AQUATIC CONSERVATION: MARINE AND FRESHWATER ECOSYSTEMS
Aquatic Conserv: Mar. Freshw. Ecosyst. (2014)
Published online in Wiley Online Library
(wileyonlinelibrary.com). DOI: 10.1002/aqc.2488

The native South American crayfishes (Crustacea, Parastacidae): state of knowledge and conservation status

MAURICIO PEREIRA ALMERÃO[a,*], ERICH RUDOLPH[b], CATHERINE SOUTY-GROSSET[c], KEITH CRANDALL[d], LUDWIG BUCKUP[a], JULIEN AMOURET[c], ANA VERDI[e], SANDRO SANTOS[f] and PAULA BEATRIZ DE ARAUJO[a]
[a]*Federal University of Rio Grande do Sul, Zoology Department, Porto Alegre, Brazil*
[b]*Department of Biological Sciences and Biodiversity, University of Los Lagos, Osorno, Chile*
[c]*University of Poitiers, Ecology and Biological Interactions, Poitiers, France*
[d]*Computational Biology Institute, George Washington University & National Museum of Natural History, Washington, DC, USA*
[e]*Entomological Section, University of La Republica, Montevideo, Uruguay*
[f]*Federal University of Santa Maria, Biology Department, Santa Maria, Brazil*

ABSTRACT

1. South America is considered a world biodiversity hotspot, especially for freshwater ecosystems where there is significant biodiversity and endemism across different taxonomic groups. Native South American crayfishes are characterized by small range distributions, high levels of endemism and specific habitat requirements.

2. Although this group may be considered one of the most threatened among South American freshwater crustaceans, IUCN Red List assessments for most of these species are 'data deficient'. IUCN assessments are technical evaluations of species risk of extinction based on biological indicators of population features, thereby providing a 'conservation status' for each species based on common and international standards.

3. Attempts to assess the conservation status of native South American crayfishes have been limited mainly owing to a lack of fundamental data and also misinterpretations of present information. Thus, a revision of the state of knowledge of native South American crayfishes (taxonomy, phylogeny and distribution, genetics, ecology and threats) was carried out coupled with IUCN assessments of conservation status.

4. Globally, the lack of information on the biology of these species is still the major obstacle to making reliable conservation status assessments. For this reason, the Data Deficient (DD) category is still appropriate for many species. However, based on the Extent of Occurrence (EOO) and severely fragmented populations, number of locations and decline in area, extent or quality of habitat, it is recommended that some species (especially *Virilastacus* spp.) should be moved to threatened categories (VU, EN or CR). The data suggest that others are appropriately in the Near Threatened (NT) category, because they almost reach the thresholds for threatened categories.

5. The assessment of conservation status is essential to support future conservation actions, especially for species allocated to threatened categories. This study has identified new species under threat and also areas for future study to provide relevant data for conservation assessment for 'data deficient' species.

Received 20 December 2013; Revised 8 June 2014; Accepted 28 June 2014

KEY WORDS: South America; native freshwater crayfishes; IUCN; fundamental data; conservation status

*Correspondence to: M. P. Almerão, UFRGS, IB, Departamento de Zoologia, Av. Bento Gonçalves, 9500, 91501-970 - Porto Alegre, Rio Grande do Sul, Brasil. E-mail:mauricioalmerao@gmail.com

INTRODUCTION

The term 'biodiversity hotspot' was coined by Myers (1989, 1990) to identify geographical regions as conservation priorities. These regions contain large numbers of endemic species in relatively small areas under significant threat of habitat loss (Myers, 1989, 1990). More generally, however, the term hotspot has been applied to a geographical area that ranks particularly high on one or more axes of species richness, phylogenetic diversity, levels of endemism, numbers of rare or threatened species, or intensity of threat. Among different biodiversity hotspots identified in the world, South America is one of the most important (Conservation International, 2013). This is especially true for South American freshwater ecosystems where endemic and species-rich groups (Tisseuil *et al.*, 2013) face a variety of threats (Dudgeon *et al.*, 2006).

South American freshwater crustaceans, in particular, deserve special attention (Lévêque *et al.*, 2005; Bond-Buckup *et al.*, 2008; De Grave *et al.*, 2008; Magris *et al.*, 2010). One such group is the native South American crayfishes belonging to the family Parastacidae. Almost all species of native South American crayfishes are burrowers living in complex underground systems in various freshwater habitats (rivers, streams, lakes and marshlands) of southern South America (Chile, Argentina, Uruguay and Brazil) (Buckup, 2003; Rudolph, 2013). All these ecosystems have been exposed to different threats (e.g. stream channelization and urbanization) (Junk *et al.*, 2013) and are therefore at risk of population decline and local extinction.

Crayfishes are the largest and among the longest-lived invertebrates in temperate freshwater environments and are often present at high density (Gherardi and Souty-Grosset, 2006). The freshwater crayfishes are important elements in the food chain of the ecosystems in which they occur. Most species feed on benthic invertebrates, detritus, macrophytes and algae in lotic and lentic waters (Reynolds and Souty-Grosset, 2012). For this reason they have been considered key species in the transference of energy between different trophic groups (Momot, 1995; Gherardi, 2007). Beyond their impact on freshwater aquatic communities, crayfishes alter the physical environment in which they occur (Richardson, 1983). Thus, they contribute to ecosystem functioning and their presence determines the wellbeing of numerous taxa that depend upon them (Horwitz, 2010).

To be effective, conservation measures require knowledge about the conservation status of species, gained through evaluating data on distribution, ecology, genetics, threats, and population dynamics (Rodrigues *et al.*, 2006). The IUCN Red List of Threatened Species (henceforth 'Red List') (IUCN; http://www.iucn.org), contains species that are at the greatest risk of extinction and promotes their conservation by 'concentrating minds on true priorities' (Rodrigues *et al.*, 2006). Conservation status is assessed using five quantitative criteria (A–E) based on biological indicators of populations such as rapid population decline or very small population size. The relative degree of threat is used to assign species to different categories (Critically Endangered - CR; Endangered - EN; or Vulnerable - VU) (IUCN Standards and Petitions Subcommittee, 2013). For native South American parastacids, conservation status assessments have been performed over the last two decades (Table 1). Recently, a new species of *Virilastacus* (*Virilastacus jarai*) was described in Chile (Rudolph and Crandall, 2012) and was not included, therefore, in these previous assessments.

Despite the effort to allocate species to the most appropriate category, it is clear that the category used most frequently (Data Deficient - DD) reflects the lack of information for making a direct, or indirect, assessment of species risk of extinction based on its distribution or population status. The aims of this work were to present an overview of current knowledge on native South American crayfishes, to provide a detailed revision of conservation status, and to propose recommendations for this group of organisms based on IUCN Red List criteria.

