7-2019

# EVOLUTION OF FRESHWATER CRABS IN LAKE MALAWI AND THE LAKE MALAWI BASIN IN THE EAST AFRICAN RIFT VALLEY

Emily Johnson
emiljohn@nmu.edu

7-2019

# EVOLUTION OF FRESHWATER CRABS IN LAKE MALAWI AND THE LAKE MALAWI BASIN IN THE EAST AFRICAN RIFT VALLEY

Emily Johnson

# EVOLUTION OF FRESHWATER CRABS IN LAKE MALAWI AND THE LAKE MALAWI BASIN IN THE EAST AFRICAN RIFT VALLEY

By

Emily C. Johnson

THESIS

Submitted to
Northern Michigan University
In partial fulfillment of the requirements
For the degree of

MASTER OF SCIENCE

College of Graduate Education and Research

August 2019

SIGNATURE APPROVAL FORM

EVOLUTION OF FRESHWATER CRABS IN LAKE MALAWI AND THE LAKE MALAWI BASIN IN THE EAST AFRICAN RIFT VALLEY

This thesis by Emily C. Johnson is recommended for approval by the student's Thesis Committee and Department Head in the Department of Biology and by the Dean of Graduate Education and Research.

| | |
|---|---|
| Neil Cumberlidge | 20 June 2019 |
| Committee Chair: | Date |
| Kurt Galbreath | 20 June 2019 |
| First Reader: | Date |
| Erich Ottem | 20 June 2019 |
| Second Reader (if required | Date |
| John Rebers | 7 July 2019 |
| Department Head: | Date |

Dr. Lisa Eckert — Date
Dean of Graduate Education and Research

ABSTRACT

EVOLUTION OF FRESHWATER CRABS IN LAKE MALAWI AND THE LAKE MALAWI BASIN IN THE EAST AFRICAN RIFT VALLEY

By

Emily C. Johnson

Lake Malawi is the southernmost lake in the East African Rift Valley and is home to a single species of freshwater crab, the Malawi Blue Crab. Little is known about species diversity and the phylogenetic relationships of the freshwater crabs of Lake Malawi, in contrast to the lake's highly-diverse cichlid fish species flocks. The Malawi Blue Crab is presently assigned to *Potamonautes lirrangensis* (Rathbun, 1904) sensu lato, which was originally described from a single female specimen from the D. R. Congo. While the distribution of *P. lirrangensis* s. l. ranges from the Congo River basin to other Rift Valley lakes, its current taxonomic status remains controversial. Detailed comparative morphological analysis coupled with preliminary DNA sequence data were used to determine the taxonomic status of the Malawi Blue Crab, and to determine the phylogenetic relationships between the Malawi Blue Crab and the freshwater crab fauna of the Lake Malawi basin, particularly the common and widespread species, *P. montivagus*. The morphological and molecular data presented here indicate that: (1) *P. lirrangensis* should be restricted to those specimens from the Middle Congo River in the D. R. Congo (referred to here as *P. lirrangensis* sensu stricto); (2) that the specimens from Lake Kivu formerly assigned to *P. lirrangensis* s. l. be recognized as a new species (*Potamonautes* sp. 1); and (3) that the Malawi Blue Crab should be properly referred to as *P. orbitospinus* (Cunnington, 1907). In addition, it is likely that the specimens from two crater lakes in southwest Tanzania initially identified as *P. montivagus* belong to a new species (*Potamonautes* sp. 2).

DEDICATION

For my mother, with love.

# ACKNOWLEDGEMENTS

I would like to extend my sincere thanks to my advisor, Dr. Neil Cumberlidge, for providing his continuous guidance, support and patience throughout my completion of this study and this program. His level of expertise in the field is truly admirable, and I am grateful for having had the chance to work with and learn from him.

I am grateful for the funding of this research through the Excellence in Education Research Program and through the Biology department at NMU. I am also thankful for the opportunity to have traveled during this study, as well as engaged and worked with so many specialists, particularly Dr. Martin Genner (University of Bristol, UK) and Dr. Paul Clark (The Natural History Museum, London, UK).

In closing, I want to express my deepest gratitude to my close friends and family members who supported me throughout this program. To Ada, whose feedback and company helped me thrive here. To Brooklyn, Theresia, and Alicia, whose consistent encouragement helped make this possible. Finally, to my sister, Adrian, who has always been a pillar of positivity and motivation within my life. Thank you for helping me remain focused on my goal.

This thesis follows the APA citation and format style.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

# INTRODUCTION

The Order Decapoda comprises 15,000 species, 6,900 of which fall within the Brachyura (true crabs). Of these, 1,450 species of crabs are found in freshwater, and more than 1,400 of these are primary freshwater crabs that complete their life cycle solely in freshwater habitats. Another 150 species of brachyuran crabs are considered to be secondary freshwater crabs, which live in freshwater for most of their life cycle but produce larval stages that need to develop in seawater. The primary freshwater crabs are assigned to five families: Pseudothelphusidae (Neotropics), Trichodactylidae (Neotropics), Potamonautidae (Afrotropics), Potamidae (Europe and Asia), and Gecarcinucidae (Asia).

Primary freshwater crabs reside in warm freshwater habitats around the world and are present in 122 countries across five zoogeographic regions, including the Afrotropical region (Cumberlidge et al., 2009). This region includes sub-Saharan continental Africa, plus the southern part of the Arabian Peninsula, as well as several offshore islands including Madagascar, Socotra, and the Seychelles Archipelago. This ancient continental landmass that was part of Gondwana has undergone numerous changes in climate, hydrology, and geology over millions of years, and the freshwater species have adapted over time as conditions have changed.

The widespread African freshwater crab genus *Potamonautes* MacLeay, 1838, includes over a hundred species (N. Cumberlidge, pers. comm.) and belongs to the family Potamonautidae which is endemic to the Afrotropical region (Cumberlidge and Daniels, 2007). While most potamonautid species are riverine, many have adapted to a variety of habitats including lakes, mountain streams, and other freshwater systems (Daniels et al., 2015). Freshwater crabs act as water quality indicators, because these species are unable to thrive in

suboptimal aquatic habitats (Cumberlidge et al., 2009). Afrotropical freshwater crabs are the largest inland aquatic invertebrates in their ecosystems and they play an important role in ecosystem functioning. They are preyed upon by larger species of mammals, birds, reptiles, and fish, making these crustaceans an essential component of the food web (Daniels and Bayliss, 2012). These species have economic significance, acting as a food source for thousands of people, as well as medical significance through their role in two parasitic diseases in humans (paragonimiasis and onchocerciasis). Despite recent advances in the field, the evolution, phylogeny and taxonomy of freshwater crabs still requires further research, and there are many gaps in our knowledge, one of which centers on Lake Malawi and its drainage basin (Sternberg et al., 1999; Daniels et al., 2015).

Lake Malawi is an East African Rift Valley lake which formed an estimated 8-4 million years ago. The shoreline of Lake Malawi lies in three countries: Malawi, Tanzania, and Mozambique (Kochey et al., 2017), but the largest area of the lake is in Malawi. In Malawi this lake is called Lake Malawi, while in Tanzania it is called Lake Nyasa, and in Mozambique it is called Lake Niassa. This lake is the third deepest and ninth largest lake in the world, and extends 570 km from north to south, 70–75 km from west to east, and has a maximum depth of about 700 m (Chidammodzi and Muhandiki, 2015). The deepest parts of the lake (greater than 250 m) become homothermal and oxygen levels become depleted (Eccles, 1974). There have been no reports of freshwater crabs being collected from depths exceeding 200 m, probably due to the anoxic conditions there.

The Lake Malawi basin receives rivers draining into it from the north in Tanzania (Ruhuhu and Songwe Rivers) from the west in Malawi (Songwe, N. Rukuru, S. Rukuru, Dwangwe, Bua, and Lilongwe Rivers), and there is a single outflow (the Shire River) from the

southernmost part of Lake Malawi (Sungani et al., 2016; Yihdego & Paffard, 2016). The Shire River system and the associated part of Lake Malawi serves as a crucial economic resource, with the lake being used for agricultural irrigation practices, hydroelectric energy generation, and recreation and fisheries (Chidammodzi and Muhandiki, 2015). Recent declines of the edible fish populations in Lake Malawi have been attributed to overfishing and the climate crisis, and if this situation persists it could leave the Malawi economy and its people vulnerable to food shortages (Limuwa et al., 2018). While the increased effects of industrialization and the climate crisis threaten the environmental integrity of the lake, additional sources of stress include deforestation, soil erosion, agricultural pollution, overfishing, poor land husbandry, and overharvesting of water for agriculture during dry seasons (Li et al., 2018).

Ancient lakes are typically associated with high levels of biological diversity, and this is true for the East African Rift Valley lakes (Daniels and Bayliss, 2012). Lake Tanganyika is the oldest and largest lake found in the East African Rift Valley, bordering Tanzania, Zambia, the Democratic Republic of the Congo, and Burundi. This lake is a globally recognized biodiversity hotspot, hosting roughly 1,500 species of animals and plants (Marijnissen et al., 2008) including nine closely related yet morphologically distinct endemic species of *Platythelphusa* and a single endemic species of *Potamonautes* (*P. platynotus*). Lake Kivu lies in two countries, Rwanda and the Democratic Republic of the Congo, and drains into Lake Tanganyika to the south via the Ruzizi River (Haberyan and Hecky, 1987; Cumberlidge and Meyer, 2011). Lake Kivu is home to four species of freshwater crabs, two of which are endemic to the lake (Cumberlidge and Meyer, 2011; Cumberlidge and Clark, 2018). Lake Malawi hosts more than 700 species of cichlid fish (most of which are endemic) but has only a single species of freshwater crab, *Potamonautes lirrangensis* (Rathbun, 1904), the Malawi Blue Crab, that is not endemic to the lake (Reed and

Cumberlidge, 2006). The lack of diversity shown by Lake Malawi's freshwater crab fauna might be attributed to a recent invasion of the lake by freshwater crabs, or it might be because the lake experienced extreme desiccation during the Pleistocene that wiped out the lake's crab populations (Kochey et al., 2017).

# CHAPTER ONE: *POTAMONAUTES LIRRANGENSIS* (RATHBUN, 1904) SENSU LATO

This species has a widespread distributional range from Central to Southern Africa (Fig. 1). The type specimen of *P. lirrangensis* is an adult female that was collected in the late 19th Century from Lirranga (now Liranga) at the junction of the Ubangi and Congo Rivers in the Democratic Republic of the Congo. Since then, specimens attributed to *P. lirrangensis* s. l. have been reported to occur in the Congo River at Kisangani, as well as in three Rift Valley Lakes (Kivu, Tanganyika, and Malawi) and in rivers close to Lake Tanganyika in Tanzania. Despite recent contributions (Bott, 1955; Cumberlidge, 1999; Reed and Cumberlidge, 2006) it is still difficult to distinguish between similar species of freshwater crabs, including species of *Potamonautes* Bott, 1970, and identification of unknown specimens to species requires identification keys that are not available for all of the Afrotropical freshwater crabs. This is the case for *P. lirrangensis*, which was described in 1895 from a single adult female from Liranga, D. R. Congo, and no additional material has been collected from there since. This means that the identification of all of the specimens subsequently included in this species from Kisangani, Tanzania, and from Lakes Kivu, Tanganyika, and Malawi have been based on a limited set of female morphological characters alone.

The taxonomic validity of this widely distributed species is the focus of the present work that compares morphological and molecular datasets from specimens collected from the entire range of *P. lirrangensis* s. l.. Distinguishing characters of this species based on the female type specimen include (i) a large pointed exorbital tooth, an extremely reduced granule-sized epibranchial tooth; (ii) a row of teeth or granules along the anterolateral margin of the carapace;

(iii) deep carapace grooves; (iv) a deep vertical sulcus on the ischium of the third maxilliped; (v) a complete and well defined sternal groove s3/s4; (vi) two large pointed teeth on the inner margin of the cheliped carpus, and a large pointed tooth on the inner margin of the cheliped merus.

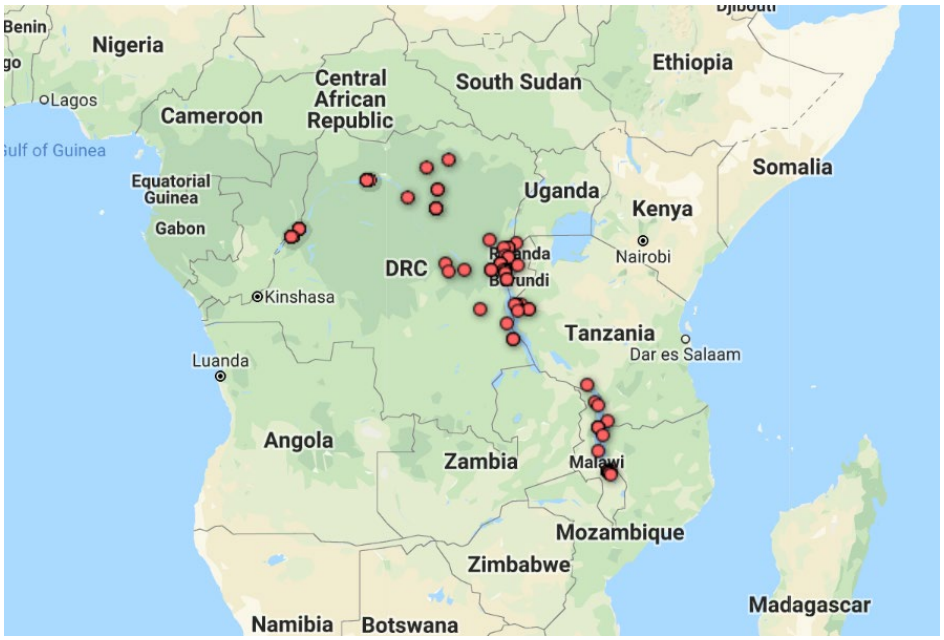



Figure 1. Map showing the distribution of *Potamonautes lirrangensis* (Rathbun, 1904) s. l.

The present study focused on morphological studies of all species found in Lake Malawi and its drainage basin. These were supplemented by molecular studies of all available species. Specimens of *P. lirrangensis* s. l. from four different areas within its range were examined here from Lake Malawi (Fig. 2), Liranga (Fig. 3), Lake Kivu (Fig. 4), and the Malagarasi River flowing into Lake Tanganyika (Fig. 5). Almost all available specimens of *P. lirrangensis* s. l. were obtained from most parts of its range from museum collections: the Natural History Museum, London, UK, the Genner Laboratory, University of Bristol, Bristol, UK, and Northern Michigan University, Marquette, MI. The aim here is to determine whether *P. lirrangensis* s. l. is

a species complex, and whether the Malawi Blue Crab from Lake Malawi actually belongs to *P. lirrangensis* or whether it belongs to a different species. Illustrations of the female type specimen from Liranga, and of other specimens from Kisangani, D. R. Congo identified as *P. lirrangensis* were used to define the characters of the specimens from these locations because I was unable to make first-hand examinations of specimens from these localities.

There have been only a few molecular studies of *P. lirrangensis* s. l. that involve specimens from Lake Malawi and from other localities in its drainage basin. Kochey et al. (2017) used mitochondrial DNA sequence markers to assess the genetic structure of the Malawi Blue Crab (which they identified as *P. lirrangensis* s. l.) at the population level within Lake Malawi by comparing samples from all parts of the lake. Those authors found strong support for there being only a single species of freshwater crab in Lake Malawi. Studies using specimens from elsewhere within the range of *P. lirrangensis* s. l. (Marijinssen et al., 2006) used 16S mitochondrial DNA data to examine the relationships of crabs from Lake Kivu, Lake Tanganyika, and Lake Malawi (that were all identified as *P. lirrangensis* s. l.) and found that the crabs from Lake Kivu belong to a separate lineage than the specimens from Lakes Malawi and Tanganyika.

## 1.1 MATERIALS AND METHODS

Measurements of carapace dimensions and walking leg lengths (p2-p5) were made using digital calipers and the values were recorded in mm (Appendices 2-3). Walking leg segments p2-p5 were measured along the midline of the merus, carpus, propodus and dactylus of p2, p3, p4 and p5. Gonopod measurements and photographs were taken using the Keyence VHX-5000 digital

microscope. Gonopod angles were calculated using the plane measurement tools of the Keyence VHX-5000. Post processing was done using Adobe Photoshop CC 2015. Species distribution maps were compiled using the programs GeoCAT and Google Fusion Tables. Localities were uploaded using comma delimited (.csv) files of previously identified specimens. Abbreviations used: cw, carapace width, distance measured across widest points of the carapace; cl, carapace length, distance spanning from anterior to posterior margin of the carapace; ch, carapace height, measures maximum height of cephalothorax; fw, front width, measures width of anterior margin between orbits; s, thoracic sternite; s3/s4, sternal sulci between adjacent thoracic sternites; p1-p5, periopods 1-5; G1, first gonopod; G2, second gonopod; coll., collected by; NMU, Northern Michigan University (Marquette, MI); NHM, Natural History Museum (London, UK); UB, University of Bristol (Bristol, UK).

