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Research Article

Molecular characterisation of the globally invasive freshwater crustacean *Cherax quadricarinatus* (von Martens, 1868) in Zambia

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Abstract

Non-indigenous species are significantly impacting ecosystems, with crayfish becoming a major concern globally. Crayfish are non-native to the African mainland and have been introduced to enhance aquaculture and for ornamental purposes. This study focuses on *Cherax quadricarinatus*, a crayfish species native to Queensland and the Northern Territory in Australia as well as to southeastern Papua New Guinea, which has established populations in Zambia's Kafue River and Lake Kariba. We used two mitochondrial markers (COI and 16S rRNA) to assess the haplotype diversity of the two populations and compared these with other non-African populations. Both COI and 16S sequences from Zambia form a major haplotype, which is common throughout the invasive range as well as parts of the native range in Australia. Our data indicates that the widespread haplotype originated from a specific and fairly large source population of Australia that spreads through human-mediated transport. The presence of the species in Zambia raises concerns about the impact of the species on various habitats and competition with native species. Thus, our study emphasizes the need for monitoring and management strategies to prevent further spread of the species in the country and abroad.

Key words: crayfish, DNA barcoding, cytochrome *c* oxidase subunit I, 16S rRNA, mitochondrial DNA, non-indigenous species (NIS), Africa

Introduction

Non-indigenous species (NIS) are among the most important anthropogenic factors that have impacted various ecosystems. Though introductions have taken place since time immemorial, the pace of these introductions has increased with modern travel and trade (Meyerson and Mooney 2007). At a global scale, the increase in NIS shows no indication of saturation (Seebens et al. 2017, 2021), and plants, arthropods, fish, and mammals are of greatest international interest and thus represent the most studied taxonomic groups in that context (Turbelin et al. 2017). In recent decades, non-native crayfish have become a major focus of biological invasion studies on a global scale (Gherardi 2007).

Crayfish has over 640 described species worldwide belonging to the two superfamilies Astacoidea and Parastacoidea (Crandall and Buhay 2008). North America, South America, Asia, Europe, and Australia is where they naturally occur but they are non-native to the African continent (Crandall and Buhay 2008). Due to their unique display of various biological, physical and commercial attributes, they have become a valuable resource in the aquaculture and ornamental industry (Lodge et al. 2012; Saoud et al. 2013). This has resulted in the wide translocation of the species to many countries worldwide, including the African continent (Belle and Yeo 2010; Madzivanzira et al. 2020, 2021; Haubrock et al. 2021; Yanai et al. 2024).

NIS of crayfish pose severe threats to biodiversity, food web structure, and physical habitat of non-native ecosystems (Nishijima et al. 2017). As both ecological engineers and keystone species, crayfish can occupy multiple trophic levels due to their omnivorous diet, which has a strong influence on the energy flow of the ecosystem and can disrupt the ecological functioning of freshwater ecosystems (O'Hea Miller et al. 2024). Additionally, non-indigenous crayfish exhibit aggressive behaviour, which is considered a key threat to native species and plays a vital role in their invasion success (O'Hea Miller et al. 2022). As a result, crayfish may be considered to be among the worst global invaders in freshwaters, with populations documented on nearly all continents except Antarctica (Lodge et al. 2012).

In Africa, there is evidence of the introduction of nine species of crayfish and the establishment of five species, namely *Astacus astacus* (Linnaeus, 1758), *Cherax quadricarinatus* (von Martens, 1868), *Faxonius limosus* (Rafinesque, 1817), *Procambarus clarkii* (Girard, 1852), and *Procambarus virginalis* Lyko, 2017 (Madzivanzira et al. 2020). Here, we report the spread of *C. quadricarinatus* in Zambia, a native species to Queensland and the Northern Territory in Australia, and southeastern Papua New Guinea (PNG) (Baker et al. 2008; Pinder et al. 2019). Occurrence data from the Global Biodiversity Information Facility (GBIF; https://www.gbif.org) show the occurrence of the species in at least 29 countries around the globe, and 5 of these countries are in Southern Africa (Figure 1).