STATE OF KNOWLEDGE

Taxonomy

In South America, the first information on crayfishes was provided by von Martens (1869)

Table 1. List of conservation status assessments of native South American crayfishes (current and suggested conservation status)

Species	List	Current conservation status (category/criteria)	Conservation status (category/criteria) suggested
Parastacus brasiliensis (von Martens, 1869)	LEFAE/RS* (Marques *et al.*, 2002) IUCN Red List (Buckup, 2010c)	Vulnerable (VU) Near Threatened (NT)	**Data Deficient (DD)**
Parastacus defossus Faxon, 1898	IUCN Red List (Buckup, 2010d)	Data Deficient (DD)	**Near Threatened (NT)**
Parastacus laevigatus Buckup and Rossi, 1980	IUCN Red List (Buckup, 2010e)	Data Deficient (DD)	**Data Deficient (DD)**
Parastacus pilimanus (von Martens, 1869)	IUCN Red List (Buckup, 2010f)	Least Concern (LC)	**Data Deficient (DD)**
Parastacus varicosus Faxon, 1898	IUCN Red List (Buckup, 2010h)	Data Deficient (DD)	**Data Deficient (DD)**
Parastacus saffordi Faxon, 1898	IUCN Red List (Buckup, 2010g)	Data Deficient (DD)	**Data Deficient (DD)**
Parastacus nicoleti (Philippi, 1882)	CCDNAC/CH**(Bahamonde *et al.*, 1998) Rudolph and Crandall (2007) IUCN Red List (Buckup, 2010a)	Vulnerable (VU) Vulnerable (VU - B1b(i, iii)) Data Deficient (DD)	**Data Deficient (DD)**
Parastacus pugnax (Poeppig, 1835)	CDNAC/CH**(Bahamonde *et al.*, 1998) Rudolph and Crandall (2007) IUCN Red List (Buckup, 2010b)	Vulnerable (VU) Vulnerable (VU - A3cd) Data Deficient (DD)	**Data Deficient (DD)**
Virilastacus araucanius (Faxon, 1914)	CCDNAC-CH**(Bahamonde *et al.*, 1998) Rudolph and Crandall (2007) IUCN Red List (Buckup, 2010j)	Data Deficient (DD) Vulnerable (VU - B1ab(iii)) Data Deficient (DD)	**Vulnerable (VU - B1ab(iii))**
Virilastacus retamali Rudolph and Crandall, 2007	Rudolph and Crandall (2007) IUCN Red List (Buckup, 2010k)	Endangered (EN - B1ab(iii)) Data Deficient (DD) Endangered (EN - B1ab(iii))	**Endangered (EN - B1ab(iii))**
Virilastacus rucapihuelensis Rudolph and Crandall, 2005	Rudolph and Crandall (2005) IUCN Red List (Buckup, 2010l)	Endangered (EN - B1ab(iii)) Data Deficient (DD)	**Critically Endangered (CR - B1ab(iii))**
Virilastacus jarai Rudolph and Crandall, 2012	Rudolph and Crandall (2012)	Critically Endangered (CR - B1ab(iii))	**Critically Endangered (CR - B1ab(iii))**
Samastacus spinifrons (Philippi, 1882)	CCDNAC-CH**(Bahamonde *et al.*, 1998) Rudolph and Crandall (2007) IUCN Red List (Buckup, 2010i)	Data Deficient (DD) Vulnerable (VU - A3cd) Data Deficient (DD)	**Data Deficient (DD)**

*Lista das espécies da fauna ameaçadas de extinção no RS.
**Categorías de conservación de decápodos nativos de aguas continentales de Chile.

with descriptions of *Astacus pilimanus* and *Astacus brasiliensis*, collected in Porto Alegre and Santa Cruz do Sul (Brazil). Following this seminal work, many others worked on the taxonomy of South American crayfish: Huxley (1879), Faxon (1898, 1914), Ortmann (1902), Riek (1971), Buckup and Rossi (1980), Hobbs (1991), and Rudolph and Crandall (2005, 2007, 2012). At present there are 13 recognized crayfish species from South America (Table 1), with eight of them in the genus *Parastacus*.

Phylogeny and distribution

The distribution of freshwater fauna is dynamic and depends not only on their movements, but also on environmental fluctuations (Ringuelet, 1961). Dynamics are influenced by orogenic and climatic activities, from the time of Gondwana to the present (Xu *et al.*, 2009). It is traditionally considered that vicariance or dispersal events are the main processes explaining the current distribution of southern hemisphere fauna (Collins *et al.*, 2011).

Freshwater crayfishes are a highly diverse group of decapod crustaceans (more than 640 species) which form a monophyletic clade in decapod phylogeny (Crandall and Buhay, 2008). There are two centres of diversification, one in the northern hemisphere comprising the Cambaridae and Astacidae, and the other in the southern hemisphere comprising the Parastacidae (Sinclair *et al.*, 2004). The family

Parastacidae comprises 15 extant genera (~170 species) of which 11 are found in Australia, New Guinea and New Zealand, three in South America and one in Madagascar (Crandall and Buhay, 2008). The 13 native South American crayfishes belong to three genera (*Parastacus*, *Virilastacus* and *Samastacus*) distributed in Uruguay, north-east and southern Argentina and central-southern Chile (Buckup, 2003; Rudolph, 2013). This group forms a distinct monophyletic clade within the broader southern hemisphere group of crayfishes (Toon *et al.*, 2010).

The first records of freshwater crayfishes in South America were made in the 18th century (Buckup, 2003). Since then, populations have been identified in several localities in Brazil, Uruguay, Argentina and Chile (Figure 1). Presumably, this distributional pattern was influenced by marine water permanence during the ingressions that occurred from the Cretaceous period to the Middle Palaeogene period (Collins *et al.*, 2011). The genus *Parastacus* seems to have a disjointed distribution in which two species (*P. brasiliensis* and *P. laevigatus*) are endemic to southern Brazil, two (*P. pugnax* and *P. nicoleti*) are endemic to Chile and the other four species (*P. saffordi*, *P. varicosus*, *P. defossus* and *P. pilimanus*) are distributed in southern Brazil, Uruguay and north-east Argentina (Morrone and Lopretto, 1994; Buckup, 2003). However, this distribution is not reflected within the *Parastacus* phylogeny. For example, closer relationships would be expected between the two Chilean species than with Uruguayan and Brazilian ones, but this has not been observed (Crandall *et al.*, 2000; Toon *et al.*, 2010). Furthermore, the genus *Samastacus* with only one species (*S. spinifrons*), occurs in Chile and Argentina and all species of the genus *Virilastacus* (*V. araucanius*, *V. retamali*, *V. rucapihuelensis* and *V. jarai*) are endemic to Chile (Rudolph, 2010).

Reproductive strategies

Gonochorism is generally the main reproductive strategy within the Parastacidae (Vogt, 2002). Nevertheless, intersex species showing supernumerary

Figure 1. Distribution map of native South American crayfishes. RS, state of Rio Grande do Sul (Brazil); SC, state of Santa Catarina (Brazil); UR, Uruguay; AR, Argentina; CH: Chile.

gonopores have been reported. Rudolph and Almeida (2000) distinguished three types of sexual systems in the South American species: protandric hermaphroditism (PH), gonochorism (G), and gonochorism with permanent intersexuality (GPI) (Table S1, Supplementary data). Protandric hermaphroditism has been detected in six species in which the male is the first sex and it is characterized by the presence of gonads of both sexes in the same individual at different times in their lives (Almeida and Buckup, 2000). It is of interest that in populations of *P. varicosus* and *S. spinifrons* different strategies have been observed. For Brazilian and Uruguayan populations of *P. varicosus* PH and GPI have been recorded, respectively, while G and GPI were detected in Chilean populations of *S. spinifrons* (Rudolph, 1999, 2002a) (Table S1).

