

FISH, FISHING AND FISHERIES

# Crayfish

Evolution, Habitat and Conservation Strategies



Felipe Bezerra Ribeiro

Editor

NOVA



**FISH, FISHING AND FISHERIES**

**CRAYFISH**

**EVOLUTION, HABITAT AND  
CONSERVATION STRATEGIES**

No part of this digital document may be reproduced, stored in a retrieval system or transmitted in any form or by any means. The publisher has taken reasonable care in the preparation of this digital document, but makes no expressed or implied warranty of any kind and assumes no responsibility for any errors or omissions. No liability is assumed for incidental or consequential damages in connection with or arising out of information contained herein. This digital document is sold with the clear understanding that the publisher is not engaged in rendering legal, medical or any other professional services.

# **FISH, FISHING AND FISHERIES**

Additional books and e-books in this series can be found on Nova's website under the Series tab.



**FISH, FISHING AND FISHERIES**

# **CRAYFISH**

## **EVOLUTION, HABITAT AND CONSERVATION STRATEGIES**

**FELIPE BEZERRA RIBEIRO**

**EDITOR**



Copyright © 2020 by Nova Science Publishers, Inc.

**All rights reserved.** No part of this book may be reproduced, stored in a retrieval system or transmitted in any form or by any means: electronic, electrostatic, magnetic, tape, mechanical photocopying, recording or otherwise without the written permission of the Publisher.

We have partnered with Copyright Clearance Center to make it easy for you to obtain permissions to reuse content from this publication. Simply navigate to this publication's page on Nova's website and locate the "Get Permission" button below the title description. This button is linked directly to the title's permission page on copyright.com. Alternatively, you can visit copyright.com and search by title, ISBN, or ISSN.

For further questions about using the service on copyright.com, please contact:

Copyright Clearance Center

Phone: +1-(978) 750-8400

Fax: +1-(978) 750-4470

E-mail: info@copyright.com.

### **NOTICE TO THE READER**

The Publisher has taken reasonable care in the preparation of this book, but makes no expressed or implied warranty of any kind and assumes no responsibility for any errors or omissions. No liability is assumed for incidental or consequential damages in connection with or arising out of information contained in this book. The Publisher shall not be liable for any special, consequential, or exemplary damages resulting, in whole or in part, from the readers' use of, or reliance upon, this material. Any parts of this book based on government reports are so indicated and copyright is claimed for those parts to the extent applicable to compilations of such works.

Independent verification should be sought for any data, advice or recommendations contained in this book. In addition, no responsibility is assumed by the Publisher for any injury and/or damage to persons or property arising from any methods, products, instructions, ideas or otherwise contained in this publication.

This publication is designed to provide accurate and authoritative information with regard to the subject matter covered herein. It is sold with the clear understanding that the Publisher is not engaged in rendering legal or any other professional services. If legal or any other expert assistance is required, the services of a competent person should be sought. FROM A DECLARATION OF PARTICIPANTS JOINTLY ADOPTED BY A COMMITTEE OF THE AMERICAN BAR ASSOCIATION AND A COMMITTEE OF PUBLISHERS.

7

Additional color graphics may be available in the e-book version of this book.

### **Library of Congress Cataloging-in-Publication Data**

Names: Bezerra Ribeiro, Felipe, Programa de Pós-Graduação em Biologia Animal, Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil, editor.

Title: Crayfish: Evolution, Habitat and Conservation Strategies

Description: New York: Nova Science Publishers, [2019] | Series: Fish, Fishing and Fisheries | Includes bibliographical references and index.

Identifiers: LCCN 2019956507 (print) | ISBN 9781536169416 (hardcover) |

ISBN 9781536169423 (adobe pdf)

*Published by Nova Science Publishers, Inc. † New York*

*“Crayfish: Evolution, Habitat and Conservation Strategies” is a book filled with important useful and intriguing information concerning these unique freshwater creatures. It offers a fresh and contemporary overview and is an essential reading for everyone involved within the field of astacology.*

Ivana Maguire  
Prof. for Zoology and Ecology,  
University of Zagreb, Croatia



# CONTENTS

<b>Preface</b>		<b>ix</b>
<b>Acknowledgments</b>		<b>xi</b>
<b>Chapter 1</b>	Diversity and Conservation Strategies of Freshwater Crayfish in South America: An Update <i>Felipe Bezerra Ribeiro, Kelly Martinez Gomes, Augusto Frederico Huber and Tainã Gonçalves Loureiro</i>	<b>1</b>
<b>Chapter 2</b>	Crayfish of New Guinea: Current Status, Exploitation and Threats <i>Jiří Patoka</i>	<b>43</b>
<b>Chapter 3</b>	Morphometric and Behavioral Divergence in <i>Procambarus acanthophorus</i> Inhabiting in a Coastal Plain Wetland and a Mountain Pine Forest <i>Leonor Mendoza-Vargas, Fabiola Galicia-Mendoza, Gabriel Arriola-Pizano, Elizabeth Guarneros-Bañuelos, Pilar Ortega, Zuly A. Sánchez-Florentino, Santa Rodríguez-Lorenzo, Edgar Flores-Soto, Bettina Sommer, Marcela Valdés-Tovar and Héctor Solís-Chagoyán</i>	<b>71</b>

<b>Chapter 4</b>	Non-Indigenous Crayfish Species: A Global Assessment and Future Perspectives <i>Tainã Gonçalves Loureiro</i>	<b>105</b>
<b>Chapter 5</b>	Biology, Ecology, Evolution, Systematics and Utilization of the Parthenogenetic Marbled Crayfish, <i>Procambarus virginalis</i> <i>Günter Vogt</i>	<b>137</b>
<b>Chapter 6</b>	Integrative Taxonomy and Cryptic Diversity in Freshwater Crayfish: An Overview <i>Felipe Bezerra Ribeiro</i>	<b>229</b>
<b>About the Editor</b>		<b>245</b>
<b>Index</b>		<b>247</b>
<b>Related Nova Publications</b>		<b>255</b>

## PREFACE

Crayfish are one of the most iconic freshwater crustacean groups of the world. They can be found in several types of environment, e.g., streams, lakes, wetlands, caves and swamp forests. They can also develop the burrowing behavior in different levels according to the occupied habitat. Some crayfish species are able to dig up to 2m depth. The wide range of biological adaptations of these crustaceans to the limnetic ecosystems is surprising, as is their high potential of some taxa become invasive species. There is a constant growing of interest in develop research with freshwater crayfish. They can also be an important food source in several countries, besides the aquarium pet trade and the cultural importance.

The present book is constituted of six chapters. The first chapter, entitled “Diversity and conservation strategies of freshwater crayfish in South America: an update”, brings an updated review of the diversity, habitat characteristics, biological features and conservation strategies for the South American native crayfish species. In addition, this chapter also brings information regarding the non-indigenous crayfish species currently found in South American and their impact to the native fauna.

The second chapter, entitled “Crayfish of New Guinea: Current Status, Exploitation and Threats”, is a review of scientifically described species of New Guinean crayfish and known information about their occurrence gathered together with the data about their exploitation by humans and

further perspectives, with an extension to the improvement of conservation practises.

The third chapter, entitled “Morphometric and behavioral divergence in *Procambarus acanthophorus* inhabiting in a coastal plain wetland and a mountain pine forest”, brings information about some aspects of biology and behavior of a burrowing crayfish species occupying different habitats in North America.

The Chapter 4 is entitled “Non-indigenous crayfish species: A global assessment and future perspectives”. This chapter summarizes the major information on alien freshwater crayfish species, including their distribution, impacts on biodiversity and management possibilities.

The Chapter 5, entitled “Biology, Ecology, Evolution, Systematics and utilization of the Parthenogenetic Marbled Crayfish, *Procambarus virginalis*”, is an extensive review of several aspects of detection history, biology, taxonomy, geographical distribution, ecology, evolution and utilization of the marbled crayfish, parthenogenetic species with high potential for bioinvasion.

Last but not least, the Chapter 6, entitled “Integrative taxonomy and cryptic diversity in freshwater crayfish: an overview”, brings information about the use of integrative methods in taxonomy of freshwater crayfish, cryptic diversity in freshwater crayfish and the implications for conservation and invasion biology, and about the molecular methods for species delimitation in crayfish studies.

There are already several published books about freshwater crayfish in global and local scales. However, the present book brings important reviews of some aspects of evolution, habitat characteristics and conservation strategies of freshwater crayfish, including native and non-indigenous species. This book is directed not only at scientists working on freshwater crayfish or decapods crustaceans in general, but also for academic professors and students with interest in discover the wonderful world of these fascinating crustaceans.



## ACKNOWLEDGMENTS

My thanks go foremost to all contributors who attended to all requests during the preparation of this book. Their expertise and willingness to invest time into the writing process of their chapters make up the value of this book. I specially thank the Bsc. Augusto F. Huber who helped in the edition of some figures and created the cover of this book and Dr. Tainã G. Loureiro and Dr. Kelly M. Gomes for their help in the writing of some of the chapters.

I also would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) of Brazil which provided the fellowship for my postdoctoral research and also the Zoology Department and the Post Graduate Program in Animal Biology (PPGBAN) of the Universidade Federal do Rio Grande do Sul (UFRGS), which gave me the opportunity to develop my research on freshwater crayfish. A special thank to my former supervisors, Professors Paula B. Araujo, Luis E. A. Bezerra, Christoph D. Schubart and Helena Matthews-Cascon for teaching me everything that I know about carcinology and invertebrate zoology and for always trust in my work.

I also would like to thank Camila Timm Wood and Emily Rockhill for English review of some chapters and Nova Publishers for the opportunity and freedom in the process to develop this book.

Finally, I would like to thank my family and lab colleagues for their patience and tolerance during the process of developing this book.

*Felipe B. Ribeiro, PhD*

In: Crayfish  
Editor: Felipe Bezerra Ribeiro

ISBN: 978-1-53616-941-6  
© 2020 Nova Science Publishers, Inc.

*Chapter 1*

**DIVERSITY AND CONSERVATION  
STRATEGIES OF FRESHWATER CRAYFISH  
IN SOUTH AMERICA: AN UPDATE**

*Felipe Bezerra Ribeiro<sup>1,\*</sup>, Kelly Martinez Gomes<sup>1</sup>,  
Augusto Frederico Huber<sup>1</sup>  
and Tainã Gonçalves Loureiro<sup>2</sup>*

<sup>1</sup>Programa de Pós-Graduação em Biologia Animal,  
Departamento de Zoologia (Laboratório de Carcinologia),  
Instituto de Biociências, Universidade Federal do Rio Grande do Sul,  
Porto Alegre, Rio Grande do Sul, Brazil

<sup>2</sup>Centre for Invasion Biology, Department of Botany and Zoology,  
Stellenbosch University, Stellenbosch,  
Western Cape, South Africa

**ABSTRACT**

Freshwater crayfish are represented by about 700 species distributed worldwide, except in continental Africa and Antarctica. They can be found

---

\* Corresponding Author's Email: fbribeiro.ufc@gmail.com.

in streams, lakes, burrows, caves and swamp forests. In South America, there are currently 19 native species distributed along three genera and two invasive species recorded. Besides, other aspects about South American crayfishes have been studied in recent years, such as: cryptic diversity, habitat characterization and requirements, distribution, biological aspects (as reproduction and behavior), threats and conservation strategies. Among the main environmental threats posed to native species, the establishment of invasive alien freshwater crayfish species stand out due to the multiple and synergic impacts they might cause. *Procambarus clarkii* is the most studied invasive species found in South America and its establishment process in Brazil was already well documented. This chapter provides an overview on native and non-native freshwater crayfish in South America in order to encourage future researchers in the field of Astacology.

**Keywords:** burrowing crayfish, conservation status, management, invasive crayfish, limnetic ecosystems

## 1. A BRIEF OVERVIEW OF FRESHWATER CRAYFISH

Freshwater crayfish are a monophyletic group of the infraorder Astacidea Latreille, 1802 (Crandall et al., 2000a, b; Toon et al., 2010) being represented by about 700 species distributed in two superfamilies, Astacoidea Latreille, 1802 and Parastacoidea Huxley, 1879 (Crandall and De Grave, 2017).

Astacoidea is distributed in the northern hemisphere and is currently represented by three families: Astacidae Latreille, 1802, Cambaridae Hobbs, 1942 and Cambaroididae Villalobos, 1855 (Crandall and De Grave, 2017). Astacidae is the second less rich family within crayfishes, being composed only by four genera and 20 species distributed in Europe and Western North America (Crandall and De Grave, 2017). Cambaridae is distributed in North America and encompass the largest number of species of freshwater crayfish, being currently represented by 14 genera and 448 species (Crandall and De Grave, 2017). The main centre of diversity of Northern hemisphere crayfish is the southeastern USA, where about 80% of cambarid species can be found (Crandall and Buhay, 2008). Cambaroididae is the less rich family, being composed only by *Cambarellus* Faxon, 1884 which encompass six

species distributed in Eastern Asia. This genus was traditionally placed within Cambaridae, but morphological and molecular phylogenetics proposed the placement of this genus in a separated family (Stern and Crandall, 2016; Crandall and De Grave, 2017).

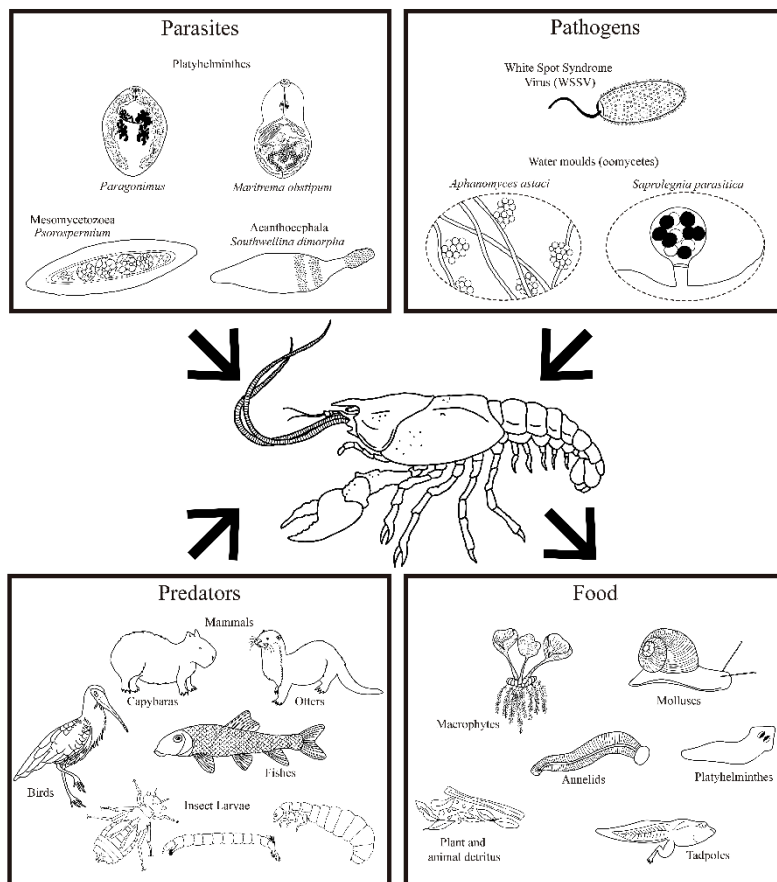


Figure 1. Trophic ecology and biotic relationships of a crayfish (*Procambarus clarkii*) evidencing its parasites and pathogens, as well as predators and food sources. Modified from Loureiro et al., (2015a).

The southern hemisphere crayfish belong to the Parastacoidea, which is represented only by the family Parastacidae Huxley, 1879. This family is currently composed of 15 genera and 196 species (Crandall and De Grave, 2017; Miranda et al., 2018). Parastacids are distributed in Australia, New Zealand, New Guinea, Madagascar and South America (Toon et al., 2010).

Freshwater crayfish play important roles in natural limnetic environment, being considered key species due to their flexible position in food webs, acting as detritivores, herbivores and predators, increasing the processing of energy and secondary production (Gherardi and Paglianti, 2004; Gherardi, 2006). These crustaceans are also important as prey source for mammals, birds, reptiles, amphibians, fish, and other invertebrates (Geiger et al., 2005; Gherardi, 2006) (Figure 1). Furthermore, the burrowing behavior, despite variable among species, also contributes to their high capacity of changing the environment in which they occur.

Considering all the potential effects that freshwater crayfish can generate in natural ecosystems, it is not surprising that local extinction or decrease on native species population size, as well as the establishment of exotic species, might significantly modify the functioning of natural environments, in a way that native populations must be preserved and the establishment of invasive species should be avoided.

## **2. DIVERSITY AND DISTRIBUTION OF FRESHWATER CRAYFISH IN SOUTH AMERICA**

South America reunite 19 native species of Parastacidae distributed in three genera, *Parastacus* Huxley, 1879, *Samastacus* Riek, 1972 and *Virilastacus* Hobbs, 1991 (Figure 2), which can be found in specific regions of Chile, Argentina, Uruguay and Brazil (Figure 3) (Buckup and Rossi, 1980; 1993; Rudolph, 2010). The genus *Parastacus* is the richest one, being currently represented by 14 species (Table 1) (Buckup and Rossi, 1980; Ribeiro et al., 2016; 2017; Ribeiro and Araujo, 2017; Huber et al., 2018; Miranda et al., 2018). *Samastacus* is monotypic, being represented only by

*Samastacus spinifrons* (Philippi, 1882) and *Virilastacus* is composed of only four species endemic to Chile (Table 1) (Rudolph, 2015; Rudolph et al., 2016).

*Parastacus* shows a disjoint distribution in the southern portion of the South American continent (Figure 3), potentially related to biogeographic factors that include the Andes mountains uplift and marine transgressions (Toon et al., 2010; Collins et al., 2011). In Brazil, 12 species are recorded from the southern region (states of Rio Grande do Sul and Santa Catarina) (Buckup and Rossi, 1980; Ribeiro et al., 2016; 2017; Miranda et al., 2018). Some of these freshwater crayfish have a restricted distribution to state of Rio Grande do Sul, such as *Parastacus brasiliensis* (von Martens, 1869), *P. promatensis* Fountoura & Counter, 2008, *P. caeruleodactylus* Ribeiro & Araujo in Ribeiro et al., 2016, *P. fluviatilis* Ribeiro & Buckup in Ribeiro et al., 2016 and *P. buckupi* Huber, Ribeiro & Araujo, 2018. Similarly, *P. pilicarpus* Huber, Ribeiro & Araujo, 2018 and *P. tuerkayi* Ribeiro, Huber & Araujo in Ribeiro et al., 2017 occur only in the state of Santa Catarina. *Parastacus laevigatus* Buckup & Rossi, 1980 is also recorded in the state of Santa Catarina, but this species has not been collected since the 1950s. Distribution data for this species are scarce and habitat information remains unknown. However, there are species with a wide geographical range, encompassing Brazil and other countries. *Parastacus varicosus* Faxon, 1898 is recorded from Brazil (state of Rio Grande do Sul and Santa Catarina), Uruguay and Argentina; *P. defossus* Faxon, 1898 from Brazil (state of Rio Grande do Sul) and Uruguay; *P. saffordi* Faxon, 1898 Brazil (state of Rio Grande do Sul and Santa Catarina) and Uruguay; and *P. pilimanus* (von Martens, 1869) in Brazil (state of Rio Grande do Sul), Uruguay and Argentina (Table 1). In Chile, two species of *Parastacus* exhibit an allopatric distribution pattern in central-south region. *Parastacus nicoleti* (Philippi, 1882) occurs in the coastal zones and *P. pugnax* (Poepigg, 1835) from the coast to the foothills of the Andes Mountains (Rudolph, 2010; 2013) (Table 1).

**Table 1. Native South American crayfish: Distribution, habitat, ecological classification (sensu Hobbs, 1942, Horwitz and Richardson, 1986), conservation status and threats.**

Species	Distribution in South America	Habitat	Ecological Classification	Threats	Conservation Status/Reference
<i>Parastacus brasiliensis</i> (von Martens, 1869)	Brazil (Rio Grande do Sul)	Stream	Tertiary burrower Type 1 a,b burrow	Urbanization, soil erosion, silting of rivers, dumping of pesticides, organic and domestic residues.	NT (Miranda et al., 2018)
<i>Parastacus buckupi</i> Huber, Ribeiro & Araujo, 2018	Brazil (Rio Grande do Sul)	Stream	Tertiary burrower Type 1 a,b burrow	Deforestation.	EN B1ab (iii) (Huber et al., 2018)
<i>Parastacus caeruleodactylus</i> Ribeiro & Araujo in Ribeiro et al., 2016	Brazil (Rio Grande do Sul)	Swamp forest	Primary burrower Type 2 burrow	Deforestation, drainage of wetlands and use of agrochemical in agriculture.	EN B1ab (iii) (Ribeiro et al., 2016)
<i>Parastacus defossus</i> Faxon, 1898	Brazil (Rio Grande do Sul), Uruguay (Montevideo)	Marshland and swamps	Primary burrower Type 2 burrow	Stream channelling and liquid waste discharged by residences, commercial properties, industry and/or agriculture.	NT (Buckup, 2010a; Almerão et al., 2014)
<i>Parastacus fluviatilis</i> Ribeiro & Buckup in Ribeiro et al., 2016	Brazil (Rio Grande do Sul)	Altitude stream	Tertiary burrower Type 1 a,b burrow	Removal of riparian vegetation and presence of exotic species (such as the rainbow trout - <i>Oncorhynchus mykiss</i> Walbaum, 1792).	EN B1ab (iii) (Ribeiro et al., 2016)



Species	Distribution in South America	Habitat	Ecological Classification	Threats	Conservation Status/Reference
<i>Parastacus laevigatus</i> Buckup & Rossi, 1980	Brazil (Santa Catarina)	Unknown	Unknown	Unknown	DD (Buckup 2010b; Almerão et al., 2014)
<i>Parastacus nicoleti</i> (Philippi, 1882)	Chile (Valdivia, Osorno and Llanquihue).	Semi-marshland and perennially green areas	Primary burrower Type 2 burrow	Deforestation.	DD (Buckup, 2010c; Almerão et al., 2014)
<i>Parastacus pilicarpus</i> Huber, Ribeiro & Araujo, 2018	Brazil (Santa Catarina)	Stream	Tertiary burrower Type 1 a,b burrow	Industrial and agricultural waste and deforestation.	DD B1ab (iii) (Huber et al., 2018).
<i>Parastacus pilimanus</i> (von Martens, 1869)	Brazil (Rio Grande do Sul), Argentina (Catamarca, Corrientes, Entre Ríos, Santa Fé) and Uruguay (Federación, Flores Rivera, Rocha, Cerro Largo).	Stream and wetland	Secondary burrower Type 2 burrow	Water course channeling, discharge of sewage domestic and industry and/or agriculture residues.	DD (Buckup, 2010d; Almerão et al., 2014)
<i>Parastacus promatensis</i> Fontoura & Conter, 2008	Brazil (Rio Grande do Sul)	Altitude stream	Tertiary burrower Unknown	Unknown	Unknown
<i>Parastacus pugnax</i> (Poepigg, 1835)	Chile (from Valdivia to Araucania)	Semi-marshland, perennially green areas	Primary burrower Type 2 burrow	Deforestation and harvesting for human consumption.	DD (Buckup, 2010e; Almerão et al., 2014)

**Table 1. (Continued)**

Species	Distribution in South America	Habitat	Ecological Classification	Threats	Conservation Status/Reference
<i>Parastacus saffordi</i> Faxon, 1898	Brazil (Rio Grande do Sul, and Santa Catarina), Uruguay (Montevideo and Maldonado).	Stream and backwater	Tertiary burrower Type 1 a,b burrow	Unknown	DD (Buckup, 2010f; Almerão et al., 2014)
<i>Parastacus tuerkayi</i> Ribeiro, Huber & Araujo in Ribeiro et al., 2017	Brazil (Santa Catarina)	Swamp forest	Primary burrower Type 2 burrow	Urban expansion.	EN B 1ab (iii) (Ribeiro et al., 2017)
<i>Parastacus varicosus</i> Faxon, 1898	Brazil (Rio Grande do Sul, Santa Catarina), Argentina (Entre Ríos) and Uruguay (Maldonado, Montevideo and Rocha)	Streams, backwater and artificial ponds	Tertiary burrower Type 1 a,b burrow	Stream channelling and liquid waste discharged by domestic residences, commercial properties, industry and/or agriculture.	DD (Buckup, 2010g; Almerão et al., 2014)
<i>Samastacus spinifrons</i> (Philippi, 1882)	Chile (from Valparaiso to Taitao peninsula) and Argentina (northeast and Nahuel-Huapi National Park).	Stream and lake	Tertiary burrower Type 1 a burrow	Exploitation for human consumption and physical alteration of habitat..	DD (Rudolph, 2013; Almerão et al., 2014)
<i>Virilastacus araucanius</i> (Faxon, 1914)	Chile (coastal strip from Concepción to Maicolpué)	Semi-marshland, perennially green areas	Primary burrower Type 2 burrow	Water pollution and substrate alteration.	VU B 1ab (iii) + 2ab (iii) (Almerão et al., 2014).

Species	Distribution in South America	Habitat	Ecological Classification	Threats	Conservation Status/Reference
<i>Virilastacus jarai</i> Rudolph & Crandall, 2012	Chile (Los Ángeles)	Fragment semi-marshland, perennially green areas	Primary burrower Type 2 burrow	Deforestation and urban expansion.	CR B1ab (ii) (Rudolph & Crandall, 2012; Almerão et al., 2014).
<i>Virilastacus retamali</i> Rudolph & Crandall, 2007	Chile (Osorno and Llanquihue)	Peatland	Secondary burrower Type 2 burrow	Deforestation and extraction of <i>Sphagnum</i> .	EN B1ab (iii) (Rudolph & Crandall 2007).
<i>Virilastacus rucapihueleensis</i> Rudolph & Crandall, 2005	Chile (Osorno)	Semi-marshland, perennially green areas (Rudolph et al., 2015)	Primary burrower Type 2 burrow	Restricted geographical range.	CR B1ab (iii) (Rudolph, 2013; Almerão et al., 2014;).

*Virilastacus* is also composed of species endemic to Chile and has a narrow geographical range in southern Chile, occurring between the coastline and the Andes Mountains (Table 1) (Figure 3) (Rudolph, 2015). In contrast, *Samastacus spinifrons* has a wide distribution in Chile (from Valparaiso to the Taitao Peninsula). Moreover, this species has a known occurrence in Argentina, being limited to a single locality, Nahuel-Huapi National Park (Aued, 2003; Rudolph, 2010).

Regarding non-indigenous crayfish species (NICS), two taxa have been introduced in South America: the Cambaridae *Procambarus clarkii* (Girard, 1852) and the Parastacidae *Cherax quadricarinatus* (von Martens, 1868) (Figure 2). *Procambarus clarkii*, also known as red swamp crayfish or Louisiana crayfish, is native to southeastern United States and northern Mexico (Hobbs et al., 1989), while *C. quadricarinatus*, known by several common names (e.g., Australian Red Claw crayfish, Tropical Blue crayfish), is indigenous to northern and northeastern Australia and southeastern Papua New Guinea (Lawrence and Jones, 2002).

Both *P. clarkii* and *C. quadricarinatus* can occupy a variety of freshwater habitats, from small and temporary streams to large lakes, have broad tolerance to environmental variability, show remarkable ecological plasticity and are considered highly invasive, representing a great threat to native species caused by competition, predation and disease transmission (Lodge et al., 2012; see session 6). Other features shared by these invasive freshwater crayfish are their rapid growth rates and early sexual maturity (Huner, 2002; Lawrence and Jones, 2002). The ensemble of such characteristics makes both species very valuable for aquaculture and aquarium trade, which are the main vectors of introduction of these two species worldwide (Lodge et al., 2012). Their introduction in South America was initially motivated by aquaculture, being *P. clarkii* the first to be introduced around the 80's, followed by *C. quadricarinatus* in the 90's.

In South America, the exotic *P. clarkii* was introduced to Brazil, Colombia, Ecuador and Venezuela (Huner, 2002; Campos, 2005; Loureiro et al., 2015a), while *C. quadricarinatus* was translocated to Argentina, Ecuador, Paraguay and Uruguay (Romero and Jimenez, 2002; Volonterio, 2009; Vazquez and López-Greco, 2007) (Figure 3). Some evidence indicates

that the introduction of both *P. clarkii* and *C. quadricarinatus* in South America was initially motivated by aquaculture companies and only later by the pet trade.

In Ecuador, for example, the red swamp crayfish was introduced for aquaculture around 1986, using a “mixed culture” in rice fields, as the model used in Louisiana (United States) at the time (Mora et al., 2004). The project was not profitable and was abandoned a few years later, probably resulting in escape or intentional introduction of individuals in other areas, in a way that current populations can now be found throughout all the coastal region (Mora et al., 2004).

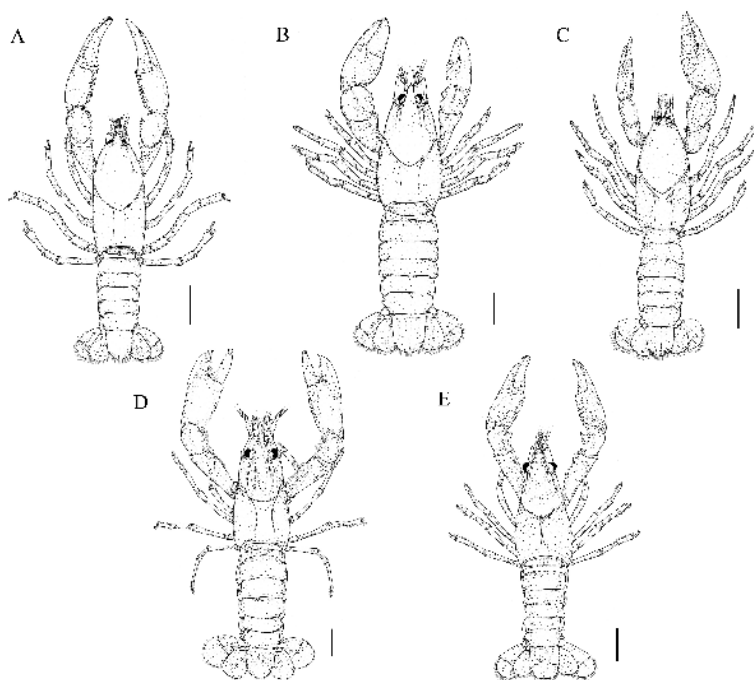


Figure 2. Native and non-indigenous crayfish genera in South America. A – *Parastacus* (illustration of *P. tuerkayi* modified from Ribeiro et al., 2017); B – *Samastacus* (illustration of *S. spinifrons* modified from Buckup and Rossi, 1991); C – *Virilastacus* (illustration of *V. rucapihuelensis* modified from Rudolph and Crandall, 2005); D – *Cherax* (illustration of *C. quadricarinatus*); E – *Procambarus* (illustration of *P. clarkii*). Scale bars = 10 mm.

The case of Ecuador is very similar to the process that happened in other places in which *P. clarkii* was introduced, like Colombia and Venezuela (Rodriguez and Suarez, 2001; Campos, 2005). In Brazil, however, the introduction history of *P. clarkii* is slightly different. The main reason for introduction was aquarium trade, since this species is not often used for culinary purposes in this country, and non-commercial use as fish bait contributed for secondary spread (Magalhães et al., 2005; Loureiro et al., 2015b).



Figure 3. Distribution map of native crayfish genera (*Parastacus*, *Samastacus* and *Virilastacus*) and the records of non-indigenous crayfish species (*Procambarus clarkii* and *Cherax quadricarinatus*) in South America.

Similarly to *P. clarkii*'s introduction process, the main motive for *C. quadricarinatus* introduction in South America was initially aquaculture and, as its farming was not as lucrative as expected, many farms were abandoned, and the exotic individuals there present started to settle and spread. The pet trade as a vector of introduction of this species is also a factor, although less important than for the red swamp crayfish. The first introduction of *C. quadricarinatus* in Ecuador, for example, was in 1992, when some businessmen came back from Australia with some specimens of this species to start harvesting for human feeding, and huge areas were constructed for this purpose; nowadays, the production of this species has decreased but is still active (Mora et al., 2004).

Information about history and vectors of the introduction of *C. quadricarinatus* in South America as well as on the invasion process are even more scarce than information for *P. clarkii*; information regarding occurrence in Argentina, Paraguay and Uruguay comes from studies focusing on biological or economical aspects and do not consider aspects of invasion ecology and potential impact (Vazquez and López-Greco, 2007; Venturiello, 2007).

Limited studies and references regarding non-indigenous crayfish species (NICS) in South America are alarming and it is possible that the range of introduction in this continent is underestimated since non-official transportation is known to occur, and most South American countries have no specific policies to avoid and control invasive freshwater crayfish introduction. Furthermore, as demonstrated by Palaoro et al., (2013), South America presents many vulnerable areas for *P. clarkii*'s establishment due to favorable climatic conditions. Last but not least, information regarding invasive dynamics and potential impacts are absent or superficial at best, and this subject should be of great importance for South American scientists that are concerned with the conservation of native species and freshwater environments since both species have major potential to generate ecological impacts and transmit diseases, as it will be mentioned in section 6.

### **3. HABITAT CHARACTERIZATION AND REQUIREMENTS OF NATIVE SOUTH AMERICAN CRAYFISH**

Freshwater crayfish can occupy several types of environments from watercourses (rivers, streams and lakes) to wetlands (swamps, marshlands and outside main drainages) (Figures 4 and 5) (Reynolds et al., 2013). The burrowing behavior of these species can be considered a singular feature that allows inhabiting a diversity of habitats and moving across heterogeneous landscapes (Johnston and Robson, 2009).

Ecological classifications based on environmental characteristics and species' biology have been proposed by some authors. Hobbs (1942) categorized North American species in primary, secondary and tertiary burrowers according to the burrowing habits. Thus, primary burrowers are considered to be species restricted to burrows and construct complex tunnels that rarely connect with open water; secondary burrowers also occupy burrows but during the rainy seasons they are found into open water; and tertiary burrowers only dig in drought periods or, occasionally, in breeding season. Nevertheless, this classification system cannot be used to assess all species, such as Australian species. Therefore, Horwitz and Richardson (1986) associated the location of burrows in different habitats and proposed three categories for Australian freshwater crayfish. Burrows of type 1 are connected to a permanent water course of surface (stream, creek and lake) and is subdivided in: type 1a - burrows in permanent water course and type 1b - burrows connected to permanent water; burrows of type 2 are connected to water table; and burrows of type 3 are those independent of the water table, where the water is from surface runoff. Both classification systems can be applied to freshwater crayfish in South America.

*Samastacus spinifrons* presents two morphotypes related to the habitats where it occurs (lakes and rivers) (Figure 5A, B) (Rudolph et al., 2016). This morphological variability was suggested as an adaptation of *S. spinifrons* to limnic environments with very different dynamics (Rudolph et al., 2016). This species is preferentially associated the watercourse, occupying regions of backwater, sheltering under rocks, between riparian vegetation or tree



roots (Rudolph, 2013). In these habitats the individual can excavate shallow burrows and unbranched tunnels for shelter (Rudolph and Almerão, 2016). Thus, *S. spinifrons* is classified as a tertiary burrower (Table 1) (sensu Hobbs, 1942) that construct type 1a burrows (Horwitz and Richardson, 1986) (Figure 5). *Parastacus brasiliensis* resembles *S. spinifrons*, inhabiting lotic waters, especially low-order streams, sheltering under stones, litter or burrows and digging at the riverbank with up to 50 cm depth (Buckup and Rossi, 1980; Fontoura and Buckup, 1989a; Miranda et al., 2018). This species, as well as *P. fluviatilis*, *P. buckupi*, *P. varicosus* and *P. saffordi* are classified as tertiary burrower and the built burrows are of type 1a, b (Figure 4A, B) (sensu Horwitz and Richardson, 1986). Potentially, *P. promatensis* share same classification as these species, however burrowing data are unknown. *Parastacus pilimanus* occupies wetlands, where the water table is close to the surface or in the outcrop of water in the field. It is classified as a species secondary burrower (Table 1) (sensu Hobbs 1942) and the burrows of type 2 (Figure 4) (sensu Horwitz and Richardson, 1986). The burrows are of type 2 and are composed to 3-7 openings, with tunnels that can reach up to 1m depth (Buckup and Rossi, 1980).

Brazilian endemic species that inhabit wetlands, as swamp forest, receive the categorization of primary burrower (Figure 4C) (sensu Hobbs, 1942) and type 2 burrows (Horwitz and Richardson, 1986). Burrows are composed of some openings, relatively complex tunnels up to 1m depth, as observed for *P. caeruleodactylus* and *P. tuerkayi* (Ribeiro et al., 2016; 2017). *Parastacus defossus* belongs to the same categorization due to similar ecological characteristics. This species occupies swamps and marshlands, where it digs galleries with a central tunnel and several ramifications, reaching up to 1.15 m depth (Noro and Buckup, 2010). In primary burrower species, it is relatively common to find adults and juveniles cohabiting the same burrow, sometimes these young individuals are from distinct brood (Noro and Buckup, 2010).

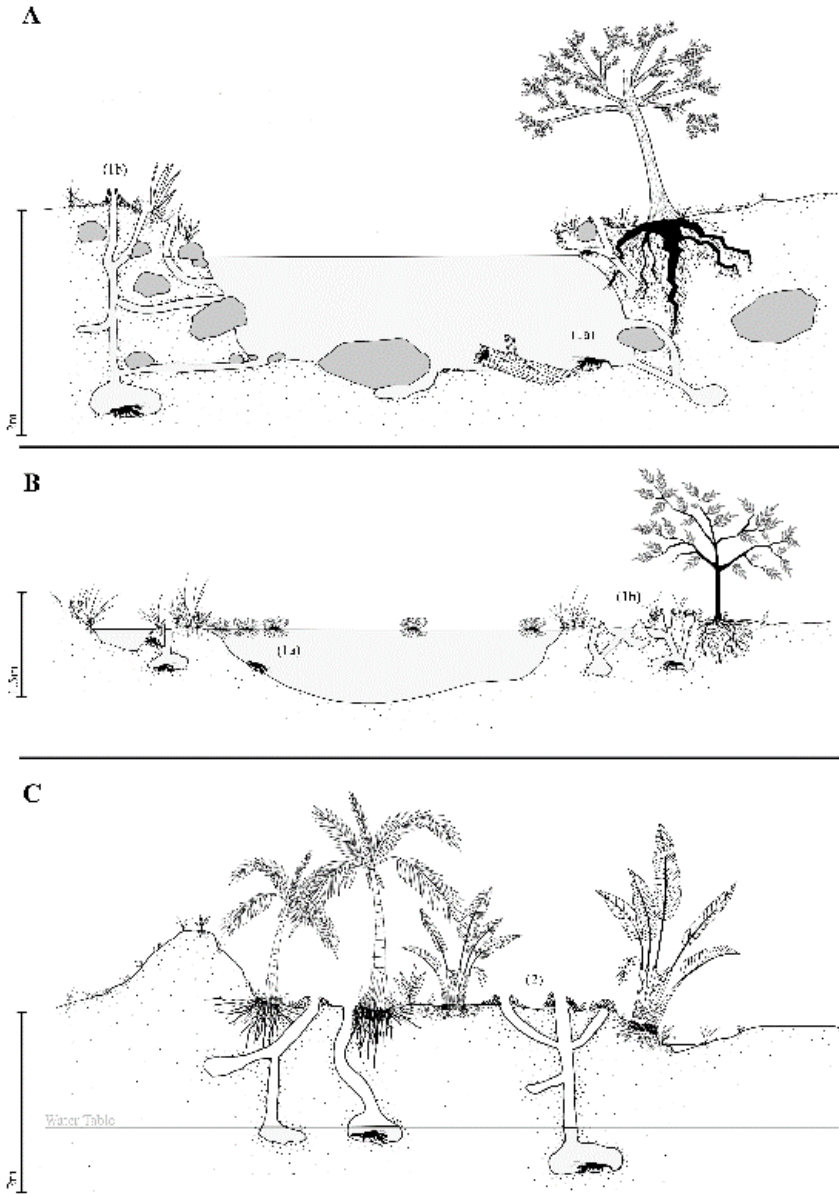


Figure 4. Habitat and burrow types of Brazilian crayfish species. A – streams; B – wetlands; C – swamp forests.

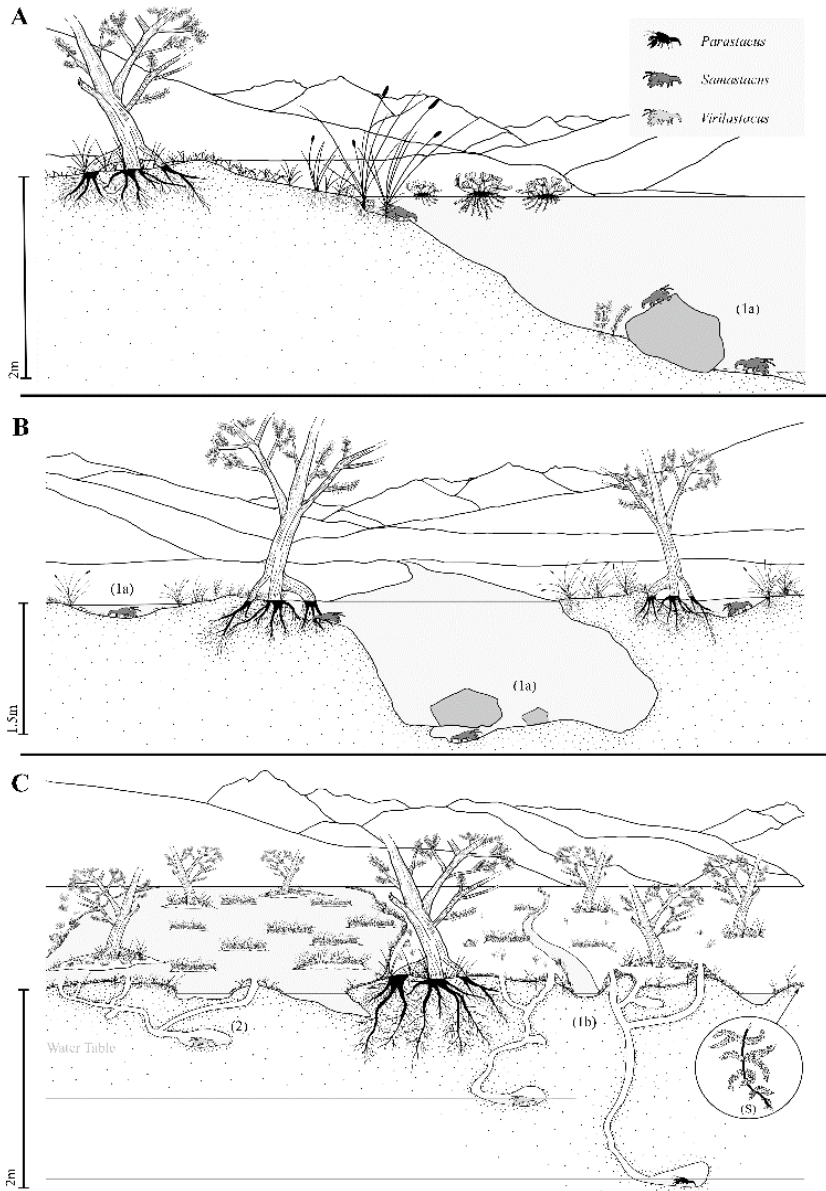


Figure 5. Habitat and burrow types of Chilean crayfish species. A – lakes; B – streams; C – “vegas” or “hualves.”

Chilean species of *Parastacus* and *Virilastacus* are recorded in semi-marshland habitats, composed of lowland evergreen forest and commonly referred to as “vegas” or “hualves” (Figure 5C) (Rudolph, 2010). This biotope is temporary or permanently flooded and soil rich in organic matter, with the presence of hydrophytic vegetation (mainly herbaceous) and *Sphagnum* moss (Rudolph and Crandall, 2012; Roig and Roig, 2004, Ramirez et al., 2014). The Chilean species of *Parastacus* are classified as primary burrowers, since the individuals remain in their galleries throughout the life cycle and the burrows are connected to the water table; and burrows are type 2 (Figure 5C). *Parastacus pugnax* excavate relatively complex burrows with moderate depth (up to 1m) according to the water table level (Rudolph, 2013). *Parastacus nicoleti* also occur in semi-marshland habitats but can occupy flooded prairies and hill peaks, up to 790 m altitude, and their complex burrows can reach up to 2 m (Fig. 5C) (Rudolph, 2013).

*Virilastacus* presents variable burrow morphology and distinct burrowing behavior among species. Nevertheless, similarities can be highlighted, such as the presence of branched tunnels and burrows not exceeding 1m depth (Rudolph and Crandall, 2012; Rudolph, 2013; 2015) (Figure 5C). *Virilastacus araucanius* construct shallow burrows and branching tunnels, many of them almost parallel to the ground surface (Rudolph, 2013). While *V. rucapihuelensis* may have burrows with multiple openings, complex tunnels that connect to the life chamber, or a single subvertical tunnel of 66 cm deep (Bedatou et al., 2010; Rudolph, 2013). Thus, this species is categorized as primary burrower and burrows of type 2, as well as *V. jarai* (Rudolph and Crandall, 2012; Rudolph, 2013, 2015). In contrast, *V. retamali* can be found in fragments of peatland in coastal Cordillera, which counts on the contribution of glaciers (Rudolph and Crandall, 2007). The burrows have few openings and shallow tunnels of up to 45cm depth, thus classified as secondary burrower (Fig. 5C) (Rudolph, 2015).

## 4. THE BIOLOGY OF NATIVE SOUTH AMERICAN CRAYFISH

### 4.1. Population Biology and Growth

Studies concerning population biology of the South American crayfish are scarce and they were focused mainly in the proportion of males and females in population (sex ratio), reproductive and recruitment period and growth/lifespan (Fontoura and Buckup, 1989a; b; Rudolph, 2002a; Noro et al., 2008; Ibarra and Arana, 2011; 2012; Yáñez-Alvarado et al., 2018).

The sex ratio of the studied native species of Parastacidae in South America were usually closer to the proportion 1 male:1 female, e.g., *P. defossus* (1:0.88) (Noro et al., 2008) and *P. brasiliensis* (1:1) (Fontoura and Buckup, 1989a). However, a larger proportion of males was recorded in populations of *S. spinifrons* from southern Chile (Rudolph, 2002b), which could be related to the high percentage of protandric hermaphrodites. In studies with crayfish the sexual ratio of 1:1 has been verified for several species but can be altered in the larger age groups due to the higher mortality of females, which may be due to the stress caused by egg laying and incubation (Momot, 1967, Prins, 1968, Abrahamsson, 1971; Price and Payne, 1984).

The reproductive period of South American Parastacidae seems to begin in late spring and keep until the mid-summer. This pattern of reproduction during spring–summer was found for *P. brasiliensis* (Fontoura and Buckup, 1989a), *P. caeruleodactylus* (Ribeiro et al., 2016; 2019), *P. defossus* (Noro and Buckup, 2008), *P. pugnax* (Rudolph, 2010), *P. varicosus* (Silva-Castiglioni et al., 2008) and *S. spinifrons* (Rudolph, 2002a). However, some species such as *Virilastacus araucanius* breed during the winter (Rudolph, 2003). The number of eggs in South American parastacids is usually small, ranging from 45 in *V. retamali* (Torres et al., 2008) to 195 in *S. spinifrons* (Bocic et al., 1988). One ovigerous female of *P. caeruleodactylus* bearing about 40 eggs was recorded by Ribeiro et al., (2016).

The growth of *P. nicoleti*, *P. pugnax* and males of *S. spinifrons* was considered isometric, while females of *S. spinifrons* showed negative allometry in past studies (Bocic et al., 1988; Ibarra and Arana, 2012; Yáñez-Alvarado et al., 2018). In relation to lifespan, the estimated longevity of South American parastacids range from 4.3 years for *P. nicoleti* (Yáñez-Alvarado et al., 2018) and *P. brasiliensis* (Fries, 1984) to 13.6 years for *P. pugnax* (Ibarra and Arana, 2012). This longevity is short in comparison with the other genera of Parastacidae, e.g., the Tasmanian giant freshwater crayfish *Astacopsis gouldi* (Clark, 1936) that can reach an average maximum age of 60 years (Lukhaup and Pekny, 2008).

## 4.2. Sexual Systems

Reproductive features of the South American crayfish are still poorly known and it is one of the most controversial features of their biology (Rudolph and Almeida, 2000). Sexual system of South American crayfish can be classified into three categories: (1) gonochorism, found in species of *Samastacus* and *Virilastacus*; (2) permanent intersexuality, found in *Parastacus*; and (3) partial protandric hermaphroditism, already recorded in *V. rucapihuelensis*, *P. nicoleti*, *P. brasiliensis*, *P. defossus* and *P. varicosus* (Rudolph and Almeida, 2000; Rudolph et al., 2007; Noro et al., 2008; Silva-Castiglioni et al., 2008; Rudolph, 2015).

Gonochorism or separation of sexes is found in *Virilastacus araucanius*, *V. jarai*, *V. retamali* and in the lake populations of *S. spinifrons* (Rudolph and Almeida, 2000; Rudolph, 2015). In all *Virilastacus* species, males have an elongated and calcified phallic papilla and the respective gonopore opens at the apical end. Females present the gonopores in the coxa of the third pair of pereopods (Hobbs, 1991; Martínez et al., 1994; Rudolph, 2015). Lake populations of *S. spinifrons* are gonochoric but 1.5 to 16% of the individuals from river populations can be intersexed (Rudolph et al., 2010).

The permanent intersexuality, i.e., the presence of male and female primary and/or secondary sexual features in the same individual is found in all species of the genus *Parastacus*. Intersexed individuals can also be found

in other parastacids, such the Australian genera *Cherax* Erichson, 1846, *Engaeus* Erichson, 1846 and *Engaewa* Riek, 1867 (Horwitz, 1988; Vasquéz and López-Greco, 2007) and in the South American genera *Samastacus* (Rudolph, 1999a, b; Rudolph and Almeida, 2000). In the genus *Parastacus*, intersexuality is characterized by the presence of supernumerary gonopores and genital ducts in the same individual (Rudolph and Almeida, 2000; Rudolph et al., 2001) and this is known since the second half of the nineteenth century (von Martens, 1869; Faxon, 1898), what was suggested to be related to a rudimentary hermaphroditism (Lönnerberg, 1898).

The partial protandric hermaphroditism in South American Parastacidae is characterized by the presence of both male and female gonads in the same individual in different stages of its life, being male the first sex (Rudolph, 1997; Rudolph and Almeida, 2000; Rudolph et al., 2001; Rudolph and Verdi, 2010). In *V. rucapuhuelensis*, Rudolph et al. (2007) recorded primary males and females and intersex specimens, besides of six distinct sexual forms according to the presence or absence of gonopores in the coxa of the pereopods 3 and 5. Hermaphroditism in *P. nicoleti* is characterized by two sexual types, primary females (in pre-puberty and puberty) and protandric hermaphrodites with male phase, transitional phase and two female phases (Rudolph, 1995). Protandric hermaphroditism was also registered in a population of *P. brasiliensis* in southern Brazil. Anatomical and histological analysis of the gonads disclosed the existence of three sexual forms, intersexed males, transitional between male and female sex, and intersexed females (Almeida and Buckup, 2000). The presence of transitional individuals suggested the existence of primary males that never change sex and the partial protandry and the existence of small females suggested that some females never go through a male phase (Almeida and Buckup, 2000). Finally, Silva-Castiglioni et al. (2008) and Noro et al. (2008) identified transitional specimens showing an ovotestis gonad in Brazilian populations of *P. varicosus* and *P. defossus*, respectively. This kind of hermaphroditism has possibly evolved in native South American crayfish as an adaptation to the burrowing habits and related to environmental conditions (Almerão et al., 2014).

### 4.3. Post-Embryonic Development and Parental Care

All freshwater crayfish are characterized by their direct development associated with posthatching brood care, with incubation of large eggs, rich in vitellus and at least three juvenile stages (Vogt, 2013). In Parastacidae, the first two juvenile stages remain attached to the female pleopods mainly by hooks in the dactylus of pereopods 4 and 5, which is an apomorphic character for Parastacoidea and correlated to the restricted mobility (Scholtz, 1975). Additional structures for fixation include the telson and anal threads (Vogt, 2013), but the first and second juvenile stages can also use the chelae of the pereopods 1 – 3 for grasping occasionally the setae of the mother's pleopods (Rudolph and Rojas, 2003). The third juvenile stage is mobile and is capable of exploratory behaviors and after return to the mother's body. This is characterized as the sheltering period (Dalosto et al., 2012; Vogt, 2013). Post-embryonic development was already described for *S. spinifrons* (as *Parastacus agassizi*) (Ringuelet, 1949; Rudolph and Iraçabal, 1994), *V. araucanius* (Faxon, 1914) (Rudolph and Rojas, 2003), *P. brasiliensis* (Noro et al., 2005), *P. caeruleodactylus* (Ribeiro et al., 2019), *P. defossus* (Barcelos, 2007), *P. nicoleti* (Rudolph and Zapata, 1986) and *P. pugnax* (Rudolph and Ríos, 1987).

Parental care in freshwater crayfish is characterized by the attachment of the eggs to the maternal pleopods with subsequent defense, ventilation and cleaning of these eggs by female, attachment of the juveniles to the maternal pleopods and the association mother-juveniles in the sheltering period of the juveniles (Vogt, 2013). Freshwater crayfish are capable to produce a glair blanket that separates the egg mass from the water, which is not seen in another freshwater decapod group (Vogt, 2013). The defense of eggs and juveniles by female is characterized mainly by the aggressive behavior and by forward bending of its pleon (Vogt, 2013). Mother-juvenile association are variable in duration. In South American parastacids, the extension of the brood protection ranges from 14 days in *P. pilimanus* (Dalosto et al., 2012) to 20 days in *V. araucanius* (Rudolph and Rojas, 2003). In *P. pilimanus*, juveniles of the third stage show exploratory behavior and return to their mother. However, in *V. araucanius*, juveniles



leave their mother immediately after moulting into the third stage without an exploratory phase (Rudolph and Rojas, 2003). The co-existence of mother and juveniles inside burrows suggest that some crayfish species can develop adaptations that allow the overlapping of generations, including high tolerance of the mother to avoid cannibalism (Dalosto et al., 2012). In strong burrowing species, mothers and juveniles can stay confined in the burrows for months showing prolonged association (Richardson, 2007).

## **5. THE BIOLOGY OF INVASION OF THE RED SWAMP CRAYFISH (*PROCAMBARUS CLARKII*) IN BRAZIL**

The red swamp crayfish was first introduced in Brazil around the 1980s, apparently motivated by aquarium and pet commerce (Huner, 1988). It was only in the 90's that a project for aquaculture was submitted to the national environmental organization, and while considering whether or not to approve it, agents came to know the potential threats and not only forbade the execution of the project but also, in 2008, the Brazilian government enacted specific legislation preventing the importation, transportation, and commercialization of *P. clarkii* in Brazil (Brasil, 2008).

Unfortunately, as demonstrated by Loureiro et al., (2015b), despite the prohibition, individuals are still being sold and even though the importation is now over or at least considerably reduced, illegal maintenance of specimens still occurs, supplying the black market. However, it is important to mention that the prevalence of the aquarium trade as the main vector of *P. clarkii*'s introduction in Brazil had some positive outcomes for the invasion process compared to the scenario of introduction and spread in other countries as China, France and Spain. The rate of introduction and propagule pressure is much lower in the case of introduction for pet trade than for aquaculture, hindering the severity of its invasion in Brazil.

In addition to the importance of aquarium trade, the secondary spread is enhanced by the usage of this freshwater crayfish as fishing bait, which is related to the prevalence of its occurrence in commercial fishing ponds and

dams. There are 17 established populations confirmed so far in Brazil, all located in the state of São Paulo, from which almost 30% are in established urban areas, where individuals are less likely to naturally disperse. Nonetheless, Anastácio et al., (2014) demonstrated that the translocation of juveniles by waterbirds is a viable way of spread. Additionally, five of the colonized locations are natural areas and the reservoirs are connected to natural streams and swamps that could act as natural dispersal corridors (Loureiro et al., 2015b).

Loureiro et al., (2019) studied for a year one of these Brazilian populations, located in an Atlantic Forest Conservation Area, and observed that the flexibility of the diet of the red swamp crayfish is fundamental for its establishment success, and also contributes to its high impact potential. The authors found that individuals had surprisingly full stomachs during the entire year and that despite feeding on both animal and plant material in similar proportions, the specific items that compose the diet vary throughout the year, with maximization of nutrient intake despite natural variations on prey availability.

It is suspected that the concentrated occurrence of this species in Brazil is just an artefact generated by the poor investigation in other areas. Its distribution seems to be highly underestimated since this species is still sold in more than 15 of the 26 Brazilian States (personal observation) and because the invasiveness risk assessment performed by Loureiro et al., (2015b) classified the red swamp crayfish as a species with high risk of establishment and impact generation in Brazil.

## **6. THREATENS AND CONSERVATION STRATEGIES FOR SOUTH AMERICAN CRAYFISH**

Freshwater crayfish species have been assessed for extinction risk, according to International Union for Conservation of Nature (IUCN) categories and criteria. An expressive number of species have been categorized as imperiled due to the urban development, pollution, damming,

climate change, harvesting, agriculture and invasive species (Richman et al., 2015). South American species have revealed that 42% of them are classified in threatened categories (i.e., Vulnerable-VU, Endangered-EN or Critically threatened-CR). Major threats, such as deforestation, wetland drainage, alien species, urbanization, water pollution and agrochemicals in agriculture, can have a direct effect on the habitat or population of freshwater crayfish (Almerão et al. 2014; Ribeiro et al., 2016; Ribeiro et al., 2017; Miranda et al. 2018; Huber et al., 2018). In addition, restricted species distribution may increase the risk of extinction and classification into threatened categories.

*Virilastacus jarai* and *V. rucapihuelensis* can be considered as an example, where the area of extent of occurrence - EOO does not exceed 100 km<sup>2</sup>, threats such as deforestation and urban expansion are present, and both species are categorized as CR (Table 1) (Rudolph 2010; Rudolph & Crandall 2012; Almerão et al., 2014). Distribution data are essential for estimating the EOO and applying IUCN assessment criterion B1, as well as habitat and threat knowledge. The frequent use of criterion B1 in extinction risk assessments is associated with the scarcity of other data, such as density and population structure (Richman et al., 2015).

The insufficiency of data to assessment have been verified for eight species in South America, being ones classified as Data Deficient - DD (Table 1). However, the placement in this category does not exclude the threats to them. Thus, *P. pugnax* is categorized as DD, but is under main threats as deforestation and harvesting for human consumption, besides a potential population decline (Rudolph, 2013; Almerão et al., 2014).

South American species categorized as VU, EN or CR have been evaluated using criterion B1, which considers distribution and population data, associated with habitat conditions, such as: a) population severely fragmented; b) continuing decline in: (ii) area of occupancy; and (iii) area, extent and/or quality of habitat. *Parastacus buckupi*, *P. caeruleodactylus*, *P. fluviatilis*, *P. tuerkayi* and *V. retamali* were evaluated as EN under B1ab (iii); *V. rucapihuelensis* with same criterion, but in CR category; *V. jarai* assessed from criterion B1ab(ii) and CR category. *Virilastacus araucanus* was classified as VU under B1ab(iii)+B2ab(iii). The criterion B2 is referred

the Area of Occupancy (AOO) of the species into EOO. Furthermore, EOO data of two species not fit the thresholds proposed by IUCN, thus they are classification as Near Threatened - NT, *P. brasiliensis* and *P. defossus*. The main threats observed for these species are the urbanization, dumping pesticides and organic residues and degradation of habitat (Almerão et al., 2014; Miranda et al., 2018).

Non-indigenous crayfish species (NICS) are known to cause multiple impacts, from the ecological to the economic and social point of view (Copp et al., 2016). Freshwater crayfish are known to be key species due to their great capability of habitat modification mainly due to their flexible position in trophic webs and due to their burrowing behavior that increases water turbidity and decreases light penetration (Angeler et al., 2001). In addition to these threats, both *P. clarkii* and *C. quadricarinatus* are known to generate other impacts on invaded ecosystems that are mainly related to predation of native species, higher competition skills for resources than most species sharing ecological niche and disease transmission (Twardochleb et al., 2013)

Freshwater crayfish are voracious and omnivorous, in a way that the consequences generated by their establishment in a community can affect different taxa and generate diverse impacts; the high rates of macrophyte consumption are related to the decrease on their abundance, diversity and changes in species composition (Matsuzaki et al., 2009); the predation of insects, mollusks, crustaceans, amphibians and fish affects the dynamics of this species and changes the food web at different levels, severely modifying trophic relationships among native species (Geiger et al., 2005).

Regarding disease transmission, the main menace is the contamination of native fauna by the fungus like *Aphanomyces astaci* Schikora, 1906 (Souty-Grosset et al., 2006). North American species are natural carriers of *A. astaci* but the Australian redclaw crayfish is a not immune host (Aquiloni et al., 2011; Hsieh et al., 2016). *Aphanomyces astaci* was responsible for the extirpation of many native European freshwater crayfish populations and although no impacts on native South American populations have been reported so far, Peiró et al. (2016) detected this pathogen's DNA in some Brazilian native crayfish species of the genus *Parastacus*, suggesting the possibility of contamination of native populations.

In order to avoid or decrease all the aforementioned impacts posed by NICS, it is of paramount importance to forbid or at least to control freshwater crayfish introduction or spread. In already colonized areas, population control, distribution area containment and mitigation of impact are also important measurements. Nonetheless, all these measures are pointless if the general public is ill-informed and scientific awareness regarding the threats offered by these species is absent. In South America specifically, this seems to be the case since little attention has been given to NICS by researchers, policy makers and managers.

Loureiro et al. (2018) suggested an easy and affordable standardized method for monitoring NICS populations that can be used to track the invasion process of NICS, at the same time it allows for comparison among locations or through time, indicating the prioritization of populations to manage. The management of NICS populations must be adjusted to each specific location and species, using the adaptive management that is based on the constant evaluation of the outcomes of each action, and relying on frequent adjustment of techniques that will increase the obtention of positive results.

## **ACKNOWLEDGMENTS**

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the fellowship provided to FBR (PDJ154614/2018-1) and the Zoology Department and the Post-Graduation Program in Animal Biology of the Universidade Federal do Rio Grande do Sul for the support provided for the research development.

## **REFERENCES**

Abrahamsson, S. A. A., (1971). Density, growth and reproduction of the crayfish *Astacus astacus* and *Pacifastacus leniusculus* in an isolated pond. *Oikos*, 22: 373-388.

- Almeida, A. O. and Buckup, L. (2000). Occurrence of protandric hermaphroditism in a population of the neotropical freshwater crayfish *Parastacus brasiliensis* (Parastacidae). *Journal of Crustacean Biology*, 20 (2): 224-230.
- Almerão, M. P., Rudolph, E., Souty-Grosset, C., Crandall, K., Buckup, L., Amouret, J., Verdi, A., Santos, S. and Araujo, P. B. (2014). The native South American crayfishes (Crustacea, Parastacidae): state of knowledge and conservation status. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 25 (2): 288-301.
- Anastácio, P. M., Ferreira, M. P., Banha, F., Capinha, C. and Rabaça, J. E. (2014). Waterbird mediated passive dispersal is a viable process for crayfish (*Procambarus Clarkii*). *Aquatic Ecology*, 48 (1): 1–10. doi: 10.1007/s10452-013-9461-0.
- Angeler, D. G., Sánchez-Carrillo, S., García, G. and Alvarez-Cobelas, M. (2001). The Influence of *Procambarus clarkii* (Cambaridae, Decapoda) on water quality and sediment characteristics in a Spanish floodplain wetland. *Hydrobiologia*, 464: 89–98. doi: 10.1023/A:1013950129616.
- Aquiloni, L., Martín, M. P., Gherardi, F. and Diéguez-Uribeondo, J. (2011). The North American Crayfish *Procambarus clarkii* is the carrier of the Oomycete *Aphanomyces astaci* in Italy. *Biological Invasions*, 13 (2): 359–67. doi: 10.1007/s10530-010-9828-2.
- Aued, M. B. (2003). Relevamiento de decápodos en el Parque Nacional Nahuel-Huapi: Distribución, abundancia e implicancias ecológicas. *Informe Técnico, Parque Nacional Nahuel-Huapi*. 18 pp. [Decapod survey in Nahuel-Huapi National Park: Distribution, abundance and ecological implications. *Technical Report, Nahuel-Huapi National Park*. 18 pp.]
- Barcelos, D. F. (2007). *Morfologia externa de Parastacus defossus Faxon, 1898* (Crustacea, Decapoda, Parastacidae). Dissertação de Mestrado. Programa de Pós-Graduação em Biologia Animal, Universidade Federal do Rio Grande do Sul, 99p. [*External morphology of Parastacus defossus Faxon, 1898* (Crustacea, Decapoda, Parastacidae). Master Dissertation. Post-graduation Program in Animal Biology, Federal University of Rio Grande do Sul].

- Bedatou, E., E. Rudolph, J. F. Genise, M. González & R. N. Melchor. (2010). Architecture of burrows of extant land crayfishes from south-central Chile. *Workshop on Crustacean Bioturbation - Fossil and Recent*. pp. 7-10.
- Bocic, V., Rudolph, E. and López, D. (1988). Biología reproductiva y dinámica poblacional del camarón de río *Samastacus spinifrons* (Philippi, 1882). *Boletín de la Sociedad de Biología de Concepción*, 59: 9-21. [Reproductive biology and population dynamics of the freshwater crayfish *Samastacus spinifrons* (Philippi, 1882). *Bulletin of the Society of Biology of Concepción*, 59: 9-21].
- Brasil. (2008). IBAMA – Instituto Brasileiro do Meio Ambiente. Portaria n° 5 de 28 de Janeiro de 2008.[IBAMA - Brazilian Institute of Environment. Ordinance n° 5 of January 28, 2008].
- Buckup, L. (2010a). *Parastacus defossus*. The IUCN Red List of Threatened Species 2010: e.T153669A4529563. <http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T153669A4529563.en>. Downloaded on 29 September 2019.
- Buckup, L. (2010b). *Parastacus laevigatus*. *The IUCN Red List of Threatened Species* 2010: e.T153700A4533698. <http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T153700A4533698.en>. Downloaded on 29 September 2019.
- Buckup, L. (2010c). *Parastacus nicoleti*. The IUCN Red List of Threatened Species 2010: e.T153697A4533324. <http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T153697A4533324.en>. Downloaded on 29 September 2019.
- Buckup, L. (2010d). *Parastacus pilimanus*. *The IUCN Red List of Threatened Species* 2010: e.T153691A4532526. <http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T153691A4532526.en>. Downloaded on 29 September 2019.
- Buckup, L. (2010e). *Parastacus pugnax*. The IUCN Red List of Threatened Species 2010: e.T153709A4535070. <http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T153709A4535070.en>. Downloaded on 29 September 2019.