It is documented that three *Cherax* species were translocated from South Africa to Zambia in the early 1990s, but only *C. quadricarinatus* was successfully established in the wild (Mikkola 1996; Douthwaite et al. 2018; Madzivanzira et al. 2020, 2021). However, the first documented introduction of *C. quadricarinatus* in Zambia's Zambezi River Basin was in 2001. The species were imported from Swaziland to two fish farms in the Kafue Flats and Lake Kariba (Douthwaite et al. 2018; Madzivanzira et al. 2020). Further reports indicate the presence of established populations of *C. quadricarinatus* in the Kafue River, Lake Kariba and most recently in Zambia's largest wetland in the upper Zambezi, the Barotse Floodplain (Nunes et al. 2016; Douthwaite et al. 2018; Madzivanzira et al. 2020, 2021).

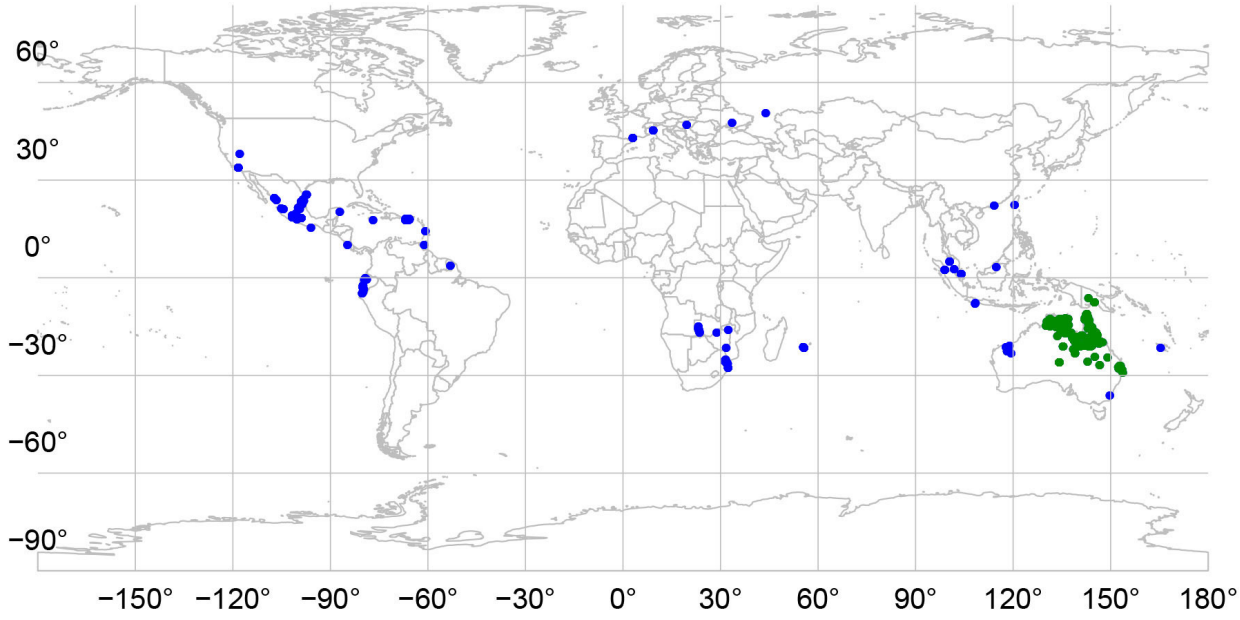

Figure 1. Global distribution of *C. quadricarinatus*. Green dots indicate the native range and blue dots indicate the invaded range. Distribution data taken from Global Biodiversity Information Facility (GBIF; https://www.gbif.org; accessed on 2024-07-16) based on 390 records (see text for details).

Mitochondrial and microsatellite markers suggest that the native *Cherax quadricarinatus* population consists of two highly divergent lineages in Australia and two other lineages in PNG (Baker et al. 2008). Other studies focusing on introduced populations evaluated the genetic variability of the species, revealing moderate allelic diversity in China (He et al. 2012) and Mexico (Max-Aguilar et al. 2021). The mitochondrial markers COI and 16S revealed a high haplotype diversity in Martinique (Baudry et al. 2020), China (Liu et al. 2021), and the Malay Peninsula (Mohd Dali et al. 2021). However, no studies inferred the origin and genetic variation of populations in Zambia or elsewhere in Africa. Here, we used two common mitochondrial barcoding markers to determine the haplotype diversity of two populations of *C. quadricarinatus* in the Kafue River and Lake Kariba, Zambia. We compare the African populations with all previously genotyped populations from both the global invasive range and the native range of the species in Australia and PNG.

