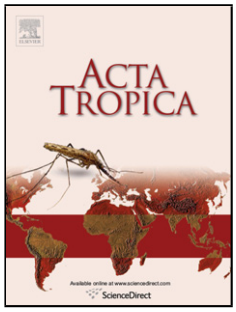
DNA barcoding of the medically important freshwater snail Physa acuta reveals multiple invasion events into Africa

Lawton, S.P., Allan, F., Hayes, P.M. and Smit, N.J.



Title: DNA barcoding of the medically important freshwater snail *Physa acuta* reveals multiple invasion events into Africa

Authors: Scott P. Lawton, Fiona Allan, Polly M. Hayes, Nico J. Smit

PII: S0001-706X(18)30235-3
DOI: https://doi.org/10.1016/j.actatropica.2018.08.027
Reference: ACTROP 4763

To appear in: *Acta Tropica*

Received date: 24-2-2018
Revised date: 22-7-2018
Accepted date: 24-8-2018

Please cite this article as: Lawton SP, Allan F, Hayes PM, Smit NJ, DNA barcoding of the medically important freshwater snail *Physa acuta* reveals multiple invasion events into Africa, *Acta Tropica* (2018), https://doi.org/10.1016/j.actatropica.2018.08.027

DNA barcoding of the medically important freshwater snail *Physa acuta* reveals multiple invasion events into Africa

Running head: BAR-CODING PHYSA ACUTA FROM AFRICA

Scott P. Lawton[1*], Fiona Allan[2], Polly M. Hayes[3], Nico J. Smit[4]

[1]Molecular Parasitology Laboratory, School of Life Sciences, Pharmacy and Chemistry, Kingston University London, Kingston upon Thames, Surrey, KT1 2EE, UK

[2]Wolfson Wellcome Biomedical Laboratories, Department of Life Sciences, Natural History Museum, London, SW7 5BD, UK

[3]Department of Biomedical Sciences, University of Westminster, London, W1W 6XH, UK

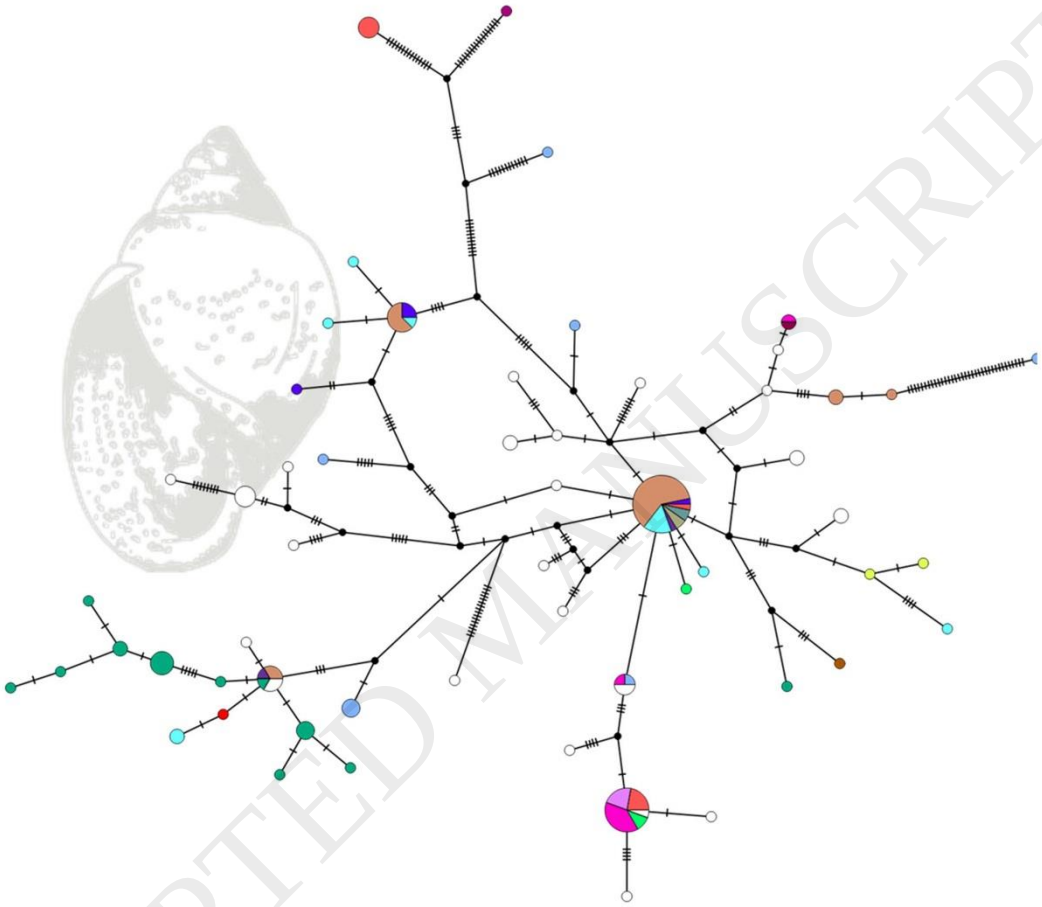
[4]Water Research Group, Unit for Environmental Sciences and Management, Potchefstroom Campus, North-West University, Potchefstroom, 2520, South Africa