- Buckup, L. (2010f). *Parastacus saffordi*. The IUCN Red List of Threatened Species 2010: e.T153637A4524122. <http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T153637A4524122.en>. Downloaded on 29 September 2019.
- Buckup, L. (2010g). *Parastacus varicosus*. The IUCN Red List of Threatened Species 2010: e.T153641A4525510. <http://dx.doi.org/10.2305/IUCN.UK.2010-3.RLTS.T153641A4525510.en>. Downloaded on 29 September 2019.
- Buckup, L. and Rossi, A. (1980). O Gênero *Parastacus* no Brasil (Crustacea, Decapoda, Parastacidae). *Revista Brasileira de Biologia*, 40: 663-681. [The genus *Parastacus* in Brazil (Crustacea, Decapoda, Parastacidae). *Brazilian Journal of Biology*, 40: 663-681].
- Buckup, L. and Rossi, A. (1993). Os Parastacidae do espaço meridional andino (Crustacea, Astacidea). *Revista Brasileira de Biologia*, 53 (2): 167-176. [The Parastacidae of the Andean Southern Space (Crustacea, Astacidea). *Brazilian Journal of Biology*, 53 (2): 167-176].
- Campos, M. R. (2005). *Procambarus (Scapulicambarus) clarkii* (Crustacea: Decapoda: Cambaridae): Una langostilla no nativa en Colombia. *Revista de la Academia Colombiana de Ciencias*, 29 (111):295-302.
- Collins, P. A., Giri, F. and Williner, V. (2011). Biogeography of the freshwater decapods in the La Plata basin, South America. *Journal of Crustacean Biology*, 31 (1): 179-191.
- Copp, G. H., Ian, Russell, C., Peeler, J., Gherardi, F., Tricarico, E., Macleod, A., Cowx, I. G., Nunn, A. D., Occhipinti-Ambrogi, A., Savini, D., Mumford, J. and Britton, R. J. (2016). European non-native species in aquaculture risk analysis scheme – a summary of assessment protocols and decision support tools for use of alien species in aquaculture. *Fisheries Management and Ecology*, 23 (1): 1-11. doi: 10.1111/fme.12074.
- Crandall, K. A. and Buhay, J. E. (2008). Global diversity of crayfish (Astacidae, Cambaridae, and Parastacidae-Decapoda) in freshwater. *Hydrobiologia*, 595: 295-301.



- Crandall, K. A. and De Grave, S. (2017). An updated classification on the freshwater crayfishes (Decapoda: Astacidae) of the world, with a complete species list. *Journal of Crustacean Biology*, 37: 615-653.
- Crandall, K. A., Harris, D. J. and Fetzner Jr, J. W. (2000a). The monophyletic origin of freshwater crayfish estimated from nuclear and mitochondrial DNA sequences. *Proceedings of the Royal Society of London: Biological Sciences*, 267 (1453): 1679-1686.
- Crandall, K. A., Fetzner Jr, J. W., Jara, C. G. and Buckup, L. (2000b). On the phylogenetic positioning of the South American freshwater crayfish genera (Decapoda: Parastacidae). *Journal of Crustacean Biology*, 20 (3): 530-540.
- Dalosto, M. M., Palaoro, A. V. and Santos, S. (2012). Mother-offspring relationship in the neotropical burrowing crayfish *Parastacus pilimanus* (von Martens, 1869) (Decapoda, Parastacidae). *Crustaceana*, 85 (11): 1305-1315.
- Dalosto, M. M., Palaoro, A. V., Souty-Grosset, C., Bueno, S. L. S., Loureiro, T. G., Almerão, M. P., Araujo, P. B. and Santos, S. (2015). One step ahead of the enemy: investigating aggressive interactions between invasive and native crayfish before the contact in nature. *Biological Invasion*, 17: 1-13.
- Faxon, W. (1898). Observations on the Astacidae in the United States National Museum and in the Museum of Comparative Zoology, with descriptions of new species. *Proceedings of the United States National Museum*, 20: 642-694.
- Fontoura, N. F. and Buckup, L. (1989). Dinâmica populacional e reprodução em *Parastacus brasiliensis* (von Martens, 1869) (Crustacea, Decapoda, Parastacidae). *Revista Brasileira de Biologia*, 49: 911-921. [Population dynamics and reproduction in *Parastacus brasiliensis* (von Martens, 1869) (Crustacea, Decapoda, Parastacidae). *Brazilian Journal of Biology*, 49: 911-921].
- Fontoura, N. F. and Buckup, L. (1989). O crescimento do *Parastacus brasiliensis* (von Martens, 1869) (Crustacea, Decapoda, Parastacidae). *Revista Brasileira de Biologia*, 49: 897-909. [The growth of *Parastacus*

- brasiliensis* (von Martens, 1869) (Crustacea, Decapoda, Parastacidae). *Brazilian Journal of Biology*, 49: 897-909].
- Fries, B. G. (1984). Observações sobre o lagostim de água doce *Parastacus brasiliensis* (von Martens, 1869) em condições de cultivo experimental em laboratório (Crustacea, Decapoda, Parastacidae). *Revista Brasileira de Biologia*, 44: 409–416. [Observations on the freshwater crayfish *Parastacus brasiliensis* (von Martens, 1869) under experimental laboratory cultivation conditions (Crustacea, Decapoda, Parastacidae). *Brazilian Journal of Biology*, 44: 409–416].
- Geiger, W., Alcorlo, P., Baltanás, A. and Montes, C. (2005). Impact of an introduced crustacean on the trophic webs of Mediterranean Wetlands. In *Issues in Bioinvasion Science*, 7: 49–73.
- Gherardi, F. and Paglianti, A. (2004). Combined effects of temperature and diet on growth and survival of young-of-year crayfish: a comparison between indigenous and invasive species. *Journal of Crustacean Biology*, 24 (1): 140-48.
- Gherardi, F. (2006). Crayfish invading Europe: the case study of *Procambarus clarkii*. *Marine and Freshwater Behaviour and Physiology*, 39 (3): 175-191.
- Hsieh, C., Huang, C. and Pan, Y. (2016). Crayfish plague *Aphanomyces astaci* detected in redclaw crayfish, *Cherax quadricarinatus* in Taiwan. *Journal of Invertebrate Pathology*, 136: 117-123.
- Hobbs, H. H., Jr. 1942. The crayfishes of Florida. University of Florida Publication, *Biological Science Series*, 3 (2): 1-179.
- Hobbs, H. H., Jass, J. O. and Huner, J. V. (1989). A review of global crayfish introductions with particular emphasis on two North American species (Decapoda, Cambaridae). *Crustaceana*, 56 (3): 299-316.
- Hobbs, H. H. (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 (4): 800-811.
- Horwitz, P. (1988). Secondary sexual characteristics of females of the freshwater crayfish genus *Engaeus* (Decapoda, Parastacidae). *Crustaceana*, 54 (1): 25-32.

- Horwitz, P. H. and Richardson, A. M. M. (1986). An ecological classification of the burrows of Australian freshwater crayfish. *Australian Journal of Marine and Freshwater Research*, 37: 237-242.
- Huber, A. F., Ribeiro, F. B. and Araujo, P. B. (2018). New endemic species of freshwater crayfish *Parastacus* Huxley, 1879 (Crustacea: Decapoda: Parastacidae) from the Atlantic forest in southern Brazil. *Nauplius*, 26: e2018015.
- Huner, J. V. (1988). *Procambarus* in North America and elsewhere. In *Freshwater Crayfish Biology Management and Exploitation*, edited by David M. Holdich and Roger S. Lowery, 239-261. London: Chapman & Hall.
- Huner, J. V. (2002). *Procambarus*. In *Biology of Freshwater Crayfish*, edited by David M. Holdich, 541-584. Oxford: Blackwell Science.
- Ibarra, M. and Arana, P. (2011). Crecimiento del camarón excavador *Parastacus pugnax* (Poepfig, 1835) determinado mediante técnica de marcaje. *Latin American journal of aquatic research*, 39(2), 378-384. [Growth of the burrowing crayfish *Parastacus pugnax* (Poepfig, 1835) determined by means of marking technique. *Latin American Journal of Aquatic Research*, 39 (2), 378-384]. doi: 10.4067/S0718-560X2011000200018
- Ibarra, M. and Arana, P. (2012). Biological parameters of the burrowing crayfish, *Parastacus pugnax* (Poepfig, 1835), in Tiuquilemu, Biobío Region, Chile. *Latin American Journal of Aquatic Research*, 40: 418-427.
- Johnston, K. and Robson, B. J. (2009). Habitat use by five sympatric Australian freshwater crayfish species (Parastacidae). *Freshwater Biology*, 54: 1629-1641. doi: 10.1111/j.1365-2427.2009.02213.x.
- Lawrence, C. and Jones, C. (2002). *Cherax*. In *Biology of Freshwater Crayfish*, edited by David M. Holdich, 645-666. Oxford: Blackwell Science.
- Lodge, D. M., Deines, A., Gherardi, F., Yeo, D. C. J., Arcella, T., Baldrige, A. K., Barnes, M. A., Chadderton, L. W., Feder, J. L., Gantz, C. A., Howard, G. W., Jerde, C. L., Peters, B. W., Peters, J. A., Sargent, L. W., Turner, C. R., Wittmann, M. E. and Zeng, Y. (2012). Global

- introductions of crayfishes: evaluating the impact of species invasions on ecosystem services. *Annual Review of Ecology, Evolution, and Systematics*, 43 (1): 49–72. doi: 10.1146/annurev-ecolsys-111511-103919.
- Loureiro, T. G., Anastácio, P. M., Araujo, P. B., Souty-Grosset, C. and Almerão, M. P. (2015a). Red swamp crayfish: biology, ecology and invasion - an overview. *Nauplius*, 23: 1-19. doi: 10.1590/S0104-64972014002214.
- Loureiro, T. G., Bueno, S. L. S., Anastácio, P. M., Almerão, M. P., Souty-Grosset, C. and Araujo, P. B. (2015b). Distribution, introduction pathway, and invasion risk analysis of the North American crayfish *Procambarus clarkii* (Decapoda: Cambaridae) in southeast Brazil. *Journal of Crustacean Biology*, 35: 88-96. doi: 10.1590/S0104-64972014002214.
- Loureiro, T. G., Anastácio, P. M., Bueno, S. L. S. and Araujo, P. B. (2018). Management of invasive populations of the freshwater crayfish *Procambarus clarkii* (Decapoda, Cambaridae): test of a population-control method and proposal of a standard monitoring approach. *Environmental Monitoring and Assessment*, 190 (9): 559. doi: 10.1007/s10661-018-6942-6.
- Loureiro, T. G., Anastácio, P. M., Bueno, S. L. S., Wood, C. T. and Araujo, P. B. (2019). Food Matters: Trophodynamics and the role of diet in *Procambarus clarkii* invasion success in an Atlantic Forest conservation area. *Limnologia*, 79: 125717. doi: doi.org/10.1016/ j.limno.2019.125717.
- Lönnerberg, F. (1898). Some biological and anatomical facts concerning *Parastacus*. *Zoologischer Anzeiger*, 21: 334-352.
- Lukhaup, C. and Pekny, R. (2008). *Süßwasserkrebse aus aller Welt*, 2. Auflage. Dähne Verlag, Ettlingen, 291p. [*Freshwater crayfish from all over the world*, 2nd edition. Dähne publishing house, Ettlingen, 291p].
- Magalhães, C., Bueno, S. L. S., Bond-Buckup, G., Valenti, W. C., da Silva, H. L. M., Kiyohara, F., Mossolin, E. C. and Rocha, S. S. (2005). Exotic species of freshwater decapod crustaceans in the State of São Paulo,

- Brazil: records and possible causes of their introduction. *Biodiversity and Conservation*, 14 (8): 1929-45. doi: 10.1007/s10531-004-2123-8.
- Martínez, R. I., Llanos, F. E. and Quezada, A. E. (1994). *Samastacus araucanius* (Faxon, 1914): aspectos morfológicos de un nuevo registro para Chile (Crustacea, Decapoda, Parastacidae). *Gayana Zoológica*, 58 (1): 9-15. [*Samastacus araucanius* (Faxon, 1914): morphological aspects of a new record for Chile (Crustacea, Decapoda, Parastacidae). *Gayana Zoology*, 58 (1): 9-15].
- Matsuzaki, S. S., Usio, N., Takamura, N. and Washitani, I. (2009). Contrasting impacts of invasive engineers on freshwater ecosystems: an experiment and meta-analysis. *Oecologia*, 158 (4): 673–86. doi: 10.1007/s00442-008-1180-1.
- Miranda, I., Gomes, K. M., Ribeiro, F. B., Araujo, P. B., Souty-Grosset, C. and Schubart, C. D. (2018). Molecular systematics reveals multiple lineages and cryptic speciation in the freshwater crayfish *Parastacus brasiliensis* (von Martens, 1869) (Crustacea: Decapoda: Parastacidae). *Invertebrate Systematics*, 32: 1265-1281.
- Momot, W. T. (1967). Population dynamics and productivity of the crayfish *Orconectes virilis*, in a marl lake. *American Midland Naturalist*, 78 (1): 55-81.
- Mora, V., Uyaguari, M. and Osorio, V. (2004). Situación actual de las especies introducidas en el Ecuador con fines acuícolas. In *Artículos de tesis de grado* edited by FIMCBOR. Guayaquil: Escuela Superior politécnica del Litoral. [Current situation of the species introduced in Ecuador for aquaculture purposes. In *Thesis articles edited by FIMCBOR*. Guayaquil: Polytechnic Superior School of the Coast].
- Noro, C. K. and Buckup, L. (2008). Estrutura populacional e biologia reprodutiva de *Parastacus defossus* (Crustacea: Decapoda: Parastacidae). *Revista Brasileira de Zoologia*, 25: 624-629. [Population structure and reproductive biology of *Parastacus defossus* (Crustacea: Decapoda: Parastacidae). *Brazilian Journal of Zoology*, 25: 624-629].
- Noro, C. K., Buckup, L. and Bond-Buckup, G. (2005). The juvenile stages of *Parastacus brasiliensis* (von martens, 1869) (Crustacea, Decapoda, Parastacidae). *Journal of Natural History*, 39 (21): 1851-1873.

- Noro, C., López-Greco, L. S. and Buckup, L. (2008). Gonad morphology and type of sexuality in *Parastacus defossus* Faxon, 1898, a burrowing, intersexed crayfish from southern Brazil (Decapoda: Parastacidae). *Acta Zoologica*, 89: 59-67.
- Noro, C. K. and Buckup, L. (2010). The burrows of *Parastacus defossus* (Decapoda: Parastacidae), a fossorial freshwater crayfish from southern Brazil. *Zoologia*, 27 (3): 341-346. doi: 10.1590/S1984-46702010000300004.
- Palaoro, A., Dalosto, M., Costa, G. and Santos, S. (2013). Niche conservatism and the potential for the crayfish *Procambarus clarkii* to invade South America. *Freshwater Biology*, 58 (7): 1379-1391. doi: 10.1111/fwb.12134.
- Peiró, D. F., Almerão, M. P., Delaunay, C., Jussila, J., Makkonen, J., Bouchon, D., Araujo, P. B. and Souty-Grosset, C. (2016). First detection of the Crayfish Plague pathogen *Aphanomyces astaci* in South America: a high potential risk to native crayfish. *Hydrobiologia*, 781 (1): 181-190. doi: 10.1007/s10750-016-2841-4.
- Price, J. O. and Payne, J. F. (1984). Size, age and population dynamics in an r-selected population of *Orconectes neglectus chaenodactylus* Williams (Decapoda, Cambaridae). *Crustaceana*, 46 (1): 29-38.
- Prins, R. (1968). Comparative ecology of the crayfish *Orconectes rusticus* and *Cambarus tenebrosus* in Doe Run, Meade County, Kentucky. *Internationale Revue der gesamten Hydrobiologie*, 53 (5): 667-714.
- Ramírez, C., San Martín, C., Vidal, O., Pérez, Y., Valenzuela, J., Solís, J. L. and Toledo, G. (2014). Tundra subantártica en la Isla Grande de Chiloé, Chile: flora y vegetación turbos de campana. *Anales Instituto Patagonia*, 42 (2): 17-37. [Sub-Antarctic tundra on the Isla Grande de Chiloé, Chile: turbo bell flora and vegetation. *Annals Instituto Patagonia*, 42 (2): 17-37].
- Reynolds, J., Souty-Grosset, C. and Richardson, A. (2013). Ecological roles of crayfish in freshwater and terrestrial habitats. *Freshwater Crayfish*, 19 (2): 197-218. doi: 10.5869/fc.2013.v19-2.197.

- Ribeiro, F. B. and Araujo, P. B. (2017). Designation of a neotype for *Parastacus nicoleti* Philippi, 1882 (Crustacea: Decapoda: Parastacidae). *Zootaxa*, 4338 (2): 393-400.
- Ribeiro, F. B., Buckup, L., Gomes, K. M. and Araujo, P. B. (2016). Two new species of South American freshwater crayfish genus *Parastacus* Huxley, 1879 (Crustacea: Decapoda: Parastacidae). *Zootaxa*, 4158 (3): 301-324.
- Ribeiro, F. B., Gomes, K. M., Huber, A. F. and Araujo, P. B. (2019). Description of the second juvenile stage of the blue-fingered burrowing crayfish *Parastacus caeruleodactylus* (Decapoda: Astacidea: Parastacidae). *Zootaxa*, 4686 (4): 581–592. doi: 10.11646/zootaxa.4686.4.7.
- Ribeiro, F. B., Huber, A. F., Schubart, D. and Araujo, P. B. (2017). A new species of *Parastacus* Huxleys, 1879 (Crustacea, Decapoda, Parastacidae) from a swamp forest in southern Brazil. *Nauplius*, 25 (e2017008): 1-13.
- Richardson, A. M. M. (2007). Behavioral Ecology of Semiterrestrial Crayfish. p. 319-338. In: Duff, J. E. and Thiel, M. *Evolutionary Ecology of Social and Sexual Systems: Crustaceans as Model Organisms*. Oxford University Press, 502p.
- Richman, N. I., Böhm, M., Adams, S. B., Alvarez, F., Bergey, E. A., Bunn, J. J. S., Burnham, Q., Cordeiro, J., Coughran, J., Dawkins, K. L., DiStefano, R. J., Doran, N. E., Edsman, L., Eversole, A. G., Füreder, L., Furse, J. M., Gherardi, F., Hamr, P., Holdich, D. M., Horwitz, P., Johnston, K., Jones, C. M., Jones, R. L., Jones, T. G., Kawai, T., Lawler, S., López-Mejía, M., Miller, R. M., Pedraza-Lara, C., Reynolds, J. D., Richardson, A. M. M., Schultz, M. B., Schuster, G. A., Sibley, P. J., Souty-Grosset, C., Taylor, C. A., Thoma, R. F., Walls, J., Walsh, T. S. and Collen, B. (2015). Multiple drivers of decline in the global status of freshwater crayfish (Decapoda: Astacidea). *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370: 1–11. doi: 10.1098/rstb.2014.0060.
- Ringuelet, R. (1949). La morfología y el mecanismo de sujeción de las crías de *Parastacus agassizi* Faxon. *Notas Museo de La Plata*, (14): 55-59.

- [The morphology and clamping mechanism of *Parastacus agassizi* Faxon. *Notes from La Plata Museum*, (14): 55-59].
- Rodríguez, G. and Suárez, H. (2001). Anthropogenic dispersal of decapod crustaceans in aquatic environments. *Interciencia*, 26 (7): 282-288.
- Roig, C. and Roig; F. (2004). Consideraciones generales. In: Blanco, D. E.; de la Balze, M. *Los Turbales de la Patagonia: Bases para su inventario y la conservación de su biodiversidad*. Wetlands International. Buenos Aires, Argentina, 154 pp. [General considerations. In: Blanco, D. E.; de la Balze, M. *The Turbales of Patagonia: Bases for its inventory and the conservation of its biodiversity*. Wetlands International Buenos Aires, Argentina, 154 pp.].
- Romero, X. and Jiménez, R. (2002). Histopathological survey of diseases and pathogens present in Redclaw Crayfish, *Cherax quadricarinatus* (Von Martens), cultured in Ecuador. *Journal of Fish Diseases*, 25 (11): 653–67. doi: 10.1046/j.1365-2761.2002.00411.
- Rudolph, E. H. (1995). Partial protandric hermaphroditism in the burrowing crayfish *Parastacus nicoleti* (Philippi, 1882) (Decapoda: Parastacidae). *Journal of Crustacean Biology*, 15: 720-732.
- Rudolph, E. H. (1997a). Intersexualidad en el camarón excavador *Parastacus pugnax* (Poeppig, 1835) (Decapoda, Parastacidae). *Investigaciones Marinas, Valparaíso*, 25: 7-18. [Intersexuality in the burrowing crayfish *Parastacus pugnax* (Poeppig, 1835) (Decapoda, Parastacidae). *Marine Investigations, Valparaíso*, 25: 7-18].
- Rudolph, E. H. (1997b). Aspectos fisicoquímicos del hábitat y morfología de las galerías del camarón excavador *Parastacus nicoleti* (Philippi 1882) (Decapoda, Parastacidae) en el Sur de Chile. *Gayana*, 61: 97–108. [Physicochemical aspects of the habitat and morphology of the galleries of the burrowing crayfish *Parastacus nicoleti* (Philippi 1882) (Decapoda, Parastacidae) in southern Chile. *Gayana*, 61: 97-108].
- Rudolph, E. H. (1999). Intersexuality in the freshwater crayfish *Samastacus spinifrons* (Philippi, 1882) (Decapoda, Parastacidae). *Crustaceana*, 72 (3): 325-337.
- Rudolph, E. H. (2002a). Sobre la biología del camarón de río *Samastacus spinifrons* (Philippi, 1882) (Decapoda, Parastacidae). *Gayana*, 66 (2):



- 147-159. [On the biology of the river crayfish *Samastacus spinifrons* (Philippi, 1882) (Decapoda, Parastacidae). *Gayana*, 66 (2): 147-159].
- Rudolph, E. H. (2002b). New records of intersexuality in the freshwater crayfish *Samastacus spinifrons* (Philippi, 1882) (Decapoda, Parastacidae). *Journal of Crustacean Biology*, 22 (2): 377-389.
- Rudolph, E. H. (2010). Sobre la distribución geográfica de las especies chilenas de Parastacidae (Crustacea:Decapoda:Astacidea). *Boletín de Biodiversidad de Chile*, 3: 32-46. [On the geographical distribution of the Chilean species of Parastacidae (Crustacea: Decapoda: Astacidea). *Biodiversity Bulletin of Chile*, 3: 32-46].
- Rudolph, E. H. (2013). A checklist of the Chilean Parastacidae (Decapoda, Astacidea). *Crustaceana*, 86 (12): 1468-1510.
- Rudolph, E. H. (2015). Current state of knowledge on *Virilastacus* species (Crustacea, Decapoda, Parastacidae). *Latin American Journal of Aquatic Research*, 43 (5): 807-818.
- Rudolph, E. H. and Zapata, L. (1986). Desarrollo embrionario y post-larval del camarón de las vegas *Parastacus nicoleti* (Philippi, 1882) en condiciones de laboratorio. *Biota*, 2: 37-50. [Embryonic and post-larval development of the Vegas' crayfish *Parastacus nicoleti* (Philippi, 1882) under laboratory conditions. *Biota*, 2: 37-50].
- Rudolph, E. and Almeida, A. (2000). On the sexuality of South American Parastacidae (Crustacea: Decapoda). *Invertebrate Reproduction and Development*, 37 (3): 249-257.
- Rudolph, E. H., Colihueque, N. and Yañez, M. (2016). Morphological and genetic analysis in morphologically divergent river and lake specimens of the freshwater crayfish *Samastacus spinifrons* (Philippi, 1882) (Decapoda, Parastacidae). *Crustaceana*, 89 (8): 877-899.
- Rudolph, E. H. and Iraçabal, J. (1994). Desarrollo embrionario y postembrionario del camarón de río *Samastacus spinifrons* (Philippi, 1882) (Decapoda, Parastacidae). *Boletín de la Sociedad de Biología de Concepción*, 65: 43-49. [Embryonic and post-embryonic development of the river crayfish *Samastacus spinifrons* (Philippi, 1882) (Decapoda, Parastacidae). *Bulletin of the Society of Biology of Concepción*, 65: 43-49].

- Rudolph, E. H. and Ríos, O. J. (1987). Desarrollo ontogenético del camarón de las vegas *Parastacus pugnax* (Poepigg, 1835), em condiciones de laboratorio. *Biota*, Chile 3: 45-48. [Ontogenetic development of the Vegas' crayfish *Parastacus pugnax* (Poepigg, 1835), in laboratory conditions. *Biota*, Chile 3: 45-48].
- Rudolph, E. & K. A. Crandall. (2007). A new species of burrowing crayfish *Virilastacus retamali* (Decapoda: Parastacidae) from the southern Chilean peatland. *Journal of Crustacean Biology*, 27 (3): 502-512.
- Rudolph, E. H. and Crandall, K. (2012). A New Species of Burrowing Crayfish *Virilastacus retamali* (Decapoda: Parastacidae) from the Southern Chile Peatland. *Journal of Crustacean Biology*, 27 (3): 502-512. doi: 10.1651/S-2681.1.
- Rudolph, E., Retamal, F. A. and Martínez, A. W. (2007). Partial protandric hermaphroditism in the burrowing crayfish *Virilastacus rucapihuelensis* Rudolph and Crandall, 2005 (Decapoda, Parastacidae). *Journal of Crustacean Biology*, 27 (2): 229-241.
- Rudolph, E., Retamal, F. A. and Martínez, A. (2010). Cultivo de camarón de río *Samastacus spinifrons*: ¿una nueva alternativa para la diversificación de la acuicultura chilena? *Latin American Journal of Aquatic Research*, 38 (2): 254-264.
- Rudolph, E. H. and Rojas, C. S. (2003). Embryonic and early postembryonic development of the burrowing crayfish, *Virilastacus araucanius* (Faxon, 1914) (Decapoda, Parastacidae) under laboratory conditions. *Crustaceana*, 76: 835-850.
- Rudolph E., Verdi, A. and Tapia, J. (2001). Intersexuality in the burrowing crayfish *Parastacus varicosus* Faxon, 1898 (Decapoda, Parastacidae). *Crustaceana*, 74: 27-37.
- Rudolph, E. and Verdi, A. (2010). Intersexuality in the burrowing crayfish, *Parastacus pilimanus* (Von Martens, 1869) (Decapoda, Parastacidae). *Crustaceana*, 83 (1): 73-87.
- Rudolph, E. H. and Crandall, K. (2012). A new species of burrowing crayfish, *Virilastacus jarai* (Crustacea, Decapoda, Parastacidae) from central-southern Chile. *Proceedings of the Biological Society of Washington*, 125 (3): 258-275. doi: 10.2988/11-39.1.

- Rudolph E. and Almerão, M. P. (2016). The native South American crayfish (Decapoda: Parastacidae). In: Tadashi, K.; Faulkes, Z.; Scholtz, G. *Freshwater crayfish: a global overview*, CRC Press, 679 pp.
- Scholtz, G. (1995). The attachment of the young in the New Zealand freshwater crayfish *Paranephrops zealandicus* (White, 1847) (Decapoda, Astacida, Parastacidae). *New Zealand Natural Sciences*, 22: 81-89.
- Silva-Castiglioni, D.; López-Greco, L.; Oliveira, G. T.; Bond-Buckup, G. 2008. Characterization of the sexual pattern of *Parastacus varicosus* (Crustacea: Decapoda: Parastacidae). *Invertebrate Biology* 127(4): 426-432.
- Souty-Grosset, C., Holdich, D. M., Noel, P. Y., Reynolds, J. D. and Haffner, P. (2006). *Atlas of Crayfish in Europe*. Paris: Museum National d'Histoire Naturelle.
- Stern, D. and Crandall, K. A. (2016). Phylogenetic estimate of the freshwater crayfish (Decapoda: Astacidea) using morphology and molecules. In: Tadashi, K.; Faulkes, Z.; Scholtz, G. *Freshwater crayfish: a global overview*. CRC Press, 679 pp.
- Toon, A., Pérez-Losada, M., Schweitzer, C. E., Feldmann, R. M., Carlson, M. and Crandall, K. (2010). Gondwanan radiation of the Southern Hemisphere crayfishes (Decapoda: Parastacidae): evidence from fossils and molecules. *Journal of Biogeography*, 37: 2275-2290. doi: 10.1111/j.1365-2699.2010.02374.x.
- Torres, P. A., Valenzuela, A. P., Retamal, F. A., Martínez, A. W. and Rudolph, E. H. (2008). Primeros datos respecto a la biología reproductiva del camarón excavador *Virilastacus retamali* Rudolph and Crandall, 2007 (Decapoda, Parastacidae). Libro Resúmenes del XXVIII Congreso de Ciencias del Mar. Viña del Mar, Chile, 26-30 de mayo, 273 pp. [First data regarding the reproductive biology of the burrowing crayfish *Virilastacus retamali* Rudolph and Crandall, 2007 (Decapoda, Parastacidae). Book Abstracts of the XXVIII Congress of Marine Sciences. Viña del Mar, Chile, May 26-30, 273 pp.].

- Twardochleb, L. A., Olden, J. D. and Larson, E. R. (2013). A global meta-analysis of the ecological impacts of nonnative crayfish. *Freshwater Science*, 32 (4): 1367-82. doi: 10.1899/12-203.1.
- Vazquez, F. J. and López-Greco, L. S. (2007). Intersex females in the red claw crayfish, *Cherax quadricarinatus* (Decapoda: Parastacidae). *Revista de Biología Tropical*, 55 (1): 25-31.
- Venturiello, J. P. (2007). *Estudio de viabilidad económica del cultivo de la Langosta Australiana de Pinzas Rojas (Cherax quadricarinatus)*. Montevideo: FAO Proyecto TCP/URU/3101.[*Study of the economic viability of the cultivation of the Australian Red Crayfish (Cherax quadricarinatus)*]. Montevideo: FAO Project TCP / URU / 3101].
- Vogt, G. (2013). Abbreviation of larval development and extension of brood care as key features of the evolution of freshwater Decapoda. *Biological Reviews*, 88 (1): 81-116.
- Volonterio, O. (2009). First report of the introduction of an Australian Temnocephalidan into the New World. *Journal of Parasitology*, 95 (1): 120-23. doi: 10.1645/GE-1726.1.
- von Martens, E. (1869). Südbrasilianische Süß-und Brackwasser-Crustaceen nach den Sammlungen des Dr. Reinh. Hensel. *Archiv für Naturgeschichte*, 35 (1): 1-37. [South Brazilian freshwater and brackish crustaceans according to the collections of Dr. Ing. Reinh. Hensel. *Archive of Natural History*, 35 (1): 1-37].
- Yáñez-Alvarado, M., Rudolph-Latorre, E. and Orellana-Olave, J. (2018). Growth of the burrowing crayfish *Parastacus nicoleti* (Philippi, 1882) (Crustacea, Decapoda, Parastacidae). *Nauplius*, 26: e2018010.

*Chapter 2*

**CRAYFISH OF NEW GUINEA:  
CURRENT STATUS, EXPLOITATION  
AND THREATS**

*Jiří Patoka\**

Department of Zoology and Fisheries, Faculty of Agrobiolgy,  
Food and Natural Resources, University of Life Sciences Prague,  
Praha, Czech Republic

**ABSTRACT**

New Guinea is the world's largest a tropical island, also reaching the highest elevation of any tropical island, and covering a significant percentage of global biodiversity. Contrary to vertebrate species, invertebrates are mostly poorly studied. The best-known group of invertebrates are butterflies. From aquatic biota, numbers of newly discovered crayfish species are increasing recently in New Guinea. These crayfish belong to the genus *Cherax* and many of them are attractively coloured. For this reason, they are harvested in the wild and exploited as ornamental animals, which are exported via Jakarta mainly to the European

---

\* Corresponding Author's E-mail: patoka@af.czu.cz.

Union and the USA. Unfortunately, our current knowledge about biology, ecology and distribution of these species is either very limited or currently simply absent and the intensive capture can be very devastating to indigenous populations of *Cherax* crayfishes. Besides the risk of overharvesting, one new threat was recently identified: crayfish plague, the infectious disease being probably lethal to New Guinean crayfishes, was found in Java, Indonesia. Since the western half of the island of New Guinea is a part of Indonesian territory, the introduction of the pathogen here via aquaculture or other pathways is possible. In this chapter, the complete list of scientifically described species of New Guinean crayfish and known information about their occurrence are gathered together with the data about their exploitation by humans and further perspectives, with an extension to the improvement of conservation practises.

**Keywords:** Parastacidae, *Cherax*, endemic species, pet trade, ornamental animal, conservation, distribution

## 1. GENERAL ASPECTS

### 1.1. Worldwide Distribution of Parastacid Crayfish

Parastacids, i.e., freshwater crayfishes from superfamily Parastacoidea and family Parastacidae, contain circa 200 taxa with exclusive native occurrence in the Southern Hemisphere (Crandall and De Grave, 2017). Australia was identified as the main centre of diversity for all parastacids, with occurrence of 7 of the 15 genera from this family (Crandall and Buhay, 2008). In addition, some parastacids are native to surrounding islands and islets including New Guinea, New Zealand and Tasmania, as well as to South America and Madagascar (Crandall and De Grave, 2017). No crayfish is indigenous to continental Africa but within the last decades, some species like *Cherax quadricarinatus* was introduced there (Nunes et al., 2017) and also to various localities in the Northern Hemisphere like Singapore (Ahyong and Yeo, 2007), Mexico (Bortolini et al., 2007), Slovenia (Jaklič and Vrezec, 2011) and Hungary (Weiperth et al., 2019), and in environmentally suitable habitats established feral populations.

## 1.2. Pet Trade with Freshwater Crayfish

In comparison with a long history of exploitation of freshwater crayfish for human consumption (Patoka et al., 2014b), pet trade or so-called ornamental crayfish keeping started in mid 1990s and has continuously increased year to year (Faulkes, 2015a; Patoka et al., 2015c). There are more than thirty species pet-traded (Patoka et al., 2014a; Faulkes, 2015b; Chucholl and Wendler, 2017). As noted Faulkes (2015a), crayfish have a potentially greater distribution in ornamental aquaria than marine crustaceans. Ornamental crayfish are kept both in indoor aquaria and outdoor private and park ponds (Patoka et al., 2017a). There are several species relatively commonly in this market, particularly *Cambarellus patzcuarensis*, *Procambarus clarkii* and *P. virginialis*, which are North American species (Patoka et al., 2014c; Faulkes, 2015b). From parastacids, *Cherax destructor* and *C. quadricarinatus* are considered the most popular species. It must be noted that many traded crayfish, especially New Guinean species, originated from capture in the field (Lukhaup, 2015; Patoka et al., 2015a). There are also scientifically undescribed endemic species advertised under commercial names only. In some cases, more than one species of ornamental crayfish are traded under the same name, and also scientific misnomers used as commercial names were recognised (Chucholl, 2013; Patoka et al., 2014a; Lukhaup, 2015).

## 1.3. New Guinea

New Guinea is the world's largest and highest tropical island with a surface area of 786,000 km<sup>2</sup> and an elevation range of the central orogenic belt up to 5030 m a.s.l. The island is situated between Australia and the equator and, not surprisingly supports a large number of both vertebrate and invertebrate species, which are in many cases endemics (Brass, 1941; Heads, 2002). Even though if many collections have been made by numerous naturalists (Bott, 1974), large areas of the island remain, relatively, both zoologically and botanically unexplored or poorly studied.

Administratively, the territory of New Guinea is divided into two political entities. The eastern part of the island comprises the mainland of independent country Papua New Guinea while the eastern half belongs to Indonesia and is divided into two provinces: West Papua (known as Irian Jaya and West Irian in the past) and Papua, with the capital city being Jayapura.

## 2. NEW GUINEAN CRAYFISHES

The New Guinean crayfishes are generally classified as moderately burrowing species (Bláha et al., 2016) although there are also some species which are most likely non-burrowing like *Cherax acherontis* (Patoka et al., 2017b). They inhabit different types of habitats, from lowlands (e.g., *C. lorentzi* and *C. warsamsonicus*) (Holthuis, 1949; Lukhaup et al., 2017) to high mountains (e.g., *C. minor* and *C. monticola*) (Holthuis, 1950, 1996); from epigeic brooks, rivers and lakes to caves in the case of troglobitic *C. acherontis* (Patoka et al., 2017b). Phylogenetically, the New Guinean members of the genus *Cherax* comprise an isolated group in comparison to Australian congeners (Munasinghe et al., 2004; Bláha et al., 2016). Historically, two subgenera within the genus *Cherax*, namely *Cherax* and *Astaconephrops*, were established (Holthuis, 1950). However, after a recent analysis of molecular data (Bláha et al., 2016), these subgenera are not currently perceived to be valid (Crandall and De Grave, 2017).

Currently, 26 crayfish species have been scientifically described to New Guinea, the vast majority of them from the Indonesian part of the island. Just two of them, *C. quadricarinatus* and *C. rhynchotus* also occur in Australia, one of which is indigenous to Misool Island, and the others are truly endemic to New Guinean (Holthuis, 1949; Austin, 1996). Most of them are endemic to a very restricted area, typically to one small lake or stream (e.g., Holthuis, 1982; Lukhaup and Pekny, 2006). Generally, data about crayfish occurrence and distribution are mostly lacking in the eastern part of the island. Nevertheless, some anecdotal reports from local people suggest that the



diversity of astacofauna in this part of New Guinea is similar to that of the western part.

## 2.1. Exploitation by Humans

Generally, information about crayfish production in Asian countries other than China are mostly not available and this is true also for New Guinea. Some local New Guinean people catch crayfish for consumption particularly when the water level has declined within the dry season, as mentioned by Lukhaup and Herbert (2008). For instance, local people living in Baliem Valley (Dani, Lani and Yali tribes) had hunted *Cherax monticola* by three-pointed arrows (Le Roux, 1948; Holthuis, 1950). Currently, they catch crayfish by angling using earthworms as bait, by hand, or using handnets; these crayfish are in part consumed by locals, and also sold to hotels in the city of Wamena as a region-specific tasty meal for tourists (Patoka J., 2017, pers. observ.). Crayfish indigenous to Wissel Lakes are hunted by local people using spears, arrows, dip nets of 1.5 to 2 m in diameter, and square nets of size 4 x 4 m. These nets are attached to a rope made from rattan. The rope is usually more than 20 m long. Nets are baited with boiled and chopped tubers of sweet potato, *Ipomoea batatas*, and taro, *Colocasia esculenta* (Holthuis, 1949).

In recent decades, the New Guinean crayfish have been, due to their attractive colouration perceived as one of the most popular ornamental crayfish worldwide. Colouration is known to be one of the main predictors of long-term availability of crayfish in the pet trade (Chucholl and Wendler, 2017). The main market with these crayfish is in Sorong, West Papua, Indonesia (Lukhaup, 2015). With the exception of *C. quadricarinatus*, the other traded species are exclusively harvested from the wild and mostly exported via wholesalers in Jakarta, Java, Indonesia, abroad, mainly to the European Union and the USA (Patoka et al., 2015c). Many of exported crayfish are advertised for sale under misnomers or commercial names; sometimes, multiple species are traded under the same name (Patoka et al., 2014a). Usually, crayfish are packed separately in small plastic boxes and

shipped on wet foam out of water and their mortality is low. However, some less than experienced suppliers from Indonesia pack 10 or more individuals into one plastic box, and the mortality of these crayfishes is much higher; in certain cases, a complete shipment with tens of wild crayfish can be lost (Patoka, J., unpublished data).

## 2.2. Astacological Expeditions in New Guinea

In contrast to the Australian astacofauna including *Cherax* species, New Guinean crayfish have been at the periphery of scientific interest for a long time, mainly due to the extremely rugged terrain with swamps, mountains and fast-flowing streams, which have made many regions of the island difficult to reach and survey (Bláha et al., 2016). Most of the early zoologists who explored enigmatic fauna of New Guinea focused on vertebrates. But, some of them found a rich diversity of decapod crustaceans like shrimps and crabs, particularly the German naturalist and cartographer Karl Benjamin Hermann von Rosenberg in 1858 and between 1869 and 1870, the Italian zoologist, botanist and ethnologist Luigi Maria d'Albertis between 1872 and 1878, also the Italian zoologist and ethnographer Lamberto Loria between 1889 and 1895, the Hungarian zoologist and ethnographer Lajos Bíró between 1896 and 1901, and the Dutch naturalists Lieven Ferdinand de Beaufort and Hendrik Albertus Lorentz in 1903 and the latter also in 1907 and between 1909 and 1910 (Holthuis, 1982).

The first crayfishes from this island were collected by Constantijn Willem Rudolf Renesse van Duivenbode, the Dutch merchant, a trader in feathers and other naturalia, collector and dealer in natural history specimens. In 1898, he sent some parastacids from the dolomites in the Charles-Louis Range to the British Museum but no further details are known (Holthuis, 1949).

The first documented crayfish species followed in 1899 when Italian zoologist Giuseppe Nobili described *Astacopsis australasiensis* from west New Guinea and *Astaconephrops albertisii* from south-western New Guinea. However, the status of the specimen identified by Nobili as *A.*

*australasiensis* is confused. Even if some authors suggested that the latter one is identical with *Cherax quadricarinatus* (Calman, 1911), it was later revised as a valid species *Cherax albertisii* by Holthuis (1949).

Later, *Cheraps aruanus* from the Aroe Islands and *Cheraps lorentzi* from north-western and West New Guinea were collected by Swiss specialist in freshwater decapods Jean Roux between 1907 and 1908 and described in 1911 (Holthuis, 1949, 1982). *Cheraps* is an outdated name probably caused as an invalid original spelling for *Cherax* (Crandall and De Grave, 2017).

Between 1910 and 1912, the German Expedition explored the eastern part of Indonesia, and in 1911, the leader and geologist Odo Deodatus Tauern collected crayfish in Misool Island. The species was later described as *Cherax misolicus* by Holthuis (1949).

Between 1920 and 1922, the Netherlands Scientific Central New Guinea Expedition caught some unknown crayfishes in pools in the upper Baliem river basin slightly west of Habbema Lake on their way to the top of Puncak Trikora (Mt. Wilhelmina). All captured crayfish were probably consumed by members of the expedition and no specimens were preserved for transport to Europe (Holthuis, 1950).

Between 1933 and 1939, the American zoologist and philanthropist Richard Archbold sponsored a series of biological expeditions to New Guinea for the American Museum of Natural History (Brass, 1941). Within the Third Archbold Expedition, he and his team used the two-engined flying-boat "Guba" and discovered in the central highland the hitherto unknown Baliem Valley (named by Archbold as the Grand Valley, where they obtained crayfish later described as *Cherax monticola* by Holthuis (1950).

In 1936, during the Bamu-Puran Expedition to the eastern half of New Guinea, I. Champion and C. T. J. Adamson visited Lake Kutubu, the second largest lake in this area. Champion and Adamson the most probably collected there one crayfish male and one female later used as the type material in the description of *Cherax papuanus* by Holthuis (1949).

In 1939, the expedition of the Royal Netherlands Geographic Society explored the region of Wissel Lakes (also known as the Paniai Lakes), a complex of three lakes: Paniai, Taga and Tigi (sometimes also called Tiri),

ca. 1650 to 1750 m a.s.l. There was found a rich endemic astacofauna partly collected by the Dutch zoologist and director of the Rijksmuseum van Natuurlijke Historie (now the Naturalis Biodiversity Center), Leiden, Netherlands, Hilbrand Boschma himself, partly obtained from local people (Holthuis, 1949, 1982).

In 1952, during the first Brongersma's expedition to the Bird's Head Peninsula, Marinus Boeseman surveyed several lakes including Wissel Lakes and Aitinjo Lake. Among others, he collected the type material used later in the description of pet traded species such as *Cherax holthuisi* (Lukhaup and Pekny, 2006).

Between 1954 and 1955, the Dutch carcinologist Lipke Bijdeley Holthuis, a phenomenal specialist on New Guinean decapods, visited for the first time the island within the second Brongersma's expedition to the Bird's Head Peninsula. Holthuis together with M. Boeseman surveyed shorelines of the Ajamaru Lakes in the Kais river drainage, and samples were stored in the Rijksmuseum van Natuurlijke Historie, where currently a large collection of New Guinean decapods exists (Holthuis, 1982). Also, certain new species were later described based on these samples: *Cherax holthuisi* and *C. boesemani* (Lukhaup and Pekny, 2006; Lukhaup and Pekny, 2008).

In 2016, the German rocker, crayfish hunter and photographer Chris Lukhaup accompanied the local guide Irianto Wahid and surveyed several waterbodies in Sorong and South Sorong Regencies, West Papua Province to clarify the distribution of some pet-traded crayfish species. Based on the material collected, three new species of *Cherax* crayfish were subsequently scientifically described: *C. warsamsonicus* (Lukhaup et al., 2017), *C. alyciae* and *C. mosesalossa* (Lukhaup et al., 2018).

In 2017, the Czech Expedition to Baliem Valley (zoologists: Jiří Patoka and Martin Bláha) found with aid of the local guide Onesius Lokobal the first troglobitic crayfish of non-North American origin, *Cherax acherontis*, in subterranean Yumugima river flowing through Hagepma cave near the city of Wamena (Patoka et al., 2017b). This finding is promising for the future because more cave crayfish species can be expected in karst systems of New Guinea.

The type material from the aforementioned expeditions has been almost exclusively deposited in the Bogor Zoology Museum, Java, Indonesia, and in the aforementioned Naturalis Biodiversity Center, Leiden, Netherlands. Some type specimens have been deposited also in the collections in the British Museum, London, UK, the Naturhistorisches Museum in Basel, Switzerland, and in the Naturmuseum Senckenberg in Frankfurt am Main, Germany.

Despite these collections, some New Guinean *Cherax* species were described based on the type material at least partly obtained from German and Czech pet markets, namely: *C. boesemani*, *C. gherardii*, *C. holthuisi*, *C. peknyi*, *C. pulcher*, and *C. snowden* (Lukhaup and Pekny, 2006; Lukhaup and Herbert, 2008; Lukhaup and Pekny, 2008; Lukhaup, 2015; Lukhaup et al., 2015; Patoka et al., 2015a, b).

### 2.3. List of Species

#### ***Cherax acherontis* Patoka, Bláha & Kouba, 2017**

This crayfish is the first truly troglobitic crayfish found outside of North America (Stern et al., 2017). It inhabits the karst cave with rich dripstone decoration located in the New Guinea Highlands, Jayawijaya Regency, Papua Province. There are at least three entrances to the cave. Close to the cave is situated Palimoro village, which is inhabited by one family of Dani people but locals do not catch these crayfish for consumption or other purposes. The cave was formed by the subterranean Yumugima river which is a tributary of Baliem river. No crayfish burrows were found in the cave. The density is relatively low, ca. 40 adults and tens of juveniles per 3 km (Patoka et al., 2017b).

Due to low food availability and lack of photostimuli, the cave environment formed certain morphological features characteristic to troglobitic crayfish in general: long and slender claws, reduced eyes, loss of body pigmentation, and long antennae. This is also true for *C. acherontis*. This crayfish uses long and thickened third maxillipeds covered with dense setae to filtrate organic particles which consumes. This adaptation is known

also in one North American troglobitic crayfish, *Troglocambarus maclanei* (Hobbs et al., 1977). This is evidence of convergent evolution (Patoka et al., 2017b). Uncalcified soft patches on lateral margins of chelae of adult males are absent. The exploitation of this creature as ornamental is not expected to occur.

### ***Cherax albertisii* (Nobili, 1899)**

This is the first New Guinean crayfish species scientifically described (Nobili, 1899). Its native range is in the Fly river drainage, south New Guinea (Holthuis, 1949).

Uncalcified soft patches on lateral margins of chelae of adult males are red. This species is traded as ornamental, although more rarely in comparison with the closely related *C. quadricarinatus* which is commonly available in the pet trade (Chucholl, 2013; Patoka et al., 2015c). Both species are morphologically not easily distinguished, being the main difference the lateral teeth on rostral margins: existing three in *C. albertisii*, while only two in *C. quadricarinatus* as described by Holthuis (1949). Nevertheless, in the same publication, Holthuis (1949) also suggested that variability in this characteristic is known and thus it is not a solid characteristic. Furthermore, the chelae are narrower and the scaphocerite is broader in *C. albertisii* than in *C. quadricarinatus* (Holthuis, 1949). Later, Austin (1996) did not find any differences between both mentioned species on the basis of electrophoretic or morphological evidence, and some authors accepted this synonymization (Coughran and Leckie, 2007). Regardless, *C. albertisii* remains accepted as a valid species in the recent revision (Crandall and De Grave, 2017). *Cherax divergence* described by Holthuis (1950) was later synonymized with *C. albertisii* as its junior synonym (Holthuis, 1996).

### ***Cherax alyciae* Lukhaup, Eprilurahman & von Rintelen, 2018**

This species is endemic to shallow creeks in the Digul river drainage in the eastern part of the Boven Digoel Regency, Papua Province, close to the border of Papua New Guinea. To improve the knowledge about the native range of this species, future collecting trips were recommended by the authors of the description (Lukhaup et al., 2018).

The most closely related species is *C. peknyi*, both species can be easily distinguished by the colouration of life individuals and by a presence of white and translucent uncalcified soft patches on lateral margins of chelae of adult males which are completely absent in *C. peknyi* (Lukhaup and Herbert, 2008; Lukhaup et al., 2018). This species is currently not traded as a pet but due to its attractive colouration, its introduction on the market can be expected.

### ***Cherax boesemani* Lukhaup & Pekny, 2008**

The first individuals of this species were collected by Marinus Boeseman in 1955 (Boeseman, 1963) who also described its native range as two Ajamaru Lakes (Jow and Semitoe also called Maroemega) located in the Bird's Head Peninsula. Later, the species was scientifically described based on the morphological comparison and matching these collected specimens and individuals imported via pet trade to Germany (Lukhaup and Pekny, 2008).

This crayfish is known as a host of temnocephalid worms (Temnocephalidae), which attach mainly to chelae, pleon and telson (Lukhaup and Pekny, 2008). Uncalcified soft patches on lateral margins of chelae of adult males are yellowish or pale to white (Lukhaup and Pekny, 2008). Both red and blue colouration morphs of this species are exploited as ornamentals (Lukhaup and Pekny, 2008; Chucholl, 2013; Patoka et al., 2014a).

### ***Cherax boschmai* Holthuis, 1949**

This species is one of the *Cherax* found being endemic to Paniai Lake and its abundance is probably low. The chelae are very slender, lacking uncalcified soft patches on the lateral margins in adult males (Holthuis, 1949). Unusually, despite the collection of two males, the largest of the four collected females was chosen as the holotype. But there is also a picture of *C. boschmai* in the description marked by Holthuis (1949) as the holotype male. The pictures/plates are of poor quality and for this reason, a future revision of the type material seems to be necessary. This species is currently not traded as a pet.

***Cherax buitendijkae* Holthuis, 1949**

This species is endemic to Paniai Lake. Based on morphological analysis, the most related species are *C. pallidus* and *C. murido*, both endemic also to this lake. Uncalcified soft patches on lateral margins of chelae of adult males are lacking. Local people hunt *C. buitendijkae* by spears, thus one can speculate that these crayfishes inhabit shallow waters at the shoreline of the lake (Holthuis, 1949). This species is currently not traded as a pet.

***Cherax communis* Holthuis, 1949**

This species is considered endemic to the Wissel Lakes region, namely Paniai and Tigi lakes and adjoining streams such as Araboe and Elegeboe rivers. This is the most common crayfish species in this region. It has a very broad and robust chelae without uncalcified soft patches on the lateral margins (Holthuis, 1949). Since *C. communis* have poorly delimited taxonomic boundaries and thus may contain some cryptic species, Holthuis (1982) suggested that it would be a species complex. Based on morphological analysis, Australian *Cherax destructor* seems to be one of the most closely related species to (Holthuis, 1949). *Cherax communis* is very rarely traded as a pet.

***Cherax gherardii* Patoka, Bláha & Kouba, 2015**

This species occurs in surrounding tributary streams to the Ajamaru Lakes, Bird's Head Peninsula, West Papua Province. A future collecting trip to improve the distribution of *C. gherardii* is recommended. This species was named in honour of Italian zoologist Francesca Gherardi and due to the incorrect original spelling, the change to species name to "*gherardiae*" was suggested later (Patoka et al., 2015b). But this is an unjustified emendation and thus perceived to be not a valid name (Crandall and De Grave, 2017).

Uncalcified soft patches on the lateral margins of chelae of adult males are pale and translucent. Based on DNA analysis, the most closely related species are *C. boesemani* and *C. pulcher* (Patoka et al., 2015a; Bláha et al., 2016). This species is traded as ornamental and its popularity seems to be rising (Vodovsky et al., 2017).



***Cherax holthuisi* Lukhaup & Pekny, 2006**

This species is endemic to Aitinjo Lake, Bird's Head Peninsula, West Papua Province. It was described based on the type material bought from local people by M. Boeseman in 1952 and later compared with one of the numerous individuals imported from Indonesia to Germany as ornamentals (Holthuis, 1982; Lukhaup and Pekny, 2006). Uncalcified soft patches on the lateral margins of the chelae of adult males are absent. Being a popular ornamental animal (Patoka et al., 2015c; Chucholl and Wendler, 2017), this was the first *Cherax* scientifically described after its introduction on the pet market. *Cherax holthuisi* usually has orange or yellow colouration, also blue individuals can be rarely found pet-traded (Lukhaup and Pekny, 2006). Moreover, a dark colouration morph traded under its commercial name *Cherax* sp. black scorpion is known (Bláha et al., 2016).

***Cherax longipes* Holthuis, 1949**

Together with *C. solus*, this species is an endemic of Tigi Lake, the second largest of the Wissel Lakes (Holthuis, 1949). Uncalcified soft patches on the lateral margins of chelae of adult males absent. *Cherax longipes* has very long and slender walking legs, in which character it differs from all other *Cherax* species. Based on the information from the collector of the type material, father H. F. Tillemans, this crayfish lives in deeper parts of the lake (Holthuis, 1949). This species is currently not traded as ornamental.

***Cherax lorentzi* Roux, 1911**

There were two originally described species later considered by Holthuis (1949) being the subspecies of one species only: *C. lorentzi lorentzi* and *C. lorentzi aruanus*. This is a typical lowland species and it has a relatively wider native range in comparison with the majority of New Guinean crayfish. It occurs between the Bird's Head Peninsula on the west to the Lorentz river on the east while the subspecies *C. lorentzi aruanus* is endemic to the Aru Island (Holthuis, 1982). Uncalcified soft patches on lateral margins of chelae of adult males are red. This species is occasionally traded as ornamental (Patoka et al., 2015c).

***Cherax minor* Holthuis, 1996**

This species is known to inhabit small shallow creeks and one small lake in the Baliem river drainage, Wurigelebur Mountain region, Papua Province, altitude 1200 to 1300 m a.s.l. In comparison with the other known New Guinean crayfish, *C. minor* is significantly smaller in body length (up to 75 mm) (Holthuis, 1996).

The type material was collected by A. Suyanto and later emphasized by Daisy Wowor, the curator of the Bogor Zoology Museum, to L. B. Holthuis who described the new species as *C. minor*. Uncalcified soft patches on the lateral margins of the chelae of adult males are strikingly pale. Morphologically, the most closely relative species is *C. monticola* (Holthuis, 1996). This species is not traded as ornamental.

***Cherax misolicus* Holthuis, 1949**

This species is endemic to the Misool island, one of the four major islands in the Raja Ampat archipelago located off the northwest tip of the Bird's Head Peninsula, West Papua Province. The type material was collected by O. Tauern in 1911 and M. A. Lieftinck in 1948, and later described as a new species by Holthuis (1949).

Uncalcified soft patches on the lateral margins of the chelae of adult males are the most probably red, because Holthuis (1949) noted, that the most closely related species is *C. lorentzi* belonging to “red claw” lineage defined by Eprilurahman (2014). Also, Roux (1914), who collected some specimens from the Misol Island, misidentified those with *C. quadricarinatus*, another “red claw” species. Contreary, Lukhaup et al., (2017) suggested that *C. misolicus* is phylogenetically and morphologically the most closely related species to *C. warsamsonicus*, the species with the patches of white colour. This contradiction must be revealed and explained in future revision. This species is currently not traded as a pet.

***Cherax mosessalossa* Lukhaup, Eprilurahman & von Rintelen, 2018**

This species is endemic to Klademak Creek drainage in Sorong, in the western part of the Bird's Head Peninsula, West Papua Province. Uncalcified soft patches on the lateral margins of the chelae of adult males

are white and translucent. Carapace surface smooth with scattered fine hairs, which are absent in its relatives, *C. misolicus* and *C. warsamsonicus* (Lukhaup et al., 2018), but see the morphological confusions in the *C. misolicus* paragraph. This species is currently not traded as a pet.

### ***Cherax monticola* Holthuis, 1950**

Along with *C. acherontis* (Patoka et al., 2017b) and *C. minor* (Holthuis, 1996), this species has a native range in Baliem Valley ca. 1700-3300 m a.s.l., Papua Province. *Cherax monticola* is called by local people “false shrimp” because it has robust claws which are an “evidence” of hybridization between shrimps and crabs (Patoka, J., 2017, pers. observ.). The type locality is the Baliem river (mentioned as Balim in the original description of the species) and some individuals were caught also in the Ibele river 15 km northeast of Habbema Lake. Even though the first individuals were found by the Netherlands Scientific Central New Guinea Expedition between 1920 and 1922, the type material was later collected within the Third Archbold Expedition between 1938 and 1939 (Holthuis, 1950).

Uncalcified soft patches on the lateral margins of the chelae of adult males are whitish. Large males have typically two spots on each chela and, uniquely within the genus, females also have small uncalcified spots on their chelae. Subadult individuals are marbled and these colouration patterns are different in adults (Patoka, unpublished data). *Cherax monticola* is the only one species of New Guinean crayfish with the complete mitochondrial genome sequenced (Gan et al., 2016). Many of the collected crayfish from both localities had attached egg capsules of temnocephalids on their body (Holthuis, 1950). This species is fished for human consumption by local people and rarely traded as ornamental.

### ***Cherax murido* Holthuis, 1949**

This species is endemic to Paniai Lake and probably inhabit shallow waters at the shoreline. Morphologically, *C. murido* is the most related to *C. buitendijkae* and *C. pallidus*. Uncalcified soft patches on the lateral margins of the chelae of adult males are absent. This species is not exploited as

ornamental but speared by local people for human consumption similarly as *C. communis* (Holthuis, 1949).

### ***Cherax quadricarinatus* (von Martens, 1868)**

This species is indigenous both to Australia and New Guinea and consists of the highly divergent Australian and New Guinean lineages (Baker et al., 2008). Uncalcified soft patches on the lateral margins of the chelae of adult males are red. Among New Guinean crayfish, *C. albertisii*, *C. lorentzi* and *C. misolicus* are considered to be the most closely related species (Holthuis, 1949). This species is very popular both for human consumption and as well as ornamental creature and has been introduced out of its native range also in the rest of Indonesian territory (Patoka et al., 2016; Patoka et al., 2018b).

### ***Cherax pallidus* Holthuis, 1949**

This species is one of the endemics of Paniai Lake. The complete type of material was obtained from local people but no crayfish was speared. Thus, Holthuis (1949) suggested that this crayfish lives in the deeper parts of the lake. Uncalcified soft patches on the lateral margins of the chelae of adult males are absent, chelae are very long and slender. Based on the morphological characteristics, the most closely related species are *C. buitendijkae* and *C. murido* (Holthuis, 1949). This species is currently not traded as ornamental.

### ***Cherax paniaicus* Holthuis, 1949**

As its specific name suggests, this is one of the endemic species of Paniai Lake. Uncalcified soft patches on the lateral margins of the chelae of adult males are absent. The related species is *C. boschmai* and *C. solus*, the most closely related species is *C. communis* (Holthuis, 1949). *Cherax paniaicus* is currently not pet-traded.

### ***Cherax papuanus* Holthuis, 1949**

This is the only one currently known species of *Cherax* with exclusive distribution in the territory of Papua New Guinea. It is an endemic of 710 m

deep Lake Kutubu (originally reported as Lake Marguerite) at 850 m a.s.l. Uncalcified soft patches on the lateral margins of the chelae of adult males are absent (Holthuis, 1949). This species is currently not traded as ornamental.

### ***Cherax peknyi* Lukhaup & Herbert, 2008**

This species is native to the Fly river drainage with a type locality in Tamu river, Western Province of Papua New Guinea. Uncalcified soft patches on the lateral margins of the chelae of adult males absent (Lukhaup et al., 2018). This species is a very popular ornamental animal (Chucholl, 2013; Patoka et al., 2015c). It is exported by wholesalers from Jakarta, Indonesia to the European, Japanese and USA pet markets (Lukhaup and Herbert, 2008). Thus, the distribution of the species is the most probably more widespread at least in the Indonesian region adjoining to the type locality.

### ***Cherax pulcher* Lukhaup, 2015**

The species was simultaneously described by Jiří Patoka, Martin Bláha and Antonín Kouba as *C. crucifer* (Patoka et al., unpublished data) but the description made by Christian Lukhaup was published first, thus the name *C. pulcher* is the only one valid (Lukhaup, 2015). This species is endemic to Hoa Creek drainage, Bird's Head Peninsula, West Papua Province. The author of the description noted that a future collecting trip is necessary to improve the knowledge of the native range of this species. Based on morphological characteristics, *C. boesemani* was considered being the most closely related species (Lukhaup and Pekny, 2008; Lukhaup, 2015).

Uncalcified soft patches on the lateral margins of the chelae of adult males are white and translucent (Lukhaup, 2015). This crayfish is exploited as ornamental and sometimes is confused by traders and keepers with *C. warsamsonicus* due to the similar colouration of both species (Lukhaup et al., 2017).

***Cherax rhynchotus* Riek, 1951**

This species was originally described by Riek (1951) from the type locality in Queensland, Australia but it is indigenous also to Fly river in southern New Guinea (Riek, 1951; Munasinghe et al., 2004). Since chelae are absent from all the type specimens except the allotype female, it is impossible to define the presence of uncalcified soft patches on the lateral margins of the chelae of adult males from the original description of the species. Nevertheless, Eprilurahman (2014) suggested that *C. rhynchotus* belongs in the “red claw” lineage together with *C. albertisii*, *C. barretti*, *C. bicarinatus*, *C. lorentzi*, *C. nucifraga*, and *C. quadricarinatus*. In the wild populations of *C. rhynchotus*, there is a large percentage of intersexual individuals as reported Riek (1951). This species is currently not traded as ornamental.

***Cherax snowden* Lukhaup, Panteleit & Schrimpf, 2015**

The species was parallelly described by Jiří Patoka, Martin Bláha and Antonín Kouba as *C. subterigneus* (Patoka et al., 2015b) which is the junior synonym. The type locality described by Lukhaup et al., (2015) is a little bit confused: the authors mentioned that *C. snowden* is known from shallow tributaries of the Oinsok river in Sawiat District in the central part of the Bird’s Head Peninsula, West Papua Province; but also that according to the information obtained from Indonesian exporters and from the local collector Irianto Wahid, all specimens originated from creeks in the “Sungai river drainage.” Since the meaning of the term “sungai” in the Indonesian language is “river,” this is the most probably a misinterpretation of information obtained from the collector or trader. Therefore, one must agree with the authors of the description who recommended future collecting trip to clarify the native range of this species which is a popular ornamental creature (Chucholl and Wendler, 2017). Uncalcified soft patches on the lateral margins of the chelae of adult males absent and the most closely related species is *C. holthuisi* (Lukhaup et al., 2015; Patoka et al., 2015b).

***Cherax solus* Holthuis, 1949**

This species is the most probably endemic to one of the Wissel Lakes, namely Tigi Lake. Since the type material comprises just one specimen, a holotype female, it is not possible to conclusively define if uncalcified soft patches on the lateral margins of the chelae of adult males are present or absent in this species. Morphologically, the most closely related species is *C. paniaicus*, some characteristics are also similar to *C. longipes* (Holthuis, 1949). This species is currently not pet-traded. It is obvious, that a future collecting trip is necessary to ascertain more data about the morphology and also the genetics of this species.

***Cherax warsamsonicus* Lukhaup, Eprilurahman & von Rintelen, 2017**

This species is endemic to the Warsamson river drainage, in the western part of the Bird's Head Peninsula, West Papua Province. Uncalcified soft patches on lateral margins of chelae of adult males are white and translucent. This crayfish is currently traded as ornamental and sometimes is confused by traders and keepers with *C. pulcher* due to the similar colouration of both species. Phylogenetically, the most closely related species is *C. misolicus* (Lukhaup et al., 2017) but there are some morphological confusions which must be explained in the future revision (see the *Cherax misolicus* paragraph above).

**2.4. Threats**

Since the abundance, population trends and complete native range of many New Guinean crayfish species are still unknown, a decline of wild populations caused by intensive capture for ornamental and other purposes can be easily overlooked and also harmful. Additionally, as any conservation measurements cannot be applied without identification of the species and subsequent monitoring, accurate determination of scientifically undescribed species is crucial (Bláha et al., 2016). Survey of distribution of crayfish within the island of New Guinea is the following step which would reveal a

risk of habitat alteration and destruction caused by human activities like deforestation or mining on mentioned species.

Besides to potential overharvesting and habitat loss, a new threat to New Guinean crayfish was recently found in Java, Indonesia: the crayfish plague pathogen (*Aphanomyces astaci*) (Putra et al., 2018). It is really alarming, even if the sensitivity of New Guinean *Cherax* crayfish has not yet been proved and verified because the lethality to all crayfish species of non-North American origin is expected (Mrugała et al., 2016; Svoboda et al., 2017 and citations herein). One North American species, *Procambarus clarkii*, was unfortunately recently found and confirmed as the carrier and vector of the pathogen in Indonesia (Putra et al., 2018). This crayfish is a popular ornamental animal in this country. Its introduction and spread to New Guinea would have a devastating impact on native fauna, not only due to crayfish plague transmission but also since *P. clarkii* is known to be able to alter food webs and habitat structure (Souty-Grosset et al., 2016). It is obvious that future monitoring of non-indigenous crayfish and crayfish plague in Indonesia including New Guinea is needful and crucial.