Materials and methods

Occurrence data

Data on the worldwide distribution of *C. quadricarinatus* was obtained from the Global Biodiversity Information Facility (GBIF; https://www.gbif.org). To ensure spatial accuracy, the *CoordinateCleaner* R package version 3.0.1 (Zizka et al. 2019) was used to detect and filter out erroneous coordinates, including impossible locations, duplicates, missing coordinates and invalid coordinates.

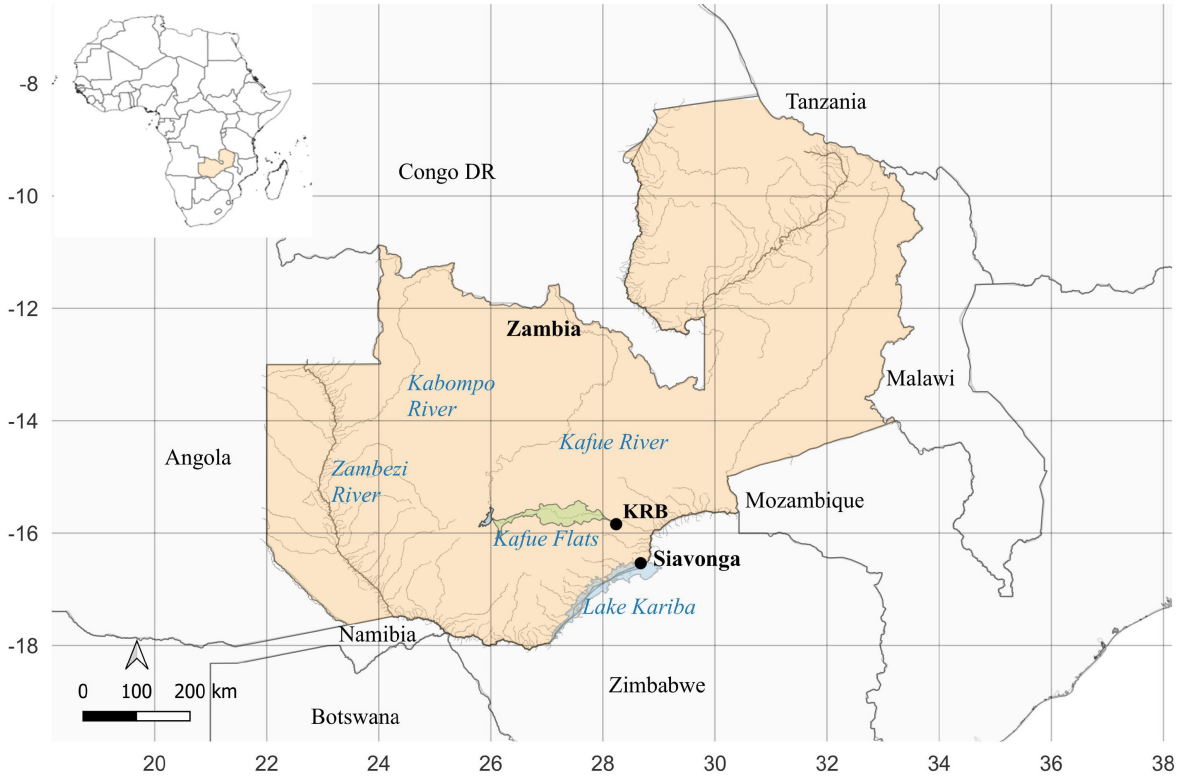

Figure 2. Sampling points (black dots) of *C. quadricarinatus* in Kafue town at Kafue River Bridge (KRB) and Siavonga at Lake Kariba, Zambezi River, Zambia.