### 1.1.2 Material Examined

***Potamonautes lirrangensis* s. l.**

**Malawi**. Lake Malawi, Cape Maclear, subadult male (cw 46.5 mm) (NHM 2010-06-CM-CM6, coll. M. Genner); Lake Malawi, Cape Maclear, subadult female (cw 50.15 mm), (NHM 2010-06-CM-CM8-CM8, coll. M. Genner); Lake Malawi, Cape Maclear, adult female (cw 52.2 mm), vi. 2010 (NHM CM13, coll. M. Genner); Cape Maclear, adult female (cw 54.4 mm), (NHM CM14, coll. M. Genner); Lake Malawi, Cape Maclear, subadult female (cw 44.1 mm), vi. 2010 (NHM CM21, coll. M. Genner); Lake Malawi, northwest coast near Nkhata Bay, 4 adult females (cw 50.9 mm with hatchlings, 57.8 mm) (NHM, coll. S. Chapman); Lake Malawi, northwest coast near Nkhata Bay, adult male (cw 54.4 mm), adult female (cw 51 mm), subadult female (cw 43.9 mm), 2 subadult males (cw 45.1, 47.9 mm) (NHM, coll. S. Chapman); Lake Malawi, northwest

coast near Nkhata Bay, adult female (cw 54.8 mm) 1961 (NHM 2011.1509, coll. Sweeney); Lake Malawi, northwest coast near Nkhata Bay, subadult male (cw 49 mm), adult female with hatchlings (cw 64.8 mm) (NHM 1954.7.26.5-6, coll. Miers); Lake Malawi, adult female (cw 61.1 mm), adult male (cw 55.4 mm), (NHM 1956.6.5.10-11, coll. G. Fryer); Lake Malawi, adult female (cw 57 mm), subadult male (cw 40 mm), 2 juveniles (cws 32.5, 33.4 mm) (NHM 1954.7.26.3-4, coll. Cunnington); Lake Malawi, north of Hudzi, 2 adult females (cws 61.1, 65.8 mm), 2 subadult females (cws 36.7, 45.1 mm), subadult male (cw 36.6 mm), 9 juveniles (NHM 1926.10.20.1-5, coll. Cristy). Lake Malawi, west coast between Ukala Bay and Ruarue, adult female (cw 52.8 mm), vi. 1896 (NHM 1908.1.31.28, coll. A. Whyte); Lake Malawi, northwest coast near Nkhata Bay, subadult male (cw 31.1 mm) (NHM 1908.1.31.16-18); Lake Malawi, Tanganyika Exp. 1896, adult male (cw 55.5 mm) (NHM 1908.1.31.27, coll. J.E.S. Moore); Lake Malawi, west coast between Ukala Bay and Ruarue, adult female (cw 53 mm), vi. 1896 (NHM 1908.1.31.28, coll. A. Whyte); Lake Malawi, Likoma, subadult male (cw 27.5 mm), 1893 (NHM 93.1.14.1, coll. J.A. Williams Esq.); Lake Malawi, 3 juvenile males (cw 11.7 to 17.9 mm), xii. 1891 (NHM 1891.12.19.1-3); Lake Malawi, Monkey Bay, adult female (cw 55-60mm) (NHM 1926.10.20.6); Lake Malawi, Cape Maclear, subadult female (cw 32.7 mm), 17.vi. 2010 (University of Bristol (UB) CM17, coll. M. Genner); Lake Malawi, Cape Maclear, subadult female (cw 37.4 mm), adult female (cw 57.6 mm), 2 adult males (cws 55.1, 54.9 mm), vi. 2010 (UB CM22, CM11, CM20, CM12, coll. M. Genner); Lake Malawi, Cape Maclear, adult female (cw 57.1 mm), subadult female (cw 33.1 mm), 26. vi. 2010 (UB CM10, CM5, coll. M. Genner); Lake Malawi, Cape Maclear, su adult male (cw 49.2 mm), vi. 2010 (UB CM4, 21/6/10, coll. M. Genner); Lake Malawi, Cape Maclear, subadult female (cw 48.3 mm) (UB CM9, coll. M. Genner); Lake Malawi, Cape Maclear, subadult female (cw 46.7 mm) (UB CM15, coll. M.

Genner); Lake Malawi, Cape Maclear, subadult female (cw 35.4 mm) (UB CM16, coll. M. Genner); Lake Malawi, Cape Maclear, subadult female (cw 45.4 mm) (UB CM24, coll. M. Genner); Lake Malawi, Cape Maclear, adult male (cw 59.4 mm) (UB CM7, coll. M. Genner); 2 adult females (cws 55.4, 54.4 mm), 2 subadult females (cws 44.9, 45.9 mm) (coll. M. Genner); Lake Malawi, Monkey Bay among rocks in sand, subadult male (cw 51.1 mm), iii. 1968, (NMU TRW 1972.04, coll. D.H. Eccles); Lake Malawi, 7.2 kilometers east northeast of Monkey Bay, Lake Malawi, subadult male (cw 46.2 mm), v. 1968 (DH Eccles) (NMU TRW 1972.05, coll. D.H. Eccles); Lake Malawi, subadult female (cw 51.1 mm) (NMU 09.1988k.1, coll. Irv. Cornfield)**. D. R. Congo**. Lake Tanganyika, Kalemie (formerly Albertville), adult female (cw 62 mm) (NHM 1919.3.8.1-3, coll. M. Dhont de Bie); Lake Kivu, D. R. Congo, subadult male (cw 46.5 mm), (172 Sci. Nat. Brussels); Lake Kivu 23, large cote, Goma base, subadult male (cw 44.5 mm) (NHM 30.xi.52, coll. I. Gordon). Kisangani, subadult male (cw 47.2 mm) (NHM 1955.6.22.65, coll. G. Browne). **Tanzania.** Mungonya River, W Kigoma, Taveta, subadult male (cw 39.5 mm), iv. 1971 (NMU TRW 1971.05, coll. T.R. Williams); Malagarazi River, Uvinza, Kigoma area, adult female (cw 80.1 mm), iv. 1971 (NMU TRW 1971.15, coll. T.R. Williams).

## 1.2 RESULTS

### *Morphological Analysis*

The results of the comparative study of diagnostic characters of specimens of *P. lirrangensis* s. l. (based on the female type from Liranga, D. R. Congo) from different localities that cover a majority of the range of this species are shown in Table 1. All individuals identified as *P.*

*lirrangensis* s. l. share the following characters: a complete postfrontal crest, a large pointed exorbital tooth, a small, low epibranchial tooth, two pointed spines on the inner margin of the cheliped carpus, and a large pointed spine on the inner margin of the chelied merus, an arched dactylus of the major cheliped, a granulated suborbital region of the carapace sidewall, and a G1 terminal article angled outwards between 47° and 52° to the longitudinal axis of the gonopod.

On the other hand, a number of differences were found between the different subpopulations of *P. lirrangensis* s. l.. For example, the Malawi Blue Crab from Lake Malawi (Fig. 6) has a distinctly toothed anterolateral margin of the carapace, walking leg p5 has distinctly elongated segments, its s3/s4 sternal groove is complete and deep throughout, and it has a deep vertical sulcus on the ischium of the third maxilliped. In contrast, the anterolateral margin of the carapace of specimens from the D.R. Congo, Lake Kivu (Figs. 7–8), and Tanzania (Fig. 9) is lined by granules rather than distinct teeth, and the s3/s4 groove and the vertical groove on the third maxilliped ischium are both faint and shallow.

Table 1. Summary of the morphological characters observed in *P. lirrangensis* s. l. from the D.R. Congo, Lake Malawi, Lake Kivu, and Tanzania. * = shared character; ? = data deficient; Dark shaded cells represent characters found in Malawi Blue Crab; Light shaded cells represent characters shared by Kigoma, Malagarasi River, Tanzania and Lake Kivu *Potamonautes* sp. 1.

| Morphological Character | Liranga, DRC | Kisangani, DRC | | Lake Tanganyika, Zambia | Lake Malawi, Malawi | | Lake Kivu, DRC | Kigoma, Malagarasi River Tanzania | |
|---|---|---|---|---|---|---|---|---|---|
| *Post-frontal Crest Complete* | * | * | | ? | * | | * | * | |
| *Exorbital tooth large pointed* | * | * | | ? | * | | * | * | |
| *Epibranchial tooth low* | * | * | | ? | * | | * | * | |
| *Carapace posterior region with deep grooves* | * | * | | ? | * | | * | * | |
| *Cheliped merus distal tooth spine-like, pointed* | * | * | | ? | * | | * | * | |
| *Cheliped carpus inner margin with two large subequal spine-like, pointed teeth* | * | * | | ? | * | | * | * | |
| *Dactylus of major cheliped arched* | * | * | | ? | * | | * | * | |
| *Branchiostegal suborbital region granulated* | * | * | | ? | * | | * | * | |
| *G1 terminal article angle 47-52 degrees* | ? | ? | | ? | * | | * | * | |
| | | | | | | | | | |
| *Carpus, propodus, dactylus of pereiopod 5 all elongated, slender* | not elongated | not elongated | | ? | * | | not elongated | not elongated | |
| *s3/s4 groove complete and deep* | ? | incomplete | | ? | * | | faint, shallow | faint, shallow | |
| *Ischium of third maxilliped with deep vertical sulcus* | smooth, lacking sulcus | smooth, lacking sulcus | | ? | * | | smooth, lacking sulcus | faint, shallow | |
| *G1 terminal article widened by large rounded lobe* | ? | * | | ? | * | | widened but no lobe | widened but no lobe | |
| *G1 terminal article tip upcurved* | ? | ? | | ? | * | | no | no | |
| *Anterolateral margin between exorbital and epibranchial teeth lined by small distinct teeth* | * | * | | ? | * | | lined by granules | lined by granules | |
| *Anterolateral margin lined by small distinct teeth* | * | lined by granules | | ? | * | | lined by granules | lined by granules | |
| *Carapace colour when alive red/brown* | Dark greenish blue/ brownish blue | * | | ? | Dark Blue | | ? | ? | |
| *DNA Sequence Data Available* | No | No | | Yes | Yes | | Yes | No | |
| *Carapace height/ Front width (ch/fw)* | ? | 1.05 | | ? | 1.0 | | 1.0 | 1.0 | |
| *Carapace length / Front width (cl/fw)* | 3.2 | 2.4 | | ? | 2.5 | | 2.4 | 2.4 | |
| *Carapace width/ Front width (cw/fw)* | 4.9 | 3.1 | | ? | 3.5 | | 3.1 | 2.9 | |

*The Malawi Blue Crab from Lake Malawi*

The postfrontal crest is complete, the anterolateral margin of the carapace is heavily and distinctly toothed, the posterior region of the carapace has deep grooves, the epibranchial tooth is low and granule-like, the exorbital tooth is large and pointed, the anterolateral margin between the exorbital and epibranchial teeth is lined by small distinct teeth, and the suborbital region of the carapace sidewall is granulated. The carapace height is equal to that of the front width (ch/fw

1.0), the carapace length is 2.5 times that of the front width (cl/fw 2.5), and the carapace width is 3.5 times that of the front width (cw/fw 3.5). Sternal sulcus s3/s4 is complete and deep, and there is a deep vertical sulcus on the ischium of the third maxilliped. The distal tooth on the inner margin of the cheliped merus is pointed and spine-like, and the inner margin of the carpus of the cheliped has two large spines. The dactylus of the cheliped of adults is pigmented (black). The carpus, propodus, and dactylus of p5 are all slender and elongated. The G1 terminal article has a large rounded lobe on the medial margin, ending in a thin upturned tip, and there are setae on the margins of the G1 terminal article and sub-terminal segment.

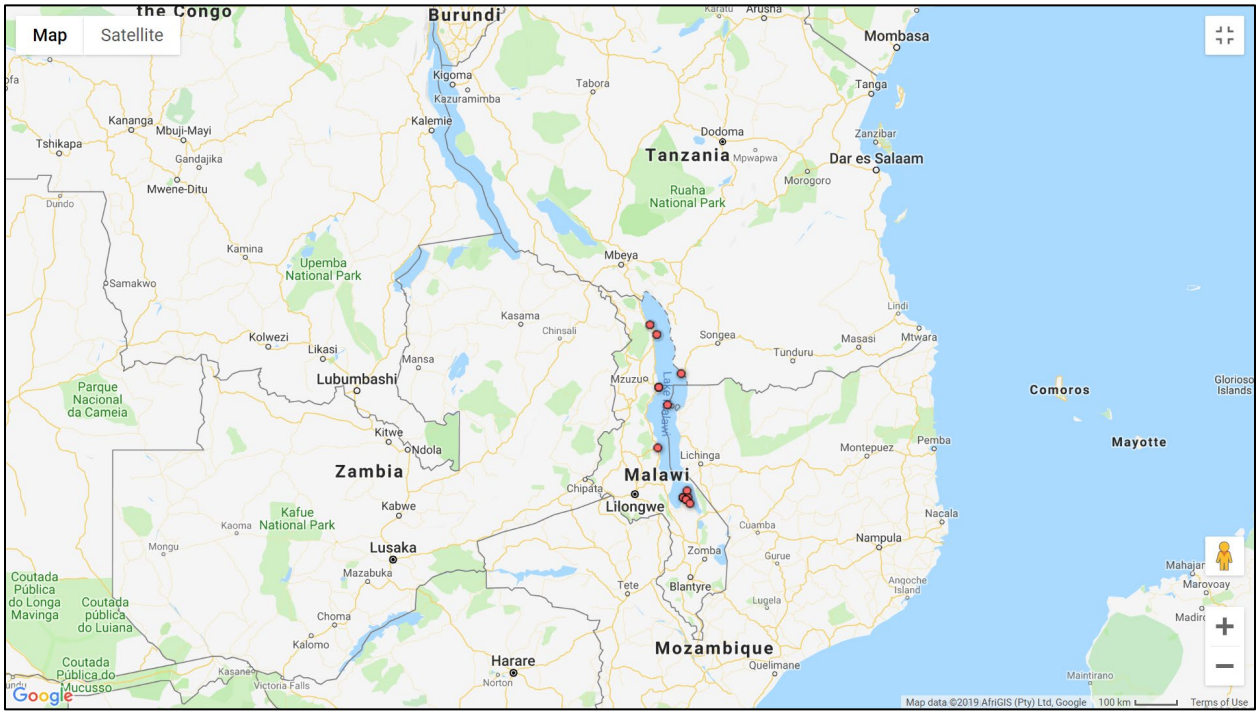



Figure 2. Map showing the distribution of the Malawi Blue Crab in Lake Malawi.

*Potamonautes lirrangensis* s. l. from Kisangani, D. R. Congo:

The postfrontal crest is complete, the exorbital tooth is large and pointed, the epibranchial tooth is low, the anterolateral margin of the carapace is toothed, and the anterolateral margin between

the exorbital and epibranchial teeth is lined by small teeth. The posterior region of the carapace has deep grooves. The distal tooth on the inner margin of the cheliped merus is pointed and spine-like, and the inner margin of the cheliped carpus has two large spines. The dactylus of the cheliped is distinctly arched. The suborbital region of the branchiostegal sidewall of the carapace is granulated. Sternal sulcus s3/s4 is faint or absent. The ischium of the third maxilliped is smooth, lacking a vertical sulcus. The G1 terminal article has a large rounded lobe on the medial margin. The carapace height is equal to the front width (ch/fw 1.0); the carapace length is about twice that of the front width (cl/fw 2.4); the carapace width is about three times the front width (cw/fw 3.1). Rathbun (1921) reported that these specimens identified as *P. lirrangensis* from Kisangani, D. R. Congo, have deep carapace sutures, and a distinctly outlined anterior mesogastric region with cervical grooves fading anteriorly; an exorbital tooth outer margin with denticulation; a small, low and indistinct epibranchial tooth; an anterolateral margin lined with sharp, acorn-shaped teeth; two sharp, large spines on the inner margin of the cheliped carpus (the posterior is smaller than the anterior spine); fingers of the chelipeds are pigmented (the color persisting in alcohol); and the cheliped fingers (dactylus and propodus) both have an irregular toothing pattern, generally alternating large and small.

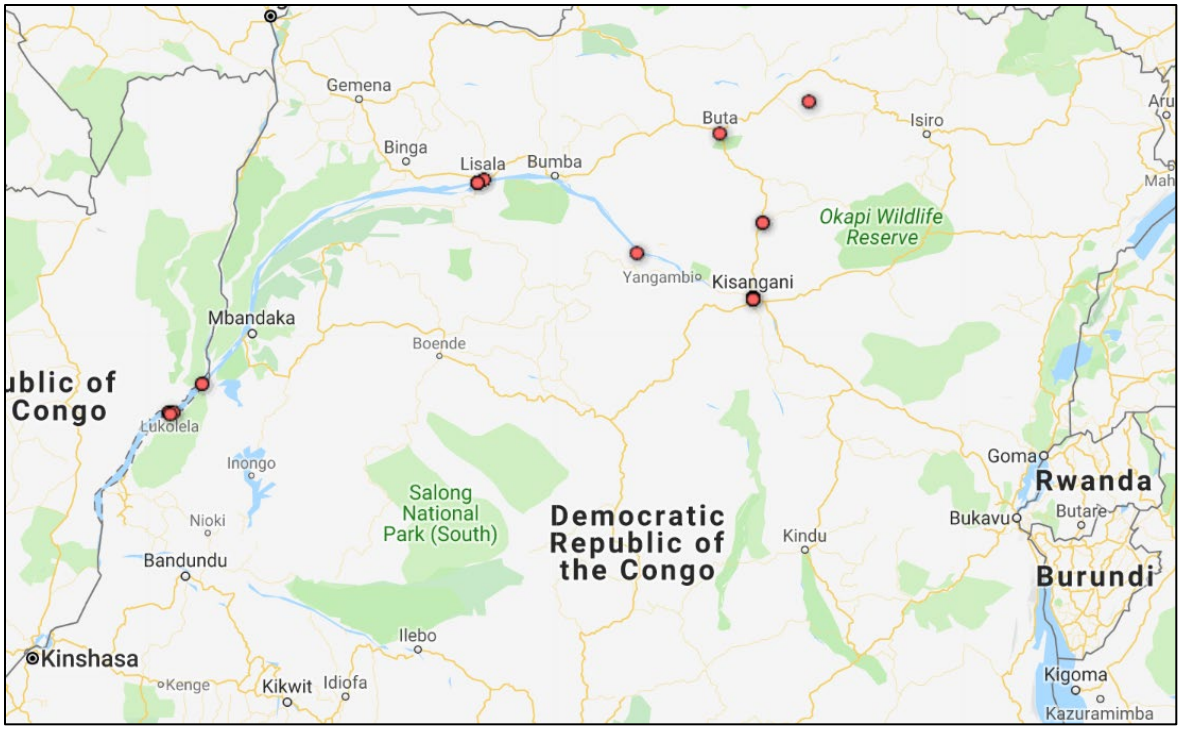



Figure 3. Map showing the distribution of *P. lirrangensis* s. s. in the Democratic Republic of the Congo.

*Potamonautes lirrangensis* s. l. from Lake Kivu

The postfrontal crest is complete, the exorbital tooth is large and pointed, the epibranchial tooth is low, the posterior region of the carapace has deep grooves, the anterolateral margin is lined with small teeth or granules, and the anterolateral margin between the exorbital and epibranchial teeth is lined by granules. The branchiostegal suborbital region is granulated. The height of the carapace is equal to the front width (ch/fw 1.0), the carapace length is approximately twice the front width (cl/fw 2.4), the carapace width is three times the front width (cw/fw 3.1), and the vertical sulcus on the ischium of the third maxilliped is faint or absent. The sternal groove s3/4 is faint and shallow, and the episternal grooves are all faint. The dactylus of the major cheliped is slightly arched, leaving a small interspace between the fingers (propodus and dactylus) when closed. The distal tooth on the inner margin of the cheliped merus is pointed and spine-like, the

inner margin of the cheliped carpus has two large spines, and the cheliped dactylus is arched. The carpus, propodus, and dactylus of p5 are not elongated. There are setae present on the margins of the G1 terminal article and sub-terminal segment, and although the G1 terminal article is widened it lacks a large lobe, and the tip is straight and does not curve upward.