### 3. CONSERVATION AND MANAGEMENT PRACTISES

Following New Guinean *Cherax* species are currently listed in the IUCN Red List of Threatened Species (version 3, May 2017, <https://www.iucnredlist.org>): *C. boesemani*, *C. holthuisi*, *C. lorentzi*, *C. monticola*, *C. papuanus*, *C. peknyi*, *C. quadricarinatus*, and *C. rhynchotus*. From this list, *C. papuanus* is classified as Vulnerable, *C. quadricarinatus* and *C. rhynchotus* as Least Concern while the others as Data Deficient. In Indonesia, Regulation No. 41/PERMEN-KP/2014 bans the import of selected 152 non-native fish species. In this law, fishes are defined as “all types of the organism in which all or part of its life cycle is in an aquatic environment.” Paradoxically, many banned species such as North American crayfish *Procambarus clarkii*, are cultured and produced in Indonesia in huge quantities because it is legal to release non-native species into the wild and indeed, local people do so for further exploitation. Despite



this, the general regulation of invasive species PERMEN LHK P94/2016 categorizes this crayfish as an invasive species not occurring in Indonesian territory (Patoka et al., 2018a; Putra et al., 2018). It is probably caused also by incorrect determination of the species by Indonesian researchers. For instance, one of the misnomers used is “*Cherax procambarus clarkii*” (see Lekatompessy and Da Costa, 2019).

Based on the aforementioned recent findings of the invasive North American crayfish and the crayfish plague pathogen in Indonesia, the legislative regulations should be improved with a special focus on conservation of the wildlife in general and of the New Guinean crayfish in particular. What is crucial, this important step must be also linked with the further education of the general public and traders as well, involving far greater communication among scientists, administrations, politicians, the pet industry, conservationists and wildlife managers including stakeholders and liaison authorities from Papua New Guinea.

## REFERENCES

- Ahyong, S. T. and Yeo, D. C. (2007). Feral populations of the Australian red-claw crayfish (*Cherax quadricarinatus* von Martens) in water supply catchments of Singapore. *Biological Invasions*, 9 (8): 943-946.
- Austin, C. (1996). Systematics of the freshwater crayfish genus *Cherax* Erichson (Decapoda: Parastacidae) in northern and eastern Australia: electrophoretic and morphological variation. *Australian Journal of Zoology*, 44 (3): 259-296.
- Baker, N., De Bruyn, M. and Mather, P. B. (2008). Patterns of molecular diversity in wild stocks of the redclaw crayfish (*Cherax quadricarinatus*) from northern Australia and Papua New Guinea: impacts of Plio-Pleistocene landscape evolution. *Freshwater Biology*, 53 (8): 1592-1605.
- Bláha, M., Patoka, J., Kozák, P. and Kouba, A. (2016). Unrecognized diversity in New Guinean crayfish species (Decapoda, Parastacidae): The evidence from molecular data. *Integrative Zoology*, 11 (6): 457-468.

- Boeseman, M. (1963). Notes on the fishes of Western New Guinea. *Zoologische Mededelingen Leiden*, 38 (14): 221-242.
- Bortolini, J. L., Alvarez, F. and Rodríguez-Almaraz, G. (2007). On the presence of the Australian redclaw crayfish, *Cherax quadricarinatus*, in Mexico. *Biological Invasions*, 9 (5): 615-620.
- Bott, R. (1974). Die Süßwasserkrabben von Neu Guinea. *Zoologische Verhandelingen*, 136 (1): 1-36.
- Brass, L. J. (1941). The 1938-39 expedition to the Snow Mountains, Netherlands New Guinea. *Journal of the Arnold Arboretum*, 22 (2): 271-295.
- Calman, W. (1911). XLIV.—Note on a crayfish from New Guinea. *Annals and Magazine of Natural History*, 8 (45): 366-368.
- Chucholl, C. (2013). Invaders for sale: trade and determinants of introduction of ornamental freshwater crayfish. *Biological Invasions*, 15 (1): 125-141.
- Chucholl, C. and Wendler, F. (2017). Positive selection of beautiful invaders: long-term persistence and bio-invasion risk of freshwater crayfish in the pet trade. *Biological Invasions*, 19 (1): 197-208.
- Coughran, J. and Leckie, S. (2007). Invasion of a New South Wales stream by the tropical crayfish, *Cherax quadricarinatus* (von Martens). In: Lunney, D., Eby, P., Hutchings, P. and Burgin, S. (editors). *Pest or Guest: the zoology of overabundance*. Royal Zoological Society of New South Wales, Mosman, NSW, Australia. p. 40-46.
- Crandall, K. A. and Buhay, J. E. (2008). Global diversity of crayfish (Astacidae, Cambaridae, and Parastacidae—Decapoda) in freshwater. *Hydrobiologia*, 595 (1): 295-301.
- Crandall, K. A. and De Grave, S. (2017). An updated classification of the freshwater crayfishes (Decapoda: Astacidea) of the world, with a complete species list. *Journal of Crustacean Biology*, 37 (5): 615-653.
- Eprilurahman, R. (2014). *Molecular taxonomy and evolution of freshwater crayfish of the genus Cherax (Decapoda: Parastacidae) from northern Australia and New Guinea*, Charles Darwin University, Northern Territory, Australia.

- Faulkes, Z. (2015a). The global trade in crayfish as pets. *Crustacean Research*, 44: 75-92.
- Faulkes, Z. (2015b). Marmorcrebs (*Procambarus fallax* f. *virginalis*) are the most popular crayfish in the North American pet trade. *Knowledge and Management of Aquatic Ecosystems*, 416: 20.
- Gan, H. M., Tan, M. H. and Austin, C. M. (2016). The complete mitogenome of the red claw crayfish *Cherax quadricarinatus* (Von Martens, 1868) (Crustacea: Decapoda: Parastacidae). *Mitochondrial DNA*, 27 (1): 385-386.
- Heads, M. (2002). Regional patterns of biodiversity in New Guinea animals. *Journal of Biogeography*, 29 (2): 285-294.
- Holthuis, L. B. (1949). Decapoda Macrura, with a revision of the New Guinea Parastacidae. *Zoological Results of the Dutch New Guinea Expedition*, 1939 (3): 289-328.
- Holthuis, L. B. (1950). Results of the Archbold expeditions. No 63 The Crustacea Decapoda Macrura collected by the Archbold New Guinea expeditions. *American Museum Novitates*, 1461: 1-17.
- Holthuis, L. B. (1982). Freshwater Crustacea Decapoda of New Guinea. In: Gressitt, J. (editor). *Biogeography and ecology of New Guinea*. Dr W. Junk Publishers, The Hague. p. 603-619.
- Holthuis, L. B. (1996) *Cherax (Astaconephrops) minor* new species, a parastacid from the mountains of Irian Jaya (W. New Guinea), Indonesia (Crustacea: Decapoda: Parastacidae). *Zoologische Mededelingen*, 70: 361-366.
- Jaklič, M. and Vrezec, A. (2011). The first tropical alien crayfish species in European waters: the redclaw *Cherax quadricarinatus* (Von Martens, 1868) (Decapoda, Parastacidae). *Crustaceana*, 84 (5-6): 651-665.
- Le Roux, C. C. F. M. (1948). *De Bergpapoea's van Nieuw-Guinea en hun woongebied [The Mountain Papuans of New Guinea and their residential area]*. EJ Brill, Leiden.
- Lekatompessy, H. S. and Da Costa, G. W. (2019). The species inventarization of freshwater lobster (*Cherax* sp.) in Tigi Lake of Widimei village, Deiyai Regency. *TABURA: Jurnal Perikanan Dan Kelautan*, 1 (1): 1-9.

- Lukhaup, C. (2015). *Cherax (Astaconephrops) pulcher*, a new species of freshwater crayfish (Crustacea, Decapoda, Parastacidae) from the Kepala Burung (Vogelkop) Peninsula, Irian Jaya (West Papua), Indonesia. *ZooKeys*, 502: 1-10.
- Lukhaup, C., Eprilurahman, R. and von Rintelen, T. (2017). *Cherax warsamsonicus*, a new species of crayfish from the Kepala Burung (Vogelkop) peninsula in West Papua, Indonesia (Crustacea, Decapoda, Parastacidae). *ZooKeys*, 660: 151-167.
- Lukhaup, C., Eprilurahman, R. and von Rintelen, T. (2018). Two new species of crayfish of the genus *Cherax* from Indonesian New Guinea (Crustacea, Decapoda, Parastacidae). *ZooKeys*, 769: 89-116.
- Lukhaup, C. and Herbert, B. (2008). A new species of crayfish (Crustacea: Decapoda: Parastacidae) from the Fly River Drainage, Western Province, Papua New Guinea. *Memoirs of Queensland Museum*, 52 (2): 213-219.
- Lukhaup, C., Panteleit, J. and Schrimpf, A. (2015). *Cherax snowden*, a new species of crayfish (Crustacea, Decapoda, Parastacidae) from the Kepala Burung (Vogelkop) Peninsula in Irian Jaya (West Papua), Indonesia. *ZooKeys*, 518: 1-14.
- Lukhaup, C. and Pekny, R. (2006). *Cherax (Cherax) holthuisi*, a new species of crayfish (Crustacea: Decapoda: Parastacidae) from the centre of the Vogelkop Peninsula in Irian Jaya (West New Guinea), Indonesia. *Zoologische Mededelingen*, 80 (1): 101-107.
- Lukhaup, C. and Pekny, R. (2008). *Cherax (Astaconephrops) boesemani*, a new species of crayfish (Crustacea: Decapoda: Parastacidae) from the centre of the Vogelkop Peninsula in Irian Jaya (West New Guinea), Indonesia. *Zoologische Mededelingen*, 82: 331-340.
- Mrugała, A., Veselý, L., Petrusek, A., Viljamaa-Dirks, S. and Kouba, A. (2016). May *Cherax destructor* contribute to *Aphanomyces astaci* spread in Central Europe? *Aquatic Invasions*, 11 (4): 459-468.
- Munasinghe, D., Burridge, C. and Austin, C. (2004). Molecular phylogeny and zoogeography of the freshwater crayfish genus *Cherax* Erichson (Decapoda: Parastacidae) in Australia. *Biological Journal of the Linnean Society*, 81 (4): 553-563.

- Nobili, G. (1899). Contribuzioni alla conoscenza della fauna carcinologica della Papuasìa, delle Molluche e dell'Australia. *Annali del Museo Civico di Storia Naturale di Genova*, 20: 230-282.
- Nunes, A. L., Zengeya, T. A., Hoffman, A. C., Measey, G. J. and Weyl, O. L. (2017). Distribution and establishment of the alien Australian redclaw crayfish, *Cherax quadricarinatus*, in South Africa and Swaziland. *Peer J*, 5: e3135.
- Patoka, J., Bláha, M., Kalous, L. and Kouba, A. (2017a). Irresponsible vendors: non-native, invasive and threatened animals offered for stocking garden ponds. *Aquatic Conservation*, 27: 692–697.
- Patoka, J., Bláha, M. and Kouba, A. (2015a). *Cherax (Astaconephrops) gherardii*, a new crayfish (Decapoda: Parastacidae) from West Papua, Indonesia. *Zootaxa*, 3964 (5): 526-536.
- Patoka, J., Bláha, M. and Kouba, A. (2015b). *Cherax (Cherax) subterigneus*, a new crayfish (Decapoda: Parastacidae) from West Papua, Indonesia. *Journal of Crustacean Biology*, 35 (6): 830-838.
- Patoka, J., Bláha, M. and Kouba, A. (2017b) *Cherax acherontis* (Decapoda: Parastacidae), the first cave crayfish from the Southern Hemisphere (Papua Province, Indonesia). *Zootaxa*, 4363 (1): 137-144.
- Patoka, J., Kalous, L. and Kopecký, O. (2014a). Risk assessment of the crayfish pet trade based on data from the Czech Republic. *Biological Invasions*, 16 (12): 2489-2494.
- Patoka, J., Kalous, L. and Kopecký, O. (2015c). Imports of ornamental crayfish: the first decade from the Czech Republic's perspective. *Knowledge and Management of Aquatic Ecosystems*, 416: 4.
- Patoka, J., Magalhães, A. L. B., Kouba, A., Faulkes, Z., Jerikho, R. and Vitule, J. R. S. (2018a). Invasive aquatic pets: failed policies increase risks of harmful invasions. *Biodiversity and Conservation*, 27 (11): 3037-3046.
- Patoka, J., Nývltová, M., Fišáková, Kalous, L., Škrdla, P. and Kuča, M. (2014b). Earliest evidence for human consumption of crayfish. *Crustaceana*, 87 (13): 1578-1585.

- Patoka, J., Petrůl, M. and Kalous, L. (2014c). Garden ponds as potential introduction pathway of ornamental crayfish. *Knowledge and Management of Aquatic Ecosystems*, 414: 13.
- Patoka, J., Wardiatno, Y., Ali, M., Yonvitner, Daisy, W., Jerikho, R., Takdir, M., Purnamasari, L., Petrůl, M., Kalous, L., Kouba, A. and Bláha, M. (2018b). Redclaw crayfish, *Cherax quadricarinatus* (von Martens, 1868), widespread throughout Indonesia. *BioInvasions Records*, 7 (2): 185-189.
- Patoka, J., Wardiatno, Y., Yonvitner, Kuřiková, P., Petrůl, M. and Kalous, L. (2016). *Cherax quadricarinatus* (von Martens) has invaded Indonesian territory west of the Wallace Line: evidences from Java. *Knowledge and Management of Aquatic Ecosystems*, 417 (39): 1-6.
- Putra, M. D., Bláha, M.; Wardiatno, Y., Krisanti, M., Jerikho, R., Kamal, M. M., Mojžišová, M., Bystřický, P. K., Kouba, A., Kalous, L., Petrušek, A. and Patoka, J. (2018). *Procambarus clarkii* (Girard, 1852) and crayfish plague as new threats for biodiversity in Indonesia. *Aquatic Conservation*, 28 (6): 1434-1440.
- Riek, E. (1951). The freshwater crayfish (Family Parastacidae) of Queensland, with an appendix describing other Australian species. *Records of the Australian Museum*, 22 (4): 368-388.
- Roux, J. (1914). Über das Vorkommen der Gattung Cheraps auf der Insel Misool. *Zoologischer Anzeiger*, 44: 97-99.
- Souty-Grosset, C., Anastácio, P. M., Aquiloni, L., Banha, F., Choquer, J., Chucholl, C. and Tricarico, E. (2016). The red swamp crayfish *Procambarus clarkii* in Europe: impacts on aquatic ecosystems and human well-being. *Limnologica*, 58: 78-93.
- Stern, D. B., Breinholt, J., Pedraza, C., López-Mejía, M., Owen, C. L., Bracken-Grissom, H., Fetzner, J. W. and Crandall, K. A. (2017). Phylogenetic evidence from freshwater crayfishes that cave adaptation is not an evolutionary dead-end. *Evolution*, 71 (10): 2522-2532.
- Svoboda, J., Mrugała, A., Kozubíková-Balcarová, E. and Petrušek, A. (2017). Hosts and transmission of the crayfish plague pathogen *Aphanomyces astaci*: a review. *Journal Fish Diseases*, 40 (1): 127-140.

- Vodovsky, N., Patoka, J. and Kouba, A. (2017). Ecosystem of Caspian Sea threatened by pet-traded non-indigenous crayfish. *Biological Invasions*, 7: 2207-2217.
- Weiperth, A., Gál, B., Kuříková, P., Langrová, I., Kouba, A. and J. Patoka. (2019). Risk assessment of pet-traded decapod crustaceans in Hungary with evidence of *Cherax quadricarinatus* (von Martens, 1868) in the wild. *North-West Journal of Zoology*, 15: e171303.





*Chapter 3*

**MORPHOMETRIC AND BEHAVIORAL  
DIVERGENCE IN *PROCAMBARUS*  
*ACANTHOPHORUS* INHABITING  
IN A COASTAL PLAIN WETLAND  
AND A MOUNTAIN PINE FOREST**

***Leonor Mendoza-Vargas<sup>1,\*</sup>, Fabiola Galicia-Mendoza<sup>1</sup>,  
Gabriel Arriola-Pizano<sup>1</sup>, Elizabeth Guarneros-Bañuelos<sup>2</sup>,  
Pilar Ortega<sup>3</sup>, Zuly A. Sánchez-Florentino<sup>4</sup>,  
Santa Rodríguez-Lorenzo<sup>5</sup>, Edgar Flores-Soto<sup>6</sup>,  
Bettina Sommer<sup>7</sup>, Marcela Valdés-Tovar<sup>4</sup>  
and Héctor Solís-Chagoyán<sup>4</sup>***

<sup>1</sup>Departamento El Hombre y su Ambiente, Universidad Autónoma  
Metropolitana Unidad Xochimilco, CDMX, México

<sup>2</sup>Departamento de Fisiología, Escuela Nacional de Ciencias Biológicas,  
Instituto Politécnico Nacional, CDMX, México

---

\* Corresponding Author's E-mail: leonormendozavargas@yahoo.com.mx.

<sup>3</sup>Departamento de Física y Química Teórica, Facultad de Química, Universidad Nacional Autónoma de México, CDMX, México

<sup>4</sup>Laboratorio de Neurofarmacología, Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, CDMX, México

<sup>5</sup>Centro de Investigación e Innovación Integral Empresarial Mar Bermejo, S.C. CDMX, México

<sup>6</sup>Departamento de Farmacología, Facultad de Medicina, Universidad Nacional Autónoma de México, CDMX, México

<sup>7</sup>Departamento de Investigación en Hiperreactividad Bronquial, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, CDMX, México

## ABSTRACT

*Procambarus acanthophorus* is an endemic secondary burrower distributed in the Gulf of Mexico slope in wetlands at the sea level. In these habitats, *P. acanthophorus* survives the annual dry season, sheltered in deep tunnels built by obeying their own burrowing behavior. Unexpectedly, populations of this crayfish species were found in a mountain pine-oak forest from Hidalgo, Mexico at 1979 m.a.s.l. Female and male crayfish collected from both the wetland and forest were maintained under laboratory conditions and hatched progeny became available. This fact allowed us to determine whether progeny from these contrasting ecosystems exhibits similitude or divergence in the growth rate as well as in ontogeny of its burrowing behavior. The morphometry and burrowing activity of 15 crayfish hatched by environment, in juvenile stage, were evaluated for 6 months. Total length and weight were measured every 2 or 3 days. On the other hand, for ethological study, juveniles were kept individually in aquaria filled with wetland's sediment. Results showed that in the progeny from Hidalgo, biometric parameters in 6-month-old crayfish were 2-fold higher than Veracruz offspring. All 10-day-old juveniles began to perform behavioral patterns related to burrowing. At the first stage, progeny from wetland made small circular grooves using its telson, whereas juveniles from the forest predominantly pushed the sediment to make grooves with their chelipeds. At the second stage, all crayfish made small balls of sediment and dispersed them in the aquarium. At the third stage, 4-week-old offspring from the forest could remove sediment and digging shallow pools; in contrast, crayfish from wetland built short tunnels with small diameter. At the fourth stage, 16-week-old progeny from Hidalgo removed more sediment to form deeper pools and

excavated small tunnels; on the other hand, offspring from the wetland made deeper tunnels with one or two interconnected entrances. The burrowing patterns performed by adult crayfish from the wetland is more like that expressed by 6-month-old progeny from Hidalgo, but shelters built by these juveniles had poor stability and burrows frequently collapsed; these refuges gained stability after they were re-built several times. Results showed divergence in both phenotypic traits and behavioral patterns. Regarding burrowing activity, the availability of adequate sediment to dig, apparently induced the execution of a consistent genetic neuronal program to develop the burrowing behavior in juveniles; however, the ecological history seemingly determined the growth rate as well as the sequence and final consolidation of this plastic behavior.

**Keywords:** morphometry, burrowing behavior, crayfish juvenile stage, divergence, forest, *Procambarus acanthophorus*, wetland

## 1. INTRODUCTION

### 1.1. Family Cambaridae Generalities

In Mexico, 57 cambarids have been described; 56 species are included in the genera *Procambarus* (Ortmann, 1905) or *Cambarellus* (Ortmann, 1905) and one remaining belongs to the genus *Orconectes* (Cope, 1872) (Álvarez et al., 2012). Hobbs (1984) hypothesized that Mexican crayfish originated in southern United States, since the largest number of *Procambarus* species in the American continent can be found in this area and a considerable number are endemic (Hobbs, 1984; 2001). He stated that ancestral species might have dispersed from north to middle America through two migratory routes: crayfish might have arrived at north-central Mexico and the Pacific slope including the states of Nayarit, Sinaloa, Jalisco and Michoacán; the second route might have followed along the Gulf of Mexico slope (Hobbs, 1984; Álvarez et al., 2012). Nowadays, three areas of distribution can be distinguished in Mexico considering the geographical relief and the reports of crayfish presence. The Gulf of Mexico slope is the most diverse area considering two criteria: the species richness and their short-range of endemism. The second most important area is the Trans-

Mexican Volcanic Belt that goes across the medial transverse axis in the country. Finally, the third zone is the Pacific slope and represents the lowest in Cambarid richness (Álvarez et al., 2012).

In Mexico, the genus *Procambarus* is the most abundant and is composed by 45 species (Armendariz, 2011). This taxon has a broad distribution range regarding the altitude gradients and ecosystems. Their populations can be found in habitats at the sea level such as wetlands or marshes, but also in different mountain forest habitats such as oak, pine-oak, pine and cloud forest in altitudes between 300 and above 3,000 m.a.s.l (Álvarez and Villalobos, 2015; Hobbs, 1989). Despite this distribution pattern, it has been considered that crayfish are benthic organisms with a poor ability to disperse and most of them have a low fecundity rate and a “K” population growth strategy (Armendariz, 2011). This might explain why most of *Procambarus* crayfish in Mexico have a restricted distribution and this can be considered as a short-range endemic taxon. Furthermore, it is unusual that populations of two species overlap inhabiting the same locality or water body (Armendariz, 2011). Only three *Procambarus* crayfish species present an extensive geographic distribution range covering several states of Mexico, maybe because of their reproductive biology and/or their ecological colonization success. These crayfish are: *P. clarkii* (Girard, 1852) that is distributed in several continents; their dispersion has been related to their introduction to aquaculture (Martín-Torrijos et al., 2018; Yi, 2018; Jin et al., 2019). Also, *P. llamasi* (Villalobos, 1954) has been collected in the states of the Yucatan peninsula, Veracruz and Campeche (Rodríguez-Serna et al., 2002; Armendariz, 2011) and meanwhile, the crayfish *P. acanthophorus* (Villalobos, 1948) shows a wide distribution as will be described below.

*Procambarus* species inhabit lotic and lentic freshwater systems such as rivers or streams (principally in pools) as well as lakes, lagoons, ditches or dams, respectively. However, they never occupy an ecological niche in the cause of rivers with fast current (Armendariz, 2011). Crayfish might be grouped in accordance to different ecological traits; for instance, they have been classified as epigeal living in continental water bodies and hypogean or stygobitic that lives in caves. Additionally, in accordance with Hobbs

(1981), they have been considered as stream dwellers, as inhabitants of lakes, ponds, or ditches, as burrowers and as cave dwellers. Regarding burrower species, they have been subgrouped in primary, secondary and tertiary. The primary burrowers spend almost their entire life underground in their built refuges; on the other hand, secondary burrowers spend much of their lives in burrows that can or not reach the water table during the dry season (Hobbs, 1981), however they move into open water during the rainy season. Tertiary burrowers live almost their entire life in open waters (Hobbs, 1981). In Mexico, a limited number of primary or secondary burrowers have been reported. Among them, *P. acanthophorus* shows burrowing behavior and digs tunnels that vary in depth and complexity with chimney (Mendoza-Vargas et al., 2016; Viccon-Pale et al., 2016).

The burrowing behavior in crayfish is a stereotyped sequence of movements and postures required to build structured refuges (Hobbs, 1981). This behavior involves the movement of several thoracic and pleonal appendages and other anatomical structures such as telson and uropods (Grow, 1981; Hobbs, 1981). In addition, the performance of stereotyped behaviors depends on neuronal circuits (Yeh et al., 1997; Edwards et al., 2003) and their modulation by neurotransmitters such as serotonin or octopamine (Yeh et al., 1996; Momohara et al., 2013), as well as by neurohormones such as melatonin (Mendoza-Vargas et al., 2018). Hence, the execution of the complex movement sequences by juvenile crayfish needs the ontogenetic development of the musculature and the neuronal circuits to display and coordinate these stereotyped behaviors (Edwards et al., 2003). For instance, the maturation of the thoracic and pleonal appendages as well as telson and uropods occurs in the post-embryonic developmental stages (Vogt, 2008); furthermore, certain neurons involved in a circuit such as the lateral giant neuron involved in the escape response of the agonist behavior, can only be used when completely developed at the beginning of the juvenile stage (Edwards et al., 1994). The corporal consolidation of muscles and appendages along the ontogeny is strongly related to the proliferation of neuronal precursors (Sullivan and Macmillan, 2001), the survival of *de novo* formed neurons (Song et al., 2007) and the establishment of the neuronal circuits that coordinate the motor patterns in

the central nervous system (Sullivan and Macmillan, 2001). These traits are necessary for crayfish to acquire complex stereotyped behaviors such as the social agonistic encounters or the burrowing of shelters (Sato and Nagayama, 2012). In this sense, the use of developed appendages in juvenile stages parallels with the plasticity of the brain and peripheral motor systems (Song et al., 2007). In addition, the biotic (ecological resources and social cues) or the abiotic environment might influence the form in which juvenile crayfish learn and improve these behaviors and this improves their brain plasticity (Sandeman and Sandeman, 2000; Patoka et al., 2019). More attention has been given to the determination of the ontogeny of the agonist behavior by which the social hierarchy is established in some species such as *P. clarkii* (Sato and Nagayama, 2012; Herberholz et al., 2016; Patoka et al., 2019); however, little information exists regarding the acquisition of the burrowing behavior by cambarid juveniles of primary (Grow, 1981) or secondary burrowers.

## **1.2. *Procambarus acanthophorus* Generalities**

*Procambarus acanthophorus* is an endemic cambarid classified as a secondary burrower (Álvarez et al., 2005; Barba-Macías et al., 2015; Mendoza-Vargas et al., 2016; Viccon-Pale et al., 2016). Information regarding the geographical distribution of this species is scarce but localities where this crayfish have been found are coastal plains and wetlands. In this regard, this mexican species has been collected in several states of Mexico's southeast such as Veracruz, in wetland ecosystems at the sea level, for instance in La Mixtequilla, Tlacotalpan (Viccon-Pale et al., 2016), Texistepec and Mandinga lagoon (Carrillo-Pavón, 2016); also in Chiapas State, where a controvertial caught was documented in Emiliano Zapata lagoon at 40 m of altitude (Hobbs, 1989); in Oaxaca State, this species was described to inhabit "El Castillo", a locality near Tuxtepec at 20 m.a.s.l. (Villalobos, 1949); finally, in the central region of Tabasco State at altitudes ranging between 0 and 80 m.a.s.l (Barba-Macías et al., 2015).

Some ecological traits of *P. acanthophorus* have been studied in the wetlands of Veracruz State (Mendoza-Vargas et al., 2016; Viccon-Pale et al., 2016). This ecosystem presents an annual fluctuation in the rain regime, with a clear rainy season during summer and a dry season the rest of the year. This weather change has profound impacts in the groundwater level. During the rainy season the soil is flooded, whereas in drought the groundwater level drops and the soil dries. This secondary burrower has adapted to this fluctuating environment and the dynamic of its populations is strongly influenced by the changes in this abiotic factor (Viccon-Pale et al., 2016). To survive, *P. acanthophorus* build deep tunnels following a complex burrowing behavior to reach the water table during the dry season. This behavior is controlled by the circadian system and is synchronized by the day-night cycle (Mendoza-Vargas et al., 2016). In addition, evidence has suggested that there is a strong relationship between the physiological regulation of the burrowing activity rhythm and the availability of adequate substrate to dig (Mendoza-Vargas et al., 2016; Mendoza-Vargas et al., 2018). Unexpectedly, we found this species living in a pine-oak forest in a mountain zone in the Sierra Madre Oriental. In this site, the necessity to dig tunnels is absent because *P. acanthophorus* inhabits perennial streams. This fact was intriguing regarding the growth and the ontogeny of the burrowing behavior displayed by this crayfish inhabiting two contrasting ecosystems, taking into account the differences in the ecological history of the predecessors.

*Procambarus acanthophorus* has been considered a potential aquaculture product as well as *P. clarkii* (Yi, 2018; Jin et al., 2019). In this way, the reproductive biology of this burrower species has been extensively studied to establish hatcheries with native breeding (Cervantes-Santiago et al., 2010). Results of these works have suggested that in captivity, *P. acanthophorus* lifespan is approximately two years and it might reach 10 cm length (from rostrum to telson). Additionally, the survival rate of the embryos in this species is close to 70%, but survival depends on the females' age. In this sense, if size is considered as an age indicator (although

imprecise), animals between 4 and 6 cm might reach an egg viability close to 100%. Contrastingly, embryos survival is not observed in eggs from females up to 6.6 cm (Cervantes-Santiago et al., 2010). Evidently, eggs' viability decreases as females' age advances.

Early development of *P. acanthophorus* has been characterized in detail by the group of Hernández-Vergara (Cervantes-Santiago et al., 2010; Hernández-Vergara et al., 2012). Whole development, from fertilization to the juvenile stage, takes approximately 25 days and 9 embryonic stages have been recognized. During this process, the eggs size augments 600 times. Hatching lasts 10 or 15 min and in this process, the chorion breaks and the post-embryonic crayfish are released. To reach the final juvenile stage, 4 post-embryonic developmental stages must be undertaken and this process lasts about 10 days (Hernández-Vergara et al., 2012). During these post-hatching stages, crayfish develop some important digging structures such as the telson, uropods, pereopods (thoracic appendages) and pleopods (pleonal appendages), but progeny remain attached to the mother's pleura or thoracic and pleonal appendages. This species present only one juvenile stage of short duration and at this developmental phase, crayfish are more independent and begin to explore the environment separated from their mother (Hernández-Vergara and Pérez-Rostro, 2012). Nonetheless, this is a burrower species that builds deep shelters that have a positive effect on soil properties used in local agriculture; furthermore, it is an important local food resource, but little attention has been paid to the study of this species' biological and ecological traits, including the ontogeny of its burrowing behavior.

This chapter focuses on two issues: the first is to confirm through taxonomic definition that specimens found in the wetland or in the mountain forest are *P. acanthophorus*. Additionally, the acquisition (ontogeny) of the behavioral patterns to build burrows in progeny hatched under laboratory conditions was analyzed. Moreover, the comparison of the sequence followed by juveniles whose parents inhabit either the ecological context of the wetland or the forest ecosystems was performed.



## 2. MATERIAL AND METHODS

### 2.1. Collection Sites

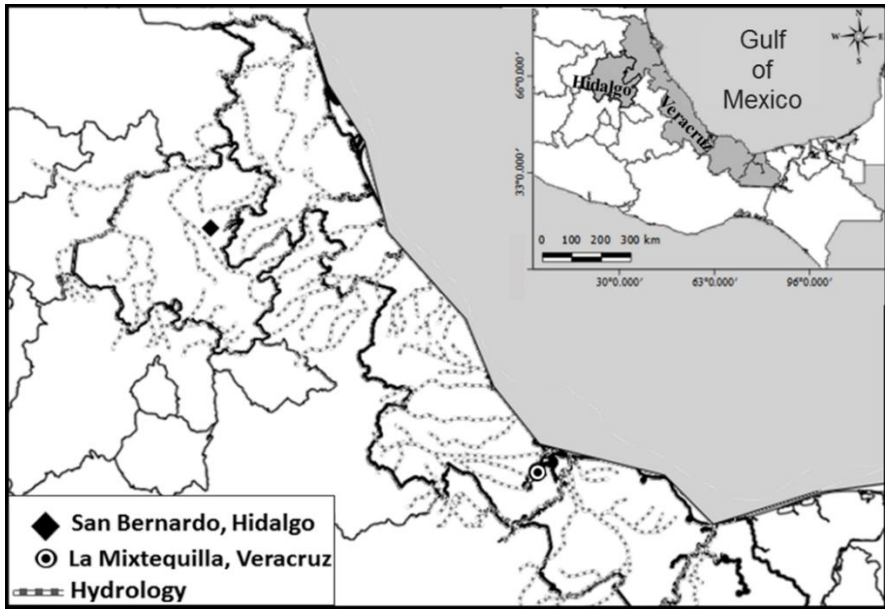


Figure 1. Collection sites in Hidalgo and Veracruz States, Mexico. The rivers close to these sites are indicated on the map.

Specimens were collected from two different sites in Mexico. The first site is located at Veracruz State in a wetland ecosystem, whereas the second site is located in Hidalgo State in a mountain pine-oak forest. Regarding the place in the coastal plain of Veracruz, the wetland “La Mixtequilla” is located in the municipality of Ignacio de la Llave ( $18^{\circ} 32' 24''$  N;  $95^{\circ} 57' 0.1''$  W) at the sea level (Figure 1). This site is a protected area in the Ramsar site “Alvarado Lagoon System” that is considered the third-largest wetland in Mexico (Comisión Nacional para el Conocimiento y el Uso de la Biodiversidad [CONABIO], 1998). The predominant climate is tropical humid, the annual mean precipitation is 1200 mm and mean temperature is  $26^{\circ}\text{C}$ . The soil types in this site are gleysols (these are formed under flood conditions produced by rising groundwater) and vertisols (Instituto Nacional

de Estadística y Geografía, 2017). The dominant vegetation in La Mixtequilla wetland is the weed *Cyperus articulatus* L. (Cyperaceae) and the shrub *Mimosa pigra* L. (Fabaceae); these plants are native and perennial species that are well represented in the neotropical Atlantic region of Mexico (Rzedowski, 1986). Adult male and female crayfish were collected during the rainy season in a periodically flooded pastureland and brought to laboratory for experimentation.

The mountain site is located at Hidalgo State (20° 37' 50.58" N, 98° 39' 59.13" W) at 1979 m.a.s.l (Figure 1). This area lies close to the San Bernardo town in Zacualtipan de los Angeles municipality, in the western side of the Sierra Madre Oriental. The predominant climate is temperate subhumid, the annual mean precipitation and temperature are 800 mm and 16°C respectively. The edaphology of the area is complex because of the geographical relief; predominant soil types are cambisols (their surface horizon could range in similar areas between 25 and 50 cm), luvisols (these might form on flat or gently sloping landscapes and are rich in organic material), as well as regosols (these form in steep sloping with a shallow surface horizon and contain large amounts of gravel). The principal vegetation type found here is pine-oak forest with predominancy of *Pinus patula*, *Quercus crassifolia*, *Q. laurina*, *Abies religiosa*, *Liquidambar styraciflua*, *Mimosa biuncifera*, *Cynodon plectostachyum*, *Digitaria decumbens*, *Muhlenbergia aff. plumbea* and *Bouteloua gracilis* (Instituto Nacional de Estadística y Geografía, 2017). Adult male and female crayfish were captured between the rocks in lentic pools of perennial streams found in the forest.

## 2.2. Taxonomic Identification

Adult male crayfish exist in two forms named I or II; the former is the primary reproductive type, with the first pair of pleopods (pleonal appendages) fully developed, large quelae and spines on the ischiopodites of the third and fourth pairs of walking legs. In this male type, the number, form and position of the apical structures in the gonopods (first pair of

pleopods) are primordial to determine the Cambarid species. Form II is a non-reproductive type with smaller quelae and no spines on the ischiopodites (Laufer et al., 2005). In addition to catch living crayfish for experimentation, Form I male crayfish were also collected in both sites for taxonomic identification. Animals were fixed in 70% ethanol to preserve the morphological traits used for taxonomy. Specimens were observed under a stereoscopic Zeiss microscope (mod. Stemi 305) and photographs were acquired by a Zeiss camera (mod. Axiocam ERc 5s) and digitallized with the Zeiss Labscope imaging software. Identification was performed following taxonomic keys defined specifically for middle America cambarids (Hobbs, 1976; Rojas, 1998; Villalobos, 1998).

### **2.3. Laboratory Conditions**

Adult female *P. acanthophorus* between 4 and 6 cm long and adult males were collected at the described sites and brought to the laboratory to obtain the progeny. Animals were kept in confinement and grouped in aquaria with sediment collected from the wetland. Under these conditions, 3 ovigerous female crayfish by site were randomly chosen for experimentation.

### **2.4. Biometric Measurements**

For biometric measurements, 10 juveniles crayfish were randomly selected every two or three days and biometric traits were measured. Total length from the rostrum to the telson was determined with a Vernier caliper (0.1mm accuracy), while weight was registered with a calibrated balance (0.001g accuracy). Data were averaged and the mean vs. the crayfish age were plotted. A correlation analysis was performed to establish the relationship between the total length and weight.

## 2.5. Burrowing Behavior Studied through Video Recordings

When progeny reached the juvenile stage and parted from their mothers, (5 animals per female; 15 animals by site) crayfish were individually and randomly separated in an aquarium of 6.5 x 4 x 3 cm (length, height, width), with wetland sediment (2 cm) at the bottom, saturated with water (0.5 cm above the sediment level). Crayfish were kept under a photoperiodic light-dark regimen (L-D 12:12, lights on at 7 am) and they were fed twice a week with pellets for shrimps (Purina™).

Ontogeny of burrowing behavior was studied by video recordings using a digital Nikon videocamera (mod. D3400). The camera was set above the aquarium to follow the displayed burrowing patterns, but also alongside it to compare the progression in complexity of the tunnels dug by juveniles. The activity was recorded daily for six months during the photophase, considering that juvenile crayfish *P. clarkii* can present a diurnal rhythm in their locomotor activity (Fuentes-Pardo et al., 2001). Recordings were analyzed to describe the main behavioral patterns acquired progressively by juvenile *P. acanthophorus*. Then, a comparison between the activities developed by progeny of crayfish collected from wetland or the pine forest was established.

## 3. RESULTS AND DISCUSSION

### 3.1. Identification

*Procambarus acanthophorus* has been found distributed in Mexico, in flooding ecosystems at the sea level in the Gulf of Mexico slope; however, some populations of this crayfish were unexpectedly found in a mountain zone in the Sierra Madre Oriental. To corroborate the identity of the crayfish collected at these sites, taxonomic keys were used. In both cases, crayfish corresponded to *P. acanthophorus* (Figures 2-9).

These crayfish presented cylindrical body like lobster, crab or hermit crab and this morphological trait corresponds to suborder Pleocyemata. In

addition, the third pair of thoracic appendages had hooks (Figure 2) and this trait corresponds to family Cambaridae.

As shown in Figure 3, ischium of the second pair of pereopods did not bear hooks and crayfish possessed pigmented body and faceted pigmented eyes.

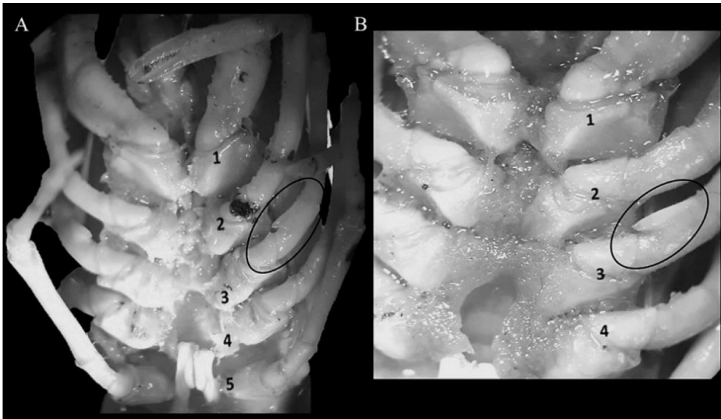


Figure 2. Ventral view of the cephalothorax. Pereiopods pairs are numbered and the hook in the third pair is highlighted by an oval. A) Crayfish collected in San Bernardo, Hidalgo; B) Crayfish collected in La Mixtequilla, Veracruz. Photographs by Fabiola Galicia-Mendoza.

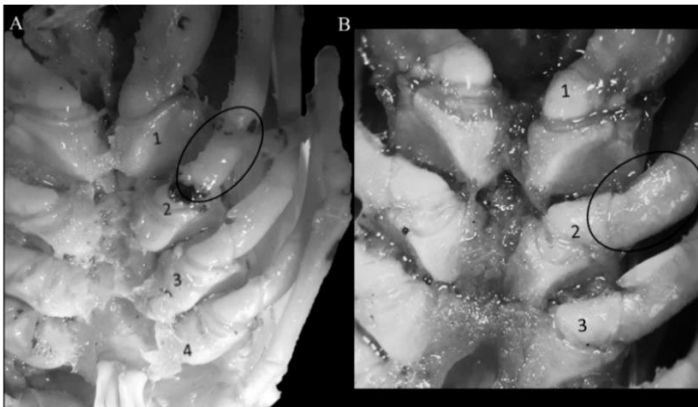


Figure 3. Ventral view of the cephalothorax. Basal segment of left pereopods of organisms collected in A) San Bernardo, Hidalgo or B) La Mixtequilla, Veracruz. Photographs by Fabiola Galicia Mendoza.

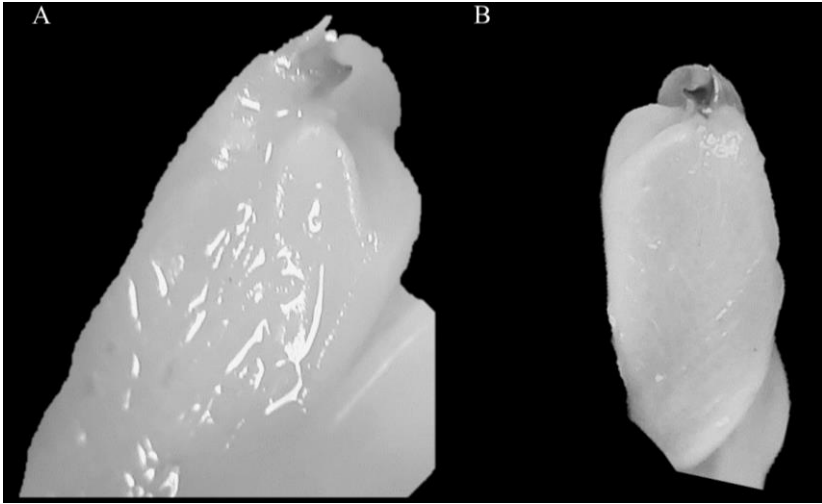


Figure 4. Lateral view of the first pair of right pleopods. A) Organism collected in San Bernardo, Hidalgo. B) Crayfish collected in La Mixtequilla, Veracruz. Photographs by Fabiola Galicia Mendoza.

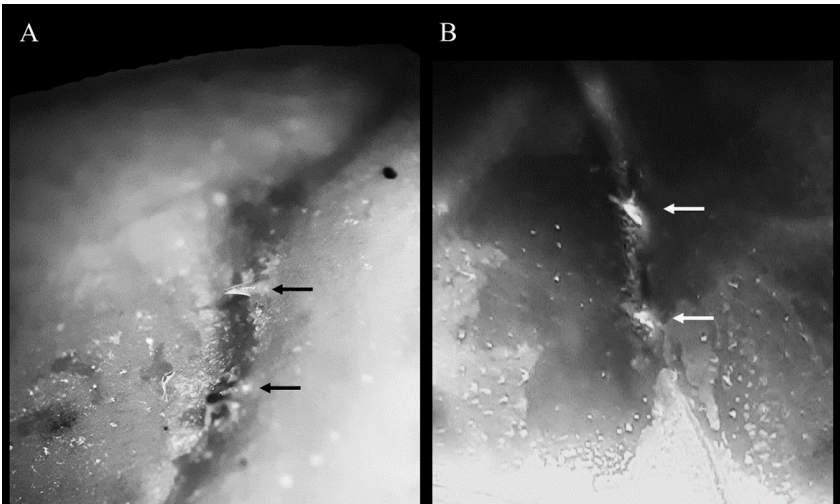


Figure 5. Lateral view of the carapace showing the presence of cervical spines. Black or white arrows indicate the spines. A) Crayfish collected in San Bernardo, Hidalgo or B) in La Mixtequilla, Veracruz. Photographs by Fabiola Galicia Mendoza.

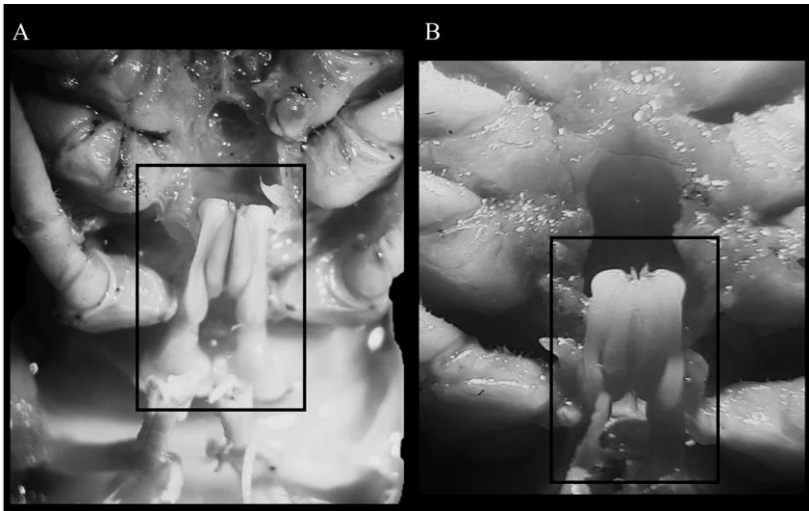


Figure 6. Ventral view of the basal portion of the first pair of pleopods. A) Crayfish collected in San Bernardo, Hidalgo or B) in La Mixtequilla, Veracruz. Photographs by Fabiola Galicia Mendoza.

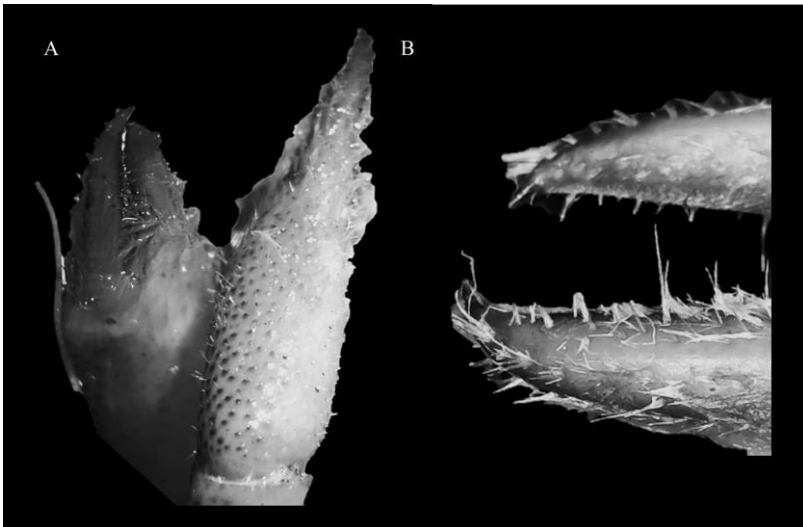


Figure 7. Lateral view of the quelae. A) Crayfish collected in San Bernardo, Hidalgo or in B) La Mixtequilla, Veracruz. Photographs by Fabiola Galicia Mendoza.

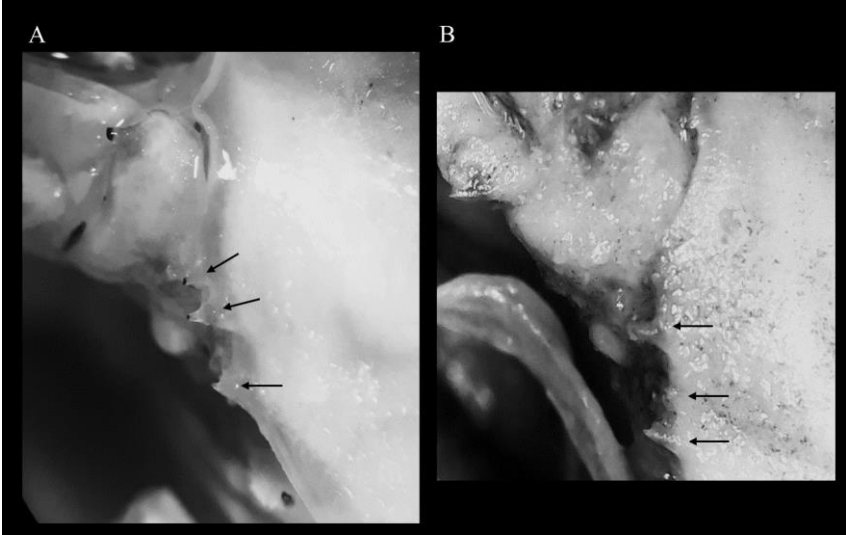


Figure 8. Lateral view of a portion of the face with suborbital angle. Arrows indicate the spines. A) Crayfish collected in San Bernardo, Hidalgo or b) in La Mixtequilla, Veracruz. Photographs by Fabiola Galicia Mendoza.

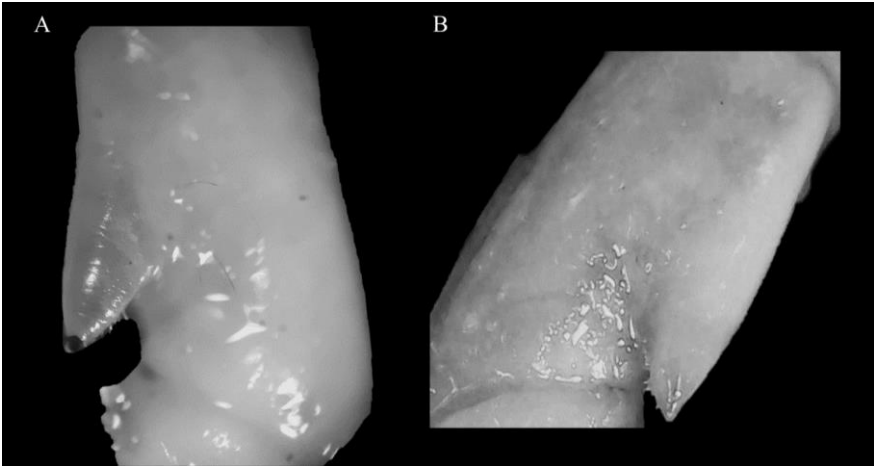


Figure 9. Caudoventral view of the basal portions of the third pereopod. A) Crayfish collected in San Bernardo, Hidalgo or B) in La Mixtequilla, Veracruz. Photographs by Fabiola Galicia Mendoza.

In these *Procambarus*, the first pair of pleopod (gonopod) terminated in more than two elements and the central projection of the first pleopod was



seldom blade-like (Figure 4). Additionally, carapace presented two cervical spines (Figure 5).

The first pair of pleopods in these crayfish was symmetrical (Figure 6) and their quelae presented conspicuously pubescent fingers and palms (Figure 7).

In addition, they possessed three spines in the suborbital angle (Figure 8) and the tip of their hooks on the ischium of the third pereopod did not extend proximally to the basioischial articulation (Figure 9).

Considering all these morphological traits, results of the taxonomic identification strongly suggest that the organisms collected in both La Mixtequilla wetland in Veracruz State and in the oak-pine forest in Hidalgo State are *P. acanthophorus*. This taxonomic determination assumes that morphological traits are adequate criteria to determine the crayfish species. Taxonomy based in morphology might not necessarily reflect adequate phylogenetic relationships between cambarids (Breinholt et al., 2012; Álvarez and Villalobos, 2015). However, this tool has been extensively used to get a precise determination of the identity of known crayfish species; even nowadays taxonomic keys are used to describe new cambarid species (Johnson, 2018; Lukhaup et al., 2018; Glon et al., 2019).

As previously mentioned, *P. acanthophorus* had been found inhabiting the coastal plains in the Gulf of Mexico slope in flooding habitats at the sea level. This crayfish has been adapted to this environment by the execution of the burrowing behavior (Viccon-Pale et al., 2016). However, some populations of this species were collected in a forest ecosystem in environmental conditions completely different regarding altitude, soil, weather and rain regime. In the wetland, crayfish live in an annual fluctuating flooding environment and during the dry season they reach the water level by building tunnels. Contrastingly, the crayfish found in the mountainous site live in perennial streams between rocks found in lentic pools with slow current. Apparently, crayfish from the forest do not need to dig burrows to survive; however, a study of population ecology is required to determine the relationship between the mentioned abiotic factors and the dynamic of its populations.

### 3.2. Biometric Comparison

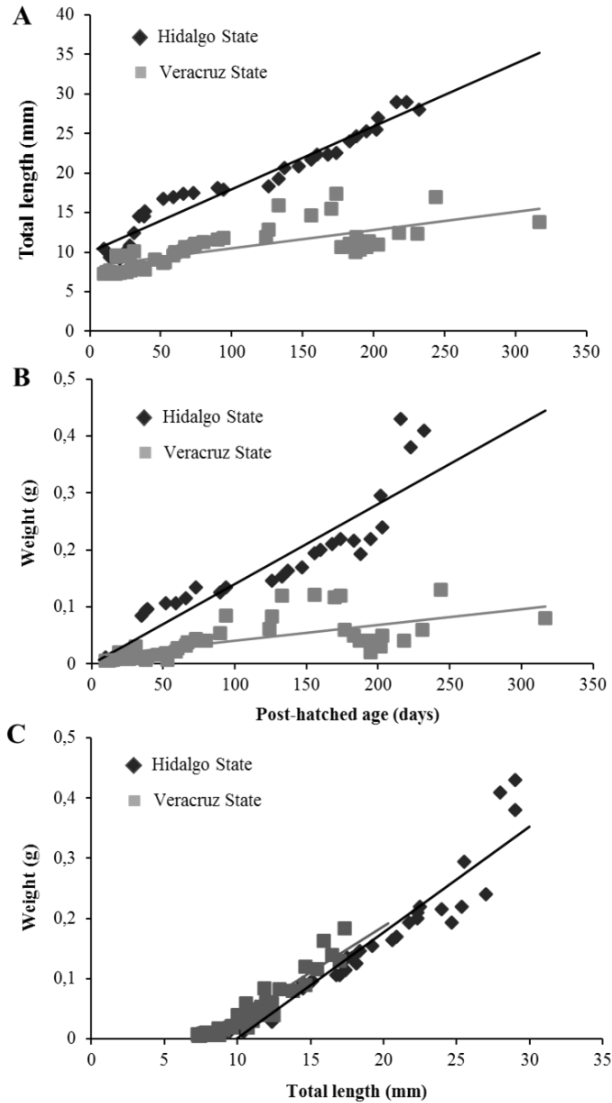


Figure 10. Biometric measurements of juvenile progeny of crayfish collected in Hidalgo State or Veracruz State, Mexico. Crayfish's age was considered from the day of hatching on. A) Linear correlation of length vs. age. B) Linear correlation of weight vs. age. C) Length vs. weight correlation.

Some crayfish species such as *P. clarkii* present a broad geographic distribution range. This fact has allowed the study on how the phenotypic expression is influenced by environment gradients by comparing morphological, physiological, behavioral or ecological traits (Evangelista et al., 2019). Because *P. acanthophorus* was found in an unexpected ecosystem, two corporal parameters were measured hypothesizing that the ecological context affects the expression of phenotypic traits in this crayfish. Biometry performed in juvenile progeny hatched under laboratory conditions showed that forest's crayfish were larger since the beginning and grew faster during their early development (Figure 10A and 10B). The length and weight of these juveniles at day 210 (7-month-old) almost doubled the values for wetland's crayfish (Figure 10A and 10B). However, the length-weight relationship showed a linear correlation between these parameters, with a similar slope between sites (Hidalgo:  $r^2 = 0.9$ ; slope = 0,017. Veracruz:  $r^2 = 0.91$ ; slope = 0.014) (Figure 10C).

Results indicated an evident difference in the growth rate of the progeny of females inhabiting the forest or wetland habitats. The morphometric divergence found in *P. acanthophorus* could involve a high level of plasticity in the expression of gene products that control the growth process. Apparently, in *P. acanthophorus* the final phenotype derived from this plasticity might be dependent on the environmental context. In addition, considering that the weight gain was a linear function of the corporal elongation in the compared groups of this crayfish, research will be necessary to determine whether changes in specific anatomical structures (such as quelaes or the pleonal segments) present an isometric or allometric growth related to total length. Examples of both growth types have been reported in crayfish species (Geddes et al., 1988; Daveikis et al., 1996; Gutiérrez-Yurrita and Montes, 1999; Seitz et al., 2005).

### 3.3. Ontogeny of the Burrowing Behavior

On the other hand, the stages to acquire the ability to dig tunnels were evaluated in *P. acanthophorus* juveniles. Four stages were described

regarding ontogeny of burrowing behavior. In the first, juvenile crayfish made small grooves when they explored the surface substrate (Figure 11).

Progeny of *P. acanthophorus* covered the post-embryonic stages approximately in 10 days. Post-embryonic crayfish stay adhered to their mothers' pleopods and pereopods (Hernández-Vergara and Pérez-Rostro, 2012). When they become juveniles, they begin exploring the environment by themselves and, at this point under our experimental conditions, they were separated and kept in an aquarium with sediment collected from the wetland. Juveniles from both collection sites initiated exploring the surface immediately after they were placed in the aquaria. They touched the sediment continuously moving their antennae and quelae. While continuing the exploration, they made small grooves with their pleonal portion and the telson. *P. acanthophorus* from Hidalgo mainly used the quelipeds to touch the soil whereas crayfish from Veracruz used the antennae in greater proportion and marked more complex trails in comparison to Hidalgo's crayfish.

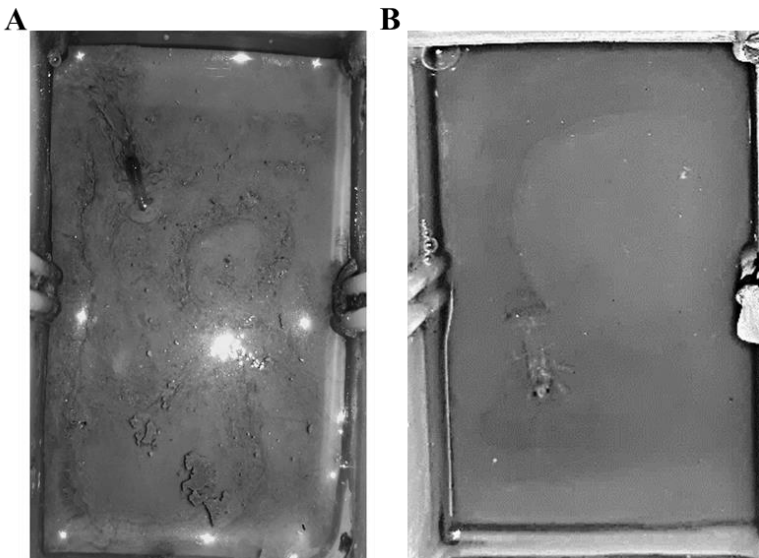


Figure 11. Ten-day-old juvenile *P. acanthophorus* made small grooves. A) An example of grooves made by progeny of females from the wetland in Veracruz State or B) from the forest in Hidalgo State.

At the second stage, crayfish began digging shallow pools and the removed sediment was dispersed in the aquarium surface. They used the chelipeds to heap up balls of sediment (Figure 12). This stage was the part of the ontogeny with more similarities between groups. It could be suggested that this behavior is involved in the learning of chimneys' construction to mark the entrance to the shelters; however, the building of chimneys was not observed at least until the crayfish were 6-month-old.

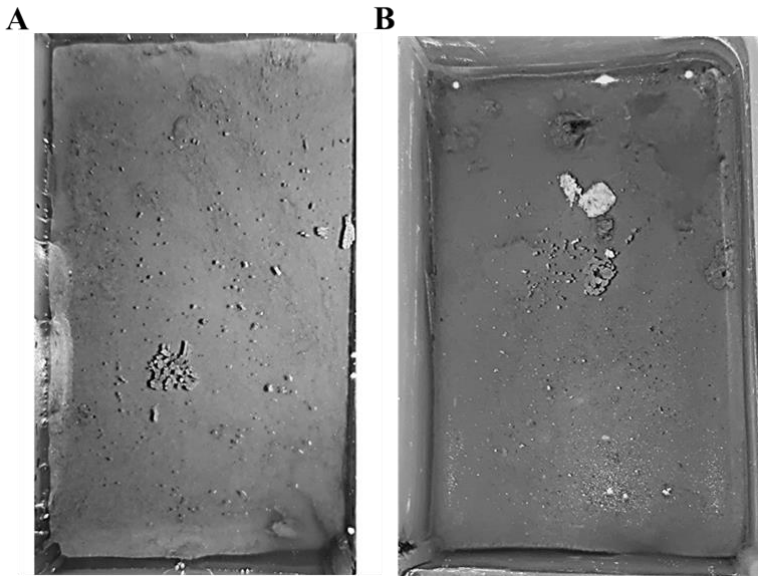


Figure 12. Juvenile *P. acanthophorus* made small balls of substrate and dispersed them in the surface of the aquarium. A) An example of balls made by progeny from Veracruz and B) from Hidalgo.

At the third stage, crayfish removed bigger amounts of sediment. Whereas *P. acanthophorus* from Veracruz made shallow pools and dug short tunnels (Figure 13A), crayfish from Hidalgo made deep pools removing the sediment from one half of the aquarium to the other side (Figure 13B). To dig, crayfish from Veracruz used the pleonal segments and the telson; meanwhile, chelipeds were the structures more used by crayfish from Hidalgo. At this stage and for the first time, crayfish from La Mixtequilla dug tunnels, although they had a short length and small diameter.

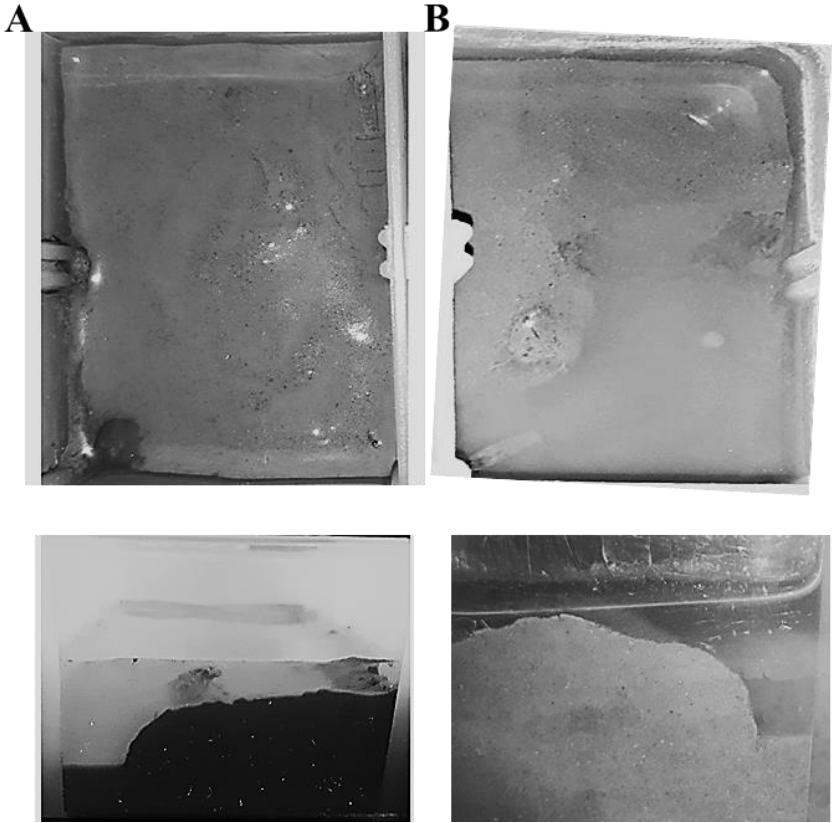


Figure 13. Juvenile *P. acanthophorus* dug tunnels or deep pools. A) An example of digging activity performed by progeny from Veracruz and B) from Hidalgo. Upper panels correspond to upper-view photographs and lower panels to lateral-view photographs.

At the fourth stage, crayfish increased the complexity of the tunnels and progeny spent some time hidden inside the refuges (Figure 14). Crayfish from Veracruz dug long tunnels with one or two interconnected entrances. In addition, in the sediment surface, these crayfish marked grooves with their abdomen also communicating the tunnel's entrances (Figure 14A). On the other hand, crayfish from Hidalgo dug deep pools and posteriorly they built tunnels of larger diameter. Refuges built by these crayfish frequently collapsed; however, after re-building the tunnel several times, they became more stable (Figure 14B).

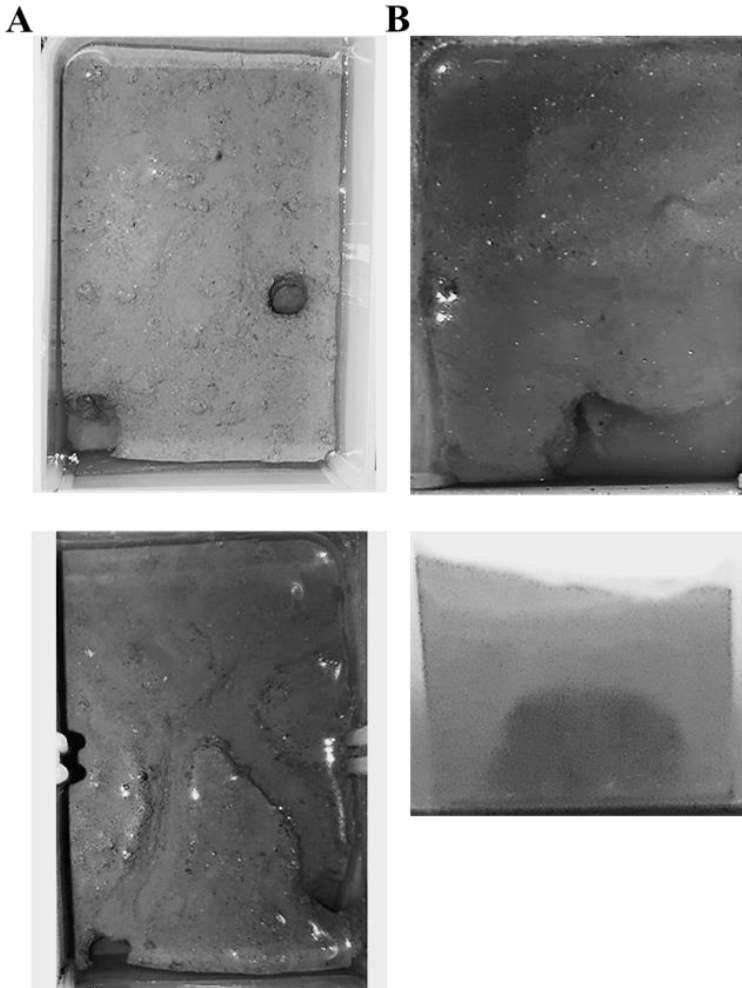


Figure 14. Juvenile *P. acanthophorus* built complex tunnels. A) an example of refuges built by progeny from Veracruz and B) from Hidalgo.

Crayfish whose parents were collected in contrasting habitats but hatched under laboratory conditions showed divergences regarding their growth rate and the burrowing behavior development. These facts were not surprising considering that parents were found inhabiting two ecosystems with remarkable abiotic differences. For instance, weather, rain regime, temperature, but most importantly, the wetland in Veracruz is a lentic habitat that presents annual flooding changes that do not generate any water current.