Study area

The study was conducted in the Kafue River and Lake Kariba (Zambezi River basin of Zambia). The Kafue River is the largest tributary of the Zambezi River that flows exclusively in Zambia. Lake Kariba is located in the middle Zambezi and is shared between Zambia and Zimbabwe. It is the largest man-made lake by volume in the world (Chao et al. 2008). Sampling was conducted in the Kafue River Bridge section (KRB) in Kafue town (latitude: −15.841528, longitude: 28.233333) and Siavonga town (latitude: −16.535178, longitude: 28.673917) at the northern shore of Lake Kariba in the Southern Province of Zambia (Figure 2). These sampling points are about 90 km apart (linear distance) and as much as 180 km apart along the major drainages.

Sample collection

Ten specimens were collected (five from each location), morphologically identified (Horwitz 1995) and preserved in 80% ethanol. The specimens were transported to the Systematics & Biodiversity Lab at the Justus Liebig University Giessen, Giessen, Germany.

DNA extraction, PCR, and sequencing

All ten specimens were morphologically identical based on key diagnostic traits. Given this uniformity, two representative specimens were used for DNA extraction for each location. DNA was extracted from the abdominal

tissue using the CTAB protocol as described by Wilke et al. (2006). Fragments of mitochondrial markers, COI and 16S were amplified using primers COF14 (Folmer et al. 1994) and COR722b (Wilke and Davis 2000) and 16Sar and 16Sbr (Palumbi et al. 1991), respectively. PCR was performed using the same settings for both markers including an initial denaturation step at 94 °C for 4 min. The PCR run for 40 cycles including denaturation (94 °C for 60 s), annealing (45 °C for 60 s for 5 cycles and another 35 cycles at 48 °C for 45 s), elongation (72 °C for 30 s), and final elongation (72°C for 3 min). Bidirectional sequencing was performed on an ABI 3730xl DNA Analyzer using the BigDye Terminator Kit (Life Technologies, LGC Genomics GmbH, Berlin, Germany). Vouchers are deposited in the University of Giessen Systematics and Biodiversity collection (UGSB 30107, UGSB 30108, UGSB 30117, and UGSB 30135).

Alignment and dataset composition

DNA electropherograms were manually edited and aligned using BioEdit version 7.2 (Hall 1999). Molecular identification of the specimen was then performed using BLAST (Altschul et al. 1990; Camacho et al. 2009). The COI dataset (658 bp length) was supplemented with all previously published relevant sequences available on GenBank including from Australia (DQ006294, HG942364), PNG (KU821427), Martinique (MW300737 to MW300837), Italy (OM728391–OM728394), Indonesia (KX377345–KX377348), Israel (OQ594417), Malaysia (MT924262, MW591032, NC022937), China (MT966719–MT966724 and MT966728–MT966753), Hungary (KY745779), and Singapore (MT891189). We also compiled a 16S dataset, because a considerably higher number of haplotypes from the native range were available on GenBank. The 16S dataset (580 bp length) included sequences from China (MG199588, MH919311–MH919314, MH919316–MH919317), Hungary (OL780444), Indonesia (KX258721–KX258724), Malaysia (OP389118–OP389121), Singapore (MT890203), PNG (EU244889–EU244890, EU244893), and Australia (AF135975, AY150034, DQ006552, EF493080, EU244879–EU244888, EU244891–EU244892, KJ920763–KJ920764).

The final 16S dataset was aligned using the MAFFT web service (Katoh and Tor 2008; Katoh and Standley 2013; https://www.ebi.ac.uk/jdispatcher/msa/mafft?stype=dna), whereas the final COI dataset was aligned by eye in BioEdit. Two haplotype networks were constructed using TCS version 1.2.1 (Clement et al. 2000) and a 95% connection limit. Uncorrected genetic p-distances were calculated using MEGA version 11 (Tamura et al. 2021).