Figure 4. Map showing the distribution of specimens initially identified as *P. lirrangensis* s. l. from Lake Kivu (D. R. Congo and Rwanda).

*Potamonautes lirrangensis* s. l. from Kigoma, Tanzania

The postfrontal crest is complete, the exorbital tooth is large and pointed, the epibranchial tooth is low, the posterior region of the carapace has deep grooves; the anterolateral margin is lined with small teeth or granules, and the anterolateral margin between the exorbital and epibranchial teeth is lined by granules. The branchiostegal suborbital region is granulated. The height of the carapace is equal to the front width (ch/fw 1.0), the carapace length is approximately twice the front width (cl/fw 2.4), the carapace width is three times the front width (cw/fw 3.1), and the

vertical sulcus on the ischium of the third maxilliped is faint or absent. The sternal groove s3/4 is faint and shallow, and the episternal grooves are all faint. The dactylus of the major cheliped is slightly arched, leaving a small interspace between the fingers (propodus and dactylus) when closed. The distal tooth on the inner margin of the cheliped merus is pointed and spine-like, the inner margin of the cheliped carpus has two large spines, and the cheliped dactylus is arched. The carpus, propodus, and dactylus of p5 are not elongated. There are setae present on the margins of the G1 terminal article and sub-terminal segment, and although the G1 terminal article is widened it lacks a large lobe, and the tip is straight and does not curve upward.


Figure 5. Map showing the distribution of specimens initially identified as *P. lirrangensis* s. l. from Tanzania.

## 1.3 DISCUSSION

### *Morphological Analysis*

The morphological results raise questions about the monophyly of *P. lirrangensis* s. l. across the range of this species, because there are significant morphological and molecular differences between the populations from the D. R. Congo and the three Rift Valley lakes (Kivu, Tanganyika, and Malawi). Despite the progress reported on here, there remains a general lack of adult male representatives of the two populations from the Congo River. The evidence indicates that *P. lirrangensis* s. l. is actually comprised of more than one closely-related species, one of which (*P. orbitospinus*) is restricted to Lake Malawi, and a locality in the southern part of Lake Tanganyika. A second former population of *P. lirrangensis* s. l. from Lake Kivu is probably a new species in the light of the morphological and molecular differences outlined here. Specifically, (1) the populations from Lake Malawi and Lake Tanganyika previously identified as *P. lirrangensis* s. l. do not belong to the same species as the type of *P. lirrangensis* from Liranga, D. R. Congo, and should properly be called *P. orbitospinus* (Cunnington, 1907), which is an available name. (2) The populations of *P. lirrangensis* from Lake Kivu do not belong to the same species as either the type of *P. lirrangensis* from Liranga, D. R. Congo, or to the species from Lakes Malawi and Tanganyika (here called *P. orbitospinus*), and should be treated as a new species.

*Potamonautes lirrangensis* s. s. from the Middle Congo, and *P. orbitospinus* from Lake Malawi, share a number of characters including a complete and distinct postfrontal crest, a spine-like exorbital tooth, a deeply grooved carapace, two sharp spines on the cheliped carpus inner margin, and a sharp spine on the inner margin of the cheliped merus. This set of characters has

been used by a number of authors to assign specimens from different parts of Central and Southern Africa to *P. lirrangensis*, despite differences in the male diagnostic characters of the chelipeds, gonopods, and sternum illustrated here (Table 1) (Balss, 1929; Rathbun, 1921; Bott, 1955; Reed and Cuumberlidge, 2006; Meyer and Cumberlidge, 2009). The detailed comparative morphological study carried out here comparing adult males from Lakes Kivu and Lake Malawi (and photographs of adult males from Kisangani) identified differences in walking leg length, sternal grooves, carapace dentition, and gonopod one that had previously not been used when assigning individuals to this species. For example, the Malawi Blue Crab from Lake Malawi (here referred to *P. orbitospinus*) has distinctly elongated p5 segments when compared to the other populations in this study. The fifth walking leg (p5) is typically the shortest of the walking legs (p2-p5) in river living species (Cumberlidge, 1999), suggesting that this elongation of the periopods may be an adaptation to life in the deeper waters of Lake Malawi.

*Potamonautes lirrangensis* s. s. from the D.R. Congo differs in some respects from *P. orbitospinus* from Lake Malawi. For example, in *P. lirrangensis* s. s. from Liranga, the vertical sulcus on the ischium of the third maxilliped is absent (vs a vertical sulcus on the ischium of the third maxilliped in *P. orbitospinus*). In addition, the anterolateral margin of the carapace of *P. lirrangensis* s. s. from Liranga is lined by large granules, whereas that of *P. orbitospinus* is lined by distinct teeth.

The anterolateral margin of the carapace of the specimens from Lake Kivu is lined by large granules, whereas that of *P. orbitospinus* is lined by distinct teeth. The s3/s4 sternal groove and the vertical groove on the third maxilliped ischium of the specimens from Lake Kivu and Kigoma are both faint and shallow, whereas in *P. orbitospinus* both of these grooves are deep. The terminal article of gonopod 1 of the specimens from Lake Kivu is slightly widened (but

lacks a rounded lobe) and ends in a straight tip, whereas that of *P. orbitospinus* is clearly widened by a distinct rounded large lobe and ends in a distinct upwardly-curved tip. Measurements from illustrations of the female type *P. lirrangensis* from Liranga and adult males from Kisangani from the literature (Rathbun, 1904, 1921) indicate that the female type of has a carapace width nearly 5 times that of the front width (cw/fw 4.9), which is the greatest difference found in carapace proportions among all of the populations of this species (but this may be a measuring error arising from distortions in the photograph provided by Rathbun, 1904). The carapace height of the specimens from Lake Kivu, Tanzania (Kigoma and Malagarasi), and Kisangani, D. R. Congo (Plate XXV, Rathbun, 1921) was equal to the front width (ch/fw 1.0), the carapace length was over 2 times the front width (cl/fw 2.4), and the carapace width was 3 times that of the front width (cw/fw 3.0). Similar proportions were found for the male Kisangani, D. R. Congo (but these were based on measurements of the photos provided by Rathbun, 1921)).

The lack of male characters for *P. lirrangensis* s. s. from Liranga, D. R. Congo makes it impossible to compare it to the adult male crabs from Lake Malawi (treated here as *P. orbitospinus*), or to adult males from Lake Kivu, and Kigoma, Tanzania. The fact that the type of *P. lirrangensis* is a dried specimen over 100 years old means that molecular data are also not available. Nevertheless, it is clear that the specimens from Lake Malawi are morphologically different from the available specimens from Lake Kivu and the Congo. The specimens from Lake Malawi (treated here as *P. orbitospinus*) and from Lake Kivu (here treated as *Potamonautes* sp. 1) are also morphologically and genetically different from each other and represent valid taxa in their own right when male characters such as gonopods, chelipeds, and sternal grooves are compared.

*Molecular Evidence*

Previous works focusing on phylogenetic analyses of species of *Potamonautes* have used a single representative of the widespread *P. lirrangensis* s. l.. For example, Daniels et al. (2015) used a specimen of *P. lirrangensis* from Lake Kivu (ZMA Crust. De. 204681) when constructing a maximum likelihood phylogeny for the Afrotropical freshwater crab fauna based on mitochondrial 12S rRNA, 16S rRNA, cytochrome c oxidase subunit I (COI), and nuclear histone 3 markers. Their tree topology placed the specimens from Lake Kivu then identified as *P. lirrangensis* s. l. as sister to *P. suprasulcatus* from Tanzania. Daniels and Klaus (2018) used COI, 16S rRNA and histone 3 in their analysis of two distantly-related endemic species of *Potamonautes* from Sao Tome and Principe. In that study, 60 species of *Potamonautes* were sequenced, and they included the same '*P. lirrangensis'* specimen from Lake Kivu which was used in the Daniels et al. (2015) tree. Their results also showed that this specimen from Lake Kivu was a sister species to *P. suprasulcatus*, and that these taxa were in turn part of a larger clade that included species from the Rift Valley which they called '*Potamonautes* Clade A'.

Kochey et al. (2017) conducted a molecular study of the Malawi Blue Crab based on specimens then identified as *P. lirrangensis* s. l. from all parts of Lake Malawi to test for diversification of this species within the lake. Those authors found low levels of nucleotide diversity for mitochondrial loci NADH dehydrogenase subunit 1 (ND1) and cytochrome b (CtyB), suggesting a recent and single colonization event of the lake, and supporting the idea that there is only a single species of freshwater crab inhabiting Lake Malawi. Marijnissen et al. (2006) used mitochondrial 12S rRNA and 16S rRNA markers from three specimens of *P. lirrangensis* from three African lakes: Lakes Malawi, Kivu, and Tanganyika (Zambia) to study relationships of East African Rift Valley freshwater crabs. That study showed that the specimens

from Lakes Malawi and Tanganyika were sister taxa in the same clade, and that the specimen from Lake Kivu belonged to a separate lineage, with branch lengths indicating that the Lake Kivu specimen represents the most basal lineage of the three (Fig. 1).


Figure 6. Malawi Blue Crab (*Potamonautes orbitospinus*) from Lake Malawi, Malawi (NHM 1908.1.31.27, cw 55.6 mm, cl 39.5 mm, ch 16.2 mm, fw 15.7 mm). Frontal view (top), dorsal and ventral views of carapace, minor and major chelipeds, G1 (left) ventral and dorsal views at x20 magnification, G1 dorsal at x50 magnification, G2 (bottom right corner) at x20 magnification.

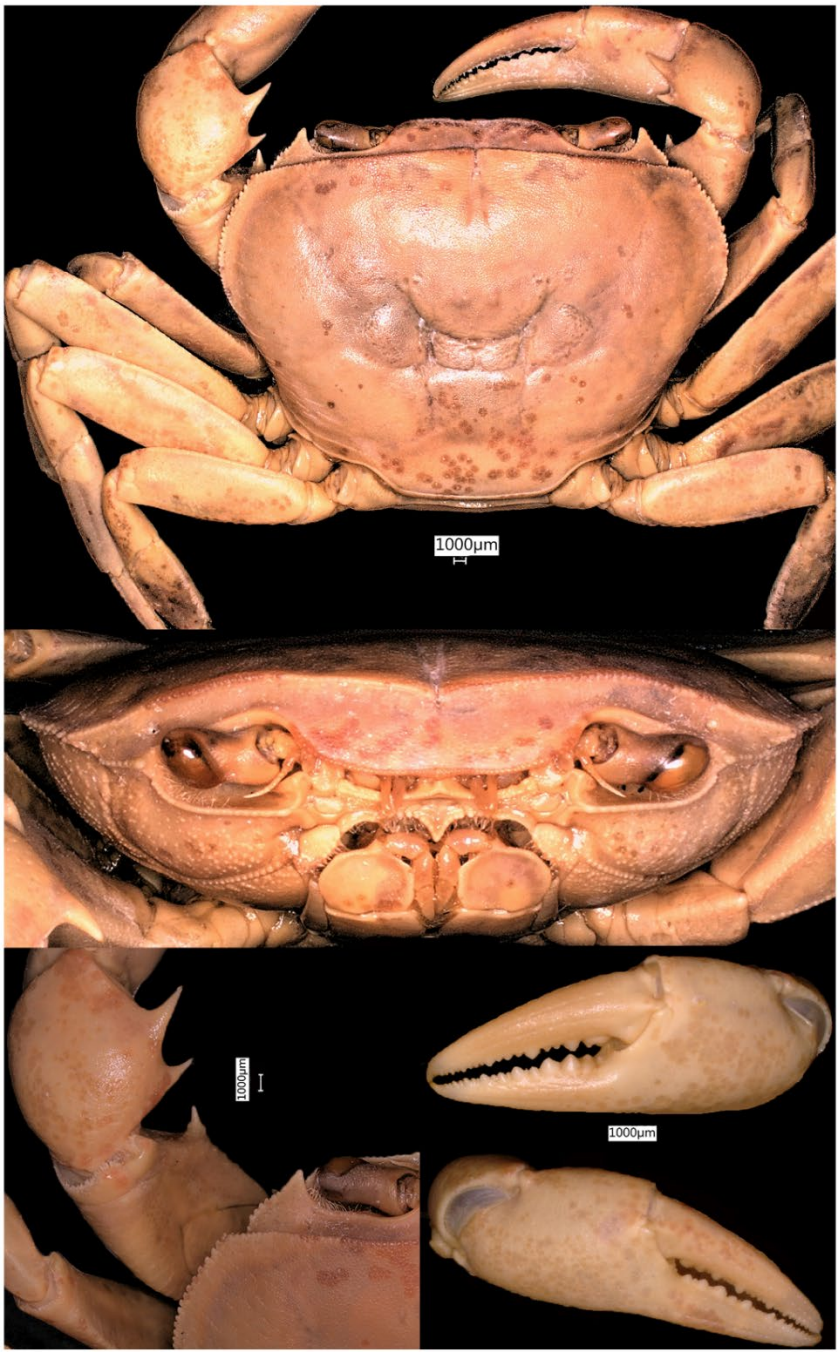



Figure 7. *Potamonautes lirrangensis* s. l. from Lake Kivu, D. R. Congo (NHM 2018.306, cw 46.5 mm, cl 36.9 mm, ch 15.1 mm, fw 15.2 mm). Dorsal view of carapace at x5 magnification (top), front view at x5 magnification, p1 carpus and merus spines, major and minor chelipeds at x5 magnification.


Figure 8. *Potamonautes lirrangensis* s. l. from Lake Kivu, D. R. Congo (NHM 2018.306, cw 46.5 mm). Ventral view (top), G1 and G2 view at x20 magnification, dorsal view at x5 magnification, G1 (right) ventral view at x20 magnification, G1 dorsal at x30 magnification, G2 at x20 magnification, G1 ventral at x50 magnification.

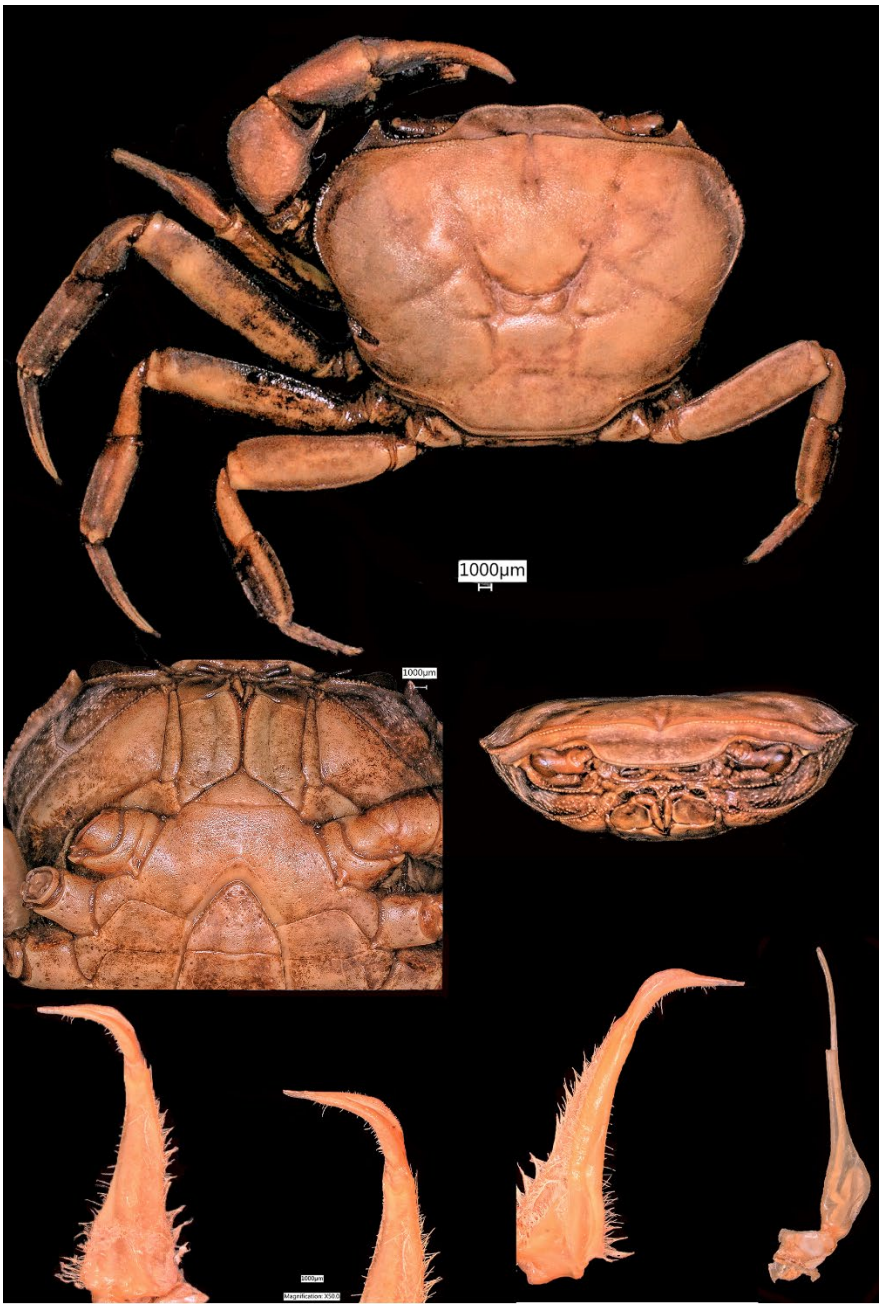



Figure 9. *Potamonautes lirrangensis* s. l. from Kigoma, Tanzania (NMU TRW 1971.05, cw 39.5 mm, cl 31.8 mm, ch 13 mm, fw 13.5 mm). Dorsal view of carapace at x5 magnification, ventral and frontal views at x10 magnification, G1 (right) dorsal view at x30 and x50 magnification, G1 ventral at x30 magnification, G2 at x30 magnification.