The rain only induces the rise and fall in the water table. In contrast, crayfish in Hidalgo's forest live in a perennial running water ecosystem. Conceivable, during the rainy season these streams increase drastically in water quantity and current velocity. One natural flood-drought cycle in the forest might represent two periods of crayfish adaptation to lentic-pools, first anticipating the natural episode of fast-running current during the rainy season and a second period during pools re-establishment after the disturbing event.

Annual floods and drought cycles (flow regime) in lotic systems have been characterized as a disturbing abiotic factor that promote adaptations in life history traits such as reproduction cycles or growth (Lytle and Poff, 2004; Bonada et al., 2007; Lytle et al., 2008; de la Fuente et al., 2018). Studies of these effects have shown many examples of these changes in benthic or nektonic animals as well as in riparian plants (Lytle and Poff, 2004). Regarding our issue, in La Mixtequilla wetland, in Veracruz State, crayfish survive by digging large tunnels to reach the water table during the dry season as was previously mentioned. A population dynamic study in this site suggests that progeny of *P. acanthophorus* hatches in January or February, approximately one month before they need to begin building their refuges (Viccón-Pale, 2016). Hence, juvenile population might gain in size and weight at a low rate during the dry season, when they are hidden inside the shelters. After 6 months, around September, when the field is flooded, 20 or 30 mm-juveniles and adult crayfish are found in open waters (Viccón-Pale, 2016). In contrast, crayfish from the forest grew faster and reached reproductive sizes in 6 months under laboratory conditions. The minimal reproductive size in *P. acanthophorus* females is 37 mm (Cervantes-Santiago et al., 2010). We can hypothesize that if population abundance of *P. acanthophorus* in the forest decreases during floods when the current-velocity increases, in the next drought period when lentic pools are re-established, this population might be recovered by a second peak of reproduction and fast growth of juveniles. However, further research is needed to determine the population ecology of *P. acanthophorus* from Hidalgo State.



Interestingly, divergence was observed also in behavior by comparing the digging activity in organisms of the same species but from two dissimilar ecosystems. Ecological pressures through biotic or abiotic factors have been related not only to life history traits but also to trophic niche and behavior (Crews, 2010; Faulk and Dolinoy 2011; Ledón-Rettig et al., 2012; Evangelista et al., 2019). Our results suggest that *P. acanthophorus* is a crayfish with a high level of phenotypic plasticity, understanding this term as the capacity of the biological system to express different phenotypes from the same genome, but matching the best phenotype with a determined ecological context (West-Eberhard, 1989; Pigliucci, 2005; Schmid and Guillaume, 2017). Epigenetic changes have been described as a mechanism that might explain the phenotypic variability regarding environment (Ledón-Rettig et al., 2012). This mechanism modulates life history traits (Elgart et al., 2015; Kappeler et al., 2017; Lo et al., 2018) but also the behavior in invertebrates and vertebrates (Anreiter et al., 2017; Maejima et al., 2018; Merlin and Liedvogel, 2019). Many questions remain elusive and require evidence to obtain a better point of view. For instance, we need to know if the signals that modulate the growth process in this crayfish present similitude or divergence in their level of expression or action regarding populations inhabiting in those contrasting environments. It would also be interesting to know if there are differences in anatomic or physiological traits such as muscle fiber composition in quelae or abdomen and/or in their functioning, as seemingly crayfish from Hidalgo dug by using the chelipeds more often, while crayfish from Veracruz preferred to use the pleonal segments to perform the burrowing behavior.

In conclusion, our data suggest that *P. acanthophorus* is a cambarid that present a broad geographical distribution and has a high level of adaptability by modifying at least morphological and behavioral traits. Additionally, it might be hypothesized that in this crayfish species, predecessors' ecological history determines both growth rate and stages to acquire the burrowing behavior.

## REFERENCES

- Álvarez, F. and Villalobos, J. L. (2015). The Crayfish of Middle America. p. 448-463. In: Kawai, T., Faulkes, Z. and G. Scholtz (eds.). *Freshwater Crayfish: A Global Overview*. First edition. CRC Press, Boca Raton, Florida, USA.
- Álvarez, F., Villalobos, J. L., Armendáriz, G. and Hernández, C. (2012). Relación biogeográfica entre cangrejos dulceacuícolas y acociles a lo largo de la zona mexicana de transición: reevaluación de la hipótesis de Rodríguez (1986). *Revista Mexicana de Biodiversidad*, 83: 1073-1083.[Biogeographic relationship between freshwater and crayfish crabs throughout the Mexican transition zone: reassessment of the Rodríguez hypothesis (1986). *Mexican Journal of Biodiversity*, 83: 1073-1083].
- Álvarez, F., Villalobos, J. L. and Robles, R. 2005. Crustáceos. In: Bueno, J., Álvarez, F. and Santiago, S. (eds). *Biodiversidad del estado de Tabasco*. Instituto de Biología, UNAM; CDMX, México. 386 pag.b[Crustaceans. In: Well, J., Álvarez, F. and Santiago, S. (eds). *Biodiversity of the state of Tabasco*. Institute of Biology, UNAM; CDMX, Mexico. 386 pag.].
- Anreiter, I., Kramer, J. M. and Sokolowski, M. B. (2017). Epigenetic mechanisms modulate differences in *Drosophila* foraging behavior. *Proceedings of the National Academy of Sciences*, 114 (47): 12518-12523.
- Armendáriz, G. Y. (2011). *Patrones de distribución y riqueza de especies de los acociles (Decapoda: Cambaridae) de México*. M.Sc. Thesis, Program in Biological Sciences, National Autonomous University of Mexico, Mexico City, Mexico, 77 pag.[*Patterns of distribution and richness of species of acociles (Decapoda: Cambaridae) of Mexico*. M.Sc. Thesis, Program in Biological Sciences, National Autonomous University of Mexico, Mexico City, Mexico, 77 pag.].
- Barba-Macías, E., Carmona-Osalde, C., Quiñones-Rodríguez, L. and Rodríguez-Serna, M. (2015). Registros nuevos de cambáridos (Crustacea: Cambaridae: Procambarus) en la cuenca del Grijalva-Usumacinta, Tabasco. *Revista Mexicana de Biodiversidad*, 86 (3): 620-

- 628.[New records of cambarids (Crustacea: Cambaridae: *Procambarus*) in the Grijalva-Usumacinta basin, Tabasco. *Mexican Journal of Biodiversity*, 86 (3): 620-628].
- Bonada, N., Rieradevall, M. and Prat, N. (2007). Macroinvertebrate community structure and biological traits related to flow permanence in a Mediterranean river network. *Hydrobiologia*, 589: 91-106.
- Breinholt, J. W., Porter, M. L. and Crandall, K. A. (2012). Testing phylogenetic hypotheses of the subgenera of the freshwater crayfish genus *Cambarus* (Decapoda: Cambaridae). *PLoS One*, 7 (9): e46105.
- Carrillo-Pavón, I. (2016). *Propuesta de manejo del manglar del Sistema Lagunar Mandinga*. B.Sc. Thesis. Graduate Program in Biological Sciences, Universidad Veracruzana, Veracruz, México. 152 pag.[Mangrove management proposal of the Mandinga Lagoon System. B.Sc. Thesis Graduate Program in Biological Sciences, Universidad Veracruzana, Veracruz, Mexico. 152 p.].
- Cervantes-Santiago, E., Hernández-Vergara, M. P., Pérez-Rostro, C. I. and Olvera-Novoa, M. A. (2010). Reproductive performance of the crayfish *Procambarus (Austrocambarus) acanthophorus* Villalobos 1948 under controlled conditions. *Aquaculture*, 308 (1-2): 66-70.
- CONABIO. 1998. *La diversidad biológica de México. Estudio del país*. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. México. [*The biological diversity of Mexico. Country study National Commission for the Knowledge and Use of Biodiversity. Mexico*].
- Crews, D. (2010). Epigenetics, brain, behavior, and the environment. *Hormones* (Athens), 9: 41-50.
- Daveikis, V. F. and Alikhan, M. A. (1996). Comparative body measurements, fecundity, oxygen uptake, and ammonia excretion in *Cambarus robustus* (Astacidae, Crustacea) from an acidic and a neutral site in northeastern Ontario, Canada. *Canadian Journal of Zoology*, 74: 1196-1203.
- de la Fuente, M., Bonada, N., Beche, L., Dahm, C. N., Mendez, P. K., Tockner, K., Uehlinger, U. and Acuna, V. (2018). Evolutionary responses of aquatic macroinvertebrates to two contrasting flow regimes. *Hydrobiologia*, 808: 353-370.

- Edwards, D. H., Fricke, R. A., Barnett, L. D., Yeh, S. R. and Leise, E. M. (1994). The onset of response habituation during the growth of the lateral giant neuron of crayfish. *Journal of Neurophysiology*, 72 (2): 890-898.
- Edwards, D. H., Issa, F. A. and Herberholz, J. (2003). The neural basis of dominance hierarchy formation in crayfish. *Microscopy Research and Technique*, 60 (3): 369-76.
- Elgart, M., Snir, O. and Soen, Y. (2015). Stress-mediated tuning of developmental robustness and plasticity in flies. *Biochimica et Biophysica Acta*, 1849 (4): 462-466.
- Evangelista, C., Olden, J. D., Lecerf, A. and Cucherousset, J. (2019). Scale-dependent patterns of intraspecific trait variations in two globally invasive species. *Oecologia*, 189 (4): 1083-1094.
- Faulk, C. and Dolinoy, D. C. (2011). Timing is everything: the when and how of environmentally induced changes in the epigenome of animals. *Epigenetics*, 6: 791-797.
- Fuentes-Pardo, B., Lara-Aparicio, M. and López de Medrano, S. (2001). On the ontogeny of the motor circadian rhythm in crayfish. *Bulletin of Mathematical Biology*, 63 (2): 353-369.
- Geddes, M. C., Mills, B. J. and Walker, K. F. (1988). Growth in the Australian freshwater crayfish, *Cherax destructor* Clark, under laboratory conditions. *Marine and Freshwater Research*, 39: 555-568.
- Glon, M. G., Thoma, R. F., Daly, M. and Freudenstein J. V. (2019). *Lacunibambarus chimera*: a new species of burrowing crayfish (Decapoda: Cambaridae) from Illinois, Indiana, Kentucky, and Tennessee. *Zootaxa*, 4544 (4): 451-478.
- Grow, L. (1981). Burrowing behaviour in the crayfish, *Cambarus diogenes diogenes* Girard. *Animal Behaviour*, 29 (2): 351-356.
- Gutiérrez-Yurrita, P. J. and Montes, C. (1999). Bioenergetics and phenology of reproduction of the introduced red swamp crayfish, *Procambarus clarkii*, in Doñana National Park, Spain, and implications for species management. *Freshwater Biology*, 42: 561-574.

- Herberholz, J., Swierzbinski, M. E., Birke, J. M. (2016). Effects of Different Social and Environmental Conditions on Established Dominance Relationships in Crayfish. *Biological Bulletin*, 230 (2): 152-164.
- Hernández-Vergara, M. and Pérez-Rostro, C. (2012). Advances in Domestication and Culture Techniques for Crayfish *Procambarus acanthophorus*. p. 217-240. In: Muchlisin, Z. (Ed.) *Aquaculture*, InTech, London, United Kingdom.
- Hobbs, H. H. (1976). *Crayfishes (Astacidae) the North and Middle America*. Environmental Protection Agency, Cincinnati, USA. 173 pag.
- Hobbs, H. H. Jr. (1981). The crayfishes of Georgia. *Smithsonian Contributions to Zoology*, 318: 1-549.
- Hobbs, H. H, Jr. (1984). On the distribution of the crayfish genus *Procambarus* (Decapoda: Cambaridae). *Journal of Crustacean Biology*, 4: 12-24.
- Hobbs, H. H., Jr. (1989). An illustrated checklist of the American crayfish (Decapoda: Astacoidae: Cambaridae: Parastacidae). *Smithsonian Contributions to Zoology*, 480: 1-236.
- Hobbs, H. H., III. (2001). Decapoda. p. 955-1001. In: Thorp, J. H.; Covich, A. P. (eds.) *Ecology and classification of North American freshwater invertebrates*. Second edition, Academic Press.
- Instituto Nacional de Estadística y Geografía (México). (2017). *Anuario estadístico y geográfico de Hidalgo 2017*. Instituto Nacional de Estadística y Geografía. México, INEGI. 671 pag.[*Hidalgo Statistical and Geographic Yearbook 2017*. National Institute of Statistics and Geography. Mexico, INEGI. 671 pag.]
- Jin, S., Jacquin, L., Xiong, M., Li, R., Lek, S., Li, W. and Zhang, T. (2019). Reproductive pattern and population dynamics of commercial red swamp crayfish (*Procambarus clarkii*) from China: implications for sustainable aquaculture management. *Peer J*, 7: e6214.
- Johnson, D. P. (2018). The subgenus *Ortmannicus* (Decapoda: Cambaridae) in Texas, with descriptions of new species. *Zootaxa*, 4468 (1): 1-63.
- Kappeler, L., Clemessy, M., Saget, S., Decourtye, L. and Le Bouc, Y. (2017). Regulation of growth: Epigenetic mechanisms? *Annals of Endocrinology*, 78 (2): 92-95.

- Laufer, H., Demir, N., Pan, X., Stuart, J. D. and Ahl, J. S. (2005). Methyl farnesoate controls adult male morphogenesis in the crayfish, *Procambarus clarkii*. *Journal of Insect Physiology*, 51 (4): 379-84.
- Ledón-Rettig, C. C., Richards, C. L. and Martin, L. B. (2012). Epigenetics for behavioral ecologists. *Behavioral Ecology*, 24 (2): 311-324.
- Lo, N., Simpson, S. J. and Sword, G. A. (2018). Epigenetics and developmental plasticity in orthopteroid insects. *Current Opinion in Insect Science*, 25: 25-34.
- Lukhaup, C., Eprilurahman, R. and von Rintelen, T. (2018). Two new species of crayfish of the genus *Cherax* from Indonesian New Guinea (Crustacea, Decapoda, Parastacidae). *Zookeys*, 769: 89-116.
- Lytle, D. A., Bogan, M. T., Finn, D. S. (2008). Evolution of aquatic insect behaviours across a gradient of disturbance predictability. *Proceedings of the Royal Society B: Biological Sciences*, 275 (1633): 453-462.
- Lytle, D. A. and Poff, N. L. (2004). Adaptation to natural flow regimes. *Trends in Ecology and Evolution*, 19 (2): 94-100.
- Maejima, H., Ninuma, S., Okuda, A., Inoue, T. and Hayashi, M. (2018). Exercise and low-level GABAA receptor inhibition modulate locomotor activity and the expression of BDNF accompanied by changes in epigenetic regulation in the hippocampus. *Neuroscience Letters*, 685: 18-23.
- Martín-Torrijos, L., Kawai, T., Makkonen, J., Jussila, J., Kokko, H. and Diéguez-Uribeondo, J. (2018). Crayfish plague in Japan: A real threat to the endemic *Cambaroides japonicus*. *PLoS One*, 13 (4): e0195353.
- Mendoza-Vargas, L., Guarneros-Bañuelos, E., Galicia-Mendoza, F., Báez-Saldaña, A., Flores-Soto, E., Alvarado, R., Valdés-Tovar, M., Benítez-King, G., Fuentes-Pardo, B. and Solís-Chagoyán, H. (2018). Involvement of Melatonin in the Functioning of the Circadian System in Crayfish. *International Journal of Molecular Sciences*, 19 (7): 2147.
- Mendoza-Vargas, L., Ortega, P., Fuentes-Pardo, B. and Viccon-Pale, J. A. (2016). Circadian rhythm in locomotor activity in the burrower crayfish *Procambarus acanthophorus* (Villalobos 1948). *Biol Rhythm Res* 47: 93-101.

- Merlin, C. and Liedvogel, M. (2019). The genetics and epigenetics of animal migration and orientation: birds, butterflies and beyond. *Journal of Experimental Biology*, 222 (Pt Suppl 1): jeb191890.
- Momohara, Y., Kanai, A. and Nagayama, T. (2013). Aminergic control of social status in crayfish agonistic encounters. *PLoS One*, 8 (9): e74489.
- Patoka, J., Kalous, L. and Bartoš, L. (2019). Early ontogeny social deprivation modifies future agonistic behaviour in crayfish. *Scientific Reports*, 9 (1): 4667.
- Pigliucci, M. (2005). Evolution of phenotypic plasticity: Where are we going now? *Trends in Ecology and Evolution*, 20: 481–486.
- Rodríguez-Serna, M., Carmona-Osalde, C., Arredondo-Figueroa, J. L. and Olvera-Novoa, M. A. (2002). Distribución geográfica actual de *Procambarus (Austrocambarus) llamasii* (Cambaridae) en la Península de Yucatán. *Hidrobiológica*, 12 (1): 1-5.[Current geographical distribution of *Procambarus (Austrocambarus) llamasii* (Cambaridae) in the Yucatan Peninsula. *Hydrobiologica*, 12 (1): 1-5].
- Rojas, Y. (1998). *Revisión taxonómica de ocho especies de Procambarus (Crustacea: Decapoda: Cambaridae) del centro de Veracruz, México*. Thesis, Facultad de Ciencias, UNAM, México. 158 pag. [*Taxonomic review of eight species of Procambarus (Crustacea: Decapoda: Cambaridae) from downtown Veracruz, Mexico*. Thesis, Faculty of Sciences, UNAM, Mexico. 158 pag.].
- Rzedowski, J. (1986). *Vegetación de México*. Limusa, CDMX México. 432 pag. [*Vegetation of Mexico*. Limusa, CDMX Mexico. 432p.].
- Sandeman, R. and Sandeman, D. (2000). “Impoverished” and “enriched” living conditions influence the proliferation and survival of neurons in crayfish brain. *Journal of Neurobiology*, 45 (4): 215-26.
- Sato, D. and Nagayama, T. (2012). Development of agonistic encounters in dominance hierarchy formation in juvenile crayfish. *Journal of Experimental Biology*, 215 (Pt 7): 1210-1217.
- Schmid, M. and Guillaume, F. (2017). The role of phenotypic plasticity on population differentiation. *Heredity*, 119 (4): 214-225.
- Seitz, R., Vilpoux, K., Hopp, U., Harzsch, S. and Maier, G. (2005). Ontogeny of the Marmorcrebs (marbled crayfish): a parthenogenetic

- crayfish with unknown origin and phylogenetic position. *Journal of experimental zoology. Part A, Comparative experimental biology*, 303 (5): 393-405.
- Song, C. K., Johnstone, L. M., Schmidt, M., Derby, C. D. and Edwards, D. H. (2007). Social domination increases neuronal survival in the brain of juvenile crayfish *Procambarus clarkii*. *Journal of Experimental Biology*, 210 (Pt 8): 1311-24.
- Song, C. K., Johnstone, L. M., Schmidt, M., Derby, C. D. and Edwards, D. H. (2007). Social domination increases neuronal survival in the brain of juvenile crayfish *Procambarus clarkii*. *Journal of Experimental Biology*, 210 (Pt 8): 1311-1324.
- Sullivan, J. M., Macmillan, D. L. (2001). Embryonic and postembryonic neurogenesis in the ventral nerve cord of the freshwater crayfish *Cherax destructor*. *Journal of Experimental Zoology*, 290 (1): 49-60.
- Viccon-Pale, J. A., Ortega, P., Mendoza-Vargas, L., Castilla-Hernández, P., López-Cuevas, A., Meléndez-Herrada, A., Rivera-Becerril, F., Vela-Correa, G. and Signoret-Poillon, M. (2016). Structure and population dynamics of the secondary burrower crayfish *Procambarus acanthophorus* from a tropical Mexican wetland. *Canadian Journal of Zoology*, 94: 479-488.
- Villalobos, A. (1948). Estudios de los cambarinos mexicanos. VII. Descripción de una nueva especie del género *Procambarus*, *Procambarus acanthophorus* n. sp. *Anales del Instituto de Biología*, 19: 175-182. [Studies of Mexican Cambarinae. VII. Description of a new species of the genus *Procambarus*, *Procambarus acanthophorus* n. sp. *Annals of the Institute of Biology*, 19: 175-182].
- Villalobos, J. (1998). *Taxonomía de los Crustáceos con énfasis en el orden Decapoda*. Instituto de Biología, Universidad Nacional Autónoma de México, Distrito Federal, México. 33 pag. [Taxonomy of Crustaceans with emphasis on the Decapoda order. Institute of Biology, National Autonomous University of Mexico, Federal District, Mexico. 33 pag.] Vogt, G. (2008). Investigation of hatching and early post-embryonic life of freshwater crayfish by in vitro culture, behavioral



- analysis, and light and electron microscopy. *Journal of Morphology*, 269 (7): 790-811.
- West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology, Evolution and Systematics*, 20: 249-278.
- Yeh, S. R., Fricke, R. A., Edwards, D. H. (1996). The effect of social experience on serotonergic modulation of the escape circuit of crayfish. *Science* 271 (5247): 366-369.
- Yeh, S. R., Musolf, B. E. and Edwards, D. H. (1997). Neuronal adaptations to changes in the social dominance status of crayfish. *Journal of Neuroscience*, 17 (2): 697-708.
- Yi, S., Li, Y., Shi, L., Zhang, L., Li, Q. and Chen, J. (2018). Characterization of Population Genetic Structure of red swamp crayfish, *Procambarus clarkii*, in China. *Scientific Reports*, 8 (1): 5586.



*Chapter 4*

**NON-INDIGENOUS CRAYFISH SPECIES:  
A GLOBAL ASSESSMENT AND  
FUTURE PERSPECTIVES**

*Tainã Gonçalves Loureiro\**

Centre of Excellence for Invasion Biology,  
Department of Botany and Zoology, Stellenbosch University,  
Stellenbosch, Western Cape, South Africa

**ABSTRACT**

Invasive species are widely recognized as a major threat to biodiversity and non-indigenous crayfish species (NICS) figure among the main threats to freshwater environments, biodiversity and ecosystem functioning. The main vector for freshwater crayfish introduction is the aquaculture and the pet trade and 28 NICS are known so far, distributed worldwide, except for Antarctica. *Procambarus clarkii*, *P. fallax*, *Cherax quadricarinatus*, *C. destructor* and *C. canii* figure as the most frequently introduced, widespread species and to which great impact is associated. Habitat degradation, transmission of diseases, modification of food webs, predation on native species and competition for resources are the most

---

\* Corresponding Author's E-mail: loureiro.tg@gmail.com.

recurrent threats associated to the establishment of NICS and the main efforts to control this species involve the elimination or reduction of populations through mechanical, chemical or biological methods although only few examples of success exist. This chapter summarizes the major information on NICS, including their distribution, impacts on biodiversity and management possibilities.

**Keywords:** invasion process, NICS, crayfish introduction, introduction pathway, impact, aquaculture, pet trade, crayfish disease, management

## **1. THE NEW PANGAEA AS A CONSEQUENCE OF BIOLOGICAL GLOBALIZATION**

Biological invasion is among the main threats to species conservation and maintenance of ecosystem services. Along with climate change and habitat fragmentation, it has been contributing to the severe global change we've been facing for the last two centuries (Jeschke et al., 2014; Simberloff et al., 2013). Species invasion can drive severe ecological changes that can further facilitate other invasion processes, in a feedback loop. Ecological changes might result in niche availability allowing the establishment of exotic species, and the settlement of invasive species might unbalance ecological dynamics. It may also affect native species abundance and diversity by predation, competition, disease transmission, etc, thus generating ecological changes that might favor a new invasion process. Hence, the causes and consequences of one (i.e., establishment or induced changes), might affect the other directly or indirectly. However, the relative importance of factors can vary depending on local factors or the species in question.

The biogeographical barriers that have both poked species evolution, as well as contained and shaped species distributions for centuries are blurring or vanishing more and more, every decade, due to human interference. Since the first nomads, humans have been transporting species of interest; with the development of transport technologies, the scale of human movement across the globe is unprecedented (Capinha et al., 2015).

The intentional or unintentional transportation of exotic species to a new habitat, however, is not the main concern of conservationists, the real threat is offered by the species that can in fact establish and become invasive. From the total of species that are translocated, only a small fraction is able to survive, establish self-sustained populations and expand, being then considered invasive species (Jeschke et al., 2014). The problem is that the amount of species being transported is so high, that even this small fraction is huge, contributing to a process called biotic homogenization, in which this re-arrangement of species distribution due to invasions and extinctions decreases the taxonomic, genetic, morphological or functional variation among communities (Baiser et al., 2012; Petsh, 2016; Turbelin et al., 2017).

## **2. NON-INDIGENOUS CRAYFISH SPECIES (NICS) AROUND THE WORLD**

Freshwater crayfishes (infraorder Astacidea) are a diverse and widespread group, being found in all continents, except Antarctica and continental Africa (Crandall and De Grave, 2017). These macroinvertebrates can be found in several types of inland water: some species dependent on running waters occur in streams and rivers, while others prefer lakes, ponds or swamps (Holdich, 2001). These decapods are the largest mobile freshwater invertebrates, have a long lifespan and are polytrophic omnivores; they frequently become dominant in aquatic habitats, thus modifying the availability of resources to other organisms both directly and indirectly (Crooks, 2002). Hence, they are considered ecosystem engineers and key components of food webs (Momot, 1995; Nyström et al., 1996).

Most species are very sensitive to changes on their natural environment; these specialist species tend to be under great threat due the well-known massive impacts on freshwater environments (Collen et al., 2014). Unlike those, a smaller group of crayfishes are very flexible regarding the type of

**Table 1. Non indigenous crayfish species around the world, their family, common name, native region, introduced range and main reference. Native and introduced range by continental subdivision**

Family	Species	Common name	Native region	Introduced range	Main reference
Astacidae	<i>Astacus astacus</i>	Noble crayfish	Europe	Europe	Kouba et al. (2014)
	<i>Austropotamobius pallipes</i>	White-clawed crayfish	Europe	Europe	Kouba et al. (2014)
	<i>Austropotamobius torrentium</i>	Stone crayfish	Europe	Europe	Kouba et al. (2014)
	<i>Pacifastacus leniusculus</i>	Signal crayfish	North America	Asia	Olden et al. (2006)
				Europe	Kouba et al. (2014)
	<i>Pontastacus leptodactylus</i>	Danube crayfish	Europe	Europe	Chucholl et al. (2012)
				North America	Olden et al. (2006)
<i>Pontastacus pachypus</i>	-	Europe	North America	Olden et al. (2006)	
			Europe	Kouba et al. (2014)	
Cambaridae	<i>Faxonius difficilis</i>	Painted crayfish	North America	North America	Olden et al. (2006)
	<i>Faxonius immunis</i>	Calico crayfish	North America	Europe	Kouba et al. (2014)
				North America	Olden et al. (2006)
	<i>Faxonius juvenilis</i>	-	North America	Europe	Kouba et al. (2014)
	<i>Faxonius limosus</i>	Spiny-cheek crayfish	North America	Europe	Kouba et al. (2014)
				North America	Olden et al. (2006)
	<i>Faxonius neglectus</i>	Gapped ringed crayfish	North America	North America	Olden et al. (2006)
	<i>Faxonius palmeri</i>	Gray-speckled crayfish	North America	North America	Olden et al. (2006)
	<i>Faxonius rusticus</i>	Rusty Crayfish	North America	North America	Olden et al. (2006)
	<i>Faxonius virilis</i>	Northern virile crayfish	North America	Europe	Kouba et al. (2014)
	<i>Procambarus acutus</i>	White River crayfish	North America	Africa	Ibrahim et al. (1997)
				Europe	Kouba et al. (2014)
				North America	Taylor et al. (2007)
<i>Procambarus alleni</i>	Everglades Crayfish	North America	North America	Taylor et al. (2007)	
			Europe	Kouba et al. (2014)	

Family	Species	Common name	Native region	Introduced range	Main reference
Cambaridae	<i>Procambarus clarkii</i>	Red swamp crayfish	North America	Africa	de Moore (2002)
				Asia	Uderbayev et al. (2017)
				Central America	Huner and Barr (1991)
				Europe	Kouba et al. (2014)
				North America	Taylor et al. (2007)
	South America	Loureiro et al. (2015)			
	<i>Procambarus cubensis</i>	-	Central America	Europe	Chucholl et al. (2012)
	<i>Procambarus virginialis</i>	Marbled crayfish	-	Africa	Kawai et al. (2009)
				Asia	Kawai and Takahata (2010)
				Europe	Kouba et al. (2014)
<i>Procambarus zonangulus</i>	Southern White River Crayfish	North America	Europe	Chucholl et al. (2012)	
Cambaroididae	<i>Cambarellus chapalanus</i>	-	North America	Europe	Chucholl et al. (2012)
	<i>Cambarellus diminutus</i>	Least crayfish	North America	Europe	Chucholl et al. (2012)
	<i>Cambarellus montezumae</i>	-	North America	Europe	Chucholl et al. (2012)
	<i>Cambarellus patzcuarensis</i>	Mexican Dwarf crayfish	North America	Europe	Chucholl et al. (2012)
	<i>Cambarellus puer</i>	Swamp Dwarf crayfish	North America	Europe	Chucholl et al. (2012)
	<i>Cambarellus shufeldtii</i>	-	North America	Europe	Chucholl et al. (2012)
	<i>Cambarellus texanus</i>	Brazos Dwarf crayfish	North America	Europe	Chucholl et al. (2012)
Parastacidae	<i>Cherax cainii</i>	Smooth marron	Australasia	Africa	de Moore (2002)
				Asia	Lawrence and Jones (2002)
				Australasia	
				North America	
				South America	
	<i>Cherax destructor</i>	Common yabby	Australasia	Asia	Uderbayev et al. (2017)
				Africa	de Moore (2002)
Australasia				Lawrence and Jones (2002)	

**Table 1. (Continued)**

Family	Species	Common name	Native region	Introduced range	Main reference
Parastacidae				Europe	Chucholl et al. (2012)
	<i>Cherax holthuisi</i>	-	Australasia	Europe	Chucholl et al. (2012)
	<i>Cherax pulcher</i>	-	Australasia	Asia	Lawrence and Jones (2002)
				Europe	
				North America	
	<i>Cherax quadricarinatus</i>	Australasian redclaw crayfish	Australasia	Africa	de Moore (2002)
				Asia	Uderbayev et al. (2017)
				Australasia	Lawrence and Jones (2002)
				Central America	
				Europe	
	North America	Lawrence and Jones (2002)			
	<i>Cherax tenuimanus</i>	Marron	Australasia	Africa	de Moore (2002)
				Asia	Lawrence and Jones (2002)
Australasia					
North America					



environment they need and the resources they use (Reynolds and Souty-Grosset, 2011; Buřič et al., 2013; Loureiro et al., 2019). These species with high ecological plasticity can thrive in a wide range of environmental conditions, and this flexibility is exactly what favors their introduction to new locations, in order to meet human needs as aquaculture, fishing and aquarism.

From the more than 650 freshwater crayfish species, 33 NICS are known so far (Table 1). The family Cambaridae, indigenous to North America, is the most diverse family within freshwater crayfishes (14 genera and 448 species) (Crandall and De Grave, 2017). It is also the taxon that comprises most alien species spread worldwide (42%, Table 1). Most of NICS come from North America (60%) as expected, since this region is considered the main center of crayfish diversity (Crandall and Buhay, 2008). Europe is the region that hosts the highest number of NICS, whereas South and Central America are the areas with the lowest numbers of NICS (Table 1). The prevalence of alien crayfish in Europe is probably a result of historic, cultural and economic factors, since crayfish are traditionally very appreciated in culinary and as pets in European countries. In Central and South America, citizens have lower average purchasing power and less people have access to these decapods as either pets or food. Additionally, this difference might also be due to lower public, political and academic awareness regarding invasive species in Central and South America.

The African continent demands special attention, since no native crayfish is known to occur in continental Africa, and only the endemic Parastacidae (genus *Astacoides* Guérin-Méneville, 1839) in the Island of Madagascar, as reminiscent distribution from Gondwana (Scholtz, 2002). Still, six NICS were introduced to Africa (Table 1) (Gherardi et al., 2011; Marufu and Nhiwatiwa, 2014; Loureiro et al., 2015a; Nunes et al., 2017) and some are considered invasive and with ongoing spread, as the red swamp crayfish, the Australian redclaw and marbled crayfish (Kawai et al., 2009; Nunes et al., 2017). Large populations of the marbled crayfish are established in Madagascar and offer important threat to endemic parastacids as well as to other taxa (Kawai et al., 2009). The red swamp crayfish is the most widespread NICS in Africa; it has been introduced into Egypt, Kenya,

South Africa, Sudan, Uganda and Zambia and there is a great chance that it will reach the largest of the African Rift lakes, the Tanganyika, which is also the second-oldest freshwater lake in the world, hosting more than 600 endemic species (Snoeks 2000; Marijnissen et al., 2008). The Australian redclaw crayfish was initially introduced into Swaziland for aquaculture, and managed to spread through the river system, being now also found in Mozambique, South Africa, Zambia and Zimbabwe, with high risk of establishment in the Okavango Delta, a World Heritage Site (Nunes et al., 2017).

### **3. SNACK OR THREAT? PET OR PEST? PATHWAYS OF FRESHWATER CRAYFISH INTRODUCTION**

As mentioned in section 1, the global trade greatly extended the capacity of species to colonize regions outside their natural range, and alien species translocation has increased with the worldwide development of aquatic, aerial and terrestrial transportation (Capinha et al., 2015). International or inter-regional transportation of species usually constitutes the vector of introduction of alien species while the secondary spread, despite in smaller scale, is also an important feature to understand the invasion process (Figure 1).

NICS are among the most broadly introduced freshwater species due to their close association with human activities (e.g., fishing, culinary, aquarism). Freshwater crayfish are appreciated as pets being sold in aquarium and pet shops, as well as considered a fine dish in culinary, thus harvested for human consumption and commonly traded internationally (Chucholl and Wendler, 2017) (Figure 1). Therefore, the pet trade and the astaciculture (freshwater crayfish farming) are the main pathways of introduction of this group, even though other factors also play important roles in the NICS invasion process.

The astaciculture is the main vector of crayfish introduction in many places. Apparently, it was the first reason for freshwater crayfish

translocation among continents. Crayfish consumption increased rapidly over the least 60 years, and around 91% of their production is based on NICS, with China as the greatest producer and exporter of this deli (Lodge et al., 2012). Australia, USA and some European countries are also important producers (Holdich et al., 1993).

The most aquacultured species for the food market are *Procambarus clarkii*, *Cherax quadricarinatus*, and *Cherax destructor* due to their fast growth, high fecundity, high resistance to disease and to variation in environmental conditions, and also due to their meat in regards to texture and taste (Marshall et al., 1988; Jones, 1995; Jerry et al., 2005).

Crayfish as pets are kept in freshwater aquariums and garden ponds, from which they can scape or be released by owners that no longer want to cultivate them (Peaey, 2010; Chucholl, 2013a) (Figure 1). A great variety of the world's entire range of crayfish species is available in the pet trade in all continents and their sales have increased dramatically in the least decades (Holdich et al., 2010; Faulkes, 2015). Thus, two main concerns can be pointed out: the potential for over exploitation of natural populations and the source of NICS introduction resulting from the release of individuals in natural habitats.

Nentwig et al. (2018) included seven NICS to the list of "More than '100 worst' alien species in Europe": *Procambarus clarkii*, *P. fallax* f. *virginalis*, *Pacifastacus leniusculus*, *Cherax quadricarinatus*, *C. destructor*, *Faxonius virilis* and *F. limosus*. Alarmingly, all these species are sold in pet trade around the world. The genus *Procambarus*, *Cambarellus* and *Cherax* include both the most commonly sold ones as well as the most worldwide introduced species (Table 1) (Soes and Koese, 2010; Chucholl, 2013a; Faulkes, 2015).

Obviously, the popularity of species and availability on market vary locally, but some trends are identified. Species that can be easily reared in aquaria, like NICS from the genus *Procambarus* and *Cambarellus*, are preferred. These easily grown species with high reproductive potential are usually cultivated by retail sellers and aquarium hobbyists, eliminating the need for purchasing or importing animals while also decreasing market price (Chucholl, 2013a). Species from the genus *Cherax*, on the other hand, tend

to have lower reproductive potential and are more difficult to cultivate, increasing their sales value.

The body color also affects NICS popularity among hobbyists due to their attractiveness (Faulkes, 2015). *Cherax peknyi*, for instance, has naturally bright colors; *Cambarellus patzcuarensis* and *Procambarus alleni* have bright colored morphs that are the ones usually available on pet trade (Faulkes, 2015).

Additionally, Chucholl (2013) and Banha et al. (2019) related crayfish size to the likelihood of NICS deliberate release because they can outgrow or overpopulate their aquaria, since crayfish fecundity is often size-dependent in a positive relation. On the other hand, crayfish size might also influence its detectability after introduction since smaller species are more difficult to find, possibly resulting in the prevalence of bigger species in lists of non-indigenous or invasive species (Chucholl, 2013a).

Despite NICS introduction due to astaciculture and pet trade, some other reasons have driven crayfish introduction in regional or local scales, contributing to what is named secondary spread or diffusion dispersal. The secondary spread happens when an introduced species increases its distribution range following initial invasion, usually in a local scale, reaching new habitats via human-mediated translocations, hitchhiking on other animals (i.e., ectozoochory) or, for aquatic species, by movement within a waterbody (e.g., downstream migration) (Erfmeier et al., 2011; O'Loughlin and Green, 2017) (Figure 1).

For NICS, human mediated secondary spread is the most commonly reported reason responsible for local spread of these species due to their use as live fishing bait (Banha and Anastácio, 2015; Loureiro et al., 2015a) (Figure 1). Live bait presents greater risk of NICS introduction due to its high risk bait release; thus, Gherardi (2010) recommends that anglers should fish in the same environment where they captured the crayfish bait.

Furthermore, NICS human-mediated secondary introduction was also motivated for biological control of plants and invertebrates (Figure 1). In Kenya, for instance, *P. clarkii*'s was used as a biological control agent of *Biomphalaria pfeifferito*, an intermediate host of human schistosomiasis

(Hofkin et al., 1991; Marshal, 2019) as well as of the mosquito *Culex quinquefasciatus*, in Egypt (Heikal et al., 2018).

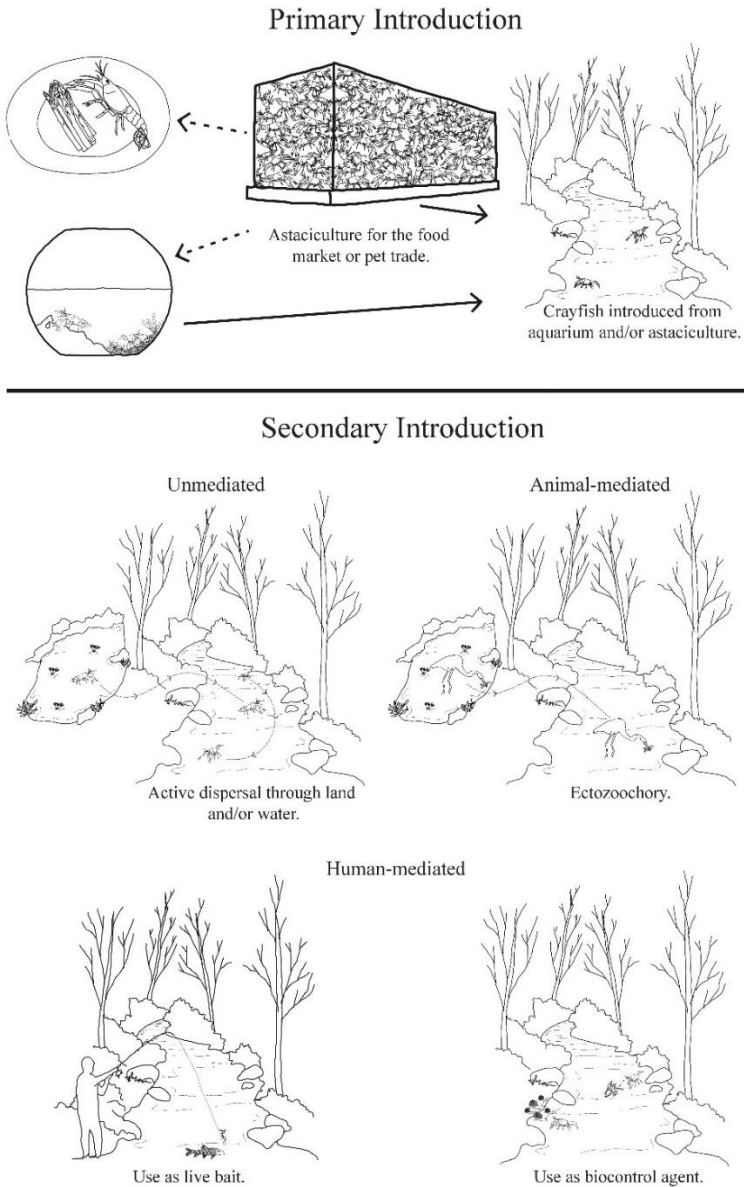


Figure 1. Non indigenous crayfish primary and secondary pathways of introduction.

The main animal vector associated to the transportation of aquatic organisms are waterbirds, whose abundance, widespread distribution, and high movement rates within and between environments, are related to the internal and external transportation of seeds and invertebrates, especially regarding species included in their diets (Figuerola and Green, 2002; Van Leeuwen and Van der Velde, 2012; Coughlan et al., 2017) (Figure 1). Anastácio et al. (2014) and Águas et al. (2014) confirmed the viability of juvenile crayfish transportation by birds (e.g., pigeons and ducks) and the probability of success is affected by water depth, crayfish size, travelled distance, and relative humidity. Thus, waterbird-mediated passive dispersal can contribute to NICS rapid spread, enhancing the local process of invasion.

Crayfish are also very active animals, and the red swamp crayfish, for example, is known to disperse at a rate of up to 4 km in a single day outside a waterbody (Gherardi and Barbaresi, 2000). Although the most common dispersion rate is up to 255 m a day for the red swamp and 461 m for the signal crayfish, it ranges from immobility for several days to large distances in less than a day (Anastácio et al., 2015).

Crayfish are also capable of active dispersion between isolated waterbodies, crossing terrestrial barriers not only by flood events or transportation by other animals, but also by walking on land (Grey and Jackson, 2012; Puky, 2014; Thomas et al., 2019), which facilitates the secondary spread (Figure 1). This terrestrial emigration in freshwater crayfish seems to be variable among species, and the extent to which each species is able to move overland is still poorly understood.

In order to decrease the number of NICS being introduced worldwide, it is of utmost importance to understand vectors and pathways of introduction and to estimate arrival rates. These data are required to develop accurate risk assessments for specific trade pathways and species, and to develop effective management plans. Hence, it is important not only to understand the motivations and vectors for NICS introduction globally, but also regionally and locally.

#### **4. NICS SECRET OF SUCCESS AND HAZARDOUSNESS**

Invasive alien species pose notable threat to the recipient environment because they impact native biota and ecosystem functioning on many levels (e.g., genetic, individual, population, community, ecological) and through different mechanisms as trophic interactions, competition, disease transmission, changes in nutrient availability, primary productivity or habitat structure (Vilá et al., 2000; Ehrenfeld, 2011; Ricciardi et al., 2013).

Understanding why some alien species become invasive while others fail and the potential impact offered by alien species are fundamental goals in invasion ecology (Parker et al. 1999). The success of establishment of a certain invader, the time that this process takes place, and the harm it offers will depend on characteristics of both the alien species and the recipient environment.

The invasion process requires at least four steps (Catford et al., 2009). The first is transportation, either intentional or unintentional (Richardson et al., 2000). In this phase, aided by human action, the species is able to transpose the first barrier that contains species natural expansion range, the geographical barrier (Blackburn et al., 2007). Species that are not transported by accident, but motivated by its relation to human needs, are usually transported in higher numbers and to a wider range of sites (Perkins et al., 2011). The relation to human needs also influences the second step: introduction. In this step, an alien species is released in the recipient ecosystem after transportation, different to the one in which that species evolved and occurs naturally (Blackburn et al., 2007). Species of interest for humans will have more frequent introduction events as well as more individuals being introduced per event, increasing the propagule pressure. NICS are strongly related to human activities as explained in section 3, and this close relationship increases the transportation rates as well as the propagule pressure, and both outcomes are strongly related to invasion success.

The third step is the establishment of the non-indigenous species in the introduced environment, and its ability to surmount the environmental barrier offered by booth biotic and abiotic features of the recipient

environment (Catford et al., 2009). In this stage, a myriad of ecological interactions might regulate the establishment of the alien species positively (e.g., biotic facilitation) or negatively (e.g., biotic resistance) (Perkins et al., 2011). Hence, the likelihood of an introduced species to become invasive is determined by the interplay of the invader's attributes, the abiotic environment, as well as biotic interactions within the novel environments (Jeschke, 2014).

Species with strategies for dealing with the challenges provided by the recipient ecosystem will have a higher chance of colonization success and NICS are known to have some of these features. Ecological or phenotypic plasticity, for example, allow individuals to cope with variable environmental condition, tolerate biotic and abiotic fluctuation and adapt to different resources (Jeschke, 2014).

Resource availability is one of the main constraints that an alien species can face in a new environment and the success of colonization is related to the ability to use a wide variety of habitats and resources (Shea and Chesson, 2002). Therefore, a wide niche breadth would be an important aptness for an invasive species to contour a potential environmental barrier. Freshwater crayfish feed on vertebrates, invertebrates, macrophytes, algae and detritus, being generally classified as polytrophic omnivorous and generalist feeders (Capelli, 1975; Correia, 2002) and thus able to shift their diet according to availability of resources (Olsson et al., 2009; Loureiro et al., 2019). The best example of diet plasticity is given by the world's most successful crayfish invader, *P. clarkii*. This freshwater crayfish is considered a "warm-water" species (Chucholl, 2013) although it nowadays has populations established in high latitudes and altitudes locations; one of the changes observed in this "cold-water" populations is the trophic ecology. Individuals will shift their preferences and also consume different food items to meet their nutritional needs (Chucholl, 2013).

The invasive signal crayfish has been shown to have doubled the niche width of the native noble crayfish in Sweden and higher ecological plasticity regarding resources use (Olsson et al., 2009).



The trophic plasticity observed in many NICS not only favors the species' adaptation to new environments but also is one of the main causes of the high threat offered by them (Shea and Chesson, 2002). The flexible position of freshwater crayfish in trophic nets of the ecosystem in which they are inserted, since they can act as both grazers (primary consumers) and predators (higher-level consumers) makes them keystone species (Reynolds et al., 2013). Keystone species are those who can broadly modify ecosystem functioning and community dynamics, mostly by feeding pressure, thus having a high potential to generate impact and affect organisms of different levels (Lodge et al., 1994; Crandall and Buhay, 2008).

The potential impact of NICS in recipient habitats that have no native crayfish species or species belonging to the same functional group can be dramatic with rapid environmental change. In recipient environments presenting equivalent functional groups, invaders and natives might show a similar impact on a local scale; however in a regional scale, invaders tend to offer greater impact due to their capacity to spread and frequent high population growth (Olssen et al., 2009).

Competition is another important interaction regulating the fitness of invasive species and the potential harm offered by their establishment (Shea and Chesson, 2002; Shurin, 2006). Crayfish compete by resources such as food and shelter, and these interactions are mediated by factors such as resource availability and population density, as well as age and size of crayfish (Huber et al., 2002; Bergman and Moore, 2003; Corkum and Cronin, 2004). The power of this interaction on controlling alien colonization is affected by the degree of agonistic behavior of the invader. Many crayfish species are known to have aggressive predisposition (Miller et al., 1992; Goessmann et al., 2000) and this behavior is related to the capability to obtain or defend resources, such as shelter and food, not only from other invertebrates but also from vertebrates like fish and amphibians (Ranta and Lindström, 1993; Carpenter, 2005; Tricarico and Gherardi, 2010).

The North American NICS *P. clarkii*, for example, demonstrated superior fight strategies and dominance of resources when competing with the native South American *Parastacus brasiliensis* (Dalosto et al., 2015).

Nonetheless, the impact of competition also affects other groups rather than only native crayfish. The exotic signal crayfish establishment in British rivers, for example, affected significantly the growth, diet and trophic position of native fish species (Wood et al., 2016).

Other biotic interactions between resident and alien species also affect the colonization potential of the foreign species, such as pathogens and the lack of mutualistic interactions (Theoharides and Dukes, 2007). These interactions constitute barriers offered by the invaded environment that are more related to the recipient habitat characteristics. Despite their great importance for understanding the complex invasion process, we will not explore them to a further extent since this section rather focuses on characteristics of NICS.

The pathogens brought by NICS are also a potential source of impact to invaded communities, and the most studied model is the fungus-like species *Aphanomyces astaci* Schikora, 1906. This species of water mold is native to North America and carried by North American NICS which are usually not affected by them (Söderhäll and Cerenius, 1999). Freshwater crayfish species native from elsewhere can be strongly affected by this oomycete and many native European populations were decimated after the introduction of North American species as *Faxonius limosus*, *F. immunis*, *F. virilis*, *Pacifastacus leniusculus* and *P. clarkii* (Holdich et al. 2009; Filipová et al. 2013; Schrimpf et al. 2013; Tilmans et al. 2014).

The last step of the invasion process is the spread and it is regulated by the dispersal barrier, related to the capacity of the specimens to move, as well as by the environmental barrier, since dispersing individuals might face challenges in a novel environment (Blackburn et al., 2007). The dispersal ability of some NICS, as mentioned in section 3, is remarkable for freshwater organisms and increases considerably their potential expansion range (Soreson et al., 2012; Hudina et al., 2017). Interspecific variation is known to occur so some species might be more prone to disperse than others. Two highly invasive NICS, *Pacifastacus leniusculus* and *Procambarus clarkii* for instance, excel as highly active and broadcasted species (Barbaresi et al., 2004; Bubb et al., 2004).

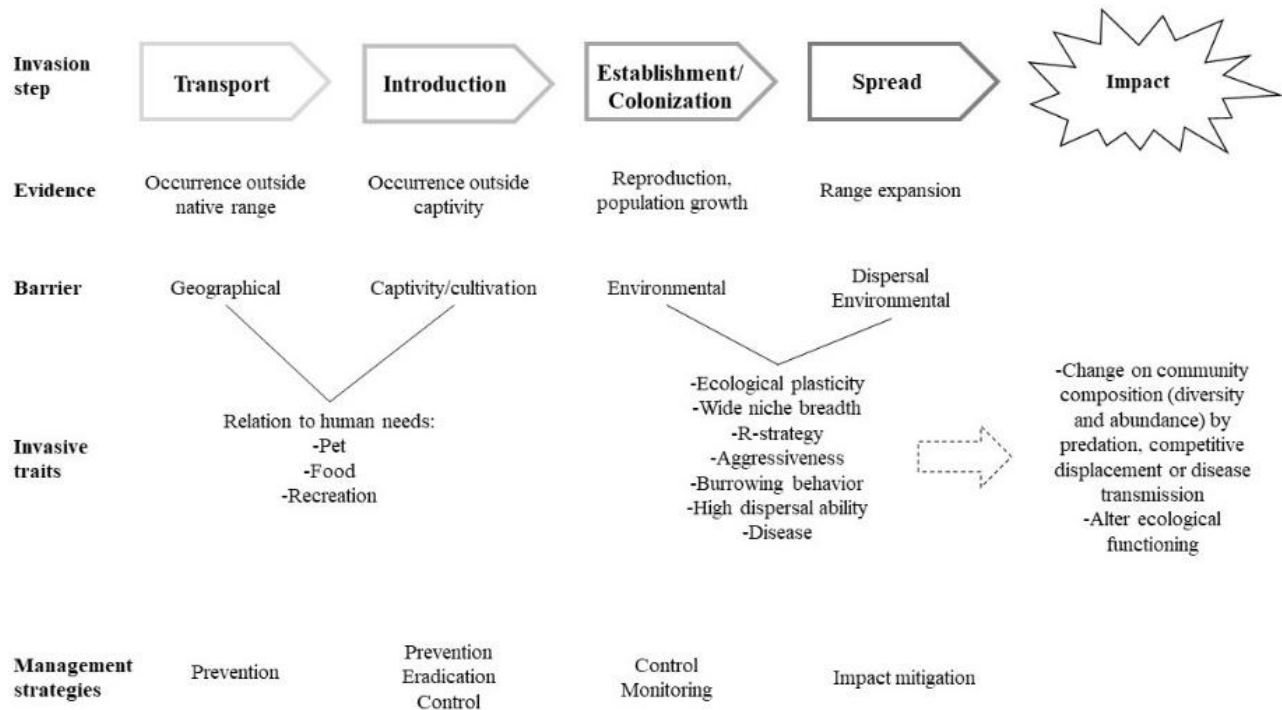


Figure 2. Schematic overview of the invasion process steps, the evidence of each stage, the barrier that need to be overcome, the non-indigenous traits that favor the overcoming, the management strategies considered more viable in each invasion step and the main impacts offered by NICS.

The most widely distributed NICS are R-selected species that produce large offspring, have high growth rates and early sexual maturity (Price and Payne, 1984). The achievement of both steps, establishment and spread, is further influenced by population growth and R-selected species tend to quickly reach high densities and have higher invasive potential (Dörr and Scalici, 2012; Sargent and Lodge, 2014).

Additionally, flexible species with faster growth and high fecundity are easier and cheaper to cultivate. All features mentioned to contribute to NICS great success in transposing the environmental barrier and establishing, are also important for selecting species that will be used for aquaculture and pet trade, reinforcing the NICS capability of surpassing the first barrier as well.

NICS share multiple invasive traits that will allow them to overcome the different barriers of the invasion process. These traits are also related to the potential impact offered by alien freshwater crayfish and to their selection as species of interest. Therefore, the same characteristics are responsible for making this species desirable by the market and unwanted by conservationist, and the lack of interaction and exchanges between these two sectors can be thought as the main responsible for NICS invasion.

## **5. CLIMATE CHANGE AND NICS INVASION**

Climate change is linked primarily to the rapid increase of CO<sub>2</sub> concentrations from pre-industrial levels of 280 ppm to present levels of roughly 400 ppm (Rhein et al., 2013). This increasing trend is expected to continue, with some predictions suggesting values between 800-1150 ppm by 2100 (Collins et al., 2013). Increasing atmospheric CO<sub>2</sub> has two major impacts: global warming and acidification. While acidification due to CO<sub>2</sub> increased concentration might be of higher concern to marine environments, in freshwater habitats, the elevation of average temperature can increase atmospheric water vapor content, alter precipitation patterns, intensity and extremes, change soil moisture and modify runoff (Woodward et al., 2015).

The expected changes in climate might lead to unpredictable chain effects that will alter species survival, distribution, abundance, community

composition and even lead to extinctions and new evolutionary scenarios, influencing the limnetic biota and ecosystems dynamics (Woodward et al., 2015). Locations that are expected to have reduced seasonal supplies to off-river habitats and perennial flow, for example, would have lower connectivity and gene flow of species could be compromised.

The numerous direct and indirect, predicted and unpredicted consequences of climate change makes this phenomenon, along with biological invasions, an important threat to conservation. Consequences of climate change can affect future and ongoing bioinvasions by altering vector strength and alien species composition. These effects could moderate the survival of exotic and native species and regulate invasion success (Hellmann et al., 2008). Studies focusing on the effects on NICS however, have been seldomly explored and the little information that has been published so far, explore possible consequences for the establishment and spread of NICS. Yet, no experimental studies have been conducted so far and knowledge is rather based in hypothesis than actual observations.

Most of NICS establishment and spread is restricted by temperature, so global warming would favor invasion in currently colder areas such as Alaska, Canada and Northern Europe (Ruokonen et al., 2018). On the other hand, Sandström et al. (2013) and Bohman et al. (2015) raised the possibility of increased pathogen pressure on NICS under a warming scenario, which could decrease invasiveness. Freshwater crayfish dispersal ability could also be hampered by the decrease in connectivity due to reduced rain and flow, decreasing the spread of NICS.

Further studies are necessary to address the role of climate change on crayfish invasion, growth, survival, community structure and diversity, leading to protective approaches that may target the most probable invaders in a changing global scenario.

## **6. NICS MANAGEMENT**

It is well known that biological invasions are generally irreversible and prevention of introduction and spread is the most secure approach

(Simberloff et al., 2013). To do that, potentially invasive or harmful species must be identified and a range of protocols, spatial autocorrelation analysis and quantitative approaches are available and suggested as pre-establishment approaches (Lodge et al. 2006; Verbruge et al., 2012; Armendáriz et al., 2017). However, as explored by Faulkes (2018), the existence of regulations to limit NICS cultivation and trade have limited success in avoiding NICS introduction across different countries mainly regarding pet-trade since aquaculture for culinary is usually strongly regulated while aquarium and pet shops, as well as hobbyists, are more difficult to supervise, animals are farmed in smaller scales and might rely on informal market (Loureiro et al., 2015b).

Many freshwater crayfish species are still being translocated and introduced worldwide and to tackle NICS invasion and define management approaches, some previous information must be known. The identification of introduction pathways and vectors can be followed by the regulation or prohibition of certain vectors (Hulme, 2015; Saul et al., 2017).

Post-establishment management measures will prioritize control, containment and eradication of invasive species (Simberloff et al., 2013). The fast identification and early response are approaches strongly related to the likelihood of success of management strategies (Gioria et al., 2016) and some methods are available to be used such as DNA barcoding (Filipová et al., 2011) and e-DNA based detection that allows the identification of target species even at low abundance levels, regardless direct observations.

After identification, the next steps are related to the response to control or eradicate the species and despite laborious, mechanical removal seems to be the best approach when considering intensive removal rather than extensive (Loureiro et al., 2018). Other approaches will include, biological control, biocides, pond drainage and fencing. Since local specificities are expected due to species characteristics as well as environmental interactions, adaptive management is recommended to allow the constant re-evaluation of the methods being used, the substitution or adequacy of non-profitable strategies and the maintenance of successful actions in a way to include local and temporal appropriate approaches.

## ACKNOWLEDGMENTS

The author is grateful to the Centre of Excellence for Invasion Biology for the fellowship provided to TGL and to the Botany and Zoology department of Stellenbosch University for the support and structure. Many thanks to Dr Felipe Bezerra Ribeiro for the invitation and to Dr Camila Timm Wood for English reviewing.

## REFERENCES

- Águas, M. F., Banha, M. and Anastácio, P. M. (2014). Can Recently-Hatched Crayfish Cling to Moving Ducks and Be Transported during Flight? *Limnologica* 48: 65-70.
- Anastácio, P. M., Banha, F., Capinha, C., Bernardo, J. M., Costa, A. M., Teixeira, A. and Bruxelas S. (2015). Indicators of Movement and Space Use for Two Co-Occurring Invasive Crayfish Species. *Ecological Indicators*, 53: 171-81.
- Anastácio, P. M., Ferreira, M. P., Banha, F., Capinha, C. and Rabaça, J. E.. (2014). Waterbird-Mediated Passive Dispersal Is a Viable Process for Crayfish (*Procambarus clarkii*). *Aquatic Ecology*, 48(1): 1-10.
- Armendáriz, G., Quiroz-Martínez, B. and Alvarez, F. (2017). Risk Assessment for the Mexican Freshwater Crayfish: The Roles of Diversity, Endemism and Conservation Status. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 27(1): 78-89.
- Baiser, B., Olden, J. D., Record, S., Lockwood, J. and McKinney, M. L. (2012). Pattern and Process of Biotic Homogenization in the New Pangaea. *Proceedings of the Royal Society B: Biological Sciences*, 279 (1748): 4772-
- Banha, F., Diniz, A. and Anastácio, P. M. (2019). Patterns and Drivers of Aquarium Pet Discharge in the Wild. *Ecological Indicators*, 106: 105513.

- Banha, F. and Anastácio, P. M. (2015). Live Bait Capture and Crayfish Trapping as Potential Vectors for Freshwater Invasive Fauna. *Limnologica*, 51: 63-69.
- Barbaresi, S., Santini, G., Tricarico, E. and Gherardi, F. (2004). Ranging Behaviour of the Invasive Crayfish, *Procambarus clarkii* (Girard). *Journal of Natural History*, 38 (22): 2821-32.
- Bergman, D. A. and Moore, P. A. (2003). Field Observations of Intraspecific Agonistic Behavior of Two Crayfish Species, *Orconectes rusticus* and *Orconectes virilis*, in Different Habitats. *The Biological Bulletin*, 205 (1): 26-35.
- Blackburn, T. M., Pyšek, P., Bacher, S., Carlton, J. T., Duncan, R. P., Jarošík, V., Wilson, J. R. U. and Richardson, D. M. (2011). A Proposed Unified Framework for Biological Invasions. *Trends in Ecology and Evolution*, 26 (7): 333-39.
- Bohman, P., Edsman L., Sandström, A., Nyström, P., Stenberg, M., Hertonsson, P. and Johansson, J. (2015). Predicting Harvest of Non-Native Signal Crayfish in Lakes—a Role for Changing Climate? *Canadian Journal of Fisheries and Aquatic Sciences*, 73 (5): 785-92.
- Capinha, C., Essl, F., Seebens, H., Moser, D. and Pereira, H. M. (2015). The dispersal of alien species redefines biogeography in the Anthropocene. *Science*, 348 (6240): 1248-1251.
- Carpenter, J. (2005). Competition for Food between an Introduced Crayfish and Two Fishes Endemic to the Colorado River Basin. *Environmental Biology of Fishes*, 72(3): 335-342.
- Catford, J. A., Jansson, R. and Nilsson, C. (2009). Reducing Redundancy in Invasion Ecology by Integrating Hypotheses into a Single Theoretical Framework. *Diversity and Distributions*, 15 (1): 22-40.
- Chucholl, C. (2012). New Alien Crayfish Species in Central Europe: Introduction pathways, life histories, and ecological impacts. PhD dissertation. der Fakultät für Naturwissenschaften der Universität Ulm, 237p.
- Chucholl, C. (2013a). Invaders for Sale: Trade and Determinants of Introduction of Ornamental Freshwater Crayfish. *Biological Invasions*, 15 (1): 125-41.



- Chucholl, C. (2013b). Feeding Ecology and Ecological Impact of an Alien ‘Warm-Water’ Omnivore in Cold Lakes. *Limnologia - Ecology and Management of Inland Waters*, 43(4): 219-29.
- Chucholl, C. and Wendler, F. (2017). Positive Selection of Beautiful Invaders: Long-Term Persistence and Bio-Invasion Risk of Freshwater Crayfish in the Pet Trade. *Biological Invasions* 19 (1): 197-208.
- Collen, B., Whitton, F., Dyer, E. E., Baillie, J. E. M., Cumberlidge, N., Darwall, W. R. T., Pollock, C., Richman, N. I., Soulsby, A. and Böhm, M. (2014). Global Patterns of Freshwater Species Diversity, Threat and Endemism. *Global Ecology and Biogeography*, 23 (1): 40-51.
- Collins, M., Knutti, R., Arblaser, J., Dufresne, J., Fichefet, T., Friedlingstein, P., Gao, X., Gutowski, W., Johns, T., Krinner, G., Shongwe, M., Teballdi, C., Weaver, A. and Wehner, M. (2013). Long-Term Climate Change: Projections, Commitments and Irreversibility. Pp. 1029–1136 in Climate Change 2013: The Physical Science Basis. IPCC reports.
- Corkum, L. D. and Cronin D. J. (2004). Habitat Complexity Reduces Aggression and Enhances Consumption in Crayfish. *Journal of Ethology*, 22 (1): 23-27.
- Correia, A. M. (2002). Niche Breadth and Trophic Diversity: Feeding Behaviour of the Red Swamp Crayfish (*Procambarus clarkii*) towards Environmental Availability of Aquatic Macroinvertebrates in a Rice Field (Portugal). *Acta Oecologica*, 23 (6): 421-29.
- Coughlan, N. E., Stevens, A. L., Kelly, T. C., Dick, J. T. A. and Jansen, M. A. K. (2017). Zoochorous Dispersal of Freshwater Bivalves: An Overlooked Vector in Biological Invasions? *Knowledge and Management of Aquatic Ecosystems*, 418 (2): 1-8.
- Crandall, K. A. and Buhay, J. E. (2008). Global Diversity of Crayfish (Astacidae, Cambaridae, and Parastacidae—Decapoda) in Freshwater. *Hydrobiologia*, 595 (1): 295-301.
- Crandall, K. A. and De Grave, S. (2017). An Updated Classification of the Freshwater Crayfishes (Decapoda: Astacidea) of the World, with a Complete Species List. *Journal of Crustacean Biology*, 37(5): 615-53.
- Crooks, J. A. (2002). Characterizing Consequences Invasions: The Role of Ecosystem Engineers. *Oikos*, 97: 153-66.

- Dalosto, M. M., Palaoro, A. V., Souty-Grosset, C., Bueno, S. L. S., Loureiro, T. G., Almerão, M. P., Araujo, P. B and Santos, S. (2015). One Step Ahead of the Enemy: Investigating Aggressive Interactions between Invasive and Native Crayfish before the Contact in Nature. *Biological Invasions*, 17 (12): 3503-3515.
- de Moor, I. (2002). Potential Impacts of Alien Freshwater Crayfish in South Africa. *African Journal of Aquatic Science*, 27(2): 125-39.
- Ehrenfeld, J. G. (2011). Ecosystem Consequences of Biological Invasions. *Annual Review of Ecology, Evolution, and Systematics*, 41 (1): 59-80.
- Erfmeier, A., Böhnke, M. and Bruelheide, H. (2011). Secondary Invasion of Acer Negundo: The Role of Phenotypic Responses versus Local Adaptation. *Biological Invasions*, 13 (7): 1599-1614.
- Faulkes, Z. (2015). The Global Trade in Crayfish as Pets. *Crustacean Research*, 44: 75-92.
- Faulkes, Z. (2018). Prohibiting Pet Crayfish Does Not Consistently Reduce Their Availability Online. *Nauplius* 26: e2018023.
- Figuerola, J. and Green, A. J. (2002). Dispersal of Aquatic Organisms by Waterbirds: A Review of Past Research and Priorities for Future Studies. *Freshwater Biology*, 47 (3): 483-94.
- Filipová, L., Grandjean, F., Chucholl, C., Soes, D. M. and Petrusek, A. (2011). Identification of Exotic North American Crayfish in Europe by DNA Barcoding. *Knowledge and Management of Aquatic Ecosystems*, 401: 1-11.
- Filipová, L., Petrusek, A., Matasova, K., Delaunay, C. and Grandjean. F. (2013). Prevalence of the Crayfish Plague Pathogen *Aphanomyces astaci* in Populations of the Signal Crayfish *Pacifastacus leniusculus* in France: Evaluating the Threat to Native Crayfish. *PLoS One*, 8 (7): e70157.
- Gherardi, F. and Barbaresi, S. (2000). Invasive Crayfish: Activity Patterns of *Procambarus clarkii* in the Rice Fields of the Lower Guadalquivir (Spain). *Archiv Für Hydrobiologie*, 150 (1): 153-168.
- Gherardi, F. (2010). Invasive Crayfish and Freshwater Fishes of the World. *Revue Scientifique et Technique (International Office of Epizootics)*, 29 (2): 241-254.