Results

The COI dataset including a total of 156 sequences revealed 28 haplotypes distributed across eleven countries (Figure 3, Supplementary material Table S1). Due to the unequal number of sequences obtained, the dataset was reduced

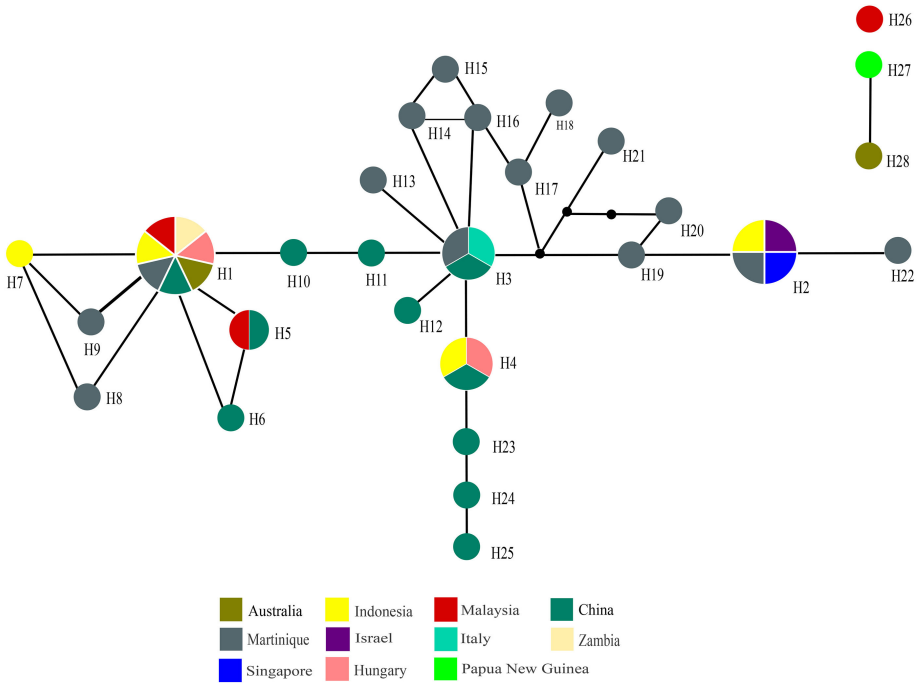

Figure 3. TCS haplotype network of *C. quadricarinatus* based on COI (658 bp, N = 156 sequences from 11 countries) showing the relationship of 28 haplotypes as represented by colour-coded circles. The size of the circle is proportional to the number of countries shared by the respective haplotype. Note that the dataset is reduced to single specimens per country per haplotype.

to a single representative per country. Haplotype 1 (from here on called H1) is a cosmopolitan haplotype that is found in seven out of eleven countries, including the native range of Australia as well as Malaysia, Indonesia, China, Martinique, and Hungary. All samples from Zambia are genetically identical and clustered within this major haplotype. Haplotype H1 is also closely related to haplotypes from four other countries, i.e. China (H5, H6, H10), Malaysia (H5), Indonesia (H7), and Martinique (H8, H9). Interestingly, a very high number of haplotypes is found in Martinique (N = 15), however, they are only separated by a few mutational steps from each other.

The native range of Australia (H28) and PNG (H27), and the invasive region of Malaysia (H28) represent isolated haplotypes that are not connected to the main haplotype network based on the 95% connection limit. Mean pairwise distances were generally low (about 1.7%), indicating close relatedness of the haplotypes, except for the divergent haplotypes H27 and H28 from the native range, which showed a considerable mean genetic distance to the remaining haplotypes of about 6.0 and 5.8%, respectively (Table S3).

In the 16S dataset including a total of 43 sequences, 13 haplotypes were identified (Figure 4, Table S2). H1 and H2 were cosmopolitan, occurring in six and five out of eight countries, respectively. Samples from Zambia were again genetically identical and clustered in the major haplotype called H1, which is shared with 5 other countries including the native range of Australia. The remaining haplotypes are mainly closely related to haplotypes H1 and H2 and are mainly only separated by a few mutational steps. Similar to the

