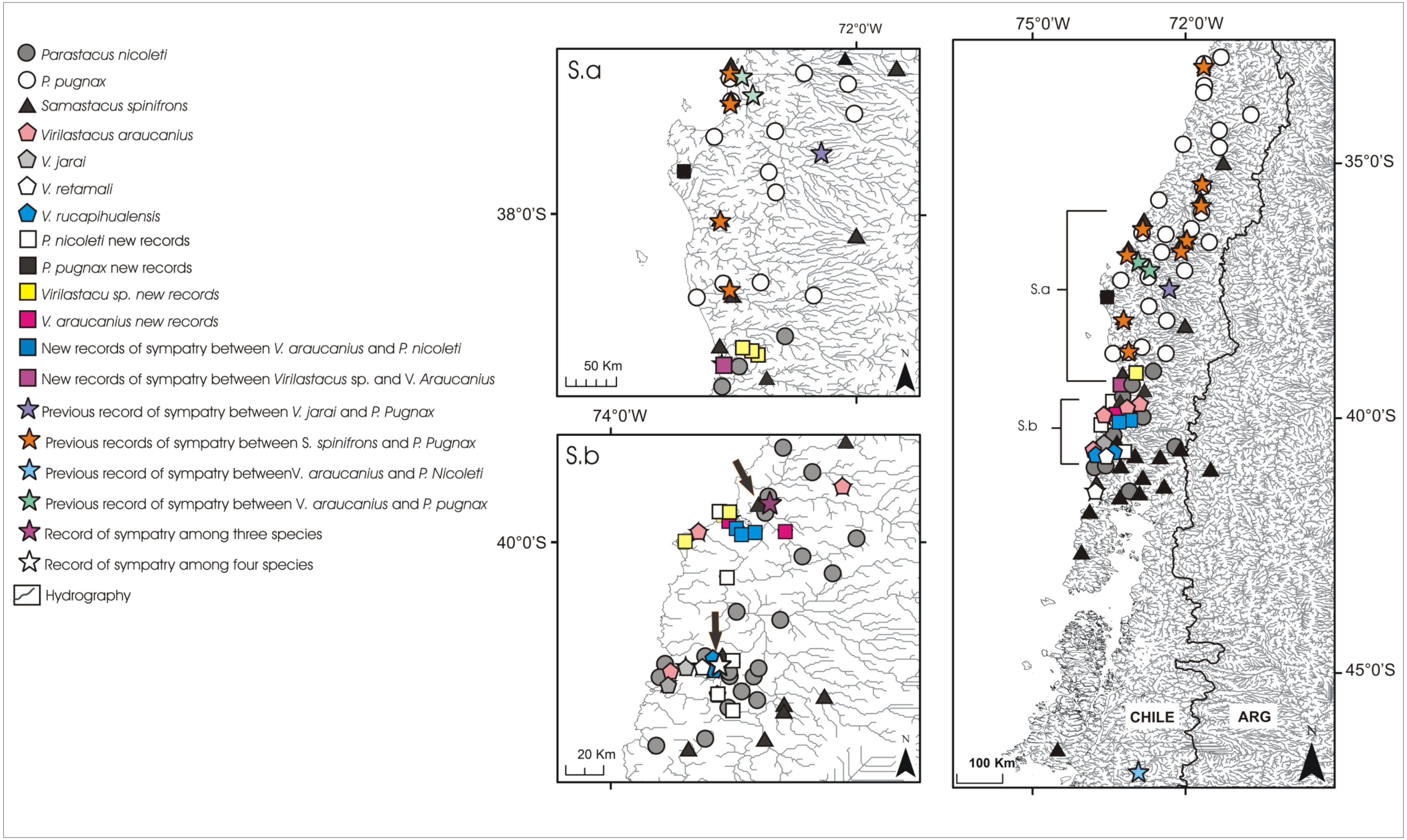

Figure 2. Distribution of species of Pacific freshwater crayfish based on previous and new records, from scientific collections and bibliography. S. a: Sympatry zone "a"; S. b: Sympatry zone "b"; the black arrows are highlighting the sympatry of three or four species in a range of up to 5 km.

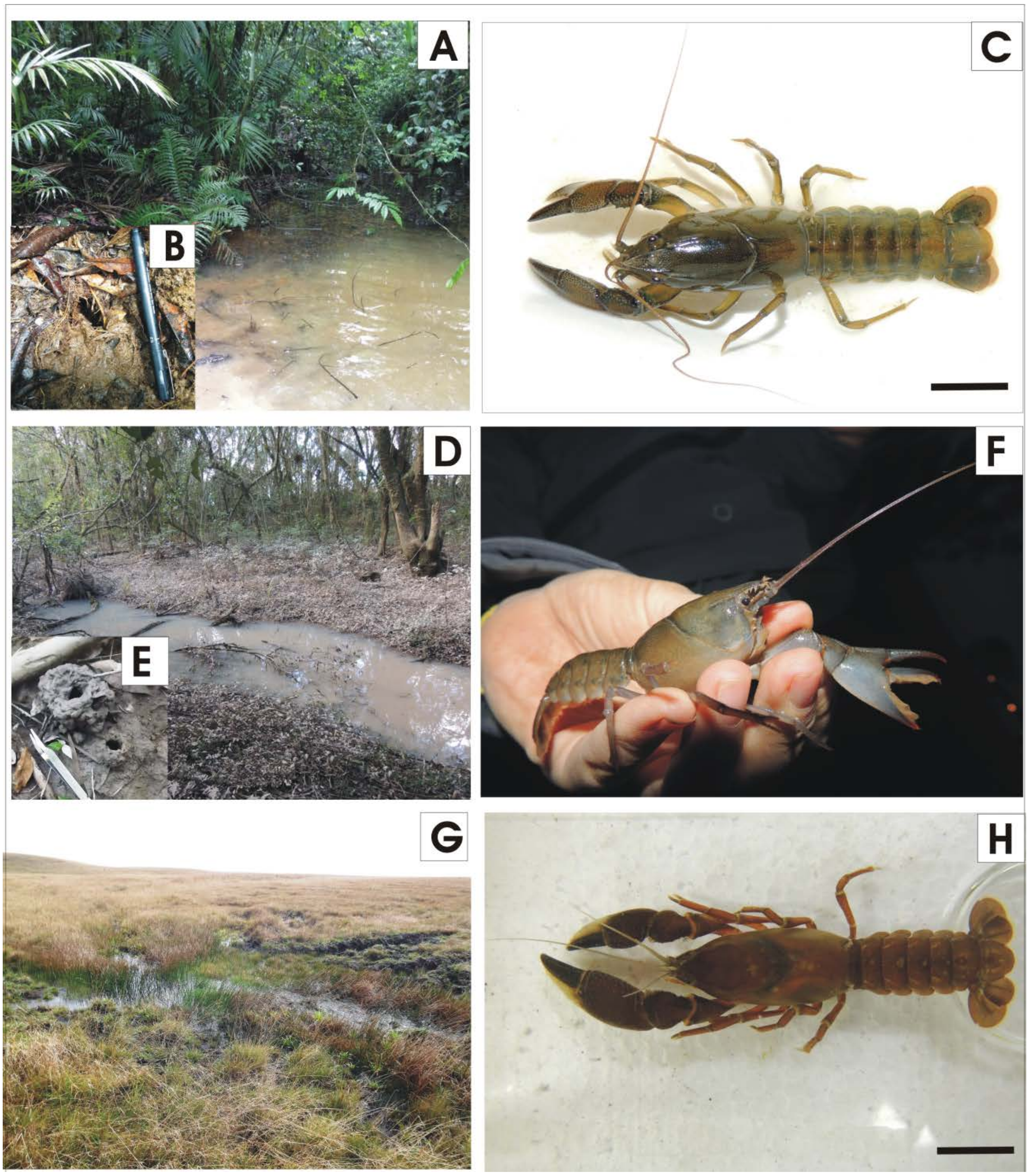

Figure 3. New occurrences records of Atlantic crayfishes in wetlands situated in Brazil according to Junk et al. (2014). **A**. Rain water fed wetlands in small depressions; **B**. Burrow with simple openings (type 2); **C**. *Parastacus saffordi* Faxon 1898; **D**. River floodplain; **E**. Burrow (type 2) with ornamented opening (chimney), height of 5 cm. **F**. *Parastacus* sp. **G**. Flooded grassland of altitude; **H**. *Parastacus fluviatilis* Ribeiro & Buckup 2016.

5.2. Capítulo II

Artigo formatado conforme a revista:

Studies on Neotropical Fauna and Environment

Gomes et al. (2017)

Species distribution modeling and climatic changes: a study with South American freshwater crayfishes

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ABSTRACT

Numerous threats to the survival of freshwater crayfish from South America have been verified. However, climate change has not yet been considered a potential threat. The aims of this study are: (1) to know the present distribution and (2) to project the effects of climate changes on the distribution of freshwater crayfishes in South America; in addition (3) to estimate the expansion or reduction in the size of geographical range for the groups of species. We used 216 occurrence sites of 21 species that were gathered in four groups: aquatic, semi-aquatic, Atlantic semi-terrestrial and Pacific semi-terrestrial. Models were performed for present and future (2070) scenarios. The suitability areas of present distribution were < 100, 000 km² for all groups and the performance of the model were considerate good to optimal. The temperature was the variable that more contributed to build the models (present and future). Potentially, it was responsible for the distribution area expansion of the species in 2070. Thus, the crayfishes can occupy higher and less disturbed regions. We suggest that immediate attention must be paid especially to the Atlantic and Pacific semi-terrestrial groups, focusing on their conservation, since their occurrence sites have environmental complexity and high fragmentation. In addition, the wetlands tend to disappear fast due to climate influences on dynamics of freshwater ecosystems.

Keywords: Parastacids; Future scenarios; Endangered species; Freshwater crustaceans

Introduction

Freshwater ecosystems comprise a diversity of habitats (surface waters, subterrenean waters, and riparian systems) that support a rich, endemic, and fragile aquatic fauna (Ward & Tockner 2001, Strayer & Dudgeon 2010). These ecosystems also provide essential services to the maintenance of biodiversity and to the human demand (Ward & Tockner 2001, Strayer & Dudgeon 2010). The interaction among vertical, horizontal, and groundwater gradients contributes to the complexity and heterogeneity of the landscape and influences the aquatic biota structure (Ward & Tockner 2001). Nevertheless, the connection between mosaics of the landscape habitat is easily lost due to human activities.

Deforestation, reservoirs and channels construction, basins transposition, wetlands drainage, excessive irrigation and water supply directly affect the integrity of freshwater systems (Tundisi 2003, GWSP 2004). These impacts on riparian zones and watersheds promote an unbalance of sediments, organic matter, water, and nutrients input to lakes and rivers (Strayer & Dudgeon 2010). Besides the worldwide environmental degradation and habitat loss, overexploited fisheries, introduction of alien species (Dudgeon 2006, Strayer & Dudgeon 2010), and climatic changes have been also considered a threat to freshwater biodiversity (Richman et al. 2015).

Climatic changes are also considered a silent threat to the dynamics of freshwater ecosystems, and, along with others anthropogenic drivers, have been considered responsible for the global warming (IPCC 2014). Since the industrial revolution, gas emissions in the atmosphere have been increasing the concentrations of dioxide carbon, methane, and nitrous oxide and are related to global climate changes (IPCC 2014). The effects of high temperatures on water global cycle are not expected to be uniform over the 21st century (Trenberth 2013). Therefore, the global warming can increase the contrast between wet and dry climates through different seasons and regions (Trenberth 2013). The intensification and the increased frequency of flood and drought events are also a projection for future scenarios (IPCC 2007). The expansion of the dominance of dry zones in subtropical regions is also expected (Trenberth 2013).

The projection of climatic scenarios has been made for several species as an attempt to predict habitats in the future in order to plan and discuss conservation measures. Regardless of the period of time used for the projection, simulations show that organisms will have reduced distribution areas (Bond et al. 2011, Zank et al. 2014, Brown et al. 2015). In species with high environmental suitability, the reduction of the distribution area may be severe (Brown et al. 2015) or even total in the next 60 years (Zank et al. 2014). Alteration in hydrological regime, temperature, organic matter concentration, and salinity can cause changes in the distribution of the freshwater biota (Bond et al. 2011, Caputi et al. 2014, Jappense et al. 2015). Two-thirds of the Australian freshwater crayfishes are at extinction risk due to poor connectivity between areas of suitable habitat (Richman et al. 2015). These species are displaced across the landscape in order to search for water resources (water table or stream) (Cook et al. 2014). Therefore, maintaining the environmental heterogeneity and connectivity are important for the maintenance of populations.

Freshwater crayfishes of family Parastacidae Huxley 1879 are restrict to the South Hemisphere, occurring in South America, Madagascar, New Zealand, New Guinea, and Australia (Crandall & Buhay 2008, Kawai & Crandall 2016). South American crayfishes occupy permanent and temporary habitats in southern Brazil (Rio Grande do Sul and Santa Catarina states), Uruguay, eastern Argentina, and central-south Chile (Buckup & Rossi 1980, 1993, Rudolph 2015, Ribeiro et al. 2016, 2017). Climate changes have not yet been considered a threat to the three native genera, *Parastacus* Huxley, 1879 (eleven species), *Virilastacus* Hobbs, 1991 (four species), and *Samastacus* Riek, 1971 (one species). Nevertheless, other threats common to aquatic ecosystem are frequently recorded (Buckup 2010 a,b,c,d,e,f,g,h, Ribeiro et al. 2016, 2017).

Three recently described species were considered Endangered according to IUCN criteria (Ribeiro et al. 2016, 2017). Criteria used for this classification were the naturally restricted distribution and the presence of threats such as rice planting, presence of rainbow trout (exotic fish - *Oncorhynchus mykiss* Walbaum, 1792), and urbanization (Ribeiro et al 2016, 2017). The lack of adequate data concerning eight species of South American crayfishes was an impediment to the evaluation of their conservation status in a recent survey (Almerão et al. 2015). This lack of information is a constant obstacle for the knowledge of the crustacean fauna worldwide, mainly in freshwater decapods (Cumberlidge et al. 2010). In addition, the presence of cryptic species may be frequent among the Atlantic species (Ribeiro, F. B. pers.comm.); this fact combined with the habitat degradation and others threats make the study of these species very challenging.

The aims of this study are: (1) to know present distribution and (2) to project the effects of climate changes on the distribution of freshwater crayfishes in South

America; in addition (3) to estimate the expansion or reduction in size of geographical range for the groups of species.

Material and Methods

Species data

Habitats occupied by South American crayfishes include lakes, springs, streams, and wetlands (Buckup & Rossi 1980, Rudolph 2010, 2013, 2015, Gomes et al. 2017 unpublished data). An interesting feature of these organisms is the burrowing behavior. All species are potentially burrowers, but the intensity of the behavior is variable among them (Berril & Chenoweth, 1982).

Samastacus spinifrons (Philippi 1882) belongs to a monotypic genus that occurs in lakes and streams in Chile and Argentina; its burrower capacity is reduced, creating shallow burrows in river banks (Rudolph 2002). The genus *Virilastacus* is found exclusively in Chilean wetlands. *Virilastacus retamali* Rudolph & Crandall (2007) is restricted to peatlands and *V. jarai* Rudolph & Crandall (2012) occurs in a single semi-marshland locality (Rudolph et al. 2015). *Virilastacus rucapihuelensis* Rudolph & Crandall (2005) and *V. araucanius* Faxon (1914) are also found in semi-marshlands ("vegas" or "hualves") and the structure of burrows are similar between the two species, formed by several openings with depth lower than 1 meter (Rudolph et al. 2015).

The genus *Parastacus* has a disjoint distribution, and according to the occurrence data, these crayfishes are absent in the extreme west of Argentina. However, a previous record of *P. pilamanus* (von Martens, 1869) in Catamarca was analyzed in the Museo Argentino de Ciencias Naturales Bernadino Rivadavia (Buenos Aires, Argentina) and was taxonomically confirmed. Nevertheless, it was not possible to attribute the geographical coordinate for this locality due to the lack of information.

From now on, we will use the term "Atlantic" in reference to species that occur in Brazil, Uruguay, and Argentina; and "Pacific" for species distributed in Chile. Pacific species comprise *P. nicoleti* (Philippi 1882) and *P. pugnax* (Poepigg 1835) which mainly inhabit marshlands (Tab. I). The burrowing behavior is strong in these species; they build deep galleries composed by several openings in the soil surface (Rudolph 2010, 2013, 2015). Atlantic species are suitable to occur in springs, streams, and wetlands of several configurations (floodplains, temporary ponds, highland grasslands, lowland forests, and peatlands) (Buckup & Rossi 1980, Gomes et al. 2017 unpublished data) (Tab. I). Some crayfish species occur mainly in permanent streams, but can also temporarily occupy wetlands (e.g. *P. brasiliensis* (von Martens 1869)). These species have moderate burrower capacity, building burrows with tunnels with depth lower than1 meter and composed by 1 to 5 openings. Other species inhabit only wetlands and have strong burrowing behavior, similar to Pacific species of *Parastacus*.

Biotic Data

The total of 326 occurrence records of 21 species of freshwater crayfish was compiled from the Coleção Científica da Universidade Federal do Rio Grande do Sul, Brazil (UFRGS), Museu de Ciência e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, Brazil (MCP-PUCRS), Museo Argentino de Ciencias Naturales Bernadino Rivadavia, Buenos Aires, Argentina (MACN-BA), and published literature. We excluded records with taxonomic uncertainties and localities without detailed information. These data were gathered according to the freshwater habitat occupied by species and the burrows classification followed what was proposed by Horwitz & Richardson (1986). Therefore, we grouped the species as follows: **(1) aquatic**, species exclusive to streams and lakes (i.e. *Samastacus spinifrons*); **(2) semi-aquatic,** species that occur in streams and wetlands associated with waterbodies or water tables (e.g. *Parastacus saffordi* Faxon 1898); and **(3) semi-terrestrial**, species that occur in different types of wetlands and always connected to the water table (e.g. *P. pugnax*) (Tab. I). We used this approach to facilitate the assessment in an environmental point of view and minimize the effects of the lack of data from the species.

Spatial autocorrelation was corrected using spatially rarefy occurrence data tools present in SDMtools (Brown, 2014) and performed in ArcGis 10.2 (ESRI 2014). This method corresponds to the Euclidian distance that randomly selects a single presence within a shared area; then, each selected record is independent and located in regions of high climatic heterogeneity (Brown, 2014). The distance value used was a minimum of 5km² and maximum of 25 km² extension. The graduated filtering method can maximize the number of localities that are spatially independent in studies with limited occurrence points (Brown, 2014).