- Gherardi, F., Britton, J. R., Mavuti, K. M., Pacini, N., Grey, J., Tricarico, E. and Harper, D. M. (2011). A Review of Allodiversity in Lake Naivasha, Kenya: Developing Conservation Actions to Protect East African Lakes from the Negative Impacts of Alien Species. *Biological Conservation*, 144 (11): 2585-2596.
- Gioria, M., Pyšek, P. and Osborne, B. A. (2016). Timing Is Everything: Does Early and Late Germination Favor Invasions by Herbaceous Alien Plants??. *Journal of Plant Ecology*, 11 (1): 4-16.
- Goessmann, C., Hemelrijk, C. and Huber, R. (2000). The Formation and Maintenance of Crayfish Hierarchies: Behavioral and Self-Structuring Properties. *Behavioral Ecology and Sociobiology*, 48(6): 418-28.
- Grey, J. and Jackson, M. C. (2012). ‘Leaves and Eats Shoots’: Direct Terrestrial Feeding Can Supplement Invasive Red Swamp Crayfish in Times of Need. *PLOS One*, 7 (8): e42575.
- Heikal, M. N., Manar, S., Shamseldean, M., Shamseldean, M. and Hamdi, S. A. (2018). The red swamp crayfish, *Procambarus clarkii* (Gerard, 1852) an invasive species in Egypt as a biocontrol agent of the mosquito, *Culex quinquefasciatus* Say, 1823. *Bioscience Research*, 15 (2): 839-849.
- Hellmann, J. J., Byers, J. E., Bierwagen, B. G. and Dukes, J. S. (2008). Five Potential Consequences of Climate Change for Invasive Species. *Conservation Biology*, 22 (3): 534-43.
- Hofkin, B. V., Koech, D. K., Oumaj, J. and Loker, E. S. (1991). The North American Crayfish *Procambarus clarkii* and the Biological Control of Schistosome-Transmitting Snails in Kenya: Laboratory and Field Investigations. *Biological Control*, 1 (3): 183-187.
- Holdich, D. M. (2001). *Biology of Freshwater Crayfish*. Wiley-Blackwell, 512 p.
- Holdich, D. M., Reynolds, J. D., Souty-Grosset, C. and Sibley P. J. (2010). A Review of the Ever Increasing Threat to European Crayfish from Non-Indigenous Crayfish Species. *Knowledge and Management of Aquatic Ecosystems*, (394–395): 11.
- Holdich, D. M. (1993). A Review of Astaciculture: Freshwater Crayfish Farming. *Aquatic Living Resources*, 6(4): 307-317.

- Huber, R., Daws, A. G., Tuttle, S. A. and Panksepp, J. B. (2002). Quantitative Behavioral Techniques for the Study of Crustacean Aggression BT. In: Wiese, K. (ed.) *The Crustacean Nervous System*. Pp. 186–201. Berlin, Heidelberg: Springer Berlin Heidelberg.
- Hudina, S., Kutleša, P., Trgovčić, K. and Duplić, A. (2017). Dynamics of Range Expansion of the Signal Crayfish (*Pacifastacus leniusculus*) in a Recently Invaded Region in Croatia. *Aquatic Invasions*, 12 (1): 67-75.
- Hulme, P. E. (2015). Invasion Pathways at a Crossroad: Policy and Research Challenges for Managing Alien Species Introductions. *Journal of Applied Ecology*, 52 (6): 1418-24.
- Jerry, D. R., Purvis, I. W., Piper, L. R. and Dennis, C. A. (2005). Selection for Faster Growth in the Freshwater Crayfish *Cherax destructor*. *Aquaculture*, 247 (1): 169-76.
- Jeschke, J. M., Bacher, S., Blackburn, T. M., Dick, J. T. A., Essl, F., Evans, T., Gaertner, M., Hulme, P. E., Kühn, I., Mrugała, M., Pergl, J., Pyšek, P., Rabitsch, W., Ricciardi, A., Richardson, D. M., Sendek, A., Vilà, M., Winter, M. and Kumschick, S. (2014). Defining the Impact of Non-Native Species. *Conservation Biology*, 28 (5):1188-1194.
- Kawai, T. and Takahata, M. (2010). *The Biology of Freshwater Crayfish*. Sapporo: Hokkaido University Press.
- Kawai, T., Scholtz, G., Morioka, S., Ramanamandimby, F., Lukhaup, C. and Hanamura, C. (2009). Parthenogenetic Alien Crayfish (Decapoda: Cambaridae) Spreading in Madagascar. *Journal of Crustacean Biology*, 29 (4): 562-67.
- Kotovska, G., Khrystencko, D., Patoka, J. and Kouba, A. (2016). East European Crayfish Stocks at Risk: Arrival of Non-Indigenous Crayfish Species. *Knowledge & Management of Aquatic Ecosystems*, (417): 37.
- Kouba, A., Petrusek, A. and Kozák, P. (2014). Continental-Wide Distribution of Crayfish Species in Europe: Update and Maps. *Knowledge and Management of Aquatic Ecosystems*, (413): 05.
- Larson, E. R., Kreps, T. A., Peters, B., Peters, J. A. and Lodge, D. M. (2019). Habitat explains patterns of population decline for an invasive crayfish. *Ecology*, 100 (5): e02659.

- Lawrence J. C. (2002). *Cherax*. In: Holdich, D. M. (ed.). *Biology of freshwater crayfish*. Oxford, UK: Blackwell Science, 702 pp.
- Leeuwen, C. H. A. and van der Velde, G. (2012). Prerequisites for Flying Snails: External Transport Potential of Aquatic Snails by Waterbirds. *Freshwater Science*, 31 (3): 963-972.
- Lodge, D. M., Deines, A., Gherardi, F., Yeo, D. C. J., Arcella, T., Baldrige, A. K., Barnes, M. A., Chadderton, W. L., Feder, J. L., Gantz, C. A., Howard, G. W., Jerde, C. L. and Zeng, Y. (2012). Global Introductions of Crayfishes: Evaluating the Impact of Species Invasions on Ecosystem Services. *Annual Review of Ecology, Evolution, and Systematics*, 43 (1): 449-472.
- Lodge, D. M., Kershner, M. W. J., Aloï, E. and Covich, A. P. (1994). Effects of an Omnivorous Crayfish (*Orconectes rusticus*) on a Freshwater Littoral Food Web. *Ecology*, 75 (5): 1265-1281.
- Lodge, D. M., Williams, S., MacIsaac, H. J., Hayes, K. R., Leung, B., Reichard, S., Mack, R. N., Moyle, P. B., Smith, M., Andow, D. A., Carlton, J. T. and McMichael, A. (2006). Biological invasions: recommendations for U.S. policy and management. *Ecological Applications*, 16 (6): 2035-2054.
- Loureiro, T. G., Anastácio, P. M., Araujo, P. B., Souty-Grosset, C. And Almerão, M. P. (2015a). Red swamp crayfish: biology, ecology and invasion - an overview. *Nauplius*, 23: 1-19.
- Loureiro, T. G., Bueno, S. L. S., Anastácio, P. M., Almerão, M. P., Souty-Grosset, C. and Araujo, P. B. (2015b). Distribution, introduction pathway, and invasion risk analysis of the North American crayfish *Procambarus clarkii* (Decapoda: Cambaridae) in southeast Brazil. *Journal of Crustacean Biology*, 35: 88-96.
- Loureiro, T. G., Anastácio, P. M., Bueno, S. L. S. and Araujo, P. B. (2018). Management of invasive populations of the freshwater crayfish *Procambarus clarkii* (Decapoda, Cambaridae): test of a population-control method and proposal of a standard monitoring approach. *Environmental Monitoring and Assessment*, 190: 559.
- Loureiro, T. G., Anastácio, P. M., Bueno, S. L. S., Wood, C. T. and Araujo, P. B. (2019). Food Matters: Trophodynamics and the role of diet in

- Procambarus clarkii* invasion success in an Atlantic Forest conservation area. *Limnologica*, 79: 125717.
- Marijnissen, S. A. E., Michel, E., Cleary, D. F. R. and McIntyre, P. B. (2008). Ecology and Conservation Status of Endemic Freshwater Crabs in Lake Tanganyika, Africa. *Biodiversity and Conservation*, 18 (6): 1555-1573.
- Marshall, B. E. (2019). Crayfish, Catfish and Snails: The Perils of Uncontrolled Biological Control. *African Journal of Aquatic Science*, 44 (1): 1-5.
- Marshall, G. A., Moody, M. W. and Hackney, C. R. (1988). Differences in Color, Texture, and Flavor of Processed Meat from Red Swamp Crawfish (*Procambarus clarkii*) and White River Crawfish (*P. acutus acutus*). *Journal of Food Science*, 53 (1): 280-281.
- Marufu, L. and Nhwatiwa, T. (2014). Invasive Australian Crayfish *Cherax quadricarinatus* in the Sanyati Basin of Lake Kariba: A Preliminary Survey. *African Journal of Aquatic Science*. 39(2): 233-236.
- Miller, J. E., Savino, J. F. and Neely, R. K. (1992). Competition for Food between Crayfish (*Orconectes virilis*) and the Slimy Sculpin (*Cottus cognatus*). *Journal of Freshwater Ecology*, 7 (2): 127-136.
- Momot, W. T. (1995). Redefining the Role of Crayfish in Aquatic Ecosystems. *Reviews in Fisheries Science*, 3 (1): 33-63.
- Nentwig, W., Bacher, S., Kumschick, S., Pyšek, P. and Vilà, M. (2018). More than '100 Worst' Alien Species in Europe. *Biological Invasions*, 20 (6): 1611-1621.
- Nunes, A. L., Zengeya, T. A., Measey, G. J. and Weyl, O. L. F. (2017). Freshwater Crayfish Invasions in South Africa: Past, Present and Potential Future. *African Journal of Aquatic Science* 42(4):309–23.
- Nyström, P., Brönmark, C. and Granéli, W. (1996). Patterns in Benthic Food Webs: A Role for Omnivorous Crayfish? *Freshwater Biology*, 36 (3): 631-46.
- Olden, J. D., McCarthy, J. M., Maxted, J. T., Fetzer, W. W. and Vander Zanden, M. J. (2006). The Rapid Spread of Rusty Crayfish (*Orconectes rusticus*) with Observations on Native Crayfish Declines in Wisconsin (U.S.A.) over the Past 130 Years. *Biological Invasions*, 8 (8): 1621-28.

- O'Loughlin, L. S. and Peter T. G. (2017). Secondary Invasion: When Invasion Success Is Contingent on Other Invaders Altering the Properties of Recipient Ecosystems. *Ecology and Evolution*, 7 (19): 7628-37.
- Olsson, K., Stenroth, P., Nyström, P. and Granéli, W. (2009). Invasions and Niche Width: Does Niche Width of an Introduced Crayfish Differ from a Native Crayfish? *Freshwater Biology*, 54 (8): 1731-40.
- Parker, I. M., Simberloff, D., Lonsdale, W. M., Goodell, K., Wonham, M., Kareiva, P. M., Williamson, M. H., Von Holle, B., Moyle, P. B., Byers, J. E. and Goldwasser, L. (1999). Impact: Toward a Framework for Understanding the Ecological Effects of Invaders. *Biological Invasions*, 1 (1): 3-19.
- Perkins, L. B., Leger, E. A. and Nowak, R. S. (2011). Invasion Triangle: An Organizational Framework for Species Invasion. *Ecology and Evolution* 1 (4): 610-25.
- Price, J. O. and Payne, J. F. (1984). Size, Age, and Population Dynamics in an R-Selected Population of *Orconectes neglectus chaenodactylus* Williams (Decapoda, Cambaridae). *Crustaceana*, 46 (1): 29-38.
- Puky, M. (2014). Invasive Crayfish on Land: *Orconectes limosus* (Rafinesque, 1817) (Decapoda: Cambaridae) Crossed a Terrestrial Barrier to Move from a Side Arm into the Danube River at Szeremle, Hungary. *Acta Zoologica Bulgarica*, 66 (SUPPL. 7): 143-46.
- Ranta, E. and Lindström, K. (1993). Body Size and Shelter Possession in Mature Signal Crayfish, *Pacifastacus leniusculus*. *Annales Zoologici Fennici*, 30 (2): 125-132.
- Reynolds, J. and Souty-Grosset, C. (2011). *Management of Freshwater Biodiversity: Crayfish as Bioindicators*. Cambridge University Press, 384 p.
- Reynolds, J., Souty-Grosset, C. and Richardson, A. (2013). Ecological Roles of Crayfish in Freshwater and Terrestrial Habitats. *Freshwater Crayfish*, 19: 197-218.
- Rhein, M., Rintoul, S., Aoki, S., Campos, E., Chambers, D., Feely, R. and Wang, F. (2013). Observations: Ocean. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth*

- Assessment Report of the Intergovernmental Panel on Climate Change. *Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, 255-315.
- Ricciardi, A., Hoopes, M. F., Marchetti, M. P. and Lockwood, J. L. (2013). Progress toward Understanding the Ecological Impacts of Nonnative Species. *Ecological Monographs*, 83 (3): 263-282.
- Ruokonen, T. J., Sjövik, R., Erkamo, E., Tulonen, J., Ercoli, F., Kokko, H. and Jussila, J. (2018). Introduced Alien Signal Crayfish (*Pacifastacus leniusculus*) in Finland – Uncontrollable Expansion despite Numerous Crayfisheries Strategies. *Knowledge & Management of Aquatic Ecosystems*, (419): 27.
- Sandström, A., Andersson, M., Asp, A., Bohman, P., Edsman, L., Engdahl, F., Nyström, P., Stenberg, M., Hertonsson, P. and Vrålstad, T. (2013). Population Collapses in Introduced Non-Indigenous Crayfish. *Biological Invasions*, 16 (9): 1961-1977.
- Sargent, L. W. and Lodge, D. M. (2014). Evolution of Invasive Traits in Nonindigenous Species: Increased Survival and Faster Growth in Invasive Populations of Rusty Crayfish (*Orconectes rusticus*). *Evolutionary Applications*, 7 (8): 949-961.
- Saul, W. C., Roy, H. E., Booy, O., Carnevali, L., Chen, H. J., Genovesi, P., Harrower, C. A., Hulme, P. E., Pagad, S., Pergl, J. and Jeschke, J. M. (2017). Assessing Patterns in Introduction Pathways of Alien Species by Linking Major Invasion Data Bases. *Journal of Applied Ecology*, 54 (2): 657-669.
- Schrimpf, A., Chucholl, C., Schmidt, T. and Schulz, R. (2013). Crayfish Plague Agent Detected in Populations of the Invasive North American Crayfish *Orconectes immunis* (Hagen, 1870) in the Rhine River, Germany. *Aquatic Invasions*, 8 (1): 103-109.
- Shea, K. and Chesson, P. (2002). Community Ecology Theory as a Framework for Biological Invasions. *Trends in Ecology and Evolution*, 17 (4): 170-76.
- Simberloff, D., Martin, J. L., Genovesi, P., Maris, V., Wardle, D. A., Aronson, J., Courchamp, F., Galil, B., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R., Tabacchi, E. and Vilà, M. (2013). Impacts of



- Biological Invasions: What's What and the Way Forward. *Trends in Ecology and Evolution*, 28 (1): 58-66.
- Snoeks, J. B. T. (2000). How Well Known Is the Ichthyodiversity of the Large East African Lakes? *Advances in Ecological Research*, 31: 17-38 Academic Press.
- Söderhäll, K. and Cerenius, L. (1999). The Crayfish Plague Fungus: History and Recent Advances. *Freshwater Crayfish*, 12: 11-35.
- Soes, D. M. and Koese. B. (2010). Invasive Freshwater Crayfish in the Netherlands: A Preliminary Risk Analysis. *EIS-Stichting european invertebrate survey Nederland*, Leiden: Bureau Waardenburg, 75 p.
- Taylor, C. A., Schuster, G. A., Cooper, J. E., DiStefano, R. J., Eversole, A. G., Horton, P. H., Hobbs, H., Robison, H. W., Skelton, C. E., Thoma, R. F. (2007). A Reassessment of the Conservation Status of Crayfishes of the United States and Canada after 10+ Years of Increased Awareness. *Fisheries*, 32 (8): 372-389.
- Theoharides, K. A. and Dukes, J. S. (2007). Plant Invasion across Space and Time: Factors Affecting Nonindigenous Species Success during Four Stages of Invasion. *New Phytologist*, 176 (2): 256-273.
- Thomas, L., Craddock, O. E., Baxter, C. and Palacio, A. (2019). Agonistic Behavior of an Invasive Crayfish (*Orconectes palmeri*) toward the Muckalee Crayfish (*Procambarus gibbus*): Effects of Residence, Size, and Cover. *Journal of Freshwater Ecology*, 34 (1): 19-26.
- Thomas, J. R., Masefield, S., Hunt, R., Wood, M. J., Hart, A. G., Hallam, J., Griffiths, S. W. and Cable, J. (2019). Terrestrial Emigration Behaviour of Two Invasive Crayfish Species. *Behavioural Processes* 167 (February): 103917.
- Tilmans, M., Mrugała, A., Svoboda, J., Engelsma, M. Y., Petie, M., Soes, D. M., Nutbeam-Tuffs, S., Oidtmann, B., Roessink, I., Petrussek, A. (2014). Survey of the Crayfish Plague Pathogen Presence in the Netherlands Reveals a New *Aphanomyces astaci* Carrier. *Journal of Invertebrate Pathology*, 120: 74-79.
- Tricarico, E. and Gherardi, F. (2010). Past Ownership Makes Crayfish More Aggressive. *Behavioral Ecology and Sociobiology*, 64 (4): 575-581.

- Turbelin, A. J., Malamud, B. D. and Francis, R. A. (2017). Mapping the Global State of Invasive Alien Species: Patterns of Invasion and Policy Responses. *Global Ecology and Biogeography*, 26 (1): 78-92.
- Uderbayev, T., Patoka, J., Beisembayev, R., Petrtyl, M., Bláha, M. and Kouba, A. (2017). Risk Assessment of Pet-Traded Decapod Crustaceans in the Republic of Kazakhstan, the Leading Country in Central Asia. *Knowledge & Management of Aquatic Ecosystems*, 418 (30): 1-7.
- Wood, K. A., Hayes, J.B., England, J. and Grey, J. (2016). Invasive Crayfish Impacts on Native Fish Diet and Growth Vary with Fish Life Stage. *Aquatic Sciences*, 79 (1): 113-125.
- Woodward, G., Perkins, D. M. and Brown. L. E. (2010). Climate Change and Freshwater Ecosystems: Impacts across Multiple Levels of Organization. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 365 (1549): 2093-2106.

*Chapter 5*

**BIOLOGY, ECOLOGY, EVOLUTION,  
SYSTEMATICS AND UTILIZATION OF THE  
PARTHENOGENETIC MARBLED CRAYFISH,  
*PROCAMBARUS VIRGINALIS***

***Günter Vogt\****

Faculty of Biosciences, University of Heidelberg,  
Heidelberg, Germany

**ABSTRACT**

The marbled crayfish, *Procambarus virginalis*, is the only obligately parthenogenetic species of the ~15,000 decapod crustaceans. This chapter describes its detection history, biology, taxonomy, geographical distribution, ecology, evolution and utilization. The marbled crayfish was detected in 1995 in the German aquarium trade. Morphological and genetic evidence suggests that it has arisen by autotriploidy from slough crayfish, *Procambarus fallax*, which is native to Florida and southern Georgia. Since marbled crayfish was neither described in the extensive biogeographical literature on crayfishes of this region nor found in respective museum

---

\* Corresponding Author's E-mail: [gunter.vogt@web.de](mailto:gunter.vogt@web.de).

collections it is thought to have originated in evolutionarily recent times, perhaps in captivity. Genetic investigations revealed that marbled crayfish is of single origin and monoclonal. Comparison of morphology, life history, genetics, reproduction, behavior, ecology and biogeography between marbled crayfish and its parent species and application of the Evolutionary Genetic Species Concept for asexuals suggests treating marbled crayfish as a separate species rather than keeping it as a parthenogenetic lineage within *Procambarus fallax*. Beginning in the late 1990s, marbled crayfish was spread via the pet trade from Germany across the world. Releases have led to the establishment of wild populations in 16 countries on three continents. In Madagascar, marbled crayfish has already invaded a considerable proportion of the country. Behavioral and ecological data suggest that marbled crayfish can compete with other crayfish species, even with much bigger ones. Despite of genetic uniformity, marbled crayfish have adapted to a wide range of habitats in tropical to cold-temperate biomes. This was apparently possible by their capability to produce different phenotypes from the same genome by epigenetic mechanisms. Because of genetic identity, high fecundity, easy rearing, the availability of a draft genome and further advantages, the marbled crayfish is increasingly being used as a laboratory model for research including development, neurobiology, behavior, reproduction, toxicology, stem cell biology, genetics, epigenetics, and invasion biology. In Madagascar, wild marbled crayfish stocks are exploited as a food commodity.

**Keywords:** biogeography, competition, ecology, evolution, invasion, marbled crayfish, parthenogenesis, research model, systematics, exploitation

## 1. INTRODUCTION

In the last two decades, the marbled crayfish, *Procambarus virginalis*, has gained considerable attention in the scientific community and the public (Hossain et al., 2018; Vogt, 2018a, b; Faulkes, 2019). It is the only obligate parthenogen among the ~700 described crayfish species (Crandall and De Grave, 2017) and even the ~15,000 decapod crustaceans (De Grave et al., 2009). Soon after its detection in the German pet trade in 1995 it became a popular aquarium animal, first in Germany (Werner, 1998) and then worldwide (Chucholl, 2013, 2016; Faulkes, 2015a, b). Because of the

apomictic parthenogenetic production of up to 650 genetically identical offspring per female and clutch and easy rearing, it is well suitable as a laboratory model for research (Vogt, 2008a, 2018b; Faulkes, 2016; Scholtz, 2016). Meanwhile, marbled crayfish is among the best investigated freshwater crayfish species (Vogt, 2018a). It is the first crayfish with a fully sequenced genome (Gutekunst et al., 2018).

Releases led to the establishment of wild populations in several European countries, Madagascar and probably Japan (Chucholl, 2016; Usio et al., 2017; Gutekunst et al., 2018; Hossain et al., 2018). These invasive populations are feared to threaten native crayfish species by competition and transmission of the crayfish plague, an oomycete disease lethal to European astacid and African, South American and Australian parastacid crayfish species (Scholtz et al., 2003; Jones et al., 2009; Keller et al., 2014; Mrugała et al., 2015; Chucholl, 2016; Vodovsky et al., 2017; Hossain et al., 2018). They may also disturb food webs in the invaded ecosystems.

This chapter reviews the detection and research history, biological features, taxonomy, geographical distribution, ecology and evolution of the marbled crayfish. It further discusses its use and potential as a research model and legal regulations.

## **2. DETECTION AND RESEARCH HISTORY**

The oldest record on marbled crayfish known to me comes from colleague Frank Steuerwald. He reported that marbled crayfish were offered at an arthropod trade fair in Frankfurt, Germany, in 1995 (Steuerwald, personal communication). Attempts to clarify its geographical provenance failed due to unspecific information of the trader. In the following years, marbled crayfish became increasingly popular among aquarists. Marbled crayfish owners soon noticed that there were no males and that single females produced offspring, which led to discussions in aquarists' blogs on whether marbled crayfish might be a self-fertilizing hermaphrodite or even a parthenogen. However, at that time parthenogenesis was unknown in freshwater crayfish and other decapod crustaceans.

The publication history of marbled crayfish started three years after its discovery, when Werner (1998) described and illustrated this enigmatic freshwater crayfish in a German book for aquarists on the rearing of freshwater decapods. He coined the German name “Marmorrebs” (i.e., marbled crayfish) due to its marbled coloration and noted that marbled crayfish are kept by many German aquarists. Lukhaup (2001) wrote the first popular scientific article on marbled crayfish in a German aquarist’s journal. He used the name *Procambarus* sp. and emphasized that consulted crayfish specialists were not able to determine this new crayfish to species level. He further noted that marbled crayfish are offered in almost any aquarium shop in Germany. Seitz (2001) collected first life history data and my lab investigated the reproductive organs histologically (Tolley, 2002; Vogt and Tolley, 2004; Vogt et al., 2004), demonstrating that all individuals were pure females, excluding hermaphroditism as the mode of reproduction. In 2002, I introduced the new crayfish to an international scientific audience at a conference on Decapoda in Corfu, Greece (Tolley and Vogt, 2002).

The first scientific article on marbled crayfish was a joint paper by zoologists from the universities of Berlin and Heidelberg and aquarists that have kept marbled crayfish since 1995 (Scholtz et al., 2003). In this paper in the renowned journal *Nature*, we demonstrated parthenogenesis of marbled crayfish and emphasized its potential for research. We further warned that releases into the wild might threaten the indigenous European crayfish fauna and negatively affect freshwater ecosystems. This article was covered by the international media introducing the marbled crayfish to a world-wide public. The first specimen in the wild was detected in the same year in a gravel pit near Karlsruhe, Baden-Wuerttemberg, Germany (Marten et al., 2004).

The publications of the following years until today can be broadly categorized into three groups (Vogt, 2018a): (1) investigations on the biological features of marbled crayfish and its development and use as new laboratory model for research, (2) occurrence and spreading of marbled crayfish in the wild, and (3) marbled crayfish in the pet trade. The first category includes papers on the morphology, physiology, molecular biology, development, neurobiology, behavior, genetics, epigenetics, stem cell biology, toxicology and evolution. The second category comprises finds

in the wild, adaptations to invaded habitats, and the role of marbled crayfish as transmitter of the crayfish plague. The third category deals with rearing of marbled crayfish and the role of the pet trade in its distribution over the world.

The annual number of publications on marbled crayfish has steadily increased since 1998. Meanwhile, more than 250 articles, book chapters, reports, and theses have been published by more than 400 scientists from different biological disciplines and countries (Vogt, 2018a; Faulkes, 2019). In a crayfish book from 2016 entitled “*Freshwater Crayfish: Global Overview*” (Kawai et al., 2016) a section of seven chapters and 157 pages was specifically devoted to marbled crayfish.

### **3. MORPHOLOGY**

The morphology of marbled crayfish and the variability of major morphological characters are rather well investigated (Kawai et al., 2009; Martin et al., 2010a; Vogt et al., 2015, 2018, 2019; Lyko, 2017a). Morphological characters were used as diagnostic features and for the comparison of marbled crayfish with its parent species, *Procambarus fallax*. The latter aspect is discussed later in the “species issue” section.

#### **3.1. Diagnostic Characters**

The marbled crayfish is a relatively slender member of the Cambaridae and consists of females only. The maximum total length (tip of rostrum to end of telson) reliably recorded is about 11.2 cm corresponding to a body weight of ~35 g (Vogt et al., 2018). Marbled crayfish are well pigmented and show variable patterns of lighter spots on darker background (Figures 1A, B, 4A). The carapace is wider than the pleon and the pleon inclusive of the telson is longer than the carapace (Figure 1A). The first antenna is longer than the body. The rostrum has a central spiky acumen and elevated lateral margins with spines at their anterior ends (Figure 2A). A median carina is

lacking. The postorbital ridge is well developed and has a cephalic spine (Figure 2A). The areola constitutes approximately one third of the carapace length and is on average 5-6 times as long as wide (Figure 1A). The suborbital angle is not acute (Figure 2A). There is one acute cervical spine and one acute branchiostegal spine (Figure 2A) on either side of the cephalothorax.

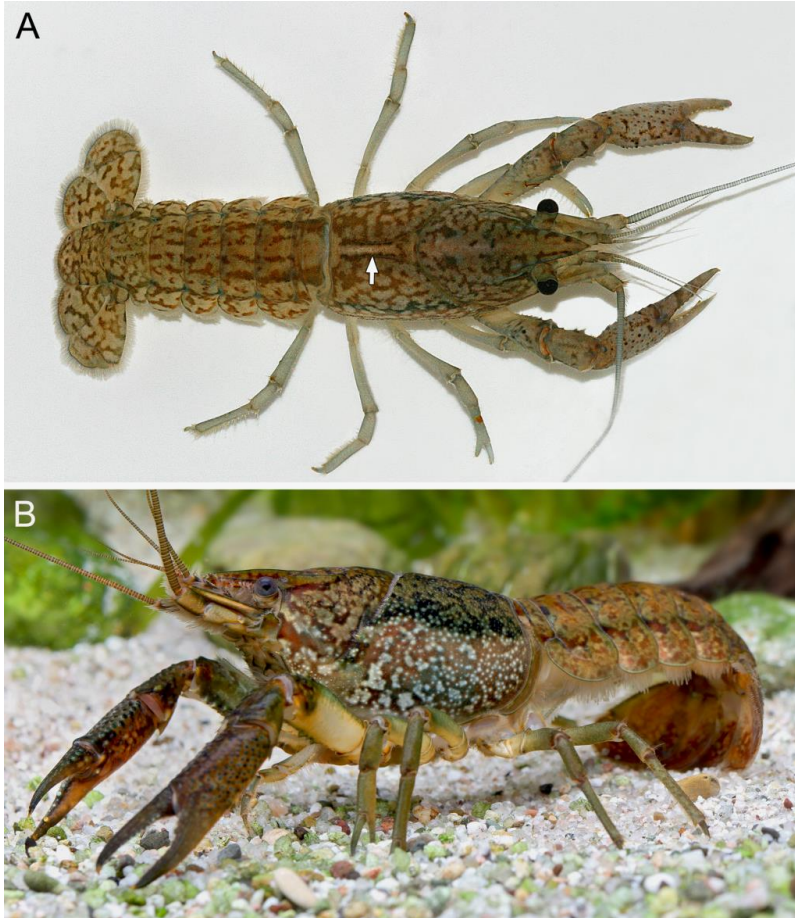


Figure 1. Habitus of marbled crayfish. A: Dorsal view of laboratory raised specimen showing eponymous marbled coloration. Arrow denotes areola (from Vogt, 2009). B: Lateral view of specimen from Lake Moosweiher, Germany (from Vogt et al., 2018).



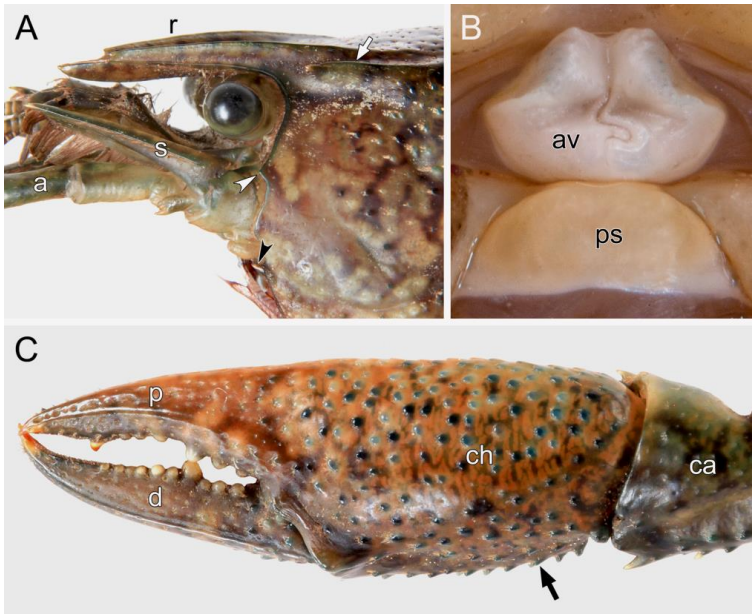


Figure 2. Morphological characters of marbled crayfish. A: Side view of cephalic region showing eye, rostrum (r), postorbital ridge (arrow), antenna (a) and scaphocerite (s). Black arrowhead, branchiostegal spine; white arrowhead, suborbital angle (from Vogt et al., 2018). B: Annulus ventralis (av) and postannular sclerite (ps) (from Vogt et al., 2018). C: Dorsal view of right cheliped. Arrow, row of tubercles on mesial margin of palm; ca, carpus; ch, chela; d, dactylus; p, propodus (from Vogt et al., 2018).

The annulus ventralis (spermatheca), a species-specific character of cambarid crayfishes, is bell-shaped, has a medial depression and is bisected by a trough leading caudally into an S-shaped sinus (Figure 2B). It is freely movable against the pre-annular sternum and is about 1.6 times as broad as long. The post-annular sclerite is dome-shaped and of similar width than the annulus ventralis (Figure 2B). The mesial margin of the palm of the chela has a row of 8-9 tubercles (Figure 2C). The lateral margin of the propodus is without spiniform tubercles and the opposable margin often shows a prominent excision (Figure 2C). The opposable margin of the propodus carries about 7 solid whitish denticles and 1 larger and corneous distal one, whereas the opposable margin of the dactylus has a row of about 10 whitish denticles (Figure 2C). The tips of the dactylus and propodus are pointed and corneous (Figure 2C).

The typical coloration of marbled crayfish consists of creamy patches on a darker background. These patches are most prominent on the lateral side of the carapace (Figures 1B, 4A). This marbled color pattern led to the name Marmorkrebs or marbled crayfish. Additionally, there are black and white spots, particularly on the pleon and chelipeds (Figure 2C). Further typical features are a median brown stripe extending dorsally from the rostrum to the caudal margin of the carapace (Figure 1A) and a broad black band dorsolaterally on the carapace extending from the cervical groove to the posterior end of the carapace (Figure 1B). Each side of the pleon has a prominent undulating black band between terga and pleura and an additional black band further dorsally (Figures 1B, 4A).

### **3.2. Variation of Morphological Characters**

The morphological characters of marbled crayfish vary considerably between laboratory raised and wild populations and within populations. The most variable characters are the shape of the rostrum and areola, the number of denticles on the dactylus and propodus of the cheliped, and the marmoration pattern on the lateral sides of the cephalothorax. The latter is specific for each individual and varies even between body sides (Figure 1A).

There are also differences between laboratory raised and wild populations concerning body proportions, background coloration and spination. Specimens raised in my laboratory had on average a longer pleon and a broader carapace (Vogt et al., 2018). The background coloration is more variable in laboratory raised than wild specimens ranging from olive to brown, reddish, ochre and bluish. In wild populations, the dorsal side is mostly dark olive, probably serving camouflage (Vogt et al., 2018). All marbled crayfish in the wild have prominent spines on the carapace, antennal basis and chelipeds (Figure 3A, B) (Chucholl and Pfeiffer, 2010; Martin et al., 2010b; Dümpelmann and Bonacker, 2012; Vogt et al., 2018). These spines are considerably reduced or lacking in laboratory raised specimens (Figure 3C, D).

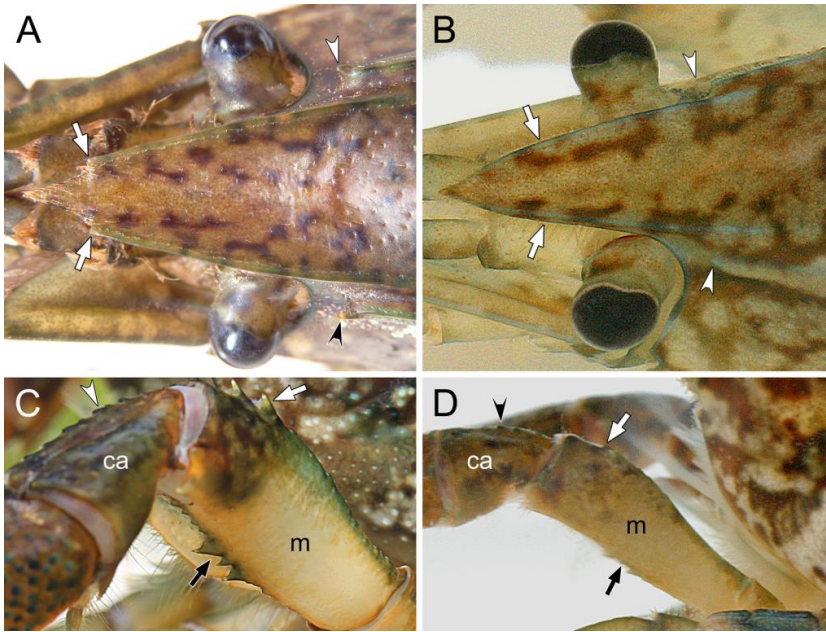


Figure 3. Differences in spination between wild (A, C) and laboratory raised (B, D) marbled crayfish. A: Lateral spines of rostrum (arrows) and anterior spines of postorbital ridges (arrowheads) in wild specimen from Lake Moosweiher (from Vogt et al., 2018). B: Absence of lateral rostral spines (arrows) and postorbital ridge spines (arrowheads) in laboratory raised specimen (from Vogt et al., 2018). C: Prominent spines (arrows) and protuberances (arrowhead) on carpus (ca) and merus (m) of wild specimen (from Vogt et al., 2018). D: Weakly developed spines (arrows) and protuberances (arrowhead) on carpus and merus of laboratory raised specimen (from Vogt et al., 2018).

#### 4. LIFE HISTORY

The life cycle of marbled crayfish consists of embryonic, juvenile and adult periods (Vogt et al., 2004; Seitz et al., 2005). The embryonic period starts with oviposition and attachment of the eggs to the pleopods and ends with hatching of the first juvenile stage. The juvenile period includes approximately 15 stages with interspersed molts. The adult life period begins with first reproduction and is characterized by alternating reproduction and growth phases that are separated by molts.

The embryos develop on the maternal pleopods (Figure 4A). The duration of embryonic development depends on water temperature and varies from about 17 to 28 days. The embryos develop directly, i.e., there are no larval stages (Vogt et al., 2004). Embryogenesis is characterized by short-germ development (Figure 4B) and stereotyped cell lineage (Figure 12A). Short-germ development is the sequential addition of body segments to the cephalon anlage from a posterior growth zone over many days.

The first two lecithotrophic juvenile stages are permanently carried on the pleopods of the mother (Figure 4C). Stage-3 juveniles, the first feeding stage, and sometimes also stage-4 juveniles adhere most of the time to the maternal pleopods for shelter but leave them regularly for feeding. The stage-3 juveniles appear as miniature adults and are fully developed with the exception of the reproductive organs. The postembryonic brooding period lasts 14-25 days, depending on environmental conditions (Vogt and Tolley, 2004; Vogt, 2008a, b). The juveniles (Figure 4E) grow step-wise at each molt and acquire successively their individual marmoration pattern. The internal and external sexual organs develop during the juvenile phase and are fully developed and functional only in the first reproducing stage.

Adult marbled crayfish have a total length of ~4-11 cm. Growth is indeterminate, which means that individuals grow until death without fixed growth limit, increasing their size at each molt. In my laboratory population generation time was mostly 6-7 months. The maximum longevity observed in the laboratory was 4.5 years but most adults lived for 2-3 years (Vogt et al., 2004; Vogt, 2008a, b, 2010).

Marbled crayfish reproduce exclusively by apomictic parthenogenesis. Consequently, all individuals are females. Mother and offspring are genetically identical (Martin et al., 2007; Vogt et al., 2008, 2015) with the exception of random mutations. In the laboratory, marbled crayfish can reproduce all year round but peaks of egg-laying were observed around spring and autumn equinoxes (Figure 5) (Vogt, 2015b). Most females in my laboratory spawned twice a year but some spawned once or three times. The maximum number of clutches per female and lifetime recorded so far was seven (Vogt, 2010). Clutch sizes varied between some 50 and 731 eggs depending on female size (Vogt, 2010; Vogt et al., 2015). The hatching rate

can be higher than 80% (Seitz et al., 2005; Vogt, 2008b). The maximum number of hatchlings per clutch was 427 in the laboratory (Vogt, 2010) and 650 in the wild (Lipták et al., 2017).

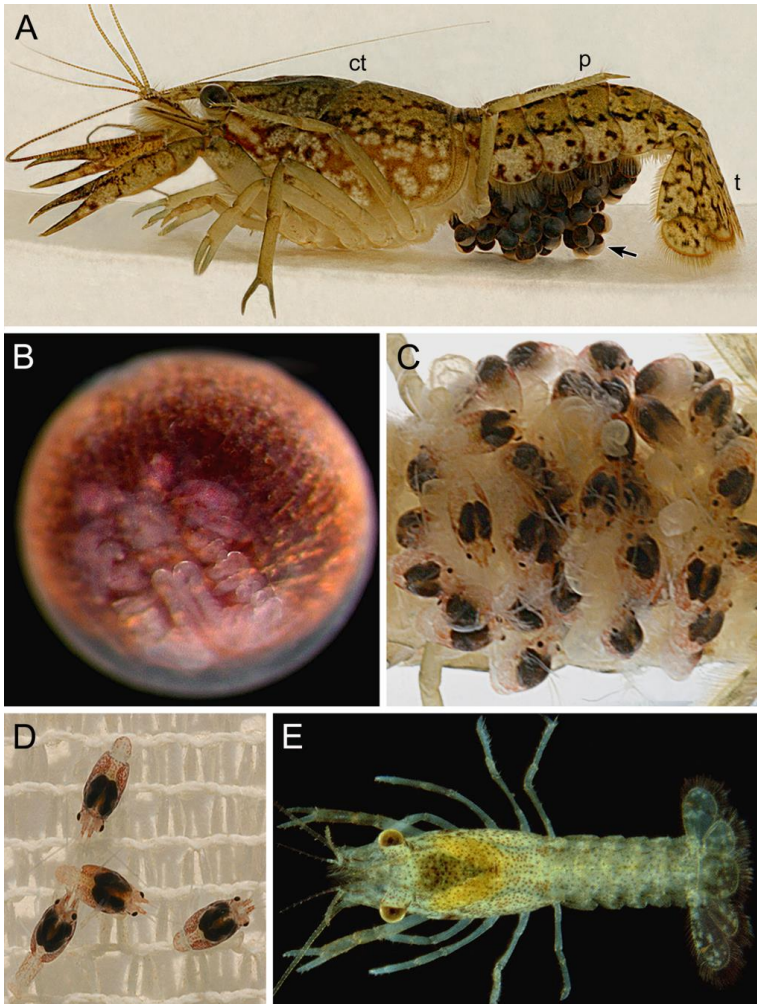


Figure 4. Life history of marbled crayfish. A: Mother with embryos on pleopods (arrow). ct, cephalothorax; p, pleon; t, telson (from Vogt and Tolley, 2004). B: Embryo at 50% development (from Vogt, 2018b). C: Stage-2 juveniles brooded on maternal pleopods (from Vogt and Tolley, 2004). D: Stage-2 juveniles raised in net culture system (from Vogt et al., 2008). E: Stage 5 juvenile (from Vogt et al., 2008).

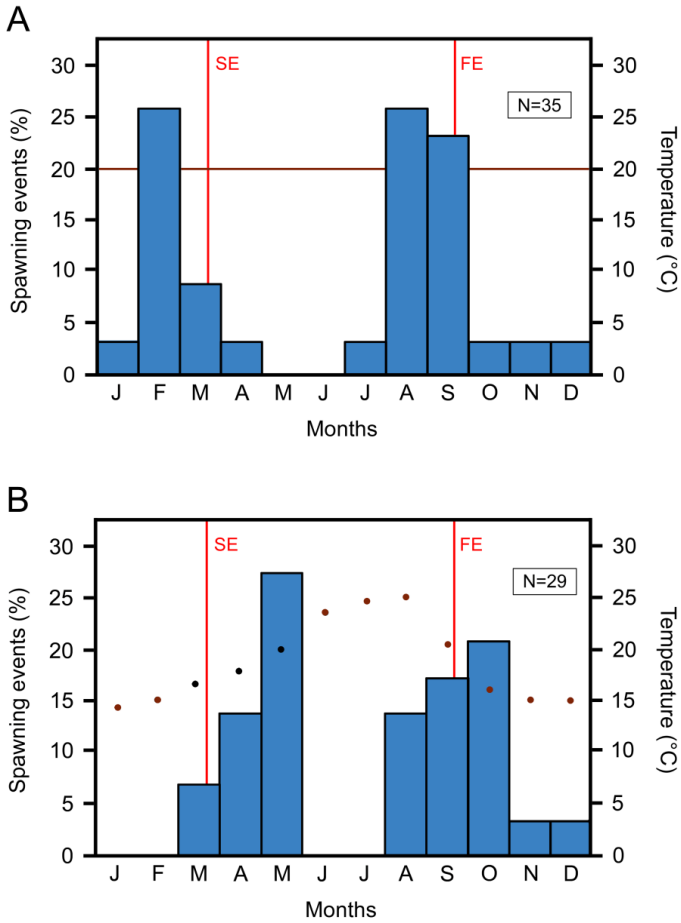


Figure 5. Annual distribution of spawnings in laboratory reared marbled crayfish. A: Rearing at constant temperature of 20°C (horizontal line). There are two prominent maxima of egg-laying, one before spring equinox (SE) and another one before fall equinox (FE). B: Rearing at seasonally fluctuating temperature ranging from ~15°C in winter to ~25°C in summer (horizontal dotted line). There are two maxima of egg-laying, one in May and another one in October (from Vogt, 2015b).

## 5. REARING

In the European Union, rearing of marbled crayfish is only allowed for research purposes (for details see “legislation section” below). In order to

prevent escapes into the wild marbled crayfish must be kept indoors in closed water systems.

Marbled crayfish can be mass cultured in commercially available stand alone aquarium systems with flow-through water management and aeration (Jimenez and Faulkes, 2010). However, due to the ability to breathe atmospheric air they can also be kept in very simple static containers without aeration (Vogt, 2008a; Vogt et al., 2015). Shelters are obligatory. Marbled crayfish are best cultured at temperatures of 18–25°C. Maximum growth is obtained at 25°C and maximum survival at 20°C (Seitz et al., 2005). They survive temperatures below 5°C and above 30°C for many weeks but mortality increases under such conditions and reproduction stops (Seitz et al., 2005; Kaldre et al., 2016). Marbled crayfish are omnivorous and can be fed throughout life with commercial pellet food, for example, Tetra WaferMix tablets. Tap water is suitable as a water source (Vogt, 2008a).

The embryos and early juvenile stages can be raised either naturally on the maternal pleopods or in vitro in net culture systems (Figure 4D) and even 12-well micro-plates (Vogt, 2007; Vogt et al., 2008). For net culture, small vessels should be filled to a water level of about 2 cm and be equipped with a fine-meshed net to enable attachment of the juveniles. Micro-plates are particularly suitable for the individual culture of embryos.

## 6. TAXONOMY

The possession of an annulus ventralis clearly indicated that marbled crayfish is a member of the American crayfish family Cambaridae (Scholtz et al., 2003). Morphological comparison and the analysis of the mitochondrial *cytochrome oxidase subunit 1 (COI)* and *12S rRNA* genes of several crayfish species revealed closest relationship of marbled crayfish to slough crayfish, *Procambarus fallax* (Hagen 1870), which is native to Florida and southern Georgia (Martin et al., 2010a). Therefore, marbled crayfish was considered as a parthenogenetic form of slough crayfish and provisionally named *Procambarus fallax* forma *virginalis* (Martin et al., 2010a).



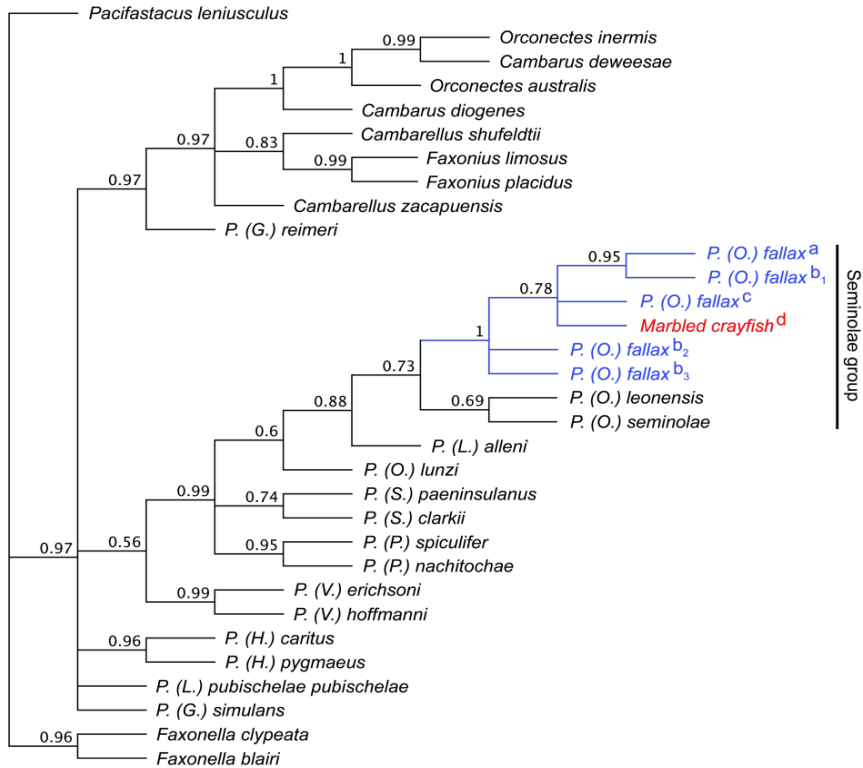


Figure 6. Taxonomy of marbled crayfish. The phylogenetic consensus tree is inferred from fragments of the mitochondrial *cytochrome c oxidase subunit I (COI)* genes. Included are marbled crayfish from Lake Moosweiher (d) and *Procambarus fallax* from the German aquarium trade (a), wild populations in the Everglades (b<sub>1</sub>-b<sub>3</sub>) and a laboratory population (c). Further included are *Procambarus* species from different subgenera (G, *Girardiella*; H, *Hagenides*; L, *Leonticambarus*; O, *Ortmannicus*; P, *Pennides*; S, *Scapulicambarus*; V, *Villalobosus*) and species of other genera of the Cambaridae. The astacidean *Pacifastacus leniusculus* was used as an outgroup. Numbers are posterior probability values. A clade with a value >0.70 is regarded as well supported. Marbled crayfish and *Procambarus fallax* form a distinct cluster and marbled crayfish is nested within *Procambarus fallax*. *Procambarus seminolae* and *Procambarus leonensis*, two further members of the Seminoiae group of the subgenus *Ortmannicus*, branch off next to the *Procambarus fallax*/marbled crayfish cluster (modified after Vogt et al., 2019).

More recent phylogenetic tree construction with *COI* sequences of marbled crayfish, *Procambarus fallax* and 25 Cambaridae from different genera confirmed that marbled crayfish belongs to the genus *Procambarus*



and the subgenus *Ortmannicus* and that it is most closely related to *Procambarus fallax* (Figure 6) (Vogt et al., 2019). Sequencing of 51 marbled crayfish from different laboratory colonies (Martin et al., 2010a; Vogt et al., 2015) and habitats from far distant sites in Germany (Martin et al., 2010b; Filipová et al., 2011; Vogt et al., 2018), Italy (Vojtkovská et al., 2014), Sweden (Bohman et al., 2013), Hungary (Löökkös et al., 2016), Japan (Usio et al., 2017) and Madagascar (Vogt et al., 2015; Gutekunst et al., 2018) revealed identical haplotypes, suggesting that marbled crayfish has a species-specific *COI* sequence without any variation.

Sequencing of 22 *Procambarus fallax* from different parts of Florida and southern Georgia (Martin et al., 2010a; Vogt et al., 2018) and the German aquarium trade (Martin et al., 2010a; Vogt et al., 2015) revealed a total of eight different *COI* sequences. These sequences showed variation in 15 base positions (Vogt et al., 2018). The uniform *COI* gene of marbled crayfish differed in 0-6 of 658 base pairs or 0-0.91% from the *COI* genes of *Procambarus fallax*. This difference is smaller than the measured intra-specific range of *Procambarus fallax* and suggests that the maternal ancestor of marbled crayfish was a *Procambarus fallax*. Sequences identical with the marbled crayfish *COI* were detected in *Procambarus fallax* museum samples coming from neighboring Alachua and Union counties in northern Florida (Vogt et al., 2018), indicating that this region may be the home of the ancestors of marbled crayfish.

Based on morphological criteria, Hobbs (1981) erected the Seminolae group of the subgenus *Ortmannicus* including *Procambarus fallax*, *Procambarus seminolae* Hobbs 1942, *Procambarus leonensis* (Hobbs 1942) and *Procambarus lunzi* (Hobbs 1940). In our *COI*-based tree, *Procambarus seminolae* and *Procambarus leonensis* branch off next to the marbled crayfish/*Procambarus fallax* cluster whereas *Procambarus lunzi* branches off a bit further away (Figure 6). The *COI* gene of marbled crayfish differs in 6.11%, 7.44% and 8.76% from the *COI* of *Procambarus leonensis*, *Procambarus seminolae* and *Procambarus lunzi*, respectively.

## 7. GENETIC FEATURES

Marbled crayfish is presently the best investigated freshwater crayfish with respect to genetics, particularly because a draft genome, transcriptome and genome-wide methylome are available (Martin et al., 2007, 2016; Vogt et al., 2008, 2015; Gatzmann et al., 2018; Gutekunst et al., 2018).

### 7.1. Chromosomes

Marbled crayfish are triploid (Vogt et al., 2015; Martin et al., 2016) having a haploid chromosome number of 92 (Martin et al., 2016). Such a high chromosome value is not unusual in the Decapoda. The signal crayfish, *Pacifastacus leniusculus trowbridgii*, was sometimes cited as the animal record holder in chromosome number (Fetzner and Crandall, 2002). The particularly high chromosome numbers and genome sizes in freshwater crayfish and other decapods (Lécher et al., 1995; Gregory and Mable, 2005) are sometimes explained by whole genome duplication events in their early evolution (Otto and Whitton, 2000; Martin et al., 2016). An argument against this polyploidization hypothesis comes from the lack of a positive correlation of chromosome number and genome size (Jeffery, 2015) suggesting that the high chromosome numbers in crayfish may at least partly result from evolutionary chromosome fragmentation.

The actual record holder in animal chromosome number is the butterfly *Polyommatus atlanticus* with a diploid chromosome number of 452 (Lukhtanov, 2015). However, since the number of chromosomes in the unfertilized eggs is 276 in the parthenogenetic marbled crayfish and only 226 in the butterfly, the animal record of chromosomes in gametes goes to the marbled crayfish.

## 7.2. Mitochondrial Genome

Analysis of the mitochondrial genome of marbled crayfish revealed a derived pattern when compared to the ground pattern of the Decapoda (Shen et al., 2013). In marbled crayfish, there are several genes inverted from the plus strand to the minus strand and vice versa by a 180° turn of a larger stretch of the mitochondrial DNA (Figure 7A). Furthermore, there is one gene translocated to another position in the same strand and three genes to other positions in the opposite strand (Shen et al., 2013). Similar deviations from the decapod ground pattern have also been found in the European lobster *Hommarus gammarus* (Nephropidae), the closest relative of freshwater crayfishes within the Astacidea (Shen et al., 2013). In contrast, the mitochondrial genome of the parastacid crayfish *Cherax destructor* was similar to the ground pattern of the Decapoda (Shen, 2012). Shen et al. (2013) concluded from these results that a big sequence inversion has occurred in the stem group of the Astacidea, has been retained in the Nephropidae and Cambaridae but has been reverted in the Parastacidae.

## 7.3. Nuclear Genome

In 2013, we have launched a project at the German Cancer Research Center (DKFZ, Heidelberg) to establish the complete genome sequence, transcriptome and genome-wide methylome of marbled crayfish (Vogt et al., 2013). The hepatopancreas and pleonal musculature of an approximately 3-year-old individual in reproduction phase from my colony was used for sequencing and assembly of the reference genome. Library generation, sequencing and pre-processing of read data was performed by a private company. It included paired end sequencing of fragments with insert sizes up to 40.000 bp on an Illumina HiSeq platform, which allowed more accurate alignment of sequences, particularly repetitive elements. Assembly of the genome was done by Julian Gutekunst in the framework of his doctoral thesis (Gutekunst, 2017).

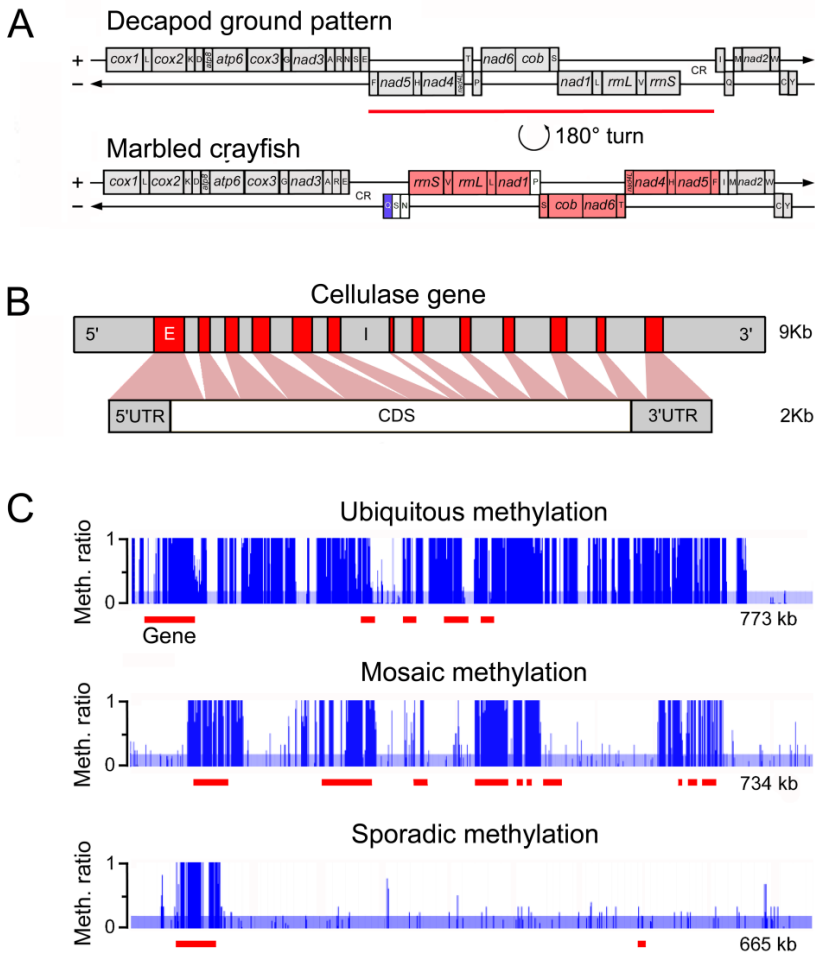


Figure 7. Genetic and epigenetic features of marbled crayfish. A: Gene arrangement in the mitochondrial genome of marbled crayfish. Genes that kept their original position in comparison to the ground pattern of the Decapoda are in grey, genes inverted to the other strand are in red, genes translocated to other positions in the same strand are in blue and genes translocated to other positions in the opposite strand are in white. Horizontal bar indicates inverted sequence stretch (redrawn and modified after Shen et al., 2013). B: Gene structure and transcript of the GH9 cellulase gene of marbled crayfish, an important component of herbivory. CDS, coding sequence; E, exon; I, intron; UTR, untranslated region (redrawn and modified after Gutekunst et al., 2018). C: Examples of ubiquitously, mosaically and sporadically methylated genomic scaffolds. Methylation ratios of CpGs are indicated by vertical bars. Methylation ratios below 0.2 are bisulfite conversion artefacts (redrawn and modified after Falckenhayn, 2016).

Meanwhile, a draft genome is published (Gutekunst et al., 2018) and can be downloaded at <http://marmorkrebs.dkfz.de/downloads/>. Benchmarking with universal single-copy orthologs indicated that the quality of the published version of the marbled crayfish genome assembly (version 0.4) is comparable to other, recently published arthropod genomes (Gutekunst et al., 2018). The genomes of several further marbled crayfish from the laboratory and the wild, males and females of *Procambarus fallax* from the German pet trade, and males and females of *Procambarus alleni* from the German pet trade were sequenced for comparison and mapped to the marbled crayfish reference genome (Gutekunst et al., 2018).

The haploid genome size of marbled crayfish was estimated to 3.3 Gb by k-mer analysis of the genome assembly and to 3.5 Gb and 3.9 Gb by flow cytometry of hemocytes using mouse and human blood cells as references, respectively (Vogt et al., 2015; Gutekunst et al., 2018). The genome size of ~3.5 Gb of marbled crayfish is an order of magnitude larger than the genomes of the common research models *Caenorhabditis elegans* (nematode, 100.2 Mb) and *Drosophila melanogaster* (fruit fly, 122.6 Mb) and in the same order of magnitude as the genomes of mouse *Mus musculus* (2.8 Gb), rat *Rattus norvegicus* (3.04 Gb) and humans (3.32 Gb). It is larger than the means of other species-rich animal groups like insects (1.6 Gb), molluscs (1.8 Gb) and fishes (1.9 Gb), close to the means of crustaceans (3.1 Gb) and mammals (3.5 Gb), but smaller than the means of amphibians (16.7 Gb) (Gregory et al., 2007; Vogt, 2017b).

The genome of marbled crayfish comprises 21,772 predicted genes incorporating a total of 86,771 exons and 65,683 introns (Gutekunst et al., 2018). This gene number is close to values published for humans, mouse, *Caenorhabditis elegans* and water flea *Daphnia pulex* but higher than in most sequenced insects (Hou and Lin, 2009; Perteza and Salzberg, 2010; Ye et al., 2017). The average gene length is 6.7 kb, with average exon and intron sizes of 0.3 kb and 2.0 kb, respectively. Repeat annotation detected 484,313 repeats covering 8.8% of the genome assembly (Gutekunst et al., 2018). They mainly consist of simple repeats, SINEs, LINEs and DNA transposons. Repeat coverage is likely to increase in future versions of the genome. The

analysis of heterozygous sequence variants revealed a global heterozygosity rate of 0.53%, which is high compared to other sequenced genomes.

The transcriptome of marbled crayfish was established from four pooled tissues (hepatopancreas, abdominal musculature, antennal gland and hematopoietic tissue) of a 2-year-old individual in growth phase from my colony. It revealed 22,338 transcripts, which corresponds roughly to the numbers of predicted genes (Falckenhayn, 2016; Gutekunst et al., 2018). Benchmarking confirmed that the quality of the transcriptome was comparable to other, recently published arthropod transcriptomes. 12,855 transcripts (57.5%) could be automatically annotated (Figure 14F).

There are not yet many individual genes of marbled crayfish analyzed in detail. An example is a cellulase gene from the glycoside hydrolase family 9, which is 9 kb long, consists of 13 exons and generates a coding sequence of 2 kb (Figure 7B) (Gutekunst et al., 2018). This endogenous and phylogenetically old cellulase is an essential enzyme for the digestion of cellulose and enables marbled crayfish to feed on plant material.

## 7.4. Epigenome

The epigenome of an organism consists of chemical changes to the DNA and histones and is crucial for gene expression (Allis and Jenuwein, 2016). Methylation of the DNA is presently the best investigated epigenetic mechanism (Edwards et al., 2017). Analysis of the methylome of marbled crayfish is expected to contribute to the understanding of the biological role of DNA methylation in invertebrates (Gatzmann et al., 2018) and the generation of different phenotypes from the same genome, a mechanism that apparently helps organisms to adapt to different environments (Vogt, 2017a).

Global DNA methylation (5-methylcytosine per total cytosine) in marbled crayfish is about 2.4% as shown by mass spectrometry (Vogt et al., 2015). This value is in the order of magnitude of zebrafish, mouse, rat, and humans (Fraga et al., 2005; Xia et al., 2015; Liu et al., 2016) but an order of magnitude higher than in most insects (Vogt, 2017b). The genomes of the

invertebrate research models *Caenorhabditis elegans* and *Drosophila melanogaster* are unmethylated (Raddatz et al., 2013).

The reference methylome of marbled crayfish, which shows the methylation of individual bases on the whole genome scale, was obtained by bisulfite sequencing on an Illumina platform of the hepatopancreas and pleonal musculature of a 2-year-old individual from my colony (Falckenhayn, 2016). Analysis of the methylation pattern of the 20 longest scaffold sequences established that about 25% of the sequences were ubiquitously methylated, 70% were mosaically methylated and 5% were sporadically methylated (Figure 7C) (Falckenhayn, 2016). Ubiquitous DNA methylation is typical of vertebrates, whereas invertebrates usually show mosaic methylation or sporadic methylation (Albalat et al., 2012; Breiling and Lyko, 2015). In the ubiquitously methylated genomes of vertebrates, more than 80% of the CpG dinucleotides are methylated and unmethylated sites are typically associated with active regulatory elements. Mosaic-type methylation is characterized by the alternation of domains of methylated and unmethylated DNA (Figure 7C).

Analysis of the methylome of marbled crayfish further revealed that 41% of the genes are heavily methylated and 26% are unmethylated (Falckenhayn, 2016). DNA methylation is CpG-specific as in other animal genomes and present in both coding genes and repeats (Falckenhayn, 2016; Gatzmann et al., 2018). Methylation was found in the coding sequences, exons, introns and 5'UTR and 3'UTR of genes. Gene body methylation was highest in evolutionarily old housekeeping genes predating the Arthropoda, genes with length of several kb and moderately expressed genes (Falckenhayn, 2016; Gatzmann et al., 2018). Repeats were generally hypomethylated and only repeats located within genes showed higher methylation levels. DNA transposons showed the highest methylation level of all repeat classes being close to the average methylation level of gene bodies. Evolutionarily old repetitive elements displayed higher methylation levels than younger ones (Falckenhayn, 2016). The integrative analysis of DNA methylation, chromatin accessibility and mRNA expression patterns revealed that gene body methylation in marbled crayfish was correlated with limited chromatin accessibility and stable gene expression. Low-methylated

genes often resided in chromatin with higher accessibility and showed increased expression variation (Gatzmann et al., 2018).

The DNA methylation toolkit of marbled crayfish consists of single homologues of the DNA methyltransferases Dnmt1 and Dnmt3 and the ten-eleven translocation methylcytosine dioxygenase Tet (Falckenhayn, 2016; Gatzmann et al., 2018), providing a full complement of enzymes for de novo and maintenance methylation, demethylation and hydroxymethylation. This pattern of single copies of the DNA methylation toolkit is relatively simple when compared to mammals, which have one Dnmt1, three Dnmt3 and three Tet (Lyko, 2018), but nevertheless, it is highly effective. It is supposed to be phylogenetically ancestral for arthropods and animals in general (Vogt, 2017b). The components of the DNA methylation system are dynamically expressed during marbled crayfish development and in adult tissues (Gatzmann et al., 2018).

## **8. BEHAVIOR**

There are only few behavioral studies published on marbled crayfish but aquarists and researchers including myself that have kept marbled crayfish for longer periods of time have collected many anecdotal data on various behavioral aspects.

### **8.1. Activity, Breathing of Atmospheric Air and Molting**

In my laboratory, marbled crayfish were mainly active during the night. During the day they hid in shelters. The activity of wild marbled crayfish has not yet been tracked by transmitters but repeated surveys in Lake Moosweiher, southwestern Germany, revealed that activity is mainly restricted to the dark period.