# CHAPTER TWO: *POTAMONAUTES MONTIVAGUS* (CHACE, 1953)

*Potamonautes montivagus* is a primarily riverine species found in the Lake Malawi basin. Newly collected specimens included in the present work have expanded the distributional range of this species to include a series of small crater lakes in southwestern Tanzania in the Lake Malawi drainage basin. An unpublished phylogeny (Fig. 10) using mitochondrial 16S rRNA, 28S rRNA, and nuclear H3 markers that included the new collections indicates that the relationships between the widespread species *P. lirrangensis* s. l. and *P. montivagus* show considerable diversification within each of these taxa. Figure 10 shows the preliminary molecular phylogeny with these species labeled by different colors (light = *P. montivagus*; dark = *P. lirrangensis* s. l.) based on the initial morphological identification of the samples. The tree groups populations of *P. lirrangensis* from Lake Malawi in their own clade, as sister to a complex lineage that includes specimens of *P. montivagus* from Tanzania, Zambia, and Malawi. The preliminary morphological identifications of the specimens were tentative because they were based on juvenile specimens which lack the well-developed characters of adult males necessary for species identification of African freshwater crabs (Cumberlidge, 1999). This preliminary phylogeny posed the question of whether specimens identified as *P. lirrangensis* s. l. and *P. montivagus* represent different morphs of the same species, or whether these two taxa include a great deal more diversity than previously thought.

In the present study, a new phylogeny was constructed based on all available 16S rRNA sequence data combining original sequences with sequences that are published from GenBank.

The tree was constructed using MrBayes with an ingroup consisting of *P. lirrangensis* s. l. from Lakes Kivu, Tanganyika, and Malawi, and specimens of *P. montivagus* from Malawi, Mozambique, and Lakes Itamba and Kyungululu in Tanzania. The sampling for the phylogeny was broadened to include other species of *Potamonautes* found in the Lake Malawi drainage basin, as well as a species of *Platythelphusa* endemic to nearby Lake Tanganyika (Table 2). Sequences were acquired from GenBank and from Dr. Martin Genner at the University of Bristol (Table 3).

Table 2. Summary of potamonautid species found in the Lake Malawi basin along with taxonomic authority and type locality of each.

| SPECIES NAME | TAXONOMIC AUTHORITY | TYPE LOCALITY |
|---|---|---|
| *Potamonautes choloensis* | (Chace, 1953) | Cholo Mountain, Malawi |
| *Potamonautes montivagus* | (Chace, 1953) | Cholo Mountain, Malawi |
| *Potamonautes obesus* | (H. Milne Edwards, 1868) | Zanzibar, Tanzania |
| *Potamonautes bayonianus* | (Brito Capello, 1864) | Mozambique |
| *Potamonautes lirrangensis* | (Rathbun, 1904) | Liranga, D.R. Congo |
| *Potamonautes bellarussus* | Daniels, Phiri, & Bayliss, 2014 | Yao Mountain, Mozambique |
| *Potamonautes mulanjeensis* | Daniels & Bayliss, 2012 | Mount Mulanje, Malawi |
| *Potamonautes suprasulcatus* | (Hilgendorf, 1898) | Mrogoro, Tanzania |

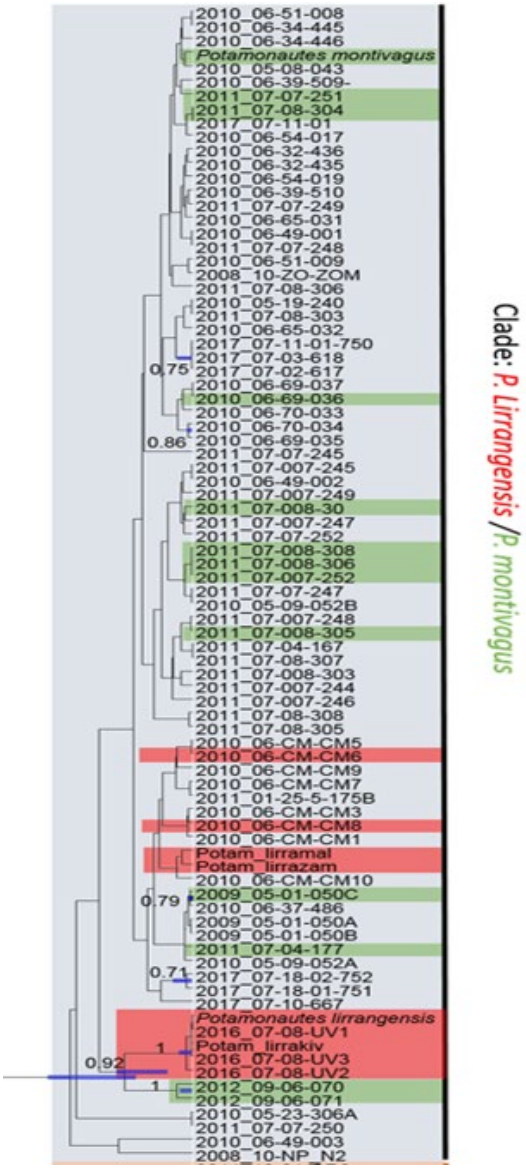



Figure 10. Preliminary phylogeny showing specimens initially identified as *P. montivagus* and *P. lirrangensis*. Phylogeny constructed using 16S, H3, and 28S markers. Specimens identified as *P. montivagus* in green, and *P. lirrangensis* in red (Martin Genner, pers. comm.). Bootstrap support values > 0.7 shown on nodes.

Table 3. Summary of species with localities, reference material, and GenBank accession numbers associated with 16S rRNA Bayesian phylogeny. The table includes a list of ingroup species of *Potamonautes* with outgroup species of *Liberonautes* and *Sudanonautes*. A species of *Platythelphusa* endemic to Lake Tanganyika was also included. Additional unpublished 16S rRNA mtDNA sequences of specimens were obtained through M. Genner at University of Bristol (Bristol, UK). *Potamonautes bellarussus* was collected in the Nyika National Park, Malawi (-10.59583333, 33.80583333); *P. choloensis* was collected from Mulanje, Malawi (-16.01583333, 35.495); *P. obesus* was collected from Chiendausiku, Malawi (-15.02063889, 35.10230556); *P. montivagus* was collected from Lake Itamba, Tanzania (-9.352222222, 33.84266667), Lake Kyungululu, Tanzania (-9.308, 33.86547222), and Zomba, Malawi (-15.38333333, 35.33333333); *P. lirrangensis* was collected from Cape Maclear, Lake Malawi, Malawi (-14.02290833, 34.84305556).

| **Species** | **Locality** | **Reference material** | **GenBank accession number** |
|---|---|---|---|
| ***Liberonautes latidactylus*** | Liberia | Daniels et al., 2015 | KP640439 |
| ***Sudanonautes floweri*** | Gabon | Daniels et al., 2015 | AY803541 |
| ***Platythelphusa armata*** | Lake Tanganyika | Marijnissen et al., 2006 | DQ203213 |
| ***Potamonautes bayonianus*** | Botswana | Daniels & Bayliss, 2012 | AY042243 |
| ***Potamonautes mulanjeensis*** | Malawi | Daniels et al., 2015 | JF799139 |
| ***Potamonautes lirrangensis* s. l.** | Lake Malawi | Marijnissen et al., 2006 | DQ203235 |
| ***Potamonautes lirrangensis* s. l.** | Lake Tanganyika | Marijnissen et al., 2006 | DQ203237 |
| ***Potamonautes lirrangensis* s. l.** | Lake Kivu | Marijnissen et al., 2006 | DQ203236 |
| ***Potamonautes lirrangensis* s. l.** | Lake Kivu | Daniels et al., 2015 | AY803534 |
| ***Potamonautes montivagus*** | Mozambique | Daniels et al., 2015 | KP640448 |
| ***Potamonautes bellarussus*** | Malawi | M. Genner, pers. comm. | Unaccessioned |
| ***Potamonautes choloensis*** | Malawi | M. Genner, pers. comm. | Unaccessioned |
| ***Potamonautes obesus*** | Malawi | M. Genner, pers. comm. | Unaccessioned |

## 2.1 MATERIALS AND METHODS

*Phylogeny in MrBayes.*

16S rRNA mtDNA sequences were aligned using Muscle in MEGA 7.0.26 (Kumar et al., 2015). Alignments were exported in NEXUS format for editing and executed in MrBayes Version 3 (Ronquist and Huilsenbeck, 2003) to build the phylogeny of the freshwater crabs of the Lake Malawi basin. MrBayes enforces Markov Chain Monte Carlo (MCMC) to compare models and to calculate posterior probabilities for the topologies (Metropolis et al., 1953; Hastings, 1970). The program was run twice for 10 million generations with a burn-in of one million samples, and a consensus tree with posterior probabilities was saved using the sumt command. The quality of the analysis was determined by viewing results in Tracer, ensuring that effective sample size values were acceptable (>200).

*Material Examined*

*Potamonautes lirrangensis*

See Chapter One.

*Potamonautes montivagus*

**Malawi**. Zomba District, 3 subadult males (cw 25.2 mm to 49.5 mm), 2 subadult females (cws 37.5, 42.9 mm), 2 adult males (cws 57.7, 67.6 mm), 8 adult females (cw 51.5 to 67.9 mm), coll. E. Lawrence (NHM 1938.6.28.9-16); Mulunguzi, subadult male (cw 48.5 mm), adult female (cw

50.4 mm), xii. 1963, coll. R. C. H. Sweeney (NHM 1969.893); Nkata Bay, 2 subadult males (cws 34.2, 47.5 mm) coll. G. Fryer (NHM 1956.65.9); Magombe, 2 juvenile males (cws 12.3, 15 mm), 2 subadult females (cws 34, 39.5 mm), 8 subadult males (cw 18.7 to 38.5 mm), coll. D. J. Lewis (NHM 2011.1541-1550); adult female (cw 52 mm), 2 subadult females (cws 28.8, 36.5 mm), subadult male (cw 42 mm), i. 1960, coll. D. J. Lewis (NHM 2011.1535-1538); Zomba, subadult female (cw 35.7 mm), (2011.1511); Zomba 610-914 m asl, subadult female (cw 25.8 mm) coll. H. H. Johnston (NHM 1894.2.2.1); Mlanje, stream flowing through Tea Estate subadult male (cw 42.1 mm), subadult female (cw 44 mm) coll. G. Fryer (NHM 1956.6.5.7-8); 2 adult females (cws 56.3, 64.9 mm), 3 subadult females (cws 26.1 to 35.4 mm), 7 subadult males (cws 15.3 to 27.7 mm), juvenile male (cw 10.6 mm), i. 1960, coll. D. J. Lewis (NHM 2011.1522-1531); Magomba, subadult female (cw 43 mm), coll. D. J. Lewis (NHM 2011.1512); Rukuru River flowing into Lake Malawi, subadult female (cw 33.2 mm) coll. A. J. Davy and W. S. Atkins (NHM 2011.1510); 3 subadult females (cws 23.2 to 33.6 mm), 3 subadult males (cws 16.7 to 20.8 mm), 3 juvenile males (cws 12.5 to 15.1 mm) coll. L. Berner (NHM 1953.3.12.3.1.5 S.); Cholo Mountain, subadult male (cw 44.1 mm), coll. J. Vincent (NHM 1938.7.5.2); Diampwe River, Central Province, 3 subadult females (cws 21.4 to 47.7 mm) (coll. G. Fryer) (NHM 1956.6.5.4-6); subadult female (cw 24.6 mm), subadult male (cw 23 mm), 1952 (NHM 1952.451 S.); northern Malawi, subadult female (cw 30.4 mm), subadult male (cw 42.2 mm), coll. Sir H. H. Johnson (NHM 1897.4.29.2-3); Lilongwe, subadult female (cw 18.5 mm), v. 2009 (UB Site 1, May 2009 #1C, sequenced); Lilongwe, subadult male (cw 23.8 mm), v. 2009 (UB Site 1, May 2009 #1D, sequenced); Matsimbe, 2 juvenile males (cws 15.7, 18.9 mm), subadult female (cw 32.2 mm), vi. 2010, coll. M. Genner (UB Site 39, 10/06/10); Tributary of Lingadzi River, juvenile male (cw 9.9 mm), iv. 2004, coll. M. Genner (tributary of Lingadzi River, Central

Malawi; 10/04/04); Zomba, juvenile male (cw 20 mm), v. 2010 (UB Site 7, 16/5/10, Zomba); Mulanje, 2 subadult males (cws 43.7, 37.5 mm), vii. 2017, coll. M. Genner (UB Site 10, 25/7/17, 667, 668); Mikolongwe, subadult male (cw 36.5 mm), v. 2010, coll. M. Genner (UB Site 9, 17/05/10). **Mozambique**. subadult male (cw 45.2 mm) (coll. H. B. Cott) (NHM 1929.3.5.14-15). **Tanzania**. Lake Itamba, adult male (cw 55.6 mm), (coll. M. Genner) (UB #305 8.3 2011-07-008-305-305); Lake Itamba, subadult male (cw 41.1 mm) (coll. M. Genner) (UB #304 8.2 2011-07-008-304-304); Lake Itamba, adult female (cw 51.6 mm), vii. 2011, (coll. M. Genner) (UB 8.6 2011-07-008-308-308); Lake Itamba, subadult male (cw 46.3 mm) (coll. M. Genner) (UB 8.5 2011-07-008-307); Lake Kyungululu, adult male (cw 56.8 mm) (coll. M. Genner) (UB 2011-07-007-252-252); adult female (cw 61 mm) (coll. M. Genner) (UB 7.8 2011-07-007-251-251); Luika, 2 subadult females (cws 42.3, 33 mm), ix. 2012 (coll. M. Genner) (UB Site 6, #1, #2, 9/2012); Rungwe, subadult male (cw 41.4 mm), vii. 2011 (coll. M. Genner) (UB Site 4, 11/7/11 4.11); Lake Itamba, adult male (cw 51 mm), vii. 2011 (coll. M. Genner) (UB #306, 8.4, 19/7/11); Matumbe Hills, southeast Tanzania above 488 m asl, subadult male (cw 38.4 mm), xi. 1989, coll. J. Kingdon (NMU TRW XI.1989); Lake Itamba, subadult female (cw 23 mm), 2 subadult males (cws 31.4, 47.8 mm), xi. 2011, coll. M. Genner (UB 22/11/11 ITA-A, ITA-B, ITA-C); Lake Itamba, subadult female (cw 44.4 mm), xi. 2011, coll. M. Genner (UB ITA C; 29/11/11); Kiunga, adult female (cw 54.2 mm), coll. M. Genner (UB Kyunga 23/22/22, 2B3); Kyungululu, 2 juvenile females, vii. 2011, coll. M. Genner (UB Kyungululu 7.1; 7.7; 8/7/11); Rungwe, subadult male (cw 36.5 mm), 2 juveniles (cws 29, 25 mm), iii. 2016, coll. M. Genner (UB Site 8, 30/03/16, [UV1, UV2, UV3]); Luika, subadult male (cw 34.3 mm), v. 2010, coll. M. Genner (UB Site 6, 15/5/10); Mlowo, subadult male (cw 23.7 mm), vii. 2017, coll. M. Genner (UB Site 2, 23/7/17). **Zambia**. Mwelalo, subadult male (cw 30.7 mm), vi. 2010, coll. M. Genner (UB Site

51, #9, 6/2010); Chipata, subadult female (cw 29.6 mm), subadult male (cw 31.3 mm), vi. 2010, coll. M. Genner (UB Site 69, #31, #33, June 2010); Muloza West, Cholo area, subadult male (cw 45.2 mm), adult female (cw 53 mm), ix. 1988, coll. M. J. Roberts (NMU 15.09.1988). **Zimbabwe**. Inyanga, from River Mare in the Eastern Highlands, 1700-1829 m asl near government trout hatchery, subadult male (cw 46.5 mm), vii. 1972, coll. T. R. Williams (NMU 7.III.1972).

*Potamonautes bellarussus*

**Tanzania.** Rungwe, subadult female (cw 40 mm), vii. 2011, coll. M. Genner (UB Site 4, 11/7/11 4.10); Rungwe, subadult male (cw 23.2 mm), v. 2010, coll. M. Genner (UB Site 7, 16/5/10); Rungwe, subadult female (cw 27.5 mm), sub adule male (cw 27 mm), ix. 2012, coll. M. Genner (UB Site 8, 4/9/12, #8.2, #8.1); Mata Mondo, adult female (cw 48.9 mm), subadult female (cw 32.5 mm), subadult male (cw 40.3 mm), ix. 2012, coll. M. Genner (UB Site 13, 6/9/12 170, 171, 172); Lipupuma, subadult male (cw 28.1 mm), ix. 2012, coll. M. Genner (UB Site 19, #19.1, 9/9/12); Malawi catchment, near Kyela Tanzania, subadult male (cw 43.5 mm), vii. 2011, coll. M. Genner (UB 2018.311); Mlowo, subadult female (cw 36 mm), vii. 2017, coll. M. Genner (UB Site 2, 23/7/17); Ruaha, 2 juveniles (damaged carapace), ii. 2011, coll. M. Genner (UB site 1, Ruaha, 1-2-11). **Malawi.** Mikolongwe, 2 subadult males (cws 40.5, 27.5 mm), ix. 2012, coll. M. Genner (UB site 9, 14/9/12, #1, #2).

*Potamonautes bayonianus*

**Zambia.** Mpulo, subadult female (cw 23.5 mm), vi. 2010, coll. M. Genner (UB Site 44, 15/6/10, #Z51, #576); Mpulo, subadult male (cw 23.3 mm), vi. 2010, coll. M. Genner (UB Site 44,

15/6/10, #Z50, #575). **Democratic Republic of the Congo.** Kisapa, adult male (cw 53.4 mm), coll. J. J. Symoens (NMU DRC 14.III.1970).

*Potamonautes obesus*

**Malawi.** Chiendausiku, adult female (cw 48 mm), i. 201, coll. M. Genner (UB Site 31, Jan 2011, 19/01/2011); Chiendausiku, subadult female (cw 23.4 mm), v. 2009, coll. M. Genner (UB Site 31, May 2009, 31A); unknown locality, subadult male (cw 25 mm), 2010, coll. M. Genner (UB #28, 2010); site 16, subadult male (cw 44.6 mm), subadult female (cw 24.1 mm), viii. 2013, coll. M. Genner (UB Site 16, #1, #2, 16/8/13); Rovuma catchment (Masai), subadult male (cw 44.8 mm), viii. 2013, coll. M. Genner (UB 2018.312). **Tanzania.** Mlingano, 2 adult females (cws 29, 31.7 mm), 2 subadult males (cws 26.5, 20.7 mm), viii. 2015, coll. M. Genner (UB Site 19, 18/8/2015, Mlingano (ML1-4)).

*Potamonautes choloensis*

**Malawi.** Mulanje, subadult male (cw 17.3 mm), subadult female (cw 16.2 mm), v. 2009, coll. M. Genner (UB Site 30; May 2009, #30F, #30H); Mbloza River, Blantyre, subadult male (cw 33.9 mm), subadult female (cw 33.2 mm) iv. 1988, coll M. J. Roberts (NMU 4.VIII.1988).