Climate data

Bio-climate data obtained from Worldclim database [www.worldclim.org (Hijmans et al. 2005)], along with the 19 variables (arc 30') used, were the same for present and future scenarios The climatic layers were evaluated by Person correlation in SDMtools (Brown 2014) and those which had I $r^2 < 0.7$ I were selected for the distribution modeling (Ranc et al. 2016). Each crayfish group had their bioclimatic variables selected: **aquatic group (**altitude, bio 3, bio 10, bio12, and bio 15); **semi-aquatic group: Atlantic semi-terrestrial** (altitude, bio 1, bio 2, bio 3, bio 6, bio 8, and bio 12); and **Pacific semi-terrestrial** (altitude, bio 3, bio 4, bio 5, bio 9, bio 12, and bio 15) (Tab. II).

Projections of climatic changes were performed for the period corresponding to the year 2070 [BCC-CSM1-1 (IPCC5)] using RCPs 6, which correspond to moderate emissions scenario (Brown et al. 2016). All bio-climatic layers were processed in ArcGis 10.2 (ESRI 2014), datum WGS84, with spatial extent variable and according to crayfish group. The processes of preparing Wordclim data to MaxEnt were made in SDMtools (Brown 2014).

Distribution models

Species distribution modeling (SDM sensu, Elith & Leathwick 2009) was performed in MaxEnt v.3.3.3k program (Phillips et al. 2006) associated with SDMtools (Brown 2014). The maximum entropy (MaxEnt) is a presence-only method which predicts the probability of distribution from incomplete information (Phillips et al. 2006). Thus, this method is used for species with limited knowledge of occurrence records (Pearson et al. 2007). The program uses a percentage of randomly chosen presence data to create the prediction model, while the other percentage is retained to test the resulting model (Phillips et al. 2006). We select 80% of occurrence sites as training data and 20% to test the resulting model.

The background extension for building the model was based on the knowledge of the distribution within the hydrographic basins, habitat, and biology of the crayfishes. So, each crayfish group has a different background extension. We adopted this measure because all crayfishes are associated with water resources (i.e. permanent waterbodies or groundwater) and the background extension can influence the accuracy of the model (Lobo et al. 2008).

The fit and model performance were assessed through k-fold cross-validation (k = 15) which considers the spatial segregation of the localities used in the construction model (Elith et al. 2011). The accuracy of the model was tested using the value of the area under curve (AUC) of receiver operating characteristic (ROC). The calculation of AUC considers the presence (corresponding to the suitable area) versus background (referent to the unsuitable area) (Phillips et al. 2006). The generated value is unique and independent of threshold which also evaluates the model performance (Swets 1988, Phillips et al. 2006). AUC values range from 0 to 1, with maximum accuracy achieving 1, and values < 0.5 corresponding to worse performances (Swets 1988, Veloz 2009). The AUC is sensitive to background extension (Lobo et al. 2008). Posteriorly, this model was projected for the year 2070.

The threshold used to define areas with greater climatic were different for the groups. For Atlantic and Pacific semi-terrestrial and semi-aquatic groups we used 0,50; and aquatic was 0,60.

The models used to compare the present and future scenarios correspond to average model generated by the MaxEnt program. The potential changes verified in the environmental projections were measured by Image J program (Rasband 2016). We selected the color threshold according to the region of interest and calculated the area size (km^2) from pixels number.

Results

The spatially rarefied analysis selected 216 sites to 21 species of freshwater crayfishes distributed in four groups. The richness of each group was as follows: nine species for the Atlantic semi-aquatic group (Tab. III), six species for the Atlantic semi-terrestrial group and five species for the Pacific semi-terrestrial group (Tab. IV), and one species for the Pacific aquatic group (Tab. V).

All groups showed regions with climatic suitability achieved by models with AUC values between 0.7 and ~1.00 (Tab. VI). The Atlantic and Pacific semi-terrestrial groups had higher AUC values, followed by the semi-aquatic and aquatic groups. Environmental variables that most contributed for the models were: mean diurnal range (bio 2), maximum temperature of warmest month (bio 5), annual precipitation (bio 12), and altitude (Tab. VII). The variables similarly contributed for each group within different scenarios. The temperature was more representative for almost all groups, except for aquatic group; and the precipitation contributed only to the prediction of the semi-aquatic group. The altitude, which was indirectly related to temperature, was the only variable that strongly contributed for *S. spinifrons* performance of model. For the semi-aquatic group, the temperature and precipitation contributed for building the model.

The high suitability areas for occurrence of most crayfishes groups were concentrated mainly in the coastal region of Brazil and Chile. The projected range did not exceed 100,000 km² (Tab. VII). The semi-aquatic group showed suitable areas along the coastal region of Rio Grande does Sul and Santa Catarina states (Brazil) and also in the central-west basins of Rio Grande do Sul, comprising an estimated distribution of 86,641 km² (Fig. 1). The west region of Argentina was not suitable for the occurrence of this group, with a threshold of ≤ 0.30. We obtained similar results for the Atlantic semi-terrestrial group, which distribution area was estimated in 42,023 km² (Fig. 2). For Chilean groups, Pacific semi-terrestrial and aquatic, the distribution was discontinuous along the coastal region and was estimated in 28,412 km² (Fig. 3) and 51,114 km² (Fig. 4), respectively.

Changes verified in the species distribution among climatic scenarios were subtle. *Samastacus spinifrons* (aquatic group) showed the greater alteration, expanding its occurrence in 14% through the Chilean territory. Atlantic semi-terrestrial, Pacific semi-terrestrial group and Atlantic semi-aquatic groups also expanded their distribution in 5.5%, ~1% and 2.4%, respectively. The distribution of these species was more restricted to coastal regions. Our predictions did not showed loss of species; however, some localities may disappear because other factors will be acting upon these species' occurrence.

Discussion

The disjointed distribution of the freshwater crayfishes motivated an analysis using groups of species instead one species only. Similarly, during the process of choosing the background extension for the semi-terrestrial group we found problems while used a background covering Atlantic and Pacific semi-terrestrial groups. The model could not predict adequate areas for the occurrence of the Atlantic semi-terrestrial. The distribution pattern and diversification process of South American crayfish have been related to Gondwana breakup and uplift of Andes (Toon et al. 2010). Besides acting as a vicariant barrier, the uplift modified drainages and regional climate, modifying the landscape of Chile (Hoorn et al. 2010, Leubert & Weigend et al. 2014, Lessmann et al. 2016). During the process of choosing the background extension for the semi-terrestrial group, we realize the influence of climate in model prediction. The use of a background covering Atlantic and Pacific semi-terrestrials groups was not adequate to make predictions for the Atlantic portion; therefore, we opted for a distinct background.

Temperature was the bioclimatic variable more representative during the running of models and it can directly affect the survival of crustaceans. Some effects are known both negative and positive effects on aquatic species such as spawning, larval phase, recruitment, growth, migration, and size at maturity (Caputi et al. 2010). The increase of temperature induces a hormonal shift in organisms, promoting an antagonist effect on growth rates (decreasing inter-molt periods and reducing the size increment at each molt) (Hartnoll 2001). The frequency of molt events can increase the risk of predation and cannibalism in crustaceans; therefore, the rapid hardening of the exoskeleton minimizes the mortality risk (Stein 1977). High temperatures can promote a stressful effect on ovarian maturation, impairing the fecundity and eggs viability (Tropea et al. 2015). In lower temperatures, the fecundity is maintained and is generally high (Aguilar-Alberola et al. 2014). Lower pH levels (< 5) also affect the survival of freshwater species because the process of exoskeleton calcification is ceased in post-molt stages (Malley 1980). In temporary habitats, changes in precipitation regimes and evaporation can increase salinity, shorten wet periods, and decrease the connectivity between pools (Tuytens et al. 2014).

High suitability areas were small in both present and future models for all groups. The lack of occurrence records for Atlantic semi-terrestrial may have decreased the prediction power. Hence, the results concerning this group should be treated with caution due to the low occurrence number. Therefore, the MaxEnt program has been considered an adequate method to predict the species distribution for presence-data only and for organisms with few presence points (Pearson et al. 2007, Merrow et al. 2013).

Predictions to 2070 were insufficient to determine the degree of vulnerability of freshwater crayfishes. The results showed subtle expansions of species distribution and a decrease of future habitat suitability. The potential displacement of the species to higher areas, observed mainly in Chilean groups, can represent a refuge to crustaceans in response to climate shifts. This displacement may happen because highlands have a milder weather and perturbations may be lower. Thus, each group has a different climatic demand, since climate changes are not homogeneous across geographical range (Zank et al. 2014). A species will only be affected if its distribution coincides with regions of higher climatic shift (Zank et al. 2014). Nevertheless, it does not mean that consequences cannot be perceived in non-projected areas.

Other factors that were not considered in our models could be acting in present and future distribution of species, such as their regional dynamic and biological features (e.g. dispersal ability) (Tuytens et al. 2014). Furthermore, the models do not predict connectivity between areas. If there is no connection between habitats, the species cannot colonize them.

Crayfishes burrowing behavior may be an important feature to minimize the effects of climate changes on its survival. This ability allows that species to inhabit several habitats types and displace across heterogeneous landscape (Johnston & Robson 2009). The areas that are around rivers were suggested as potential habitats for crayfish in future projections and can be important regions for the maintenance of populations. When there are disturbances within or near the stream, these areas are used as refuge (March & Robinson 2006).

Chilean wetlands and floodplains had decreased climatic potential for crayfish occurrence. These environments can disappear if changes projected in configuration actually occur, such as reduction of riparian forest and woodlands and expansion of terrestrial areas (Colloff et al. 2016). Similar scenarios are predicted to glaciers peatland in Chile; the retreat of habitats might extinct restricted populations from this environment (Quenta et al. 2016), such as *Virilastacus retamali* (Rudolph et al. 2015).

Species of genera *Virilastacus* and *Parastacus* inhabit different types of wetlands and are already at extinction risk by habitat degradation and narrow range criteria (Almerão et al. 2015, Ribeiro et al. 2016, 2017). In addition, approximately 64—71% of the wetlands have already been lost and this reduction is more accentuated in inland territories than in coastal wetlands (Davidson 2014).

The semi-aquatic and aquatic groups have association with permanent water bodies and the water stress may not be the first effects that should be considered; instead, shifts in the catchment structure of water bodies and vegetation are predicted to be the main consequences (Dyer et al. 2014). In temporary habitats, shifts in their dynamic may affect the length of flood and reduce the habitat available for species (Hulsmans et al. 2008). In permanent or seasonal environments, the human consumption and environmental transformations to obtain access to water may be aggravated with changes in precipitation and evaporation (Dyer et al. 2014).

South American crayfishes suffered strong influence of temperature during the performance of distribution modelling. Nonetheless, other factors can be important for species distribution and knowledge about their vulnerability (e.g. soil type and urbanization). The limitations in performing distribution analysis of South American species are the lack of data with similar refinement, as well as the total lack of occurrence data. Most species are subsampled and present restrict distribution. We suggest that immediate attention must be paid specially to the Atlantic and Pacific semi-terrestrial groups, focusing on their conservation, since their occurrence sites have environmental complexity, high fragmentation, and rapid degradation (Almerão et al. Junk et al. 2014, Davidson 2014).

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Tables, Figures and Captions

Table I. Distribution and habitat type of the freshwater crayfish in South America, according to their groups. **UFRGS:** Coleção Científica da Universidade Federal do Rio Grande do Sul, Brazil. **MACN-BA:** Museo Argentino de Ciencias Naturales Bernadino Rivadavia, Buenos Aires, Argentina. **Buckup & Rossi 1980:** Buckup L, Rossi A. 1980. O Gênero *Parastacus* no Brasil (Crustacea, Decapoda, Parastacidade). Rev. Bras. Biol., 40: 663–681. **Gomes et al.:** Gomes KM, Ribeiro FB, Rudolph EH, Araujo PB. 2017. Distribution, sympatry and habitat characterization of South America crayfishes (Crustacea: Decapoda: Parastacidae). Studies on Neotropical Fauna and Environment. Unpublished. **Ribeiro et al. 2016:** Ribeiro FB, Buckup L, Gomes KM, Araujo PB. 2016. Two new species of South American freshwater crayfish genus *Parastacus* Huxley, 1879 (Crustacea: Decapoda: Parastacidae). Zootaxa, 30, 4158(3): 301–24. DOI 10.11646/zootaxa.4158.3.1. **Ribeiro et al. 2017:** Ribeiro FB, Huber AF, Schubart CD, Araujo PB. 2017. A new species of *Parastacus* Huxley, 1879 (Crustacea, Decapoda, Parastacidae) from a swamp forest in southern Brazil. Nauplius, 25: 1–14. DOI 10.1590/2358-2936e2017008. **Rudolph 2002:** Rudolph EH. 2002. Sobre la biología del camarón de río *Samastacus spinifrons* (Philippi, 1882) (Decapoda, Parastacidae). Gayana, 66(2): 147–159. **Rudolph 2010:** Rudolph EH. 2010. Sobre la distribución geográfica de las especies chilenas de Parastacidae (Crustacea: Decapoda: Astacidea). Boletín de Biodiversidad de Chile, 3: 32–46. **Rudolph 2013:** Rudolph EH. 2013. *Parastacus pugnax* (Poeppig, 1835) (Crustacea, Decapoda, Parastacidae): conocimiento biológico, presión extractiva y perspectivas de cultivo. Lat. Am. J. Aquat. Res., 41(4): 611–632. **Rudolph 2015:** Rudolph EH. 2015. Current state of knowledge on *Virilastacus* species (Crustacea, Decapoda, Parastacidae). Lat. Am. J. Aquat. Res., 43(5): 807–818.

Table II. List of 19 bioclimatic layers from Worldclim — Global Climate Data (Hijmans et al. 2005) used for the distribution modeling of the South American crayfishes.

Table III. Occurrence records of the freshwater crayfishes selected from the spatially rarefy for semi-aquatic group. **UFRGS**: Coleção Científica da Universidade Federal do Rio Grande do Sul, Brazil. **MCP-PUCRS**: Museu de Ciência e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, Brazil. **MACN-BA**: Museo Argentino de Ciencias Naturales Bernadino Rivadavia, Buenos Aires, Argentina.

Table IV. Occurrence records of the freshwater crayfishes selected from the spatially rarefy for two groups: Atlantic semi-terrestrial and Pacific semi-terrestrial. **UFRGS**: Crustacean Collection of Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil. **Rudolph 2010:** Rudolph EH. 2010. Sobre la distribución geográfica de las especies chilenas de Parastacidae (Crustacea: Decapoda: Astacidea). Boletín de Biodiversidad de Chile, 3: 32–46.

Table V. Occurrence records of the freshwater crayfishes selected from the spatially rarefy for aquatic group - *Samastacus spinifrons*. **Rudolph 2010:** Rudolph EH. 2010. Sobre la distribución geográfica de las especies chilenas de Parastacidae (Crustacea: Decapoda: Astacidea). Boletín de Biodiversidad de Chile, 3: 32–46.

Table VI. Results summary of the distribution modeling (present and future scenarios) for freshwater crayfishes groups (semi-aquatic; Atlantic semi-terrestrial and Pacific semi-terrestrial; and aquatic). Area size of distribution in the present and in 2070 (expansion or reduction of area in future), as well as AUC values according to modelled scenarios.

Table VII. AUC mean with standard deviation (sd); and the percentage contribution of two most important bioclimatic variables to the distribution models of each crayfishes group in South America (semi-aquatic; Atlantic semi-terrestrial and Pacific semi-terrestrial; and aquatic). Alt: Altitude; Bio 1: Annual Mean Temperature; Bio 2: Mean Diurnal Range, calculated from Mean of monthly (max temp - min temp); bio12: Annual Precipitation (Hijmans et al. 2005).

Figure 1. Occurrence records of freshwater crayfishes, present and future projections of semi-aquatic group, and distribution expansion projected for 2070 (2.4%).

Figure 2. Occurrence records of freshwater crayfishes, present and future projections of Atlantic semi-terrestrial group, and distribution expansion projected for 2070 (5.5%).

Figure 3. Occurrence records of freshwater crayfishes, present and future projections of Pacific semi-terrestrial group, and distribution expansion projected for 2070 (~ 1%).

Figure 4. Occurrence records of freshwater crayfishes, present and future projections of Pacific aquatic group, and distribution expansion projected for 2070 (14%).

Table I. Distribution and habitat type of the freshwater crayfish in South America, according to their groups.

UFRGS: Coleção Científica da Universidade Federal do Rio Grande do Sul, Brazil. MACN-BA: Museo Argentino de Ciencias Naturales Bernadino Rivadavia, Buenos Aires, Argentina. **Buckup & Rossi 1980:** Buckup L, Rossi A. 1980. O Gênero *Parastacus* no Brasil (Crustacea, Decapoda, Parastacidade). Rev. Bras. Biol., 40: 663–681. **Gomes et al.:** Gomes KM, Ribeiro FB, Rudolph EH, Araujo PB. 2017. Distribution, sympatry and habitat characterization of South America crayfishes (Crustacea: Decapoda: Parastacidae). Unpublished. **Ribeiro et al. 2016:** Ribeiro FB, Buckup L, Gomes KM, Araujo PB. 2016. Two new species of South American freshwater crayfish genus *Parastacus* Huxley, 1879 (Crustacea: Decapoda: Parastacidae). Zootaxa, 30, 4158(3): 301–24. DOI 10.11646/zootaxa.4158.3.1. **Ribeiro et al. 2017:** Ribeiro FB, Huber AF, Schubart CD, Araujo PB. 2017. A new species of *Parastacus* Huxley, 1879 (Crustacea, Decapoda, Parastacidae) from a swamp forest in southern Brazil. Nauplius, 25: 1–14. DOI 10.1590/2358-2936e2017008. **Rudolph 2002:** Rudolph EH. 2002. Sobre la biología del camarón de río *Samastacus spinifrons* (Philippi, 1882) (Decapoda, Parastacidae). Gayana, 66(2): 147–159. **Rudolph 2010:** Rudolph EH. 2010. Sobre la distribución geográfica de las especies chilenas de Parastacidae (Crustacea: Decapoda: Astacidea). Boletín de Biodiversidad de Chile, 3: 32–46. **Rudolph 2013:** Rudolph EH. 2013. *Parastacus pugnax* (Poeppig, 1835) (Crustacea, Decapoda, Parastacidae): conocimiento biológico, presión extractiva y perspectivas de cultivo. Lat. Am. J. Aquat. Res., 41(4): 611–632. **Rudolph 2015:** Rudolph EH. 2015. Current state of knowledge on *Virilastacus* species (Crustacea, Decapoda, Parastacidae). Lat. Am. J. Aquat. Res., 43(5): 807–818.