When the oxygen content in the water is low, marbled crayfish go to the water surface, roll themselves on the side and propel a mixture of water and atmospheric air through the exposed gill chamber (Vogt, 2008a). Berried



females even leave the water completely under conditions of low oxygen and fan the carried embryos and brooded juveniles in atmospheric air for a few minutes. These behaviors enable marbled crayfish to survive in waters of very low oxygen concentration as long as they can reach the water surface.

In the laboratory, most specimens molted during the day in the open arena of the aquarium when their conspecifics rested in the shelters. Since molting specimens are unable to fight or flee, this time selection probably reduced the likelihood of becoming cannibalized.

## **8.2. Spawning and Brooding Behaviors**

The spawning and brooding behaviors of marbled crayfish follow the scheme described for other cambarid species (Andrews, 1904; Mason, 1970; Gherardi, 2002; Reynolds, 2002) except that the eggs are not fertilized.

In the days before spawning marbled crayfish clean the underside of the pleon and the pleopods, the attachment sites of the eggs. For spawning, they form a pouch by bending the pleon towards the underside of the cephalothorax, fill this pouch with a gelatinous secretion and then release the eggs into it (Seitz, 2001; Seitz et al., 2005; Kato et al., 2016). The gelatinous mass is derived from so-called glair glands located in the sterna and pleura of the pleon and the tail fan (Seitz et al., 2005; Kato et al., 2016; Vogt, 2018c). These glands appear 2-3 weeks before spawning and are good indicators of forthcoming egg-laying. The gelatinous mass differentiates into an inner mass of relatively low viscosity and a parchment-like structure at the contact zone with the water, resembling the flysheet of a tent. In the sexually reproducing Cambaridae, the eggs are fertilized in this fertilization tent by sperm that is mobilized from the annulus ventralis (Andrews, 1904; Zehnder, 1934; Mason, 1970; Reynolds, 2002; Vogt, 2002).

The fertilization tent is an autapomorphy of all freshwater crayfish and is neither found in freshwater shrimps and freshwater crabs nor in clawed lobsters, the marine sister clade of freshwater crayfish. This structure is maintained for several hours and seems to have multiple functions. It probably protects the soft and highly labile fresh eggs (and sperm in sexually

reproducing species) from the osmotic stress of freshwater. A further function is apparently the prevention of egg loss during the complicated and long-lasting egg attachment procedure, which includes a series of very specific rolling and turning movements of the spawning female (Andrews, 1904; Zehnder, 1934; Mason, 1970; Thomas, 1991).

Berried females of marbled crayfish hide in shelters and are more aggressive than unberried females. They either completely stop feeding or take up only small amounts of food. They clean the eggs with their 4th and 5th pereopods, aerate the eggs and juveniles regularly by shaking of the pleopods, and remove decaying eggs.

### **8.3. Agonistic Behaviors**

In the laboratory, marbled crayfish showed the typical spectrum of agonistic crayfish behaviors including avoidance (retreat), threat (approach with outspread chelae), strike (sudden thrust of outstretched chelae against one another) and fight as defined in Gherardi (2002) and Lundberg (2004). Takahashi et al. (2019) revealed that large specimens usually defeated smaller opponents except when the small opponents owned high quality shelters. The aggressiveness and competitiveness of marbled crayfish in comparison to other crayfish species is discussed in the “ecology” section below.

Interestingly, despite genetic identity and the absence of males marbled crayfish established social hierarchies when reared in groups (Vogt et al., 2008; Farca Luna et al., 2009). The dominant, which was usually the biggest female in a group, showed aggressive behaviors and the subordinates showed avoiding behaviors. Juveniles displayed initially no agonistic behaviors and started to establish social hierarchies from about juvenile stage 7 when their claws became suitable for fighting (Vogt et al., 2008). When a dominant was removed from a group and grouped together with considerably larger specimens its dominant behavior was rapidly reverted to subordinate behavior.

## 9. BIOGEOGRAPHY AND ECOLOGY

All marbled crayfish populations in the wild originate from releases since the year 2003. Marbled crayfish was not found in the native range of its parent species, *Procambarus fallax*, which led to the hypothesis that it may have arisen in captivity (details in “evolution” section below). There are only few publications on the ecology of marbled crayfish but some populations are presently under intense investigation.

### 9.1. Geographical Distribution

Marbled crayfish were detected in the wild in 14 European countries, Madagascar and Japan (Table 1, Figure 8). In Europe, they were found in one site in Austria (Salzburg ORF, 2018), Croatia (Cvitanić, 2017), Denmark (CPH Post Online, 2019), Estonia (Estonian Research Council, 2018) and Romania (Pârvulescu et al., 2017), two sites in the Czech Republic (Patoka et al., 2016), Italy (Nonnis Marzano et al., 2009; Vojtkovská et al., 2014), the Netherlands (Soes and Koese, 2010), Sweden (Bohman et al., 2013) and the Ukraine (Novitsky and Son, 2016), four sites in Hungary (Lökkös et al., 2016) and Slovakia (Lipták et al., 2016; Liptak et al., 2017), 5 sites in Malta (Deidun et al., 2018) and 22 sites in Germany (Chucholl et al., 2012; Chucholl, 2016; Lyko, 2017a). In Japan, marbled crayfish were found in two localities (Kawai, 2017; Usio et al., 2017) and in Madagascar in >30 sites (Jones et al., 2009; Kawai et al., 2009; Gutekunst et al., 2018; Andriantsoa et al., 2019) (Figure 8). There are further reports on marbled crayfish in garden ponds in Germany and Slovakia (Stloukal, 2009; Chucholl, 2016), which I have not included in Table 1 and Figure 8.

Some finds in the wild consisted of single specimens, some of a few specimens and some of dozens to hundreds of individuals. Not all finds reflect established populations (Figure 8). In Europe, there are at least 31 established populations, and in Madagascar there is obviously a large area of about 100,000 km<sup>2</sup> inhabited by marbled crayfish. In 20 cases the actual population status is unclear (Table 1, Figure 8), because follow-up studies

were not performed. This number includes the two sites in Japan, in which only 1 and 2 specimens were found (Tadashi Kawai, pers. comm.). Marbled crayfish have apparently disappeared from River Märstaån and a pond in Skara in Sweden (Patrik Bohman, pers. comm.) and two sites in the Netherlands (Menno Soes, pers. comm.).

The European populations are mostly restricted to individual, isolated water bodies, mainly ponds and lakes. However, in Berlin there is apparently a system of several lakes (Hundekehlesee, Grunewaldsee, Krumme Lanke and Schlachtensee) and their connecting canals invaded (Linzmaier, 2016; Stefan Linzmaier, pers. comm.). The same holds for Lake Hévíz, its outflow and several downstream canals in the West-Balaton region in Hungary (Lökkös et al., 2016). Several populations of marbled crayfish were also found in close proximity to each other in the Váh-Nitra river system in Slovakia (Lipták et al., 2016).



Figure 8. Finds of marbled crayfish in the wild (as of August 13, 2019). Black dots, established populations; red dots, population status unclear; white dots, probably extinct. BE, Belgium; BH, Bosnia and Herzegovina; CH, Switzerland; CZ, Czech Republic; DK, Denmark; ES, Estonia; HR, Croatia; LV, Latvia; LT, Lithuania; MD, Moldova; M, Montenegro; NL, Netherlands; K, Kosovo; R, Russia; SK, Slovakia; SL, Slovenia; T, Turkey. The European populations are presently restricted to narrow geographical areas whereas in Madagascar marbled crayfish has spread over some 100.000 km<sup>2</sup>. See text and Table 1 for references.

**Table 1. Marbled crayfish finds in the wild (as of August 13, 2019)**

Country	Location	Coordinates	Year of detection	Population status, found specimens	Reference
Austria	Lake Karlsbader Weiher, Salzburg	47°50'17.3"N; 13°00'39.4"E	2018	Unclear, several specimens	Salzburg ORF (2018)
Croatia	Lake Šoderica	46°14'18.1"N; 16°54'49.9"E	2013	Established	Cvitanić (2017)
Czech Republic	Pond in Přátelství Park, Prague	50°07'21"N; 14°29'43" E	2015	Established	Patoka et al. (2016)
	Radovesická spoil heap near Břlína	50°33'08"N; 13°48'53"E	2016	Established	Patoka et al. (2016)
Denmark	Karup stream, Skive, Jutland	~56°33'54"N; 09°01'55"E	2019	Unclear, 1 specimen	CPH Post Online (2019)
Estonia	Cooling canal of Narva power plant	59°16'24.9"N; 27°54'54.0"E	2017	Established	Estonian Research Council (2018)
Germany	Lake near Giften, Lower Saxony	52°12'55"N; 09°49'33"E	2010	Established	Chucholl et al. (2012)
	River in Exten, Lower Saxony	52°10'14"N; 09°05'48"E	2009	Unclear, several specimens	Chucholl et al. (2012)
	Rhine harbour, Duisburg, North Rhine-Westfalia	51°27'36"N; 06°43'24"E	2009	Unclear, several specimens	Chucholl et al. (2012)
	Lake Krumme Lanke, Berlin	52°27'07.9"N; 13°13'57.3"E	2009	Established	Linzmaier (2016)
	Lake Hundeklehsee, Berlin	52°28'56.2"N; 13°15'31.4"E	2016	Established	Stefan Linzmaier (pers. comm.)
	Pond in Klepzig, Saxony-Anhalt	51°28'58.0"N; 12°07'13.7"E	2010	Established	Wendt (2011)
	Lake Dillinger See, Saarland	49°21'16.3"N; 06°42'17.1"E	2011	Unclear, 1 specimen	Potel (2011)
	Brook Burbach, Saarland	49°15'17.4"N; 06°56'37.6"E	2019	Unclear, 1 juvenile	Steffen Potel (pers.comm.)
	Brook near Uebigau, Saxony	51°20'17"N; 13°33'33"E	2009	Unclear, 1 specimen	Martin et al. (2010b)
	Lake Singliser See, Hesse	51°03'33"N; 09°18'22"E	2010	Established	Dümpelmann and Bonacker (2013)
Ditch, Mörfelden, Hesse	49°58'43"N; 08°36'59"E	2008	Unclear, 3 specimens	Chucholl et al. (2012)	

**Table 1. (Continued)**

Country	Location	Coordinates	Year of detection	Population status, found specimens	Reference
Germany	River Rhine, Bingen, Rhineland-Palatine	~49°58' N; 07°53' E	2011	Unclear, 1 specimen	Chucholl et al. (2012)
	Lake Reilinger See, Baden-Wuerttemberg	49°17'48.1"N; 08°32'40.6"E	2017	Established	Lyko (2017a)
	Gravel pit, Eggenstein, Baden-Wuerttemberg	49°5'19"N; 08°22'17"E	2003	Unclear, 1 specimen	Marten et al., 2004
	Lake Epplesee, Baden-Wuerttemberg	48°32'36"N; 09°09'14"E	2011	Established	Chucholl et al., (2012)
	Lake Moosweiher and outflow, Freiburg, Baden-Wuerttemberg	48°01'53"N; 07°48'18"E	2009	Established	Chucholl and Pfeiffer, (2010)
	Lake Flückiger See, Freiburg, Baden-Wuerttemberg	48°01'02"N; 07°81'84"E	2015	Established	Seitz (2015)
	Gravel pit Schuttern, Baden-Wuerttemberg	48°24'07.2"N; 07°51'39.5"E	2018	Unclear, 1 specimen	Christian Günter (pers. comm.)
	Pond in Denzlingen, Baden-Wuerttemberg	48°04'05.5"N; 07°53'51.5"E	2015	Established	Christian Günter (pers. comm.)
	Lake Matschelsee, Baden-Wuerttemberg	48°24'21.6"N; 07°48'59.0"E	2017	Established	Michael Pfeiffer (pers. comm.)
	Brook near Weissingen, Bavaria	48°26'46"N; 10°09'28"E	2008	Unclear, 1 specimen	Chucholl et al. (2012)
	Lake Murner See, Bavaria	49°20'57"N; 12°12'16"E	2019	Established	Frank Lenich, pers. comm.
Hungary	Thermal Lake Hévíz	46°47'14.1"N; 17°11'34.2"E	2014	Established	Lökkös et al. (2016)
	Északiárapasztó-csatorna canal	near Lake Heviz	2014	Established	Lökkös et al. (2016)
	Páhoki-övcatorna canal	near Lake Heviz	2013	Established	Lökkös et al. (2016)
	Egysített-övcatorna canal	near Lake Heviz	2014	Established	Lökkös et al. (2016)

Country	Location	Coordinates	Year of detection	Population status, found specimens	Reference
Italy	Canale Maestro della Chiana, Tuscany	43°16'50.64"N; 11°50'11.98"E	2008	Unclear, 1 specimen	Nonnis Marzano et al. (2009)
	Po di Maistra Channel, Po delta	44°59'2.80"N; 12°22'32.35"E	2009	Unclear <sup>1</sup> , 3 specimens	Vojkovská et al. (2014)
Japan	River in Sapporo	~43°03'46"N; 141°22'15"E	2009	Probably extinct <sup>2</sup> , 1 specimen	Kawai and Takahata (2010); Kawai (2017)
	Outflow stream of Matsubara Izumi along Shigenobu River	33°47'12.8"N; 132°47'21.8"E	2016	Unclear, 2 specimens	Usio et al. (2017)
Madagascar	37 sites near Antananarivo; brick pits, drainage ditches, rice fields and fish ponds	~18°53'29"S; 47°30'27"E	2007	Established	Jones et al. (2009)
	Ikopa and Ampasimbe River systems	~18°56'S; 47°35'E	2007	Established	Kawai et al. (2018)
	29 sites throughout Madagascar; ponds, lakes, rivers, rice fields, channels and marshes	17°37'49"S; 48°29'56.8"E to 23°30'47.3"S; 44°30'37.3"E	2016–2018	Established	Gutekunst et al. (2018); Andriantsoa et al. (2019)
Malta	Water reservoir at Pembroke, Island of Malta	35°55'48.3"N; 14°28'47.8"E	2017	Established	Deidun et al. (2018)
	Ta' Sarraflu pond, Island of Gozo	36°02'11.8"N; 14°11'56.6"E	2016	Established	Deidun et al. (2018)
	Ghajn il-Papri pond, Island of Gozo	36°01'21.9"N; 14°17'16.6"E	2016	Established	Deidun et al. (2018)
	Il-Hofra pond, island of Gozo	36°01'15.1"N; 14°16'10.0"E	2016	Established	Deidun et al. (2018)
	Wied l-Lunzjata, watercourse, island of Gozo	36°02'03.8"N; 14°13'52.9"E	2016	Established	Deidun et al. (2018)

**Table 1. (Continued)**

Country	Location	Coordinates	Year of detection	Population status, found specimens	Reference
Netherlands	Vlij canal in Dordrecht	~51°48'45.9"N; 04°41'21.6"E	2004	Extinct <sup>3</sup> , several specimens	Soes and van Eekelen, (2006); Soes and Koese (2010)
	Middelburg, in garden pond and on land	~51°30'12"N; 03°35'58"E	2014	Extinct <sup>3</sup> , 2 specimens	Soes and Koese (2016)
Romania	4 ponds in Băile Felix	46°59'20.1"N; 21°58'43.3"E	2017	Established	Părvulescu et al. (2017)
Slovakia	Leopoldov gravel pit	48°27'17.6"N; 17°47'04.4"E	2014	Established	Lipták et al. (2016)
	Koplotovce gravel pits	48°28'22.7"N; 17°48'13.8"E	2010	Established	Jansky and Mutkovič (2010); Lipták et al. (2016)
	Opatovce thermal stream	48°46'01.6"N; 18°34'39.2"E	2014	Established	Lipták et al. (2016)
	Chorvátske rameno channel in Bratislava	48°05'58.6"N, 17°07'50.2"E	2016	Established	Lipták et al. (2017)
Sweden	River Märstaån	59°37'23.5"N; 17°51'54"E	2012	Extinct <sup>4</sup> , 13 specimens	Bohman et al. (2013)
	Pond in Skara	58°23'24"N; 13°10'56.3"E	2013	Extinct <sup>4</sup> , 1 specimen	Bohman et al. (2013)
Ukraine	Quarry in Dnepropetrovsk	48°30'19.15"N; 35°06'8.56" E	2014	Established	Novitsky and Son (2016)
	Ornamental ponds in Odessa	46°26'53.85"N; 30°45'12.54"E	2015	Unclear, 3 specimens	Novitsky and Son (2016)

<sup>1</sup>, Elena Tricarico, pers. comm., Feb. 17, 2019; <sup>2</sup>, Tadashi Kawai, pers. comm., Feb. 18, 2019; <sup>3</sup>, Menno Soes, pers. comm., Feb. 25, 2019; <sup>4</sup>, Patrik Bohman, pers. comm., Feb. 16, 2019. Approximate coordinates of the area (~) are given if no precise coordinates were given by the finders.



A few marbled crayfish including a female with hatchlings were found in two sites in the Rhine river, the biggest river in Germany (Chucholl et al., 2012). Furthermore, three adults were detected in the delta of the Po river in Italy (Vojtkovská et al., 2014), and many specimens were found in a side channel of the Danube river in Slovakia (Lipták et al., 2016). Should marbled crayfish establish populations in these big rivers there are hardly any limits to their spread across Central and Eastern Europe.

## **9.2. Biomes and Habitats**

Marbled crayfish occur in tropical, subtropical and warm and cold temperate biomes (Chucholl et al., 2012; Gutekunst et al., 2018; Andriantsoa et al., 2019). The Madagascar population is distributed over humid, sub-humid and sub-arid climate zones and warm-temperate highland regions above 1000 m altitude (Gutekunst et al., 2018; Andriantsoa et al., 2019).

Marbled crayfish were found in oligotrophic to eutrophic lakes, brooks and rivers, swamps and rice fields, and acidic (pH 3.9) and polluted waters (Jones et al., 2009; Kawai et al., 2009; Chucholl et al., 2012; Dümpelmann and Bonacker, 2012; Bohman et al., 2013; Gutekunst et al., 2018; Andriantsoa et al., 2019). They were not yet found in brackish water, but laboratory experiments revealed that they can survive salt concentration up to 18 ppt for more than 80 days. However, growth and reproduction stops at 6 ppt (Vesely et al., 2017).

Marbled crayfish occur in water bodies of variable temperatures. The warmest habitat reported so far is thermal Lake Hévíz in Hungary with water temperatures of 38°C in summer and 22°C in winter (Lökkös et al., 2016). Lake Moosweiher in Germany has summer temperatures of 26°C and winter temperatures of 4°C (Chucholl and Pfeiffer, 2010). The coldest habitat in which different life stages of marbled crayfish were found is river Märstaån in Sweden, which due to its continuous flow does not freeze in winter, reaching water temperatures around 0°C (Bohman et al., 2013). Marbled crayfish were observed crawling on the bottom in 0-2°C cold water.

However, this population is apparently vanished (Patrik Bohman, pers. comm.).

Marbled crayfish can dig simple burrows and walk over land (Jones et al., 2009; Chucholl et al., 2012). They probably do not burrow by preference, but rather when the water table is lowered in the dry season (Jones et al., 2009).

### **9.3. Population Structure**

In central Europe, berried individuals can be found throughout most of the year, but there seem to be two major annual recruitment periods as observed in the laboratory. In Lake Moosweiher egg-carrying and juvenile-carrying marbled crayfish were even found in December and January at water temperatures of 5-7°C (Buri, 2015; Christian Günter, pers. comm.). A survey on the reproduction of marbled crayfish in Lake Šoderica (Croatia), revealed that egg-carrying marbled crayfish were particularly frequent in June and October/November (Cvitanić, 2017).

Size-frequency distribution analysis of the population in Lake Moosweiher revealed 4 cohorts in late summer/early autumn and some larger specimens (Wolf, 2014), probably representing two different years of reproduction and some survivors from earlier years. Comparison of the peak carapace lengths of 17, 26, 33 and 40 mm with laboratory data suggests approximate ages of 6, 12, 18 and 24 months (Vogt, 2010).

Andriantsoa et al. (2019) have recently analyzed five populations from humid, sub-humid, dry and sub-arid bio-climatic regions in Madagascar (Figure 9). The size-frequency distribution graphs of four of these populations coming from a pond, river, lake and rice field are shown in Figure 9. Standardized catch per hour differed considerably between sites, ranging from 154 animals in a midland river at Ihosy (~700 m a.s.l.) to <20 specimens in a highland rainforest river at Andragaroa (~1150 m a.s.l.), reflecting differences in population densities. Marbled crayfish were on average significantly larger and heavier in the Ihosy river when compared to the other sites.

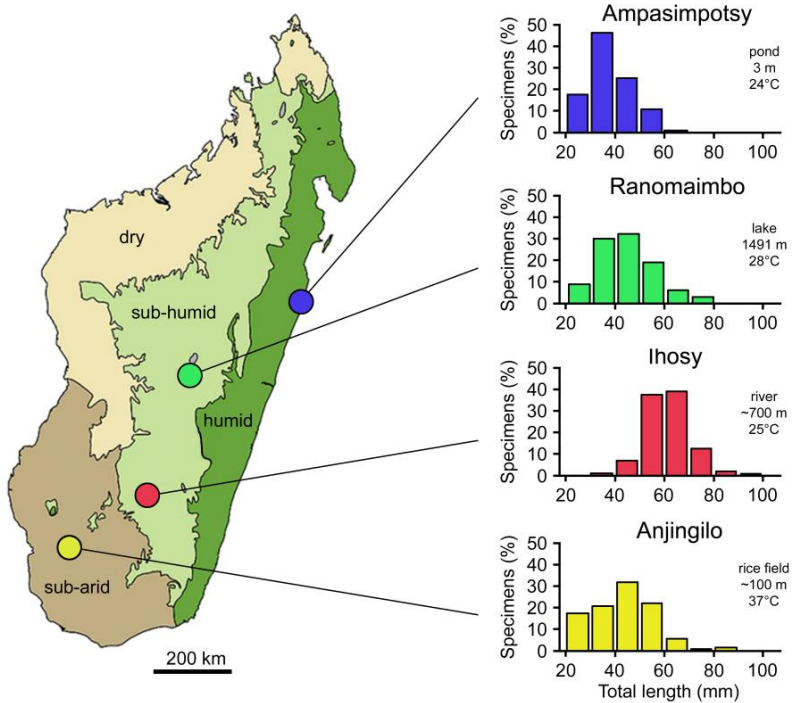


Figure 9. Size-frequency distribution of differently adapted populations of marbled crayfish in Madagascar. The analyzed populations were from different water bodies in different bio-climatic regions. Values on right side of graphs give altitude and water temperature measured at the time of sampling (8-10 a.m.) in 10 cm water depth. The particularly high water temperature in Anjingilo was caused by thermal water. Marbled crayfish were significantly ( $p < 0.05$ , Kruskal–Wallis one-way analysis of variance) larger in the Ihosy river than in the other sites (redrawn and modified after Andriantsoa et al., 2019).

#### 9.4. Food, Predators and Potential Impacts on Invaded Ecosystems

In Malta, marbled crayfish preyed on the gastropod *Cornu aspersum*, tadpoles of the painted frog *Discoglossus pictus* and Bedriaga's frog *Pelophylax bedriagae*, adults of the western mosquito fish *Gambusia affinis*, adults of the scarlet darter nymph *Crocothemis erythrae*, and larvae of the lesser drone fly *Eristalinus taeniops* (Deidun et al., 2018). Most of the

feeding took place early in the morning. Later during the day marbled crayfish were observed under reeds and vegetation in a largely inactive state. When disturbed, they retreated in the mud. In Madagascar, marbled crayfish preyed on *Biomphalaria pfeifferi*, the intermediate host of *Schistosoma* flatworms that cause schistosomiasis (Andriantsoa et al., 2019). Kawai et al. (2009) found mainly vegetable material in the digestive tract of Malagassy marbled crayfish.

In Malta, the long-tailed field mouse (*Apodemus sylvaticus*), the brown rat (*Rattus norvegicus*) and the North African hedgehog (*Erinaceus algirus*) were observed to prey on marbled crayfish in shallow, restricted water where the crayfish were easily accessible from land. The non-native Bedriaga's frog was feeding on sub-adults. The common chameleon (*Chamaeleo chamaeleon*) and the praying mantis (*Mantis religiosa*) occasionally preyed on marbled crayfish along the margins of the ponds on Gozo. In Lake Moosweiher, marbled crayfish has established a vivid population despite of the presence of crayfish competitors (see below) and effective crayfish predators like catfish (*Silurus glanis*), pike (*Esox lucius*), perch (*Perca fluviatilis*), eel (*Anguilla anguilla*) and great crested grebe (*Podiceps cristatus*) (Vogt et al., 2019).

Liptak et al. (2019) provided first insights into the trophic ecology of marbled crayfish in a lentic ecosystem. They studied an oligotrophic gravel pit at Leopoldov (south-western Slovakia) to identify food web interactions using analysis of  $^{13}\text{C}$  and  $^{15}\text{N}$  isotopes. The surface area of the study site is ca. 13 ha and maximum depth is 7 m. Maximum water temperature in summer is 25°C and minimum water temperature in winter is 4°C. The shoreline is surrounded by terrestrial vegetation, including trees, and most of the bottom is covered by submerged macrophytes and algae. Marbled crayfish used mostly allochthonous detritus (30%), algae (25%) and autochthonous detritus (21%) as food sources. Zoobenthos and macrophytes contributed 9% and 14%, respectively. Marbled crayfish was found to be an important food source for top fish predators but marginal for omnivorous fish. The study indicates that marbled crayfish can utilize energy from the bottom of the food web and transfer it to higher trophic levels. Since marbled crayfish can exploit various trophic levels on the one hand and be prey for

top level organisms on the other hand it has the potential to significantly modify food webs and ecosystems.

## 9.5. Invasiveness and Competition with Other Crayfish Species

Chucholl (2016) calculated an almost double Freshwater Invertebrate Invasiveness Scoring Kit score for marbled crayfish when compared to its parent species, making marbled crayfish a high risk species for Central Europe and also the USA once released into the wild (Feria and Faulkes, 2011). The co-existence of marbled crayfish and spiny-cheek crayfish *Faxonius limosus* in several German lakes and comparison of the competitiveness of marbled crayfish and other crayfish species in laboratory experiments indicate that marbled crayfish can over-invade resident crayfish populations and successfully compete with them.

In Lake Moosweiher, a mesotrophic gravel pit of 7.6 ha near Freiburg, southwestern Germany, marbled crayfish lives sympatrically with *Faxonius limosus*, an invasive crayfish that was introduced to Central Europe in 1890. *Faxonius limosus* is native to the north-eastern USA and is thus naturally adapted to temperate climate. It grows to 12 cm TL and has similar egg numbers per clutch as marbled crayfish but reproduces sexually (Đuriš et al., 2006; Kozák et al., 2006). *Faxonius limosus* is present in Lake Moosweiher probably since about 1990 (Michael Pfeiffer, personal communication) but marbled crayfish only since 2009 (Chucholl and Pfeiffer, 2010). Catching from April 07 to December 24, 2014 revealed a total of 467 marbled crayfish and 270 spiny-cheek crayfish. Interestingly, spiny-cheek crayfish were predominant in April and May, whereas marbled crayfish was predominant in November and December (Figure 10A). Marbled crayfish established a vivid population in Lake Moosweiher but did not replace *Faxonius limosus* because both species still co-exist in the lake.

Linzmaier et al. (2018) compared the behavioral traits of marbled crayfish from aquaria and the field and spiny-cheek crayfish *Faxonius limosus* from the field. They measured agonistic encounters, activity levels, and the response to a simulated threat in laboratory trials. The authors found

that marbled crayfish were on average more aggressive than spiny-cheek crayfish, even against larger opponents (Figure 10B). Marbled crayfish from the field were less active than those from aquaria, but there was no difference in aggressiveness. Marbled crayfish often “froze” (stopped moving and ducked) in response to a simulated threat, whereas spiny-cheek crayfish reacted either offensively or retreated. The authors concluded that the success of marbled crayfish in establishing new populations despite the presence of resident crayfish could be influenced by their behavioral flexibility in addition to their higher reproductive potential.

Jimenez and Faulkes (2011) conducted size-matched intra- and interspecific pairings of marbled crayfish and red swamp crayfish, *Procambarus clarkii*, another potent world-wide invader of freshwater ecosystems. They revealed that marbled crayfish were as likely to win a fight as *Procambarus clarkii*. Contests between *Procambarus clarkii* and marbled crayfish began significantly faster than contests between two marbled crayfish. These results suggest that marbled crayfish have the potential to compete with other crayfish species on the same level as *Procambarus clarkii*. Hossain et al. (2019) principally confirmed this result. Mature marbled crayfish dominated over size-matched females of red swamp crayfish in 100% and against males in 60% of the pairings. Premature marbled crayfish dominated in more than 75% of the trials. Agonistic behavior and intensity of fights significantly dropped after establishment of dominance. The co-occurrence of marbled crayfish and *Procambarus clarkii* in Ta’ Sarrafllu pond, Gozo Island, Malta (Deidun et al., 2018) confirms that marbled crayfish can compete with the red swamp crayfish also in natural environments.

Forř et al. (2019) examined agonistic encounters among juveniles of marbled crayfish, signal crayfish *Pacifastacus leniusculus* and yabby *Cherax destructor* and found that signal crayfish showed the highest potential to establish dominance. Marbled crayfish was the least aggressive and least successful of the species tested. Sympatric populations of marbled crayfish with one of these much bigger growing crayfish species were not yet found. However, in a water reservoir at Pembroke on Malta Island,

marbled crayfish lives together with the much bigger narrow-clawed crayfish *Pontastacus leptodactylus* (Deidun et al., 2018).

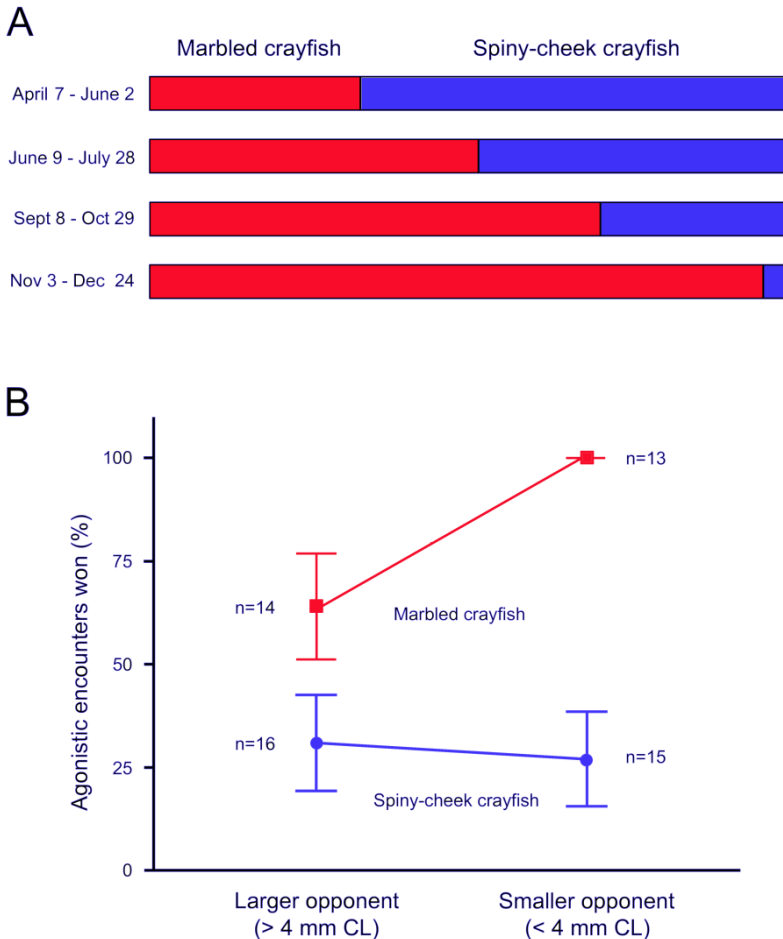


Figure 10. Coexistence and competition of marbled crayfish and spiny-cheek crayfish, *Faxonius limosus*. A: Relative abundance (%) of active marbled crayfish and spiny-cheek crayfish in Lake Moosweiher, southern Germany, at different times of the year (data from Günter, 2014; Lehninger, 2014; Wolff, 2014; Buri, 2015). B: Agonistic encounters won by marbled crayfish and spiny-cheek crayfish with opponents of unequal size of the other species. The left side shows the outcomes against larger opponents and the right side against smaller opponents (redrawn and modified after Linzmaier et al., 2018).

The enormous success of marbled crayfish in Madagascar is probably related to three factors: dispersal by humans, favorable climate, and the absence of crayfish and freshwater crab competitors in most of the invaded areas. Madagascar is home to seven endemic parastacid crayfish species (Jones et al., 2007) and 12 endemic species of freshwater crabs (Cumberlidge and Sternberg, 2002), which are generally very strong competitors of freshwater crayfish. The areas presently inhabited by marbled crayfish are mainly outside of the ranges of the indigenous freshwater crayfish and crabs (Cumberlidge and Sternberg, 2002; Jones et al., 2007; Gutekunst et al., 2018) so that they had not to compete with them during invasion. However, Andriantsoa et al. (2019) found sympatric populations of marbled crayfish and native crayfish *Astacoides betsileoensis* in a river at Andragararoa, with *Astacoides granulimanus* in a channel in Sahavondronina, and with the crab *Hydrotelphusa agilis* in a river at Andragararoa, suggesting that they can principally compete with both the much bigger native crayfish species and the aggressive native freshwater crabs.

## 10. EVOLUTION

In this section, I will discuss whether marbled crayfish has originated by autopolyploidy or allopolyploidy and whether it has arisen in the wild or in captivity. I will further examine its evolutionary age and future evolution.

### 10.1. Origination by Autopolyploidy or Allopolyploidy

The generation of allopolyploids (hybrids) by fusion of the genomes of two closely related species is much more common in animals than the origination of autopolyploids from a single species (Gregory and Mable, 2005). In contrast to autopolyploidy, hybridization is known for freshwater crayfish including the genus *Procambarus* (Cesaroni et al., 1992; Perry et al., 2001; Zuber et al., 2012). Autopolyploids usually look similar to their



parent species (Lewis, 1967; Soltis et al., 2007), whereas hybrids often display features of both parent species or intermediate features as shown for the crayfish hybrids *Faxonius rusticus* x *Faxonius propinquus* (Perry et al., 2001) and *Faxonius rusticus* x *Faxonius sanbornii* (Zuber et al., 2012).

Marbled crayfish do neither show morphological nor genetic signs of hybridization, suggesting autotriploidization as the most likely mode of their origin. The morphological characters of marbled crayfish and *Procambarus fallax* are very similar (Kawai et al., 2009; Martin et al., 2010a; Vogt et al., 2015, 2018). Even the annulus ventralis, which is species-specific in the Cambaridae, is identical. The uniform *COI* sequence of marbled crayfish was identical to the *COI* sequence of some specimens of *Procambarus fallax* collected in northern Florida (Vogt et al., 2018) as described above, indicating that the mother of the first marbled crayfish was a *Procambarus fallax* female.

Unlike mitochondrial markers, which are exclusively inherited by the female, nuclear genetic markers are suitable to show if a second species was involved in the generation of marbled crayfish. Martin et al. (2016) reported on the identity of *elongation factor 2* genes in marbled crayfish and *Procambarus fallax* but differences to *Procambarus alleni*, *Procambarus clarkii*, *Procambarus acutus* and *Procambarus liberorum*, supporting the autopolyploidy hypothesis. Particularly suitable for addressing the autopolyploid or allopolyploid question was the microsatellite PclG-02, which is uniformly tri-allelic in marbled crayfish showing the fragment lengths combination 267 bp/271 bp/303 bp. In *Procambarus fallax*, *Procambarus alleni* and *Procambarus clarkii* this microsatellite was mono-allelic or di-allelic and varied among specimens as is expected for diploid and sexually reproducing species (Vogt et al., 2015, 2018). We have recently found all of the three typical marbled crayfish alleles in *Procambarus fallax*, albeit in different combinations typical of diploid sexual species, but not in *Procambarus alleni* and *Procambarus clarkii* (Vogt et al., 2015, 2018) suggesting that a second species was not necessary to generate marbled crayfish. Further nuclear gene sequences should be compared between marbled crayfish, *Procambarus fallax* and other *Procambarus* species,

particularly of the Seminolae group, to make the autopolyploidy hypothesis waterproof.

The next question to be addressed concerns the number of parent individuals of *Procambarus fallax* that might have given rise to the first marbled crayfish. Autopolyploidization of a single diploid genome cannot yield microsatellite loci with three alleles of different fragment lengths as observed for PclG-02 except if there are additional Indel mutations. However, tri-allelic loci can arise without any mutation if an unreduced oocyte with genotype AB is fertilized by a sperm with genotype C and if subsequent meiosis is inhibited (Figure 11). Alternatively, a tri-allelic locus can emerge by fusion of a reduced genotype A oocyte with an unreduced BC sperm.

Meiosis in freshwater crayfish and other decapods starts in the ovarian oocytes and is then arrested in prophase I. Meiosis continues shortly after spawning and fertilization of the oocytes and includes extrusion of polar bodies I and II (Yano, 1988; Hertzler, 2005; Sellars et al., 2010; Kato et al., 2016). The physical presence of males is not necessary for fertilization because mating usually occurs days, weeks or even months before egg laying. The sperm is stored in the female's spermatheca (annulus ventralis) or in externally attached spermatophores, depending on crayfish family.

In shrimp, fish and bivalve aquaculture, autotriploids are artificially produced by the inhibition of either polar body I or polar body II extrusion in fertilized eggs by an abrupt cold or heat shock or by exposure to 6-dimethylaminopurine (Sellars et al., 2006, 2010; Piferrer et al., 2009). Inhibition of polar body I extrusion can reveal mono-allelic, di-allelic and tri-allelic gene loci whereas inhibition of polar body II extrusion can only yield mono-allelic and di-allelic loci. These triploidization experiments may serve as an illustrative example on how marbled crayfish could have emerged from a mated *Procambarus fallax* female by inhibition of meiosis I, perhaps by a heat or cold shock during storage or transportation.

Autotriploidization can have multiple evolutionary consequences. Autopolyploids often show enhanced fitness traits (Xiang et al., 2006; Lavania et al., 2012) as observed in marbled crayfish, which grows bigger,

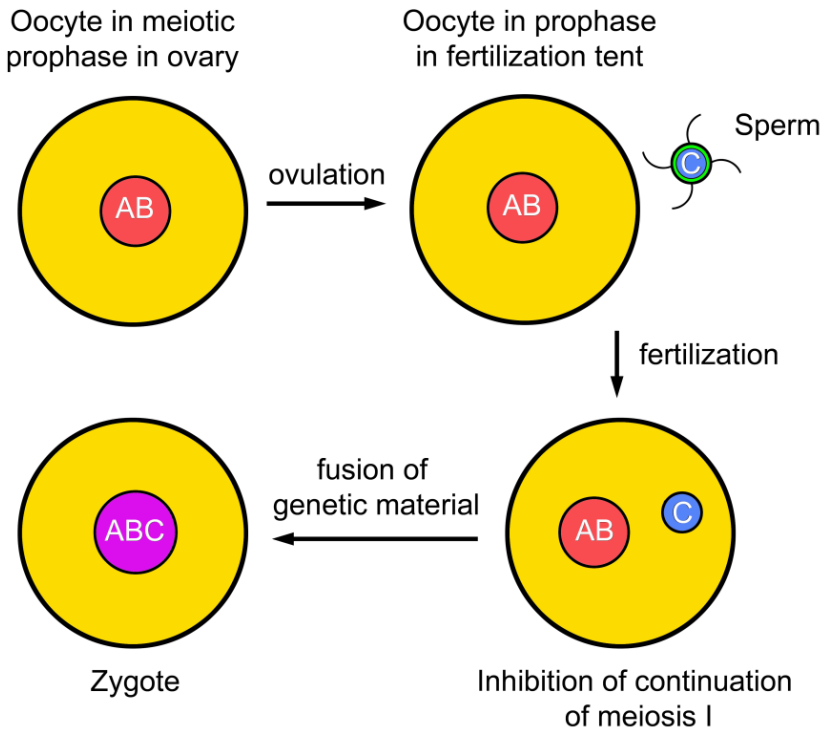


Figure 11. Hypothesis on the origination of marbled crayfish from *Procambarus fallax*. An oocyte of a *Procambarus fallax* female in meiotic prophase with genotype AB was fertilized by a sperm with genotype C. Continuation of meiosis was then suppressed by a temperature shock. The genetic material of the female and male pronuclei fused leading to a triploid ABC zygote that has given rise to the first marbled crayfish.

is more fecund and lives longer than its parent species (for detail see “species issue” section below). Unlike in hybrids, there are no new genes introduced into the genome of autopolyploids, and therefore, the enhanced fitness must be related to gene dosage alteration and changes of gene expression (Osborn et al., 2003). Beyond these immediate effects the triploid genome may enable new evolutionary trajectories that were previously impossible. For example, gene redundancy could facilitate adaptive divergence of duplicated genes, increasing the long-term genomic flexibility of autotriploids (Parisod et al., 2010).

## 10.2. Evolutionary Age

The lack of evidence for marbled crayfish in the voluminous monographs on the crayfishes of Florida and Georgia (Hobbs, 1942, 1981) may suggest that marbled crayfish has originated in evolutionarily recent times, perhaps in the second half of the last century. If marbled crayfish had existed for thousands of years in the home range of its parent species it should be widespread and frequent enough not to escape detection because it is highly fecund, grows bigger than *Procambarus fallax* and consists of females only. This idea is supported by Feria and Faulkes (2011) who predicted with climate and habitat based species distribution models that marbled crayfish could inhabit a considerably larger geographical area than *Procambarus fallax* when present in the southern states of the USA.

However, larger pure female samples and specimens exceeding the upper size limit of *Procambarus fallax* of 9 cm total length (Vogt et al., 2019), which could represent overlooked and wrongly determined marbled crayfish, were neither mentioned in the literature nor found by us in museum collections (Vogt et al., 2018, 2019). Analysis of the *Procambarus fallax* samples listed in the online catalogue of the Smithsonian Institution National Museum of Natural History (USNM), Washington, D.C. (<https://collections.nmnh.si.edu/search/iz/>) revealed only few pure female lots with >3 specimens (7 of 300 lots), which could be marbled crayfish. However, unisex lots with >3 females or males were also present in the collections of other Floridian crayfish species suggesting that the pure female lots of *Procambarus fallax* derived from random natural accumulations of *P. fallax* females rather than marbled crayfish.

Of the 2299 sexed *Procambarus fallax* in the USNM collection sampled from the beginning of the 20th century until 1990 in different counties and habitats throughout the entire distribution range (Figure 12) 55.33% were females and 44.67% were males (Vogt, 2019a). Its closest relatives *Procambarus seminolae* (n=801) and *Procambarus leonensis* (n=150) revealed similar female proportions of 53.93% and 54.67%, respectively.

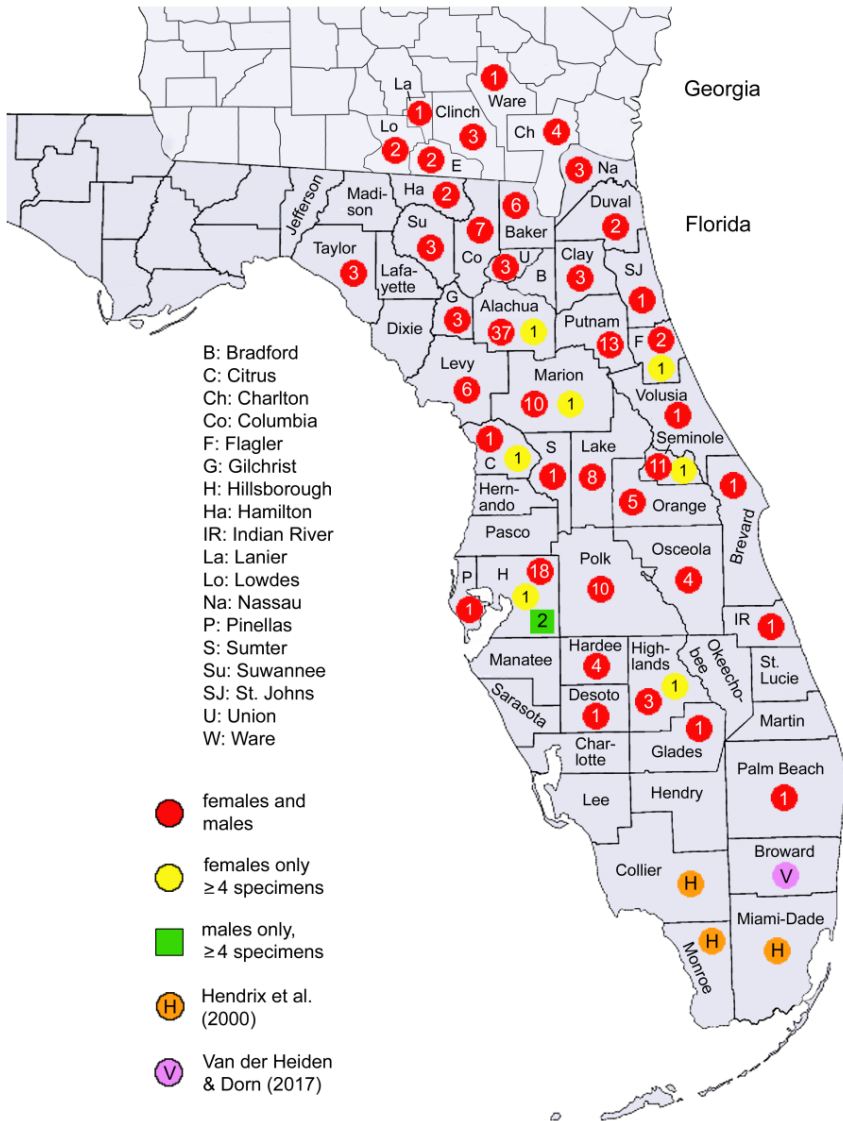


Figure 12. Geographical origin and gender distribution of the samples of *Procambarus fallax* in the collection of the USNM. The numbers in the circles indicate the number of lots collected in the respective counties. A total of 194 lots included both males and females. Pure female samples with >3 specimens were found in 7 counties and pure male samples >3 specimens were found in 1 county. The circles marked by H and V indicate counties sampled by Hendrix et al. (2000) and Van der Heiden and Dorn (2017). These samples included both females and males.

Moreover, of 1702 sexed *Procambarus fallax* collected in the Everglades by van der Heiden and Dorn (2017), 51.2% were females and 48.8% were males. These data demonstrate that the sex ratios of *Procambarus fallax* in the USNM collection are within the normal range of sexually reproducing freshwater crayfish populations in Florida. If marbled crayfish had existed for thousands of years together with *P. fallax* and had erroneously been collected as *P. fallax* because of the close morphological similarity of both crayfish, then the sex ratio in the *P. fallax* collection should be significantly biased towards females, because they would contain males and females of *P. fallax* in approximately equal proportions plus females of marbled crayfish. However, because this is obviously not the case, the marbled crayfish must be regarded as evolutionarily young.

### 10.3. Origin in Nature or Captivity

There are two possible scenarios on the site of origin of the marbled crayfish. Firstly, marbled crayfish may have arisen in the native range of *Procambarus fallax* by spontaneous autotriploidization and concomitant transition from sexual to parthenogenetic reproduction. Spontaneous autotriploidy of the whole genome is not too rare in animals but mostly remains undetected and has no consequences at the population level because triploids are usually sterile. For example, 91 of 5142 progeny of Atlantic salmon *Salmo salar* from Norwegian farms were shown to be spontaneous, sterile autotriploids (Glover et al., 2015). Saura et al. (1993) have developed a model that explains how triploidy and parthenogenesis can arise through a single event, resulting in a reproductively isolated and fertile new lineage or species. An alternative scenario with an intermediate diploid parthenogen was discussed by Martin et al. (2016).

The second scenario suggests that marbled crayfish may have originated in captivity, for example by a heat or cold shock in a sensitive phase of egg development during transportation or storage. We have earlier favored this hypothesis (Vogt et al., 2015) because there is no evidence for marbled crayfish populations in the biogeographic and ecological literature on the

crayfishes of Florida and southern Georgia (Hobbs, 1942, 1972, 1981, 1989; Hendrix et al., 2000; Lukhaup, 2003; VanArman, 2003, 2011; Van der Heiden, 2012; Van der Heiden and Dorn, 2017; Manteuffel-Ross et al., 2018). Marbled crayfish would thus be an “anecozoon,” i.e., an animal that has originated in captivity, lacking natural primary populations. Another example of a new animal species that has originated in captivity is the lizard *Aspidoscelis neavesi* that has arisen in the laboratory by hybridization between a triploid, parthenogenetic female of *Aspidoscelis exsanguis* and a diploid, gonochoristic male of *Aspidoscelis inornata* (Cole et al., 2014).

Although the site and conditions of the origin of the marbled crayfish remain unresolved, we have some evidence on the area where its ancestors may have come from. All genetic markers (*COI* and microsatellites) that characterize marbled crayfish were found in *Procambarus fallax* populations from neighboring Alachua and Union counties in northern Florida (Vogt et al., 2018), albeit in different combinations typical of diploid and sexually reproducing species. They were not found in specimens from the Everglades and southern Georgia, the southernmost and northernmost parts of the range of *P. fallax*, identifying the area of the Alachua and Union counties as the most likely home of the parents of the first marbled crayfish.

#### 10.4. Future Evolution

In Madagascar, marbled crayfish has invaded an area that is comparable in size to the home range of its parent species, *Procambarus fallax*, in the southeastern USA. Thus, marbled crayfish represents already a numerically large evolutionary unit. There is also no doubt that marbled crayfish can reproduce and propagate in the colder climate of Central Europe because there are more than 30 established populations with thousands of specimens. These European populations will probably form one large population in future, which will evolve independently from the Malagasy population due to geographic isolation.

Evolutionary theory predicts short-term success of obligate parthenogens but their long-term extinction (Bell, 1982; Henry et al., 2012).

The early success is usually explained by the saved costs for male production that can be invested in population growth. Apparently, marbled crayfish is presently in this early phase of expansion. The long-term extinction of parthenogens is explained by the accumulation of deleterious mutations and the absence of genetic variability, which is thought to impede adaptation to changing environments and resistance against diseases (Butlin, 2002; Henry et al., 2012; Martin, 2016).

The sketched dead-end scenario seems to apply to many but not all obligate animal parthenogens. Some parthenogens including marbled crayfish can apparently generate phenotypic diversity from the same genetic template by epigenetic mechanisms, keeping genetically uniform populations in the game of life when the environmental conditions change (Vogt, 2015a, 2017a). There are examples of evolutionarily very successful obligate parthenogens in the animal kingdom like the darwinulid ostracods among the Crustacea and the bdelloid rotifers that propagated without sex for almost 100 million years and generated 35 and 360 extant asexual species, respectively (Butlin, 2002; Mark Welch et al., 2009; Schön et al., 2009). Perhaps, marbled crayfish follows this evolutionary path.

## 11. SPECIES ISSUE

The following section discusses whether the marbled crayfish should be considered as a parthenogenetic lineage of *Procambarus fallax* named *Procambarus fallax* forma *virginalis* as suggested by Martin et al. (2010a) or as a separate species named *Procambarus virginalis* as suggested by Vogt et al. (2015). Our suggestion was based on mating experiments and the observation of differences in life history parameters and genetic markers between marbled crayfish and *Procambarus fallax*. Later, one of the authors of the Vogt et al. (2015) article formally described the marbled crayfish as a separate species (Lyko, 2017a) without adding new arguments. Unfortunately, the species description published by Lyko has several shortcomings such as the absence of a determination key, an implausible genetic marker claimed to be exclusive for marbled crayfish, and imprecise



drawings of taxonomically relevant morphological characters like the annulus ventralis. Because of the general importance of considering marbled crayfish as the first asexual species of the ~15.000 decapod crustaceans and the availability of new morphological, life history and genetic data I will here discuss the species issue of marbled crayfish in detail.

### **11.1. Problems Associated with the Erection of Polyploid and Asexual Species**

In the last decade, there was an intense debate on whether polyploids and asexuals like marbled crayfish should be described as new species or kept as cytotypes within their parent species (Vogt, 2019b). Soltis et al. (2007) advocated for describing polyploids as separate species when they represent distinct evolutionary units and fulfill the criteria of one or more species concepts, despite morphological similarities with their parent species (for species concepts see Wheeler and Meier, 2000; De Quiroz, 2007; Soltis et al., 2007). They further emphasized that the traditional practice to include morphologically similar cytotypes in one species may be convenient but obscures insights into evolution and speciation processes and hinders conservation.

Plant biologists have already described numerous polyploid species including autopolyploids (Eriksson et al., 2017). Barker et al. (2016) estimated that there may be some 50,000 undescribed polyploid species in addition to the 350,000 named species in the flowering plants. Zoologists are more hesitant to place morphologically similar diploids and polyploids in different species but there are already some examples. For instance, Schmid et al. (2015) demonstrated that many of the diploid-polyploid anuran populations are a mixture of diploid and polyploid cryptic species rather than of different cytotypes of the same species.

Like polyploids, asexuals do not fit into classical species concepts because these were developed for sexual reproducers (reviewed in Mayr, 1963, 1996; Wheeler and Meier, 2000; Coyne and Orr, 2004). However, Barraclough et al. (2003) emphasized the need to investigate speciation in

asexuals to answer central questions of evolution such as the predominance of sexual reproduction in animals. Asexuals are an important component of the biodiversity, particularly in plants (De Meeûs et al., 2007). Well known purely asexual higher animal taxa are the bdelloid rotifers and ostracod crustaceans (Mark Welch et al., 2009; Schön et al., 2009). Further examples of formally described asexual animal species are some mites (Ros et al., 2008) and lizards (MacCulloch et al., 1997). For example, the lizard *Darevskia rostombekowi* is obligately parthenogenetic like marbled crayfish.

## 11.2. Arguments against Raising Marbled Crayfish to Species Rank

Arguments that speak against raising marbled crayfish from “forma” to species rank may come from the close similarity of morphological traits and mitochondrial genes in marbled crayfish and *Procambarus fallax*. However, the morphological similarity is no disqualifier because there are numerous morphologically very similar cryptic animal species described that were only identified by genetic differences or marked life history differences (Hebert et al., 2004; Bickford et al., 2006; Mills et al., 2017). The similarity of the mitochondrial genomes of marbled crayfish and *Procambarus fallax* is also no disqualifier. It rather supports the hypothesis that marbled crayfish has arisen from a female *Procambarus fallax* in relatively recent evolutionary times.

Colleagues advocating for treating marbled crayfish as a parthenogenetic lineage of *Procambarus fallax* rather than as a separate species usually refer to the examples of water flea *Daphnia pulex* and New Zealand mud snail *Potamopyrgus antipodarum*, which have sexual and parthenogenetic populations in a single species. However, the obligately parthenogenetic populations of these species do neither meet the single origin criterion nor the reproductive or geographic isolation criterion of the Evolutionary Genetics Species Concept developed by Barraclough et al. (2003) and Birky and Barraclough (2009) for asexuals. In *Potamopyrgus*

*antipodarum*, triploid lineages originated many times from sexually reproducing populations and coexist with their sexual ancestors (Neiman et al., 2011). The same holds for *Daphnia pulex*, which includes cyclic parthenogenetic and obligately parthenogenetic populations. The obligate parthenogens have evolved multiple times from the cyclic parthenogens, occur together with them and cross-breed occasionally with them (Hebert and Finston, 2001; Dufresne, 2011).

### 11.3. Arguments for Raising Marbled Crayfish to Species Rank

In order to test whether it is justified to consider marbled crayfish as a separate species we have applied to all data the Evolutionary Genetic Species Concept for asexuals (Barraclough et al., 2003; Birky and Barraclough, 2009). This concept requires single origin of the new species, discrete clusters of phenotypes and genotypes in the neo-species and parent species, and reproductive or geographic isolation.

Comparison of mitochondrial *COI* genes, *12S rRNA* genes and microsatellites of marbled crayfish from far distant geographical regions revealed identical sequences in all marbled crayfish as detailed in the “taxonomy” section. The identity of these commonly used mitochondrial and nuclear markers, which showed considerable variation in *Procambarus fallax* and other freshwater crayfish species (Hulák et al., 2010; Williams et al., 2010; Da Silva et al., 2011), demonstrates monoclonality of marbled crayfish. Gutekunst et al. (2018) have recently compared ~20% of the entire DNA sequences of 10 marbled crayfish from Madagascar, German lakes and different laboratories with the reference genome and revealed a total of only 416 single nucleotide variants (SNV). The differences of individuals to the reference genome were 128-219 SNVs, which roughly corresponds to the normal mutation rate in animals. These data strongly support single origin of marbled crayfish. It is well known that the descendants of the first parthenogenetic marbled crayfish that appeared in Germany spread via aquarists and the pet trade, first in Germany and later throughout the world. Specimens of this homogeneous “world-wide aquarium population” were

repeatedly released into the wild, explaining easily monoclonality of all marbled crayfish populations in Europe, Africa and Asia.

Marbled crayfish and *Procambarus fallax* are similar with respect to morphological characters but differ markedly with respect to fitness traits. We found no qualitative morphological feature that would unambiguously enable discrimination of the two crayfish. The taxonomically relevant morphological traits, coloration and major body proportions varied considerably within marbled crayfish and *Procambarus fallax*, complicating the recognition of morphological differences. In contrast, other phenotypic features like body size, fertility and longevity differ markedly between marbled crayfish and *Procambarus fallax*. Marbled crayfish reach considerably larger maximum body sizes (~11 versus 9 cm total length), produce significantly larger maximum clutch sizes (~730 versus 330 eggs) and live on average longer (~2-3 versus 1-2 years) than *Procambarus fallax* (Vogt et al., 2019). The larger clutch sizes are due to bigger growth (egg number is positively correlated with body size) and ~40% increased fertility in equally sized specimens (Vogt et al., 2019). A further important factor that contributes to higher fertility of marbled crayfish populations is parthenogenesis, because all population members are egg-producing females.

Marbled crayfish and *Procambarus fallax* are ecologically similar in as far as they inhabit very diverse habitats and can stand harsh and seasonally fluctuating environmental conditions. Both crayfish also show effective survival adaptations to heavy predation pressure and competition with other crayfish species. However, they differ in population dynamics (Vogt et al., 2019) and invasive potential (Chucholl, 2016), which is probably related to differences in life history traits. These data indicate that the distinct phenotypic cluster criterion is fulfilled for several traits including body size, fertility, longevity, population structure and invasiveness.

There are close similarities in the mitochondrial genomes between marbled crayfish and *Procambarus fallax* but striking differences with respect to some nuclear genomic features, suggesting that the distinct genetic clusters requirement is fulfilled as well. The nuclear genomic differences concern ploidy level, haploid genome size, microsatellite pattern and DNA

methylation level. Ploidy is 2N in *Procambarus fallax* and 3N in marbled crayfish, and the haploid genome size is ~10% smaller in marbled crayfish when compared to *Procambarus fallax* (Martin et al., 2010a; Vogt et al., 2015; Gutekunst et al., 2018). Loss of DNA during or after polyploidization is not uncommon. For example, in synthetic autopolyploids of the plant *Phlox drummondii* an immediate loss of 17% of total DNA has been recorded followed by a further reduction of up to 25% upon the third generation (Parisod et al., 2010). The degree of DNA loss in marbled crayfish is not trivial because it corresponds to several times the entire genome of the well known genetics model *Drosophila melanogaster*. Even if only non-coding sequences were lost it might have had significant consequences for gene regulation via alteration of the chromatin structure.

Marbled crayfish and *Procambarus fallax* also differ markedly with respect to genome-wide DNA methylation (2.4% versus 2.9%), an important epigenetic modification of the DNA, influencing gene regulation, phenotype variation and environmental adaptation (Jaenisch and Bird, 2003; Lyko et al., 2010; Vogt, 2017a). The reduction of DNA methylation by 20% in marbled crayfish may have led to pronounced changes of gene expression when compared to *Procambarus fallax*. Together with differences in ploidy and related gene dosage alteration these epigenetic differences may be causative of the enhanced fitness traits and higher invasiveness of marbled crayfish.

Our crossbreeding experiments (Vogt et al., 2015, 2019) with marbled crayfish females and *Procambarus fallax* males revealed that both mated readily but the offspring was always pure marbled crayfish as revealed by microsatellite analysis, suggesting reproductive isolation. Reproductive isolation is probably due to cytogenetic incompatibility rather than behavioral and mechanical barriers because mating behaviors and the morphology of the sperm receptacles are very similar in marbled crayfish and *Procambarus fallax*. Probably, marbled crayfish and *Procambarus fallax* are also geographically isolated because there is no evidence for the presence of marbled crayfish in the native range of *Procambarus fallax* as discussed above.

Diagnosability is another important criterion for the erection of a new species. This criterion is fulfilled for marbled crayfish, since it can unambiguously be identified and distinguished from *Procambarus fallax* by the tri-allelic microsatellite *PclG-02*. A determination key is given in the following (from Vogt et al., 2019):

1. Presence of annulus ventralis	Cambaridae, 2
Absence of annulus ventralis	Astacidae, Parastacidae
2. Annulus ventralis freely moveable, dactyl of chela distinctly longer than mesial margin of palm, tubercles on mesial surface of latter well developed	<i>Procambarus</i> , 3
Otherwise	other genera
3. Marbled coloration and bell-shaped annulus ventralis	<i>P. fallax</i> , <i>P. virginalis</i> , 4
Otherwise	other <i>Procambarus</i> species
4. Males present, specimens >7 cm TL rare, maximum size ~9 cm TL, microsatellite <i>PclG-02</i> mono-allelic or di-allelic with various fragment lengths	<i>P. fallax</i>
Males absent, specimens >7 cm TL frequent, maximum size ~11 cm TL, microsatellite <i>PclG-02</i> generally tri-allelic with fragment lengths of 267, 271 and 303 bp	<i>P. virginalis</i>

If one had only the morphology of small and medium sized specimens of marbled crayfish and *Procambarus fallax* and their mitochondrial genes for decision-making, there would be no reason for treating marbled crayfish as a separate species. However, if one considers monoclonality of marbled crayfish, reproductive isolation in laboratory experiments, considerable differences in fitness traits, nuclear genomic features and some ecological characteristics, and the present knowledge on geographic distribution, then it is justified to assign marbled crayfish and slough crayfish to different species named *Procambarus virginalis* and *Procambarus fallax*, respectively. All criteria of the Evolutionary Genetic Species Concept for asexuals and the diagnosability requirement for the erection of a new species are well met. Following this argumentation, marbled crayfish would be the

first unisexual and the first polyploid species of the almost 15.000 decapod crustaceans.

## 12. UTILIZATION

Marbled crayfish should not be used for aquaculture but due to several advantageous properties it should be used as a laboratory research model.

### 12.1. Aquaculture and Fisheries

Marbled crayfish should not be used for aquaculture because of the potential threat of escapes and negative effects on native crayfish populations and invaded ecosystems. It is meanwhile confirmed that marbled crayfish can carry and transmit the crayfish plague (Keller et al., 2014). Moreover, there is no need for a new aquaculture crayfish species because several crayfish that grow much bigger than marbled crayfish are already well established in the market. Examples are the noble crayfish, *Astacus astacus*, narrow-clawed crayfish, *Astacus leptodactylus*, yabby, *Cherax destructor*, marron, *Cherax tenuimanus*, red claw crayfish, *Cherax quadricarinatus*, signal crayfish, *Pacifastacus leniusculus*, and red swamp crayfish, *Procambarus clarkii* (Holdich, 1993).

Marbled crayfish must not be used as live bait for fisheries due to the potential threats mentioned above. Existing wild populations may be exploited for human consumption, which may help to limit these populations. However, fished specimens must not be translocated to other places to start new populations. In Madagascar, marbled crayfish has become an abundant and popular food commodity in its distribution range (Figure 13A-G). It is fished from rivers and lakes and cultured in rice fields (Andriantsoa et al., 2019). For local people, marbled crayfish represents a cheap protein source and an attractive source of income (Figure 13F).

## 12.2. Use as Research Model

Marbled crayfish are increasingly being used as a laboratory model for research (Vogt, 2019c). Particular advantages are easy rearing, the production of high numbers of genetically identical offspring and the generation of epigenetically different individuals from the same genetic template. Presently, more than a dozen laboratories in different countries use marbled crayfish for investigating various biological topics.

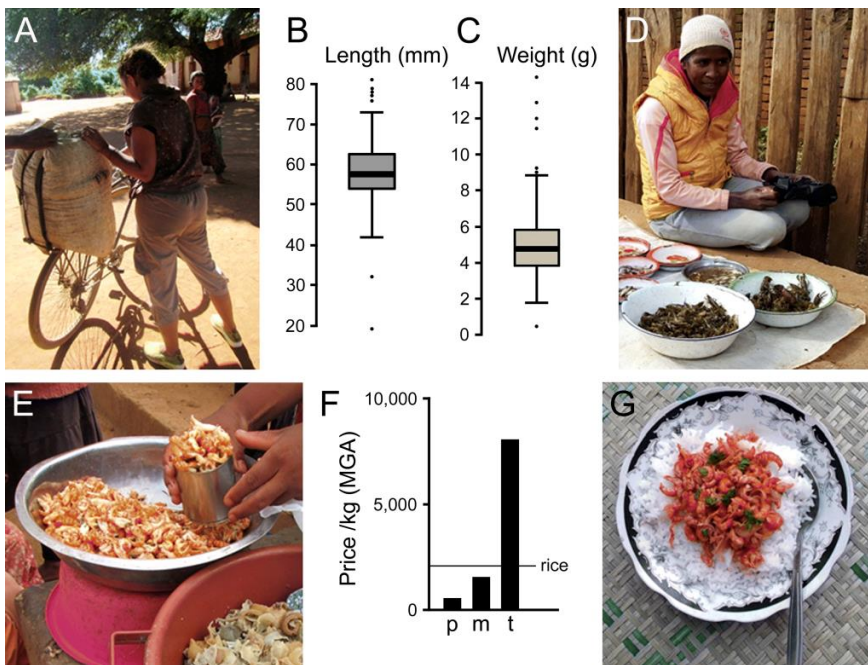


Figure 13. Use of marbled crayfish as food commodity in Madagascar. A: Commercial distribution of live marbled crayfish in large bags. B: Total length of 200 arbitrarily sampled crayfish from a commercial distribution bag. C: Weight of the crayfish in B. D: Market sale of live marbled crayfish. E: Market sale of processed tail meat of marbled crayfish. F: Prices of marbled crayfish in comparison to rice. p, purchasing price of marbled crayfish; m, market price of live crayfish; t, price of tail meat; MGA, Malagasy Ariary (all illustrations from Andriantsoa et al., 2018; graphs modified).



### 12.2.1. Model for Reproduction

The obligately parthenogenetic marbled crayfish and its gonochoristic parent species *Procambarus fallax* are well suitable to identify the genetic and hormonal causes of parthenogenesis. There are ongoing projects on this topic in at least two research groups. Forthcoming findings could be helpful to develop monosex aquacultures in commercially interesting decapods (Levy et al., 2019).