*Potamonautes suprasulcatus*

**Tanzania.** Nenguruwe stream, west of Amani, East Usambara Mts., adult male (cw 60.3 mm) coll. J. N. Raybould (NMU 09.1965.1); Nyandeo stream near Kilombero (between Mikumi and Kierege, near great Ruaha R) south of Kilosa, adult male (cw 73.5 mm), iv. 1966, coll. J. N. Raybould (NMU TRW 1966.07).

## 2. 2 RESULTS

### *Morphological Comparative Studies*

Specimens initially identified as *Potamonautes montivagus* from Lake Kyungululu and Lake Itamba, Tanzania, other localities in Tanzania, localities in Malawi (outside of Lake Malawi), northeastern Zambia, and Zimbabwe share the following characters (Fig. 11). The postfrontal crest is complete, the exorbital tooth is low, the epibranchial tooth is absent, the anterolateral margin of the carapace is smooth or has fine granulations, the proximal tooth on the inner margin of the cheliped carpus is shorter than the distal tooth, the vertical sulcus on the ischium of the third maxilliped is deep, the sternal sulcus s3/s4 is deep at the sides and shallow in the center, and the G1 terminal article has a rounded lobe on the medial margin and ends with an upturned tip. The diagnostic characters of *P. montivagus* used here (Fig. 12) were based on the holotype of this species published by Chace (1953, Fig. 3).

A summary of the results of the morphological analysis comparing *P. montivagus* with *P. lirrangensis* s. l. and Malawi basin freshwater crab fauna are compiled in Table 4. Shared morphological characters observed in all species include two large spines on the inner margin of the cheliped carpus, a pointed distal tooth on the cheliped merus, and a major cheliped with an arched dactylus. Additional shared characters between *P. lirrangensis* s. l. and *P. montivagus* include a complete and distinct postfrontal crest, deep grooves on the posterior region of the carapace, a granulated branchiostegal sidewall suborbital region, elongated p5 segments (in

crater lake *P. montivagus* specimens), an exorbital tooth with a granulated margin, a deep vertical sulcus on the ischium of the third maxilliped, and G1 terminal article with a large rounded lobe on the medial margin that ends in an upcurved tip. However there are a number of morphological difference between these two taxa. For example, *P. lirrangensis* s. l. has a large spine-like pointed exorbital tooth and distinct toothing or granulation along the anterolateral margin of the carapace, while *P. montivagus* has a low blunt exorbital tooth and a smooth anterolateral margin (Figs. 12–13). The carapace proportions (ch/fw, cl/fw, and cw/fw) of *P. lirrangensis* s. l. (carapace height, length and width) are different than those of *P. montivagus* (Table 4).

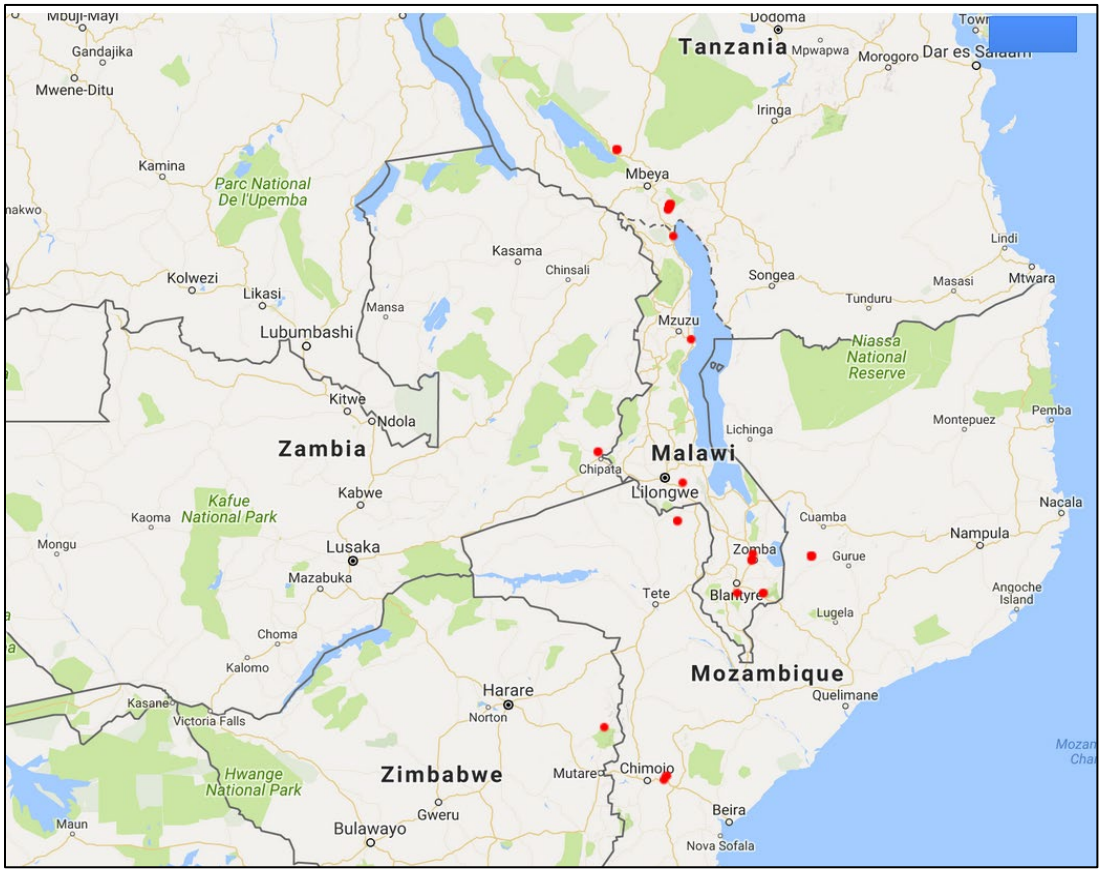


Figure 11. Map showing the distribution of specimens initially identified as *Potamonautes montivagus* in Malawi, Mozambique, Zimbabwe, Zambia and Tanzania.

Table 4. Morphological comparisons made between *P. lirrangensis* s. l., *P. montivagus*, and other species of freshwater crabs found in the Lake Malawi drainage basin. * = shared character; ? = data deficient; dark shaded cells represent characters found in the Malawi Blue Crab. The morphological characters shown here are also shared by specimens of *P. montivagus* from a number of localities, but the carapace proportions and gonopod angles were measured for Lake Itamba only.

| Morphological Character | *Potamonautes orbitospinus* (Lake Malawi, Malawi) | *Potamonautes montivagus* (Lake Itamba, Tanzania) | | *Potamonautes choloensis* | *Potamonautes obesus* | *Potamonautes bayonianus* | *Potamonautes bellarussus* | *Potamonautes suprasulcatus* | *Potamonautes mulanjeensis* |
|---|---|---|---|---|---|---|---|---|---|
| *Post-frontal Crest Complete* | * | * | | faint meeting al margins | * | * | * | * | * |
| *Exorbital tooth large pointed* | * | low | | low | low, pointed | low | low | low | low |
| *Epibranchial tooth low* | * | low granule | | small, distinct | small, distinct | distinct | absent | low granule | * |
| *Carapace posterior region with deep grooves* | * | * | | shallow | shallow | shallow | * | * | * |
| *Cheliped merus distal tooth spine-like, pointed* | * | * | | * | * | * | * | * | * |
| *Cheliped carpus inner margin with two large subequal spine-like, pointed teeth* | * | * | | * | * | * | * | * | * |
| *Dactylus of major cheliped arched* | * | * | | * | * | * | * | * | * |
| *Branchiostegal suborbital region granulated* | * | * | | smooth | * | * | * | smooth | ? |
| *G1 terminal article angle 47-52 degrees* | * | 56 | | no | no | no | no | no | ? |
| | | | | | | | | | |
| *Carpus, propodus, dactylus of pereiopod 5 all elongated, slender* | * | * | | not elongated | not elongated | not elongated | not elongated | not elongated | ? |
| *s3/s4 groove complete and deep* | * | incomplete in center | | * | complete, faint medially | * | complete, faint medially | complete, faint medially | * |
| *Ischium of third maxilliped with deep vertical sulcus* | * | * | | absent | absent | absent | absent | absent | faint, distinct |
| *G1 terminal article widened by large rounded lobe* | * | * | | no | no | no | no | no | no |
| *G1 terminal article tip upcurved* | * | * | | no | no | * | no | no | * |
| *Anterolateral margin between exorbital and epibranchial teeth lined by small distinct teeth* | * | * | | faint granules | * | fine granules | * | * | ? |
| *Anterolateral margin lined by small distinct teeth* | * | Smooth/ faint granules | | smooth/ faint flat granules | fine granules | smooth | faint granules | * | granulated |
| *DNA Sequence Data Available* | Yes | yes | | yes | no | | yes | | yes |
| *Carapace height/ Front width (ch/fw)* | 1.0 | 1.3 | | 1.0 | 1.1 | 1.0 | 1.0 | 1.1 | 1.0 |
| *Carapace length / Front width (cl/fw)* | 2.5 | 2.8 | | 2.3 | 2.2 | 2.2 | 2.5 | 2.6 | 2.0 |
| *Carapace width/ Front width (cw/fw)* | 3.5 | 3.7 | | 3.0 | 2.9 | 2.9 | 3.3 | 3.6 | 3.1 |

*Potamonautes choloensis* from Malawi (cw 41.6 mm, cl 29.8 mm, fw 13.3 mm). The diagnostic characters of *P. choloensis* used here were based on the holotype of this species published by Chace (1953, Fig. 1). The postfrontal crest is faint but complete and meets the anterolateral margins of the carapace, the exorbital tooth and the epibranchial tooth are both small but distinct, the anterolateral margin of the carapace is faintly granulated, the anterolateral margin between

the exorbital and epibranchial teeth is faintly granulated, and the posterior region of the carapace has faint shallow grooves. The carapace height is equal to the front width (ch/fw 1.0), the carapace length is about twice that of the front width (cl/fw 2.3), and the carapace width is 3 times the front width (cw/fw 3.0). The distal tooth on the inner margin of the cheliped merus is pointed, the inner margin of the cheliped carpus has two spine-like teeth unequal in size, and the dactylus of the major cheliped is arched. The branchiostegal sidewall suborbital region is smooth. The ischium of the third maxilliped is smooth, lacking a sulcus, and the s3/s4 sternal groove is complete and deep. Carapace proportions were calculated using museum specimen (NMU 4.VII.1988, male, cw 33.9 mm, 26.5 mm, ch 11.7 mm, fw 11.3 mm).

*Potamonautes obesus* from Tanzania (NHM 2018.312, cw 44.8 mm, cl 34.3 mm, ch 17.1 mm, fw 15.5 mm). The postfrontal crest is complete, the exorbital tooth is low and pointed, the epibranchial tooth is small and distinct, the posterior region of the carapace has only faint grooves, the anterolateral margin of the carapace is lined with fine granules, the anterolateral margin between the exorbital and epibranchial teeth is lined by small teeth, the carapace height is higher than the front width (ch/fw 1.1), the carapace length is about twice that of the front width (cl/fw 2.2), and the carapace width is about 3 times the front width (cw/fw 2.9). The distal tooth on the inner margin of the cheliped merus is pointed, the inner margin of the cheliped carpus has two spine-like teeth unequal in size, and the dactylus of major cheliped is arched with a distinct gape. The branchiostegal sidewall suborbital region is granulated, the ischium of the third maxilliped is smooth and lacks a sulcus, and sternal groove s3/s4 is complete but faint in the center.

*Potamonautes bayonianus* from the D. R. Congo (NMU DRC 14.III.1970, cw 53.4 mm, cl 40.8 mm, ch 18.3 mm, fw 18.6 mm). The postfrontal crest is complete, the exorbital tooth is

low and pointed, the epibranchial tooth is small and distinct, the posterior region of the carapace has only faint grooves, the anterolateral margin of the carapace is lined with fine granules, the anterolateral margin between the exorbital and epibranchial teeth is lined by small teeth, the carapace height is equal to the front width (ch/fw 1.0), the carapace length is about twice that of the front width (cl/fw 2.1), and the carapace width is about 3x the front width (cw/fw 2.9). The distal tooth on the inner margin of the cheliped merus is pointed, the inner margin of the cheliped carpus has two spine-like teeth unequal in size, and the dactylus of major cheliped is arched with a distinct gape. The ischium of the third maxilliped is smooth and lacking a sulcus, and sternal groove s3/s4 is complete and deep.

*Potamonautes bellarussus* from Tanzania (NHM 2018.311, cw 43.5 mm, cl 33.4 mm, ch 13.8 mm, fw 13 mm). The postfrontal crest is complete, the exorbital tooth is low and pointed, the epibranchial tooth is a small granule, the posterior region of the carapace has deep grooves, the anterolateral margin of the carapace is lined with fine granules, the anterolateral margin between the exorbital and epibranchial teeth is lined by small teeth, the carapace height is equal to the front width (ch/fw 1.0), the carapace length is about 2.5 times that of the front width (cl/fw 2.5), and the carapace width is about 3 times that of the front width (cw/fw 3.3). The distal tooth on the inner margin of the cheliped merus is pointed, the inner margin of the cheliped carpus has two spine-like teeth unequal in size, and the dactylus of major cheliped is arched with a distinct gape. The branchiostegal sidewall suborbital region is granulated, the ischium of the third maxilliped is smooth and lacking a sulcus, and sternal groove s3/s4 is complete but faint in the center.

*Potamonautes suprasulcatus* from Tanzania (NMU 09.1965.1, cw 60.3 mm, cl 42.4 mm, ch 17.6 mm, fw 16.6 mm). The postfrontal crest is complete, the exorbital tooth is low and

pointed, the epibranchial tooth is small and distinct, the posterior region of the carapace has deep grooves, the anterolateral margin of the carapace is lined with fine granules, the anterolateral margin between the exorbital and epibranchial teeth is lined by small teeth, the carapace height is higher than the front width (ch/fw 1.1), the carapace length is about 2.5 times that of the front width (cl/fw 2.6), and the carapace width is about 3.5 times that of the front width (cw/fw 3.6). The distal tooth on the inner margin of the cheliped merus is pointed, the inner margin of the cheliped carpus has two spine-like teeth unequal in size, and the dactylus of major cheliped is arched with a distinct gape. The branchiostegal sidewall suborbital region is granulated, the ischium of the third maxilliped is smooth and lacking a sulcus, and sternal groove s3/s4 is complete but faint in the center.

*Potamonautes mulanjeensis* from Malawi (cw 38.5 mm, cl 25.4 mm, ch 12.9 mm, fw 12.4 mm). The diagnostic characters of *P. mulanjeensis* used here were based on the holotype of this species published by Daniels and Bayliss (2012, Fig. 5). The postfrontal crest is complete, the exorbital tooth is low and pointed, the epibranchial tooth is small and distinct, the posterior region of the carapace has deep grooves, the anterolateral margin of the carapace is lined with fine granules, the anterolateral margin between the exorbital and epibranchial teeth is lined by small teeth, the carapace height is equal to the front width (ch/fw 1.0), the carapace length is about twice that of the front width (cl/fw 2.0), and the carapace width is about three times that of the front width (cw/fw 3.1). The distal tooth on the inner margin of the cheliped merus is pointed, the inner margin of the cheliped carpus has two spine-like teeth unequal in size, and the dactylus of major cheliped is arched with a distinct gape. The branchiostegal sidewall suborbital region is granulated, the ischium of the third maxilliped has a deep vertical sulcus, and sternal groove s3/s4 is complete and deep.

*Results of the Phylogenetic Analysis*

The results of the Bayesian analysis are shown in Fig. 14. *Sudanonautes floweri* and *Liberonautes latidactylus* were used as outgroups. *Potamonautes bellarussus* falls out on a separate lineage than the other Lake Malawi basin species. *Potamonautes choloensis* and *P. obesus* are the most basal species from the Rift Valley potamonautids in reference to branch lengths. The Lake Malawi basin freshwater crab fauna appears on its own clade of the phylogeny, while *Platythelphusa armata* is sister to the *P. lirrangensis* and *P. montivagus* clade (0.9 posterior probability). Sequences of *P. lirrangensis* s. l. from Lake Kivu are sister to each other with full node support. There is also strong support for the clade showing *P. montivagus* from Malawi and Mozambique as a sister group to *P. lirrangensis* s. l. from Lakes Malawi and Tanganyika (Zambia). The specimens from the Tanzanian crater lakes (Itamba and Kyungululu) initially identified here as *P. montivagus* were positioned on a separate clade to that occupied by the other specimens of *P. montivagus* from Malawi and Mozambique, and appear to be a different (and new) species of *Potamonautes.*

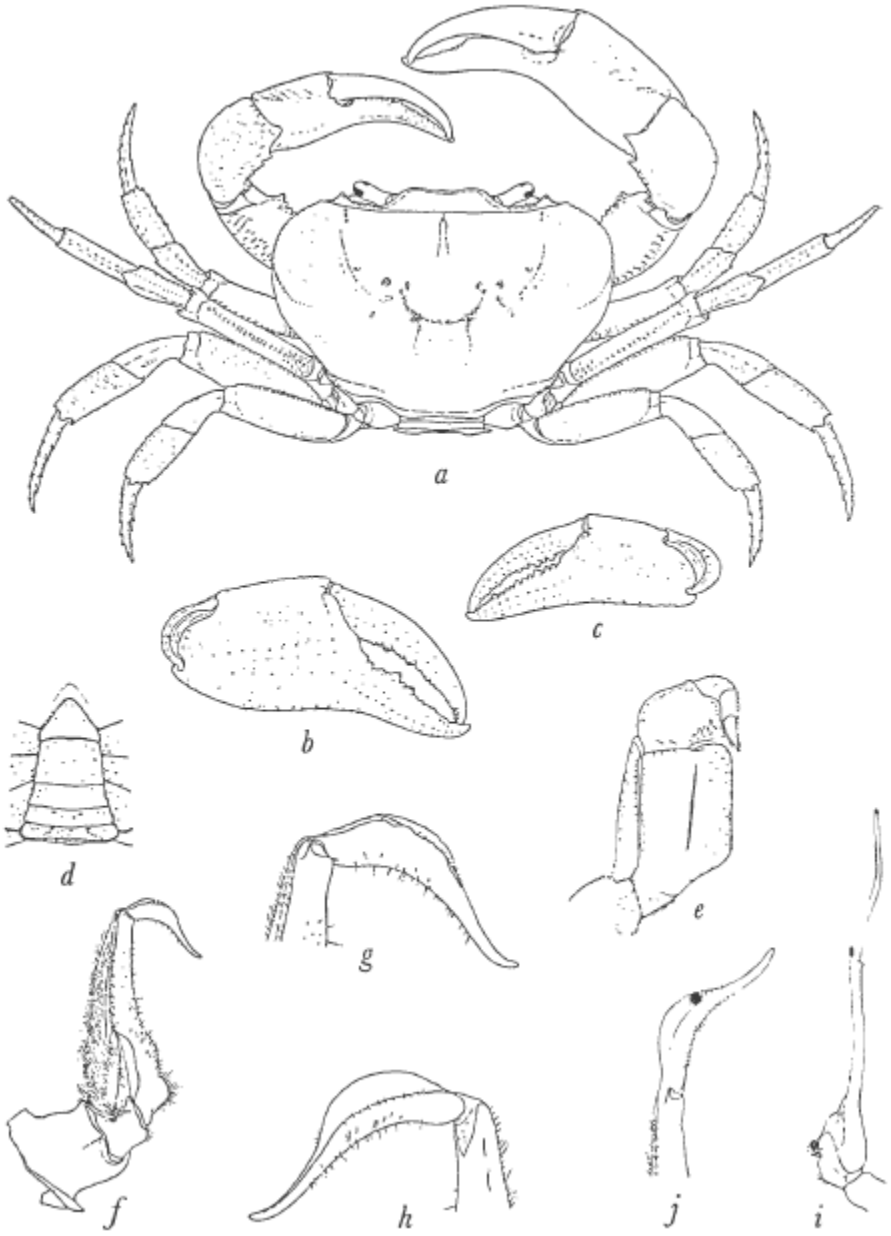



Figure 12. Illustration of the holotype of *P. montivagus* from Chace (1953) (M.C.Z. 12611, cw 65.3 mm). a. dorsal view of carapace, b-c. major and minor chelipeds, d. sternum, e. third maxilliped with vertical sulcus on ischium, f-g. ventral views of gonopod 1, h. dorsal view of terminal article of gonopod 1 displaying rounded lobe, i. gonopod 2, j. superior view of gonopod 1.