Although intersexuality in crustaceans may be caused by different factors (Ford, 2012), it seems to be a more transitory state of non-functional hermaphroditism. Two models have been developed for hermaphroditic species: the size–advantage model (reproductive success of one sex is enhanced in individuals of a particular size) and the gene dispersal model (the limits of population dispersal can affect population structure) (Ghiselin, 1969). For these crayfish, while there is a lack of population data to corroborate the size–advantage model, their burrowing life-style limits dispersal and the former is therefore likely to be a better explanation of the evolution of hemaphroditism. Clearly, hermaphroditism has evolved in native South American crayfishes with some plasticity possibly related to environmental conditions.

Habitat and population

Native South American crayfishes are generally found in rivers, streams, lakes and marshlands (Buckup, 2003; Rudolph, 2013) (Table S1). Many of them are burrowers in marshland areas away from open water (Rudolph and Crandall, 2007; Noro and Buckup, 2010) (e.g. *P. defossus* - Figure 2, and *V. retamali* - Figure 3) (Table S1) and some others

Figure 2. Species and habitat. A, *Parastacus defossus*, B, marshland area at Costa do Cerro, Lami, state of Rio Grande do Sul, Brazil (30°11'41''S - 51° 06'00''W); C, detail of chimney (black arrow); D, ready burrow casts. Courtesy of Clarissa Köhler Noro.

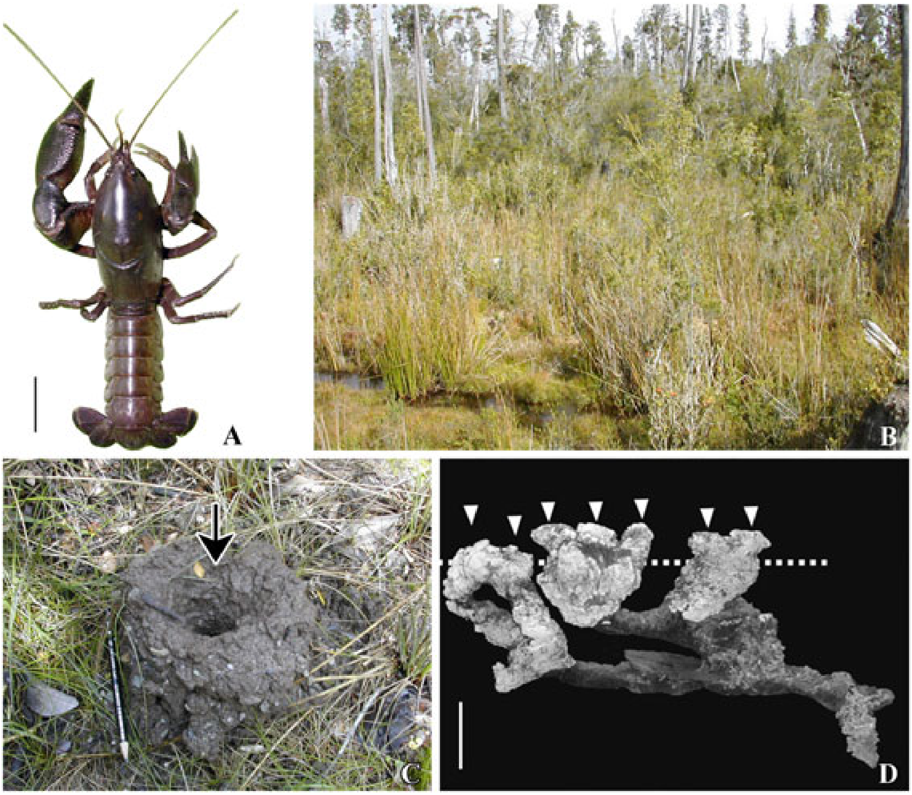

Figure 3. Species and habitat. A, *Virilastacus retamali*, B, marshland area at Estaquilla Alto, Chile (41° 25'S; 73° 46''W); C and D, detail of chimney (black arrow); D, ready burrow casts showing openings (white arrows). Courtesy of Emilio Bedatou.

burrow in the banks of rivers (Fontoura and Buckup, 1989; Buckup, 2003; Rudolph, 2013) (e.g. *P. brasiliensis* – Figure 4) or lakes (e.g. *S. spinifrons* - Figure 5) (Table S1). They construct galleries formed by a central tunnel with multiple branches (and some chambers) that connect the underground water to the soil surface by one or more openings (recognized by the chimneys opening to the surface). Burrows may reach a depth of ~1.5 m. Chimneys are conical towers formed by deposition of sediment removed by the crayfishes during the burrowing process. (Rudolph and Crandall, 2007; Noro and Buckup, 2010).

Present knowledge of population features of the South American parastacids varies greatly (Table S1). For most species there are no population data, but for three species at least one survey of population features has been carried out using different approaches. For example, ~2600 individuals of *P. brasiliensis* and ~3512 of *P. pugnax* were sampled over 1 year using a mark–recapture method (Fontoura and Buckup, 1989; Ibarra and Arana, 2012) (Table S1), and a direct capture method was used to estimate a population of ~766 individuals of *P. defossus* captured over 18 months (Noro and Buckup, 2008).

Genetic diversity

Despite the lack of information on genetic diversity of native South American crayfishes, some inferences may be drawn based on ecological and life-history similarities with other parastacids. It is assumed that their burrowing life-style is responsible for the poor dispersal ability of some species. Consequently, it is expected that populations have been historically isolated (low gene flow), resulting in significant population sub-structure within those species. This isolation may be amplified by habitat fragmentation of freshwater wetlands (see 'Main threats'). These assumptions are supported by observations on other burrowing and non-burrowing parastacids (Crandall, 1997; Nguyen *et al.*, 2005; Dawkins *et al.*, 2010; Miller *et al.*, 2013).

Figure 4. Species and habitat. A, *Parastacus brasiliensis*; B and C, brook at Morro do Cerro Negro, Mariana Pimentel, state of Rio Grande do Sul (30° 21'11''S; 51°35'02''W); D, E detail of chimney (black arrow). Courtesy of Daiana da Silva Castiglioni.