Levy et al. (2017) sequenced the gene of the insulin-like androgenic gland hormone (IAG), which is responsible for masculinization in decapods, from marbled crayfish and revealed identity of the open reading frame with the IAG in *Procambarus fallax*. Interestingly, IAG was not only expressed in the androgenic gland and testis of *Procambarus fallax* but also in the muscle tissue of both sexes of *Procambarus fallax* and in marbled crayfish females indicating novel functions of IAG in addition to its role as masculinization inducing factor.

Gutekunst (2017) searched for 93 meiosis genes in the genome of marbled crayfish and found 84 of them complete or fragmented. Evidence was lacking for *Rec8* and *Msh5*. *Rec8* protein is a meiosis-specific component of the cohesin complex that bind sister chromatids in preparation for the meiotic divisions. The *Msh5* gene encodes a member of the mutS proteins that are involved in meiotic recombination processes.

### 12.2.2. Model for Development

Marbled crayfish was repeatedly used to study development in freshwater crayfish. Alwes and Scholtz (2006) investigated its early development with respect to morphology, cell lineage and segment formation (Figure 14A). They established a staging system for embryonic development in freshwater crayfish based on externally visible blastoderm formation, gastrulation, segment formation and differentiation of limb buds and eyes. The authors also compared ontogenetic processes between marbled crayfish and other crustacean groups.

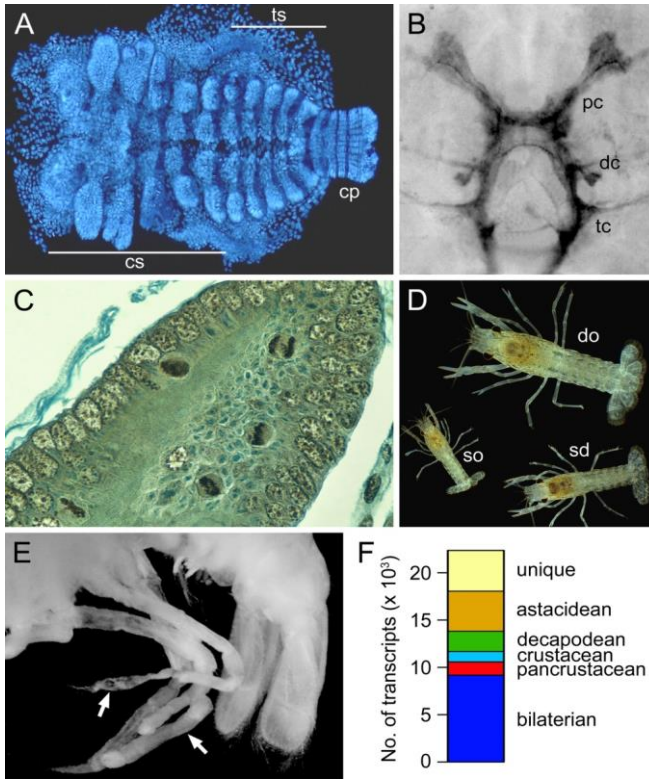


Figure 14. Marbled crayfish as research model. A: Development: Isolated and stained embryo at 50% development illustrating short germ development and stereotyped cell lineage. The embryo consists of the cephalic segments (cs), the anterior thoracic segments (ts) and the caudal papilla (cp) that will produce the still missing segments (from Vogt, 2018b; original from Alwes and Scholtz, 2006). B: Neurobiology: Synapsin-labelling of the developing brain showing protocerebrum (pc), deutocerebrum (dc) and tritocerebrum (tc) (from Vogt, 2018b; original from Vilpoux et al., 2006). C: Stem cell biology: Stem cell niche at blind end of hepatopancreas tubule including mitotic stages. These stem cells give rise to all hepatopancreatic cell types (from Vogt, 2008a). D: Behavior: Development of differences in behavior and growth among genetically identical clutch-mates. Five size-matched clutch-mates were kept together for 34 days. At the end of the experiment, the group had differentiated into one offensive dominant (do), one subdominant (sd) and three defensive subordinates (so). The dominant grew much faster than the subdominant and subordinates although food was available in excess and not monopolized (from Vogt et al., 2008). E: Toxicology: Malformation of walking legs (arrows) in stage-3 juveniles after exposure of the embryos to methyl testosterone. F: Genetics: Transcripts of marbled crayfish classified by lineage. The graph shows bilaterian-specific, pancrustacean-specific, crustacean-specific, decapod-specific, crayfish-specific and marbled crayfish-specific proportions (redrawn and modified after Gutekunst et al., 2018).

Vilpoux et al. (2006) established that the naupliar protocerebrum, deutocerebrum, tritocerebrum and mandibular neuromeres emerge simultaneously in marbled crayfish (Figure 14B). After this “naupliar brain” has formed, there is a time lag before the more caudal neuromeres develop sequentially in an anterior-posterior gradient. The authors discussed similarities and differences of brain formation between crayfish and insects.

Vogt (2008b) investigated structures involved in hatching, attachment of juveniles to their mother, exploration of the environment, and searching and processing of food. The results yielded deeper insights into the relatively hidden early life period of freshwater crayfish. Hatching was prepared by chemical weakening of the egg shell and completed by levering actions of the hatchling’s appendages. It was secured by a telson thread that kept the hatchling linked to the mother. Molting of stage-1 juveniles was secured by an anal thread that resulted from delayed molting of the hindgut. Feeding started in juvenile stage 3, when the mouthparts and gastric mill were fully developed.

Jirikowski et al. (2010) used marbled crayfish to exemplify poorly understood muscle development in Crustacea. They investigated the development and differentiation of muscle tissue and its relation to mesoderm-forming cells by different imaging tools. The authors identified single muscle precursor cells that serve as starting points for developing muscular units. They further demonstrated significant developmental advance in head appendage muscles and posterior longitudinal trunk muscle strands compared to other muscle tissues.

### *12.2.3. Model for Neurobiology*

Sintoni et al. (2007) studied the emergence of *Engrailed*-expressing cells in the embryonic brain of marbled crayfish. They found a group of six *Engrailed*-expressing neurons in the optic anlagen, which are supposed to be involved in establishing the primary axon scaffold of the brain. This report provided the first example for a cluster of homologous brain neurons in crustaceans and insects.

Rieger and Harzsch (2008) monitored histamine-immunoreactive neurons in the ventral nerve cord of marbled crayfish. They revealed that

histaminergic neurons arise at around 60% of embryonic development and persist into adulthood, indicating that they do not serve as pioneer neurons for establishing the neuronal scaffold. The authors compared patterns of histaminergic neurons in marbled crayfish and other crustaceans and evaluated their possible role as evolutionarily valuable character.

Sintoni et al. (2012) investigated neurogenesis in early life stages and adults of marbled crayfish with S-phase-specific markers combined with immunohistochemistry, dye-injection and pulse chase experiments. They found that the lateral and medial proliferation zones of the neurogenic system in the deutocerebrum are largely responsible for the production of new neurons in the early post-embryonic stages. The neurogenic niche plays a subordinate role at that time but gradually becomes the dominant and only site for the generation of new olfactory neurons in adults.

The groups of Wolfgang Stein and Andrés Vidal-Gadea at Illinois State University, Normal, IL have started a project to study the relationships between genes, neurophysiology and behaviors (Benson et al., 2017; Gährs, 2018). Benson et al. (2017) injected *innexin-4* dsDNA into juvenile marbled crayfish to suppress expression of the *innexin-4* gene. The innexins are structural components of the gap junctions and build transmembrane channels that facilitate cell-to-cell communication. The authors observed reduced walking behavior and reduced tail flip escape responses after suppression of the *innexin-4* gene.

#### 12.2.4. Model for Behavior

Marbled crayfish has already been used for studying several aspects of behavior. Vogt et al. (2008) revealed that marbled crayfish establish social hierarchies despite the absence of genetic variation. Size-matched clutch-mates of indifferent agonistic behaviors differentiated into dominants, subdominants and subordinates that showed different behaviors and growth. The dominants showed increasingly offensive behaviors and the subordinates showed avoiding or defensive behaviors. The dominants grew much faster than the subordinates although food was available in excess and not monopolized (Figure 14D).

Farca Luna et al. (2009) studied circadian regulation of agonistic behavior in marbled crayfish. Marbled crayfish showed all typical agonistic behavioral elements of freshwater crayfish and formed social hierarchies. They showed high frequencies of agonistic encounters during the first hour of cohabitation but low levels of agonistic activities after formation of hierarchies. Agonistic activity was entrained to periods of exactly 24 h under 12 h light: 12 h dark condition, and peaks of agonistic activity coincided with light-to-dark and dark-to-light transitions. After switching to constant darkness, agonistic activity followed a rhythmicity of more than 24 hours. The authors concluded that locomotion and agonistic social interactions are rhythmic behaviors that are controlled by light-entrained endogenous pacemakers.

Kasuya and Nagayama (2016) used backward escape swimming of marbled crayfish to study habituation. The application of rapid mechanical stimuli to the rostrum elicited backward swimming by rapid flexion of the pleon. When stimulation was repeated within 10 second intervals the tailflip reaction did not occur as the result of habituation. During a second series of experiments with similar stimuli previous experience of habituation was remembered.

Jackson and van Staaden (2019) investigated effects of the psychostimulant D-amphetamine sulfate on marbled crayfish and found that locomotion was significantly impacted by drug concentration. The authors concluded that marbled crayfish is a suitable animal model for studying the mechanisms of drug addiction and behavioral epigenetics.

#### *12.2.5. Model for Genetics*

Experimental data suggest that marbled crayfish is well suitable to investigate the genetic specification of decapods and the genetic basis of reproduction, immune defense and molting in arthropods (Vogt, 2018b). Marbled crayfish is also suitable to investigate more general topics like the genetic underpinning of omnivory, adaptation to fresh water, sexual system shifts, behavioral variation, the evolution of functionally diverse gene families, and resistance to cancer (Vogt, 2018b). Two examples are given in the following.

Crustaceans comprise about 66,900 species (Ahyong et al., 2011) and are particularly diverse with respect to morphology, physiology, longevity, life history and ecology (Martin and Davis, 2001). The “Crustacea” are also evolutionarily interesting because the insects, the largest animal group on earth (~1 million described species), have evolved from an ancient crustacean group, forming the Pancrustacea together with the crustaceans (Regier et al., 2005). The Decapoda comprise some 14,800 species (Ahyong et al., 2011) and the freshwater crayfish comprise ~700 species (Crandall and De Grave, 2017). Comparison of the transcriptome of marbled crayfish with those of other crustaceans, insects and vertebrates revealed that about 41% of the marbled crayfish transcripts belong to the bilaterian core, 4% to the Pancrustacea, 5% to the Crustacea, 15% to the Decapoda and 16% to the Astacidea. Approximately 19% were unique to marbled crayfish (Figure 14F) (Falckenhayn, 2016; Gutekunst et al., 2018).

Decapod crustaceans are highly resistant to tumor formation (Vogt, 2008c). Incidences of tumors are much lower than in fish although decapods live in the same environments and have similar life spans as fish. For example, skin tumors in marine fishes occur in 1-30% of specimens, depending on species and population (Mawdesley-Thomas, 1971), and zebrafish, which have a life span of 2-3 years, show neoplasias in approximately 10–15% of 1.5-2 year old specimens (Spitsbergen and Kent, 2003). Marbled crayfish have approximately the same life span but I have never found tumors although I have intensely searched for them (Vogt, 2008c). Understanding the genetic underpinning of the resistance to environmentally-induced and age-related cancer in marbled crayfish may initiate the development of new cancer therapies in humans at best.

Several laboratories are presently trying to generate transgenic marbled crayfish for research, in which specific genes are knocked out or modified, e.g., by CRISPR-Cas9, to reveal their functions. These manipulations require special permits and safety laboratories. Of course, genetically manipulated marbled crayfish must not be released into the wild.

### *12.2.6. Model for Epigenetics*

Epigenetics deals with variation of gene expression that is not encoded in the DNA sequence and their consequences for the expression of the phenotype. Epigenetic mechanisms, among them DNA methylation, play a crucial role in many fields of biology. For example, they are important for the evolution of organisms, their adaptation to the environment, and their susceptibility and resistance to diseases (Jaenisch and Bird, 2003; Feinberg and Irizarry, 2010; Tollefsbol, 2017; Vogt, 2017b). Marbled crayfish is an ideal model for epigenetics, because it can generate a broad spectrum of phenotypes from the same genome and has well methylated DNA. It is well suitable to investigate the involvement of epigenetic mechanisms in gene regulation, cell type identity, stem cell determination, regeneration, aging, disease susceptibility, environmental adaptation, and evolution (Vogt, 2018b). In the following, I will discuss three central questions of epigenetics to which marbled crayfish could significantly contribute, namely the production of phenotypic variability by stochastic epigenetic variation, the adaptation to different environments by epigenetic phenotype variation, and the transgenerational inheritance of epigenetic signatures.

The phenotypic variability of genetically identical marbled crayfish is apparently caused by epigenetic differences that arise stochastically during development or that are induced by environmental signals (Vogt et al., 2008). Stochastic developmental variation (SDV) can best be investigated in clutch-mates reared in the same environment. Extensive investigations with the marbled crayfish and other organisms revealed that SDV affects practically all aspects of life (Vogt, 2015a). The spectrum of variation is usually higher for biochemical and physiological parameters than for morphological traits. Particularly broad ranges of SDV in marbled crayfish were observed with respect to coloration and behavior (Vogt et al., 2008). For example, the marmoration pattern on the lateral side of the carapace of marbled crayfish is so variable that it is specific for each specimen. Marmoration as such is obviously genetically determined, because uniformly colored marbled crayfish were not yet found, but the marmoration pattern is epigenetically determined. This pattern originates during juvenile

development and remains then rather constant in the adults. It is not inherited to the next generation,

There is evidence from literature that epigenetic variation may help animals to adapt to environmental challenges by broadening the range of phenotypes in a population (Skinner et al., 2014; Leung et al., 2016; Vogt, 2017a). This strategy seems particularly advantageous for clonal lineages, small invasive groups and genetically depauperate populations and may explain controversial ecological issues like the invasion paradox (Sax and Brown, 2000) and the general purpose genotype (Massicotte and Angers, 2012). The monoclonal marbled crayfish seems particularly suitable to investigate this topic in detail because it has invaded different geographical regions in Europe and Madagascar since 2003 and has adapted to a wide spectrum of habitats despite the virtual absence of genetic variability (Jones et al. 2009; Chucholl, 2016; Vogt, 2017a; Gutekunst et al., 2018). First comparison of global DNA methylation between marbled crayfish from German laboratory lineages and Lake Moosweiher revealed higher values in laboratory-raised than wild specimens (Vogt, 2017a). More meaningful information on the potential involvement of DNA methylation in environmental adaptation is expected from the comparison of the genome-wide methylomes of differently adapted marbled crayfish that is presently done in the Lyko lab in Heidelberg.

The transgenerational inheritance of epigenetic marks that are acquired during an individual's life is among the most controversial topics of modern biology (Jablonka and Raz, 2009; Crews and Gore, 2014; Allis and Jenuwein, 2016). Marbled crayfish could contribute some new aspects to this theme from the perspective of parthenogenetic animals, which have not been considered so far. In mammals, the DNA methylation marks are largely erased and reprogrammed in the primordial germ cells and the zygote and early cleavage stages (Seisenberger et al., 2012; Petell et al., 2016). In zebrafish, the methylomes of the gametes are less intensely demethylated and there are significant differences between males and females (Jiang et al., 2013; Potok et al., 2013). The paternal methylome is largely maintained throughout early embryogenesis, whereas the maternal methylome is maintained only until the 16-cell stage and then progressively reprogrammed



by losses and gains of methylation markers (Jiang et al., 2013). Comparison of DNA methylation in marbled crayfish between primordial germ cells, oogonia, oocytes, freshly spawned eggs and the following cleavage stages should reveal whether their DNA methylation pattern is erased and reestablished as in mammals, reorganized by the parallel losses and gains of methylation marks like in zebrafish, or not significantly changed between generations.

#### *12.2.7. Model for Stem Cell Biology*

Marbled crayfish were already used to investigate various stem cell systems in the decapod crustaceans including the E-cells in the distal ends of the hepatopancreas tubules (Figure 14C) (Vogt, 2012) and the neurogenic niche of the olfactory brain (Sintoni et al., 2012). A particular advantage of these stem cell systems is their persistent activity until high age. Unlike mammals and most insects, freshwater crayfish continue to grow in the adult life period and regenerate lost appendages. Their stem cell systems must have co-evolved with this indeterminate type of growth suggesting possession of unknown and beneficial features that might open up new vistas in stem cell biology.

#### *12.2.8. Model for Biogerontology*

Vogt (2009, 2010) examined the suitability of marbled crayfish for research on aging and longevity. Experimental data revealed that this species is particularly suitable to investigate the dependency of aging and longevity from non-genetic factors such as stochastic developmental variation, allocation of metabolic resources, damage and repair, caloric restriction and social stress. Marbled crayfish are also well applicable to examine alterations of the epigenetic code with increasing age (Stubbs et al., 2017). As representative of the sparsely investigated indeterminately growing animals, marbled crayfish may significantly contribute to evolutionary theories of aging and longevity. Research on aging in marbled crayfish and its relatives is of practical relevance for crustacean fisheries and aquaculture and may someday offer starting points for the development of novel anti-aging interventions in humans.

### 12.2.9. Model for Toxicology

Marbled crayfish have repeatedly been used for toxicological studies. Toxicologists prefer to use genetically homogeneous lineages and species easy to rear. Marbled crayfish fulfill these requirements and are good representatives for aquatic macro-invertebrates in ecotoxicological studies.

Vogt (2007) exposed developing eggs of marbled crayfish to  $17\alpha$ -methyl testosterone and found that a concentration of  $100 \mu\text{g/L}$  caused prolonged embryonic development, reduced hatching success and growth of the juveniles, and induced severe malformations of the appendages in juveniles (Figure 14E).

Rubach et al. (2011) used marbled crayfish as a representative of freshwater Decapoda to study toxicity of the insecticide chlorpyrifos. The LC<sub>50</sub> (concentration lethal for 50% of specimens) after 96 h exposure was  $12.9 \mu\text{g/L}$  in adults and  $1.55 \mu\text{g/L}$  in juveniles. Velisek et al. (2014) exposed marbled crayfish to  $144 \mu\text{g/L}$  and  $4.320 \mu\text{g/L}$  of the herbicide prometryne and monitored effects on mortality, early development, growth rate and histopathology. All concentrations caused decreases of weight and histopathological changes in the gills. Crayfish exposed to the high concentration additionally showed delayed ontogeny.

Marenkov et al. (2017) revealed that exposure to  $0.1 \text{ mg/L}$  zinc and  $0.01 \text{ mg/L}$  cadmium resulted in lower survival compared to the control. Moreover, cadmium exposed specimens did not reproduce. The cells of the antennal gland (excretory organ) decreased considerably in size in both metal treatments.

### 12.2.10. Model for Ecology

Marbled crayfish is well suitable as a model for invasion biology because its invasions are relatively recent and ongoing. These invasive populations allow studying of the mechanisms that enable founder population to survive and proliferate in a new environment. They can further be used to investigate adaptations to new food sources and new predator threats. Finally, marbled crayfish invasions are suitable to study the temporal, qualitative and quantitative aspects of transformation of food webs and ecosystems by an invader.

Marbled crayfish live in several German lakes together with the spiny-cheek crayfish *Faxonius limosus* as described in the “ecology” section and have meanwhile also reached the ranges of the native *Astacoides* species in Madagascar (Andriantsoa et al., 2019). Therefore, there are good systems available to study competition between introduced and resident crayfish species and potential replacement of the residents by the invaders.

#### 12.2.11. Model for Evolution

Since marbled crayfish is monoclonal and apomictic parthenogenetic it is well suitable for studying animal genome evolution over time. Gutekunst et al. (2018) compared already whole genomes of various laboratory and wild populations and identified several hundred mutations. Lyko (2017b) suggested studying genome evolution in marbled crayfish as a paradigm of genome evolution in human tumor development.

Marbled crayfish is also useful to investigate if differently adapted populations have evolved different epigenetic profiles and if epigenetic differences with phenotypic effects can end up in genetic differences as hypothesized earlier (Vogt, 2017a), for example by deamination of 5-methylcytosine into thymine. Such facilitated mutations might genetically fix phenotypes that have initially been induced epigenetically. On the long range marbled crayfish may also serve as a paradigm on how new asexual species evolve from a single monoclonal stem population if they are geographically separated (Madagascar versus Europe).

### 13. COMMON NAMES AND LEGAL REGULATIONS

Marbled crayfish is treated as a separate biological unit in naming and legal regulations. In contrast to its parent species *Procambarus fallax*, which has only English common names (slough crayfish or deceitful crayfish) there are many common names for marbled crayfish in different languages (listed in alphabetical order): jarad albahr alrahamii (Arabic), 大理石螯蝦 - dàlǐshí áo xiā (Chinese), rak mramorový (Czech), mramorni rak (Croatian), marmerkreeft (Dutch), marbled crayfish (English),

marmorumita kankro (Esperanto), marmorvähk (Estonian), marmorirapu (Finnish), écrevisse marbrée (French), Marmorkrebs (German), márványrák (Hungarian), gamberi d'acqua dolce marmorizzato (Italian), マーモクレス - māmokurebusu (Japanese), 대리석무늬가재- daeliseogmunuigajae (Korean), foza orana (Malagassy), marmorkrebs (Norwegian), marmorkrebs (Malta), rak marmurkowy (Polish), lagosta mármore (Portuguese), racul marmorat (Romanian), мраморный рак - mramornyy rak (Russian), rak mramorovaný (Slovak), marmornati škarjar (Slovenian), cangrejo mármol (Spanish), marmorkräfta (Swedish) and мармуровий рак - marmurovyy rak (Ukrainian). This multitude of common names reflects the worldwide attention to this unusual crayfish.

There are already several legal regulations in different countries on the import, export, keeping, utilization and release of marbled crayfish. For example, in the European Union marbled crayfish is listed among the invasive alien species of EU concern according to EU Regulation No. 1143/2014 (European Parliament and Council of the European Union, 2014; European Commission, 2017). This regulation prohibits keeping and breeding of marbled crayfish within the Union, placing on the market and releasing into the environment. Keeping for scientific purposes is allowed. In the United States there are different regulations depending on state. For example, Missouri has added marbled crayfish to the prohibited species list and Tennessee designated it as “Class V wildlife,” meaning it can only be kept by zoos (Faulkes, 2019). In Madagascar, the transportation and release of wild marbled crayfish is prohibited (Ministry of Agriculture, Animal Husbandry and Fishing, 2008).

## ACKNOWLEDGMENTS

Many thanks to Michael Pfeiffer and Christian Günter (Gobio Bureau, March-Hugstetten, Germany), Frank Lenich (Regensburg, Germany), Stefan Linzmaier (Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany), Steffen Potel (Saarbrücken, Germany), Menno

Soes (Bureau Waardenburg, Culemborg, Netherlands), Elena Tricarico (Università degli Studi di Firenze, Italy), Patrik Bohman (Institute of Freshwater Research, Drottningholm, Sweden) and Tadashi Kawai (Central Fisheries Research Institution, Hokkaido, Japan) for information on the actual status of marbled crayfish populations in the wild.

## REFERENCES

- Ahyong, S. T., Lowry, J. K., Alonso, M., Bamber, R. N., Boxshall, G. A., Castro, P., Gerken, S., Karaman, G. S., Goy, J. W., Jones, D. S., Meland, K., Rogers, D. C. and Svavarsson, J. (2011). Subphylum Crustacea Brünnich, 1772. *Zootaxa*, 3148: 1-237.
- Albalat, R., Martí-Solans, J. and Cañestro, C. (2012). DNA methylation in amphioxus: From ancestral functions to new roles in vertebrates. *Briefings in Functional Genomics*, 11: 142-155.
- Allis, C. D. and Jenuwein T. (2016). The molecular hallmarks of epigenetic control. *Nature Reviews Genetics*, 17: 487-500.
- Alwes, F. and Scholtz, G. (2006). Stages and other aspects of the embryology of the parthenogenetic Marmorkrebs (Decapoda, Reptantia, Astacida). *Development, Genes and Evolution*, 216: 169-184.
- Andrews, E. A. (1904). Breeding habits of crayfish. *American Naturalist*, 38: 165-206.
- Andrews, E. A. (1906). Egg-laying of crayfish. *American Naturalist*, 40: 343-356.
- Andriantsoa, R., Tönges, S., Panteleit, J., Theissinger, K., Coutinho Carneiro, V., Rasamy, J. and Lyko, F. (2019). Ecological plasticity and commercial impact of invasive marbled crayfish populations in Madagascar. *BMC Ecology*, 19: article 8.
- Barker, M. S., Arrigo, N., Baniaga, A. E., Li, Z. and Levin, D. A. (2016). On the relative abundance of autopolyploids and allopolyploids. *New Phytologist*, 210: 391-398.
- Barracough, T. G., Birky, C. W. Jr. and Burt, A. (2003). Diversification in sexual and asexual organisms. *Evolution*, 57: 2166-2172.

- Bell, G. (1982). *The Masterpiece of Nature: The Evolution and Genetics of Sexuality*. University of California Press, Berkeley, CA.
- Benson, A., Städele, C., Gähns, C., Vidal-Gadea, A. and Stein, W. (2017). Behavioral consequences of RNA-mediated suppression of innexin expression in marbled crayfish. *Abstracts of the Illinois State University Graduate Student Symposium, March 2017*. Illinois State University, Normal, IL.
- Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., Ingram, K. K. and Das, I. (2006). Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution*, 22: 148-155.
- Birky, C. W. Jr. and Barraclough, T. G. (2009). Asexual speciation. In: *Lost Sex: The Evolutionary Biology of Parthenogenesis* (Schön, I., Martens, K. and van Dijk, P., ed.), pp. 201-216. Springer, Dordrecht, Netherlands.
- Bohman, P., Edsman, L., Martin, P. and Scholtz, G. (2013). The first Marmorcrebs (Decapoda: Astacida: Cambaridae) in Scandinavia. *BioInvasions Records*, 2: 227-232.
- Breiling, A. and Lyko, F. (2015). Epigenetic regulatory functions of DNA modifications: 5-methylcytosine and beyond. *Epigenetics & Chromatin*, 8: article 24.
- Buri, D. B. M. (2015). *Population Study of the Invasive Species Marmorcrebs (Procambarus fallax forma virginalis) in the Lake Moosweiher in Freiburg im Breisgau, South-Western Germany (03.11.–24.12.2014)*. Bachelor Thesis. University of Freiburg, Freiburg, Germany.
- Butlin, R. (2002). The costs and benefits of sex: New insights from old asexual lineages. *Nature Reviews Genetics*, 3: 311-317.
- Cesaroni, D., Allegrucci, G. and Sbordoni, V. (1992). A narrow hybrid zone between two crayfish species from a Mexican cave. *Journal of Evolutionary Biology*, 5: 643-659.
- Chucholl, C. (2013). Invaders for sale: Trade and determinants of introduction of ornamental freshwater crayfish. *Biological Invasions*, 15: 125-141.

- Chucholl, C. (2016). Marbled crayfish gaining ground in Europe: The role of the pet trade as invasion pathway. In: *Freshwater Crayfish: Global Overview* (Kawai, T., Faulkes, Z. and Scholtz, G., ed.), pp. 83-114. CRC Press, Boca Raton, FL.
- Chucholl, C. and Pfeiffer, M. (2010). First evidence for an established Marmorkrebs (Decapoda, Astacida, Cambaridae) population in Southwestern Germany, in syntopic occurrence with *Orconectes limosus* (Rafinesque, 1817). *Aquatic Invasions*, 5: 405-412.
- Chucholl, C., Morawetz, K. and Groß, H. (2012). The clones are coming – strong increase in Marmorkrebs [*Procambarus fallax* (Hagen, 1870) f. *virginalis*] records from Europe. *Aquatic Invasions*, 7: 511-519.
- Cole, C. J., Taylor, H. L., Baumann, D. P. and Baumann, P. (2014). Neaves' Whiptail Lizard: The first known tetraploid parthenogenetic tetrapod (Reptilia: Squamata: Teiidae). *Breviora*, 539 (1): 1-20.
- Coyne, J. A. and Orr, H. A. (2004). *Speciation*. Sinauer Associates, Sunderland, MA.
- CPH Post Online (2019). *Self-fertilising invasive crayfish discovered in Denmark*. Copenhagen, Denmark, February 15, 2019.
- Crandall, K. A. and De Grave, S. (2017). An updated classification of the freshwater crayfishes (Decapoda: Astacidea) of the world, with a complete species list. *Journal of Crustacean Biology*, 37: 615-653.
- Crews, D. and Gore, A. C. (2014). Controversies and debate of generational epigenetic inheritance. In: *Transgenerational Epigenetics: Evidence and Debate* (Tollefsbol, T. O., ed.), pp. 369-390. Academic Press, Waltham, MA.
- Cumberlidge, N. and Sternberg, R. V. (2002). The freshwater crabs of Madagascar (Crustacea, Decapoda, Potamoidea). *Zoosystema*, 24: 41-79.
- Cvitančić, M. (2017). *Reproduktivni ciklus invazivne vrste mramornog raka Procambarus fallax (Hagen, 1870) f. virginalis u jezeru Šoderica*. Diploma Thesis. University of Zagreb, Zagreb, Croatia. [*Reproductive cycle of the marbled crayfish, Procambarus fallax (Hagen, 1870) f. virginalis, in Lake Šoderica*].

- Da Silva, J. M., Creer, S., dos Santos, A., Costa, A. C., Cunha, M. R., Costa, P. O. and Carvalho, G. R. (2011). Systematic and evolutionary insights derived from mtDNA COI barcode diversity in the Decapoda (Crustacea: Malacostraca). *PLoS One*, 6: article e19449.
- De Grave, S., Pentcheff, N. D., Ahyong, S. T., Chan, T.-Y., Crandall, K. A., Dworschak, P. C., Felder, D. L., Feldmann, R. M., Fransen, C. H. J. M., Goulding, L. Y. D., Lemaitre, R., Low, M. E. Y., Martin, J. W., Ng, P. K. L., Schweitzer, C. E., Tan, S. H., Tshudy, D. and Wetzer, R. (2009). A classification of living and fossil genera of decapod crustaceans. *Raffles Bulletin of Zoology (Supplement)*, 21: 1-109.
- Deidun, A., Sciberras, A., Formosa, J., Zava, B., Insacco, G., Corsini-Foka, M. and Crandall, K. A. (2018). Invasion by non-indigenous freshwater decapods of Malta and Sicily, central Mediterranean Sea. *Journal of Crustacean Biology*, 38: 748-753.
- De Meeûs, T., Prugnotte, F. and Agnew, P. (2007). Asexual reproduction: Genetics and evolutionary aspects. *Cellular and Molecular Life Sciences*, 64: 1355-1372.
- De Quiroz, K. (2007). Species concepts and species delimitation. *Systematic Biology*, 56: 879-886.
- Dufresne, F. (2011). The history of the *Daphnia pulex* complex: Asexuality, hybridization, and polyploidy. In: *Phylogeography and Population Genetics in Crustacea* (Held, C., Koenemann, S. and Schubart, C. D., ed.), pp. 217-232. Crustacean Issues 19. CRC Press, Boca Raton, FL.
- Dümpelmann, C. and Bonacker, F. (2012). Erstnachweis des Marmorkrebses *Procambarus fallax* f. *virginialis* (Decapoda: Cambaridae) in Hessen. *Forum Flusskrebse*, 18: 3-12.
- Ďuriš, Z., Drozd, P., Kozák, P. and Polícar, T. (2006). Biometry and demography of the invasive crayfish *Orconectes limosus* in the Czech Republic. *Bulletin Français de la Pêche et de la Pisciculture*, 380/381: 1215-1228.
- Edwards, J. R., Yarychkivska, O., Boulard, M. and Bestor, T. H. (2017). DNA methylation and DNA methyltransferases. *Epigenetics & Chromatin*, 10: article 23.



- Eriksson, J. S., Blanco-Pastor, J. L., Sousa, F., Bertrand, Y. J. K. and Pfeil, B. E. (2017). A cryptic species produced by autopolyploidy and subsequent introgression involving *Medicago prostrata* (Fabaceae). *Molecular Phylogenetics and Evolution*, 107: 367-381.
- Estonian Research Council (2018). The marbled crayfish have established themselves in Narva power plant. Retrieved from [https://www.eurekalert.org/pub\\_releases/2018-06/erc-tmc060618.php](https://www.eurekalert.org/pub_releases/2018-06/erc-tmc060618.php) (January 10, 2019).
- European Commission (2017). *Invasive Alien Species of Union Concern*. Publications Office of the European Union, Luxembourg.
- European Parliament and Council of the European Union (2014). Regulation (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species. *Official Journal of the European Union*: L 317/35.
- Falckenhayn, C. (2016). *The Methylome of the Marbled Crayfish Procambarus virginalis*. Doctoral Thesis. University of Heidelberg, Heidelberg, Germany.
- Farca Luna, A. J., Hurtado-Zavala, J. I., Reischig, T. and Heinrich, R. (2009). Circadian regulation of agonistic behavior in groups of parthenogenetic marbled crayfish, *Procambarus* sp. *Journal of Biological Rhythms*, 24: 64-72.
- Faulkes, Z. (2015a). Marmorkrebs (*Procambarus fallax* f. *virginalis*) are the most popular crayfish in the North American pet trade. *Knowledge and Management of Aquatic Ecosystems*, 416: 20.
- Faulkes, Z. (2015b). The global trade in crayfish as pets. *Crustacean Research*, 44: 75-92.
- Faulkes, Z. (2016). Marble crayfish as a new model organism and a new threat to native crayfish conservation. In: *Freshwater Crayfish: Global Overview* (Kawai, T., Faulkes, Z. and Scholtz, G., ed.), pp. 31-53. CRC Press, Boca Raton, FL.
- Faulkes, Z. (2019). Marmorkrebs.org: Advancing marbled crayfish research. Retrieved from <http://faculty.utrgv.edu/zen.faulkes/marmorkrebs/> (January 10, 2019).

- Feinberg, A. P. and Irizarry, R. A. (2010). Stochastic epigenetic variation as a driving force of development, evolutionary adaptation, and disease. *Proceedings of the National Academy of Sciences of the U.S.A.*, 107 (Supplement 1): 1757-1764.
- Feria, T. P. and Faulkes, Z. (2011). Forecasting the distribution of Marmorkrebs, a parthenogenetic crayfish with high invasive potential, in Madagascar, Europe, and North America. *Aquatic Invasions*, 6: 55-67.
- Fetzner, J. W. and Crandall, K. A. (2002). Genetic variation. In: *Biology of Freshwater Crayfish* (Holdich, D. M., ed.), pp. 291-326. Blackwell, Oxford, UK.
- Filipová, L., Grandjean, F., Chucholl, C., Soes, D. M. and Petrussek, A. (2011). Identification of exotic North American crayfish in Europe by DNA barcoding. *Knowledge and Management of Aquatic Ecosystems*, 401: article 11.
- Fořt, M., Hossain, S., Kouba, A., Buřič, M. and Kozák, P. (2019). Agonistic interactions and dominance establishment in three crayfish species non-native to Europe. *Limnologica*, 74: 73-79.
- Fraga, M. F., Ballestar, E., Paz, M. F., Ropero, S., Setien, F., Ballestar, M. L., Heine-Suñer, D., Cigudosa, J. C., Urioste, M., Benitez, J., Boix-Chornet, M., Sanchez-Aguilera, A., Ling, C., Carlsson, E., Poulsen, P., Vaag, A., Stephan, Z., Spector, T. D., Wu, Y. Z., Plass, C. and Esteller M. (2005). Epigenetic differences arise during the lifetime of monozygotic twins. *Proceedings of the National Academy of Sciences U.S.A.*, 102: 10604-10609.
- Gährs, C. (2018). Transgenic crustaceans: Adaptation of modern molecular technology to the study of neural function in decapods. In: *Program and Abstracts of the 20th Genes, Brain, and Behavior Meeting*, 59. Mayo Civic Center, Rochester, MN.
- Gatzmann, F., Falckenhayn, C., Gutekunst, J., Hanna, K., Raddatz, G., Coutinho Carneiro, V. and Lyko, F. (2018). The methylome of the marbled crayfish links gene body methylation to stable expression of poorly accessible genes. *Epigenetics & Chromatin*, 11: article 57.

- Gherardi, F. (2002). Behaviour. In: *Biology of Freshwater Crayfish* (Holdich, D. M., ed.), pp. 258-290. Blackwell Science, Oxford, UK.
- Glover, K. A., Madhun, A. S., Dahle, G., Sørvik, A. G. E., Wennevik, V., Skaala, Ø., Morton, H. G., Hansen, T. J. and Fjelldal, P. G. (2015). The frequency of spontaneous triploidy in farmed Atlantic salmon produced in Norway during the period 2007–2014. *BMC Genetics*, 16: article 37.
- Gregory, T. R. and Mable, B. K. (2005). Polyploidy in animals. In: *The Evolution of the Genome* (Gregory, T. R., ed.), pp. 427-517. Elsevier Academic Press, Amsterdam, Netherlands.
- Gregory, T. R., Nicol, J. A., Tamm, H., Kullman, B., Kullman, K., Leitch, I. J., Murray, B. G., Kapraun, D. F., Greilhuber, J. and Bennett, M. D. (2007). Eukaryotic genome size databases. *Nucleic Acids Research*, 35 (Database Issue): D332-D338.
- Günter, C. (2014). *Populationsuntersuchung des gebietsfremden Marmorkrebses Procambarus fallax forma virginalis im Moosweiher in Freiburg im Breisgau (07.04.–02.06.2014)*. Bachelor Thesis. University of Freiburg, Freiburg, Germany. [*Population Investigation on the Non-native Marbled Crayfish, Procambarus fallax forma virginalis, in Lake Moosweiher in Freiburg, Breisgau (April 07 to June 02, 2014)*].
- Gutkunst, J. (2017). *Clonal Genome Evolution of the Marbled Crayfish, Procambarus virginalis*. Doctoral Thesis. University of Heidelberg, Heidelberg, Germany.
- Gutkunst, J., Andriantsoa, R., Falckenhayn, C., Hanna, K., Stein, W., Rasamy, J. R. and Lyko, F. (2018). Clonal genome evolution and rapid invasive spread of the marbled crayfish. *Nature Ecology & Evolution*, 2: 567-573.
- Hebert, P. D. N. and Finston, T. L. (2001). Macrogeographic patterns of breeding system diversity in the *Daphnia pulex* group from the United States and Mexico. *Heredity*, 87: 153-161.
- Hebert, P. D. N., Penton, E. H., Burns, J. M., Janzen, D. H. and Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the U.S.A.*, 101: 14812-14817.

- Hendrix, A. N., Armstrong, D. and Grue, C. (2000). *Everglades Crayfish Final Report*. South Florida Ecosystem Restoration Program. U. S. Department of the Interior, Washington, DC.
- Henry, L., Schwander, T. and Crespi, B. J. (2012). Deleterious mutation accumulation in asexual *Timema* stick insects. *Molecular Biology and Evolution*, 29: 401-408.
- Hertzler, P. L. (2005). Cleavage and gastrulation in the shrimp *Penaeus (Litopenaeus) vannamei* (Malacostraca, Decapoda, Dendrobranchiata). *Arthropod Structure & Development*, 34: 455-469.
- Hobbs, H. H. Jr. (1942). The crayfishes of Florida. *University of Florida Publications, Biological Science Series*, 3(2): 1-179.
- Hobbs, H. H. Jr. (1972). *Crayfishes (Astacidae) of North and Middle America*. Biota of Freshwater Ecosystems. Identification Manual 9. Environmental Protection Agency, Washington, DC.
- Hobbs, H. H. Jr. (1981). The crayfishes of Georgia. *Smithsonian Contributions to Zoology*, 318: 1-549.
- Hobbs, H. H. Jr. (1989). An illustrated checklist of the American crayfishes (Decapoda: Astacidae, Cambaridae, and Parastacidae). *Smithsonian Contributions to Zoology*, 480: 1-236.
- Holdich, D. M. (1993). A review of astaciculture: Freshwater crayfish farming. *Aquatic Living Resources*, 6: 307-317.
- Hossain, S., Patoka, J., Kouba, A. and Buřič, M. (2018). Clonal crayfish as biological model: A review on marbled crayfish. *Biologia*, 73: 841-855.
- Hossain, S., Kubec, J., Kouba, A., Kozák, P. and Buřič, M. (2019). Still waters run deep: Marbled crayfish dominates over red swamp crayfish in agonistic interactions. *Aquatic Ecology* 53: 97-107.
- Hou, Y. and Lin, S. (2009). Distinct gene number-genome size relationships for eukaryotes and non-eukaryotes: Gene content estimation for dinoflagellate genomes. *PLoS ONE*, 4: article e6978.
- Hulák, M., Kašpar, V., Kozák, P., Buřič, M., Filipová, L. and Petrušek, A. (2010). Cross-species amplification of microsatellite markers in the invasive spiny-cheek crayfish (*Orconectes limosus*): Assessment and application. *Journal of Applied Genetics*, 51: 73-78.

- Jablonka, E. and Raz, G. (2009). Transgenerational epigenetic inheritance: Prevalence, mechanisms, and implications for the study of heredity and evolution. *Quarterly Review of Biology*, 84: 131-176.
- Jackson, C. J. and van Staaden, M. (2019). Characterization of locomotor response to psychostimulants in the parthenogenetic marbled crayfish (*Procambarus fallax* forma *virginalis*): A promising model for studying the neural and molecular mechanisms of drug addiction. *Behavioural Brain Research*, 361: 131-138.
- Jaenisch, R. and Bird, A. (2003). Epigenetic regulation of gene expression: How the genome integrates intrinsic and environmental signals. *Nature Genetics (Supplement)*, 33: 245-254.
- Jansky, V. and Mutkovič, A. (2010). Rak *Procambarus* sp. (Crustacea: Decapoda: Cambaridae) – prvý nález na Slovensku. *Acta Rerum Naturalium Musei Nationalis Slovenici*, 56: 64-67. [Crayfish *Procambarus* sp. (Crustacea: Decapoda: Cambaridae) - first found in Slovakia].
- Jeffery, N. W. (2015). *Genome Size Diversity and Evolution in the Crustacea*. Dissertation. University of Guelph, Guelph, Canada.
- Jiang, L., Zhang, J., Wang, J.-J., Wang, L., Zhang, L., Li, G., Yang, X., Ma, X., Sun, X., Cai, J., Zhang, J., Huang, X., Yu, M., Wang, X., Liu, F., Wu, C. I., He, C., Zhang, B., Ci, W. and Liu, J. (2013). Sperm, but not oocyte, DNA methylome is inherited by zebrafish early embryos. *Cell*, 153: 773-784.
- Jimenez, S. A. and Faulkes, Z. (2010). Establishment and care of a colony of parthenogenetic marbled crayfish, Marmorokrebs. *Invertebrate Rearing*, 1: 10-18.
- Jimenez, S. A. and Faulkes, Z. (2011). Can the parthenogenetic marbled crayfish Marmorokrebs compete with other crayfish species in fights? *Journal of Ethology*, 29: 115-120.
- Jirikowski, G., Kreissl, S., Richter, S. and Wolff, C. (2010). Muscle development in the marbled crayfish – insights from an emerging model organism (Crustacea, Malacostraca, Decapoda). *Development, Genes and Evolution*, 220: 89-105.

- Jones, J. P. G., Andriahajaina, F. B., Hockley, N. J., Crandall, K. A. and Ravoahangimalala, O. R. (2007). The ecology and conservation status of Madagascar's endemic freshwater crayfish (Parastacidae; *Astacoides*). *Freshwater Biology*, 52: 1820-1833.
- Jones, J. P. G., Rasamy, J. R., Harvey, A., Toon, A., Oidtmann, B., Randrianarison, M. H., Raminosoa, N. and Ravoahangimalala, O. R. (2009). The perfect invader: A parthenogenic crayfish poses a new threat to Madagascar's freshwater biodiversity. *Biological Invasions*, 11: 1475-1482.
- Kaldre, K., Meženin, A., Paaver, T. and Kawai, T. (2016). A preliminary study on the tolerance of marble crayfish *Procambarus fallax* f. *virginialis* to low temperature in Nordic climate. In: *Freshwater Crayfish: A Global Overview* (Kawai, T., Faulkes, Z. and Scholtz, G., ed.), pp. 54-62. CRC Press, Boca Raton, FL.
- Kasuya, A. and Nagayama, T. (2016). Habituation of backward escape swimming in the marbled crayfish. *Zoological Science*, 33: 6-12.
- Kato, M., Hiruta, C. and Tochinai, S. (2016). Behavior of chromosomes during parthenogenetic oogenesis in Marmorkrebs *Procambarus fallax* f. *virginialis*. *Zoological Science*, 33: 426-430.
- Kawai, T. (2017). Observation on mandible and gill morphology in *Pacifastacus leniusculus* and *Cherax quadricarinatus* with a review of the introduction of alien crayfish into Japan. *Freshwater Crayfish*, 23: 29-39.
- Kawai, T. and Takahata, M. (ed.) (2010). *Biology of Crayfish*. Hokkaido University Press, Sapporo, Japan. (in Japanese).
- Kawai, T., Scholtz, G., Morioka, S., Ramanamandimby, F., Lukhaup, C. and Hanamura, Y. (2009). Parthenogenetic alien crayfish (Decapoda: Cambaridae) spreading in Madagascar. *Journal of Crustacean Biology*, 29: 562-567.
- Kawai, T., Faulkes, Z. and Scholtz, G., ed. (2016). *Freshwater Crayfish: A Global Overview*. CRC Press, Boca Raton, FL.
- Keller, N. S., Pfeiffer, M., Roessink, I., Schulz, R. and Schrimpf, A. (2014). First evidence of crayfish plague agent in populations of the marbled

- crayfish (*Procambarus fallax* forma *virginalis*). *Knowledge and Management of Aquatic Ecosystems*, 414: article 15.
- Kozák, P., Buřič, M. and Polícar, T. (2006). The fecundity, time of egg development and juvenile production in spiny-cheek crayfish (*Orconectes limosus*) under controlled conditions. *Bulletin Français de la Pêche et de la Pisciculture*, 380+381: 1171-1182.
- Lavania, U. C., Srivastava, S., Lavania, S., Basu, S., Misra, N. K. and Mukai, Y. (2012). Autopolyploidy differentially influences body size in plants, but facilitates enhanced accumulation of secondary metabolites, causing increased cytosine methylation. *Plant Journal*, 71: 539-549.
- Lécher, P., Defaye, D. and Noel, P. (1995). Chromosomes and nuclear DNA of Crustacea. *Invertebrate Reproduction and Development*, 27: 85-114.
- Lehninger, M. (2014). *Populationsuntersuchung des gebietsfremden Marmorkrebses Procambarus fallax forma virginalis im Moosweiher in Freiburg im Breisgau (09.06.–28.07.2014)*. Bachelor Thesis. University of Freiburg, Freiburg, Germany. [Population Investigation on the Non-Native Marbled Crayfish, *Procambarus fallax forma virginalis*, in Lake Moosweiher in Freiburg, Breisgau (June 09 to July 28, 2014)].
- Leung, C., Breton, S. and Angers, B. (2016). Facing environmental predictability with different sources of epigenetic variation. *Ecology and Evolution*, 6: 5234-5245.
- Levy, T., Rosen, O., Simons, O., Alkalay, A. S. and Sagi, A. (2017). The gene encoding the insulin-like androgenic gland hormone in an all-female parthenogenetic crayfish. *Plos One*, 12: article e0189982.
- Levy, T., Aflalo, E. D. and Sagi, A. (2019). Sex control in cultured decapod crustaceans. In: *Sex Control in Aquaculture* (Wang, H.-P., Pifferer, F. and Chen, S. L., ed.), pp. 689-704. Wiley-Blackwell, Oxford, UK.
- Lewis, H. (1967). The taxonomic significance of autopolyploidy. *Taxon*, 16: 267-271.
- Linzmaier, S. M. (2016). Vom Aquarium in den See: Marmorkrebsen gelingt der Sprung in die Freiheit. *Verbund Journal*, 106 (August 2016): 14-15. [From the aquarium to the lake: Marbled crayfish manages to leap into freedom].

- Linzmaier, S. M., Goebel, L. S., Ruland, F. and Jeschke, J. M. (2018). Behavioral differences in an over-invasion scenario: Marbled vs. spiny-cheek crayfish. *Ecosphere*, 9: article e02385.
- Lipták, B., Mrugała, A., Pekárik, L., Mutkovič, A., Gruľa, D., Petrusek, A. and Kouba, A. (2016). Expansion of the marbled crayfish in Slovakia: Beginning of an invasion in the Danube catchment? *Journal of Limnology*, 75: 305-312.
- Lipták, B., Mojžišová, M., Gruľa, D., Christophoryová, J., Jablonski, D., Bláha, M., Petrusek, A. and Kouba, A. (2017). Slovak section of the Danube has its well-established breeding ground of marbled crayfish *Procambarus fallax* f. *virginalis*. *Knowledge and Management of Aquatic Ecosystems*, 418: article 40.
- Lipták, B., Veselý, L., Ercoli, F., Bláha, M., Buřič, M., Ruokonen, T. J. and Kouba, A. (2019). Trophic role of marbled crayfish in a lentic freshwater ecosystem. *Aquatic Invasions*, 14: 299–309.
- Liu, Y., Zhang, Y., Tao, S., Guan, Y., Zhang, T. and Wang, Z. (2016). Global DNA methylation in gonads of adult zebrafish *Danio rerio* under bisphenol A exposure. *Ecotoxicology and Environmental Safety*, 130: 124-132.
- Lökkös, A., Müller, T., Kovács, K., Várkonyi, L., Specziár, A. and Martin, P. (2016). The alien, parthenogenetic marbled crayfish (Decapoda: Cambaridae) is entering Kis-Balaton (Hungary), one of Europe's most important wetland biotopes. *Knowledge and Management of Aquatic Ecosystems*, 417: article 16.
- Lukhaup, C. (2001). *Procambarus* sp. – Der Marmorkrebs. *Aquaristik Aktuell*, 7+8: 48-51. [*Procambarus* sp. – The marbled crayfish].
- Lukhaup, C. (2003). *Süßwasserkrebse aus aller Welt*. Dähne Verlag, Ettlingen, Germany. [Freshwater crayfish from all over the world].
- Lukhtanov, V. A. (2015). The blue butterfly *Polyommatus* (*Plebicula*) *atlanticus* (Lepidoptera, Lycaenidae) holds the record of the highest number of chromosomes in the non-polyploid eukaryotic organisms. *Comparative Cytogenetics*, 9: 683-690.
- Lundberg, U. (2004). Behavioural elements of the noble crayfish, *Astacus astacus* (Linnaeus, 1758). *Crustaceana*, 77: 137-162.



- Lyko, F. (2017a). The marbled crayfish (Decapoda: Cambaridae) represents an independent new species. *Zootaxa*, 4363: 544-552.
- Lyko, F. (2017b). Ein Krebs für die Krebsforschung. *Biologie in unserer Zeit*, 47: 172–177. [A crayfish for cancer research].
- Lyko, F. (2018). The DNA methyltransferase family: A versatile toolkit for epigenetic regulation. *Nature Reviews Genetics*, 19: 81-92.
- Lyko, F., Foret, S., Kucharski, R., Wolf, S., Falckenhayn, C. and Maleszka, R. (2010). The honey bee epigenomes: Differential methylation of brain DNA in queens and workers. *PLoS Biology*, 8: article e1000506.
- MacCulloch, R. D., Murphy, R. W., Kupriyanova, L. A. and Darevsky, I. S. (1997). The Caucasian rock lizard *Lacerta rostombekovi*: A monoclonal parthenogenetic vertebrate. *Biochemical Systematics and Ecology*, 25: 33-37.
- Manteuffel-Ross, T. M., Stolen, E. and Hinkle, C. R. (2018). Abundance and habitat associations of two Florida crayfishes, *Procambarus paeninsulanus* (Faxon, 1914) and *P. fallax* (Hagen, 1870) (Decapoda: Astacoidea), assessed with N-mixture modeling. *Journal of Crustacean Biology*, 38: 285-294.
- Marenkov, O., Kovalchuk, J., Shapovalenko, Z., Naboka, O., Nesterenko, O. and Dzhobolda, B. (2017). Parameters of the histological adaptation of Marmorkrebs *Procambarus fallax* f. *virginalis* (Decapoda, Cambaridae) to zinc and cadmium ions pollution. *World Scientific News*, 90: 189-202.
- Mark Welch, D. B., Ricci, C. and Meselson, M. (2009). Bdelloid rotifers: Progress in understanding the success of an evolutionary scandal. In: *Lost Sex: The Evolutionary Biology of Parthenogenesis* (Schön, I., Martens, K. and van Dijk, P., ed.), pp. 259-280. Springer, Dordrecht, Netherlands.
- Marten, M., Werth, C. and Marten, D. (2004). Der Marmorkrebs (Cambaridae, Decapoda) in Deutschland – ein weiteres Neozoon im Rheineinzugsgebiet. *Lauterbornia*, 50: 1-23. [The marbled crayfish (Cambaridae, Decapoda) in Germany – another neozoon in the catchment of the Rhine river]

- Martin, P. (2016). Parthenogenesis: Mechanisms, evolution, and its relevance to the role of marbled crayfish as model organism and potential invader. In: *Freshwater Crayfish: A Global Overview* (Kawai, T., Faulkes, Z. and Scholtz, G., ed.), pp. 63-82. CRC Press, Boca Raton, FL.
- Martin, J. W. and Davis, G. E. (2001). *An Updated Classification of the Recent Crustacea*. Science Series 39. Natural History Museum of Los Angeles County, Los Angeles, CA.
- Martin, P., Kohlmann, K. and Scholtz, G. (2007). The parthenogenetic Marmorkrebs (marbled crayfish) produces genetically uniform offspring. *Naturwissenschaften*, 94: 843-846.
- Martin, P., Dorn, N. J., Kawai, T., van der Heiden, C. and Scholtz, G. (2010a). The enigmatic Marmorkrebs (marbled crayfish) is the parthenogenetic form of *Procambarus fallax* (Hagen, 1870). *Contributions to Zoology*, 79: 107-118.
- Martin, P., Shen, H., Füllner, G. and Scholtz, G. (2010b). The first record of the parthenogenetic Marmorkrebs (Decapoda, Astacida, Cambaridae) in the wild in Saxony (Germany) raises the question of its actual threat to European freshwater ecosystems. *Aquatic Invasions*, 5: 397-403.
- Martin, P., Thonagel, S. and Scholtz, G. (2016). The parthenogenetic Marmorkrebs (Malacostraca: Decapoda: Cambaridae) is a triploid organism. *Journal of Zoological Systematics and Evolution Research*, 54: 13-21.
- Mason, J. C. (1970). Egg-laying in the Western North American crayfish, *Pacifastacus trowbridgii* (Stimpson) (Decapoda, Astacidae). *Crustaceana*, 19: 37-44.
- Massicotte, R. and Angers, B. (2012). General-purpose genotype or how epigenetics extend the flexibility of a genotype. *Genetics Research International*, 2012: article 317175.
- Mawdesley-Thomas, L. E. (1971). Neoplasia in fish: A review. In: *Current Topics in Comparative Pathobiology, Vol. 1* (Cheng, T. C., ed.), pp. 88-170. Academic Press, New York, NY.
- Mayr, E. (1963). *Animal Species and Evolution*. Harvard University Press, Cambridge, MA.

- Mayr, E. (1996). What is a species, and what is not? *Philosophy of Science*, 63: 262-277.
- Mills, S., Alcántara-Rodríguez, J. A., Ciroso-Pérez, J., Gómez, A., Hagiwara, A., Hinson Galindo, K., Jersabek, C. D., Malekzadeh-Viayeh, R., Leasi, F., Lee, J.-S., Mark Welch, D. M., Papakostas, S., Riss, S., Segers, H., Serra, M., Shiel, R., Smolak, R., Snell, T. W., Stelzer, C.-P., Tang, C. Q., Wallace, R. L., Fontaneto, D. and Walsh, E. J. (2017). Fifteen species in one: Deciphering the *Brachionus plicatilis* species complex (Rotifera, Monogononta) through DNA taxonomy. *Hydrobiologia*, 796: 39-58.
- Ministère de l'Agriculture, de l'Élevage et de la Pêche (2008). Arrêté No 16825/2008. Ministère de l'Agriculture, de l'Élevage et de la Pêche, Antananarivo, Madagascar. [Enactment No 16825/2008].
- Mrugała, A., Kozubíková-Balcarová, E., Chucholl, C., Cabanillas Resino, S., Viljamaa-Dirks, S., Vukić, J. and Petrussek, A. (2015). Trade of ornamental crayfish in Europe as a possible introduction pathway for important crustacean diseases: Crayfish plague and white spot syndrome. *Biological Invasions*, 17: 1313-1326.
- Neiman, M., Paczesniak, D., Soper, D. M., Baldwin, A. T. and Hehman, G. (2011). Wide variation in ploidy level and genome size in a New Zealand freshwater snail with coexisting sexual and asexual lineages. *Evolution*, 65: 3202-3216.
- Nonnis Marzano, F., Scalici, M., Chiesa, S., Gherardi, F., Piccinini, A. and Gibertini, G. (2009). The first record of the marbled crayfish adds further threats to fresh waters in Italy. *Aquatic Invasions*, 4: 401-404.
- Novitsky, R. A. and Son, M. O. (2016). The first records of Marmorkrebs [*Procambarus fallax* (Hagen, 1870) f. *virginalis*] (Crustacea, Decapoda, Cambaridae) in Ukraine. *Ecologica Montenegrina*, 5: 44-46.
- Osborn, T. C., Pires, J. C., Birchler, J. A., Auger, D. L., Chen, Z. J., Lee, H.-S., Comai, L., Madlung, A., Doerge, R. W., Colot, V. and Martienssen, R. A. (2003). Understanding mechanisms of novel gene expression in polyploids. *Trends in Genetics*, 19: 141-147.
- Otto, S. P. and Whitton, J. (2000). Polyploid incidence and evolution. *Annual Review of Genetics*, 34: 401-437.

- Parisod, C., Holderegger, R. and Brochmann, C. (2010). Evolutionary consequences of autopolyploidy. *New Phytologist*, 186: 5-17.
- Pârvulescu, L., Togor, A., Lele, S.-F., Scheu, S., Șinca, D. and Panteleit, J. (2017). First established population of marbled crayfish *Procambarus fallax* (Hagen, 1870) f. *virginalis* (Decapoda, Cambaridae) in Romania. *BioInvasions Records*, 6: 357-362.
- Patoka, J., Buřič, M., Kolář, V., Bláha, M., Petrtýl, M., Franta, P., Tropek, R., Kalous, L., Petrusek, A. and Kouba, A. (2016). Predictions of marbled crayfish establishment in conurbations fulfilled: Evidences from the Czech Republic. *Biologia*, 71: 1380-1385.
- Perry, W. L., Feder, J. L. and Lodge, D. M. (2001). Implications of hybridization between introduced and resident *Orconectes* crayfishes. *Conservation Biology*, 15: 1656-1666.
- Perteu, M. and Salzberg, S. L. (2010). Between a chicken and a grape: Estimating the number of human genes. *Genome Biology*, 1: article 206.
- Petell, C. J., Alabdi, L., He, M., San Miguel, P., Rose, R. and Gowher, H. (2016). An epigenetic switch regulates de novo DNA methylation at a subset of pluripotency gene enhancers during embryonic stem cell differentiation. *Nucleic Acids Research*, 44: 7605-7617.
- Piferrer, F., Beaumont, A., Falguière, J.-C., Flajšhans, M., Haffray, P. and Colombo, L. (2009). Polyploid fish and shellfish: Production, biology and applications to aquaculture for performance improvement and genetic containment. *Aquaculture*, 293: 125-156.
- Potel, S. (2011). Marmorkrebs im Saarland! Umweltmagazin Saar 4/2011: 8. [Marbled crayfish in the Saarland].
- Potok, M. E., Nix, D. A., Parnell, T. J. and Cairns, B. R. (2013). Reprogramming the maternal zebrafish genome after fertilization to match the paternal methylation pattern. *Cell*, 153: 759-772.
- Raddatz, G., Guzzardo, P. M., Olova, N., Fantappiè, M. R., Rampp, M., Schaefer, M., Reik, W., Hannon, G. J. and Lyko, F. (2013). Dnmt2-dependent methylomes lack defined DNA methylation patterns. *Proceedings of the National Academy of Sciences of the U.S.A.*, 110: 8627-8631.

- Regier, J. C., Shultz, J. W. and Kambic, R. E. (2005). Pancrustacean phylogeny: Hexapods are terrestrial crustaceans and maxillopods are not monophyletic. *Proceedings of the Royal Society London B*, 272: 395-401.
- Reynolds, J. D. (2002). Growth and reproduction. In: *Biology of Freshwater Crayfish* (Holdich, D. M., ed.), pp. 152-191. Blackwell, Oxford, UK.
- Rieger, V. and Harzsch, S. (2008). Embryonic development of the histaminergic system in the ventral nerve cord of the Marbled Crayfish (Marmorokrebs). *Tissue and Cell*, 40: 113-126.
- Ros, V. I., Breeuwer, J. A. and Menken, S. B. (2008). Origins of asexuality in *Bryobia* mites (Acari: Tetranychidae). *BMC Evolutionary Biology*, 19: article 153.
- Rubach, M., Crum, S. and Van den Brink, P. (2011). Variability in the dynamics of mortality and immobility responses of freshwater arthropods exposed to chlorpyrifos. *Archives of Environmental Contamination and Toxicology*, 60: 708-721.
- Salzburg ORF (2018). Erstmals Marmorokrebse in Teich gefunden. <https://salzburg.orf.at/news/stories/2943915/> (27.10.2018) [First find of marbled crayfish in pond].
- Saura, A., Lokki, J. and Suomalainen, E. (1993). Origin of polyploidy in parthenogenetic weevils. *Journal of Theoretical Biology*, 163: 449-456.
- Sax, D. F. and Brown, J. H. (2000). The paradox of invasion. *Global Ecology and Biogeography*, 9: 363-371.
- Schmid, M., Evans, B. J. and Bogart, J. P. (2015). Polyploidy in Amphibia. *Cytogenetics and Genome Research*, 145: 315-330.
- Scholtz, G. (2016). Happy birthday! The first decade of Marmorokrebs research – results and perspectives. In: *Freshwater Crayfish: Global Overview* (Kawai, T., Faulkes, Z. and Scholtz, G., ed.), pp. 3-12. CRC Press, Boca Raton, FL.
- Scholtz, G., Braband, A., Tolley, L., Reimann, A., Mittmann, B., Lukhaup, C., Steuerwald, F. and Vogt, G. (2003). Parthenogenesis in an outsider crayfish. *Nature*, 421: 806.
- Schön, I., Rossetti, G. and Martens, K. (2009). Darwinulid ostracods: Ancient asexual scandals or scandalous gossip? In: *Lost Sex: The*

- Evolutionary Biology of Parthenogenesis* (Schön, I., Martens, K. and van Dijk, P., ed.), pp. 217-240. Springer, Dordrecht, Netherlands.
- Seisenberger, S., Peat, J. R., Hore, T. A., Santos, F., Dean, W. and Reik, W. (2012). Reprogramming DNA methylation in the mammalian life cycle: Building and breaking epigenetic barriers. *Philosophical Transactions of the Royal Society London B*, 368: article 20110330.
- Seitz, R. (2001) *Lebensdaten und Reproduktionsbiologie des Marmorkrebses (Crustacea, Decapoda)*. Diplomarbeit. Experimentelle Tierökologie, Universität Ulm, Ulm, Germany. [*Life History Data and Reproductive Biology of the Marbled Crayfish (Crustacea, Decapoda)*].
- Seitz, F. (2015). *Agonistische Interaktion zweier gebietsfremder Flusskrebsarten, Orconectes limosus (Rafinesque, 1817) und Procambarus fallax (Hagen, 1870) forma virginalis*. Bachelor's Thesis. University of Freiburg, Freiburg, Germany. [*Agonistic interaction of two alien crayfish species, Orconectes limosus (Rafinesque, 1817) und Procambarus fallax (Hagen, 1870) forma virginalis*].
- Seitz, R., Vilpoux, K., Hopp, U., Harzsch, S. and Maier, G. (2005). Ontogeny of the Marmorkrebs (marbled crayfish): A parthenogenetic crayfish with unknown origin and phylogenetic position. *Journal of Experimental Zoology A*, 303: 393-405.
- Sellars, M. J., Degnan, B. M. and Preston, N. P. (2006). Production of triploid Kuruma shrimp, *Marsupenaeus (Penaeus) japonicus* (Bate) nauplii through inhibition of polar body I, or polar body I and II extrusion using 6-dimethylaminopurine. *Aquaculture*, 256: 337-345.
- Sellars, M. J., Li, F., Preston, N. P. and Xiang, J. (2010). Penaeid shrimp polyploidy: Global status and future direction. *Aquaculture*, 310: 1-7.
- Shen, H. (2012). *Mitogenomic Analysis of Decapod Phylogeny (Crustacea, Malacostraca)*. Doctoral Thesis. Humboldt-University Berlin, Berlin, Germany.
- Shen, H., Braband, A. and Scholtz, G. (2013). Mitogenomic analysis of decapod crustacean phylogeny corroborates traditional views on their relationships. *Molecular Phylogenetics and Evolution*, 66: 776-789.
- Sintoni, S., Fabritius-Vilpoux, K. and Harzsch, S. (2007). The Engrailed-expressing secondary head spots in the embryonic crayfish brain:

- Examples for a group of homologous neurons in Crustacea and Hexapoda? *Development, Genes and Evolution*, 217:791-799.
- Sintoni, S., Benton, J. L., Beltz, B. S., Hansson, B. S. and Harzsch, S. (2012). Neurogenesis in the central olfactory pathway of adult decapod crustaceans: Development of the neurogenic niche in the brains of procambarid crayfish. *Neural Development*, 7: article 1.
- Skinner, M. K., Gurrerero-Bosagna, C., Muksitul Haque, M., Nilsson, E. E., Koop, J. A. H., Knutie, S. A. and Clayton, D. H. (2014). Epigenetics and the evolution of Darwin's finches. *Genome Biology and Evolution*, 6: 1972-1989.
- Soes, M. (2016). Onderzoek voorkomen marmerkreeft in Middelburg. Bureau Waardenburg, Culemborg, Netherlands. [Research to prevent marbled crayfish in Middelburg].
- Soes, M. and Koese, B. (2010). Invasive freshwater crayfish in the Netherlands: A preliminary risk analysis. Interim Report EIS2010-01. Bureau Waardenburg, Culemborg, Netherlands.
- Soes, M. and van Eekelen, R. (2006). Rivierkreeften, een oprukkend probleem? *De Levende Natuur*, 107: 56–59. [Freshwater crayfish, an increasing problem?].
- Soltis, D. E., Soltis, P. S., Schemske, D. W., Hancock, J. F., Thompson, J. N., Husband, B. C. and Judd, W. S. (2007). Autopolyploidy in angiosperms: Have we grossly underestimated the number of species? *Taxon*, 56: 13-30.
- Spitsbergen, J. M. and Kent, M. L. (2003). The state of the art of the zebrafish model for toxicology and toxicologic pathology research – advantages and current limitations. *Toxicology and Pathology*, 31 (Supplement): 62-87.
- Stloukal, E. (2009). Recent distribution of non-indigenous (sic) crayfish species in Slovakia. *Folia faunistica Slovaca*, 14: 119-122.
- Stubbs, T. M., Bonder, M. J., Stark, A.-K., Krueger, F., BI Ageing Clock Team, von Meyenn, F., Stegle, O. and Reik, W. (2017). Multi-tissue DNA methylation age predictor in mouse. *Genome Biology*, 18; article 68.