*Correspondence: Scott Lawton, Molecular Parasitology Laboratory, School of Life Sciences, Kingston University, Kingston upon Thames, Surrey, KT1 2EE, UK

Tel.: +44 20 841 72103; Fax.: +44 20 841 72887

E-mail: s.p.lawton@kingston.ac.uk

Graphical Abstract



Highlights:

- First DNA barcoding analyses of African isolates of *Physa acuta*
- No distinct African specific lineages identified in *P. acuta*
- African isolates of *P. acuta* appears to have multiple origins as a result of both direct and secondary invasion events

Abstract

The medically important freshwater snail *Physa acuta* is highly invasive and has been reported in several freshwater environments across Africa. To identify species and provide initial insights into the origins of *P. acuta* into African fresh water environments standard molecular barcoding analyses, using the mitochondrial cytochrome c oxidase subunit I gene (COI), was performed on *P. acuta* isolates from Angola, Burundi and South Africa. Phylogenetic analyses Isolates from Africa could not be distinguished from *P. acuta* populations from other countries using Comparisons of COI sequences between isolates of *P. acuta* showed there to be no geographically specific clusters and the African isolates were distributed across four distinct unrelated clades suggesting several independent invasion events. Haplotype analyses indicated that there were a high number of haplotypes with low variation between them, which led to significant differences in AMOVA analyses between countries. This was further evidence of multiple invasion events suggesting multiple novel haplotypes being continually and independently introduced to each country. This approach not only provides initial insight into the invasion of Africa by *P. acuta* but a molecular method to monitor and manage the use of an agent of biological control.

Keywords: Africa; Biological control; DNA Barcoding; Invasion; *Physa acuta*

1. Introduction

Physa acuta (Draparnaud, 1805) (syn. *Physella acuta*) represents a paradox for medical malacology as it acts as an intermediate host of several human food borne trematode diseases, including echinostomiasis and fascioliasis (Dreyfuss et al., 2002; El–Bahy, 1997; Kanev, 1994), but can also replace snail species such as *Bulinus* and *Biomphalaria* which are responsible for the transmission of urinogenital and hepato-intestinal schistosomiasis throughout Africa (Dobson, 2004; Gashaw et al., 2008). Although thought to originate in North America, *P. acuta* has successfully invaded a range of natural and artificial freshwater habitats across the world and is considered to have become cosmopolitan over the past 200 years, invading Europe in the 19th century and further expanding into Asia, Australia, Africa and South America; partly attributed to the global aquarium and ornamental garden trade (Albrecht et al., 2009; Appleton, 2003; De Kock and Walmarans, 2007; Madsen and Frandsen, 1989).

It is the highly competitive nature of *P. acuta* that makes it a potential biological control agent against schistosomiasis as several studies have already shown that accidentally established populations of *P. acuta* replace native freshwater snail species throughout Africa (De Kock and Walmarans, 2007; Dillon et al., 2009; Dobson, 2004; Gashaw et al., 2008). This was particularly illustrated in the Vaal River system of South Africa, where *P. acuta* has become the most abundant snail species and has completely replaced several native species including *Bulinus tropicus*, previously considered to be the most widespread fresh water snail in the country (De Kock and Walmarans, 2007). This success results from *P. acuta*'s rapid generation time and high reproductive plasticity, including selfing, mixed mating and out crossing in either or both sexual roles, thus increasing population numbers rapidly and overwhelming other snail populations (Dillon et al., 2009). Importantly, Gashaw et al. (2008) also illustrated reduced growth rates and egg outputs in *Biomphalaria pfeifferi* grown in the

presence of *P. acuta*, suggesting a role for some form of chemical inhibitor secreted by the physid species. Similarly, *P. acuta* has also been shown to be refractory to schistosome infection, in miracidial choice experiments with *Biomphalaria alexandrina* from Egypt. *Schistosoma mansoni* miracidia were shown to infect *P. acuta* but could not reach patency suggest the snail could be a potential sink for the parasite (Yousif et al., 1998)