Species	Group	Country (ies)	Habitat	Database
***Parastacus* sp. 1**	Atlantic semi-aquatic	Brazil (RS)	Streams and wetlands associated with low-order watercourse	Gomes KM. pers. obs.

***Parastacus* sp. 2**	Atlantic semi-aquatic	Brazil (RS)	Streams banks	Gomes KM. pers. obs.
***Parastacus* sp. 3**	Atlantic semi-aquatic	Brazil (RS) and Argentina	Streams, floodplains, wetlands associated with streams, small pools within forest and temporary puddle	Gomes et al. 2017 (unpublished data)
***Parastacus* sp. 4**	Atlantic semi-aquatic	Brazil (RS)	Streams	UFRGS
***Parastacus* sp. 5**	Atlantic semi-aquatic	Brazil (SC)	Streams	UFRGS
***Parastacus* sp. 6**	Atlantic semi-aquatic	Argentina	Streams	MACN-BR
P. brasiliensis	Atlantic semi-aquatic	Brazil (RS)	Springs and streams (low order)	Buckup & Rossi 1980, Gomes et al. 2017 (unpublished data)
P. pilimanus	Atlantic semi-aquatic	Brazil (RS) and Argentina	Streams, floodplain river and flooded field	Buckup & Rossi 1980, Gomes et al. 2017 (unpublished data)
P. saffordi	Atlantic semi-aquatic	Brazil (RS and SC), Uruguay and Argentina	Streams, wetlands associated with streams and small pools within forest	Buckup & Rossi 1980, Gomes et al. 2017 (unpublished data)

***Parastacus* sp. 7**	Atlantic semi-terrestrial	Brazil (RS)	Peatland	Gomes KM and Ribeiro FB pers. obs.
***Parastacus* sp. 8**	Atlantic semi-terrestrial	Brazil (SC)	Swamp forest	Gomes KM and Ribeiro FB pers. obs.
P. defossus	Atlantic semi-terrestrial	Brazil (RS) and Uruguay	Swamp forest, flooded field, springs, floodplain and wetlands near to streams.	Buckup & Rossi 1980, Gomes et al. 2017 (unpublished data)
P. caeruleodactylus	Atlantic semi-terrestrial	Brazil (RS)	Swamp forest	Ribeiro et al. 2016
P. laevigatus	Atlantic semi-terrestrial	Brazil (SC)	Wetlands	This species was evaluated by body characteristics similar to burrower species
P. tuerkayi	Atlantic semi-terrestrial	Brazil (SC)	Flooded grassland	Ribeiro et al. 2017
P. nicoleti	Pacific semi-terrestrial	Chile (south-central)	Semi-marshland, perennially green areas	Rudolph 2010
P. pugnax	Pacific semi-terrestrial	Chile (south-central)	Semi-marshland, perennially green areas	Rudolph 2013
Virilastacus araucanius	Pacific semi-terrestrial	Chile (south-central)	Semi-marshland, perennially green areas	Rudolph 2015

V. jarai	Pacific semi-terrestrial	Chile	Semi-marshland fragment, perennially green areas	Rudolph 2015
V. retamali	Pacific semi-terrestrial	Chile	Geogenous peatlands	Rudolph 2015
Samastacus spinifrons	Pacific aquatic	Argentina and Chile	River and Lakes	Rudolph 2002

Table II. List of 19 bioclimatic layers from Worldclim – Global Climate Data (Hijmans et al. 2005) used for distribution modeling of the South American crayfishes.

Bioclimatic variables	Meanings
Alt	Altitude
Bio 1	Annual Mean Temperature
Bio 2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
Bio 3	Isothermality [(BIO2/BIO7) (* 100)]
Bio 4	Temperature Seasonality (standard deviation *100)
Bio 5	Max Temperature of Warmest Month
Bio 6	Min Temperature of Coldest Month
Bio 7	Temperature Annual Range (BIO5-BIO6)
Bio 8	Mean Temperature of Wettest Quarter
Bio 9	Mean Temperature of Driest Quarter
Bio 10	Mean Temperature of Warmest Quarter
Bio 11	Mean Temperature of Coldest Quarter
Bio 12	Annual Precipitation
Bio13	Precipitation of Wettest Month
Bio 14	Precipitation of Driest Month
Bio 15	Precipitation Seasonality (Coefficient of Variation)
Bio 16	Precipitation of Wettest Quarter
Bio 17	Precipitation of Driest Quarter
Bio 18	Precipitation of Warmest Quarter
Bio 19	Precipitation of Coldest Quarter

Table III. Occurrence records of the freshwater crayfishes selected from the spatially rarefy for semi-aquatic group. **UFRGS**: Coleção Científica da Universidade Federal do Rio Grande do Sul, Brazil. **MCP-PUCRS**: Museu de Ciência e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, Brazil. **MACN-BA**: Museo Argentino de Ciencias Naturales Bernadino Rivadavia, Buenos Aires, Argentina.

Semi-aquatic group	**Geographic**	**Coordinates**	**Database**
Parastacus spn 1	29º52’7.55”S	50º17’58.16”W	UFRGS
Parastacus spn 2	30º29’4.92”S	52º4’10.92”W	UFRGS
Parastacus spn 3	30º21’1.40”S	51º1’44.47”W	UFRGS
Parastacus spn 3	30º21’27.86” S	53º26’57.88”W	UFRGS
Parastacus spn 3	33º9’4.32”S	59º20’3.23”W	UFRGS
Parastacus spn 4	29º36’1.08”S	54º10’36.84”W	UFRGS
Parastacus spn 4	29º31’52”S	53º23’57.12”W	UFRGS
Parastacus spn 5	28º7’51.03”S	9º23’30.71”W	UFRGS
Parastacus spn 6	30º31’9”S	58º21’11.60”W	MACN-BA
P. brasiliensis	29º56’32” S	51º42’49” W	MCP-PUCRS
P. brasiliensis	29º48’26” S	50º53’49”W	MCP-PUCRS
P. brasiliensis	30º12’53” S	51º10’35”W	MCP-PUCRS
P. brasiliensis	30º21’38.16” S	51º41’8.16”W	UFRGS
P. brasiliensis	30º30’24.84” S	51º29’20.04”W	UFRGS
P. brasiliensis	30º30’24.84” S	51º29’20.04”W	UFRGS
P. brasiliensis	30º2’56” S	50º59’7.01”W	UFRGS
P. brasiliensis	29º36’7.20” S	52º16’4.80”W	UFRGS
P. brasiliensis	30º19’59.99” S	51º22’39”W	UFRGS
P. brasiliensis	29º24’4.97” S	51º56’39.84”W	UFRGS
P. pilimanus	28º7’43.97” S	55º31’10.85”W	UFRGS
P. pilimanus	29º55’14.34” S	55º46’2.06”W	UFRGS
P. pilimanus	29º38’33” S	53º32’2”W	UFRGS
P. pilimanus	29º43’5.34” S	53º43’40.69”W	UFRGS
P. pilimanus	30º26’50.78” S	53º39’32.14”W	UFRGS
P. pilimanus	30º10’17” S	53º34’44”W	MCP-PUCRS
P. pilimanus	28º2’53” S	55º10’58”W	MCP-PUCRS
P. pilimanus	27º58’2” S	55º19’57”W	MCP-PUCRS
P. pilimanus	32º34’25.01” S	52º33’58.18”W	UFRGS
P. pilimanus	30º53’60” S	55º40’0”W	MACN-BA
P. pilimanus	31º42’57.01” S	60º7’2.4”W	MACN-BA
P. pilimanus	30º13’26.73” S	57º39’25.51”W	MACN-BA
P. pilimanus	28º34’11.29” S	56º0’57.26”W	MACN-BA
P. pilimanus	32º49’55.83” S	54º45’50.59”W	UFRGS
P. pilimanus	29º39’38.45” S	50º12’49.93”W	UFRGS
P. saffordi	28º32’15” S	49º54’52”W	UFRGS
P. saffordi	28º23’17.96” S	49º26’57.97”W	UFRGS
P. saffordi	34º54’5.4” S	54º49’43.4”W	MACN-BA
P. saffordi	29º44’25.51” S	51º6’9.14”W	UFRGS
P. saffordi	28º50’48.64” S	49º27’39.72”W	UFRGS

P. saffordi	26º13’26.96” S	48º50’9.60”W	UFRGS
P. saffordi	29º55’36” S	51º13’0”W	MCP-PUCRS
P. saffordi	31º15’21.52” S	50º58’57.5”W	UFRGS
P. saffordi	30º10’0” S	53º49’60”W	MACN-BA
P. saffordi	34º53’40.71” S	56º9’22.77”W	MACN-BA
P. saffordi	32º31’5.34” S	59º6’16.70”W	MACN-BA
P. saffordi	29º24’30.11” S	49º57’21.27”W	UFRGS

Table IV. Occurrence records of the freshwater crayfishes selected from the spatially rarefy for two groups: Atlantic semi-terrestrial and Pacific semi-terrestrial. **UFRGS**: Crustacean Collection of Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil. **Rudolph 2010:** Rudolph EH. 2010. Sobre la distribución geográfica de las especies chilenas de Parastacidae (Crustacea: Decapoda: Astacidea). Boletín de Biodiversidad de Chile, 3: 32–46.

Atlantic semi-terrestres species	**Geographic Coordinate**		**Database**
Parastacus sp.7	30º5’49.92”S	50º51’7.2”W	UFRGS
Parastacus sp.7	29º53’38.04” S	50º16’53.4” W	UFRGS
Parastacus sp.8	28º50’48.48” S	49º27’39.6” W	UFRGS
P. defossus	30º1’9.84” S	51º3’15.84” W	UFRGS
P. defossus	29º53’36.96” S	51º9’36” W	UFRGS
P. defossus	30º6’5.76” S	51º41’39.12” W	UFRGS
P. defossus	30º21’11.88” S	51º35’2.76” W	UFRGS
P. defossus	30º12’25.2” S	51º10’0.84” W	UFRGS
P. defossus	29º43’6.6” S	53º43’44.04” W	UFRGS
P. defossus	34º55’18.12” S	56º9’51.12” W	UFRGS
P. caeruleodactylus	29º22’58.08” S	49º50’20.76” W	UFRGS
P. laevigatus	26º12’30.96” S	48º50’44.88” W	UFRGS
P. tuerkayi	26º48’10.8” S	48º37’2.28” W	UFRGS

Pacific semi-terrestres species	**Geographic Coordinate**		**Database**
P. nicoleti	73º2’60” S	39º21’0” W	Rudolph (2010)
P. nicoleti	73º13’1.2” S	39º34’1.2” W	Rudolph (2010)
P. nicoleti	73º4’58.8” S	39º40’59.88” W	Rudolph (2010)
P. nicoleti	72º49’58.8” S	39º58’58.8” W	Rudolph (2010)
P. nicoleti	73º13’1.2” S	40º22’1.2” W	Rudolph (2010)
P. nicoleti	73º5’60” S	41º25’58.8” W	Rudolph (2010)
P. nicoleti	72º37’58.8” S	39º4’58.8” W	Rudolph (2010)
P. nicoleti	73º4’58.8” S	40º4’58.8” W	Rudolph (2010)
P. nicoleti	73º24’0” S	40º19’58.8” W	Rudolph (2010)
P. nicoleti	72º12’0” S	40º34’1.2” W	Rudolph (2010)
P. nicoleti	73º19’1.2” S	40º37’58.8” W	Rudolph (2010)
P. nicoleti	73º27’0” S	40º38’60” W	Rudolph (2010)
P. nicoleti	73º32’60” S	40º55’58.8” W	Rudolph (2010)
P. nicoleti	73º46’58.8” S	40º58’1.2” W	Rudolph (2010)
P. pugnax	71º37’58.8” S	33º2’60” W	Rudolph (2010)
P. pugnax	71º17’60” S	32º55’0.12” W	Rudolph (2010)
P. pugnax	71º37’58.8” S	33º27’0” W	Rudolph (2010)
P. pugnax	72º2’20.4” S	34º38’2.4” W	Rudolph (2010)
P. pugnax	71º19’59.88” S	34º21’0” W	Rudolph (2010)
P. pugnax	71º19’58.8” S	34º40’59.88”W	Rudolph (2010)
P. pugnax	71º40’0.12” S	35º25’59.88” W	Rudolph (2010)

P. pugnax	71º42’0” S	35º42’0” W	Rudolph (2010)
P. pugnax	71º33’54” S	35º47’2.4” W	Rudolph (2010)
P. pugnax	71º49’59.88” S	36º8’60” W	Rudolph (2010)
P. pugnax	71º58’59.88” S	36º23’60” W	Rudolph (2010)
P. pugnax	72º5’60” S	36º36’0” W	Rudolph (2010)
P. pugnax	73º15’0” S	37º15’0” W	Rudolph (2010)
P. pugnax	72º43’0.12” S	37º47’60” W	Rudolph (2010)
P. pugnax	72º50’31.2” S	38º36’0” W	Rudolph (2010)
P. pugnax	72º22’59.88” S	38º43’0.12”W	Rudolph (2010)
P. pugnax	70º39’0” S	30º1’59.88” W	Rudolph (2010)
P. pugnax	70º43’0.12” S	34º2’60”W	Rudolph (2010)
P. pugnax	72º46’59.88” S	36º4’0.12”W	Rudolph (2010)
P. pugnax	72º31’59.88” S	36º16’0.12”W	Rudolph (2010)
P. pugnax	72º49’55.2” S	36º21’0”W	Rudolph (2010)
P. pugnax	72º22’59.88” S	36º23’60”W	Rudolph (2010)
P. pugnax	72º40’59.88” S	36º28’59.88”W	Rudolph (2010)
P. pugnax	71º31’59.88” S	36º32’60”W	Rudolph (2010)
P. pugnax	72º34’59.88” S	36º37’59.88”W	Rudolph (2010)
P. pugnax	72º16’0.12” S	36º37’59.88”W	Rudolph (2010)
P. pugnax	72º28’0.12” S	36º43’59.88”W	Rudolph (2010)
P. pugnax	72º1’59.88” S	36º53’60”W	Rudolph (2010)
P. pugnax	72º55’59.88” S	37º10’59.88”W	Rudolph (2010)
P. pugnax	72º43’0.12” S	37º15’0” W	Rudolph (2010)
P. pugnax	72º32’60” S	37º32’60” W	Rudolph (2010)
P. pugnax	73º9’0” S	38º40’0.12”W	Rudolph (2010)
P. pugnax	73º25’0.12” S	38º43’59.88”W	Rudolph (2010)
Virilastacus araucanius	73º1’0.12” S	36º46’0.12”W	Rudolph (2010)
V. araucanius	72º55’0.12” S	36º55’59.88”W	Rudolph (2010)
V. araucanius	73º15’0” S	39º47’50”W	Rudolph (2010)
V. araucanius	73º25’58.8” S	39º52’59.88”W	Rudolph (2010)
V. araucanius	73º43’59.88” S	40º36’0”W	Rudolph (2010)
V. araucanius	73º12’0” S	39º19’0.12”W	Rudolph (2010)
V. araucanius	72º54’0” S	39º43’59.88”W	Rudolph (2010)
V. araucanius	73º34’59.88” S	39º57’0” W	Rudolph (2010)
V. jarai	72º18’37.08” S	37º26’39.84”W	Rudolph (2010)
V. retamali	73º34’0.12” S	40º34’59.88”W	Rudolph (2010)
V. retamali	73º46’0.12” S	41º25’0.12”W	Rudolph (2010)

Table V. Occurrence records of the freshwater crayfishes selected from the spatially rarefy for aquatic group - *Samastacus spinifrons*. **Rudolph 2010:** Rudolph EH. 2010. Sobre la distribución geográfica de las especies chilenas de Parastacidae (Crustacea: Decapoda: Astacidea). Boletín de Biodiversidad de Chile, 3: 32–46.

Aquatic species	**Geographic**	**Coordinate**	**Database**
Samastacus spinifrons	71º38’15.8”S	33º5’45.6”W	Rudolph (2010)
S. spinifrons	71º15’0”S	34º58’60”W	Rudolph (2010)
S. spinifrons	71º40’0”S	35º25’60”W	Rudolph (2010)
S. spinifrons	71º42’0”S	35º42’0”W	Rudolph (2010)
S. spinifrons	72º48’14.4”S	36º7’1.2”W	Rudolph (2010)
S. spinifrons	71º58’60”S	36º23’60”W	Rudolph (2010)
S. spinifrons	72º5’60”S	36º36’0”W	Rudolph (2010)
S. spinifrons	73º7’0”S	36º40’0”W	Rudolph (2010)
S. spinifrons	73º13’0”S	38º1’60”W	Rudolph (2010)
S. spinifrons	73º5’60”S	38º43’0”W	Rudolph (2010)
S. spinifrons	72º0’0”S	38º10’60”W	Rudolph (2010)
S. spinifrons	73º13’0”S	39º10’0”W	Rudolph (2010)
S. spinifrons	72º47’60”S	39º27’0”W	Rudolph (2010)
S. spinifrons	73º16’0”S	39º49’60”W	Rudolph (2010)
S. spinifrons	73º30’0”S	40º34’60”W	Rudolph (2010)
S. spinifrons	72º5’60”S	40º36’0”W	Rudolph (2010)
S. spinifrons	72º58’60”S	40º43’60”W	Rudolph (2010)
S. spinifrons	73º12’0”S	40º46’0”W	Rudolph (2010)
S. spinifrons	72º30’0”S	40º46’0”W	Rudolph (2010)
S. spinifrons	73º37’0”S	41º0’0”W	Rudolph (2010)
S. spinifrons	72º49’60”S	41º10’0”W	Rudolph (2010)
S. spinifrons	73º45’0”S	41º15’0”W	Rudolph (2010)
S. spinifrons	72º25’0”S	41º19’60”W	Rudolph (2010)
S. spinifrons	72º54’0”S	41º28’0”W	Rudolph (2010)
S. spinifrons	73º16’60”S	41º32’60”W	Rudolph (2010)
S. spinifrons	73º53’13.56”S	41º32’60”W	Rudolph (2010)
S. spinifrons	74º1’60”S	42º37’60”W	Rudolph (2010)
S. spinifrons	73º54’0”S	42º40’0”W	Rudolph (2010)
S. spinifrons	74º30’0”S	46º30’0”W	Rudolph (2010)
S. spinifrons	71º30’0”S	41º0’0”W	Rudolph (2010)

Table VI. Results summary of the distribution modeling (present and future scenarios) for freshwater crayfishes groups (semi-aquatic; Atlantic semi-terrestrial and Pacific semi-terrestrial; and aquatic). Area size of distribution in the present and in 2070 (expansion or reduction of area in future), as well as AUC values according to modelled scenarios.

