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*Review*

# Current state of knowledge on *Virilastacus* species (Crustacea, Decapoda, Parastacidae)

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**ABSTRACT.** The genus *Virilastacus* was created in 1991 to accommodate *Parastacus araucanius* Faxon, 1914. At present, *Virilastacus* comprises four burrowing species, three of which were described at the beginning of the XXI century, and biological knowledge about these species is mainly limited to taxonomic and distributional aspects. This review compiles published information about these species, together with other data available to the author in order to update the current state of biological knowledge and, in turn, to promote the conservation of these species. An upgraded diagnosis of the genus *Virilastacus* is provided, together with information related to each species with regard to: distinctive morphological traits, geographic distribution, habitat, burrow morphology, burrowing behavior, body size, sexual system, and state of conservation. Some aspects related to morphological adaptations to their burrowing life style, phylogenetic affinities and main threats to conservation are also discussed. It is concluded that biological knowledge about these four species is scarce and fragmentary; furthermore, they are currently under threat as a result of anthropogenic activities that are degrading and fragmenting their habitat.

**Keywords:** *Virilastacus*, burrowing crayfish, morphology characters, geographic distribution, sexual system, habitat, conservation status, Chile.

# Estado de conocimiento de las especies de *Virilastacus* (Crustacea, Decapoda, Parastacidae)

**RESUMEN.** El género *Virilastacus* fue creado en 1991 para ubicar a *Parastacus araucanius* Faxon, 1914. Actualmente *Virilastacus* reúne a cuatro especies excavadoras, tres de ellas descritas a comienzos del siglo XXI, cuyo conocimiento biológico se circunscribe principalmente a aspectos taxonómicos y distribución. Esta revisión recopila la información publicada de estas especies, junto a otros datos accesibles al autor, para actualizar el conocimiento biológico y a la vez promover su conservación. Se proporciona una diagnosis actualizada del género, y de cada especie se entrega información sobre: rasgos morfológicos distintivos, distribución geográfica, hábitat, morfología de las galerías, comportamiento excavador, tamaño corporal, sistema sexual y estado de conservación. También se comentan algunas de las adaptaciones morfológicas a su estilo de vida excavador, sus afinidades filogenéticas y las principales amenazas a su conservación. Se concluye que el conocimiento biológico de estas cuatro especies es escaso y fragmentario, y que ellas se encuentran amenazadas por actividades antropogénicas que están degradando y fragmentando su hábitat.

**Palabras clave:** *Virilastacus*, camarones excavadores, caracteres morfológicos, distribución geográfica, sistema sexual, hábitat, estado de conservación, Chile.

## INTRODUCTION

At the beginning of the XX century, 10 species of the family Parastacidae had been described for South America, all grouped within one genus: *Parastacus*. Riek (1971) separated them into the genera *Samastacus* and *Parastacus*; he assigned two species [*Parastacus spinifrons* (Philippi, 1882) and *Parastacus araucanius* Faxon, 1914] to the genus *Samastacus*, in view of the following characteristics: P1 dactyls moving horizontally and phallic papillae being relatively long, articulated tubular projections. The other species, whose

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dactyls move vertically and phallic papillae are only small non-articulated protuberances, remained within *Parastacus*. Additionally, Riek (1971) characterized these genera in ecological terms: the *Parastacus* species as burrowers and inhabitants of underground waters, while the *Samastacus* species were characterized as weak burrowers, inhabiting rivers and lakes. After a period of 69 years, during which the only knowledge about *Samastacus araucanius* (Faxon, 1914) was based on the type specimen, Jara (1983) collected a second specimen, a male captured in the Botanical Gardens of the Universidad Austral de Chile (Valdivia), cohabitating with *Parastacus nicoleti* (Philippi, 1882). Rudolph & Rivas (1988) collected the third representative of this species, also a male in Hualqui (Concepción) cohabiting with *Parastacus pugnax* (Poeppig, 1835). These discoveries provided sufficient evidence to exclude the occurrence of *S. araucanius* in open waters, as had been suggested in Faxon's (1914) description of the location where the type material was obtained: "in a waterfall in Corral". Based on this evidence, as well as on morphological differences [which were already mentioned by Jara (1983) and Rudolph & Rivas (1988)], Hobbs (1991) separated these two species into the genera *Samastacus* (*S. spinifrons*) and *Virilastacus* (*V. araucanius*); this author also provided diagnoses of the three South American genera of Parastacidae. Crandall *et al.* (2000) validated these three genera, based on the sequencing of 500 nucleotides of the 16S mitochondrial DNA gene in seven of the ten species of South American parastacids. Rudolph & Crandall (2005, 2007, 2012) described three new species of *Virilastacus*, extended their geographic range, and confirmed that all the species of this genus were burrowers. In the present review, the scarce information published on the *Virilastacus* species is compiled and systematized, together with other data available to the author, with the aim to update the biological knowledge about this species and, in turn, to promote effective conservation measures.