Main threats

Freshwater ecosystems are facing accelerated degradation globally leading to a growing number of freshwater extinctions (Ricciardi and Rasmussen, 1999). For the South American freshwater crayfish, IUCN Red List assessments have identified many threats (Buckup, 2010a, b, c, d, e, f, g, h, i, j, k, l). For example, in Brazil *Parastacus* species are subjected to widespread threats from stream channelization and by pollution from urban areas, industry and agriculture, and some of these have been identified as localized and particular threats for some species (Buckup, 2010c, d, e, f, g, h). In Chile, impacts include siltation caused by deforestation to enable livestock grazing and crop production (Buckup, 2010a, b). There are also localized threats such as harvesting for human consumption, using crayfishes as bait for salmon fishing, management of river-bank vegetation and aquatic macrophytes, physical alterations to the river channel by the extraction of groundwater (Rudolph, 2002b; Buckup, 2010b, i, j; Ibarra and Arana, 2011) and the removal of *Sphagnum* moss, which is the specific habitat of two species (*V. araucanius* and *V. retamali*), for commercial purposes (Buckup, 2010j, k).

Alien species have been identified by IUCN as major threats causing biodiversity loss (IUCN Standards and Petitions Subcommittee, 2013) and have documented impact on endemic crayfishes (Lodge *et al*., 2012; Sousa *et al*., 2013). For example, in Europe many native crayfishes are threatened by invasives, through competition and dissemination of crayfish plague (a disease caused *Aphanomyces astaci*) (Holdich *et al*., 2009).

In South America, there are records of non-native crayfishes in different countries (Mendoza *et al*., 2011). One of these, *Procambarus clarkii* (Girard, 1852) occurs naturally in North America (north-eastern Mexico and in south-central USA) (Hobbs, 1972). In South America, this species has been recorded in Ecuador and Brazil (Magalhães *et al*., 2005; Torres and Álvarez, 2012) and

Figure 5. Species and habitat: (A) *Samastacus spinifrons*; (B) River Llico, Chile (41°10'19''S; 73°40'29.36''W); C, lagoon Quilque, Chile (42°39'28.25'S; 74°03'51.83''W); D, River Hueyusca, Chile (41°07'S; 73°37'W).

information generated using the Species Distribution Models (SDMs) reveals other areas suitable for species occupation, especially in southern South America (Paraguay, Chile, Argentina, Uruguay and Brazil) (Palaoro *et al*., 2013). Other non-native species have been introduced from Oceania (Lawrence and Jones, 2002): *Cherax quadricarinatus* (von Martens, 1868), *Cherax tenuimanus* Smith, 1912 and *Cherax cainii* Austin, 2002 (Austin and Bunn, 2010; Mendoza *et al*., 2011). They have been cultivated in commercial farms in Ecuador, Paraguay, Colombia, Peru, Uruguay, Argentina and Chile, but there are also some records of feral populations in some sites (Mendoza *et al*., 2011). All three species have the potential to spread and consequently become invasive (Jaklic and Vrezec, 2011).

IUCN CONSERVATION STATUS

IUCN uses two geographic concepts to define the current distribution of species and both are in criterion B: the Extent of Occurrence (EOO) and the Area of Occupancy (AOO) (IUCN Standards and Petitions Subcommittee, 2013). According to IUCN guidelines, criterion B has been designed to identify populations with restricted distributions that are also severely fragmented, undergoing continuing decline, or exhibiting extreme fluctuations (in the present or near future) (IUCN Standards and Petitions Subcommittee, 2013).

The EOO is defined as the area contained within the shortest continuous boundary that can be drawn to encompass all the known, inferred or projected sites of present occurrence of a taxon (IUCN Standards and Petitions Subcommittee, 2013). To calculate the EOO, different methods have been proposed (Gaston and Fuller, 2009); often IUCN uses the minimum convex polygon method (IUCN Standards and Petitions Subcommittee, 2013). The other IUCN concept within criterion B is the AOO, defined as the area within its EOO actually occupied by a taxon. There are different methods for estimating AOOs, including those that use inferences from the presence of known appropriate habitat (ecological modelling) (Gaston and Fuller, 2009; IUCN Standards and Petitions Subcommittee, 2013).

For native South American crayfishes, the EOO could be estimated and used for categorization within criterion B for almost the entire group, except *P. saffordi*, *P. varicosus* and *P. laevigatus* owing to taxonomic status issues with these three species that need to be resolved before an effective assessment can be made. The AOO has never been calculated for South American crayfishes, perhaps for at least two reasons: (1) problems in adapting some IUCN criteria to invertebrates (Cardoso *et al*., 2011), especially for freshwater organisms (Simaika and Samways, 2010); (2) globally, there is a lack of ecological information for characterizing species habitat and thereby understanding the factors that influence geographical distribution (Payne, 1986). These points are important when dealing with problems of scale in AOO estimation (IUCN Standards and Petitions Subcommittee, 2013) and they must be taken into account in order to calculate this parameter.

Rudolph (2010) and Rudolph and Crandall (2012) using minimum convex polygons estimated the EOO for seven Chilean species: *Parastacus pugnax* (EOO = 73 000 km^2), *P. nicoleti* (EOO = 13 000 km^2), *S. spinifrons* (EOO = 155 000 km^2), *V. araucanius* (EOO = 12 000 km^2), *V. retamali* (EOO = 3200 km^2), *V. rucapihuelensis* (EOO = 99 km^2) and *V. jarai* (EOO < 100 km^2). In some cases there are considerable discrepancies between these estimates and those estimated in IUCN assessments using the same method. For *S. spinifrons*, *V. araucanius* and *V. rucapihuelensis*, the EOOs estimated in IUCN assessments were 13 813 km^2, 315 km^2 and 5000 km^2, respectively. In the first two cases, EOOs estimated by Rudolph (2010) (155 000 km^2 and 12 000 km^2, respectively) were much larger. For *S. spinifrons*, this difference may be higher because in this estimate (155 000 km^2), only Chilean territory was considered (this species also occurs in Argentina) (Rudolph, 2010). For *V. araucanius*, probably only some populations were considered in IUCN assessments (EOO = 315 km^2). In the last case, *V. rucapihuelensis* is an endemic species with only five populations known for coastal Cordillera, Osorno Province, southern Chile (EOO = 99 km^2) (Rudolph, 2010). However, in the IUCN assessment the EOO was estimated at 5000 km^2 (Buckup, 2010l). Comparing geographic coordinates of these populations published by Rudolph (2010) and the species range map in the IUCN assessment, the latter includes areas where this species has never been recorded. Despite some discrepancies, for *P. pugnax* and *P. nicoleti* estimates were not significantly different. The EOOs estimated in IUCN assessments were 60 000 km^2 and 9623 km^2, respectively. Because both IUCN estimates are lower than those published by Rudolph (2010) (73 000 km^2 and 13 000 km^2, respectively), probably not all populations were considered in the IUCN assessment (Buckup, 2010a, b).

For Brazilian *Parastacus* species, the EOO estimates should be viewed with caution. Databases have been revised and populations have been resampled (many collections were made in the 1970s, 1980s and 1990s). There are two particularly controversial estimates: *P. brasiliensis* (EOO = 6632 km^2) and *P. varicosus* (EOO = 1127 km^2). Based on current knowledge and using the Geocat program (a rapid geospatial analysis used by IUCN in the Red Listing process) (Bachman *et al*., 2011), the EOO for *P. brasiliensis* was estimated as 91 584 km^2. In the IUCN assessment, some new populations were not considered in the calculation of the species' geographic range. Also, in the IUCN assessment, the EOO for *P. defossus* and *P. pilimanus* were not estimated. However, using the Geocat program both were estimated as 21 336 km^2 and 177 380 km^2, respectively.