It is unclear if the introduction of *P. acuta* into Africa was the result of a single invasion event from North America, or multiple and secondary invasion events from elsewhere (De Kock and Walmarans, 2007). Several studies in other countries have tried to address this issue by using tenuous shell and genital morphological characteristics, as well as population reproductive compatibilities, to link physid populations to each other (Dillon et al., 2002; Dillion, 2009). Although these studies illustrated that established invasive populations of *P. acuta* were not reproductively isolated from those in North America, they did not provide any indication of the origins of invasive populations. Similarly, general identification of *P. acuta* has been challenging, as with many snail species, traditional identification is often erroneous as it depends on highly variable morphological features including shell shape and structure, the anatomy of reproductive systems, colourisation and skin patterning. In fact, in Al-Bdairi et al. (2014) study of freshwater snails of Iraq they illustrated that the distribution of *P. acuta* had been under estimated because it had historically been identified as *Bulinus truncatus*, as both species not only share habitat but also several of the same gross shell morphologies. More recently molecular approaches have been employed to identify and monitor the spread of medically important freshwater snails which has resulted not only in a deeper understanding of disease transmission patterns but also significant improvement in species identification. This is particularly true for the snail genera *Radix* (Lawton et al., 2015) and *Bulinus* (Kane et al., 2008) where DNA barcoding using sequences of the mitochondrial gene cytochrome c oxidase

subunit 1 (COI) was employed to identify morphologically indistinguishable snails, but also to provide initial insights into the relationships between distinct snail populations.

To date, there are only a few studies on the molecular identification of physid snails despite their medical and environmental importance. Wethington and Lydeard (2007) performed the most comprehensive phylogenetic study to date of physid snails using mitochondrial markers, including COI, illustrating the absolute requirement for molecular tools for identification, showing that in fact *P. acuta* could not be genetically distinguished from morphologically different species including *Physa integra*, *Physa virgata*, *Physa cubensis* and *Physa heterostropha*. More recently, work on the invasion of South America by *P. acuta* has overcome the issues in identification by utilising COI barcoding, highlighting a potential invasion event into Bolivia, Peru and Chile from the Caribbean (Albrecht et al., 2009; Collado, 2017). To date there have been no molecular studies to address the invasion of Africa by *P. acuta* and the current study is the first of its kind to use mitochondrial COI barcoding techniques on physid snails from Africa and aimed to accurately identify African isolates of *P. acuta*. Secondly, this study aimed to use COI DNA sequence data to provide initial insights into the potential origins of isolates sampled in Angola, Burundi and South Africa. Finally, the COI DNA sequences were used to estimate differentiation between geographical isolates *P. acuta* to highlight any major divergence events associated between the African isolates and those found elsewhere.

2. Materials and Methods

2.1 Collection of snail material

Physid snail populations were collected in October 2012 from the Vaal River (-26.943751º; 27.183524º) downstream of the town Parys, South Africa, in December 2013 in Angola (-8.57986º, 13.65779º) Caxito canal (Centre) (Allan et al., 2017) and in March 2015 in Burundi (3.031666667º; 29.38305556º) Ruke in a stream. Snails were collected using scoops and/or by

hand from aquatic vegetation along the river margins and were removed from reeds, algae, leaves or other plant debris using forceps. All snails were identified using shell morphological characteristics as a single species, *Physa acuta*. These were stored in 1L plastic containers filled with water from the site and taken to the laboratory. All snail material was eventually stored in 100% molecular grade ethanol for DNA extraction at a later date.