**Family Parastacidae Huxley, 1879**

Genus *Virilastacus* Hobbs, 1991

Diagnosis

Rostrum short. Carapace lacking spines, tubercules and postorbital ridges; anterolateral portion of branchiocardiac groove clearly separated from the portion subparallel to cervical groove, which is located close to upper third portion of cephalothorax. Viewed dorsally, cervical groove V-shaped, except in *V. retamali*. Pleon lacking spines and tubercles; pleura of first abdominal segment distinct from and partly overlapped by that of the second abdominal segment. Telson without transverse suture and wholly calcified; posterior half with dorsomedian longitudinal groove. Ventral surface of ischipodite of third maxilliped bearing a median longitudinal band of tubercules; inside half of this surface with tufts of rigid setae; distolateral end of podomere rounded, except in *V. jarai*; merus lacking spines or tubercules; exopodite reaches distal end of merus. Caudal molar process of mandible quadricuspid in *V. araucanius* and *V. jarai;* tricuspid in *V. rucapihuelensis* and *V. retamali*; nodular cusp on proximal margin of cuspidal triangle. P1 chelae dimorphic, with almost completely tuberculated palms, but lacking spines or large tubercules; ventrolateral borders tuberculated to slightly subtoothed; carpus lacking large tubercules medially or ventrally, when upper surface held in a horizontal plane, dactyl moving obliquely in *V. rucapihuelensis* and *V. jarai*, and subhorizontally in *V. araucanius* and *V. retamali*. No occurrence of male and female gonopores in the same individual, except in *V. rucapihuelensis*. Male genitalia with a semi-rigid, tubular, thin, articulated, and very long phallic papilla extending forward from coxae in very close proximity to each other; lacking male cuticle partition, except in *V. rucapihuelensis*. Sternite XIII with an anterior medial plate, and posterior orifice. Viewed caudally, lateral processes of sternite XIV separated by a pronounced vertical fissure.

Type species. *Parastacus araucanius* Faxon, 1914: 553

Gender: Male

Etymology. From the Latin *virilis* = masculine; so-called because of its comparatively long phallic papilla (Hobbs, 1991; Rudolph & Crandall, 2012).

***Virilastacus araucanius* (Faxon, 1914)** (Fig. 1a)

Common name: Dwarf crayfish

Synonymy

*Parastacus araucanius* Faxon, 1914: 353, pp. 4, Figs. 1-3; Van Straelen, 1942: 9; Holthuis, 1952: 84; Bahamonde & López, 1963: 126 and 127, maps 1 and 2; Jara, 1983: R-163.

*Samastacus araucanius* Riek, 1971: 135; Manning & Hobbs, 1977: 159; Rudolph & Rivas, 1988: 73, Fig. 1; Hobbs, 1989: 80, Fig. 374; Buckup & Rossi, 1993: 167, Figs 11-13; Martínez *et al.*, 1994: 9, Figs. 1-11.

**Distinctive morphological characteristics**

Cephalothorax smooth, coloration: olive green. Small eyes. Rostrum short, reaching distal end of middle podomere of antennal flagellum; dorsally excavated. Rostral carina long and slightly prominent. Cervical groove weakly "V" shaped. Areola narrow and extended. Antennal scale short with one small distolateral spine. Basal podomere of antennula lacking

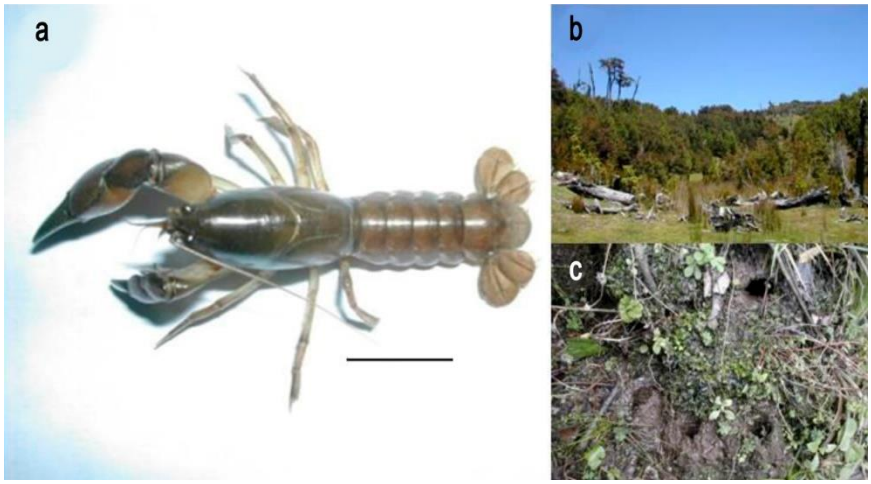


**Figure 1.** *Virilastacus araucanius* (Faxon, 1914). a) Dorsal view of male. Scale bar = 17.9 mm, b) partial view of habitat, c) overhead view of chimneys. Photos: E. Rudolph.

spines. Opposable margin of propodite without pilosity, bearing 11 to 18 teeth; dactyl moving sub horizontally, opposable margin bearing nine to 15 teeth. Individuals with female or male gonopores. Elongated phallic papilla reaches base of lateral process of XII body segment. In males, P5 coxae lack cuticle partition. Pleon coloration light brown. Telson subrectangular, dorsomedian longitudinal groove, and prominent spine on each lateral border (Faxon, 1914; Riek, 1971; Hobbs, 1991; Rudolph & Crandall, 2012). Species relatively small. The largest specimen caught is a female, measuring 28.7 mm cephalothorax length (CL) (74.8 mm total length). The smallest specimen collected is also a female, 18.3 mm CL (Martínez *et al.*, 1994). In the case of males, maximum and minimum sizes recorded are 26.0 and 19.0 mm CL respectively (Faxon, 1914; Jara, 1983). A summary of distinctive morphological features of the four species of *Virilastacus* is provided in Table 1.