The EOOs estimated for *P. brasiliensis* (91 584 km^2) and for *P. pilimanus* (177 380 km^2) were much higher than the lower threshold for threatened categories (< 20 000 km^2). In the first case, the Near Threatened (NT) category (current IUCN category) could not be justified because the EOO estimated is far from the lower threshold. Another possible category would be Least Concern (LC), but this category is used for widespread and abundant taxa (IUCN Standards and Petitions Subcommittee, 2013). Moreover, LC is used when there is no immediate threat to the survival of the species (IUCN Standards and Petitions Subcommittee, 2013). Many general threats have been identified for *Parastacus* species (see Main Threats section) (Buckup, 2010a, b, c, d, e, f, g, h, i, j, k, l). Thus, to place any *Parastacus* species in this category is not a good solution. For both species (*P. brasiliensis* and *P. pilimanus*), the Data Deficient (DD) category may be the most appropriate (Table 1). *Parastacus defossus* is the opposite case where the lower threshold for threatened categories ($< 20\,000\,km^2$) is almost achieved ($EOO = 21\,336\,km^2$). Here, NT could be justified (Table 1). For Brazilian and Uruguayan *Parastacus* species, many collections were made in the 1970s, 1980s and 1990s and were never resampled. Owing to extensive, rapid degradation of South American freshwater wetlands (Junk *et al*., 2013) many populations may now be locally extinct. Thus, monitoring populations is important to verify reliability of the current EOO and AOO estimations and population stability and viability.

Despite the better information available for the Chilean *Parastacus* species, it is not enough to make a complete assessment based on EOOs. For example, the EOO for *P. nicoleti*, calculated in an IUCN assessment ($9623\,km^2$), was not considered and the species was allocated to the Data Deficient (DD) category. Even with the EOO estimated by Rudolph (2010) ($13\,000\,km^2$), using criterion B both estimates meet the threshold for the Vulnerable (VU) category. However, to assess a species as VU, two of three subcriteria must be met: (a) Severely fragmented, or Number of locations; (b) Continuing decline in any of: (i) extent of occurrence; (ii) area of occupancy; (iii) area, extent and/or quality of habitat; (iv) number of locations or subpopulations; (v) number of mature individuals; (c) Extreme fluctuations in any of: (i) extent of occurrence; (ii) area of occupancy; (iii) number of locations or subpopulations; (iv) number of mature individuals. Using the available information for *P. nicoleti*, the only possibility is to allocate it under subcriterion b-iii. For these reasons the DD category should not be changed (Table 1).

A second case is illustrated by the example of *P. pugnax*. This species has two EOO estimates ($60\,000\,km^2$ and $73\,000\,km^2$, IUCN assessment and Rudolph, 2010, respectively). Here, the same argument is used as above for other *Parastacus* species to discourage the use of the LC category. This species is facing population declines as a result of deforestation for agriculture (livestock grazing and crops) causing reductions in habitat quality through sedimentation (Buckup, 2010d). There are also some local populations that have been harvested for human consumption and for use as bait in salmon fishing (Buckup, 2010b). Thus, DD is still a suitable category (Table 1).

For *Virilastacus* species, categorization based on criterion B (and subcriteria) is less problematic. For *V. araucanius* and *V. retamali* EOO thresholds for Vulnerable (VU) and Endangered (EN) ($< 20\,000\,km^2$ and $< 5000\,km^2$, respectively), and for *V. jarai* the EOO threshold for Critically Endangered (CR) ($< 100\,km^2$) are achieved. In all cases, the same subcriteria were met: (a) number of locations ≤ 10 and ≤ 5 (for VU and EN, respectively); and (b) population in continuing decline (especially in area, extent and/or quality of habitat) (Rudolph and Crandall, 2005). Thus, for *V. araucanius* and *V. retamali* the category suggested is Vulnerable (VU - ab(iii)) and Endangered (EN - ab(iii)), respectively. *Virilastacus rucapihuelensis* is more complicated because EOO estimates and the number of locations suggest different threat categories. The EOO estimate was $99\,km^2$, i.e. below the CR threshold, but the number of locations was estimated as ≤ 5 (suggesting the EN category) (Rudolph, 2010). It is important to note that the subcriterion b(iii) may be applied (population in continuing decline in area, extent and/or quality of habitat). In those cases where more than one category of threat is achieved, IUCN recommends that the higher category should be used (IUCN Standards and Petitions Subcommittee, 2013). Thus, based on criterion B the category suggested for *V. rucapihuelensis* is CR - ab(iii) (Table 1).

Within all five IUCN criteria some population feature is always considered to estimate extinction risk (IUCN Standards and Petitions Subcommittee, 2013): population and population size (criteria A, C and D), mature individuals (criteria A, B, C and D), generation length (or generation) (criteria A, C and E). These features are essential for forecasting other population aspects such as reduction (criterion A), continuing decline (criteria B and C) and extreme fluctuations (criteria B and C) (IUCN Standards and Petitions Subcommittee, 2013). Here, the main problem is that for almost all species there are no estimates of population size, and in some cases these estimates are based on only one population (see section Habitat and population and Table S1). One way of tackling this problem could be to sample the largest number of populations per species to obtain an average number of individuals per species, or using burrow openings as a population indicator. Another question concerns the number of mature individuals against which population decline is estimated. In cases where the size at first sexual maturity is known (e.g. *P. brasiliensis* ~31 mm Cephalotoracic Length/CL - Table S1), this could be used as a reference to estimate the number of mature individuals in populations.

IUCN uses the term 'location' with respect to threats. This concept is defined as a geographically or ecologically distinct area in which a single threatening event can rapidly affect all individuals of the taxon present (IUCN Standards and Petitions Subcommittee, 2013). The size of the location depends on the area covered by the threatening event and may include part of one or many subpopulations (IUCN Standards and Petitions Subcommittee, 2013). For native South American crayfishes, the two most plausible threats have been identified: habitat loss (stream channelization, liquid waste discharged, river-bank modifications and groundwater extraction) and harvesting (Buckup, 2010a, b, c, d, e, f, g, h, i, j, k, l). However, the number of locations (used within criterion B and D) has not been identified. The definition of 'location' is highly ambiguous taking into account the great variety of factors that could constitute a threat (Martín, 2009). Moreover, there is some confusion between the concept of location and subpopulation, because they are potentially closely linked and sometimes may be difficult to apply (Rivers *et al.*, 2010). Here, the main problem is still the considerable lack of information (especially ecological data) for estimating the extent of a particular threat within the distribution of a species. Also, more detailed information is needed about the characteristics of particular threats. For example, ecological data about invasive crayfish are essential for understanding the extent of this kind of threat (Meineri *et al.*, 2014; Sousa *et al.*, 2013). Thus, it remains difficult to apply the concepts of location to this group.