Figure 13. Specimen initially identified as *P. montivagus* from Lake Kyungululu, Tanzania (NHM 2018.310, cw 56.8 mm). Dorsal view (top), frontal and ventral views of carapace, right and left gonopods 1 and 2 at x20 magnification, major (right) cheliped, G1 (right) dorsal and ventral views x30 magnification, G1 ventral x50 magnification, G2 x20 magnification (bottom right).

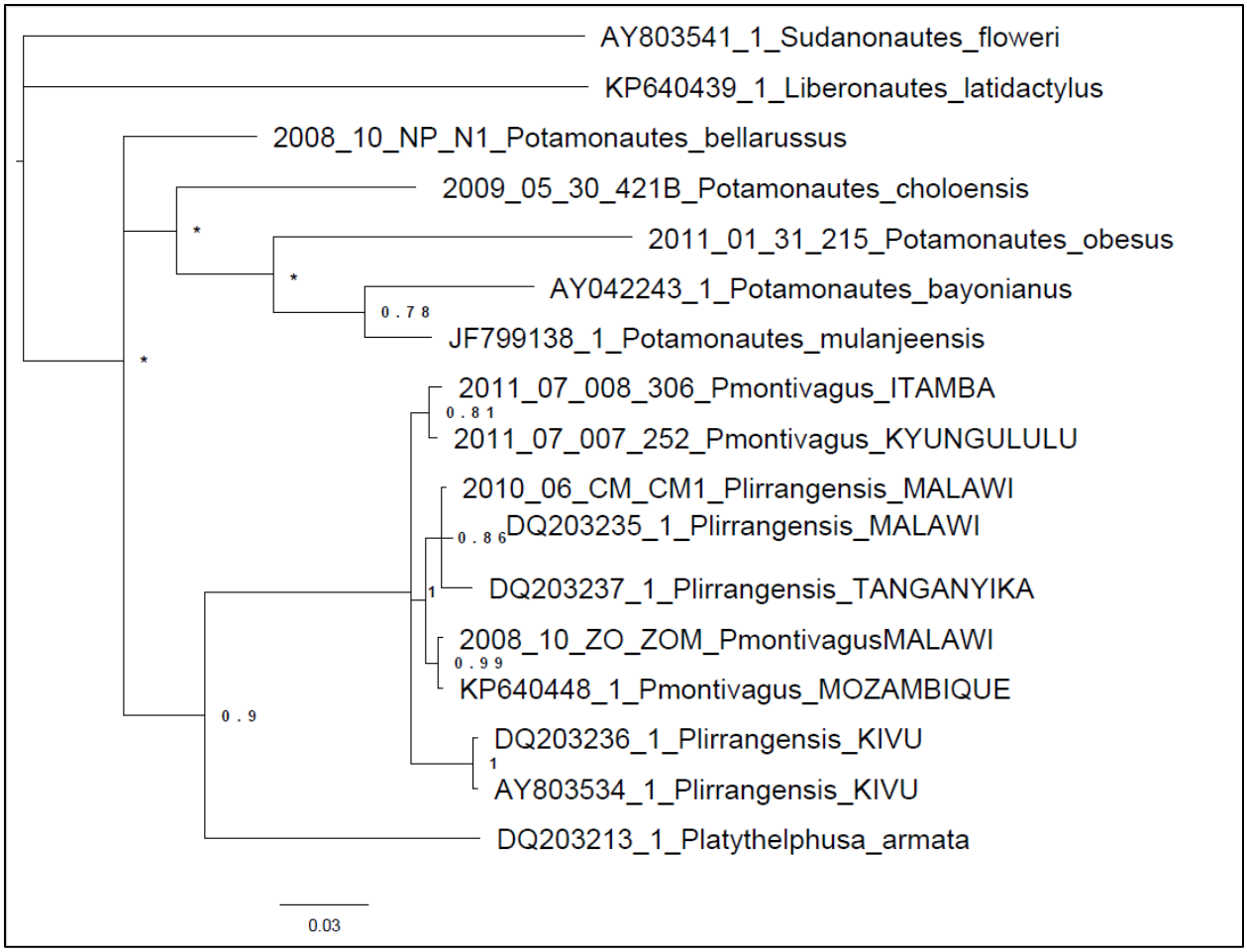



Figure 14. Bayesian tree topology (MrBayes) derived from 16S rRNA mtDNA sequences for species of *Potamonautes* from Lake Malawi and the Lake Malawi basin. Branch lengths proportional to the number of base changes per site. The Lake Tanganyika endemic species of *Platythelphusa* was also included, and *Liberonautes* and *Sudanonautes* were used as outgroups. The numbers above the nodes denote the Bayesian posterior probabilities. Taxon labels indicate GenBank/sequence ID, species name, and locality (for *P. lirrangensis* s. l. and *P. montivagus*). Only posterior probability values > 0.75 are shown, while values < 0.75 denoted with an asterisk (*).

# CHAPTER THREE: CONCLUSIONS

The phylogenetic relationships shown in the MrBayes 16S tree (Fig. 14) indicate that the ingroup taxa of this study form a polytomy with three main clades. Despite the lack of resolution at this level, there is enough detail available in the tree topology to resolve some of the key questions raised here regarding the taxonomic validity of *P. lirrangensis* s. l. and *P. montivagus*. The first clade of this polytomy includes the specimens from the two crater lakes in Tanzania (Lakes Kyungululu and Itamba) that were initially identified as *P. montivagus*, but that are now treated here as a new species of *Potamonautes* (sp. 2).

The second clade groups the specimens from Lake Kivu initially identified as *P. lirrangensis* s. l. that are treated here as a new species of *Potamonautes* (sp. 1). And the third clade consists of two lineages, one that includes the Malawi Blue Crab from Lake Malawi and the specimens from southern Lake Tanganyika that were initially identified as *P. lirrangensis* s. l. but which are treated here as *P. orbitospinus* (Cunnington, 1907). The second lineage in this third clade includes specimens from Malawi and Mozambique that were identified initially as *P. montivagus*, and are treated here as this species.

The molecular tree shown in Fig. 10 includes greater sampling and shows more detail of the diversification within *P. montivagus* in different localities across its range. However, many of the specimens are juveniles and subadults that proved difficult to identify morphologically, but which are grouped together on the molecular tree as part of the lineage representing *P. montivagus*. The MrBayes 16S tree also provides support for the continued recognition of five

other species found in the Lake Malawi drainage basin: *P. bellarussus, P. choloensis, P. obesus, P. bayonianus,* and *P. mulanjeensis.*

These topologies suggest a possible scenario for a speciation event between *P. orbitospinus* and *P. montivagus* that is linked to habitat. On the evolution of these sister taxa from a common riverine ancestor, one branch (*P. orbitospinus*) entered Lake Malawi and evolved several adaptations for lake life, while the other group (*P. montivagus*) remained in the riverine habitats in the surrounding catchment and accumulated fewer evolutionary changes. the The Malawi Blue Crab faces considerable predation pressure from a number of predatory fish species such as catfish and larger-mouthed cichlids (Kidd et al., 2003), predatory birds (Johnston, 1989), and otters (Mgoola & Msiska, 2017) associated with the lake. This intense predation pressure may have favored the evolution of the Malawi Blue Crab's additional sharp spines on its carapace and chelipeds that serve to deter potential predators. In addition, *P. montivagus* that remained in the riverine environment has a higher, more inflated carapace (ch.fw 1.3) (associated with episodic air-breathing), while that of the lake-living fully aquatic *P. orbitospinus* (ch/fw 1.0) is flatter. For *P. montivagus*, more time spent on land would provide an increased potential to escape aquatic predators, and to respond to drops in water level or the seasonal drying out of its habitat.

The highly variable and widespread riverine species, *P. montivagus*, is generally dark brown in color, its carapace and chelipeds have smooth margins that lack large pointed spines, and its walking legs are not noticeably elongated compared to other river-living species. On the other hand, the lake-dwelling *P. orbitospinus* has a bright blue body coloration with bright white margins, its carapace and chelipeds have toothed margins with large pointed spines, and its walking legs are noticeably elongated compared to those of *P. montivagus*. In addition, lake-

living specimens previously identified as *P. montivagus* from the two Tanzanian crater lakes (Itamba and Kyungululu) studied here are colored bright red and have elongated walking leg segments (p2-p5) similar to that seen in the lake-living *P. orbitospinus* from Lake Malawi (and different from the walking legs of *P. montivagus* from the rivers of Malawi). Elongated leg segments of *P. orbitospinus* may reflect adaptations to locomotion in muddy-bottomed lakes, whereas the shorter leg segments of *P. montivagus* and other species of *Potamonautes* found in the Lake Malawi basin would allow better locomotion between the rocks of the beds of fast-flowing rivers and streams. Interestingly, the species from the Tanzanian crater lakes does not have a heavily toothed carapace and spiny cheliped margins (as does the lake-living *P. orbitospinus*). Furthermore, the gonopod morphology (G1 terminal article shape) of *P. orbitospinus* of Lake Malawi is very similar to that of *P. montivagus*, and of the Tanzanian crater lake species of *Potamonautes*, and it is other carapace and pereiopod characters that are different between these three taxa. In addition, the red and blue coloration in these lake-living crabs might reflect adaptations to life at different depths in the lake, similar to those seen in species of *Pundamilia* cichlids found in Lake Victoria, another of the Rift Valley great lakes. These fish possess a gene which has been interpreted as an adaptation to habitats with contrasting light conditions varying at different depths or water clarity (Terai et al., 2017). The blue species of cichlid lives in clear, shallow waters while the red species of cichlid lives in deeper, murkier waters. A similar color adaptation may have evolved in the lake living crabs in the Lake Malawi basin, with the Malawi Blue Crab being adapted to the blue-tinged shallow waters of the lakes, while the red colored Tanzanian crater lake crabs may be adapted to the red-tinged deeper waters.

Lake Tanganyika hosts nine species of the genus *Platythelphusa* which have adaptations to the lake and are endemic to this habitat. These species are heavily armored, possess spines or granules on the external angles of the front margin of the carapace, have two to four large forward-pointing teeth on the anterolateral margin, three sharp carpal teeth on the cheliped, and sharp spines on the dactyli of p2-p5 (Cumberlidge et al., 1999). In addition three species of Platythelphusa have elongated p5 segments (*P. tuberculata*, *P. polita*, and *P. conculcata*). While *P. orbitospinus* does not exhibit the level of toothing on the carapace seen in these platythelphusids, the Lake Malawi specimens possess noticeably pointed teeth on the exorbital tooth and the cheliped carpus and merus. Only one species of *Platythelphusa (P. tuberculata*) has distinctly elongated waling legs that are seen in *P. orbitospinus*. These lake characteristics were not seen in *P. lirrangensis* s. l. from Lake Kivu (i.e., *Potamonautes* sp. 1), which may be because these crabs have only recently entered the lake environment, or it may be due to a lower predation pressure associated with the relatively small size of Lake Kivu (compared to Lakes Malawi and Tanganyika).

# REFERENCES


Bott, R. 1955. Die Süßwasserkrabben von Africa (Crust., Decap.) und ihre Stammesgeschichte *Annales du Musee Royal du Congo Belge C. (Zoologie)* 3 (sér. 3, 3) 1 (3): 209–352.

Brito-Capello, F. de. 1864. Descripçao de tres especies novas de Crustaceos da Africa occidentale. Observaçoes acerca do Penaeus bocagei Johnson, especie nova dos mares de Portugal. *Memorias da Academia Real das Sciencias. Lisboa* 3: 1–11.

Chace, F.A. 1953. Zoological results of a fifth expedition to east Africa. *Bulletin of the Museum of Comparative Zoology*, 110: 425–443.

Chidammodzi C, Muhandiki V 2015. Development of indicators for assessment of Lake Malawi Basin in an Integrated Lake Basin Management (ILBM) framework. *International Journal of the Commons*, 9(1): 209–236.

Cumberlidge, N. 1999. The freshwater crabs of West Africa: family Potamonautidae. Collection Faune et Flore Tropicales 35: Paris: IRD, 1–382.

Cumberlidge, N. & Daniels, S.R. 2008. A conservation assessment of the freshwater crabs of southern Africa (Brachyura: Potamonautidae). *African Journal of Ecology*, 46: 74–79.

Cumberlidge, N. & Clark, P.F. 2018. Albertine Rift Valley endemics: three new species of freshwater crabs (Brachyura: Potamoidea: Potamonautidae) from Uganda, the Democratic Republic of the Congo, and Rwanda. *Journal of Natural History*, 52(25–26): 1637–1656.

Cumberlidge, N. & Meyer, K.S. 2011. The freshwater crabs of Lake Kivu (Crustacea: Decapoda: Brachyura: Potamonautidae). *Journal of Natural History*, 45(29-30): 1835–1857.

Cumberlidge, N., Hobbs, H.H., & Lodge, D.M. 2015. Class Malacostraca, Order Decapoda. In: Thorp J, Rogers DC. (Eds.), Ecology and General Biology: Thorp and Covich Freshwater Invertebrates. Academic Press, 797–847.

Cumberlidge, N., Ng, P.K.L., Yeo, D., Magalhães, C., Campos, M., Alvarez, F., Naruse, T., Daniels, S., Esser, L., Attipoe, F.Y.K., Clotilde-Ba, F., Darwall, W., Mcivor, A., Baillie, J., Collen, B., & Ram, M. 2009. Freshwater crabs and the biodiversity crisis: importance, threats, status, and conservation challenges. *Biological Conservation*, 142: 1665–1673.

Cumberlidge, N., von Sternberg, R., Bills, I.R., Martin, H. 1999. A revision of the genus *Platythelphusa* A. Milne-Edwards, 1887 from Lake Tanganyika, East Africa (Decapoda: Potamoidea: Platythelphusidae). *Journal of Natural History*, 33: 1487–1512.

Cunnington, W.A. 1907. Zoological Results of the Third Tanganyika Expedition, conducted by Dr. W. A. Cunnington, 1904-1905 – Report on the Brachyurous Crustacea. *Proceedings of the Zoological Society of London,* 1907: 258–276.

Daniels, S.R. & Bayliss, J. 2012. Neglected refugia of biodiversity: mountainous regions in Mozambique and Malawi yield two novel freshwater crab species (Potamonautidae: Potamonautes). *Zoological Journal of the Linnean Society*, 164(3): 498–509.

Daniels, S.R., Phiri, E.E., Bayliss, J. 2014. Renewed sampling of inland aquatic habitats in southern Africa yields two novel freshwater crab species (Decapoda: Potamonautidae: Potamonautes). *Zoological Journal of the Linnean Society,* 171: 356–369.

Daniels, S.R., Phiri, E.E., Klaus, S., Albrecht, C., & Cumberlidge, N. 2015. Multilocus phylogeny of the Afrotropical freshwater crab fauna reveals historical drainage connectivity and transoceanic dispersal since the Eocene. *Systematic Biology*, 64: 549–567.

Eccles, D.H. 1974. An outline of the physical limnology of Lake Malawi (Lake Nyasa). *Limnology and Oceanography*, 19(5): 730–742.

Hastings, W. K. 1970. Monte Carlo sampling methods using Markov chains and their applications. *Biometrika*, 57:97–109.

Hilgendorf, F. 1898. Die Land- und Süßwasser-Dekapoden Ostafrikas. In: Die Thierwelt Deutsch Ost-Afrikas, 4(7): 1–37.

Johnston, D.W. 1989. Feeding ecology of pied kingfishers on Lake Malawi, Africa. *Biotropica,* 21(3): 275–277.

Kidd, K.A., Bootsma, H.A., Hesslein, R.H., Lockhart, W.L., & Hecky, R.E. 2003. Mercury concentrations in the food web of Lake Malawi, East Africa. *Journal of Great Lakes Research,* 29(2): 258–266.

Kochey, J.K., Daniels, S.R., Plagge, C., Mehrabi, S., Hartmann, L., Schrenk, F., Plath, M., Klaus, S. 2017. Genetic differentiation of the Malawi blue crab reflects Pleistocene desiccation of Lake Malawi (Brachyura, Potamonautidae: *Potamonautes lirrangensis* (Rathbun, 1904)). *Hydrobiologia*, 1–11.

Kumar, S., Stecher, G., Tamura, K. 2015. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0. *Molecular Biology and Evolution*, 33(7): 1870–1874.

Li, J., Brown, E.T., Crowe, S.A., & Katsev, S. 2018. Sediment geochemistry and contributions to carbon and nutrient cycling in a deep meromictic tropical lake: Lake Malawi (East Africa). *Journal of Great Lakes Research*, 44(6): 1221–1234.

Limuwa, M.M., Sitaula, B.K., Njaya, F., & Storebakken, T. 2018. Evaluation of Small-Scale Fishers' Perceptions on Climate Change and Their Coping Strategies: Insights from Lake Malawi. *Climate,* 6(2): 34.