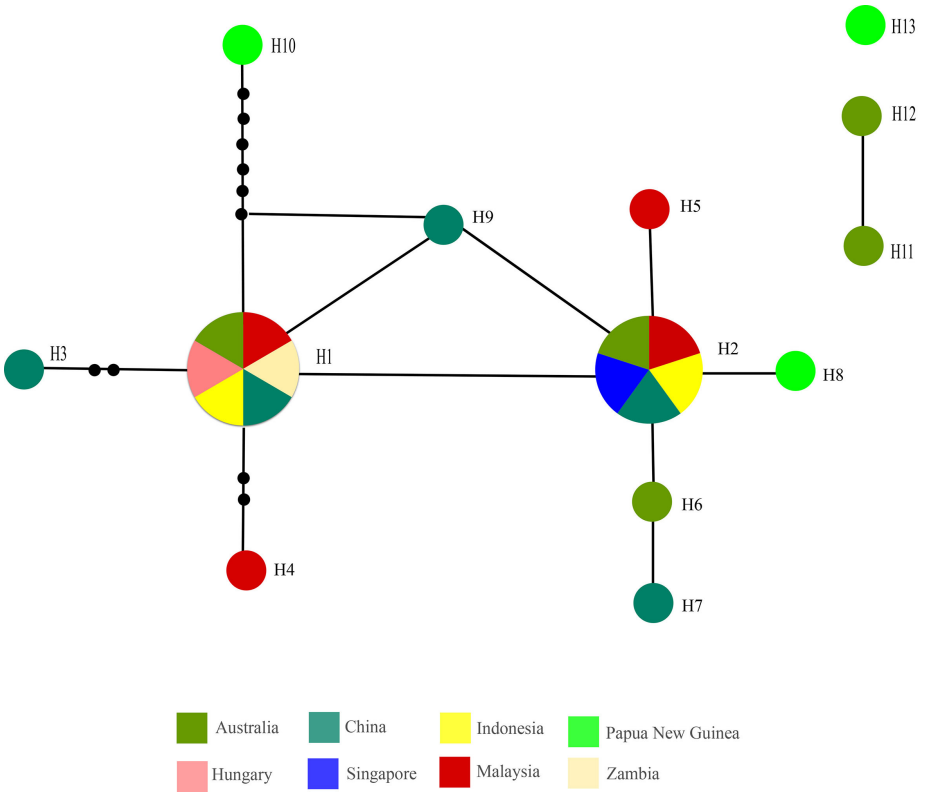

Figure 4. TCS haplotype network of *C. quadricarinatus* based on 16S (580 bp, N = 43 sequences from 8 countries) showing the relationship of 13 haplotypes as represented by a colour-coded circle. The size of the circle is proportional to the number of countries shared by the respective haplotype. Note that the dataset is reduced to single specimens per country per haplotype.

COI haplotype network, we also observed divergent haplotypes from the native range (H10–H13) that are either separated by a considerable number of mutational steps (H10) or that represent haplotypes not connected to the main haplotype network based on the 95% connection limit (H11–H13). Similar to the COI dataset, the mean pairwise distances were generally low (about 1.8%), except for the divergent haplotypes H11, H12, and H13 from the native range, which showed a considerable mean genetic distance to the remaining haplotypes of about 3.5%, 3.0%, and 4.4%, respectively (Table S4).

Discussion

Haplotype diversity and geographic distribution

For both COI and 16S, all samples from Zambia belong to a single and widespread haplotype 1 (Figure 5), which is also found in the native range of Australia. This supports the hypothesis that only crayfish from eastern Australia have been exported overseas for culture (He et al. 2012). Thus, the widespread distribution of major haplotypes may support this translocation history and indicate a successful invasive expansion of a single or very few lineages from the original gene pool. In this respect, *C. quadricarinatus* resembles invasion patterns seen in other crayfish species globally such as