CONSERVATION ACTIONS

The assessment of conservation status is essential to support species conservation (Rodrigues *et al.*, 2006). Some of the native South American crayfishes were categorized as VU, EN and CR (especially Chilean species) and here conservation action plans may be discussed and proposed. Some crayfish species are considered 'charismatic' or 'iconic' species that fascinate or attract the interest of humans, as well as being ecologically significant and, consequently, may be designated as 'heritage' or 'flagship' species. The designation sometimes provides a rationale for their protection and conservation and also for their habitat and associated species. If protected or the object of conservation interest, they may come to typify the entire landscape unit. Their protection also confers protection on other species in the same habitat, including those more cryptic or less well known; i.e. they may be useful 'umbrella' species (Wilcox, 1984; Caro and O'Doherty, 1999). In this context, a flagship or heritage species is acting as an umbrella to conserve the associated species in the environment, even if this concept is questionable (Andelman and Fagan, 2000).

On the other hand, the majority of native South American crayfishes were categorized as DD and this is the main reason why there are no species-specific conservation measures in place. However, it is important to emphasize that this category indicates that the data are inadequate for determining the degree of threat faced by a taxon, not necessarily that the taxon is not threatened. Here, more fundamental data are needed before meaningful conservation action plans can be put in place.

ACKNOWLEDGEMENTS

This work has been financially supported by CAPES/COFECUB (international consortium Brazil – France) (Project: Freshwater limnetic ecosystems and conservation of freshwater crayfish of the genus *Parastacus* in southern Brazil – 8209/2012). PBA is grateful to CNPq (National Council for Scientific and Technological Development) for a research fellowship and MPA is grateful to CAPES (Coordination of Improvement of Higher Level or Education Personnel) for fellowship 2485/2011. We are grateful to EMAE Erasmus Mundus – European Master in Applied Ecology (EMMC – EMAE, University of Poitiers, France) (FPA 2008 – 00092 / 001 FRAME MUNDB 123) for financial support to MPA, CSG and PBA.

REFERENCES

Almeida AO, Buckup L. 2000. Occurrence of protandric hermaphroditism in a population of the neotropical freshwater crayfish *Parastacus brasiliensis* (Parastacidae). *Journal of Crustacean Biology* **20**: 224–230.

Andelman SJ, Fagan WF. 2000. Umbrellas and flagships: efficient conservation surrogates or expensive mistakes? *Proceedings of the National Academy of Sciences* **97**: 5954–5959.

Austin CM, Bunn J. 2010. *Cherax cainii*. In IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. Available from http://www.iucnredlist.org [accessed November 2013].

Bachman S, Moat J, Hill AW, de la Torre J, Scott B. 2011. Supporting Red List threat assessments with GeoCAT: geospatial conservation assessment tool. *ZooKeys* **150**: 117–126.

Bahamonde N, Carvacho A, Jara C, López M, Ponce F, Retamal MA, Rudolph E. 1998. Categorías de conservación de decápodos nativos de aguas continentales de Chile. *Boletín del Museo Nacional de Historia Natural* **47**: 91–100.

Bond-Buckup G, Jara CG, Perez-Losada M, Buckup L, Crandall KA. 2008. Global diversity of crabs (Aeglidae: Anomura: Decapoda) in freshwater. *Hydrobiologia* **595**: 267–273.

Buckup L. 2003. Família Parastacidae. In *Manual de identificação dos Crustacea Decapoda de água doce do Brasil*, Melo GAS (ed). Editora Loyola: São Paulo; 117–141.

Buckup L. 2010a. *Parastacus nicoleti*. In IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. Available from http://www.iucnredlist.org [accessed November 2013].

Buckup L. 2010b. *Parastacus pugnax*. In IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. Available from http://www.iucnredlist.org [accessed November 2013].

Buckup L. 2010c. *Parastacus brasiliensis*. In IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. Available from http://www.iucnredlist.org [accessed November 2013].

Buckup L. 2010d. *Parastacus defossus*. In IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. Available from http://www.iucnredlist.org [accessed November 2013].

Buckup L. 2010e. *Parastacus laevigatus*. In IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. Available from http://www.iucnredlist.org [accessed November 2013].

Buckup L. 2010f. *Parastacus pilimanus*. In IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. Available from http://www.iucnredlist.org [accessed November 2013].

Buckup L. 2010g. *Parastacus saffordi*. In IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. Available from http://www.iucnredlist.org [accessed November 2013].

Buckup L. 2010h. *Parastacus varicosus*. In IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. Available from http://www.iucnredlist.org [accessed November 2013].

Buckup L. 2010i. *Samastacus spinifrons*. In IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. Available from http://www.iucnredlist.org [accessed November 2013].

Buckup L. 2010j. *Virilastacus araucanius*. IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. Available from http://www.iucnredlist.org [accessed November 2013].

Buckup L. 2010k. *Virilastacus retamali*. In IUCN 2013. Red List of Threatened Species. Version 2013.2. Available from http://www.iucnredlist.org [accessed November 2013].

Buckup L. 2010l. In UCN 2013. IUCN Red List of Threatened Species. Version 2013.2. Available from http://www.iucnredlist.org [accessed November 2013].

Buckup L, Rossi A. 1980. O gênero *Parastacus* no Brasil (Crustacea, Decapoda, Parastacidae). *Revista Brasileira de Biologia* **40**: 663–681.

Cardoso P, Borges PAV, Triantis KA, Ferrández MA, Martín JL. 2011. Adapting the IUCN Red List criteria for invertebrates. *Biological Conservation* **144**: 2432–2440.

Caro TM, O'Doherty G. 1999. On the use of surrogate species in conservation biology. *Conservation Biology* **13**: 805–814.

Collins PA, Giri F, Williner V. 2011. Biogeography of the freshwater decapods in the La Plata Basin, South America. *Journal of Crustacean Biology* **31**: 179–191.

Conservation International. 2013. http://www.conservation.org [03 January 2013].

Crandall KA. 1997. Genetic variation within and among crayfish species. *Freshwater Crayfish* **11**: 135–145.

Crandall KA, Buhay JE. 2008. Global diversity of crayfish (Astacidae, Cambaridae, and Parastacidae–Decapoda) in freshwater. *Hydrobiologia* **595**: 295–301.

Crandall KA, Fetzner JW, Jara CG, Buckup L. 2000. On the phylogenetic positioning of the South American freshwater crayfish genera (Decapoda: Parastacidae). *Journal of Crustacean Biology* **20**: 530–540.

Dawkins KL, Furse JM, Wild CH, Hughes JM. 2010. Distribution and population genetics of the threatened freshwater crayfish genus *Tenuibranchiurus* (Decapoda: Parastacidae). *Marine and Freshwater Research* **61**: 1048–1055.

De Grave S, Cai Y, Anker A. 2008. Global diversity of shrimps (Crustacea: Decapoda: Caridea) in freshwater. *Hydrobiologia* **595**: 287–293.