## Geographic distribution

*V. araucanius* has been recorded from areas surrounding Concepción (36°46'22"S, 73°03'47"W), Valdivia (39°48′30″S, 73°14′30″W), and Maicolpué (40°36'27,74"S, 73°44'01,44"W) (Faxon, 1914; Jara, 1983; Rudolph & Rivas, 1988; Hobbs, 1991; Martínez *et al.*, 1994; Bahamonde *et al.*, 1998). This discontinuous distribution may only be apparent, since it coincides with the presence of university research centers in these three areas (Jara *et al.*, 2006). Nevertheless, recordings of *V. araucanius* in Maicolpué (Bahamonde *et al.*, 1998) should be reviewed, given that they may correspond to specimens of either *V. rucapihuelensis* or *V. retamali*, described after the studies of Bahamonde *et al.* (1998), in a location (Rucapihuel) situated only 15 km from Maicolpué. Finally, recordings of *V. araucanius* suggest that it is distributed between the coast and the Coastal Cordillera mountain range; the extent of occurrence is estimated at 11.571,64 km$^2$ (Ministerio del Medio Ambiente, 2013a).

## Habitat

*V. araucanius* inhabits underground waters in topographic basins with evergreen lowland forest associations. Almost all recordings of this species occur in these biotopes, commonly referred to as "vegas" or "hualves" (Fig. 1b). Only the discovery of *V. araucanius* in the Botanical Gardens of the Universidad Austral de Chile in Valdivia (Jara, 1983; Hobbs, 1991) indicates its presence in flatter zones, subject to considerable anthropic intervention. Specimens of this species have also been found coexisting with *P. nicoleti* (Jara, 1994; Jara *et al.*, 2006) in the same Botanical Gardens. A similar situation occurs in the locality of Hualqui (46º56´S, 72º55´W), where specimens of *V. araucanius* have been found cohabiting with *P. pugnax* (Rudolph & Rivas, 1988).

## Burrow morphology

The burrows constructed by *V. araucanius* are shallow (<1 m), but quite complex, with multiple ramifications, many of them almost parallel to the surface, which complicates their capture, whether manually or by suction methods. In winter, *V. araucanius* constructs mud "chimneys" 2.0-6.0 cm high, located around the entrance orifices of their burrows (Jara, 1994) (Fig. 1c). According to the classification of burrowing crayfish

proposed by Hobbs (1942), *V. araucanius* would be a primary burrower, since it builds complex burrows are not connected to permanent water bodies, and the entire life cycle of this species occurs inside the burrows.

### Sexual system

Descriptions of external sexual characteristics, together with some anatomical analyses of gonads and gonoducts, suggest that *V. araucanius* is a gonochoric species. Adult females have ellipsoidal gonopores, partially surrounded by setae and covered by a non-calcified membrane. These characteristics, suggesting the occurrence of functional gonopores, are very similar to those observed in *V. rucapihuelensis* adult females (Rudolph *et al.*, 2007). Males have an elongated, calcified phallic papilla ($\overline{X}$ = 3.1 ± 0.4 mm; n = 12) and the respective gonopore opens at the apical end (Rudolph & Rivas, 1988; Hobbs, 1991; Martínez *et al.*, 1994; Rudolph & Almeida, 2000).

### Conservation status

Bahamonde *et al.* (1998) categorized *V. araucanius* as *Insufficiently Known* throughout its entire geographic range. Nevertheless, they warned that water pollution and substrate modification within its distribution area could cause negative effects on the conservation of these populations. Rudolph & Crandall (2007) classified the species as *Vulnerable* through its geographic distribution range, based on the B1ab (iii) criteria of the IUCN Red List (2001). Buckup (2010a) classified it as *Data Deficient*. The Ministerio de Medio Ambiente (2013a) described it as *Vulnerable*, in accordance with the B1ab (iii) + 2ab (iii) criteria of the IUCN Red List (2001). Finally, Almerao *et al.* (2014) also endorsed this latter categorization.

***Virilastacus rucapihuelensis* Rudolph & Crandall, 2005** (Fig. 2a)

Common name: Vega crayfish

Synonym: *Virilastacus araucanius* Rudolph & Rojas, 2003: 835, Figs. 1-8

### Distinctive morphological characteristics

Cephalothorax with small tubercles only in anteroventral regions of branchiostegites. Small eyes. Rostrum short, reaching distal margin of basal podomere of antennal flagellum; dorsally concave. Rostral carina long and prominent. Epistome antero-median lobe resembles a triangle. Cervical groove "V" shaped. Basal podomere of antennula with small spine. Dorsal surface of P1 carpus with faint median groove, opposable margin of propodite bearing 5 to 9 teeth with pilosity on both sides, but only of their proximal group, dactyl moving obliquely. Abdominal pleura ventral


**Figure 2.** *Virilastacus rucapihuelensis* Rudolph & Crandall, 2005. a) Dorsal view of specimen. Scale bar = 14.0 mm, b) partial view of habitat. Photos: E. Rudolph.

margins almost straight. Individuals with supernumerary gonopores. In adult females, pleura of second pleomere with wide anteroventral flap, weakly calcified. Males with slightly elongated phallic papillae that reach the base of the P4 coxae. In males, P5 coxae with cuticle partition. Telson subrectangular, lateral margins almost parallel, each of them with a small, blunt spine. Light brown body coloration (Rudolph & Crandall, 2005, 2012). This species is slightly larger than *V. araucanius*. The largest specimen collected (33.6 mm CL) is an intersex individual in female phase and the smallest, a primary female with 4.4 mm CL (Rudolph *et al.*, 2007) (Table 1).