2.2 DNA extraction, PCR amplification and sequencing

A total of 30 individuals (Angola n=8; Burundi n=4; South Africa n=18) were used for molecular analysis to provide accurate identification of species and to identify the origins of the African population. Owing to the small size of these snails, the whole organism was used for DNA extraction after the shell had been removed. Whole genomic DNA was extracted using the manufacturer's protocols for animal tissue DNA extraction with the Qiagen DNeasy tissue kit (Qiagen Inc.). The DNA barcoding region of the COI gene was amplified using primers and protocols as described by Folmer et al. (1994). The single gene loci of COI was used due to a large amount of comparable data available from other studies of *P. acuta*, representing wide geographical coverage relative to other that of other markers. PCR reactions were performed using 12.5 µl of DreamTaq^{TM} PCR master mix (2X DreamTaq buffer, 0.4mM of each dNTP, 4mM $MgCl_2$), 1 µM of each primer, and 1-2 ng/µl of DNA, with final reactions made up to 25 µl with PCR grade water. Reactions were performed using a Veriti 96 well thermal cycler (Applied Biosystems^{TM}) PCR machine and 5 µl of each amplicon was visualised on a 1% agarose gel stained with gel red (Bioline). The remaining 20 µl of positive PCR products were sequenced at the DNA sequencing facility of the Natural History Museum, London, using fluorescent dye terminator sequencing kits (Applied Biosystems^{TM}), these reactions were performed using the same primers as used for the PCR reaction and then run on an Applied Biosystems 3730KL automated sequencer.

2.3 DNA sequence alignment, phylogenetic analysis and molecular taxonomic assessment of Physid snails

Resultant COI sequences were 655bp in length and were assembled and edited using Bioedit (Hall, 1999). In order to definitively identify the African physid snails as *Physa acuta* (accession number: MH649321 – MH649350) the sequences generated in the current study were aligned with comparative COI sequences of different physid species within the '*P. acuta*' species group as defined by Wethington and Lydeard (2007) phylogenetic analysis of Physidae. Where possible all published COI sequences for species within the "*Physa acuta"* group were used with the majority of sequences used being defined as the species *Physa acuta* (Supplementary data 1). Standard DNA barcoding approaches were employed to accurately identify the African physid snails. Initially, sequence alignments were performed using MUSCLE (http://www.ebi.ac.uk) and phylogenetic analysis was used to identify molecular operational taxonomic units (MOUT) based on species clustering into distinct well supported clades. Phylogenetic reconstruction using both neighbour joining (NJ) and maximum likelihood (ML) analyses were performed using MEGA7 (Tamura *et al*., 2011) on a final alignment of 545bp representing the data set with the most robust and comparable content allowing analysis within and between species of physids. Typical DNA barcoding NJ analysis was performed under the conditions of the Kimura's 2-parameter (K2P) evolution model and detailed evolutionary history was inferred using the ML phylogenetic analysis under the conditions of the Hasegawa-Kishino-Yano model over a gamma distribution and taking account of invariable sites (HKY+G+I). The model was identified because it had the lowest Baysesian information criterion scores relative to the other models tested. In both cases of phylogenetic reconstruction *B. truncatus, Bulinus nasutus* and *Bulinus africanus* were used as outgroups and nodal support was assessed using bootstraps calculated from 1000 replicates during tree construction.

As it is well recognised that species clustering can vary between phylogenetic algorithms the Automatic Barcode Gap Discovery (ABGD) method (http://wwwabi.snv.jussieu.fr/public/abgd/) was employed using default settings to sort the individual sequences of the "*Physa acuta*" group into genetic clusters and hypothetical species. Finally, uncorrected *p*-distances were calculated between each physid species based on the total number of transition and transversion. In order to define separate and discrete species groups the standard 3% nucleotide divergence as used in the ABGD algorithm was used.

2.4 Diversity and evolutionary association of African Physa acuta with other populations

Owing to the challenge of defining species of Physidae snails comparisons of the African *P. acuta* where only compared to published COI sequences from individuals already putatively identified as *P. acuta*. For completeness published sequences were taken from *P. acuta* populations from North and South America, Europe and Asia in order to identify any evolutionary relationship that the African isolates may have to other global populations and to provide insights into potential invasion events. Simple comparisons between geographical isolates of *P. acuta* were performed using standard measures of molecular diversity utilising DnaSP v5 (Librado and Rozas, 2009). Unique haplotypes were identified using comparative analysis within an alignment containing *P. acuta* sequences generated in this study and those published in studies on snails from other localities (supplementary data 1). The evolutionary interrelationships between unique *P. acuta* haplotypes was assessed by constructing the most parsimonious haplotype network with TCS as implemented in PopArt (Leigh and Bryant, 2015). Finally, to identify any significant genetic differentiation between *P. acuta* isolates analysis of molecular variance (AMOVA) was performed also in PopArt where genetic distance was considered between haplotypes and the frequency of such haplotypes between populations.