Dudgeon D, Arthington AH, Gessner MO, Kawabata ZI, Knowler DJ, Lévêque C, Naiman RJ, Prieur-Richard AH, Soto D, Stiassny MLJ *et al*. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews of the Cambridge Philosophical Society* **81**: 163–182.

Faxon W. 1898. Observations on the Astacidae in the 776 Proceedings of the Biological Society of Washington United States National Museum and in the Museum of Comparative Zoology, with descriptions of new species. *Proceedings of the United States National Museum* **20**: 643–694.

Faxon W. 1914. Notes on the crayfishes in the United States National Museum and the Museum of Comparative Zoology, with descriptions of new species and subspecies, to which is appended a catalogue of the known species and subspecies. *Memoirs of the Museum of Comparative Zoology at Harvard College* **10**: 351–427.

Fontoura NF, Buckup L. 1989. Dinâmica populacional e reprodução em *Parastacus brasiliensis* (von Martens, 1869) (Crustacea, Decapoda, Parastacidae). *Revista Brasileira de Biologia* **49**: 911–921.

Ford AT. 2012. Intersexuality in Crustacea: an environmental issue? *Aquatic Toxicology* **108**: 125–129.

Gaston KJ, Fuller RA. 2009. The sizes of species' geographic ranges. *Journal of Applied Ecology* **46**: 1–9.

Gherardi F. 2007. *Biological Invaders in Inland Waters: Profiles, Distribution, and Threats*. Springer: Dordrecht, Netherlands.

Gherardi F, Souty-Grosset C. 2006. European crayfish as heritage species – linking research and management strategies to conservation and socio-economic development. *Bulletin Francais de la Peche et de la Pisciculture* **380–381**: 566.

Ghiselin MT. 1969. The evolution of hermaphroditism among animals. *The Quarterly Review of Biology* **44**: 189–208.

Hobbs HH. 1972. Biota of freshwater ecosystems, identification manual 9: crayfishes (Astacidae) of North and Middle America. Water pollution control research series. US Environmental Protection Agency, Washington, DC.

Hobbs HH. 1991. A new generic assignment for a South American crayfish (Decapoda, Parastacidae) with revised diagnoses of the South American genera and comments on the parastacid mandible. *Proceedings of the Biological Society of Washington* **104**: 800–811.

Holdich DM, Reynolds JD, Souty-Grosset C, Sibley PJ. 2009. A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowledge and Management of Aquatic Ecosystems* **394–395**: 11.

Horwitz P. 2010. The conservation status of freshwater crayfish: the basis for concern, listing and recovery processes, and community involvement. Seventeenth International Symposium on Freshwater Crayfish, Kuopio, Finland.

Huxley TH. 1879. *The Crayfish – an Introduction to the Study of Zoology*. Kegan Paul: London.

Ibarra M, Arana PM. 2011. Crecimiento del camarón excavador *Parastacus pugnax* (Poeppig 1835) determinado mediante técnica de marcaje. *Latin American Journal of Aquatic Research* **39**: 378–384.

Ibarra MA, Arana PM. 2012. Biological parameters of the burrowing crayfish, *Parastacus pugnax* (Poeppig, 1835), in Tiuquilemu, Bio Bio Region, Chile. *Latin American Journal of Aquatic Research* **40**: 418–427.

IUCN Standards and Petitions Subcommittee. 2013. Guidelines for Using the IUCN Red List Categories and Criteria. Version 10. Prepared by the Standards and Petitions Subcommittee. Available from http://www.iucnredlist.org/documents/RedListGuidelines.pdf [accessed November 2013].

Jaklic M, Vrezec A. 2011. The first tropical alien crayfish species in European waters: the redclaw *Cherax quadricarinatus* (von Martens, 1868) (Decapoda, Parastacidae). *Crustaceana* **84**: 651–665.

Junk WJ, Piedade MTF, Lourival R, Wittmann F, Kandus P, Lacerda LD, Bozelli RL, Esteves FA, Nunes da Cunha C, Maltchik L *et al*. 2013. Brazilian wetlands: their definition, delineation, and classification for research, sustainable management, and protection. *Aquatic Conservation: Marine and Freshwater Ecosystems* **24**: 5–22.

Lawrence C, Jones C. 2002. *Cherax*. In *Biology of Freshwater Crayfish*, Holdich DM (ed). Blackwell: Oxford; 635–669.

Lévêque C, Balian EV, Martens K. 2005. An assessment of animal species diversity in continental waters. *Hydrobiologia* **542**: 39–67.

Lodge DM, Deines A, Gherardi F, Yeo DCJ, Arcella T, Baldridge AK, Barnes MA, Chadderton WL, Feder JL, Gantz CA *et al*. 2012. Global introductions of crayfishes: evaluating the impact of species invasions on ecosystem services. *Annual Review of Ecology, Evolution, and Systematics* **43**: 449–472.

Magalhães C, Bueno SLS, Bond-Buckup G, Valenti WC, Silva HLM, Kiyohara F, Mossolin EC, Rocha SS. 2005. Exotic species of freshwater decapod crustaceans in the state of Sao Paulo, Brazil: records and possible causes of their introduction. *Biodiversity and Conservation* **14**: 1929–1945.

Magris RA, Bond-Buckup G, Magalhães C, Mantelatto FL, Reid JW, Maria L, Loureiro AE, Coelho PA, Santana W, Buckup L *et al*. 2010. Quantification of extinction risk for crustacean species : an overview of the National Red Listing process in Brazil. *Nauplius* **18**: 129–135.

Marques AAB, Fontana CS, Vélez E, Bencke GA, Schneider M, Reis RE. 2002. Lista das espécies da fauna ameaçadas de extinção no Rio Grande do Sul. FZB/MCT-PUCRS/PANGEA, Porto Alegre, Brasil.

Martín JL. 2009. Are the IUCN standard home-range thresholds for species a good indicator to prioritise conservation urgency in small islands? A case study in the Canary Islands (Spain). *Journal for Nature Conservation* **17**: 87–98.

Meineri E, Rodriguez-Perez H, Hilaire S, Mesleard F. 2014. Distribution and reproduction of *Procambarus clarkii* in relation to water management, salinity and habitat type in the Camargue. *Aquatic Conservation: Marine and Freshwater Ecosystems*. **24**: 312–323.

Mendoza R, Rodríguez G, Castillo S. 2011. Riesgo de dispersión y posibles impactos de los acociles australianos del género *Cherax* en México. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (Conabio), Tlalpan, México.

Miller AD, Van Rooyen A, Sweeney OF, Whiterod NS, Weeks AR. 2013. The development of 10 novel polymorphic microsatellite markers through next generation sequencing and a preliminary population genetic analysis for the endangered Glenelg spiny crayfish, *Euastacus bispinosus*. *Molecular Biology Reports* **40**: 4415–4419.

Momot WT. 1995. Redefining the role of crayfish in aquatic systems. *Review of Fisheries Science* **3**: 33–63.