### Geographic distribution

This species has been reported from five nearby sites in the Coastal Cordillera of the province of Osorno, southern Chile: Rucapihuel (40°35'00.64"S, 73°34'42.96"W), Coiguería (40°35'17.62"S, 73°32'10.00"W), Carrico (40°35'34.14"S, 73°31'19.70"W), Contaco (40°36'01.50"S, 73°31'00.97"W), and Loma de la Piedra (40°40'13.59"S, 73°30'51.42"W) (Rudolph & Crandall, 2005; Grosso & Peralta, 2009). The extent of

**Table 1.** Morphological characters useful for distinguish the four presently identified species of Virilastacus (Modified from Rudolph & Crandall, 2012).

| Character | *V. araucanius* | *V. rucapihuelensis* | *V. retamali* | *V. jarai* |
|---|---|---|---|---|
| Rostrum | | | | |
| Dorsodistal surface | Excavated | Concave | Concave | Concave |
| Rostral carina | Long and not very prominent | Long and prominent | Short and prominent | Short and little prominent |
| Eyes | Small | Small | Large | Small |
| Antennal scale | Short. Distolateral spine small | Short. Distolateral spine small | Long. Distolateral spine large | Short. Distolateral spine small |
| Basal Podomere Antennula | Spine absent | Small spine | Large spine | Large spine |
| Mandible | | | | |
| Cephalic molar process | Molariform | Dentiform | Molariform | Molariform |
| Caudal molar process | Quadricuspide | Tricuspide | Tricuspide | Quadricuspide |
| Epistome | | | | |
| Anteromedial lobe | Resembles a rhombus | Resembles a triangle | Resembles a rhombus | Resembles a rhombus |
| Posterior plate | With anterolateral tubercles small | With anterolateral tubercles small | With anterolateral tubercles large | With anterolateral tubercles large |
| Ischium of Maxilliped 3 | | | | |
| Ventral surface | With a band of small, blunt tubercules and scarce pilosity | With a band of small, blunt tubercles and scarce pilosity | With a band of large, prominent tubercles and abundant pilosity | With a band of small, blunt tubercles and abundant pilosity |
| External distal border | Without extension | Without extension | Without extension | With a large extension |
| Opposable Propo margin | | | | |
| Pilosity | Absent | Only on both sides of the proximal group of teeth | On both sides of the entire row of teeth | Throughout the dorsal side and only on the basal zone of the ventral side |
| Number of teeth | Between 11 and 18 | Between 5 and 9 | Between 13 and 17 | Between 11 and 22 |
| Dactylus | | | | |
| Movement | Subhorizontal | Oblique | Subhorizontal | Oblique |
| Number of teeth | Between 9 and 15 | Between 5 and 10 | Between 10 and 15 | Between 9 and 15 |
| Precervical cephalothorax | Dorsal ridges absent | Dorsal ridges absent | With 4, smooth dorsal ridges | With 4 smooth dorsal ridges, or with 2, or absent |
| Areola | Narrow and extended | Narrow and extended | Wide and short | Wide and extended |
| Cervical groove | Weakly V-shaped | Strongly V-shaped | U-shaped | Strongly V-shaped |
| P4 Coxae | Close together | Widely separated | Close together | Close together |
| Gonopores | Female or male | Supernumerary | Female or male | Female or male |
| Phallic Papillae | In close proximity, long and thin | Widely separated, more robust and shorter | Very close together, very long and thin | Very close together, long and thin |
| Male Cuticle Partition | Absent | Present | Absent | Absent |
| Pleomere pleura | | | | |
| Somite 1 | With a small anterior lobe, partially overlapped by the S2 pleuron | Anterior lobe absent, not overlapped by S2 pleuron | With a small anterior lobe, partially overlapped by the S2 pleuron | With a small anterior lobe, total or partially overlapped by S2 pleuron |
| Somite 2 of the adult females | Flap absent | Flap present | Flap absent | Flap absent |
| Telson | | | | |
| Form | Subretangular | Subretangular | Subtriangular | Subtriangular |
| Lateral spines | Prominent and sharp | Small and blunt | Prominent and sharp | Small and sharp |
| Habitat | Semi-marshland, perennially green areas | Semi-marshland, perennially green areas | Peatlands | Fragment semi-marshland, perennially green areas |

their occurrence covers 39.3 km$^2$ (Ministerio de Medio Ambiente, 2013b).

## Habitat

*V. rucapihuelensis* inhabits underground waters in biotopes locally called "vegas" or "hualves" (Rudolph & Crandall, 2005) (Fig. 2b). The soil profile of the "vegas" located in Rucapihuel includes two layers composed of fine clay sand. The upper layer comprises abundant iron oxides, the lower layer (80 cm below the soil surface) is of sandy gravel. In the winter, the phreatic level is close to or above the surface, and in summer, it descends as far as 1.0 or 1.5 m below the surface (Bedatou *et al.*, 2010). After carrying out year-round monthly recordings of some physicochemical parameters of the water inside the burrows, Martínez (2005) verified that temperature fluctuated between 11 y 19°C, pH between 4.1 and 5.3, and dissolved oxygen between 2.6 and 6.5 mg $L^{-1}$; on the other hand, total hardness remained constant at 17.8 ppm de $CaCO_3$.