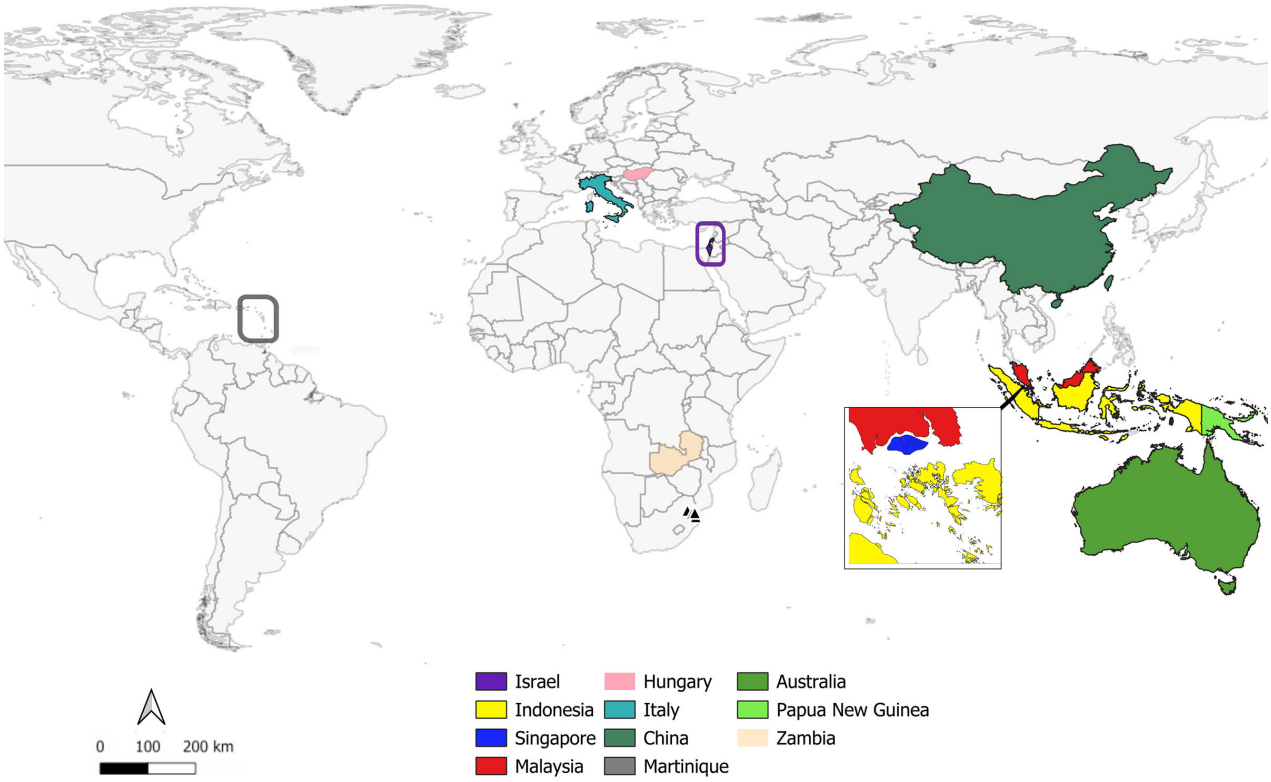

Figure 5. Global distribution of the main haplotypes H1 identified in the COI and 16S datasets of *C. quadricarinatus* that is also shared by the samples from Zambia from this study. Indonesian samples originate from West Java, while Malaysian samples are from the Malay Peninsula. The black triangles in South Africa represent the presumed source population for Zambia.

P. clarkii (Oficialdegui et al. 2019), which also indicates the presence of a single widespread haplotype and a few other haplotypes shared between countries.

The Zambian populations are supposed to originate from populations from South Africa and Eswatini (former Swaziland) (Douthwaite et al 2018). The first recorded introduction of *C. quadricarinatus* from Australia to South Africa dates back to 1988 and served for aquaculture research purposes (Nunes et al. 2017). However, permits for its use were not issued due to the species invasiveness concern. Thus, due to regulatory restrictions in South Africa because of the purported impacts, farmers continued their ventures in the 1990s in Eswatini's Sand River Dam, from where the species escaped and spread into South African waters through the Komati River (de Moor 2004; Nunes et al. 2017; Weyl et al. 2020), and eventual translocation and establishment in Zambia (Mikkola 1996; Douthwaite et al. 2018; Madzivanzira et al. 2020, 2021). The genetic composition and relationship of the presumed source population of Zambian *C. quadricarinatus* can only be traced when samples from South Africa and Eswatini become available.