Morrone JJ, Lopretto EC. 1994. Distributional patterns of freshwater Decapoda (Crustacea: Malacostraca) in southern South America: a panbiogeographic approach. *Journal of Biogeography* **21**: 97–109.

Myers N. 1989. Threatened biotas: 'hot spots' in tropical forests. *The Environmentalist* **8**: 1–20.
Myers N. 1990. The biodiversity challenge: expanded hotspots analysis. *The Environmentalist* **10**: 243–256.
Nguyen TT, Burridge CP, Austin CM. 2005. Population genetic studies on the Australian freshwater crayfish, *Cherax destructor* (Crustacea: Parastacidae) using allozyme and RAPD markers. *Aquatic Living Resources* **18**: 55–64.
Noro CK, Buckup L. 2008. Estrutura populacional e biologia reprodutiva de *Parastacus defossus* (Crustacea: Decapoda: Parastacidae). *Revista Brasileira de Zoologia* **25**: 624–629.
Noro CK, Buckup L. 2010. The burrows of *Parastacus defossus* (Decapoda: Parastacidae), a fossorial freshwater crayfish from southern Brazil. *Zoologia* **27**: 341–346.
Ortmann AE. 1902. The geographical distribution of freshwater decapods and its bearing upon ancient geography. *Proceedings of the American Philosophical Society* **41**: 267–400.
Palaoro AV, Dalosto MM, Costa GC, Santos S. 2013. Niche conservatism and the potential for the crayfish *Procambarus clarkii* to invade South America. *Freshwater Biology* **58**: 1379–1391.
Payne JF. 1986. Factors influencing patterns of crayfish distribution. *Freshwater Crayfish* **6**: 100–110.
Reynolds JD, Souty-Grosset C. 2012. *Management of Freshwater Biodiversity: Crayfish as Bioindicators*. Cambridge University Press: Cambridge.
Ricciardi A, Rasmussen JB. 1999. Extinction rates of North American freshwater fauna. *Conservation Biology* **13**: 1220–1222.
Richardson AMM. 1983. The effect of the burrow of a crayfish on the respiration of the surrounding soil. *Soil Biology and Biochemistry* **15**: 239–242.
Riek EF. 1971. The freshwater crayfishes of South America. *Proceedings of the Biological Society of Washington* **84**: 129–136.
Ringuelet RA. 1961. Rasgos fundamentales de la zoogeografía de la Argentina. *Physis* **22**: 151–170.
Rivers MC, Bachman SP, Meagher TR, Lughadha EN, Brummit NA. 2010. Subpopulations, locations and fragmentation: applying IUCN red list criteria to herbarium specimen data. *Biodiversity and Conservation* **19**: 2071–2085.
Rodrigues ASL, Pilgrim JD, Lamoreux JF, Hoffmann M, Brooks TM. 2006. The value of the IUCN Red List for conservation. *Trends in Ecology and Evolution* **21**: 71–76.
Rudolph EH. 1999. Intersexuality in the freshwater crayfish *Samastacus spinifrons* (Philippi, 1882) (Decapoda, Parastacidae). *Crustaceana* **72**: 325–337.
Rudolph EH. 2002a. New records of intersexuality in the freshwater crayfish species *Samastacus spinifrons* (Decapoda, Parastacidae). *Journal of Crustacean Biology* **22**: 377–389.
Rudolph EH. 2002b. Sobre la biología del camarón de río *Samastacus spinifrons* (Philippi 1882) (Decapoda, Parastacidae). *Gayana* **66**: 147–159.
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 EH. 2013. Freshwater Malacostracans in Chilean Inland Waters: a checklist of the Chilean parastacidae (Decapoda, Astacidea). *Crustaceana* **86**: 1468–1510.
Rudolph EH, Almeida A. 2000. On the sexuality of South American Parastacidae (Crustacea, Decapoda). *Invertebrate Reproduction and Development* **37**: 249–257.
Rudolph EH, Crandall KA. 2005. A new species of burrowing crayfish, *Virilastacus rucapihuelensis* (Crustacea: Decapoda: Parastacidae), from southern Chile. *Proceedings of the Biological Society of Washington* **118**: 765–776.
Rudolph EH, Crandall KA. 2007. A new species of burrowing crayfish *Virilastacus retamali* (Decapoda: Parastacidae) from the southern Chilean peatland. *Journal of Crustacean Biology* **27**: 502–512.
Rudolph EH, Crandall KA. 2012. A new species of burrowing crayfish, *Virilastacus jarai* (Crustacea, Decapoda, Parastacidae) from central-southern Chile. *Proceedings of the Biological Society of Washington* **125**: 258–275.
Simaika JP, Samways MJ. 2010. Large-scale estimators of threatened freshwater catchment species relative to practical conservation management. *Biological Conservation* **143**: 311–320.
Sinclair EA, Fetzner JWJ, Buhay J, Crandall KA. 2004. Proposal to complete a phylogenetic taxonomy and systematic revision for freshwater crayfish (Astacidea). *Freshwater Crayfish* **14**: 21–29.
Sousa R, Freitas FE, Mota M, Nogueira AJ, Antunes C. 2013. Invasive dynamics of the crayfish *Procambarus clarkii* (Girard, 1852) in the international section of the River Minho (NW of the Iberian Peninsula). *Aquatic Conservation: Marine and Freshwater Ecosystems* **23**: 656–666.
Tisseuil C, Cornu JF, Beauchard O, Brosse S, Darwall W, Holland R, Hugueny B, Tedesc PA, Oberdorff T. 2013. Global diversity patterns and cross-taxa convergence in freshwater systems. *Journal of Animal Ecology* **82**: 365–376.
Toon A, Pérez-Losada M, Schweitzer CE, Feldmann RM, Carlson M, Crandall KA. 2010. Gondwanan radiation of the southern hemisphere crayfishes (Decapoda: Parastacidae): evidence from fossils and molecules. *Journal of Biogeography* **37**: 2275–2290.
Torres E, Álvarez F. 2012. Genetic variation in native and introduced populations of the red swamp crayfish *Procambarus clarkii* (Girard, 1852) (Crustacea, Decapoda, Cambaridae) in Mexico and Costa Rica. *Aquatic Invasions* **7**: 235–241.
Vogt G. 2002. Functional anatomy. In *Biology of Freshwater Crayfish*, Holdich DM (ed). Blackwell: Oxford; 53–151.
von Martens E. 1869. Südbrasilische Süss-und Brackwasser Crustaceen nach den Sammlungen des Dr. *Reinh. Hensel*. *Archiv für Naturgeschicthe* **35**: 1–37.
Wilcox BA. 1984. In situ conservation of genetic resources: determinants of minimum area requirements. In *National Parks, Conservation, and Development: The Role of Protected Areas in Sustaining Society*, McNeely JA, Miller KR (eds). Smithsonian Institution Press: Washington; 639–647.
Xu J, Pérez-Losada M, Jara CG, Crandall KA. 2009. Pleistocene glaciation leaves deep signature on the freshwater crab *Aegla alacalufi* in Chilean Patagonia. *Molecular Ecology* **18**: 904–918.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web site.