## Burrow morphology and burrowing behavior

The burrow morphology is variable. Some have several relatively complex entrance orifices (diameter 2.5-3.5 cm) with multiple ramifications in the subsoil. Some of these connected to a terminal chamber with a slightly larger diameter than the tunnel, situated between 1 and 1.2 m below the surface. Others are blind tunnels (Type 1 burrow, Fig. 3a). Other burrows have only one subvertical tunnel (3 to 4.5 cm diameter and up to 66 cm depth) with a few blind tunnels emerging from the uppermost section. The lower terminal section of this system is a sub-horizontal chamber with a slightly wider diameter than the tunnel (Bedatou *et al.*, 2010) (Type 2 burrow, Fig. 3b). *V. rucapihuelensis* forms small pellets (8-10 mm maximum diameter) from the excavated material, and these pellets are deposited in the winter around the entry orifices of the burrows, forming "chimneys" of up to 12 cm height (Rudolph & Crandall, 2005) (Fig. 3c), these burrows are usually inhabited by one specimen; nevertheless, in spring-summer, it may be possible to find a female with a variable number of recently released juveniles in some of these burrows. According to Hobbs´ (1942) classification, aspects such as excavating complex burrows, distanced from permanent water bodies, together with no recordings of specimens outside the burrows, suggest that this species can be considered as a primary burrower.

## Sexual system

*V. rucapihuelensis* presents partial protandric hermaphroditism with primary males and females (Rudolph *et al.*, 2007). Depending on the presence or absence of gonopores in the P3 and P5 coxae; externally, six sexual forms may be distinguished. Anatomical analyses of the gonads and gonoducts of these sexual forms enabled us to ascertain the presence of three basic sexual types: primary males, primary females, and intersex specimens. These latter comprise one male-phase and two female-phase forms, that would originate from male-phase intersex individuals (Rudolph *et al.*, 2007).

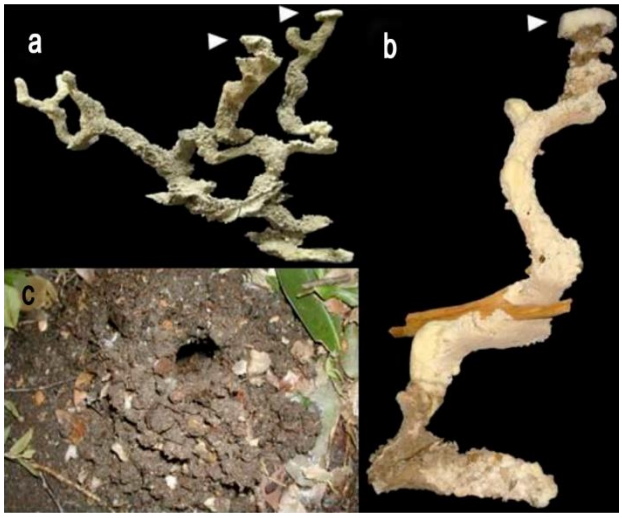


**Figure 3.** *Virilastacus rucapihuelensis* Rudolph & Crandall, 2005. a) Lateral view of mould type 1 burrow, b) lateral view of mould type 2 burrows, c) overhead view of chimney. Arrowheads = surface entrances. Photos: a) and b) E. Bedatou; c) E. Rudolph.

## Fecundity

The species produced up to 74 eggs, incubated by a primary female of 27.9 mm CL. The lowest fecundity (three eggs) observed was in a primary female of 23.1 mm CL (Rudolph *et al.*, 2007).

## Conservation status

Rudolph & Crandall (2007), based on the B1 ab (iii) criteria of the IUCN Red List (2001), classified *V. rucapihuelensis* as *Endangered* in its entire distribution range, while Buckup (2010b) classified it as *Data Deficient*. The Ministerio de Medio Ambiente (2013b) classified it as *Endangered*, maintaining that it meets the B1 ab (iii) + 2ab (iii) criteria of the IUCN Red List (2001). Finally, Almerao *et al.* (2014) suggested that *V. rucapihuelensis* is *Critically Endangered*, considering that it falls within the B1 ab (iii) criteria associated with this category.


**Figure 4.** *Virilastacus retamali* Rudolph & Crandall, 2007. a) Dorsal view of specimen. Scale bar = 12.0 mm, b) partial view of habitat, c) lateral view of chimney. Photos: E. Rudolph.

***Virilastacus retamali* Rudolph & Crandall, 2007** (Fig. 4a)

Common name: Peatland crayfish

**Distinctive morphological characteristics**

Precervical cephalothorax with four smooth ridges. Eyes comparatively large. Rostrum short reaches distal margin of middle podomere of antennal flagellum; dorsally concave. Rostral carina short and prominent. Cervical groove "U" shaped. Areola wide and short. Antennal scale long with large distolateral spine. Epistome anteromedian lobe resembling a rhombus. Basal podomere of antennula with large spine. Opposable margin of P1 propodite bearing 13 to 17 teeth, with pilosity on both sides of the row of teeth. Dactyl moving subhorizontally. Abdominal pleura with rounded ventral margins. Telson with converging lateral margins resembling a triangle, with prominent, sharp marginal spines. Individuals with female or male gonopores. Phallic papillae very elongated, reaching as far as the base of the P3 coxae. Males without cuticular partition in P5 coxae. Cephalothorax dark-olive green and pleon light olive green (Rudolph & Crandall, 2007, 2012). Species is small (17.9-30.0 mm CL; $\bar{X}$ = 21.1 ± 3.0 mm CL; n = 21), but the only size data available derives from the type series specimen (Rudolph & Crandall, 2007) (see Table 1).