	Present			¤	**Future**		
Group	Presence sites (n)	Range area (km²)	AUC training	AUC test	% area reduction or expansion	AUC training	AUC test
Semi-aquatic	45	86,641	0.895	0.817	Expansion of 2.4%	0.887	0.932
Atlantic semi-terrestrial	13	42,023	0.984	0.878	Expansion of 5.5%	0.979	0.932
Pacific semi-terrestrial	58	28,412	0.929	0.949	Reduction of 1%	0.932	0.928
Aquatic	30	51,114	0.842	0.796	Expansion of 14%	0.824	0.963

Table VII. AUC mean with standard deviation (sd); and the percentage contribution of two most important bioclimatic variables to the distribution models of each crayfishes group in South America (semi-aquatic; Atlantic semi-terrestrial and Pacific semi-terrestrial; and aquatic). Alt: Altitude; Bio 1: Annual Mean Temperature; Bio 2: Mean Diurnal Range, calculated from Mean of monthly (max temp - min temp); bio12: Annual Precipitation (Hijmans et al. 2005).

Group	AUC mean	Alt	Bio 1	Bio 2	Bio 12
Semi-aquatic	0.862 (0.099)	-	-	58	47
Atlantic semi-terrestrial	0.968 (0.032)	17.4	-	73	-
Pacific semi-terrestrial	0.905 (0.039)	-	45	21.6	-
Aquatic	0.744 (0.198)	83	-	-	-

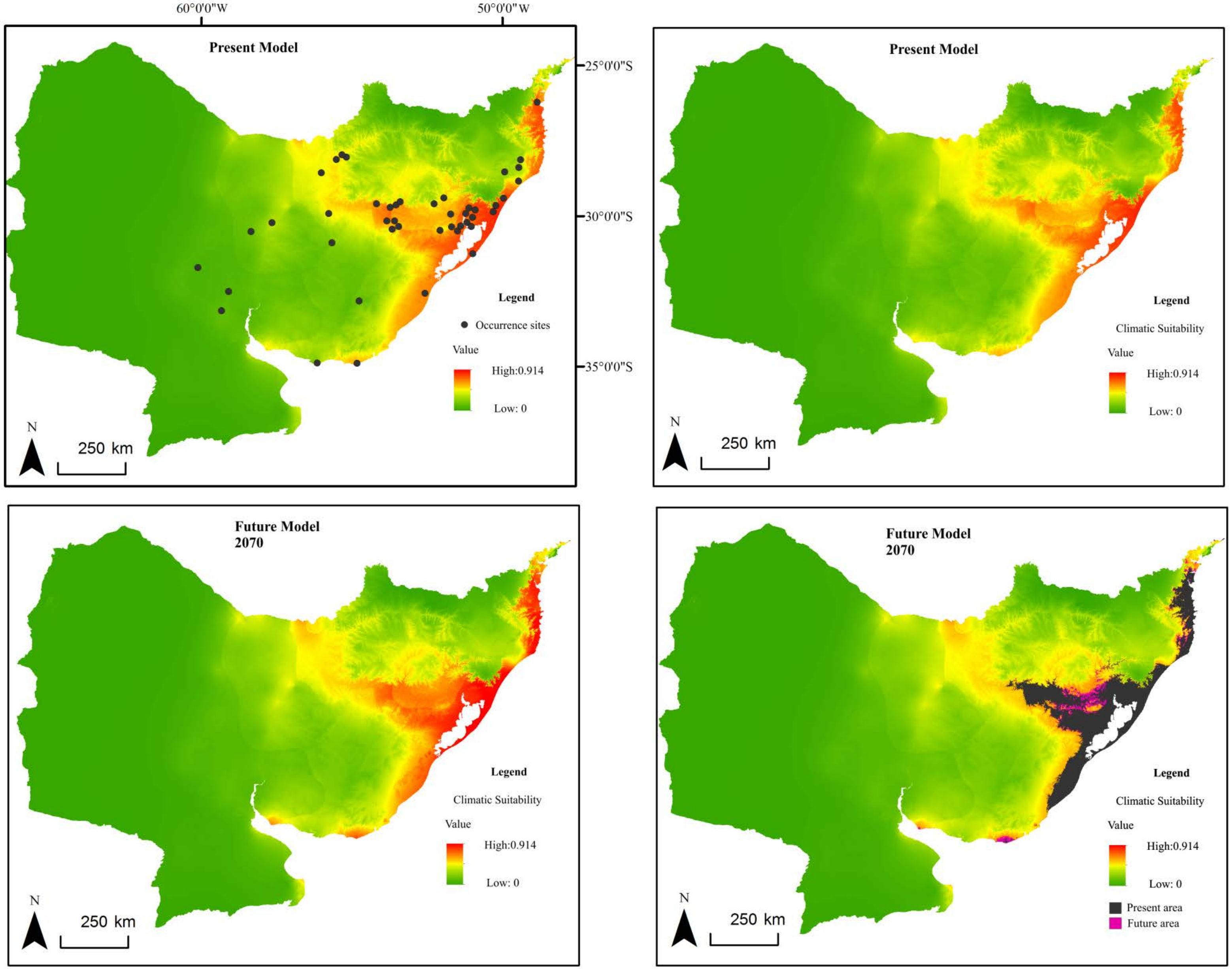

Figure 1. Occurrence records, present and future projections of semi-aquatic group, and distribution expansion projected for 2070 (2.4%).

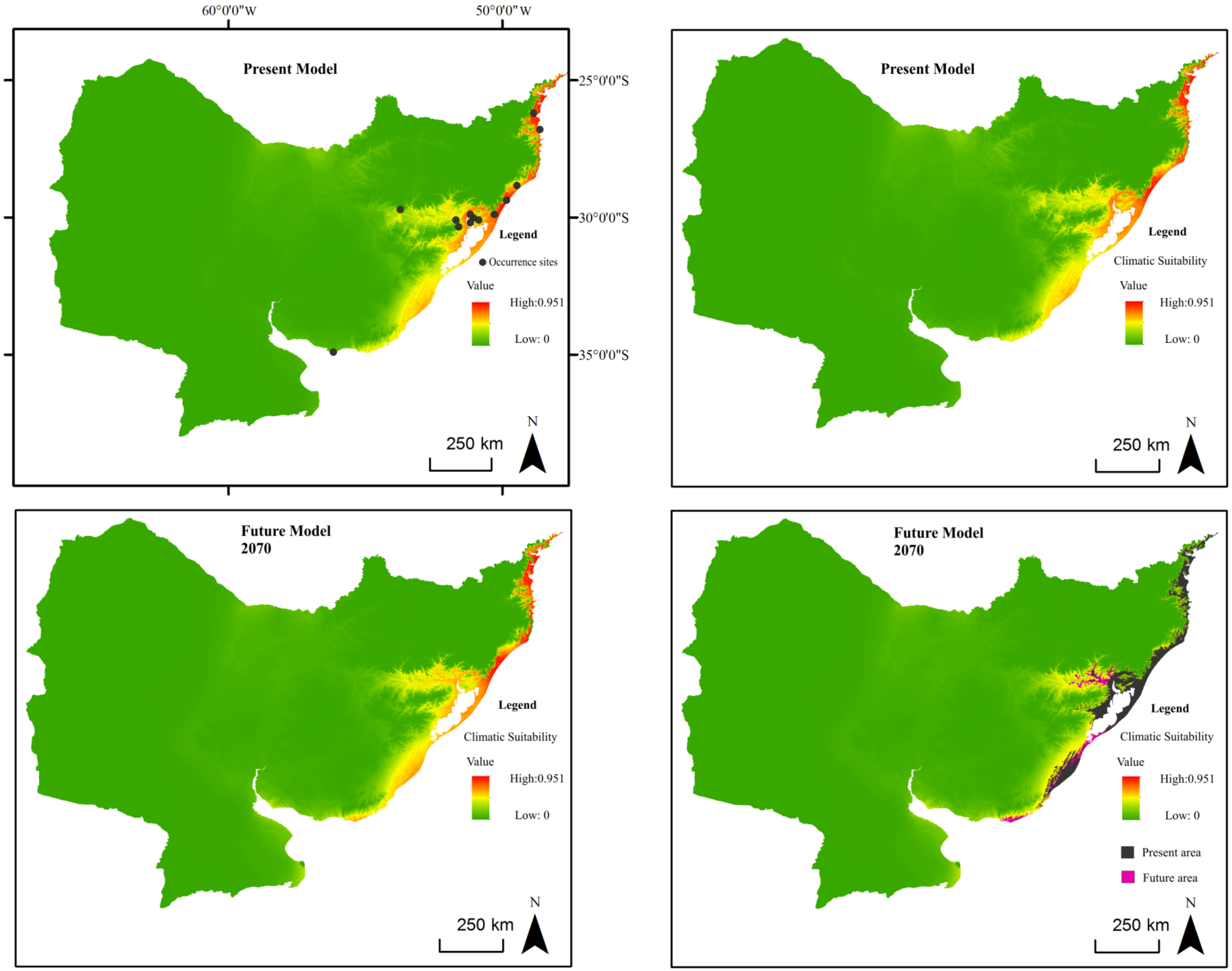

Figure 2. Occurrence records, present and future projections of Altlantic semi-terrestrial group, and distribution expansion projected for 2070 (5.5%).

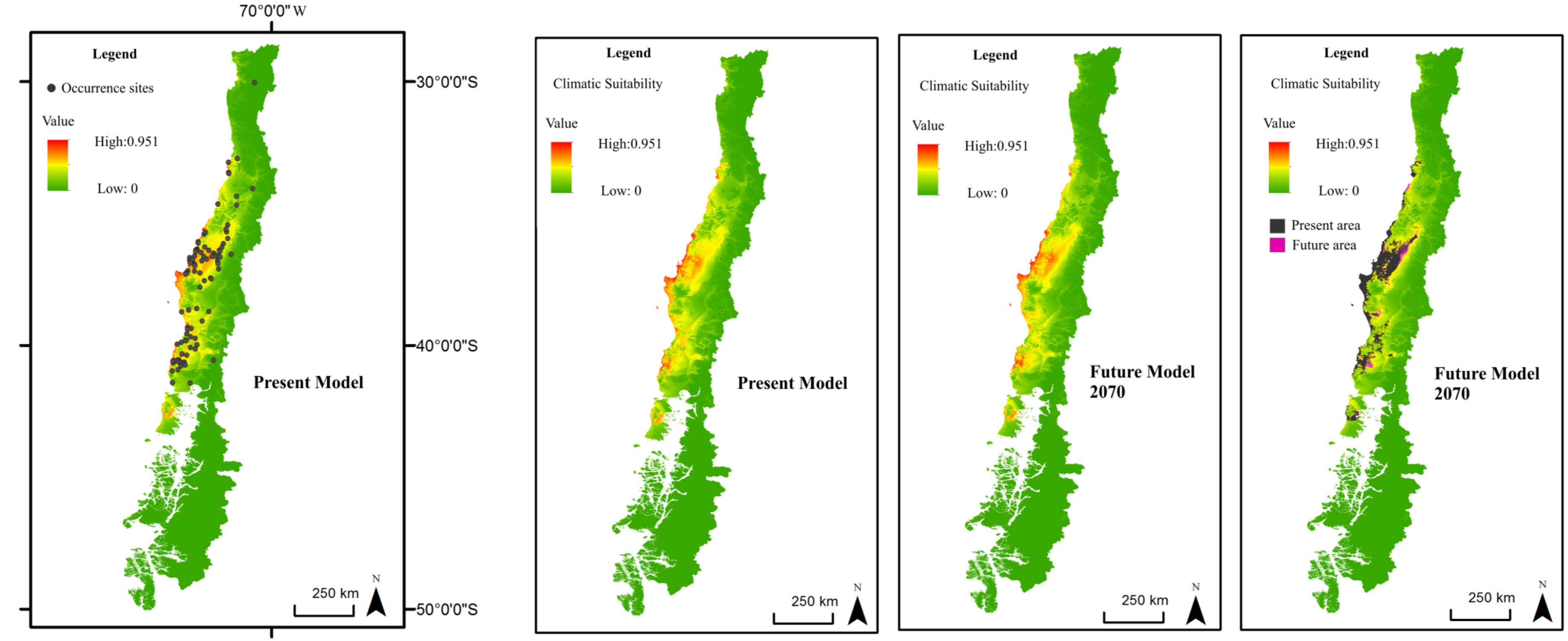

Figure 3. Occurrence records, present and future projections of Pacific semi-terrestrial group, and distribution expansion projected for 2070 (~ 1%).

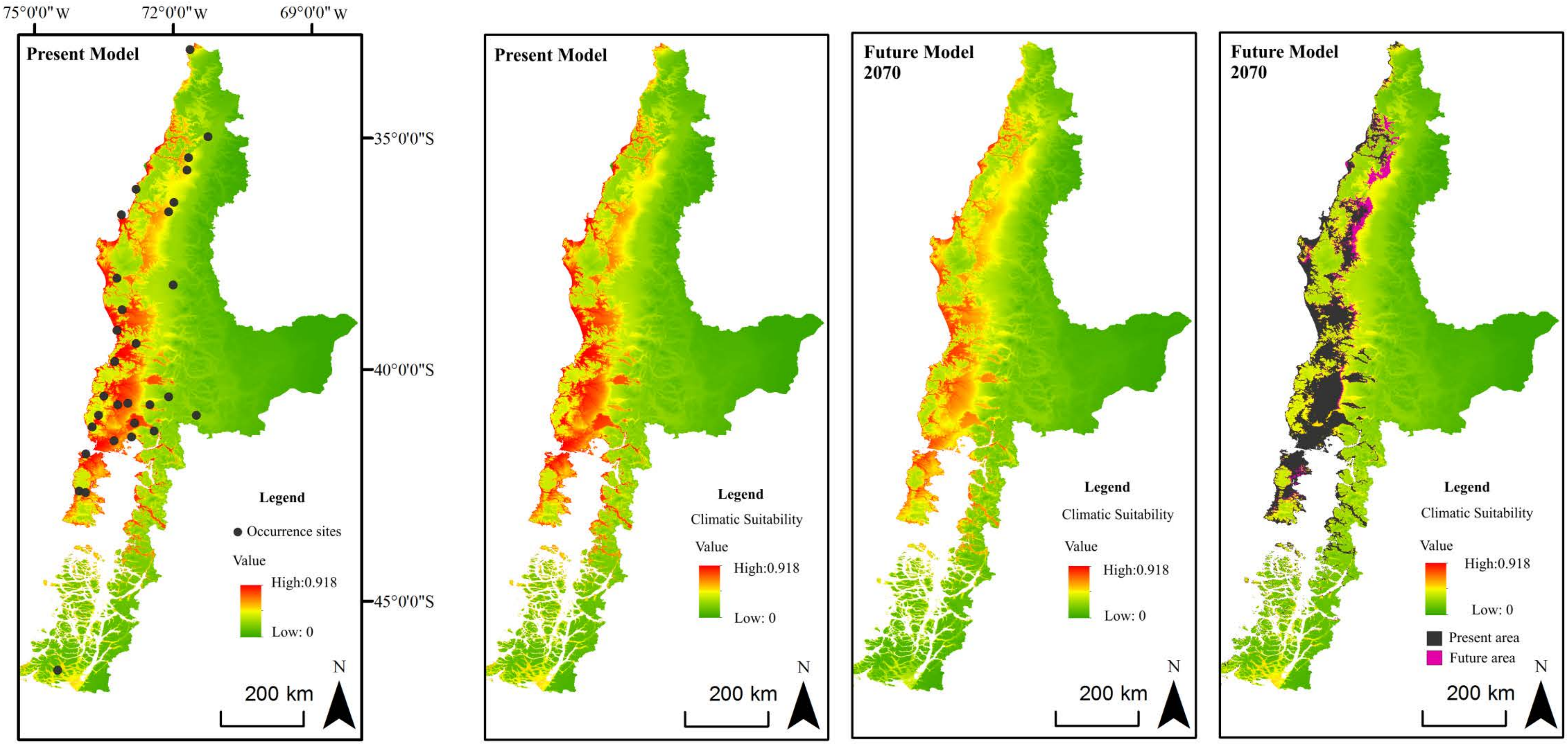

Figure 4. Occurrence records, present and future projections of Pacific aquatic group, and distribution expansion projected for 2070 (14%).

5.3 Capítulo III

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Taxonomy lags behind: Genetic diversityand significant evolutionary lineages in the endemic crayfish species *Parastacus brasiliensis* (von Martens, 1869) (Crustacea, Decapoda, Parastacidae)and its implications for conservation

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Abstract

1. The characterizationof intraspecific genetic diversity in species of the South American crayfish genus *Parastacus* Huxley, 1879is done for the first time, in this case among populations of the *Parastacus brasiliensis*(von Martens, 1869) as currently defined.

2. Phylogenetic reconstructions based on mitochondrial and nuclear markers indicate the existence of five evolutionary significant units, of which only one can be considered as*P. brasiliensis* sensu stricto.

3. One of the other four lineages corresponds to the subspecies *Parastacus brasiliensis promatensis* Fontoura & Conter, 2008, which is here elevated to species level.

4. Genetic distances among *P. brasiliensis* sensu stricto and the remaining lineages “A”, “B” and “C” increase with geographical distances, suggesting isolation by distance as an important driver of diversification and eventually speciation in these burrowing crayfishes.

4. According to this study, *P. brasiliensis* occurs mainly in Guaíba LakeBasin and the studied populations Guaíba I and II show limited connectivity and gene flow, probably due to habitat fragmentation.

5. On the basis of genetic and distribution data,the conservation status of *P. brasiliensis* as Near Threatened (NT)is confirmed. These findings lead us to encourage the establishment of preservation areas for the isolated Guaíba II population. The importance of preserving these distinct gene pools in order to maintain species genetic diversity is emphasized.

Introduction

Human activities have threatened and eliminated a considerable number of species and populations in nature that, in turn, have affected efficient ecosystem functioning (Cardinale *et al.*, 2006). Freshwater ecosystems, in particular, are critical to biodiversity by housing nearly 10% of all described species (Strayer & Dudgeon, 2010). Nevertheless, they are under severe pressure from multiple impacts, such as overexploitation, water pollution, flow modification, destruction or degradation of habitat, and invasion by exotic species (Dudgeon *et al.*, 2006; Almerão *et al.*, 2014; Loureiro *et al.*, 2015a, b). From all global conservation efforts, relatively little attention is given to freshwater ecosystems, in which the role of invertebrate species remains underestimated even in protected areas (Richman *et al.*, 2015).