The extent of spread and ecological impacts in Zambia

Cherax quadricarinatus is present in a variety of freshwater habitats in Zambia. Douthwaite et al. (2018) and Madzivanzira et al. (2020) indicate that these introductions occurred via fish farms in the upper Kafue River

catchment, the Kafue Flats and the Kariba Dam. Eilitta et al. (2023) reports several previously unknown sites where the species is currently present, i.e. the Kabompo River, the Nwambeshi River, the Kwando River, the Luena River, the Matebele Plains, and the Chingola Reservoir. In addition, Nunes et al. (2016) and Madzivanzira et al. (2021) reported a growing population in the Barotse Floodplains, with the species continuing to spread. It was previously reported that individuals of the species were present 95 km upstream in Lukulu from a point of introduction in Lealui, Mongu, and 100 km downstream in Senanga. With an average rate of spread estimated at 49 ± 29 km per year downstream and 12 ± 7 km per year upstream Madzivanzira et al. (2020, 2021). Currently, the species was detected at 113.96 km downstream in Senanga and 54.80 km upstream in Lukulu, with the estimated rate of spread approximated at 53.92 km per year downstream and 27.4 km per year upstream (Nawa et al. 2024).

Although the crayfish bring some economic benefits, we cannot ignore the impact on fishermen, who suffer losses due to the spoilage of fish caught in gill nets and the destruction of fishing gear (Douthwaite et al. 2018; Situmbeko et al. 2020; Eilitta et al. 2023). Furthermore, crayfish have profound ecological impacts on various ecosystems around the world (Geiger et al. 2005; Rodríguez-Barreras et al. 2020; Gherardi 2006, 2007). A close relative of *C. quadricarinatus*—*Procambarus clarkii*—spreading across East Africa gives an insight into the ecological damage crayfish can cause in African freshwaters. Gherardi et al. (2011) reported the drastic changes in community composition and food web in Lake Naivasha, where the species preys on the indigenous river crabs (*Potamonautes loveni*) and acts as transmitters of pathogens (Foster and Harper 2006). The species have also shown profound impacts on the lake's macrophytes by consumptive and non-consumptive destruction. Local knowledge about the effects and the extent of damage in Zambian waters is anecdotal. Reports on Lake Kariba indicate destruction of spawning grounds for fish, loss of macrophytes and modification of the benthic ecosystem. Studies indicate *C. quadricarinatus* to be an efficient predator of *Melanoides tuberculata* and *Bulinus globosus* gastropods under laboratory conditions (Monde et al. 2017) and on non-native *Tarebia granifera* and *M. tuberculata* as well as native *Neritina punctulata* gastropods in Martinique (Baudry et al. 2024). This factor may potentially contribute to the decline of gastropod populations and macroinvertebrates in various habitats in the invaded Zambezi River Basin. New strategies for mitigation or stopping the spread are thus urgently needed. However, the idea of the species as a food source or other forms of utilization such as feed or for sale for many Zambians is difficult to implement. Studies have shown high disposal of the species by fishermen in Itezhi-Tezhi, Siavonga, Kafue and Sinazongwe as the species have limited or no market locally (Situmbeko et al. 2020; Eilitta et al. 2023).

Our study highlighted the limited yet significant haplotype diversity of the species population in Zambia and its relatedness to other countries. Although the observed genetic variability may be limited based on the current data, *C. quadricarinatus* has demonstrated ecological adaptability observed from its spread across various regions. Our findings underscore the necessity of proactive monitoring and development of management strategies to mitigate the further spread of the species. Urgent action is thus required to address the increasing distribution of *C. quadricarinatus*. We recommend that current regulations should be tightened to (a) ban the import and sale of live crayfish species, (b) monitor the trapping and sale of crayfish for consumption purposes, and (c) achieve full licencing, with approved rearing and transport of live crayfish.

Authors' contribution

NM and CA: research conceptualization; NM and HM: sample design and methodology; NM and HM: investigation and data collection; NM, BS and CA: data analysis and interpretation; CA: funding provision; NM: writing – original draft; NM, HM, BS, and CA: writing – review and editing.

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Ethics and permits

Export permit from the Department of Fisheries, Zambia, permit number 0954904.

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Supplementary material

The following supplementary material is available for this article:

Table S1. COI haplotypes and their associated sequences.

Table S2. 16S haplotypes and their associated sequences.

This material is available as part of online article from:
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