**Geographic distribution**

This parastacid, recorded in two Coastal Cordillera localities in the provinces of Osorno and Llanquihue, southern Chile: Rucapihuel (40º35'00.08"S, 73º34' 44.3"W), and Estaquilla (41º25'15.93"S, 73º46'51. 74"W) (Rudolph & Crandall, 2007) extends its occurrence approximately 3.200 km$^2$ (Rudolph, 2010).

**Habitat**

Both of these *V. retamali* populations inhabit geogenous peatlands, *i.e.*, depend on rainwater and superficial underground waters to supply hydric needs (Kulzer & Cook, 2001). These peatlands originated in a small endorreic basin generated by the holocenic deglacialization where, over the course of time, organic matter has been deposited (Grignola & Ordoñez, 2002). This organic material originated from the partially decomposed, loosely compacted vegetal remains of the genus *Sphagnum*, mixed with woody fragments, gramineous and humus particles, accumulated in an anoxic environment, highly saturated with water all year round (Grignola & Ordoñez, 2002) (Fig. 4b). On capturing the type series, (17 December 2002), the water analyzed inside the burrows had a pH of 4.7, dissolved oxygen of 2.8 mg L$^{-1}$, constant hardness of 17.8 ppm of $CaCO_3$, and a temperature of 12.5ºC (Rudolph & Crandall, 2007).

**Burrow morphology**

*Virilastacus retamali* excavates shallow burrows (45 cm depth approximately) with few ramifications. A mold made with polyester resin (Bedatou *et al.*, 2010) revealed six main entrances, with diameters ranging from 3.0 to 3.5 cm. Fifteen centimeters below the surface, three of these entrances converged into a short, subhorizontal tunnel; one of its ends projected slightly upwards to form a short blind tunnel; the other extreme is connected to a main sub-horizontal tunnel. The remaining three entrances converged at a depth of 30 cm, at the other end of the main tunnel. In the winter, the species constructs "chimneys" reaching an average height of 3.9 cm (SD = ± 0.729; n = 8) (Fig. 4c). Unlike the other species of the genus, *V. retamali* has been observed and captured outside its burrows, in nearby

surface pools. Based on these observations and according to Hobbs´s criteria (1942), *V. retamali* can be categorized as a secondary burrowing species (Rudolph & Crandall, 2007).

**Sexual system**

No external morphological evidence of intersexuality has been detected in the type series (Rudolph & Crandall, 2007), suggesting that this species is gonochoric. Females smaller than 20.0 mm CL have strongly calcified semi-ellipsoidal gonopores, while females larger than 20.0 mm CL have apparently functional ellipsoidal gonopores, given that they are only partially calcified and surrounded by abundant pilosity. Males have a very elongated phallic papilla ($\overline{X}$ = 3.5 ± 0.5 mm) (Rudolph & Crandall, 2007).

**Conservation status**

Rudolph & Crandall (2007) classified *V. retamali* as an *Endangered* throughout its entire geographic range, given that it would meet the B1 ab(iii) criteria of the IUCN (2001) Red List for this category. Nevertheless, Buckup (2010c) classified it as *Data Deficient*, while Almerao *et al.* (2014) supported the endangered species classification of Rudolph & Crandall (2007).

***Virilastacus jarai* Rudolph & Crandall, 2012 (Fig. 5a)**

Common name: Angeline crayfish

**Distinctive morphological characteristics**

Precervical cephalothorax with four, two or lacking smooth dorsal ridges. Eyes small. Rostrum short, reaching distal margin of the middle podomere of antennal flagellum; dorsally concave. Rostral carina short, weakly prominent. Epistome anteromedial lobe resembling a rhombus. Cervical groove "V" shaped. Areola wide and extended. Opposable margin of P1 propodite bearing 11 to 22 teeth with pilosity along the length of dorsal side, while only on basal part of ventral side. Dactyl moving obliquely. Abdominal pleura with straight ventral margins. Telson subtriangular with a small, sharp spine in each lateral margin. Individuals with female or male gonopores. Phallic papillae elongated, reaching base of lateral process of XII body segment. Males lacking cuticular partition in P5 coxae. Cephalothorax and P1 chelipeds olive green. Pleon and caudal fan light brown (Rudolph & Crandall, 2012). It is a small species; size in the type series ranges from 6.7 to 24.8 mm CL ($\overline{X}$ = 17.5± 6.1 mm) (Rudolph & Crandall, 2012) (see Table 1).

**Geographic distribution**

*Virilastacus jarai* has only been recorded in the type locality, a fragment of wetland situated in the "El Porvenir" sector (37º26'39.84"S, 72º18'37.12"W), 1.5 km northwest of the town of Los Ángeles in central-southern Chile (Rudolph & Crandall, 2012).

**Habitat**

The species inhabits the underground waters of a fragment of semi-marshland, located in a topographic basin of 861 $m^2$ at 152 m above sea level. This area is flooded for six months of the year (May to October) and the phreatic level remains below the surface in spring-summer (Fig. 5b). The soil, characterized by a large accumulation of organic material, in addition to the high percentage of moisture originating from partially decomposed vegetal remains. On capturing the type series (13 June 2010), analysis of the water inside the burrows was as follows: dissolved oxygen = 4.9 mg $L^{-1}$, temperature = 14.1ºC, pH = 6.5, and constant hardness of 53.4 ppm of $CaCO_3$ (Rudolph & Crandall, 2012).