Habitat change is considered the major threat to biodiversity (Dobson, Bradshaw & Baker, 1997) and increases the risk of species extinction (Fahrig, 2002). The effects of this alteration may be further intensified, following landscape or habitat fragmentation (Ewers & Didham, 2006) and also caused by the presence of invasive alien species (see Gherardi, 2013 for review). However, the impact of habitat change on each population ultimately depends of the individual species' life history strategies (Ewers & Didham, 2006).Therefore, reliable information about taxa distribution and their geographical structuring is essential, when dealing with species conservation. Genetic data are useful for investigating the molecular diversity in populations of widespread species (Trontelj, Machino & Sket, 2005; Dawkins *et al.*, 2010; Burnham, Koenders & Horwitz, 2012) and to indicate possible loss of gene flow in consequence of habitat fragmentation (Dixo *et al.*, 2009; Miller *et al.*, 2014), which is mainly observed among populations of sedentary species (Dehais *et al.*, 2010). This approach facilitates the selection of priority populations for conservation (Dawkins & Furse, 2012).

Many species of freshwater crayfish are threatened, despite their function as keystone species in aquatic ecosystems (Richman *et al.*, 2015). Crayfish species have been recognized as important for the ecology of stream communities, due to their omnivorous life style (Richardson, 1983; Reynolds, Souty-Grosset & Richardson, 2013), including predation (Parkyn *et al.*, 1997; Nyström and Perez, 1998) and sediment bioturbation (Parkyn *et al.*, 1997; Statzner *et al.*, 2003), increasing organic matter processing rates (Parkyn *et al.*, 1997). In terrestrial ecosystems, crayfishes contribute with several ecological services, among them increasing soil respiration rates and facilitating the growth of fungi and rootlets (Richardson, 1983; Reynolds *et al.*, 2013). The decrease in these benefits are often better observed when the burrowers are removed from their respective habitats (Coughran & Furse, 2012).

Freshwater crayfishes of the family Parastacidae Huxley, 1879 are widely distributed throughout the Southern Hemisphere. In South America they occur in Chile, Argentina, Uruguay and Brazil and are represented by species belonging to the genera *Parastacus* Huxley, 1879, *Samastacus* Riek, 1971, and *Virilastacus* Hobbs, 1991 (Toon *et al.*, 2010; Ribeiro *et al.*, 2016). Almost all the native crayfish species of South America are burrowers, living in complex underground systems in or near different freshwater habitats (rivers, streams, lakes and wetlands) (Buckup, 2003; Rudolph, 2013).These habitats are increasingly exposed to different threats, e.g. stream channelization and urbanization (Junk *et al.*, 2013) that increase the risk of population decline and local extinctions.

This ongoing degradation and habitat loss have been the main reasons for classifying the endemic Brazilian crayfish *Parastacus brasiliensis* (von Martens, 1869) as *Vulnerable* (VU) (Marques *et al.*, 2002) and *Near Threatened* (NT) (Buckup, 2010) in recent assessments. In addition, some species-specific particularities may increase the vulnerability, among these the restricted occurrence in the basins forming the Central Depression of Rio Grande do Sul (including tributaries of the Guaíba Lake) (Buckup, 1999) and their association with mainly first-order rivers (Fontoura & Buckup, 1989). Nevertheless, Almerão *et al.* (2014) suggested *Data Deficient* (DD) as the most adequate classification, due to the lack of information regarding population size, area of occupancy, and extension of threats within the distribution of the species. The adoption of DD criteria does not mean that the taxon is not threatened, but underlines the need for adequate data to determine the real degree of threat and possible conservation plans (Almerão *et al.*, 2014).

In the present study, the phylogenetic relationships among populations of the species previously identified as *P. brasiliensis* was investigated, the patterns of genetic diversity and structure along the distribution of the species were described, and the connectivity and possible demographic changes based on mitochondrial and nuclear markers were evaluated. Finally, we provide insights on habitat characterization and current conservation status of the species based on IUCN Red List criteria.

Methods

Taxonomic history

Parastacus brasiliensis is a freshwater crayfish of moderate size (average: 28.4 mm cephalothorax length) that inhabits lotic and lentic regions of streams, but mostly shallow portions with reduced flow, where the individuals remain under litter or in burrows at the watercourse margins (Buckup & Rossi, 1980; Fontoura & Buckup, 1989) (Fig. 1). This species was first classified within the genus *Astacus* Fabricius, 1775 as

Astacus brasiliensis von Martens, 1869, along with *A. pilimanus* von Martens, 1869, based on specimens collected by Reinhold Hensel in the state of Rio Grande do Sul, Brazil. However, Huxley (1879) analyzed the branchial structure of species described by von Martens (1869) and created the genus *Parastacus* to encompass both species.

A review of South American Parastacidae by Riek (1971) considered *P. brasiliensis* a junior synonym of *P. pilimanus* without providing a convincing morphological explanation. Buckup & Rossi (1980) maintained the separation of these two species, based on morphological and morphometric analyses of 40 specimens of each species, from several localities. Taking into account that von Martens (1869) did not select a holotype, Buckup & Bond-Buckup (1994) examined the syntype series and designated the largest specimen as the lectotype and the others as paralectotypes, defining Porto Alegre as the type-locality.

Fourteen years later, Fontoura & Conter (2008) described *Parastacus brasiliensis promatensis* as a subspecies recorded exclusively from the locality of São Francisco de Paula, Rio Grande do Sul, Brazil. This designation was based on a morphometric analysis of individuals previously identified as *P. brasiliensis*.

Parastacus brasiliensis is similar to *P. pilimanus* in size and general body shape. However, it differs by having a slightly concave rostrum surface, a dactylus with dorso-proximal rows of squamous tubercles,wider areola, a larger abdomen, and lack setal cover on the cutting edge of cheliped fingers (Buckup & Rossi, 1980). Nevertheless, a taxonomic revision with the addition of new characters is necessary to better define the species, since we observed morphological variations in specimens identified as *P. brasiliensis*, indicating the possibility that *P. brasiliensis* consists of more than one species.

Species sampling

Samplings were carried out from September 2012 to August 2014 in a total of 177 localities, encompassing several hydrographical basins in the states of Rio Grande do Sul and Santa Catarina, Brazil (Fig. 2). A combination of capture methods was employed, due to the habitat complexity and the species burrowing behaviour. We used a vaccum pump, dip nets, and Polyvinyl Chloride (PVC) traps closed with a wire screen and baited with cattle liver and cat food (see also Fontoura & Buckup, 1989). In addition, burrows were excavated by hand. The traps were installed in the late afternoon, immersed in flowing water to attract the crayfishes, and removed the next morning. In total, 49 new specimens were caught.

Additional samples previously identified as *P. brasiliensis* were obtained from the following scientific collections:

- Crustacean Collection of the Zoology Department of the Universidade Federal do Rio Grande do Sul (UFRGS);
- Museum of Natural Science of Unidade Integrada Vale do Taquari de Ensino Superior (MCP-UNIVATES);
- Museum of Science and Technology of Pontifícia Universidade Católica do Rio Grande do Sul (MCP-PUCRS);
- Museum für Naturkunde Berlin (ZMB);
- and Museu de Zoologia of Universidade do Vale do Rio dos Sinos (MZU-UNISINOS).

All specimens were used for the distribution analysis, and selected specimens were used forthe genetic analysis. Species identifications were confirmed on the basis of morphological characters from available references (Buckup & Rossi, 1980).

Occurrence records of *P. brasiliensis* were plotted on a map with hydrographic basins of the state of Rio Grande do Sul (SEMA, 2010) to calculate the percentage occupied by the species within each basin.

DNA Extraction, Amplification, and Sequencing

Muscle tissue was extracted from the walking legs or chelipeds from fresh and museum specimens. These were labelled as genetic voucher specimensand kept in the reference collection (UFRGS) (Table 1). In addition, we used GenBank sequences of this and other species of the genus *Parastacus* (*P. brasiliensis:* AF1552451, AF1752441; *P. defossus*: AF1752431, EU290991; *P. pilimanus*: AF275247, FJ966039) in order to phylogenetically contextualize our target group and allow comparison with other intrageneric genetic distances.

All other sequences used in this study were generated from our own extractions. The analysis was based on DNA sequences corresponding to the mitochondrial genes 16S rRNA (16S), cytochromeoxidase subunit I (Cox1), and the nuclear 28S rRNAgene (28S). The utility of the mitochondrial markers 16S and Cox1 for both phylogenetic and population studies has been demonstrated for over a decade and they are a common choice in phylogenetic studies in decapods (see Schubart, Neigel & Felder, 2000; Mantelatto, Robles & Felder, 2007; Vergamini, Pileggi & Mantelatto, 2011; Rajković *et al.*, 2012; Maguire *et al.*, 2014). Nuclear genes are highly conserved andhave different evolution rates, allowing to infer phylogenetic relationships in a broader range, including relationships among closely related species and populations (Hwang & 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 & Reuschel, 2009; Rossi & Mantelatto, 2013; Tsang *et al.*, 2014).

Total genomic DNA was extracted from tissue subsamples using the Puregene kit (Qiagen). Polymerase chain reaction (PCR) and performedusing published primer sets for the mitochondrial genes 16S - 16L2 (5'-TGC CTG TTT ATC AAA AAC AT-3')(Schubart, 2009) and 1472 (5'-AGA TAG AAA CCA ACC TGG-3')(Crandall & Fitzpatrick, 1996), cytochromeoxidase subunit I - COL1b (5'-CCW GCT GGD GGW GGD GAY CC-3') and COH16 (5'-CAT YWT TCT GCC ATT TTA GA-3') and the nuclear 28S rRNA - 28D2L (5'-TAC CGT GAG GGA AAG YTG AAA-3)' and 28H2 (5'-CGA TTT GCA CGT CAG AAT TGC T-3') (Thiercelin & Schubart, 2014). The PCR conditions were: initial denaturation at 94°C for 4 min, followed by 40 cycles of 95°C for 45 s, 48or 50°Cfor 45 s annealing, 72°C for 75 s elongation, 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 & Schubart (2014). 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).

Sequences were proofread with Chromas 2.23 (Technelysium Pty Ltd., 2005), automatically aligned with Clustal W (Thompson, Higgins & Gibson, 1994) implemented in BioEdit 7.0.5 (Hall, 1999) and unspecific readingcorrected manually when required. Primer regions, poorly aligned regions, and large indels in sequence data were removed following recommendations by GBlocks (Castresana, 2000), making the final alignment suitable for phylogenetic analysis (Castresana, 2000). The absence of stop codons in Cox1 was confirmed using the software Artemis (Rutherford *et al.*,2000) in order to reduce the possibility of including pseudogenes (Song *et al.*, 2008).

Sequences were finally blasted in GenBank and compared with the available *Parastacus*assemble (Accession numbers: KU258522 toKU258636).

Phylogeny and Population analyses

For the phylogenetic reconstructions of the different markers, a Maximum Likelihood analyses (ML) with RAxML was performed, as proposed by Stamatakis (2006), implemented at the CIPRES portal (Miller, Pfeiffer & Schwartz, 2010), usingthe GTR+G+I substitution model. Alignments of the two mitochondrial markers were concatenated into a single dataset; missing data were designated as question marks in the alignment. In addition, we also conducted ML phylogenetic analyses for each gene fragment separately to reveal any possible discordance in the relationships among the studied lineages. The consistency of topologies was measured by the bootstrap method (1000 bootstrap pseudoreplicates), and confidence values above 50% were reported.

In order to estimate intra- and interspecific divergence rates, genetic distances were also calculated for each gene by pairwise comparisons using uncorrected p-distanceswith the software Mega 6.0 (Tamura *et al.*, 2013).Due to the lower mutation rates of 16S and 28S sequences, population structure analyses,as described below, were not performed with results from these markers. For the Cox1 gene, the number of haplotypes was calculated in DnaSP5.10 (Librado & Rozas, 2009) and haplotype and nucleotide diversities were calculated for each population using Arlequin 3.5 (Excoffier & Lischer, 2010). Haplotype networks were constructed based on the statistical parsimony method implemented in TCS (Version 1.21) (Clement, Posada & Crandall, 2000) with previous data preparation in DnaSP. Possible effects of natural selection and past demographic change as bottlenecks and population expansions were calculated with the Tajima's D index (Tajima, 1989) and visualized as mismatch distributions

(Slatkin & Hudson, 1991; Rogers & Harpending, 1992; Schneider & Excoffier, 1999) in Arlequin.

Analyses of molecular variance (AMOVA) (Excoffier, Smouse & Quattro, 1992) were performed in Arlequin to determine the distribution of genetic variation. Analyses were run based onpairwise differences (considering Guaíba I and "II"together as a single population) and the significance was tested using a nonparametric permutation procedure (Excoffier *et al.*, 1992) with 10,000 permutations. Finally, the Mantel test, implemented in Alleles in Space (AIS) (Miller, 2005), was used with 5000 permutations to test the isolation-by-distance among individuals.

Conservation status

The extinction risk was estimated from the value of extent of occurrence (EOO) with sub-criterion B1, as established in the International Union for Conservation of Nature - IUCN guidelines (2016). The EOO represents the area that encompasses the occurrence sites of a species and/or potential habitats known, inferred or projected, within the shortest continuous imaginary boundary (IUCN, 2016). The delimitation of Ottobacias shape level 5 and 6 (specific stretches in the hydrographic systems) (ANA, 2006) was used to draw a polygon, encompassing occurrence data and potential areas for the presence of the species, as defined from the available knowledge on the habitat of *P. brasiliensis*.

Results

Species identification and distribution

The morphological examination of 161 lots and 348 specimens previously identified as *Parastacus brasiliensis* required re-identifications, revealing that some of them belong to other species. The sampling revealed 20 localities with the presence of *P. brasiliensis* in Guaíba Lake and two adjacent basins (Sinos and Gravataí). Thus, considering the results of morphology and molecular analysis (see below), the species in its original definition has an occurrence covering the entire basin of Gravataí, Guaíba and Pardo and parts of the basins of Baixo Jacuí (48.5%), Taquari-Antas (50.4%), Caí (8.2%), Camaquã (5.5%) and Sinos (76.0%) in the Guaíba hydrographic region. Therefore, *P. brasiliensis* can be considered an endemic species to the Brazilian state of Rio Grande do Sul.

Genetic diversity

DNA sequences were obtained from a total of 70 individuals adding up to 1840 base pairs (bp) from three gene fragments (41 sequences of 16S with ~500 bp,46 sequences of Cox1with 600bp after cropping, 28 sequences of 28S with ~740bp) were used. From the concatenated phylogram, as well as onthe phylograms constructed using the single gene trees, [either mitochondrial (16S, Cox1) or nuclear 28S markers] (Figs 3 and 4), it becomes evident that *Parastacus brasiliensis promatensis* is lineage that is a distinct from *Parastacus brasiliensis* sensu stricto. Therefore, we here with elevate the subspecies to species level: *P. promatensis* Fontoura & Conter, 2008, new rank.

Also the remaining populations of *Parastacus brasiliensis* sensu lato currently known, revealed to be composed of distinct lineages, each one with distribution restricted to one (Sinos and Camaquã) or two (Baixo Jacuí + Vacacaí-VacacaíMirim) neighbouring hydrographic basins. The subdivision into distinct lineages is well supported in the concatenated treeand recognizable in the 16S and Cox1 phylograms, while in the 28S tree such a structure is not strongly marked and closer basins cluster together (Figs. 3 and 4).With respect to the position of *P. defossus* and/or *P. pilimanus*, the species *P. brasiliensis* would be polyphyletic unless the divergent lineages are excluded from the species.

The cluster formed mainly by the Guaíba Lake (Guaíba I: Mariana Pimentel population and II: Porto Alegre populations), but also by Caí, Taquari-Antas and Sinos (individuals from the localities of Dois Irmãos, Gravataí and Taquara) basins constitute a monophyletic group according to both mitochondrial markers (Fig. 3).Considering that the type series of *P. brasiliensis* was described from Porto Alegre (and rivers and tributaries of the Guaíba basin) (Buckup &Bond-Buckup, 1994) and both mitochondrial markers support the monophyly of the group, only individuals clustered in the Guaíba clade considered as *P. brasiliensis* sensu stricto were taken into account for further population genetic analysis. Lineages distinct from *P. brasiliensis* s. str. were named as separate lineages “A” (Baixo Jacuí + Vacacaí), “B” (Sinos) and “C” (Camaquã) (Fig. 3).

In general, distance analyses revealed lower intraspecific variation than interspecific. *Parastacus brasiliensis* s. str. data ranged from 0 to 1.9% for 16S, 0 to 2.4% for Cox1, and 0 to 1.0% for 28S. Populations from Guaíba I showed, in average, higher variation than those from Guaíba II for both mitochondrial markers (16S: 0.7 and 0.1%; Cox1: 1.5 and 0.4%, respectively) while for 28S, variation was the same within the groups (0.1%).

Genetic distances estimated between *P. brasiliensis* s.str. and close lineages analyzed here in ranged from 2.2% (*Parastacus* sp.B-Sinos) to 4.4% (*Parastacus* sp.C-Camaquã) for the 16S gene. Divergences between *P. brasiliensis*s.str.and closely related lineages were even higher for the Cox1 gene, ranging from 5.0% (*P.*sp. B-Sinos) to 17.9% (*P. promatensis*) (see Tables 2, 3 and 4 for further comparisons). In comparison to the mitochondrial markers, genetic distances between *P. brasiliensis* and closely related lineages based on the 28S gene were much lower, ranging from 0.1% (*Parastacus* sp. B-Sinos, *Parastacus* sp. C-Camaquã) to 2.6% (*P. promatensis*).

When compared to other speciesof*Parastacus*, the highest pairwise distance was obtained for *P. brasiliensis*s. str.–*P. nicoleti*(11.7%), while the smallest value was obtained for *P. brasiliensis*s. str.– *P. defossus* (4.5%), both for 16S gene (data not shown). For the 28S gene, the highest pairwise distance was obtained for *P. brasiliensis–P.pugnax* (1.1%) while the smallest value was obtained for *P. brasiliensis* – *P. varicosus* (0.1%) (data not shown).