Marijnissen, S.A., Michel, E., Kamermans, M., Olaya-Bosch, K., Kars, M., Cleary, D.F., van Loon, E.E., Dolmen, P.G.R., & Menken, S.B. 2008. Ecological correlates of species differences in the Lake Tanganyika crab radiation. In Patterns and Processes of Speciation in Ancient Lakes (pp. 81–94). Springer, Dordrecht.

Marijnissen, S.A.E., Michel, E., Daniels, S.R., Erpenbeck, D., Menken, S.B.J., & Schram, F.R. 2006. Molecular evidence for recent divergence of Lake Tanganyika endemic crabs (Decapoda: Platythelphusidae). *Molecular Phylogenetics and Evolution*, 40(2): 628–634.

Metropolis, N., Rosenbluth, A.W., Rosenbluth, M.N., Teller, A.H., & Teller, E. 1953. Equations of state calculations by fast computing machines. *The Journal of Chemical Physics*, 21: 1087–1091.

Mgoola, W.O., & Msiska, H.G. 2017. The status and distribution of the clawless otter (Aonyx capensis) in Vwaza Marsh Wildlife Reserve and Nyika National Park, Northern Malawi. IUCN Otter Specialist Group Bulletin, 34(1): 3–17.

Milne-Edwards, A. 1868. Description de quelques Crustacés nouveaux provenant des voyages de M. Alfred Grandidier a Zanzibar et a Madagascar. *Nouvelles Archives du Museum d'Histoire naturelle*, Paris IV: 69– 92, pl. XIX–XXI.

Rathbun, M.J. 1904. Les crabes d'eau douce (Potamonidae). *Nouvelles Archives du Museum d'Histoire naturelle*, Paris, 4(6): 255–312.

Rathbun, M.J. 1921. The brachyuran crabs collected by the American Museum Congo Expedition 1909-1915. (Ecological notes by H. Lang). *Bulletin American Museum of Natural History*, 43: 397–468.

Reed, S.K. & Cumberlidge, N. 2006. Taxonomy and biogeography of the freshwater crabs of Tanzania, East Africa (Brachyura: Potamoidea: Potamonautidae, Platythelphusidae, Deckeniidae). *Zootaxa*, 1262: 1–139.

Ronquist, F. & Huelsenbeck, J.P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19:1572–1574.

von Sternberg, R., Cumberlidge, N., Rodriguez, G. 1999. On the marine sister groups of the freshwater crabs (Crustacea: Decapoda: Brachyura). *Journal of Zoological Systematics and Evolutionary Research*, 37: 19–38.

Sungani, H., Ngatunga, B.P., & Genner, M.J. 2016. Migratory behaviour shapes spatial genetic structure of cyprinid fishes within the Lake Malawi catchment. *Freshwater Biology*, 61: 1062–1074.

Terai, Y., Miyagi, R., Aibara, M., Mizoiri, S., Imai, H., Okitsu, T., Wada, A., Takahashi-Kariyazono, S., Sato, A., Tichy, H, & Mrosso, H.D. 2017. Visual adaptation in Lake Victoria cichlid fishes: depth-related variation of color and scotopic opsins in species from sand/mud bottoms. *BMC evolutionary biology*, 17(1): 200.

Yihdego, Y. & Paffard, A. 2016. Hydro-engineering solution for a sustainable groundwater management at a cross border region: case of Lake Nyasa/Malawi basin, Tanzania. *Geo-Engineering*, 7: 23.

APPENDIX A

GONOPOD MEASUREMENTS FOR *P. LIRRANGENSIS* S. L. FROM LAKE MALAWI (MALAWI), LAKE KIVU (D. R. CONGO), AND KIGOMA, TANZANIA.

Also included are measurements for *P. montivagus* from Lake Itamba, Tanzania. Measurements taken from largest male representatives from each locality using Keyence digital microscope and recorded in mm.

| | ***P. orbitospinus* Lake Malawi (Malawi)** | ***P. lirrangensis* s. l. Lake Kivu, DR Congo** | ***P. lirrangensis* s. l. Kigoma, Tanzania** | ***P. montivagus* Lake Itamba (Tanzania)** |
|---|---|---|---|---|
| ***G1 Subterminal segment (mm)*** | 8.1 | 7.5 | 7.8 | 8.9 |
| ***G1 Terminal article (mm)*** | 4 | 4.36 | 3.9 | 4.3 |
| ***Total*** | 12.1 | 11.86 | 11.7 | 13.2 |
| ***TA/SS*** | 0.49 | 0.58 | 0.5 | 0.47 |
| ***G1 Terminal article angle*** | 50° | 52° | 47° | 56° |
| | | | | |
| ***G2 Subterminal segment (mm)*** | 10.5 | 9.5 | 6 | 10.2 |
| ***G2 Terminal article (mm)*** | 6.7 | 6.9 | 3.6 | 7.2 |
| ***Total*** | 17.2 | 16.4 | 9.6 | 17.4 |
| ***TA/SS*** | 0.64 | 0.73 | 0.6 | 0.71 |

# APPENDIX B

PROPORTIONAL SIZE OF LEG SEGMENTS FOR WALKING LEGS P2-P5 FOR *P. LIRRANGENSIS* S. L. FROM LAKE MALAWI (MALAWI), LAKE KIVU (D. R. CONGO), AND KIGOMA, TANZANIA.

Measurements taken manually using calipers before calculating proportions. Largest male representatives used, when applicable. Measurements taken from published photographs of specimens from Liranga, Kisangani, and (one of two) Lake Kivu localities.

| P5/FW | **Lirranga, DR Congo (CW 53.9)** | | **Kisangani, DR Congo (CW 64)** | | Lake Kivu, DR Congo (CW 46.5) | Lake Kivu, DR Congo (CW 55) (PHOTO) | **Mean** | | Malagarasi, Tanzania (CW 80.1) | Kigoma, Tanzania (CW 39.5) | **Mean** | | Lake Malawi, Malawi (CW 59) | Lake Malawi, Malawi (CW 54.7) | Lake Malawi, Malawi (CW 51.1) | Lake Malawi, Malawi (CW 51.1) | **Mean** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Merus | **1.2** | | **1.2** | | 1.11 | 1.10 | **1.1** | | 1.11 | 1.20 | **1.2** | | 1.35 | 1.51 | 1.47 | 1.26 | **1.4** |
| Carpus | **0.7** | | **0.6** | | 0.75 | 0.56 | **0.7** | | 0.71 | 0.74 | **0.7** | | 0.76 | 0.88 | 0.86 | 0.81 | **0.8** |
| Propodus | **0.8** | | **0.7** | | 0.76 | 0.71 | **0.7** | | 0.78 | 0.74 | **0.8** | | 0.88 | 1.01 | 0.96 | 0.89 | **0.9** |
| Dactylus | **0.8** | | **0.6** | | 0.72 | 0.63 | **0.7** | | 0.72 | 0.73 | **0.7** | | 1.00 | 0.89 | 0.91 | 0.80 | **0.9** |
| | | | | | | | | | | | | | | | | | |
| P4/FW | **LIRRANGA** | | **KISANGANI** | | KIVU | KIVU (PHOTO) | | | TAN (MAL) | TAN (KIGOMA) | | | ORBITO | MALAWI | MALAWI | MALAWI | |
| Merus | | | **1.5** | | 1.45 | | **1.5** | | 1.40 | 1.43 | **1.4** | | 1.94 | 1.88 | 1.90 | | **1.9** |
| Carpus | | | **0.7** | | 0.77 | | **0.8** | | 0.75 | 0.73 | **0.7** | | 0.88 | 0.94 | 0.91 | | **0.9** |
| Propodus | | | **0.9** | | 0.95 | | **1.0** | | 0.88 | 0.81 | **0.8** | | 1.35 | 1.32 | 1.21 | | **1.3** |
| Dactylus | | | **1.0** | | 1.01 | | **1.0** | | 0.98 | 0.96 | **1.0** | | 1.29 | 1.30 | 1.25 | | **1.3** |
| | | | | | | | | | | | | | | | | | |
| P3/FW | **LIRRANGA** | | **KISANGANI** | | KIVU | KIVU (PHOTO) | | | TAN (MAL) | TAN (KIGOMA) | | | ORBITO | MALAWI | MALAWI | MALAWI | |
| Merus | | | **1.5** | | 1.64 | | **1.6** | | 1.54 | 1.61 | **1.6** | | 2.00 | 1.94 | 1.94 | 1.65 | **1.9** |
| Carpus | | | **0.6** | | 0.70 | | **0.7** | | 0.81 | 0.81 | **0.8** | | 0.88 | 0.91 | 0.90 | 0.82 | **0.9** |
| Propodus | | | **0.9** | | 1.03 | | **1.0** | | 0.97 | 0.86 | **0.9** | | 1.18 | 1.22 | 1.16 | 1.09 | **1.2** |
| Dactylus | | | **1.1** | | 1.05 | | **1.0** | | 0.72 | 0.97 | **0.8** | | 1.24 | 1.21 | 1.16 | 1.08 | **1.2** |
| | | | | | | | | | | | | | | | | | |
| P2/FW | **LIRRANGA** | | **KISANGANI** | | KIVU | KIVU (PHOTO) | | | TAN (MAL) | TAN (KIGOMA) | | | ORBITO | MALAWI | MALAWI | MALAWI | |
| Merus | | | | | 1.20 | | **1.2** | | 1.23 | 1.17 | **1.2** | | 1.65 | 1.58 | 1.53 | 1.36 | **1.5** |
| Carpus | | | | | 0.67 | | **0.7** | | 0.68 | 0.63 | **0.7** | | 0.71 | 0.78 | 0.76 | 0.69 | **0.7** |
| Propodus | | | | | 0.70 | | **0.7** | | 0.70 | 0.59 | **0.6** | | 0.82 | 0.88 | 0.91 | 0.82 | **0.9** |
| Dactylus | | | | | 0.82 | | **0.8** | | 0.83 | | **0.8** | | 0.71 | 0.95 | 0.99 | 0.89 | **0.9** |

# APPENDIX C

## MEASUREMENTS OF LEG SEGMENTS FOR WALKING LEGS P2-P5 FOR SPECIMENS OF *P. LIRRANGENSIS* S. L. FROM LAKE MALAWI, LAKE KIVU, TANZANIA, D. R. CONGO.

Measurements taken manually along midline of each segment using calipers and recorded in mm. Published photographs of specimens were measured for the samples from lake Kivu (Bott,1955), Liranga, and Kisangani (Rathbun, 1921). The holotype from Lake Malawi (Cunnington, 1907) was measured directly.

| **KIVU 2018.306, cw 46.5** | Periopod 1 | P2 | P3 | P4 | P5 | FW | P5/FW |
|---|---|---|---|---|---|---|---|
| Merus | 19.5 | 18.2 | 24.9 | 22.1 | 16.9 | 15.2 | 1.1 |
| Carpus | 14.2 | 10.2 | 10.6 | 11.7 | 11.4 | 15.2 | 0.8 |
| Propodus | 34.8 | 10.7 | 15.7 | 14.5 | 11.5 | 15.2 | 0.8 |
| Dactylus | 20.3 | 12.5 | 15.9 | 15.3 | 10.9 | 15.2 | 0.7 |

| **KIVU BOTT XVI PHOTO** | cw 55, fw 16 | P2 | P3 | P4 | P5 | FW | P5/FW |
|---|---|---|---|---|---|---|---|
| Merus | | | | | 17.6 | 16 | 1.1 |
| Carpus | | | | | 9 | 16 | 0.6 |
| Propodus | | | | | 11.3 | 16 | 0.7 |
| Dactylus | | | | | 8 | 16 | 0.5 |

| **MALAWI 2018.309** | Periopod 1 | P2 | P3 | P4 | P5 | FW | P5/FW | cw 54.7 |
|---|---|---|---|---|---|---|---|---|
| Merus | 26.9 | 24.4 | 29.9 | 29 | 23.2 | 15.4 | 1.5 | |
| Carpus | 18.8 | 12 | 14 | 14.4 | 13.6 | 15.4 | 0.9 | |
| Propodus | 52.9 | 13.6 | 18.8 | 20.4 | 15.5 | 15.4 | 1.0 | |
| Dactylus | 29.8 | 14.7 | 18.6 | 20 | 13.7 | 15.4 | 0.9 | |

| **MALAWI NMU1972.04** | Periopod 1 | P2 | P3 | P4 | P5 | FW | P5/FW | cw 51.1 |
|---|---|---|---|---|---|---|---|---|
| Merus | 22.2 | 21.4 | 27.1 | 26.6 | 20.6 | 14 | 1.5 | |
| Carpus | 16.2 | 10.6 | 12.6 | 12.7 | 12.1 | 14 | 0.9 | |
| Propodus | 37.5 | 12.7 | 16.2 | 17 | 13.5 | 14 | 1.0 | |
| Dactylus | 25.9 | 13.8 | 16.2 | 17.5 | 12.8 | 14 | 0.9 | |

| **TANZANIA 1971.15** | P1 | P2 | P3 | P4 | P5 | FW | P5/FW | Malagarasi |
|---|---|---|---|---|---|---|---|---|
| Merus | 31.3 | 32 | 40 | 36.5 | 28.8 | 26 | 1.1 | cw 80.1 |
| Carpus | 27.1 | 17.6 | 21 | 19.4 | 18.4 | 26 | 0.7 | |
| Propodus | 71 | 18.3 | 25.1 | 22.8 | 20.2 | 26 | 0.8 | |
| Dactylus | 44.7 | 21.5 | 25.4 | 25.4 | 18.8 | 26 | 0.7 | |

| **TANZANIA 1971.05 male** | P1 | P2 | P3 | P4 | P5 | FW | P5/FW | kigoma |
|---|---|---|---|---|---|---|---|---|
| Merus | 13 | 15.8 | 21.7 | 19.3 | 16.2 | 13.5 | 1.2 | cw 39.5 |
| Carpus | 11.2 | 8.5 | 10.9 | 9.9 | 10 | 13.5 | 0.7 | |
| Propodus | broken | 8 | 11.6 | 10.9 | 10 | 13.5 | 0.7 | |
| Dactylus | 15.5 | broken | 13.1 | 13 | 9.8 | 13.5 | 0.7 | |

| **NMU 09.1988k.1, cw 51.1** | P1 | P2 | P3 | P4 | P5 | FW | P5/FW |
|---|---|---|---|---|---|---|---|
| Merus | 20.5 | 21.7 | 26.2 | both abse | 20 | 15.9 | 1.3 |
| Carpus | 14.7 | 11 | 13 | | 12.9 | 15.9 | 0.8 |
| Propodus | 38.8 | 13 | 17.4 | | 14.2 | 15.9 | 0.9 |
| Dactylus | 22.5 | 14.1 | 17.2 | | 12.7 | 15.9 | 0.8 |

| P. orbitospinus holotype photo L. Malawi | | | (Cunnington, 1907) | | | | |
|---|---|---|---|---|---|---|---|
| (cw 56.9 mm) | P1 | P2 | P3 | P4 | P5 | FW | P5/FW |
| Merus | 0 | 28 | 34 | 33 | 23 | 17 | 1.4 |
| Carpus | 20 | 12 | 15 | 15 | 13 | 17 | 0.8 |
| Propodus | 45 | 14 | 20 | 23 | 15 | 17 | 0.9 |
| Dactylus | 31 | 12 | 21 | 22 | 17 | 17 | 1.0 |

| Female TYPE PHOTO, Lirranga (cw 53.8, fw 14 mm) | | | | | P5 | FW | |
|---|---|---|---|---|---|---|---|
| MERUS | | | | | 28.7 | 24.2 | 1.2 |
| CARPUS | | | | | 16.6 | 24.2 | 0.7 |
| PROPODUS | | | | | 18.2 | 24.2 | 0.8 |
| DACTYLUS | | | | | 18.2 | 24.2 | 0.8 |

| RATHBUN1921 PHOTO PLATEXXV cw 62, CL 48.1mm | | | | | P5 | FW | |
|---|---|---|---|---|---|---|---|
| Merus | KISANGANI CONGO | | | | 22.7 | 18.8 | 1.2 |
| Carpus | | | | | 10.8 | 18.8 | 0.6 |
| Propodus | | | | | 13.5 | 18.8 | 0.7 |
| Dactylus | | | | | 12 | 18.8 | 0.6 |

APPENDIX D

PROPORTIONAL SIZE OF LEG SEGMENTS FOR WALKING LEGS P2-P5 IN *P. MONTIVAGUS* FROM CHOLO MOUNTAIN IN MALAWI AND FROM TWO CRATER LAKES IN SOUTHWEST TANZANIA.