**Burrowing behavior**

The species excavates shallow (<1 m), but complex burrows, not connected to lothic or lenthic waters (Rudolph & Crandall, 2012). In winter, it also constructs "chimneys" around the entrance orifices of the burrows (Fig. 5c). Outside the burrows, no specimens have been found, which suggest that the entire life cycle of *V. jarai* occurs inside the burrows. According to the Hobbs´s (1942) criteria, the species can be considered as a primary burrower.

**Sexual system**

The revision of the type series revealed the occurrence of an intersex individual; however, this is not sufficient evidence to maintain that this is a transitional stage of an eventual sex change or, even less likely, that the species presents some form of hermaphroditism. Consequently, the evidence available suggests that *V. jarai* would be a gonochoric species (Rudolph & Crandall, 2012).

**Conservation status**

Rudolph & Crandall (2012) categorized *V. jarai* as *Critically Endangered*. This conclusion was based on the B1ab (ii) criteria of the IUCN Red List (2001) for this category, *i.e.*, an estimated extent of occurrence of less than 100 $km^2$, only known recording in one location, and a projected decline in habitat quality. The *V. jarai* habitat has been subject to deforestation in the recent past to clear land for agricultural purposes. At present, this land has been divided up and the topography modified to accommodate building development projects resulting from the rapid expansion of the town of Los Ángeles. Almerao *et al.* (2014) endorsed categorizing *V. jarai* as a critically endangered species.

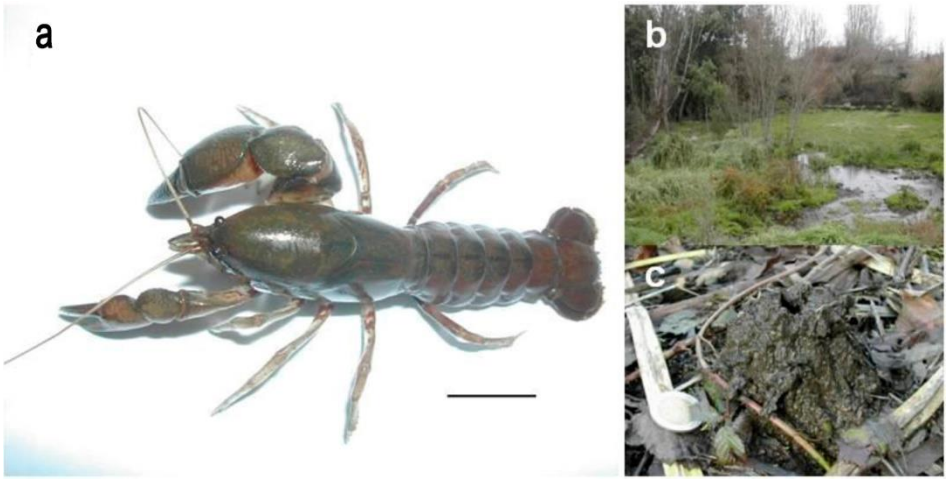


**Figure 5.** *Virilastacus jarai* Rudolph & Crandall, 2012. a) Dorsolateral view of specimen. Scale bar = 9.0 mm, b) partial view of habitat, c) lateral view of chimney. Photos: E. Rudolph.

## REMARKS

The four *Virilastacus* species are endemic to Chile and have a restricted geographic range, distributed between the coastline and the Coastal Cordillera, from Concepción (36º46'S) to Estaquilla (41º25'S), except for *V. jarai*, whose presence has only been recorded in one location, to the east of the coastal Cordillera (Fig. 6). Furthermore, within this coastal fringe, populations present a clearly discontinuous distribution, associated with wetlands. These species are burrowers, and although their burrows are shallow (<1.5 m), have multiple ramifications and are much elaborated (Bedatou *et al.*, 2010). The most significant morphological adaptations to this life style include the following aspects: (1) body without large protuberances, facilitating their movement inside the tunnels; (2) highly developed cephalothorax, in comparison to the scarcely developed pleon; (3) cephalothorax taller than broad, which increases the volume of the branchial chamber and makes it possible to house larger branchia than those species that inhabit open waters; (4) P1 chelae relatively large and vertically orientated, and (5) reduced eye size (Rudolph, 1997; Reynolds *et al.*, 2013). They also share some biological characteristics of all freshwater astacids (Families Astacidae, Cambaridae and Parastacidae) such as: low fecundity, direct development with hatching in the juvenile stage, extended parental care up to the second juvenile stage, as well as omnivorous feeding habits (Rudolph & Rojas, 2003; Rudolph, 2013). Like the other species of these three families, they are very important functional elements in the linmic ecosystems, both as prey and as consumers (Jara *et al.*, 2006; Almerao *et al.*, 2014). Nevertheless,