Population structure and connectivity

Based on a 600 bp Cox1 fragment of unambiguous sequences, 14 haplotypes (h) were recognized for *P. brasiliensis* sensu stricto, which resulted in a total haplotype diversity of 0.84. Among the haplotypes, ten (71.42%) corresponded to single individuals, and four (28.58%) were shared. The frequencies of haplotypes in different populationswere heterogeneous: Guaíba I showed higher haplotype and nucleotide diversity than Guaíba II (Hd: 0.93, π:0.01462 and Hd: 0.29, π: 0.00364, respectively) (Table 5). Both populations were separated by a minimum of 16 mutational steps and therefore are here shownas two distinct haplotype networks. There were no haplotypes shared among different locations and/or among populations (Fig.6).

Analysis of molecular variance (AMOVA) indicated that specimens within the *P. brasiliensis* populations have the lowest percentage of variation (21.45%), whereas the variation among populations was high (78.75%). Because of the high diversity among and low diversity within populations, genetic differentiation was very high (ϕ_{ST}= 0.78) (Table 6). Moderate, but significant positive correlation was observed between pairwisegenetic and geographic distances (r = 0.5665,p=0.0009, Mantel test, Fig.S1, in

Supporting Information), suggesting that dispersal is restricted betweenboth populations.

Analysis of mismatch distribution resulted in a ragged profile for Guaíba I, suggesting repeated contractions followed by expansions events (neutral selection), corroborated by the negative Tajima D (D = -0.08957; p = 0.50100). On the other hand, the unimodal curve observed for Guaíba II, together with the significant value of Tajima D (D = -2.02610; p = 0.00300), matched the one resulting from a single colonization event, probably after a genetic bottleneck.

Taxonomic implications

The genetic approach used to document the variability among *P. brasiliensis* populations suggests that the variability goes beyond the population level. Therefore, we elevate the subspecies *Parastacus brasiliensis promatensis* to full species level: *P. promatensis* Fontoura & Conter, 2008, new rank. Furthermore, three genetically distinct lineages may be considered as three new species. The description of these new species will be conducted elsewhere (Ribeiro et al., in prep.).

Conservation Status

The new EOO estimated for the endemic crayfish *P. brasiliensis* sensu stricto comprises 41,000 km^2.Considering the current data, this represents only 38% of the original EOO (104,000 km^2,referring to data from scientific collections predating this study) (Fig.S2, Supporting Information).Therefore, the new threat category established for the species is Near Threatened, according to the distribution data.

Most of the occurrences registeredby us were surrounded by urban patches, characterizing fragmentation and decline of habitat. Other local threats identified were human habitations on the watercourse margins, dump of domestic wastewater, plumbing, drainage and watercourse deviation. Such interventions in the environment directly affect the habitat of the species and were frequent to Guaíba I group. In rural areas, the use of agrochemicals and the removal of riparian vegetation for agricultural and livestock activities were frequent, and observed for both groups, Guaíba I and Guaíba II.

Discussion

Genetic variation in Brazilian *Parastacus brasiliensis* sensu lato

Genetic diversity has been widely recognized as an important component of biodiversity evaluations (Ehrlich & Wilson, 1991; Humphries, Williams & Wright, 1995). In the present study, the results obtained in the analyses of genetic diversity allowed us to document the hidden diversity in the taxon *Parastacus brasiliensis*. The most important implication of these findings is the recognition and conservation of the involved species. Unrecognized diversity within *P. brasiliensis* is not entirely surprising as it seems to be recurrent in crayfish groups (e.g. Baker *et al*., 2004, Fratini *et al*., 2005; Trontelj *et al*., 2005; Filipová *et al*., 2010; Larson *et al*., 2012; Maguire *et al*., 2014). Additionally, the existence of at least two distinct lineages was previously recognized with the establishment of the subspecies *P. brasiliensis promatensis* by (Fontoura & Conter (2008).

Based on the phylogenetic reconstructions and observed valuesof evolutionary divergence (p-distances), *P. brasiliensis* and *P. promatensis* differ in 4.1% (16S rRNA) and 17.9% (Cox1) (Tables 2 and 3). These values are higher or in a similar high range than those reported for several crayfishspecies in which more than one independent evolving lineage was suggested (3.5to 4.5% for 16S;~ 7.0% for Cox1) (Grandjean *et al*., 2000; Fratini *et al.*, 2005; Zaccara *et al.*, 2004; Maguire *et al.*, 2014). Results from a more conserved marker (28S) also support *P. promatensis* as a distinct species, diverging in 2.6% from *P. brasiliensis*. Further morphological and ecological differences confirm the distinction of both species (Ribeiro *et al.*, in preparation).

Besides *P. promatensis*, we highlight the existence of three additional distinct lineages inhabiting other hydrographic basins than Guaíba Lake, with no overlapping areas or mixed populations between lineages. Populations within the same or neighbouring hydrographical basins are genetically similar (i.e. Group "A"; Fig.3, Tables 2 to 4), suggesting restricted gene flow among geographically separated groups of populations or lineages. Phylogenetic reconstructions and a taxonomic revision of South American representatives of *Parastacus* are being conducted and may clarify these relations (Ribeiro *et al.*, in preparation).

From a phylogeographic perspective, very little is known about the diversification process of several taxa inhabiting the temperate grasslands and the corresponding freshwaters of the Pampas, a South American ecoregion in which the state of Rio Grande do Sul is included (Langone, Camargo & de Sá, 2016). Nevertheless, behavioural and ecological studies show that burrowing crayfishes have limited dispersal abilities and high parental care (Baumart *et al.*, 2015; Palaoro, del Valle & Thiel, 2015), attributes that incites to consider isolation by distance as an important driver of species diversification in these crayfishes (i.e. reduced gene flow that facilitates isolation, resulting in divergence and subsequent speciation) (Avise, 2009).

***Parastacus brasiliensis*: population genetic structure between and within basins**

The phylogenetic reconstructions showed that *P. brasiliensis* sensu stricto is constituted of two populationsthat inhabit mainly the Guaíba Lake Basin (but also Taquari-Antas and Sinos basins, in restricted localities). However, the high degree of genetic differentiation between these populations (ϕ_{st}= 0.78) strongly suggests the restriction of gene flow between Guaíba I and II. High genetic structure and geographic subdivision within populations were reported for several crayfish species worldwide and is often explained by their typical patchy distribution, low dispersal ability, and small effective population sizes (Gouin, Grandjean & Souty-Grosset, 2006; Diéguez-Uribeondo *et al.*, 2008; Dawkins *et al.*, 2010; Koizumi *et al.*, 2012; Gross *et al.*, 2013).

While Guaíba Iharboursindividual haplotypes representing several locations, Guaíba IIconsists of two haplotypes separated by one mutation (representatives from Mariana Pimentel), and one haplotype from Porto Alegre, separated from the first two by ten mutational steps (Fig. 6), indicating highly localized sub-populations and limited connection within and specially between Guaíba I and II populations.Low genetic diversity within a population, as observed in Guaíba II, is often related to small effective population size, probably accompanied by founder effects or genetic bottlenecks (Koizumi *et al.*, 2012). Considering the low dispersal capability of the species, a founder effect is the more likely possibility, confirmed by the unimodal profile detected by mismatch analysis (Fig. 7).

It is also worth mentioningthat Guaíba II is located in a more distant portion of the Guaíba Lake basin, and the high genetic divergence observed between both populations may be a consequence of isolation-by-distance (IBD) (Fig. 8). IBD in general can promote a loss of intra-population genetic diversity and an increased inter-population diversity, which may both negatively affect the long-term viabilityof a group or population (Amos & Harwood, 1998).

Implications for Conservation and Management

The favoured habitat of sampled *P. brasiliensis* was similar to that described by Buckup & Rossi (1980) and Fontoura & Buckup (1989), where the authors report for Guaíba I and Guaíba II populations, respectively. Populations of lineages "A", "B" and "C" occupy a similar habitat to that of *P. brasiliensis* sensu stricto, while *P. promatensis* differs by its occurrence in a stream located at 850m altitude in Mampituba basin, which belongs to the coastal hydrographic region.

The analysis of distribution data using EOO with the B criterion is a widely used measure in the evaluation of invertebrates, including freshwater crustaceans (Cumberlidge *et al.,* 2009; Magris *et al.*, 2010; Lewis & Senior, 2011; Richman *et al.*, 2015). Moreover, 79% of the global diversity of threatened crayfishes was assessed by the B1 criterion combined with decline or fluctuation and only ~ 3% species presented sufficient data to application of the A criterion, which uses population size (Richman *et al.*, 2015). Similarly, South American crayfishes were evaluated exclusively by the B1 criterion, and high fragmentation and continuing decline in area, extension of occurrence and/or habitat quality were used to justify the threat category established (Almerão *et al.*, 2014). Primary data of distribution and conservation status are essential for making conservation and investment decisions (Darwall *et al.*, 2011).

Endemism is a common trait among crayfishes, since 98% of the species globally evaluated to extinction risk were registered in a single country (Richman *et al.*, 2015). Despite the endemicity of *P. brasiliensis* in the state of Rio Grande do Sul and its restricted distribution to five of the eight basins that compose the Guaíba hydrographic region, the EOO estimatedislarger than the threshold established for the threatened category (i.e. 20,000km^2). Thus, we define the Near Threatened category as the most appropriate, but the possibility of category change for *P. brasiliensis* to Threatened in the near future is not unlikely, since several menaces exist. Moreover, the occurrence sites surrounded by intense urbanization showed high fragmentation of habitat.

The Guaíba hydrographic region harbours 61% of the population of the state of Rio Grande do Sul (Fundação Estadual de Proteção Ambiental Henrique Luiz Roessler–FEPAM, 2010). In the urban areas, extensive environmental problems have been associated with high urban and industrial concentration, such as dump of domestic wastewater, industrial residues, domestic waste, and air pollution due to industries and vehicles. In rural regions, soil erosion, silting of rivers, dump of pesticides and organic residues, especially animal waste into the rivers, have been recognized as the main disturbances (FEPAM, 2010).

Despite the common threats mentioned above, some particular problems can be highlighted for each hydrographic basin, as contamination of superficial waters by cyanide, and the presence of mercury in the fluvial sediment (Sinos basin) (Cerveira *et al.*, 2011; Rodrigues *et al.*, 2011), intense coal mining and presence of mutagenic agents in soils under the influence of coal wastes (Baixo Jacuí) (Silva Júnior & Vargas, 2014), and the decrease in water quality during the last 20 years due to the presence of a petrochemical complex in the Caí basin (Terra *et al.*, 2006, 2008).

Conservation strategies for *P. brasiliensis*, as for any other organism, should aim to increase the population size, but also to promote the maintenance of genetically distinct lineages, each representing a unique evolutionary history and an important biodiversity reservoir (Bertocchi *et al.*, 2008). In this context, the genetic approach brought another interpretation forthe distribution of data and necessary decisions about target populations for conservation. The Guaíba II group showed to be an important local genetic pool, distinct from Guaíba I, located in an isolated portion of the basin where there is only a private protected area that does not include any record of *P.*

brasiliensis. Both factors highlight the fragility of this population facing threats and stress, and reinforce the need for monitoring and adoption of conservation measures for this representative section of the Guaíba basin.

In contrast, in the area of occurrence of Guaíba I, nine protection areas of different types (Natural Parks and Private Reserves of Natural Patrimony) already exist and in at least two of these areas crayfishes were found.

Conservation actions herein proposed for *P. brasiliensis* comprise: 1) minimize the damaging effects on crayfish habitats, mainly riparian deforestation, plumbing and water contamination. 2) Assess the extinction risk and potential threats to each hydrographic basin with occurrence of *P. brasiliensis* with standardized sampling (because each basin is affected by specific threats and in different intensities, and the species must not necessarily be under threats in all drainages).This information can furthermore subsidize management and conservation proposals. 3) Encourage the creation of protection areas in different drainages with occurrence of *P. brasiliensis,* for the preservation of the genetic diversity. Special attention should be given to the Guaíba II population that is currently not inside a protected area.

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Supporting Information

Figure S1. Result of Mantel Test correlation between geographic distance and genetic distance in *Parastacus brasiliensis* s. str. sampling locations.

Figure S2. Reduction of potential distribution of *Parastacus brasiliensis* s. str. (von Martens, 1869) in the state of Rio Grande do Sul, Brazil.

Table 1. List of specimens, hydrographic basins, sampling localities, respective geographical coordinates and collection numbers used in the present study. UFRGS: Carcinological Colection of Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, RS; MZU: Zoological Museum of Universidade do Vale do Rio dos Sinos, RS; RHG: Hydrographic Region of Guaíba; RHL: Hydrographic Region of Litoranean; RHU: Hydrographic Region of Uruguai.

Table 2. Genetic divergence matrix (p-distances) of the mitochondrial gene 16S rRNA among evolutionary lineages of *Parastacus* “A”, “B” and “C” designate distinct, but undescribed species of *Parastacus*, followed by respective hydrographic basins.

Table 3. Genetic divergence matrix (p-distances) of the mitochondrial gene Cox1 among species of *Parastacus*. “A”, “B” and “C” designate distinct but undescribed *Parastacus* species, followed by respective hydrographic basins.

Table 4. Genetic divergence matrix (p-distances) of the nuclear gene 28S among species of *Parastacus*. “A”, “B” and “C” designate distinct but undescribed *Parastacus* species, followed by respective hydrographic basins.

Table 5. Distribution of haplotypes, based on the Cox1 gene, detected in *Parastacus brasiliensis*. N: number of individuals, Hd: haplotype diversity, π: nucleotide diversity.

Table 6. Analysis of molecular variance (AMOVA) in *P. brasiliensis* (*: significant values). **Figure 1**. *Parastacus brasiliensis* s. str. (von Martens, 1869). A. Live specimen; B. Habitat, first order stream found in Mariana Pimentel, Rio Grande do Sul, Guaíba Basin; C. Habitat, a spring near agriculture area; D. Habitat, watercourse near urban area, burrow in the margin; E. and F. Burrows with chimney, scale 5 cm; G. simple opening chimney with subtle edge; H. Burrow simple openings without chimney.

Figure 2. *Parastacus brasiliensis* s. str. (von Martens, 1869). Distribution of samples of specimens used: new sampled localities, material examined from scientific collections and museums and DNA extractions, in the states of Rio Grande do Sul and Santa Catarina, Brazil.

Figure 3. Phylogram for populations of *Parastacus*, based on Maximum Likelihood analysis (ML) of a concatenated dataset (16S and Cox1).Complete (A) and simplified (B) versions. For locations, see Table 1. Numbers represent identification of individuals. GenBank sequences are represented by (*); 1000 bootstraps. Black circles correspond to bootstrap values > 50%, white circles correspond to bootstrap values > 70% and values <50% are not shown.

Figure 4. Phylogram for populations of *Parastacus*, based on Maximum Likelihood analysis (ML) of 28S nDNA gene sequences.Complete (A) and simplified (B) versions. For locations, see Table 1. Numbers represent identification of individuals. GenBank sequences are represented by (*). 1000 bootstraps. Bootstrap values under 50% are not shown.

Figure 5. Distribution of lineages “A”, “B” and “C” groups and *Parastacus promatensis* Fontoura & Conter 2008 in hydrographic basins of state of Rio Grande do Sul, Brazil.

Figure 6. Haplotype network of *Parastacus brasiliensis* s. str. constructed with TCS 1.21 with a connection limit of 95%, derived from Cox1 mtDNA (600 bp). A) Guaíba I and B) Guaíba II and respective pairwise mismatch distribution.

Table 1. List of specimens, hydrographic basins, sampling localities, respective geographical coordinates and collection numbers used in the present study. UFRGS: Carcinological Colection of Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, RS; MZU: Zoological Museum of Universidade do Vale do Rio dos Sinos, RS; RHG: Hydrographic Region of Guaíba; RHL: Hydrographic Region of Litoranean; RHU: Hydrographic Region of Uruguai.

Hydrographic Basin	**Localities identification**	**Coordinates**	**Collection Number**
BaixoJacuí (RHG)	19 Barão do Triunfo	30.33°S; 51.68°W	UFRGS 4886
	67, 69, 88 Faxinal do Soturno	29.58°S; 53.50°W	UFRGS 5793, 5796
	23 Pântano Grande	30.17°S; 52.40°W	UFRGS 208
	20 São Jerônimo	30.48°S; 52.06°W	UFRGS 5339
	31 São Sebastião do Caí-Harmonia	29.55°S; 51.38°W	UFRGS 2191
Caí (RHG)	26 Dois Irmãos	29.60°S, 51.09°W	UFRGS 2346
Camaquã (RHL)	50-52 Dom Feliciano	30.54°S; 52.12°W	UFRGS 5485, 5493, 5497
Gravataí (RHG)	16 Gravataí	29.87°S; 51.03°W	UFRGS 3709
Lago Guaíba (RHG)	79, 85 Alagado (Porto Alegre)	30.10°S; 51.19°W	UFRGS 5759
	7-13, 73-77, 86 Mariana Pimentel	30.34°S; 51.56°W	UFRGS 4886, 4927, 5756, 5758, 5764
	78 Morro Santana (Porto Alegre)	30.06°S; 51.12°W	UFRGS 5757
	98-102 Morro do Osso (Porto Alegre)	30.11°S; 51.23°W	UFRGS 5947
	81-83 Padres (Porto Alegre)	30.09°S; 51.17°W	UFRGS 5762
	80 Piquete (Porto Alegre)	30.09°S; 51.18°W	UFRGS 5760
	96 Quinta da Estância Grande (Gravataí)	30.04°S; 51.23°W	UFRGS 5946
	92-94 Renascença (Porto Alegre)	30.09°S; 51.19°W	UFRGS 5860, 5861
	65 Sertão Santana	30.45°S; 51.59°W	UFRGS 4924
	95 Sítio do Mato (Porto Alegre)	30.11°S; 51.14°W	UFRGS 5868
	17, 28 Viamão	30.13°S; 51.12°W	UFRGS 1354
Santa Maria (RHU)	72 Rosário do Sul	30.16°S; 54.53°W	UFRGS 1848
Sinos (RHG)	1-6 Caraá	29.40°S; 50.41°W	UFRGS 6142
	103, 104 Gravataí	29.80°S; 50.96°W	UFRGS 5948
	53 Sapucaia do Sul	29°55°S; 51.17°W	MZU 192
	33 Taquara	29.65°S; 50.78°W	UFRGS 2694
Taquari-Antas (RHG)	18 Venâncio Aires	29.58°S; 52.19°W	UFRGS 4793
Tramandaí (RHL)	14 Maquiné	29.54°S; 50.23°W	UFRGS 3896
	21, 41, 43, 105, 106 São Francisco de Paula	29.50°S; 50.22°W	UFRGS 4161, 4151, 4153, 5949
Vacacaí-VacacaíMirim (RHG)	66, 68, 87, 89 Silveira Martins	29.65°S; 53.62°W	UFRGS 5141, 5778, 5794

Table 2. Genetic divergence matrix (p-distances) of the mitochondrial gene 16S rRNA among evolutionary lineages of *Parastacus* "A", "B" and "C" designate distinct, but undescribed speciesof *Parastacus*, followed by respective hydrographic basins.