Table showing the proportions of leg segment lengths (against front width). Specimens from Lakes Itamba and Kyungululu in Tanzania here identified as a new species (*Potamonautes* sp. 2).

| P5/FW | Itamba 2018.308 (CW 51.2) | Itamba 2018.307 (CW 55.2) | **Mean** | | **Kyungululu 2018.310 (CW 56.8)** | | **Cholo Mtn., Malawi (Chace, 1953) photo** |
|---|---|---|---|---|---|---|---|
| Merus | 1.4 | | **1.4** | | **1.6** | | **1.4** |
| Carpus | 0.9 | | **0.9** | | **0.9** | | **0.7** |
| Propodus | 0.9 | | **0.9** | | **1.0** | | **0.8** |
| Dactylus | 1.0 | | **1.0** | | **1.0** | | **0.8** |
| | | | | | | | |
| P4/FW | | | | | | | |
| Merus | 1.8 | 1.6 | **1.7** | | **1.9** | | **1.6** |
| Carpus | 0.9 | 0.9 | **0.9** | | **1.0** | | **0.8** |
| Propodus | 1.1 | 1.1 | **1.1** | | **1.2** | | **0.9** |
| Dactylus | 1.3 | 1.2 | **1.2** | | **1.4** | | **1.1** |
| | | | | | | | |
| P3/FW | | | | | | | |
| Merus | 1.8 | 1.8 | **1.8** | | **2.0** | | **1.6** |
| Carpus | 0.9 | 0.9 | **0.9** | | **1.0** | | **0.8** |
| Propodus | 1.1 | 1.0 | **1.1** | | **1.2** | | **0.9** |
| Dactylus | 1.2 | 1.2 | **1.2** | | **1.3** | | **0.8** |
| | | | | | | | |
| P2/FW | | | | | | | |
| Merus | 1.6 | 1.5 | **1.5** | | **1.7** | | **1.2** |
| Carpus | 0.8 | 0.8 | **0.8** | | **0.9** | | **0.6** |
| Propodus | 0.8 | 0.8 | **0.8** | | **0.9** | | **0.7** |
| Dactylus | 1.0 | 1.0 | **1.0** | | **1.1** | | **0.9** |

# APPENDIX E

## MEASUREMENTS AND PROPORTIONAL SIZE OF LEG SEGMENTS FOR SPECIES OF *POTAMONAUTES* FOUND IN THE LAKE MALAWI BASIN.

Measurements were made along the midline of each segment using digital calipers and all results are recorded in mm. The relative proportions of each segment (against front width) are shown on the right of the green column.

| ***P. choloensis (CW 33.9, FW 11.3)*** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **NMU 4.VII.1988** | P2 | P3 | P4 | P5 | | | P2/FW | P3/FW | P4/FW | P5/FW |
| Merus | 12.9 | 17.8 | 17 | 13.6 | | Merus | 1.1 | 1.6 | 1.5 | 1.2 |
| Carpus | 6.8 | 8.2 | 8.9 | 7.8 | | Carpus | 0.6 | 0.7 | 0.8 | 0.7 |
| Propodus | 7 | 9.6 | 10.5 | 7.8 | | Propodus | 0.6 | 0.8 | 0.9 | 0.7 |
| Dactylus | 8.3 | 10.2 | 10.7 | 7.6 | | Dactylus | 0.7 | 0.9 | 0.9 | 0.7 |
| | | | | | | | | | | |
| ***P. obesus (CW 44.7, FW 15.6)*** | | | | | | | | | |
| **2018.312** | P2 | P3 | P4 | P5 | | | P2/FW | P3/FW | P4/FW | P5/FW |
| Merus | 16.1 | 20.2 | 18.3 | 15.8 | | Merus | 1.0 | 1.3 | 1.2 | 1.0 |
| Carpus | 8.6 | 9.5 | 8.9 | 8.6 | | Carpus | 0.6 | 0.6 | 0.6 | 0.6 |
| Propodus | 8.5 | 10.2 | 10.5 | 8.9 | | Propodus | 0.5 | 0.7 | 0.7 | 0.6 |
| Dactylus | 11.3 | 13 | 12 | 8 | | Dactylus | 0.7 | 0.8 | 0.8 | 0.5 |
| | | | | | | | | | | |
| ***P. bayonianus (CW 53.4, FW 18.6)*** | | | | | | | | | |
| **NMU DRC 14.III.1970** | P2 | P3 | P4 | P5 | | | P2/FW | P3/FW | P4/FW | P5/FW |
| Merus | 20 | 25.2 | 24 | 19.3 | | Merus | 1.1 | 1.4 | 1.3 | 1.0 |
| Carpus | 11.8 | 14 | 13.5 | 12.4 | | Carpus | 0.6 | 0.8 | 0.7 | 0.7 |
| Propodus | 12.2 | 14.4 | 14.3 | 12 | | Propodus | 0.7 | 0.8 | 0.8 | 0.6 |
| Dactylus | 14 | 16.6 | 16.8 | 12.3 | | Dactylus | 0.8 | 0.9 | 0.9 | 0.7 |
| | | | | | | | | | | |
| ***P. bellarussus (CW 43.1, FW 13.4)*** | | | | | | | | | |
| **2018.311** | P2 | P3 | P4 | P5 | | | P2/FW | P3/FW | P4/FW | P5/FW |
| Merus | 16.4 | 21.2 | 19.7 | 14.9 | | Merus | 1.2 | 1.6 | 1.5 | 1.1 |
| Carpus | 8.5 | 10.7 | 11.1 | 10 | | Carpus | 0.6 | 0.8 | 0.8 | 0.7 |
| Propodus | 8.3 | 10.8 | 11 | 8.3 | | Propodus | 0.6 | 0.8 | 0.8 | 0.6 |
| Dactylus | 10.7 | 13.3 | 12.5 | 9 | | Dactylus | 0.8 | 1.0 | 0.9 | 0.7 |
| | | | | | | | | | | |
| ***P. suprasulcatus (CW 60.3, FW 16.6)*** | | | | | | | | | |
| **NMU 09.1965.1** | P2 | P3 | P4 | P5 | | | P2/FW | P3/FW | P4/FW | P5/FW |
| Merus | 22.7 | 26.1 | 26.8 | 20.6 | | Merus | 1.4 | 1.6 | 1.6 | 1.2 |
| Carpus | 12.3 | 13.6 | 14.6 | 13.8 | | Carpus | 0.7 | 0.8 | 0.9 | 0.8 |
| Propodus | 11.2 | 14 | 15.1 | 12.5 | | Propodus | 0.7 | 0.8 | 0.9 | 0.8 |
| Dactylus | 15.1 | 17 | 18.3 | 14 | | Dactylus | 0.9 | 1.0 | 1.1 | 0.8 |

APPENDIX F

MEASUREMENTS OF LEG SEGMENTS FOR *P. MONTIVAGUS* FROM MALAWI AND SPECIES OF *POTAMONAUTES* FROM TWO TANZANIAN CRATER LAKES.

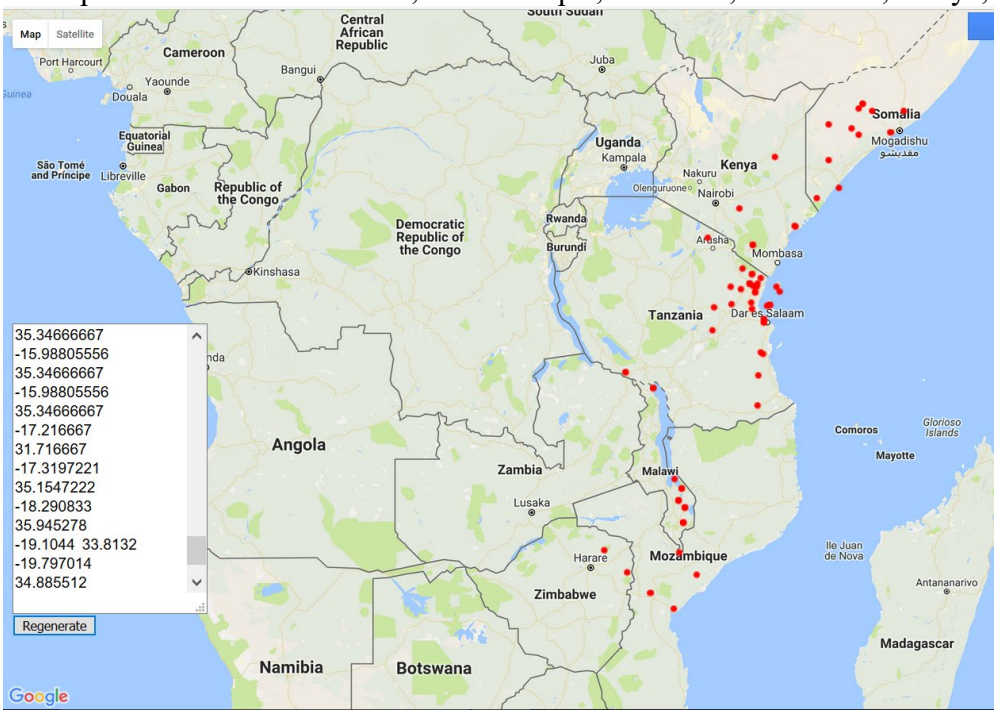
Specimens from Lakes Itamba and Kyungululu in Tanzania were here identified as a new species (*Potamonautes* sp. 2). Measurements were made along the midline of each segment using digital calipers and all results are recorded in mm.

| Largest male representatives; | Leg measurements in mm | | | | | |
|---|---|---|---|---|---|---|
| **ITAMBA 2018.308** | Periopod 1 | P2 | P3 | P4 | P5 | FW |
| Merus | 22 | 22 | 25.8 | 25 | 19.5 | 14 |
| Carpus | 17.3 | 11.1 | 13 | 12.8 | 12.5 | |
| Propodus | 45.3 | 11.2 | 14.8 | 15 | 13.1 | |
| Dactylus | 28.5 | 14.5 | 17.1 | 17.5 | 13.9 | |
| | | | | | | |
| **ITAMBA 2018.307** | Periopod 1 | P2 | P3 | P4 | P5 | FW |
| Merus | 24.4 | 23 | 26.5 | 24.7 | absent | 15.1 |
| Carpus | 17 | 12.2 | 13.8 | 14.2 | | |
| Propodus | 40.1 | 12.5 | 15.8 | 16.8 | | |
| Dactylus | 25.1 | 15.8 | 17.5 | 17.6 | | |
| | | | | | | |
| **KYUNGULULU 2018.310** | Periopod 1 | P2 | P3 | P4 | P5 | FW |
| Merus | 26.5 | 24.6 | 28.7 | 26.6 | 22.2 | 14.2 |
| Carpus | 20.3 | 12.5 | 14.5 | 14.6 | 13.2 | |
| Propodus | 53.5 | 13.4 | 16.7 | 16.7 | 13.7 | |
| Dactylus | 32.1 | 15.2 | 18.8 | 19.3 | 14.5 | |
| | | | | | | |
| **Cholo Mtn. (Chace, 1953)** | Periopod 1 | P2 | P3 | P4 | P5 | FW |
| Merus | | 21 | 27 | 28 | 23 | 17 |
| Carpus | | 11 | 13 | 14 | 12 | |
| Propodus | | 12 | 15 | 15 | 13 | |
| Dactylus | | 16 | 13 | 18 | 14 | |

APPENDIX G

MAP SHOWING THE DISTRIBUTION OF *POTAMONAUTES OBESUS* (H. MILNE EDWARDS, 1868).

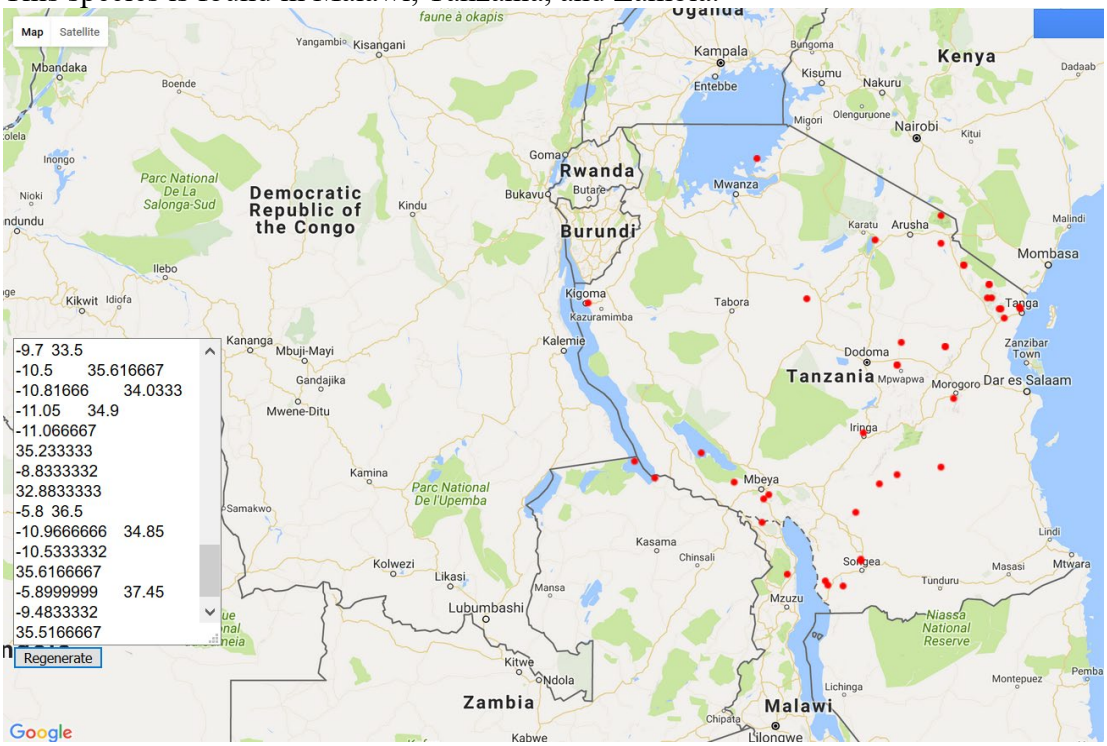
This species is found in Malawi, Mozambique, Tanzania, Zimbabwe, Kenya, and Somalia.



APPENDIX H

MAP SHOWING THE DISTRIBUTION OF *POTAMONAUTES SUPRASULCATUS* (HILGENDORF, 1898).

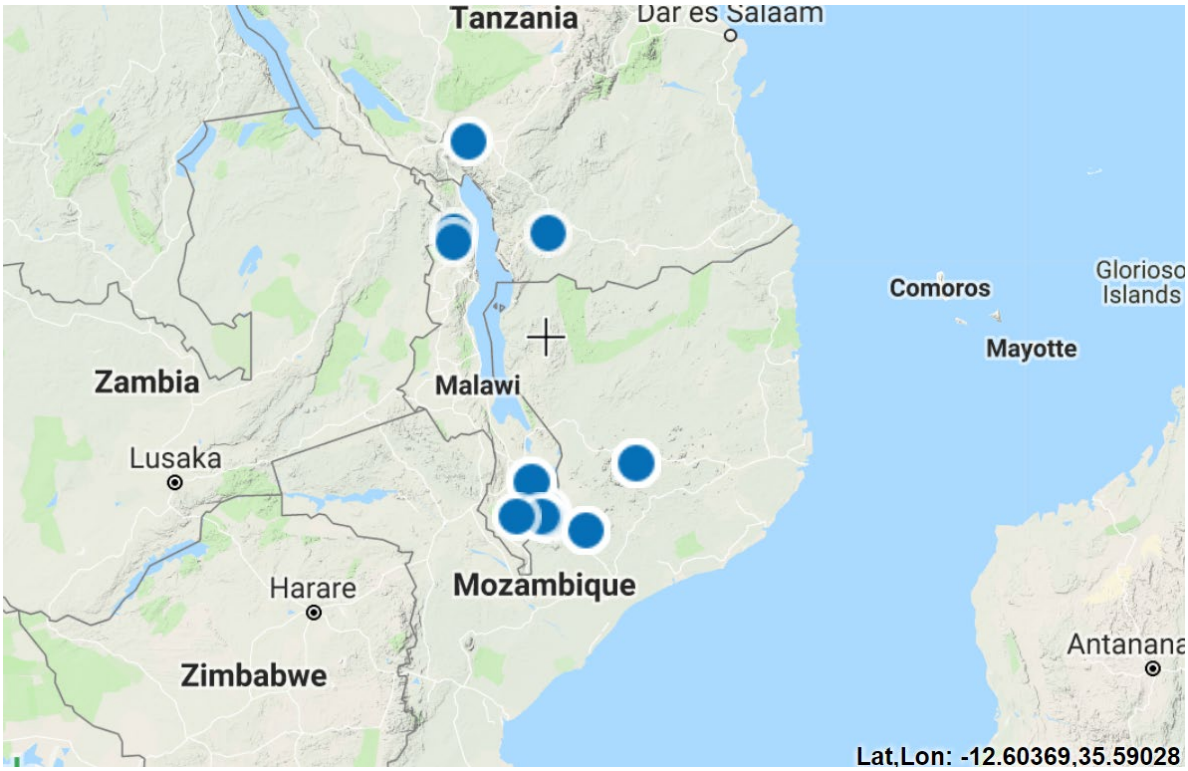
This species is found in Malawi, Tanzania, and Zambia.



APPENDIX I

MAP SHOWING THE DISTRIBUTION OF *POTAMONAUTES CHOLOENSIS* (CHACE, 1953).

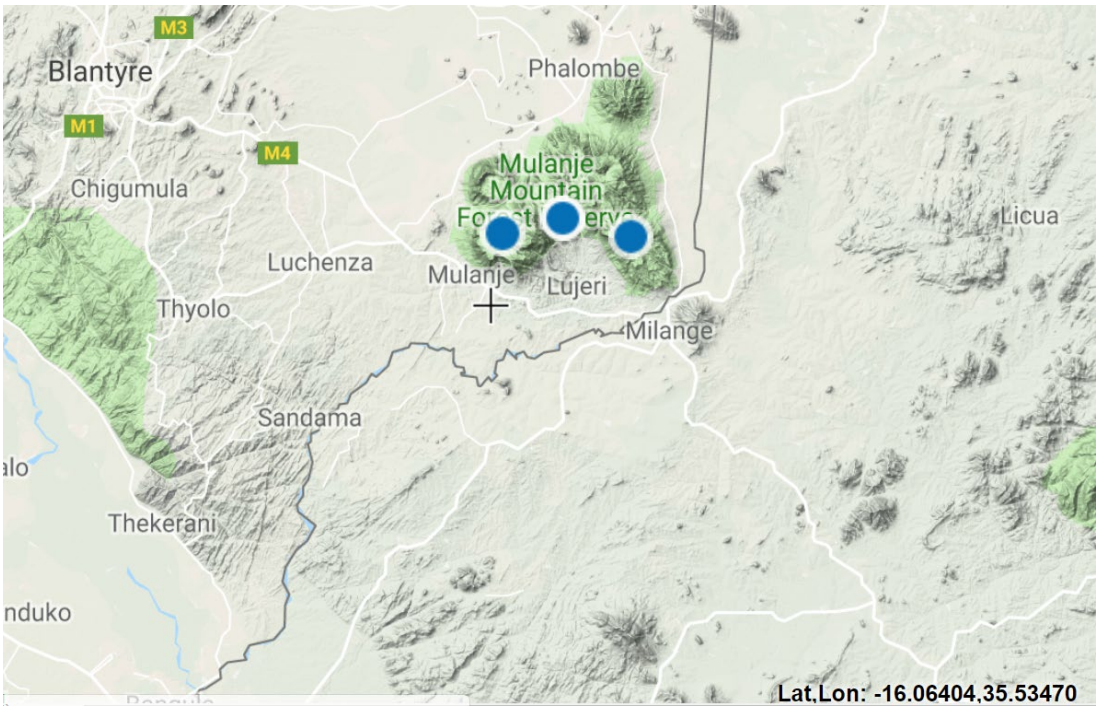
This species is found in Malawi, Tanzania, and Mozambique.



APPENDIX J

MAP SHOWING THE DISTRIBUTION OF *POTAMONAUTES MULANJEENSIS* (DANIELS & BAYLISS, 2012).

This speciesis only known from Mount Mulanje in Malawi.



APPENDIX K

MAP SHOWING THE DISTRIBUTION OF POTAMONAUTES BELLARUSSUS (DANIELS, PHIRI, & BAYLISS, 2014).

This species is found in Mozambique and Tanzania.