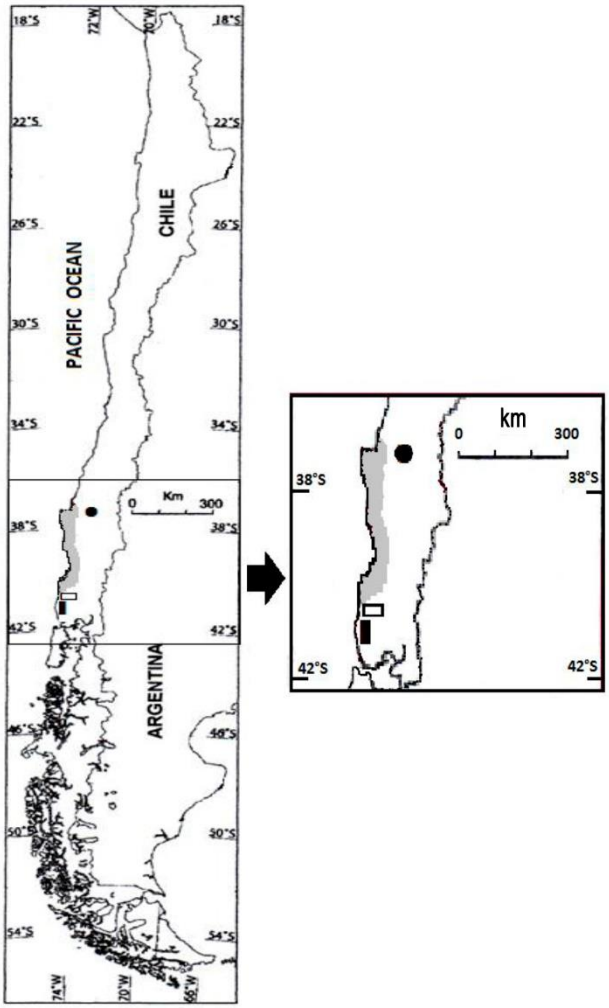


**Figure 6.** Geographical distribution of *Virilastacus* species. Gray fringe = *Virilastacus araucanius*; ● *Virilastacus jarai*; □ *Virilastacus rucapihuelensis*; ▌*Virilastacus retamali*.

biological knowledge about these species is limited mainly to taxonomic and distributional aspects, with some information about their sexual system. Three of the four species have separate sexes while *V. rucapihuelensis* presents partial protandric hermaphroditism, with primary males and females (Rudolph *et al.*, 2007). Similarly, some information has been acquired about their phylogenetic affinities (Rudolph & Crandall, 2007, 2012). Reconstruction of phylogenetic relationships revealed the monophyly of the *Virilastacus* genus and of each one of its species. Furthermore, they showed that there is clear genetic differentiation between *V. jarai* and the other species of the genus. *Virilastacus jarai* is situated in a different basal clade with respect to the clade of the other three species, with *V. araucanius* being the species phylogenetically closest to *V. jarai*. Moreover, the incubation period of *V. rucapihuelensis* and probably that of the other three species extends from mid-winter to mid-summer (from July to February) (Rudolph *et al.*, 2007). Recently, the Ministerio del Medio Ambiente (2013a, 2013b) and Almerao *et al.* (2014), based on the IUCN (2001) Red List criteria, have reevaluated and updated the state of conservation of Chilean and South American parastacids, respectively. Notwithstanding these updates, no effective measures for their protection have been implemented yet and, as a result, the conservation of the *Virilastacus* species is still under threat. Included among these threats are: (1) drainage of the "vegas" for forestry, farming and livestock development; (2) use of chemicals (agricultural fertilizers and pesticides); (3) clearing of vegetation and subsequent replacement of vegetation coverage; (4) free-range pig farming, which removes the original vegetation and compacts the soil, and (5) cattle farming, with trampling and destruction of the burrows. These threats are degrading, fragmenting, and ultimately decreasing the geographical extension of their habitat. Furthermore, certain intrinsic characteristics of parastacids [*i.e.*, slow growth, low fecundity, delayed sexual maturity and long periods of embryonic and early post-embryonic development (Holdich, 1993; Rudolph, 2013)], together with the restricted geographic range and scarce mobility of the *Virilastacus* species, render them particularly vulnerable to the aforementioned threats. At present, the Chilean parastacids in general, and the *Virilastacus* species in particular, are not threatened by invasive exotic species (Rudolph, 2013). However, this threat could materialize in the near future, if we consider that *Procambarus clarkii* (Girard, 1852), a species native to North America and with considerable adaptive plasticity, has been introduced successfully to almost all continents, and has already been recorded in Colombia, Ecuador and Brazil (Valencia-López *et al.*, 2012; Almerao *et al.*, 2014). Furthermore, recent studies revealed numerous areas in the southern cone of South America (Argentina, Paraguay, Uruguay, and Chile) suitable for *P. clarkii* occupation (Palaoro *et al.*, 2013). Fortunately, these four species are not under threat from fishery activities for human consumption, because they are small species whose edible part (the pleon) is underdeveloped, thus, meat yield is very meager; these species also construct very complex burrows and considerable effort is required to capture them. Finally, part of their geographic range is located to the south of the Toltén River (39°S), where the local inhabitants have no tradition of consuming these types of crustaceans. Could this lack of socio-economic significance account for the lack of legislation regulating their protection? Probably not considering that other Chilean parastacids exposed to elevated extraction pressure for human consumption purposes (*i.e.*, *Samastacus spinifrons* and *Parastacus pugnax*) and neither is protected. Minimum biological knowledge (distribution range, habitat type, life style, size, incubation period, and state of conservation) necessary to establish regulations is now available. Legislation in this respect, together with regulating compliance with the law, would greatly contribute towards the conservation of Chilean parastacids. It is believed that protection of these species can be achieved, considering that the Ministry of Environment has announced a series of measures in both the National Plan of Action on Climate Change and the draft law for the creation of a Biodiversity and Protected Areas Service.

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