- Takahashi, K., Yamaguchi, E., Fujiyama, N. and Nagayama, T. (2019). The effects of quality of shelters and prior residence on Marmorcrebs (marbled crayfish). *Journal of Experimental Biology*, 222: article jeb197301.
- Thomas, W. J. (1991). Aspects of egg attachment in *Austropotamobius pallipes* (Lereboullet, 1858) (Decapoda, Astacidae). *Crustaceana*, 61: 287-293.
- Tollefsbol, T. (2017). *Handbook of Epigenetics: The New Molecular and Medical Genetics*, 2nd edition. Academic Press, New York, NY.
- Tolley, L. (2002). *The Marbled Crayfish: A Study of Reproduction, Maternal Care Adaptations and Development*. Bachelor's thesis. University of Manchester, Manchester, UK.
- Tolley, L. and Vogt, G. (2002). Reproduction of the marble crayfish, a presumptive parthenogenic decapod. *8th Colloquium Crustacea Decapoda Mediterranea*, Corfu, Greece, September 2-6, 2002. Abstracts, p. 97.
- Usio, N., Azuma, N., Sasaki, S., Oka, T. and Inoue, M. (2017). New record of Marmorcrebs from western Japan and its potential threats to freshwater ecosystems. *Cancer*, 26: 5-11. [in Japanese with English abstract].
- VanArman, P. G. (2003). *Biology and Ecology of Epigeal Crayfish that Inhabit Everglades Environments: Procambarus alleni (Faxon) and Procambarus fallax (Hagen)*. PhD Thesis. Nova Southeastern University, Dania Beach, FL.
- VanArman, P. G. (2011). Role of native crayfish, *Procambarus alleni* (Faxon) and *Procambarus fallax* (Hagen) in Everglades food webs: A literature review and conceptual model. *Florida Scientist*, 74: 100-125.
- Van der Heiden, C. (2012). *Population Distribution, Habitat Selection, and Life History of the Slough Crayfish (Procambarus fallax) in the Ridge-Slough Landscape of the Central Everglades*. PhD Thesis. Florida Atlantic University, Boca Raton, FL.
- Van der Heiden, C.A. and Dorn, N. J. (2017). Benefits of adjacent habitat patches to the distribution of a crayfish population in a hydro-dynamic wetland landscape. *Aquatic Ecology*, 51: 219-233.



- Velisek, J., Stara, A., Koutnik, D., Zuskova, E. and Kouba, A. (2014). Effect of prometryne on early life stages of marbled crayfish (*Procambarus fallax* f. *virginalis*). *Neuroendocrinology Letters*, 35 (Supplement 2): 93-98.
- Veselý, L., Hrbek, V., Kozák, P., Buřič, M., Sousa, R. and Kouba, A. (2017). Salinity tolerance of marbled crayfish *Procambarus fallax* f. *virginalis*. *Knowledge and Management of Aquatic Ecosystems*, 418: article 21.
- Vilpoux, K., Sandeman, R. and Harzsch, S. (2006). Early embryonic development of the central nervous system in the Australian crayfish and the marbled crayfish (Marmorkrebs). *Development, Genes and Evolution*, 216: 209-223.
- Vodovsky, N., Patoka, J. and Kouba, A. (2017). Ecosystem of Caspian Sea threatened by pet-traded non-indigenous crayfish. *Biological Invasions*, 19: 2207-2217.
- Vogt, G. (2002). Functional anatomy. In: *Biology of Freshwater Crayfish* (Holdich, D. M., ed.), pp 53-151. Blackwell, Oxford, UK.
- Vogt, G. (2007). Exposure of the eggs to 17 $\alpha$ -methyl testosterone reduced hatching success and growth and elicited teratogenic effects in postembryonic life stages of crayfish. *Aquatic Toxicology*, 85: 291-296.
- Vogt, G. (2008a). The marbled crayfish: A new model organism for research on development, epigenetics and evolutionary biology. *Journal of Zoology*, 276: 1-13.
- Vogt, G. (2008b). Investigation of hatching and early post-embryonic life of freshwater crayfish by in vitro culture, behavioral analysis, and light and electron microscopy. *Journal of Morphology*, 269: 790-811.
- Vogt, G. (2008c). How to minimize formation and growth of tumours: Potential benefits of decapod crustaceans for cancer research. *International Journal of Cancer*, 123: 2727-2734.
- Vogt, G. (2009). Research on aging and longevity in the parthenogenetic marbled crayfish, with special emphasis on stochastic developmental variation, allocation of metabolic resources, regeneration, and social stress. In: *Handbook on Longevity: Genetics, Diet & Disease* (Bentley, J. V. and Keller, M. A., ed.), pp. 353-372. Nova Science Publishers, Hauppauge, NY.

- Vogt, G. (2010). Suitability of the clonal marbled crayfish for biogerontological research: A review and perspective, with remarks on some further crustaceans. *Biogerontology*, 11: 643-669.
- Vogt, G. (2012). Hidden treasures in stem cells of indeterminately growing bilaterian invertebrates. *Stem Cell Reviews and Reports*, 8: 305–317.
- Vogt, G. (2015a). Stochastic developmental variation, an epigenetic source of phenotypic diversity with far-reaching biological consequences. *Journal of Biosciences*, 40: 159-204.
- Vogt, G. (2015b). Bimodal annual reproduction pattern in laboratory-reared marbled crayfish. *Invertebrate Reproduction and Development*, 59: 218-223.
- Vogt, G. (2017a). Facilitation of environmental adaptation and evolution by epigenetic phenotype variation: Insights from clonal, invasive, polyploid, and domesticated animals. *Environmental Epigenetics*, 3: article dx002.
- Vogt, G. (2017b). Evolution of epigenetic mechanisms in animals and their role in speciation. In: *Handbook of Epigenetics: The New Molecular and Medical Genetics*, 2nd ed. (Tollefsbol, T. O., ed.), pp. 409-426. Academic Press, New York, NY.
- Vogt, G. (2018a). Annotated bibliography of the parthenogenetic marbled crayfish, *Procambarus virginalis*, a new research model, potent invader and popular pet. *Zootaxa*, 4418: 301-352.
- Vogt, G. (2018b). Investigating the genetic and epigenetic basis of big biological questions with the parthenogenetic marbled crayfish: A review and perspective. *Journal of Biosciences*, 43: 189-223.
- Vogt, G. (2018c). Glair glands and spawning in unmated crayfish: A comparison between gonochoristic slough crayfish and parthenogenetic marbled crayfish. *Invertebrate Zoology*, 15: 215-220.
- Vogt, G. (2019a). Estimating the evolutionary age of marbled crayfish from museum samples. *Journal of Natural History*: in press.
- Vogt, G. (2019b). How should we treat autopolyploid and parthenogenetic animals taxonomically? *Zootaxa*: in press.
- Vogt, G. (2019c). Genetisch gleich und doch verschieden: Vorteile und Nutzen des Marmorkrebses für die Forschung. *Forum Flusskrebse*, 30:

- 3-24. [Genetically identical and yet different: Advantages and benefits of marbled crayfish for research].
- Vogt, G. and Tolley, L. (2004). Brood care in freshwater crayfish and relationship with the offspring's sensory deficiencies. *Journal of Morphology*, 262: 566-582.
- Vogt, G., Tolley, L. and Scholtz, G. (2004). Life stages and reproductive components of the Marmorkrebs (marbled crayfish), the first parthenogenetic decapod crustacean. *Journal of Morphology*, 261: 286-311.
- Vogt, G., Huber, M., Thiemann, M., van den Boogaart, G., Schmitz, O. J. and Schubart, C. D. (2008). Production of different phenotypes from the same genotype in the same environment by developmental variation. *Journal of Experimental Biology*, 211: 510-523.
- Vogt, G., Raddatz, G., Falckenhayn, C., Hanna, K., Musch, T. and Lyko, F. (2013). Ökologische Anpassung invasiver Arten durch epigenetische Mechanismen: ein Teilaspekt des Marmorkrebs-Projekts am Deutschen Krebsforschungszentrum Heidelberg. In: *Tagungsband Internationale Flusskrebstagung*. Biologische Station StädteRegion Aachen, Aachen, pp. 109-113. [Ecological adaptation of invasive species by epigenetic mechanisms: A part of the marbled crayfish project at the German Cancer Research Center (DKFZ) in Heidelberg].
- Vogt, G., Falckenhayn, C., Schrimpf, A., Schmid, K., Hanna, K., Panteleit, J., Helm, M., Schulz, R. and Lyko, F. (2015). The marbled crayfish as a paradigm for saltational speciation by autopolyploidy and parthenogenesis in animals. *Biology Open*, 4: 1583-1594.
- Vogt, G., Lukhaup, C., Pfeiffer, M., Dorn, N. J., Williams, B. W., Schulz, R. and Schrimpf, A. (2018). Morphological and genetic characterization of the marbled crayfish, including a determination key. *Zootaxa*, 4524: 329-350.
- Vogt, G., Dorn, N. J., Pfeiffer, M., Lukhaup, C., Williams, B. W., Schulz, R. and Schrimpf, A. (2019). The dimension of biological change caused by autotriploidy: A meta-analysis with the triploid *Procambarus virginalis* and its diploid parent *Procambarus fallax*. *Zoologischer Anzeiger*, 281: 53-67.

- Vojkovská, R., Horká, I., Tricarico, E. and Ďuriš, Z. (2014). New record of the parthenogenetic marbled crayfish *Procambarus fallax* f. *virginalis* from Italy. *Crustaceana*, 87: 1386-1392.
- Wendt, W. (2011). Erstnachweis des invasiven Marmorkrebses, *Procambarus fallax* (HAGEN, 1870) f. *virginalis*, für Sachsen. *Forum Flusskrebse*, 15: 39-42. [First evidence of the invasive marbled crayfish, *Procambarus fallax* (HAGEN, 1870) f. *virginalis*, for Saxony].
- Werner, U. (1998). *Garnelen, Krebse und Krabben im Süßwasseraquarium*. Aqualog Verlag, Möhrfelden-Walldorf, Germany. [*Shrimps, Crayfishes and Crabs in the Freshwater Aquarium*].
- Wheeler, Q. D. and Meier, R., ed. (2000). *Species Concepts and Phylogenetic Theory*. Columbia University Press, New York, NY.
- Williams, B. W., Davis, C. S. and Coltman, D. W. (2010). Isolation and characterization of nine polymorphic microsatellite loci in the northern crayfish (*Orconectes virilis*). *Conservation Genetic Resources*, 2: 235-237.
- Wolf, W. (2014). *Populationsuntersuchung des invasiven Marmorkrebses (Procambarus fallax forma virginalis) im Moosweiher in Freiburg im Breisgau (08.09.-29.10.2014)*. Bachelor Thesis. University of Freiburg, Freiburg, Germany. [*Population Investigation on the Invasive Marbled Crayfish (Procambarus fallax forma virginalis) in Lake Moosweiher in Freiburg, Breisgau (Sept. 08 to Oct. 29, 2014)*].
- Xia, L., Ma, S., Zhang, Y., Wang, T., Zhou, M., Wang, Z. and Zhang, J. (2015). Daily variation in global and local DNA methylation in mouse livers. *PLoS ONE*, 10: article e0118101.
- Xiang, J., Li, F., Zhang, C., Zhang, X., Yu, K., Zhou, L., Zhang, X. and Wu, C. (2006). Evaluation of induced triploid shrimp *Penaeus (Fenneropenaeus) chinensis* cultured under laboratory conditions. *Aquaculture*, 259: 108-115.
- Yano, I. (1988). Oocyte development in the kuruma prawn *Penaeus japonicus*. *Marine Biology*, 99: 547-553.
- Ye, Z., Xu, S., Spitze, K., Asselman, J., Jiang, X., Ackerman, M. S., Lopez, J., Harker, B., Raborn, R. T., Thomas, W. K., Ramsdell, J., Pfrender, M. E. and Lynch, M. (2017). A new reference genome assembly for the

microcrustacean *Daphnia pulex*. *Genes Genomes Genetics*, 7: 1405-1416.

Zehnder, H. (1934). Über die Embryonalentwicklung des Flusskrebsses. *Acta Zoologica*, 15: 261-408. [On the embryonic development of freshwater crayfish].

Zuber, S. T., Muller, K., Laushman, R. H. and Roles, A. J. (2012). Hybridization between an invasive and a native species of the crayfish genus *Orconectes* in North-Central Ohio. *Journal of Crustacean Biology*, 32: 962-971.



*Chapter 6*

**INTEGRATIVE TAXONOMY AND CRYPTIC  
DIVERSITY IN FRESHWATER CRAYFISH:  
AN OVERVIEW**

*Felipe Bezerra Ribeiro*\*

Programa de Pós-Graduação em Biologia Animal,  
Departamento de Zoologia (Laboratório de Carcinologia),  
Instituto de Biociências, Universidade Federal do Rio Grande do Sul,  
Porto Alegre, Rio Grande do Sul, Brazil

**ABSTRACT**

Integrative taxonomy proposes a pluralistic approach to species description and other taxonomic works, combining the traditional taxonomy morphology-based with other tools such as molecular markers, ecological and population data, distribution and others. This approach has been useful in the study of biodiversity, especially for the evaluation and recognizing of cryptic species, which are defined when two or more species are classified as a single nominal species because they are morphologically poorly distinguishable. The discovery of cryptic species has profound

---

\* Corresponding Author's Email: [fbribeiro.ufc@gmail.com](mailto:fbribeiro.ufc@gmail.com).

implications for evolution, biogeography and conservation of taxa. Cryptic diversity was already identified in several crayfish species, including invading populations. The use of integrative methods, including molecular tools in the delimitation of crayfish species is bringing advances in the description of biodiversity, as well as in establishment of conservation and managements decisions.

**Keywords:** systematics, cryptic species, Parastacoidea, Astacoidea, burrowing crayfish

## 1. INTEGRATIVE TAXONOMY

Taxonomy is the branch of the Biological Sciences responsible for the identification, description, classification and nomenclature of extant and extinct species and superior taxa, playing a fundamental role in conservation and applied research (Wilson, 2004). Taking into account that species are the basic unit of biodiversity, identifying and recognizing of species are an important and difficult task, once several species' concepts were already proposed (for review see de Queiroz, 2007). However, the taxonomic impediment or taxonomic crisis, i.e., the scarce number of jobs for taxonomists and the few resources and funding for taxonomic works, results sometimes in a low number of publications and impedes the development of knowledge, especially for groups that still lacks specialists (Wheeler, 2004).

Traditional taxonomy is morphology-based and thousand species were described since the advent of Linnaean nomenclature in 1758 (Zhang, 2008). However, over the years, traditional taxonomy has been revitalized with the development of some approaches such as DNA sequencing, online access to museum collections, electron microscopy techniques, and computed tomography and taxonomic databases (e.g., Crayfish and Lobster Taxonomy Browser, Zoobank, Encyclopaedia of Life – EOL, The Global Biodiversity Information Facility – GBIF and the World Register of Marine Species – WoRMS) (Graham et al., 2004; Fetzner Jr., 2005; Knapp, 2008; Polaszek et al., 2008; Pyle et al., 2008; Pyle and Michel, 2008; Vogler and Monaghan, 2007; Faulwetter et al., 2013).



The incorporation of an integrative multidisciplinary framework has been growing in taxonomic studies. The term Integrative Taxonomy was first proposed by Dayrat (2005) to describe a pluralistic approach to species description. However, Valdecasas et al., (2008) confronted some guidelines proposed by Dayrat (2005) and showed that taxonomy has been integrative for most of its history and that an integrative approach does not mean the indiscriminate pooling of any source of data. The combined use of traditional morphology with other methodologies, such as molecular tools (mitochondrial and nuclear DNA), ecological and behavioral data, cytogenetics, chemical characteristics, among other approaches, is the goal of integrative taxonomy (Pires and Marinoni, 2010; Schlick-Steiner et al., 2010; Will et al., 2005). This approach can be applied when morphological information is not sufficient to delimit species, e.g., the case of cryptic species; or when morphology is sufficient, but the other methods may complement the information (Schlick-Steiner et al., 2010). Applying various methods gives a better interpretation of the processes that promote speciation. Thus, an integrated taxonomy does not replace the traditional taxonomy but increases or decreases the accuracy of species descriptions.

## **2. CRYPTIC SPECIES**

### **2.1. Overview of Cryptic Diversity in Freshwater Crayfish**

Cryptic species are defined when two or more species are classified as a single nominal species because they are morphologically poorly distinguishable (Bickford et al., 2006). The discovery of cryptic species has profound implications for taxonomic questions, evolution, biogeography and conservation of taxa (Bickford et al., 2006).

Molecular studies reveal that cryptic species are common and not restricted to specific taxonomic groups or ecosystems (Pfenninger and Schwenk, 2007). However, multiple cryptic species complexes have been identified in freshwater invertebrates (Taylor and Hebert, 1992; Taylor et

al., 1998; Witt and Hebert, 2000; Baker et al., 2004; Wellborn and Cothran, 2004; Westram et al., 2013). For freshwater crustaceans, some taxa that exhibit little morphological variation over long distances have been studied and it was showed that they constitute in fact cryptic species (Taylor et al., 1998; Witt and Hebert, 2000). Molecular studies have been increasing the number of species of freshwater crustaceans, recognizing that many lineages are cryptic species complexes whose component taxa ordinarily exhibit more restricted distributions than the entire complex (Taylor et al., 1998; Westram et al., 2013; Dawkins et al., 2017; Miranda et al., 2018).

Among freshwater crustaceans, crayfish (“Astacida”) are the dominant decapods in many limnetic ecosystems, playing important roles in the community dynamics (Reynolds et al., 2013; Richardson, 2007). Cryptic diversity has already been documented in northern hemisphere crayfish genera of the families Astacidae: *Astacus* Fabricius, 1775, *Austropotamobius* Skorikov, 1907, and *Pacifastacus* Bott, 1950 (Fratini et al., 2005; Trontelj et al., 2005; Mathews et al., 2008; Cataudella et al., 2010; Larson et al., 2012; Klobučar et al., 2013; Maguirre et al., 2014; Helms et al., 2015); Cambaridae: *Cambarus* Erichson, 1846, *Orconectes* Cope, 1872; Cambaroididae: *Cambaroides* Faxon, 1884; and from the southern hemisphere Parastacidae: *Cherax* Erichson, 1846, *Engaeus* Erichson, 1846, *Geocharax* Clark, 1936, *Gramastacus* Riek, 1972 and *Tenuibranchiurus* Riek, 1951, *Astacopsis* Huxley, 1879 and *Parastacus* Huxley, 1879 (Austin et al., 2003; Dawkins et al., 2017; Schultz et al., 2007; Sinclair et al., 2010; Miranda et al., 2018) (Table 1).

Within Astacidae, cryptic species were already found in the stone crayfish *Austropotamobius torrentium* (von Paula Schrank, 1803) (Klobučar et al., 2013). This fact is also supported by morphological data (Maguirre et al., 2017). One of the cryptic lineages of the stone crayfish was identified as a new species and the remaining lineages still need further revisions (Pârvulescu, 2019).

In the Japanese crayfish, the cambaroidid *Cambaroides japonicus* (De Haan, 1841 [in De Haan, 1833-1850]), two very distinct cryptic lineages were identified through molecular markers (Koizumi et al., 2012) (Table 1). These lineages had experienced contrasting demographic

processes as rapid expansion vs. slow stepwise range expansion, following differential drainage topologies and past climate events (Koizumi et al., 2012).

**Table 1. Examples of some cryptic species within freshwater crayfish families**

Family	Species	Number of evolutionary distinct lineages	Reference
Astacidae	<i>Astacus leptodactylus</i>	2	Maguirre et al., (2014)
	<i>Austropotamobius torrentium</i>	7	Klobučar et al., (2013)
	<i>Pacifastacus leniusculus</i>	4	Larson et al., (2012)
Cambaridae	<i>Cambarus englishi</i>	5	Helms et al., (2015)
	<i>Cambarus hali</i>	9	Helms et al., (2015)
	<i>Faxonius virilis</i>	4	Mathews et al., (2008)
		5	Filipová et al., (2010)
Cambaroididae	<i>Cambaroides japonicus</i>	2	Koizumi et al., (2012)
Parastacidae	<i>Astacopsis gouldi</i>	2	Sinclair et al., (2011)
	<i>Cherax tenuimanus</i>	2	Austin (1979; 1986); Austin and Knott (1996); Nguyen et al., (2002),
	<i>Parastacus brasiliensis</i>	8	Miranda et al., (2018)
	<i>Tenuibranchiurus glypticus</i>	6 within <i>Tenuibranchiurus</i> and two within the new genus	Dawkins et al., (2017)

In Cambaridae, the virile crayfish, *Faxonius virilis* (Hagen, 1870) (as *Orconectes virilis*), populations were already widely studied (Fetzner et al., 1997; Mathews et al., 2008; Filipová et al., 2010). Mathews et al., (2008) showed that populations identified in the USA as the virile crayfish represent, together with additional related species, a highly diversified cryptic species complex. Filipová et al., (2010) included invading populations of the virile crayfish from Europe in their analysis and results

confirm that these populations belong to the same lineage and are distinct from the North American ones. The native distribution of European lineage still remains unknown, as no information is available on the origin of founders of these populations (Filipová et al., 2010).

In Parastacidae, genetically diverse groups have previously been identified mainly for the Australasian genera (Table 1). The analysis of the monotypic *Tenuibranchiurus* (Dawkins et al., 2010) using two mitochondrial gene regions (cytochrome oxidase subunit 1 – COI and the 16S rDNA – 16S) and three nuclear gene regions (glyceraldehyde-3-phosphate dehydrogenase – GAPDH; histone-3 – H3; and arginine kinase – AK) identified genetically diverse groups and supported the recognition of two genera, with six species within *Tenuibranchiurus* and two within the new genus (Dawkins et al., 2017). In the giant Tasmanian freshwater crayfish *Astacopsis gouldi* Clark, 1936, a new cryptic lineage from north-east Tasmania was identified and requires further investigation and may be of extremely high conservation value (Sinclair et al., 2011) (Table 1).

For the South American endemic *Parastacus*, cryptic diversity was recorded for *P. brasiliensis* (von Martens, 1869), indicating that this species consists of at least eight different lineages (Miranda et al., 2018) (Table 1). The integrative approach using morphology, genetics and habitat characteristics also provided the elevation of the subspecies *P. brasiliensis promatensis* to full species status, *Parastacus promatensis* Fontoura & Conter, 2008 (Miranda et al., 2018).

## **2.2. Cryptic Crayfish Species and the Implications for Conservation and Invasion Biology**

Integrative taxonomy approaches are playing an important role in conservation biology by identifying biodiversity that may not be evident only by morphology-based taxonomy. The inclusion of molecular tools is fundamental to the recognizing of genetic diversity in cryptic species or evolutionary significant units (ESUs) within species (Burnham and Dawkins, 2013; Sinclair et al., 2010). ESUs are genetically differentiated

populations that have high priority for conservation priority (Legge et al., 1996). The genetic diversity is an important component in the evaluation of biodiversity, once the definition of boundaries for management units within species is necessary (Cataudella et al., 2010; Burnham and Dawkins, 2013). Conservation and management decisions should be based in solid knowledge about taxonomy, distribution and genetic characteristics of the species and populations (Burnham and Dawkins, 2013).

The stone crayfish, *A. torrentium*, for example, comprises morphologically cryptic but molecularly distinct taxa (Klobučar et al., 2013). Considering the relatively small geographical areas occupied by this species, the lineages of this species complex should be given the highest conservation priority to avoid local extinctions. The loss of biodiversity caused by local extinctions contributes to the loss of significant information regarding past geological and climatic events (Koizumi et al., 2012).

Regarding non-indigenous species, studies on genetic diversity are important when analyzing the origin of biological invasions. In invading populations, genetic variability is influenced by the history of native populations of the species as well as historical and demographical characteristics of its introduction to the area (Estoup and Guillemaud, 2010). The genetic analyses of invaders' populations improve knowledge about biodiversity of the taxon in its original distribution areas (Filipová et al., 2010).

### **3. MOLECULAR METHODS FOR SPECIES DELIMITATION IN CRAYFISH STUDIES**

Species identification is critical to biological research, including studies of evolution, conservation, biogeography and taxonomy. Species can be defined on the basis of various operational criteria (Luo et al., 2018). Species description and identification within freshwater crayfish has traditionally been undertaken through morphological examination. However, molecular tools have long been employed to addressing taxonomic and systematic

questions in the last decades (Austin, 1996; Austin and Knott, 1996; Austin and Ryan, 2002; Miranda et al., 2018).

Several methods for validating freshwater crayfish species were based on the comparison of intra and interspecific genetic distances (Schultz et al., 2007; Ribeiro et al., 2017; Miranda et al., 2018). However, methods using the Phylogenetic Species Concept (de Queiroz, 2007), identifying minimal phylogenetic units as the Operational Taxonomic Units (OTUs) (Luo et al., 2018).

Molecular species delimitation has been employed in specific genetic analysis of populations or as part of an integrative taxonomic approach to species identification (Larson et al., 2016; Dawkins et al., 2017; Miranda et al., 2018). The main molecular methods already used in species delimitation for freshwater crayfish are listed below.

### **3.1. The Poisson Tree Process (PTP) Method**

The Poisson Tree Process (PTP) method proposes species boundaries that are consistent with the Phylogenetic Species Concept. The main advantage of this method is that it models speciation in terms of the number of substitutions (Zang et al., 2013).

### **3.2. General Mixed Yule Coalescent Method (GMYC, Single Threshold Algorithm)**

The Generalized Mixed Yule Coalescent (GMYC) method was proposed by Pons et al., (2006) method and consists in a likelihood approach for delimiting species by fitting within- and between-species branching models to reconstructed gene trees (Fujisawa and Barraclough, 2013).

### **3.3. Automatic Barcode Gap Discovery (ABGD)**

The ABGD method was proposed by Puillandre et al., (2012) and is one of the most computationally efficient. ABGD is based on genetic distances computed from a single locus rather than an explicit species concept and requires a priori specification of an intraspecific distance threshold (Puillandre et al., 2012).

### **3.4. *k*/θ Method**

This method defines species groups based on specimens/populations that form clusters (i.e., clades) that are separated by deep genetic gaps that must be due to diversifying selection or long-term physical isolation instead of genetic drift (Apte et al., 2007; Dawkins et al., 2017).

## **CONCLUSION**

Taxonomy needs to be pluralistic to improve species discovery and description. The use of molecular methods, such as DNA sequencing, to identify hidden diversity has grown rapidly in the last decades. This made possible to analyze many populations in a relative quick and budget way. Molecular tools also provide additional advantages because they can be non-lethal, and samples can be stored for long periods of time. The integration of traditional morphology and molecular approaches also brings important contributions to conservation and the comprehension of the biological invasion process. Molecular methods can also be used in delimitation of species, helping in validation of freshwater crayfish species and the identification of cryptic species.

## ACKNOWLEDGMENTS

The author is grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the fellowship provided (PDJ154614/2018-1) and to the Programa de Pós-graduação em Biologia Animal and the Departamento de Zoologia of the Universidade Federal do Rio Grande do Sul for the research support.

## REFERENCES

- Apte, S., Smith, P. J. and Wallis, G. P. (2007). Mitochondrial phylogeography of New Zealand freshwater crayfishes, *Paranephrops* spp. *Molecular Ecology*, 16: 1897-1908.
- Austin, C. M. (1979). *Biochemical systematics of the genus Cherax (Decapoda: Parastacidae)*. Honours Thesis. University of Western Australia, Perth, Australia.
- Austin, C. M. (1986). *Electrophoretic and morphological systematic studies of the genus Cherax (Decapoda: Parastacidae) in Australia*. PhD Thesis. University of Western Australia, Perth, Australia.
- Austin, C. M. (1996). Systematics of the freshwater crayfish genus *Cherax* Erichson (Decapoda: Parastacidae) in northern and eastern Australia: electrophoretic and morphological variation. *Australian Journal of Zoology*, 44: 259-296.
- Austin, C. M. and Knott, B. (1996). Systematics of the freshwater crayfish genus *Cherax* Erichson (Decapoda: Parastacidae) in south-western Australia: electrophoretic, morphological and habitat variation. *Australian Journal of Zoology*, 44: 223-258.
- Austin, C. M. and Ryan, S. G. (2002). Allozyme evidence for a new species of freshwater crayfish of the genus *Cherax* Erichson (Decapoda: Parastacidae) from the south-west of Western Australia. *Invertebrate Systematics*, 16: 357-367.



- Baker, A. M., Hughes, J. M., Dean, J. C. and Bunn, S. E. (2004). Mitochondrial DNA reveals phylogenetic structuring and cryptic diversity in Australian freshwater macroinvertebrate assemblages. *Marine and Freshwater Research*, 55(6): 629-640.
- Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., Ingram, K. K. and Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, 22: 148-155.
- Burnham, Q. and Dawkins, K. L. (2013). The role of molecular taxonomy in uncovering variation within crayfish and the implications for conservation. *Freshwater crayfish*, 19(1): 29-37.
- Cataudella, R., Paolucci, M., Delaunay, C., Ropiquet, A., Hassanin, A., Balsamo, M. and Grandjean, F. (2010). Genetic variability of *Austropotamobius italicus* in the Marches region: implications for conservation. *Aquatic Conservation*, 20: 261-268.
- Dayrat, B. (2005). Towards integrative taxonomy. *Biological Journal of the Linnean Society*, 85: 407-415.
- de Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology*, 56: 879-886.
- Dawkins, K. L., Furse, J. M., Wild, C. H. and Hughes, J. M. (2010). Distribution and population genetics of the freshwater crayfish genus *Tenuibranchiurus* (Decapoda: Parastacidae). *Marine and Freshwater Research*, 61: 1048-1055.
- Dawkins, K. L., Furse, J. M., Wild, C. H. and Hughes, J. M. (2017). A novel genus and cryptic species harboured within the monotypic freshwater crayfish genus *Tenuibranchiurus* Riek, 1951 (Decapoda: Parastacidae). *Peer J*, 5: e3310.
- Estoup, A. and Guillemaud, T. (2010). Reconstructing routes of invasion using genetic data: why, how and so what? *Molecular Ecology*, 19: 4113-4130.
- Faulwetter, S., Vasileiadou, A., Kouratoras, M., Dailianis, T and Arvanitidis, C. (2013). Micro-computed tomography: introducing new dimensions to taxonomy. *Zookeys*, 263: 1-45.

- Fetzner Jr., J. W. (2005). *The crayfish and lobster taxonomy browser: A global taxonomic resource for freshwater crayfish and their closest relatives*.
- Fetzner, J. W., Sheehan, R. J. and Seeb, L. W (1997) Genetic implications of broodstock selection for crayfish aquaculture in the Midwestern United States. *Aquaculture*, 154: 39-55.
- Filipová, L., Holdich, D. M., Lesobre, J., Grandjean, F. and Petrusek, A. 2010. Cryptic diversity within the invasive virile crayfish *Orconectes virilis* (Hagen, 1870) species complex: new lineages recorded in both native and introduced ranges. *Biological Invasions*, 12: 983–989.
- Fratini, S., Zaccara, S., Barbaresi, S., Grandjean, F., Souty-Grosset, C., Crosa, G. and Gherardi, F. (2005). Phylogeography of the threatened crayfish (genus *Austropotamobius*) in Italy: implications for its taxonomy and conservation. *Heredity*, 94: 108-118.
- Fujisawa, T. and Barraclough, T. G. (2013). Delimiting Species Using Single-Locus Data and the Generalized Mixed Yule Coalescent Approach: A Revised Method and Evaluation on Simulated Data Sets. *Systematic Biology*, 62 (5): 707-724.
- Graham, C. H., Ferrier, S., Huettman, F., Moritz, C. and Peterson, T. (2004). New developments in museum-based informatics and applications in biodiversity analysis. *Trends in Ecology and Evolution*, 19: 497-503.
- Helms, B. S., Suciú, S. K., Vaught, R. C. and Santos, S. R. (2015). Cryptic diversity within two endemic crayfish species of the Southeastern US revealed by molecular genetics and geometric morphometrics. *Hydrobiologia*, 755: 283-298.
- Knapp, S. (2008). Taxonomy as a team sport. In: Wheeler, Q. D. (Ed.) *The New Taxonomy*. CRC Press, Taylor and Francis Group, p. 33-53.
- Klobučar, G., Podnar M., Jelić, M., Franjević, D., Faller, M., Štambuk, A., Gottstein, S., Simić, V. and Maguire, I. (2013). Role of the Dinaric Karst (western Balkans) in shaping the phylogeographic structure of the threatened crayfish *Austropotamobius torrentium*. *Freshwater Biology*, 58: 1089-1105.
- Koizumi I., Usio, N., Kawai, T., Azuma, N. and Masuda, R. (2012) Loss of Genetic Diversity Means Loss of Geological Information: The

- Endangered Japanese Crayfish Exhibits Remarkable Historical Footprints. *PLoS ONE*, 7 (3): e33986.
- Larson, E. R., Abbott, C. L., Usio, N., Azuma, N., Wood, K. A., Herborg, L. M. and Olden, J. D. (2012). The signal crayfish is not a single species: cryptic diversity and invasions in the Pacific Northwest range of *Pacifastacus leniusculus*. *Freshwater Biology*, 57(9): 1823-1838.
- Larson, E. R., Castelin, M., Williams, B. W., Olden, J. D. and Abott, C. L. (2016). Phylogenetic species delimitation of the genus *Pacifastacus*. *PeerJ*, 4: E1915.
- Legge, J., Roush, R., Desalle, R., Vogler, A. and May, B; (1996). "Genetic Criteria for Establishing Evolutionarily Significant Units in Cryan's Buckmoth." *Conservation Biology*, 10 (1): 85-98.
- Luo, A., Ling, C., Ho, S. Y. W. and Zhu, C-D. (2018). Comparison of Methods for Molecular Species Delimitation across a Range of Speciation Scenarios. *Systematic Biology*, 67 (5): 830-846.
- Maguire, I., Podnar, M., Jelic, M., Štambuk, A., Schrimpf, A., Schulz, H. and Klobucar, G. (2014). Two distinct evolutionary lineages of the *Astacus leptodactylus* species-complex (Decapoda: Astacidae) inferred by phylogenetic analyses. *Invertebrate Systematics*, 28: 117-123.
- Mathews, L. M., Adams, L., Anderson, E., Basile, M., Gottardi, E. and Buckholt, M. A. (2008). Genetic and morphological evidence for substantial hidden biodiversity in a freshwater crayfish species complex. *Molecular Phylogenetics and Evolution*, 48: 126-135.
- Miranda, I., Gomes, K. M., Ribeiro, F. B., Araujo, P. B., Souty-Grosset, C. and Schubart, C. D. (2018). Molecular systematics reveals multiple lineages and cryptic speciation in the freshwater crayfish *Parastacus brasiliensis* (von Martens, 1869) (Crustacea: Decapoda: Parastacidae). *Invertebrate Systematics*, 32: 1265-1281.
- Nguyen, T. T. T., Meewan, M., Ryan, S. and Austin, C. M. (2002). Genetic diversity and translocation in the marron, *Cherax tenuimanus* (Smith): implications for management and conservation. *Fisheries Management and Ecology*, 9 (3): 163-173.
- Padial, J. M., Miralles, A., De La Riva, I. and Vences, M. (2010). The integrative future of taxonomy. *Frontiers in Zoology*, 7(16): 1-14.

- Pârvulescu, L. (2019). Introducing a new *Austropotamobius* crayfish species (Crustacea, Decapoda, Astacidae): A Miocene endemism of the Apuseni Mountains, Romania. *Zoologischer Anzeiger*, 279: 94-102.
- Pfenninger, M. and Schwenk, K. (2007) Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evolutionary Biology*, 7: 121.
- Pires, A. C. and Marioni, L. (2010). DNA barcoding and traditional taxonomy unified through Integrative Taxonomy: a view that challenges the debate questioning both methodologies. *Biota Neotropica*, 10 (2): 339-346.
- Polaszek, A., Pyle, R. and Yanega, D. (2008) Animal names for all: ICZN, ZooBank, and the New Taxonomy. In: Wheeler, Q. D. (Ed.). *The New Taxonomy*. CRC Press, Boca Raton, pp. 129–142.
- Pons, J., Barraclough, T. G.; Gomez Zurita, J., Cardoso, A., Duran, D. P., Hazell, S., Kamoun, S., Sumlin, W. D. and Vogler, A. P. (2006). Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology*, 55(4): 595-609.
- Puillandre, N., Lambert, A., Brouillet, S. and Achaz, G. (2012). ABGD, automatic barcode gap discovery for primary species delimitation. *Molecular Ecology*, 21: 1864-1877.
- Pyle, R. L., Earle, J. L. and Greene, B. D. (2008). Five new species of the damselfish genus *Chromis* (Perciformes: Labroidei: Pomacentridae) from deep coral reefs in the tropical western Pacific. *Zootaxa*, 1671: 3-31.
- Pyle, R. L., Michel, E. (2008). ZooBank: Developing a nomenclatural tool for unifying 250 years of biological information. *Zootaxa*, 1950: 39-50.
- Reynolds, J., Souty-Grosset, C. and Richardson, A. (2013). Ecological Roles of Crayfish in Freshwater and Terrestrial Habitats. *Freshwater Crayfish*, 19: 197-218.
- Ribeiro, F. B., Huber, A. F., Schubart, D. and Araujo, P. B. (2017). A new species of *Parastacus* Huxleys, 1879 (Crustacea, Decapoda, Parastacidae) from a swamp forest in southern Brazil. *Nauplius*, 25: e2017008.

- Richardson, A. M. M. (2007). Behavioral Ecology of Semiterrestrial Crayfish. p. 319-338. In: Duff, J. E. and Thiel, M. *Evolutionary Ecology of Social and Sexual Systems: Crustaceans as Model Organisms*. Oxford University Press, 502p.
- Schultz, M. B., Smith, S. A., Richardson, A. M. M., Horwitz, P., Crandall, K. and Austin, C. (2007). Cryptic diversity in *Engaeus* Erichson, *Geocharax* Clark and *Gramastacus* Riek (Decapoda: Parastacidae) revealed by mitochondrial 16SrDNA sequences. *Invertebrate Systematics*, 21: 569-587.
- Sinclair, E. A., Madsen, A., Walsh, T., Nelson, J. and Crandall, K. A. (2011). Cryptic genetic divergence in the giant Tasmanian freshwater crayfish *Astacopsis gouldi* (Decapoda: Parastacidae): implications for conservation. *Animal Conservation*, 14: 87-97.
- Taylor, D. J. and Hebert, P. D. N. (1992) *Daphnia galeata mendotae* as a cryptic species complex with interspecific hybrids. *Limnology and Oceanography*, 37: 658-665.
- Taylor, D. J., Finston, T. L. and Hebert, P. D. N. (1998). Biogeography of a widespread freshwater crustacean: pseudocongruence and cryptic endemism in the North American *Daphnia laevis* complex. *Evolution*, 52: 1648-1670.
- Trontelj, P., Machino, Y. and Sket, B. (2005). Phylogenetic and phylogeographic relationships in the crayfish genus *Austropotamobius* inferred from mitochondrial COI gene sequences. *Molecular Phylogenetics and Evolution*, 34: 212–226.
- Valdecasas, A., Williams, D. and Wheeler, Q. D. 2008. 'Integrative taxonomy' then and now: a response to Dayrat (2005). *Biological Journal of the Linnean Society. Linnean Society of London* 93(1):211–216.
- Vogler, A. P. and Monaghan, M. T. (2007). Recent advances in DNA taxonomy. *Journal of Zoological Systematics and Evolutionary Research*, 45(1): 1-10.
- Wellborn, G. A. and Cothran, R. D. (2004) Phenotypic similarity and differentiation among sympatric cryptic species in a freshwater amphipod species complex. *Freshwater Biology*, 49: 1-13.

- Westram, A. J., Jokela, J. and Keller, I. (2013). Hidden Biodiversity in an Ecologically Important Freshwater Amphipod: Differences in Genetic Structure between Two Cryptic Species. *PLOS One*, 8 (8): e69576.
- Will, K. W., Mishler, B. D. and Wheeler, Q. D. (2005). The perils of DNA barcoding and the need for integrative taxonomy. *Systematic Biology*, 54(5): 844-851.
- Wilson, E. O. 2004. Taxonomy as a fundamental discipline. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 359: 739.
- Witt, J. D. S. and Hebert, P. D. N. (2000). Cryptic species diversity and evolution in the amphipod genus *Hyaella* within central glaciated North America: a molecular phylogenetic approach. *Canadian Journal of Fisheries and Aquatic Sciences*, 57: 687-698.
- Zhang, Z. Q. (2008) Contributing to the progress of descriptive taxonomy. *Zootaxa*, 1968: 65-68.
- Zhang, J., Kapli, P., Pavlidis, P. and Stamatakis, A. (2013). A general species delimitation method with applications to phylogenetic placements. *Bioinformatics*, 29 (22): 2869-2876.

## ABOUT THE EDITOR

***Felipe Bezerra Ribeiro, PhD***

Postdoctoral Researcher Fellow

Zoology Department,

Universidade Federal do Rio Grande do Sul (UFRGS), Brazil

E-mail: fbribeiro.ufc@gmail.com

*Felipe Bezerra Ribeiro* is a PhD in Animal Biology from the Universidade Federal do Rio Grande do Sul (UFRGS), has a Master's degree in Tropical Marine Sciences, and Bachelor of Biological Sciences from the Universidade Federal do Ceará (UFC). He is member of the Brazilian Society of Carcinology (SBC) and the International Association of Astacology (IAA). He is currently a postdoctoral researcher (CNPq grantee) associated with the Department of Zoology of UFRGS and a collaborating professor in the same department and the Postgraduate Program in Animal Biology (PPGBAN), where he teaches disciplines concerning Zoology and develop research in the area of crustacean systematics and ecology, with emphasis on the taxa Astacidea (freshwater crayfish), Anomura (hermit crabs) and Isopoda (parasitic isopods).





# INDEX

## A

adaptation, 14, 21, 51, 68, 94, 119, 182,  
187, 195, 197, 198, 208, 215, 224, 225  
adult, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61,  
73, 81, 94, 100, 145, 158, 199, 214, 221  
agonistic, 76, 101, 119, 160, 171, 172, 194,  
195, 207, 210  
allopolyploidy, 174  
Andes Mountains, 5, 10  
anecozoon, 181  
annulus ventralis, 143, 149, 159, 175, 176,  
183, 188  
antenna, 141, 143  
apomictic, 139, 146, 201  
aquaculture, 10, 11, 13, 23, 30, 35, 44, 74,  
77, 99, 105, 106, 111, 112, 122, 124,  
176, 189, 199, 218, 240  
aquarium, 111, 112  
areola, 142, 144  
Argentina, 4, 5, 7, 8, 10, 13, 38  
asexual, 182, 183, 184, 201, 204, 210, 217,  
220  
astaciculture, 112, 114, 210  
astacid, 139

Astacidae, 2, 30, 31, 64, 97, 99, 108, 127,  
188, 210, 216, 222, 232, 233, 241, 242  
Astacoidea, 2, 215, 230  
Australian redclaw, 26, 64, 67, 111  
Austropotamobius torrentium, 108, 232,  
233, 240  
autopolyploidy, 174, 175, 207, 213, 218,  
225

## B

behavior, 2, 22, 72, 75, 77, 91, 95, 96, 97,  
119, 138, 140, 160, 172, 192, 194, 195,  
197, 207  
biodiversity, 38, 43, 65, 68, 105, 184, 212,  
229, 230, 234, 235, 240, 241  
biogeography, 126, 138, 230, 231, 235  
biological invasion, 123, 235, 237  
biology, 14, 20, 29, 34, 36, 39, 42, 44, 74,  
77, 102, 131, 137, 192, 197, 198, 200,  
218, 223, 234  
biotic homogenization, 107  
brain, 76, 97, 101, 102, 192, 193, 199, 215,  
221

Brazil, 1, 2, 4, 5, 6, 7, 8, 10, 12, 21, 23, 24, 30, 33, 34, 35, 36, 37, 131, 229, 242, 245  
 burrow(s), 2, 6, 7, 8, 9, 14, 15, 16, 17, 18, 23, 29, 33, 36, 51, 73, 75, 78, 87, 168  
 burrower, 6, 7, 8, 9, 15, 18, 75, 77, 78, 100  
 burrowing, 2, 4, 14, 15, 18, 21, 23, 26, 31, 33, 36, 37, 38, 40, 41, 42, 46, 72, 73, 75, 77, 78, 82, 87, 90, 93, 95, 98, 230  
 burrowing behavior, 4, 14, 18, 26, 72, 73, 75, 77, 78, 82, 87, 90, 93, 95  
 burrowing crayfish, 2, 31, 33, 37, 38, 40, 41, 42, 98, 230

## C

*C. canii*, 105  
 Cambaridae, 2, 10, 28, 30, 32, 34, 36, 64, 73, 83, 96, 97, 98, 99, 101, 108, 109, 111, 127, 130, 131, 133, 141, 149, 150, 153, 159, 175, 188, 204, 205, 206, 210, 211, 212, 214, 215, 216, 217, 218, 232, 233  
 Cambaroididae, 2, 109, 232, 233  
 captivity, 77, 138, 161, 174, 180  
 carapace, 84, 87, 141, 144, 168, 197  
 cellulase, 154, 156  
 cephalothorax, 83, 142, 144, 147, 159  
 chela, 57, 143, 188  
 cheliped, 143, 144  
*Cherax*, 10, 11, 12, 21, 32, 33, 38, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 98, 100, 102, 105, 109, 110, 113, 114, 130, 131, 132, 153, 172, 189, 212, 232, 233, 238, 241  
*Cherax destructor* (*C. destructor*), 45, 54, 66, 98, 102, 105, 109, 113, 130, 153, 172, 189  
*Cherax quadricarinatus* (*C. quadricarinatus*), 10, 11, 12, 13, 26, 32, 38, 42, 44, 45, 46, 47, 49, 52, 56, 58, 60,

62, 63, 64, 65, 67, 68, 69, 105, 110, 113, 132, 189, 212  
 Chile, 4, 5, 7, 8, 9, 10, 19, 29, 33, 35, 36, 38, 39, 40, 41, 42  
 chimneys, 91  
 chromosome, 152  
 climate change, 25, 106, 123  
 climate zone, 167  
 clutch, 139, 147, 171, 186, 192, 194, 197  
 coloration, 140, 142, 144, 186, 188, 197  
 competition, 10, 26, 105, 106, 117, 119, 138, 139, 173, 186, 201  
 conservation, vii, viii, 2, 6, 13, 28, 34, 38, 44, 61, 63, 106, 123, 131, 183, 204, 207, 212, 230, 231, 234, 235, 237, 239, 240, 241, 243  
 conservation status, 2, 6, 28, 212  
 crayfish disease, 106  
 crayfish introduction, 13, 27, 32, 105, 106, 112, 114  
 crayfish juvenile stage, 73  
 crayfish plague, 44, 62, 63, 68, 139, 141, 189, 213  
 crossbreeding experiment, 187  
 cryptic diversity, viii, 2, 234, 239, 241  
 cryptic species, 54, 183, 207, 210, 229, 230, 231, 232, 233, 234, 237, 239, 243  
 culture, 11, 102, 147, 149, 223  
 cytochrome oxidase, 149, 234

## D

dactylus, 22, 143, 144  
 dead-end scenario, 182  
 Decapoda, 28, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 63, 64, 65, 66, 67, 96, 97, 98, 99, 100, 101, 102, 127, 130, 131, 133, 140, 152, 153, 154, 196, 200, 203, 204, 205, 206, 210, 211, 212, 214, 215, 216, 217, 218, 220, 222, 238, 239, 241, 242, 243

determination key, 182, 188, 226  
 detritus, 118, 170  
 development, 22, 24, 27, 39, 40, 42, 75, 78, 89, 93, 106, 112, 138, 140, 146, 147, 158, 180, 191, 192, 193, 194, 196, 197, 199, 200, 201, 208, 212, 213, 219, 223, 227, 230  
 distribution, 2, 5, 10, 24, 25, 27, 39, 44, 45, 46, 50, 54, 58, 59, 61, 73, 74, 76, 89, 95, 96, 99, 101, 106, 107, 111, 114, 116, 122, 137, 139, 141, 148, 168, 169, 178, 179, 188, 189, 190, 208, 221, 223, 229, 234, 235  
 divergence, 52, 72, 73, 89, 95, 177, 243  
 DNA, 26, 31, 54, 65, 124, 128, 153, 155, 156, 157, 158, 185, 186, 187, 197, 198, 203, 204, 207, 208, 210, 211, 213, 214, 215, 217, 218, 219, 220, 222, 226, 230, 231, 237, 239, 242, 243, 244

## E

ecological classification, 6, 33  
 ecology, 3, 13, 34, 36, 44, 65, 87, 94, 117, 118, 131, 137, 138, 139, 160, 161, 170, 196, 201, 212, 245  
 ecosystem, 34, 77, 79, 87, 89, 94, 105, 106, 107, 117, 118, 119, 170, 214  
 ecosystem engineers, 107  
 egg, 19, 22, 57, 78, 146, 148, 159, 160, 168, 171, 176, 180, 186, 193, 213, 222  
 embryo, 192  
 endemic species, 15, 33, 44, 45, 58, 112, 174  
 epigenetics, 101, 138, 140, 195, 197, 216, 223  
 epigenome, 98, 156  
 establishment, 2, 4, 13, 24, 26, 67, 75, 94, 106, 112, 117, 119, 120, 122, 123, 124, 138, 139, 172, 208, 218, 230

Europe, 2, 32, 41, 49, 66, 68, 108, 109, 110, 111, 113, 123, 126, 128, 130, 132, 161, 167, 168, 171, 181, 186, 198, 201, 205, 208, 214, 217, 233  
 European Union (EU), 44, 47, 148, 202, 207  
 evolution, 42, 52, 63, 64, 106, 137, 138, 139, 140, 152, 161, 174, 183, 184, 195, 197, 201, 209, 211, 216, 218, 221, 224, 230, 231, 235, 244  
 evolutionary significant units (ESUs), 234  
 exploitation, 44, 45, 52, 62, 113, 138  
 extinction, 4, 24, 25, 181  
 extinction risk, 24, 25

## F

*Faxonius limosus* (*F. limosus*), 108, 120, 171, 173, 201  
*Faxonius virilis* (*F. virilis*), 108, 113, 120, 233  
*Faxonius virilis* and *F. limosus*, 113  
 fecundity, 74, 97, 113, 114, 122, 138, 213  
 female, 19, 20, 21, 22, 49, 60, 61, 80, 81, 82, 139, 146, 160, 167, 175, 176, 177, 178, 179, 181, 184, 213  
 fertilization tent, 159  
 fight, 119, 159, 160, 172  
 Florida, 32, 96, 137, 149, 151, 175, 178, 180, 181, 210, 215, 222, 223  
 food, 3, 4, 26, 51, 62, 78, 105, 107, 111, 113, 118, 119, 138, 139, 149, 160, 170, 189, 190, 192, 193, 194, 200, 222  
 food web, 4, 26, 62, 105, 107, 139, 170, 201, 222  
 forest, viii, 6, 8, 15, 18, 33, 37, 72, 73, 74, 77, 78, 79, 80, 82, 87, 89, 90, 94, 242

## G

general purpose genotype, 198  
 generation time, 146

genetic analyses, 235  
 genetic distances, 236, 237  
 genetics, 61, 101, 138, 140, 152, 187, 234, 239, 240  
 genome, 57, 95, 138, 139, 152, 153, 154, 155, 156, 157, 176, 177, 180, 185, 186, 187, 191, 197, 198, 201, 209, 210, 211, 217, 218, 227  
 genotype, 176, 177, 216, 225  
 geographical range, 5, 9, 10  
 Georgia, 99, 137, 149, 151, 178, 181, 210  
 Germany, 51, 53, 55, 134, 137, 138, 139, 140, 142, 151, 158, 161, 163, 164, 167, 171, 173, 185, 202, 204, 205, 207, 209, 213, 214, 216, 220, 226  
 gleysols, 79  
 Gonochorism, 20  
 growth, 10, 19, 20, 27, 31, 32, 72, 74, 77, 89, 93, 94, 95, 98, 99, 113, 119, 120, 122, 123, 145, 146, 149, 156, 167, 182, 186, 192, 194, 199, 200, 223  
 growth rate, 10, 72, 89, 93, 95, 122, 200

## H

habitat, 2, 5, 6, 8, 25, 26, 38, 62, 93, 106, 107, 117, 120, 167, 178, 215, 223, 234, 238  
 habituation, 98, 195  
 hatchling, 193  
 hepatopancreas, 153, 156, 157, 192, 199  
 hermaphrodite, 139  
 hermaphroditism, 20, 21, 28, 38, 40, 140  
 Hungary, 44, 69, 133, 151, 161, 162, 164, 167, 214  
 hybrid, 205

## I

impact, 13, 24, 27, 34, 62, 105, 106, 117, 119, 120, 122, 203

indigenous, 10, 26, 32, 44, 46, 47, 58, 60, 108, 111, 115, 140, 174  
 innexin-4, 194  
 insecticide, 200  
 insulin-like androgenic gland hormone, 191, 213  
 integrative taxonomy, 231, 239, 244  
 intersexuality, 20, 39  
 introduction pathway, 34, 68, 106, 124, 131, 217  
 invasion, 13, 23, 27, 34, 64, 106, 112, 114, 116, 117, 120, 121, 122, 123, 124, 131, 138, 174, 198, 200, 205, 214, 219, 239  
 invasion paradox, 198  
 invasion process, 13, 23, 27, 106, 112, 117, 120, 121, 122  
 invasive crayfish, 2, 130, 171, 205, 206  
 invasive species, 2, 4, 25, 32, 63, 98, 106, 107, 111, 114, 118, 119, 124, 129, 225  
 invasiveness, 24, 123, 186, 187

## J

Japan, 100, 139, 151, 161, 162, 165, 203, 212, 222  
 juvenile, 22, 36, 37, 72, 75, 78, 82, 88, 89, 90, 94, 101, 102, 116, 145, 146, 147, 149, 160, 163, 168, 193, 194, 197, 213

## L

laboratory model, 138, 139, 140, 190  
 Lake Hévíz, 162, 164, 167  
 Lake Moosweiher, 142, 145, 150, 158, 164, 167, 168, 170, 171, 173, 198, 204, 209, 213, 226  
 lecithotrophic, 146  
 legal regulation, 139, 201, 202  
 life history, 94, 95, 138, 140, 182, 184, 186, 196  
 limnetic ecosystems, 2, 232

lineages, 35, 58, 185, 198, 200, 204, 217,  
232, 233, 234, 235, 240, 241  
longevity, 20, 146, 186, 196, 199, 224

## M

Madagascar, 4, 44, 111, 130, 138, 139, 151,  
161, 162, 165, 167, 168, 169, 170, 174,  
181, 185, 189, 190, 198, 201, 202, 203,  
205, 208, 212, 217  
management, 2, 27, 97, 98, 99, 106, 116,  
121, 124, 131, 149, 207, 235, 241  
Marmorkrebs, 65, 101, 140, 144, 202, 203,  
204, 205, 207, 208, 211, 212, 214, 215,  
216, 217, 218, 219, 220, 222, 223, 225  
meiosis, 176, 177, 191  
methylole, 152, 153, 156, 157, 198, 209,  
211  
Mexico, 10, 44, 64, 72, 73, 74, 75, 76, 79,  
82, 87, 88, 96, 97, 99, 101, 102, 209  
microsatellite, 175, 176, 186, 187, 188, 211,  
226  
molecular biology, 140  
molt, 146  
monoclonal, 138, 198, 201, 215  
morphological variation, 63, 232, 238  
morphology, 18, 28, 36, 38, 41, 61, 87, 138,  
140, 141, 187, 188, 191, 196, 212, 229,  
230, 231, 234, 237  
morphometry, 72  
muscle development, 193  
museum collection, 138, 178, 230

## N

native, 2, 4, 10, 12, 13, 19, 21, 26, 28, 30,  
31, 36, 41, 44, 52, 53, 55, 57, 58, 59, 60,  
61, 62, 67, 77, 80, 105, 106, 108, 111,  
117, 118, 119, 120, 123, 137, 139, 149,  
161, 170, 171, 174, 180, 187, 189, 201,  
207, 208, 209, 222, 227, 234, 235, 240

neurobiology, 138, 140  
New Guinea, 4, 10, 43, 44, 45, 46, 47, 48,  
49, 50, 51, 52, 55, 56, 57, 58, 59, 60, 61,  
62, 63, 64, 65, 66, 100  
New Pangea, 106  
nomenclature, 230  
non-indigenous, 10, 11, 12, 13, 62, 69, 105,  
114, 117, 121, 206, 223, 235  
non-indigenous crayfish species (NICS), 10,  
12, 13, 26, 27, 105, 106, 107, 111, 112,  
113, 114, 116, 117, 118, 119, 120, 121,  
122, 123, 124  
non-indigenous species, 117, 235

## O

offspring, 31, 72, 122, 139, 146, 187, 190,  
216, 225  
ontogeny, 72, 75, 77, 78, 90, 91, 98, 101,  
200  
oocyte, 176, 177, 211  
ornamental animal, 43, 44, 55, 59, 62

## P

*P. fallax* f. *virginialis*, 113  
*Pacifastacus leniusculus*, 27, 108, 113, 120,  
128, 130, 133, 134, 150, 152, 172, 189,  
212, 233, 241  
Pancrustacea, 196  
parastacid, 19, 20, 21, 22, 32, 44, 45, 48, 65,  
111, 139, 153, 174  
Parastacidae, 4, 10, 19, 20, 21, 22, 28, 30,  
31, 32, 33, 35, 36, 37, 38, 39, 40, 41, 42,  
44, 63, 64, 65, 66, 67, 68, 99, 100, 109,  
110, 111, 127, 153, 188, 210, 212, 232,  
233, 234, 238, 239, 241, 242, 243  
Parastacids, 4, 44  
Parastacoidea, 2, 4, 22, 44, 230  
*Parastacus*, 4, 5, 6, 7, 8, 11, 12, 15, 18, 20,  
22, 25, 26, 28, 29, 30, 31, 32, 33, 34, 35,

36, 37, 38, 39, 40, 41, 42, 119, 232, 233, 234, 241, 242

*Parastacus brasiliensis* (*P. brasiliensis*), 5, 6, 15, 28, 31, 32, 35, 36, 119, 233, 241

parthenogenesis, 138, 139, 140, 146, 180, 186, 191, 225

pet trade, 11, 13, 23, 44, 45, 47, 50, 52, 53, 64, 65, 67, 105, 106, 112, 113, 114, 122, 138, 140, 155, 185, 205, 207

phenotype, 89, 95, 187, 197, 224

physiology, 140, 196

pleon, 22, 53, 141, 144, 147, 159, 195

pleopod, 86

population biology, 19

population structure, 25, 186

predator, 200

*Procambarus acanthophorus*  
(*P. acanthophorus*), 71, 72, 73, 74, 75, 76, 77, 78, 81, 82, 87, 89, 90, 91, 92, 93, 94, 95, 99, 100, 102

*Procambarus clarkii* (*P. clarkii*), 2, 3, 10, 11, 12, 13, 23, 26, 28, 32, 34, 36, 45, 62, 68, 74, 76, 77, 82, 89, 98, 99, 100, 102, 103, 105, 109, 113, 114, 118, 119, 120, 125, 126, 127, 128, 129, 131, 132, 172, 175, 189

*Procambarus fallax* (*P. fallax*), 65, 105, 113, 137, 141, 149, 150, 151, 155, 161, 175, 176, 177, 178, 179, 180, 181, 182, 184, 185, 186, 187, 188, 191, 201, 204, 205, 206, 207, 209, 211, 212, 213, 214, 215, 216, 217, 218, 220, 222, 223, 226

*Procambarus virginalis* (*P. virginalis*), 45, 109, 137, 138, 182, 188, 207, 209, 224, 226

propodus, 143, 144

## R

rearing, 138, 139, 140, 141, 148, 190

red swamp crayfish, 10, 11, 13, 23, 24, 68, 98, 99, 103, 111, 116, 129, 172, 189, 210

release, 62, 113, 114, 159, 202

reproduction, 2, 19, 27, 31, 94, 98, 138, 140, 145, 149, 153, 167, 168, 180, 184, 195, 206, 219, 224

reproductive isolation, 187, 188

research history, 139

research model, 138, 139, 155, 157, 189, 192, 224

rostrum, 77, 81, 141, 143, 144, 145, 195

## S

*Samastacus*, 4, 8, 10, 11, 12, 14, 20, 21, 29, 35, 39, 40

*Samastacus spinifrons*, 5, 8, 10, 14, 29, 39, 40

secondary burrower, 14, 15, 18, 72, 75, 76, 77, 102

sediment, 28, 72, 81, 82, 90, 91, 92

sex ratio, 19, 180

short germ development, 192

size-frequency distribution, 168

slough crayfish, 137, 149, 188, 201, 224

Slovakia, 161, 162, 166, 167, 170, 211, 214, 221

social hierarchy, 76

South America, 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 19, 20, 21, 22, 24, 25, 26, 27, 28, 30, 31, 32, 36, 37, 39, 41, 44, 109, 111, 119, 139, 234

spawning, 159, 160, 176, 224

species concepts, 183

species description, 182, 229, 231

species identification, 236

sperm, 159, 176, 177, 187

spine, 142, 143

stem cell biology, 138, 140, 199

stochastic developmental variation, 199, 224

stone crayfish, 232, 235  
 systematics, 35, 138, 230, 238, 241, 245

## T

taxonomic crisis, 230  
 taxonomic impediment, 230  
 taxonomy, 64, 81, 137, 139, 185, 217, 229,  
 230, 231, 234, 235, 239, 240, 241, 242,  
 243, 244  
 telson, 22, 53, 72, 75, 77, 78, 81, 90, 91,  
 141, 147, 193  
*Tenuibranchiurus*, 232, 233, 234, 239  
 testosterone, 192, 200, 223  
 threats, 2, 6, 23, 25, 26, 27, 68, 105, 106,  
 189, 200, 217, 222  
 toxicology, 138, 140, 221  
 transcriptome, 152, 153, 156, 196  
 transgenerational inheritance, 197, 198  
 transgenic, 196  
 transmitter, 141  
 tri-allelic, 175, 176, 188  
 triploidization, 176

tumor, 196, 201

## U

Uruguay, 4, 5, 6, 7, 8, 10, 13  
 utilization, 137, 202

## V

Váh-Nitra river system, 162  
 ventral nerve cord, 102, 193, 219  
 video recordings, 82  
*Virilastacus*, 4, 8, 9, 10, 11, 12, 18, 19, 20,  
 25, 39, 40, 41

## W

wetland, 7, 25, 28, 72, 73, 76, 78, 79, 81,  
 82, 87, 89, 90, 93, 94, 102, 214, 223  
 wild population, 60, 61, 138, 139, 144, 150,  
 189, 201

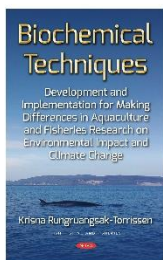




## Related Nova Publications

### BIOCHEMICAL TECHNIQUES: DEVELOPMENT AND IMPLEMENTATION FOR MAKING DIFFERENCES IN AQUACULTURE AND FISHERIES RESEARCH ON ENVIRONMENTAL IMPACT AND CLIMATE CHANGE

**AUTHOR:** Krisna Rungruangsak-Torrissen



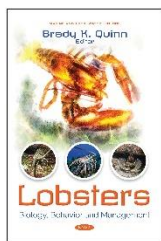
**SERIES:** Fish, Fishing and Fisheries

**BOOK DESCRIPTION:** This extraordinary book is the result of over three decades of Dr. Krisna Rungruangsak-Torrissen's career at the Institute of Marine Research in Norway. The book provides new insights into a series of growth mechanisms in aquatic living resources through the digestion and utilization of dietary protein for growth and maturation.

**HARDCOVER ISBN:** 978-1-53613-175-8  
**RETAIL PRICE:** \$160

### LOBSTERS: BIOLOGY, BEHAVIOR AND MANAGEMENT

**EDITOR:** Brady K. Quinn



**SERIES:** Marine and Freshwater Biology

**BOOK DESCRIPTION:** This book contains chapters describing studies of the larval biology, behavior and habitat preferences, and fisheries management of fished clawed and spiny lobster species.

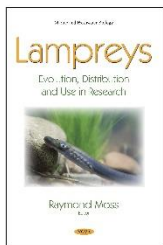
**SOFTCOVER ISBN:** 978-1-53615-711-6  
**RETAIL PRICE:** \$95

*To see a complete list of Nova publications, please visit our website at [www.novapublishers.com](http://www.novapublishers.com)*

## Related Nova Publications

### LAMPREYS: EVOLUTION, DISTRIBUTION AND USE IN RESEARCH

**EDITOR:** Raymond Moss



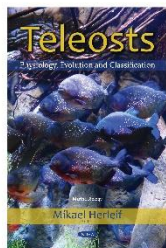
**SERIES:** Marine and Freshwater Biology

**BOOK DESCRIPTION:** *Lampreys: Evolution, Distribution and Use in Research* begins by summarizing the available knowledge about the biology, ecology, distribution, monitoring of occurrence and action plans/recovery programmes for the aforementioned species.

**SOFTCOVER ISBN:** 978-1-53614-638-7  
**RETAIL PRICE:** \$82

### TELEOSTS: PHYSIOLOGY, EVOLUTION AND CLASSIFICATION

**EDITOR:** Mikael Herleif



**SERIES:** Marine and Freshwater Biology

**BOOK DESCRIPTION:** The authors begin this compilation by analyzing catalase and peroxidase enzymes in different Black Sea teleosts related to their taxonomic, physiological and ecological position and evaluation of the anthropogenic impact on these antioxidant enzymes in fish tissues.

**SOFTCOVER ISBN:** 978-1-53613-660-9  
**RETAIL PRICE:** \$82

To see a complete list of Nova publications, please visit our website at [www.novapublishers.com](http://www.novapublishers.com)