	1	**2**	**3**	**4**
1. *P. brasiliensis* s. str.	0			
2. *P. Promatensis*	0.041	0		
3. *Parastacus* sp (A)-Baixo Jacuí+Vacacaí	0.041	0.033	0	
4. *Parastacus* sp (B)-Sinos	0.022	0.032	0.026	0
5. *Parastacus* sp (C)-Camaquã	0.044	0.040	0.038	0.032

Table 3. Genetic divergence matrix (p-distances) of the mitochondrial gene Cox1 among species of *Parastacus*. "A", "B" and "C" designate distinct but undescribed *Parastacus* species, followed by respective hydrographic basins.

	1	**2**	**3**	**4**
1. *P. brasiliensis* s. str.	0			
2. *P. promatensis*	0.179	0		
3. *Parastacus* sp (A)-Baixo Jacuí	0.146	0.138	0	
4. *Parastacus* sp (B)-Sinos	0.050	0.180	0.142	0
5. *Parastacus* sp (C)-Camaquã	0.129	0.182	0.140	0.117

Table 4. Genetic divergence matrix (p-distances) of the nuclear gene 28S among species of *Parastacus*. "A", "B" and "C" designate distinct but undescribed *Parastacus* species, followed by respective hydrographic basins.

	1	**2**	**3**	**4**
1. *P. brasiliensis* s. str.	0			
2. *P. promatensis*	0.026	0		
3. *Parastacus* sp (A)-Baixo Jacuí+Vacacaí	0.016	0.024	0	
4. *Parastacus* sp (B)-Sinos	0.001	0.026	0.016	0
5. *Parastacus* sp (C)-Camaquã	0.001	0.027	0.017	0.001

Table 5. Distribution of haplotypes, based on the Cox1 gene, detected in *Parastacus brasiliensis*. N: number of individuals, Hd: haplotype diversity,π: nucleotide diversity.

Population	**Haplotypes (h)**	**N**	**Hd**	π
Guaíba I	H1: 18 Taquara	1		
	H2: 20 Porto Alegre	1		
	H3: 82, 83 Padres, Porto Alegre	2		
	H4: 93 Renascença, Porto Alegre	1		
	H5: 81 Padres, Porto Alegre	1		
	H6: 79 Alagado, Porto Alegre	1		
	H7: 78 Morro Santana, Porto Alegre	1		
	H8: 96 Gravataí	1		
	H9: 103, 104 Gravataí	2		
	H10: 98, 100-102 Morro do Osso, Porto Alegre	4		
	H11: 95 Sítio do Mato, Porto Alegre	1		
	Total:	16	0.93	0.01462
Guaíba II	H12: 13 Mariana Pimentel	1		
	H13: 7-12, 75-77, 86 Mariana Pimentel,	10		
	65 Sertão Santana	1		
	H14: 94 Renascença, Porto Alegre	1		
	Total	13	0.29	0.00364

Table 6. Analysis of molecular variance (AMOVA) in *P. brasiliensis* (*: significant values).

Structure	**Source of variation**	**%**	**Fixation index**	**P***
Absent	Among populations	78.75	ϕ_{st}= 0.78546	<0.001
	Within populations	21.45		

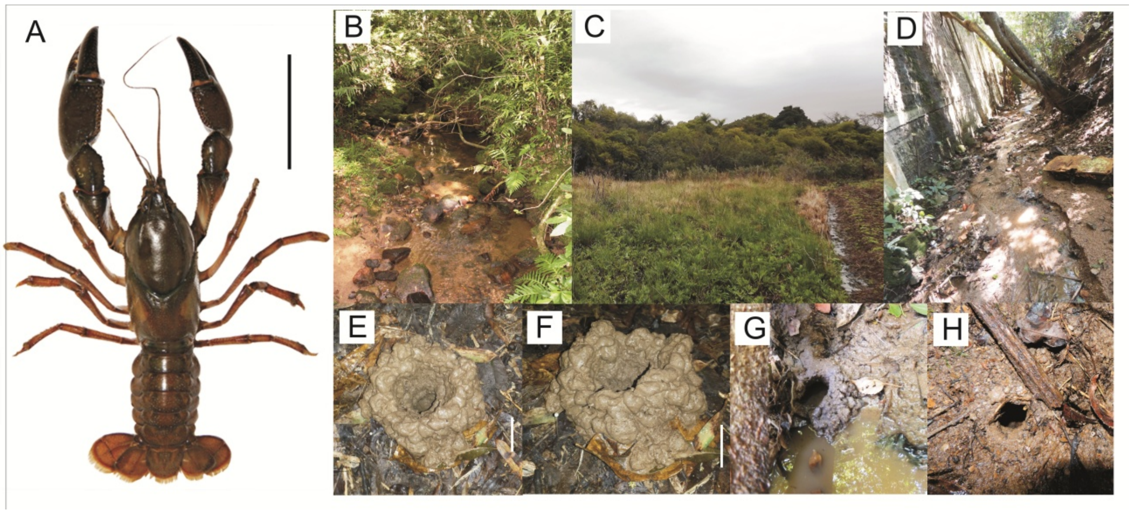


Figure 1. *Parastacus brasiliensis* s. str. (von Martens, 1869). A. Live specimen; B. Habitat, first order stream found in Mariana Pimentel, Rio Grande do Sul, Guaíba Basin; C. Habitat, a spring near agriculture area; D. Habitat, watercourse near urban area, burrow in the margin; E. and F. Burrows with chimney, scale 5 cm; G. simple opening chimney with subtle edge; H. Burrow simple openings without chimney.

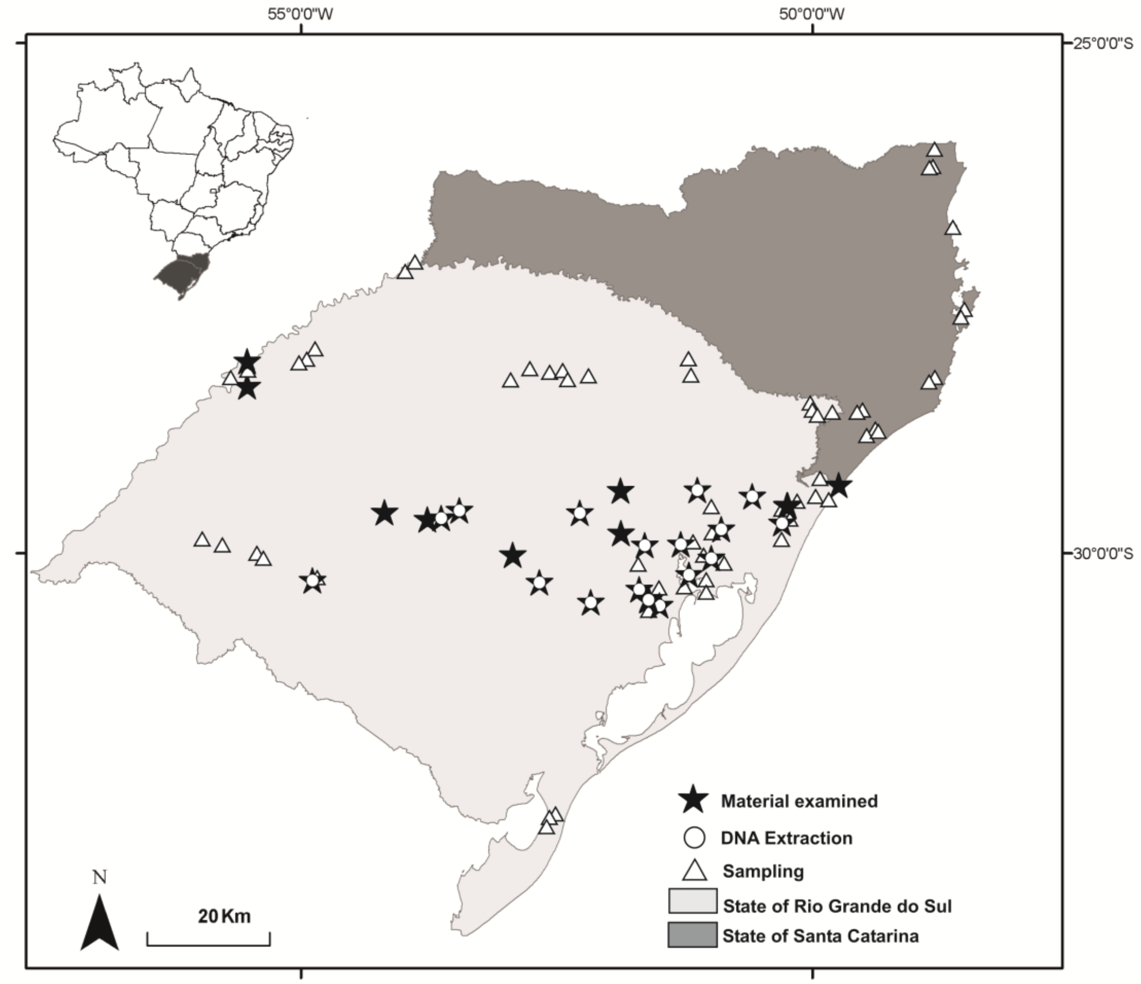
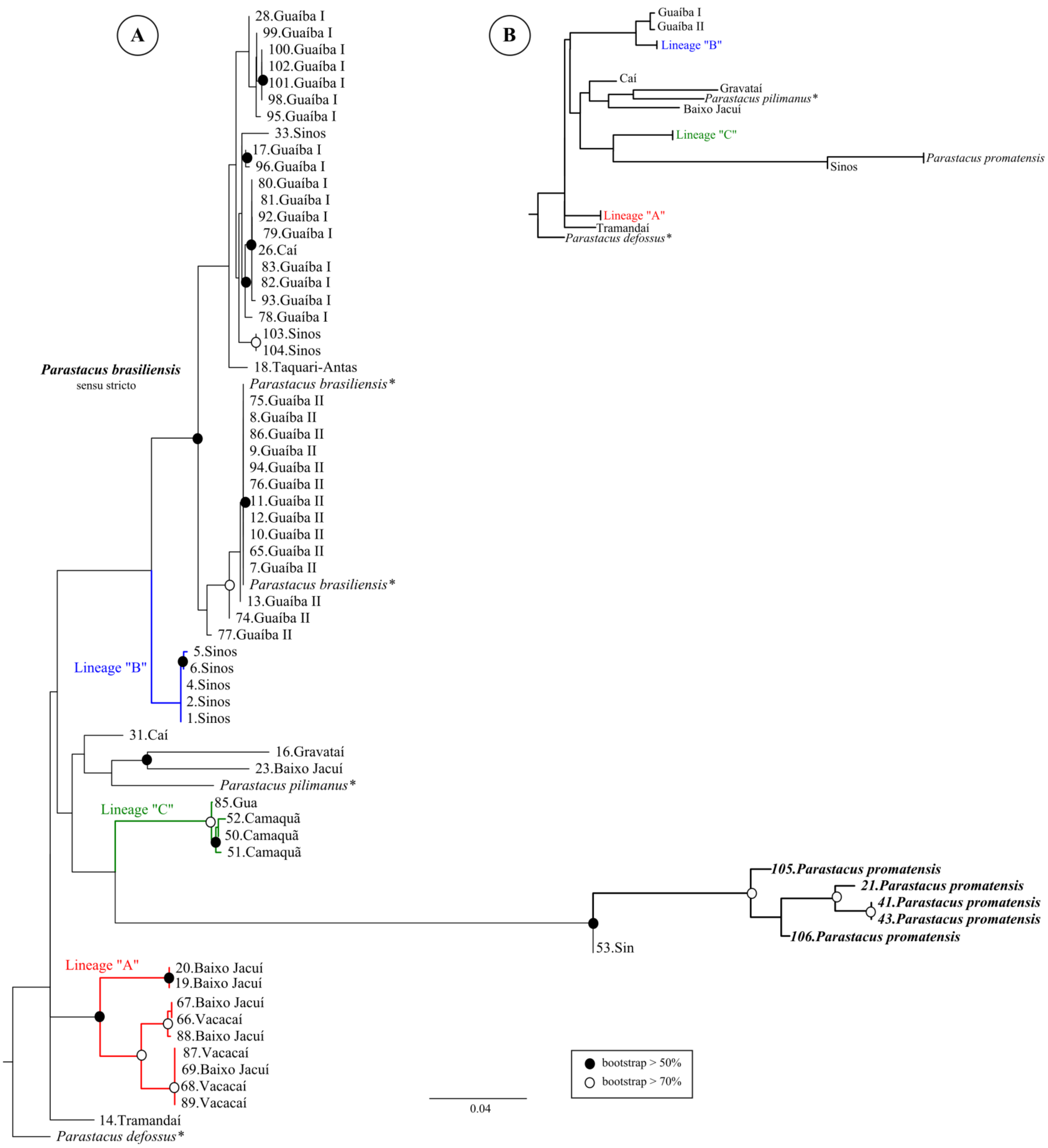


Figure 2. *Parastacus brasiliensis* s. str. (von Martens, 1869). Distribution of samples of specimens used: new sampled localities, material examined from scientific collections and museums and DNA extractions, in the states of Rio Grande do Sul and Santa Catarina, Brazil.



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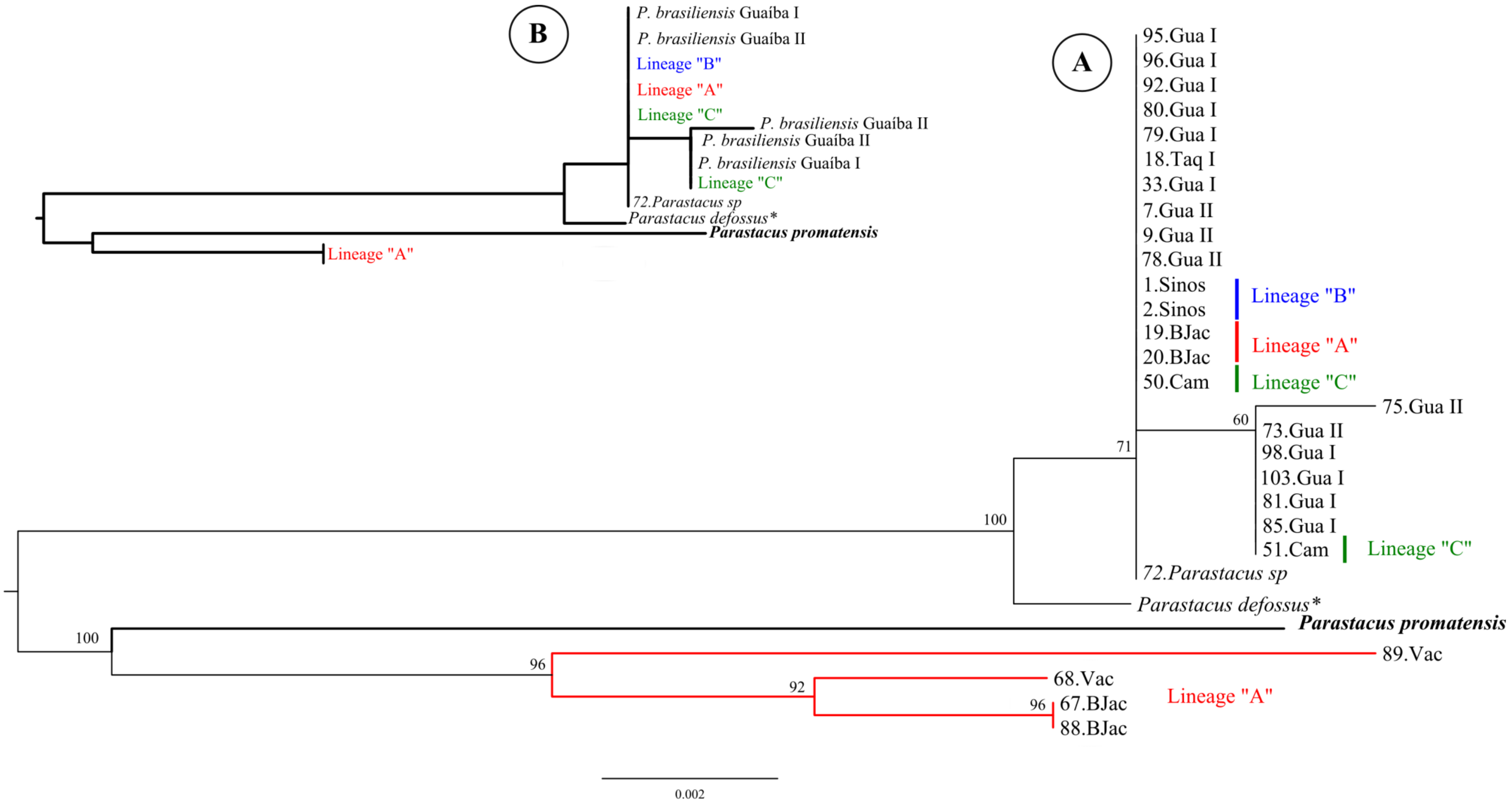

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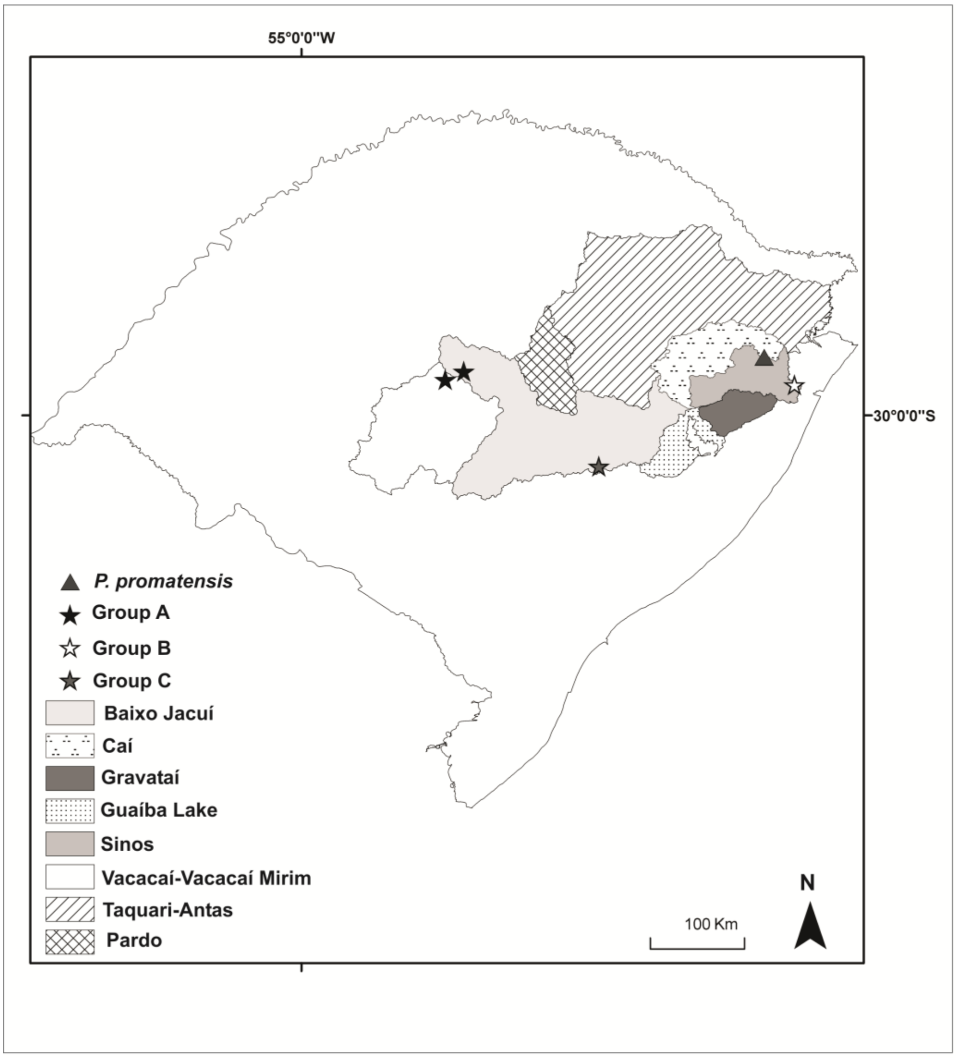

Figure 5. Distribution of lineages "A", "B" and "C" groups and *Parastacus promatensis* Fontoura & Conter 2008 in hydrographic basins of state of Rio Grande do Sul, Brazil.

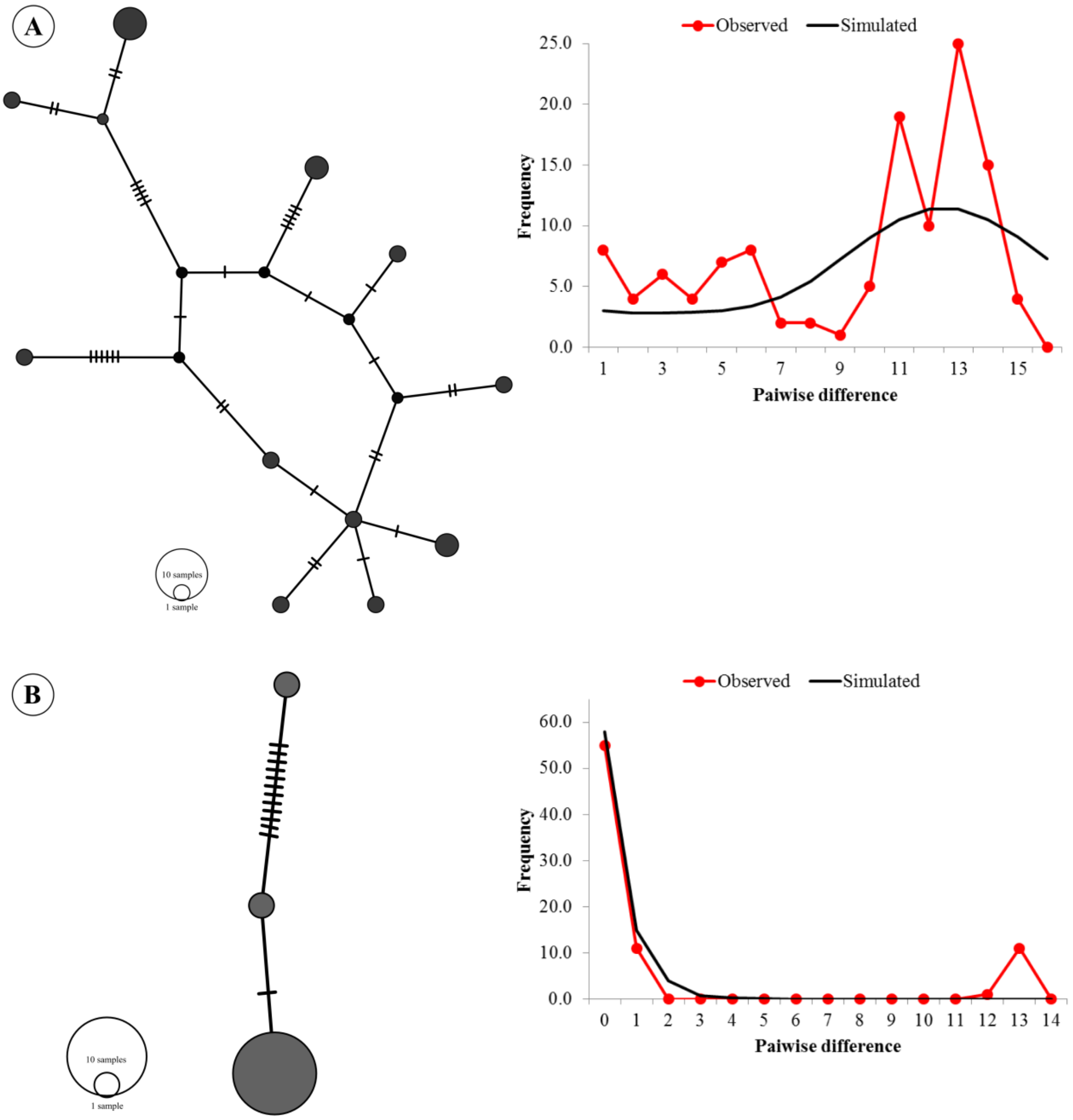

Figure 6. Haplotype network of *Parastacus brasiliensis* s. str. constructed with TCS 1.21 with a connection limit of 95%, derived from Cox1 mtDNA (823 bp). A) Guaíba I and B) Guaíba II and respective pairwise mismatch distribution.

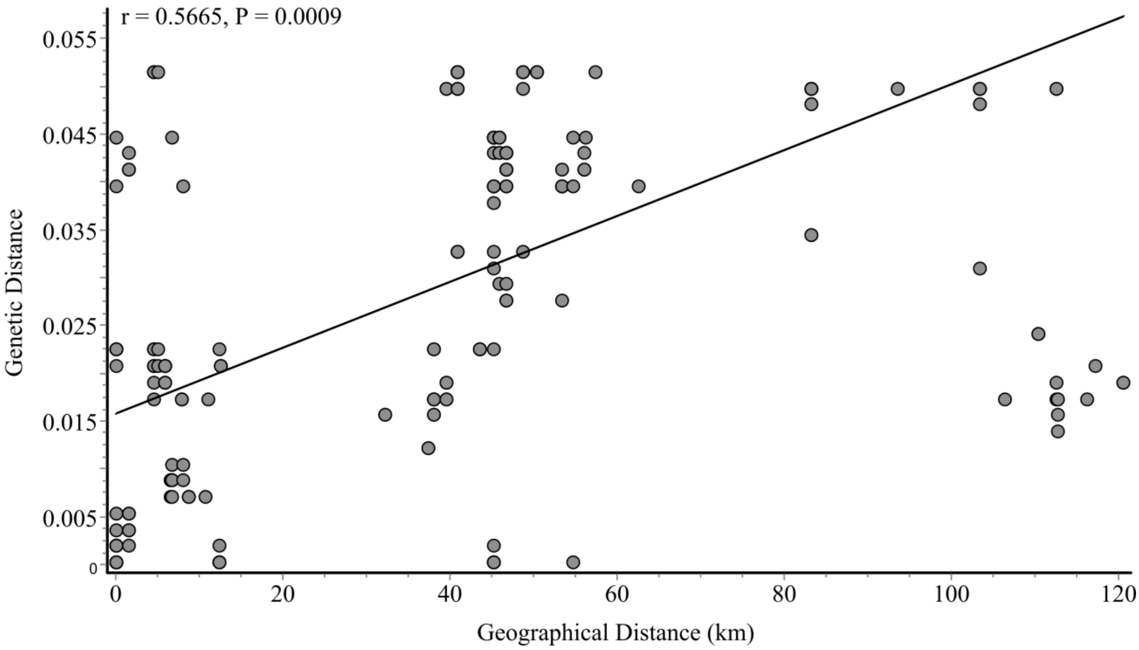

Figure 7. Result of Mantel Test correlation between geographic distance and genetic distance in *Parastacus brasiliensis* s. str. sampling locations.

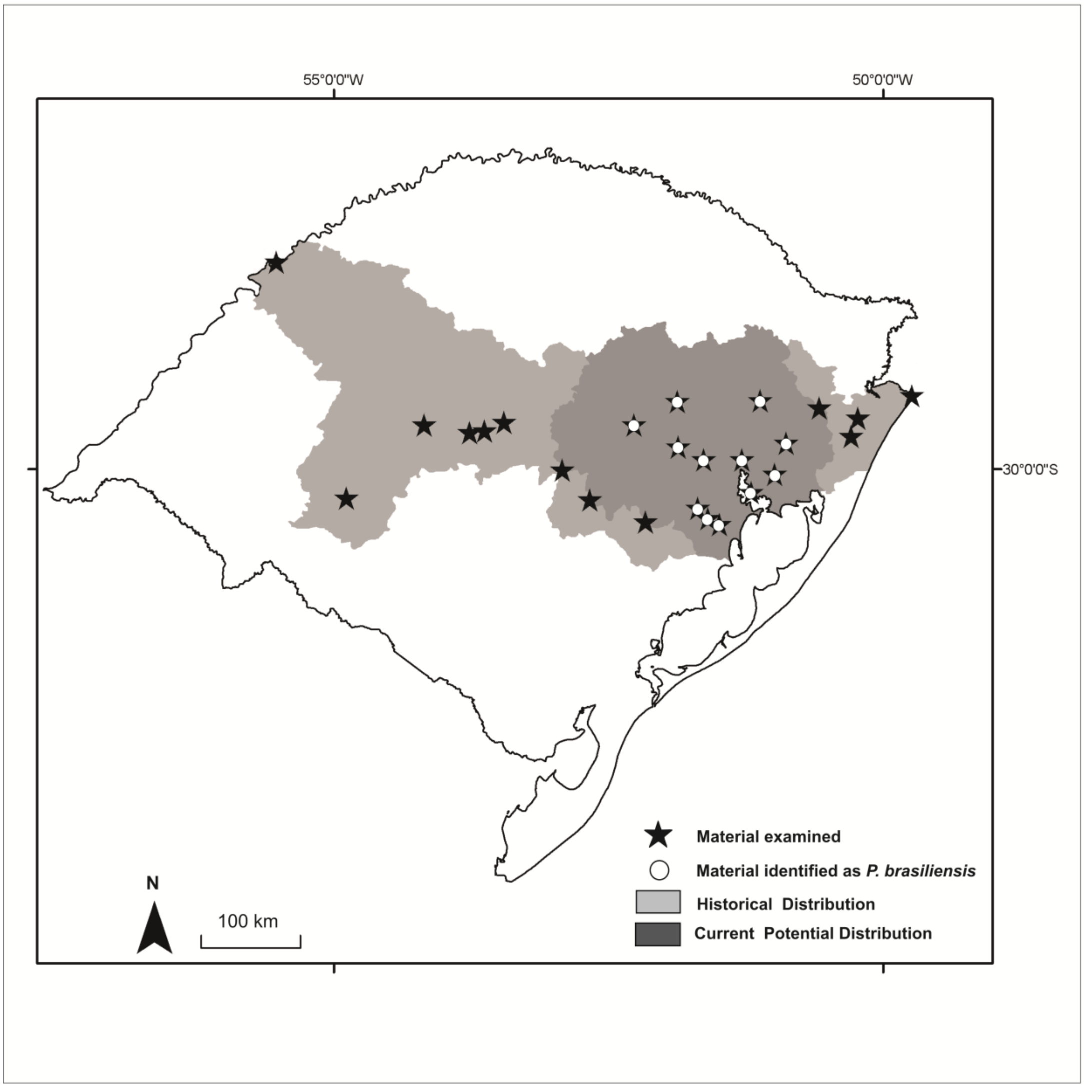

Figure 8. Reduction of potential distribution of *Parastacus brasiliensis* s. str. (von Martens, 1869) in the state of Rio Grande do Sul, Brazil.

6. Considerações Finais

O conhecimento adquirido acerca dos lagostins não se resume aos três capítulos que a compõem a tese. A vivência de campo durante os três anos de coletas realizadas nos estados do Rio Grande do Sul e Santa Catarina, contribuiu para incrementar a compreensão deste complexo grupo de crustáceos de água doce. Foi também a construção de um conhecimento que reconhece o potencial dos lagostins como uma ferramenta para análise de paisagem, mudanças no hábitat e urbanização.

Novas fronteiras foram abertas e trabalhos já estão sendo realizados tendo como ponto de partida dados oriundos da tese. Um deles refere-se às análises de paisagem com estimativas de ambientes potenciais a ocorrência dos lagostins de água doce e predições da perda do hábitat em Porto Alegre e foi recentemente realizado durante um trabalho de conclusão de curso na UFRGS (http://www.lume.ufrgs.br/bitstream/handle/10183/142149/000991097.pdf?sequence=1). As investigações sobre a genética de populações têm também apresentado resultados surpreendentes com outras espécies de lagostins, apontando para uma diversidade críptica subestimada.

A observação em campo, as análises de distribuição e requerimentos ecológicos das espécies apontam para a proposta de uma classificação das espécies de lagostins da América do Sul, considerando o habitat ocupado, tipo de toca construída e morfologia do corpo. Observou-se que a classificação existente não inclui as particularidades das espécies estudadas aqui. A necessidade da nova classificação vai além do ponto de vista acadêmico, uma vez que, ainda antes de ser elaborada formalmente as informações obtidas tem se mostrado exequível e com potencial aplicabilidade no reconhecimento das espécies em campo, a partir dos seus vestígios.

As primeiras idas a campo foram as mais difíceis. Contudo, a intensa divulgação sobre a existência dos lagostins trouxe-nos como retorno a colaboração de pesquisadores e cidadãos comuns que forneceram informações valiosas e que permitiram a observação detalhada dos ambientes e coleta dos animais. As informações compartilhadas sobre a localização desses crustáceos permitiu a descoberta de novas espécies (Anexo I – Informativos para divulgação). Se por um lado o anho acadêmico é de valor inestimável, a oportunidade de ensinar, explicar e dar um retorno à população acerca da importância biológica e ecológica dos lagostins é, no mínimo, de grandeza equivalente.

Os resultados expressos na presente tese tem também potencial de utilização como base a políticas de conservação dos lagostins, em especial porque as ameaças locais estão mapeadas para a maior parte dos pontos de ocorrência das espécies. A conservação desses crustáceos está intimamente relacionada à preservação dos ambientes que ocupam, independentemente do hábitat. Nas áreas urbanas, foram encontrados lagostins em trechos de riachos, caracterizados por acúmulo de lixo e alta demanda de matéria orgânica, praças, quintais de casas e hortas. Nos fragmentos de áreas úmidas o pisoteio de pessoas e animais, as drenagens dessas áreas e uso de pesticidas na lavoura são as principais perturbações observadas. Além disso, a população tem uma visão distorcida sobre os banhados, tratando-os como um ambiente sujo e um obstáculo à construção civil e a agricultura. Até quando essas populações resistirão com um numero viável de indivíduos para sua manutenção? Nesse sentido e, dados os cenários atuais e futuros,, a educação ambiental, aliada às políticas de conservação, sempre será um caminho viável para a conservação das espécies, pois cobrar o cuidado com o meio ambiente sem conhecê-lo é um caminho muito mais difícil e com resultados que não perduram.

7. Anexos

Folder de divulgação dos lagostins de água doce no Rio Grande do Sul.

Tu já viu um Lagostim ?

Ribeiro, F.B

Lagostim de água doce

Os lagostins de água doce são crustáceos, também chamados popularmente de caranguejo, camarão e pitú, que ocorrem no RS e SC.
Em Porto Alegre, os registros dos lagostins são mais comuns do que se imagina.

Onde são encontrados?

Áreas úmidas
(banhados e várzeas)

Cursos d'água
(sangas, arroios e rios)

Vestígios

Tipo de Tocas

Tipo chaminé, altura de 2-34cm

Tipo simples

~1.6 m de profundidade

Esquema dos túneis escavados pelos lagostins e as setas indicam as várias aberturas na superfície do solo.

Qual a sua importância?

Alimentam-se de

Plantas

Larva de insetos

Lagostim

2 cm

Consumidos por

Aves

Peixes

Anfíbios

Pequenos mamíferos

- Participam da cadeia alimentar
- Promovem a aeração do solo

Como tu podes ajudar

Se tu tiveres conhecimento da presença ou dos vestígios, por favor, contate-nos, pois a tua informação irá contribuir para trabalhos que estão sendo desenvolvidos no laboratório de Carcinologia da UFRGS, além de colaborar com a conservação das espécies de lagostins de água doce.

Contato

Email: procurolagostins@gmail.com - Kelly
Telefone: 51 - 33087698
- Lab. de Carcinologia/ UFRGS

WANTED

DEAD OR ALIVE

Os lagostins de água doce são crustáceos do gênero *Parastacus*, restritos a alguns países da América do Sul. No Brasil, ocorrem nos estados do Rio Grande do Sul e Santa Catarina. São encontrados sob pedras e folhas nos cursos d'água e em tocas construídas nas barrancas dos rios. Além disso, podem habitar as regiões de banhado, onde também constroem suas tocas (ver figura abaixo).

Se você ver ou coletar esses indivíduos, junto ao seu material de pesquisa, contate-nos , por favor !

Parastacus brasiliensis

Contato:
Lab. Carcinologia UFRGS
Telefone: 51 33087698
k.marttinez@gmail.com- Kelly

Normas de formatação dos capítulos I e II.

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Jerling, H. L. (1994). Feeding ecology of mesozooplankton in the Sundays River Estuary (PhD thesis). University of Port Elizabeth, South Africa.

For European directives: Council of the European Communities (2000). Directive 2000/60/EC of the European Parliament and the Council of 23rd October 2000 establishing a framework for community action in the field of water policy. *Official Journal of the European Communities* (L327), pp. 1-72.

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