3. Results

3.1 DNA Barcoding and Molecular Identification of Physa acuta from Africa

Both NJ and ML analyses showed there to be six major clades/groupings between species with all the African *P. acuta* clustering within a major clade which contained all other published *P. acuta* sequences (Fig 1). The basal subclades within the major *P. acuta* clade were primarily formed from individuals from the USA, Canada or Mexico, however, it is important to note that sequences representing *P. acuta* from the USA or Canada occurred in every subclade thereafter. The African *P. acuta* fell into four distinct clades and which had no major geographical specificity. Interestingly, all four of the African clades also contained individuals from the New World and/or Europe (Fig 1). To ensure that the identification of *P. acuta* from Africa was accurate *p*-distances between isolates of *P. acuta* from other parts of the globe were compared showing there to be a relatively high divergence of 2.9% between sequences but below the typical 3% for species differentiation used for COI DNA barcoding. This was also supported by the ABGD method of delineating species based on DNA barcodes which defined six major groups/clusters specified to be species. These clusters were identical to those identified in the phylogenetic analyses and did not distinguish the African *P. acuta* from any other isolates sampled from other localities. This cluster analysis of the barcodes validated the molecular identification of *P. acuta* form Africa confirming species identification and the utility of the technique.

3.2 African Physa acuta and its relationship with other populations

Statistically parsimonious networks were constructed to identify any genealogical relationships between different geographical isolates of *P. acuta* and revealed there to be no major geographical population structuring with several haplotypes being shared between geographically distinct countries (Fig 2i). Haplotypes from the USA appeared to be most common, either being shared with haplotypes from other countries or at least being closely related. The four distinct African clade were also present within clade 1 containing haplotypes

from Angola and Mexico, clade 2 containing haplotypes from South Africa and the Netherlands, clade 3 containing haplotypes from South Africa, USA, Greece, Egypt, Iran and Macedonia, and lineage 4 containing haplotypes from Angola, Burundi, Canada, Chile, Cuba and the USA (Fig 2i). Haplotypes appeared to be unique to locality in the African subset except for a single haplotype in clade 4 which was shared by Angola and Burundi as well as by *P. acuta* from USA, Chile and Cuba. The South African *P. acuta* appeared to have a greater affinity with populations from Europe, North Africa and the Middle East and did not share a haplotype with individuals from Angola or Burundi. In African clade 3 a single haplotype was shared between the USA, Greece, Macedonia and South Africa and all other haplotypes within the lineage appear to have radiated from it (Fig 2i).

A high number of haplotypes with low numbers of segregating sites were identified across the global data set of *Physa acuta* with the majority of the haplotypes only differing by 1 – 4 mutations (Fig 2ii). This was a pattern that was replicated when the haplotypes from the African *P. acuta* were compared with 4 mutations being between haplotypes being the most common (Fig 2ii). The AMOVA analysis on the global population set revealed there to be significant differences between populations when each population was considered from a continental perspective ($^{Global}\Phi_{ST}$ = 0.32201, *P* = 0.001, *P* <0.01). Similarly, when only the African countries were compared using the AMOVA significant differences between the populations were identified ($^{Africa}\Phi_{ST}$ = 0.45360, P = 0.002, P <0.01).

4. Discussion

Physa acuta is probably the most successful cosmopolitan invasive fresh water snail with a global distribution resulting from intensive introductions probably mediated by the aquarium trade (Albrecht *et al*., 2009). *Physa acuta* is notoriously challenging to identify with several authors having discussed the issues of identification based on tenuous morphological traits (Gustafson et al., 2014). In the current study, the application of molecular COI barcode

accurately identified *P. acuta* from populations sampled in Angola, Burundi and South Africa with the phylogenetic analyses clustering the African snails with reference sequences of other *P. acuta* form across the globe. This was further confirmed by the *p*-distance comparisons and the ABGD analyses which illustrated that the African *P. acuta* did not have a divergence any greater that 3% relative to any other of the *P. acuta* isolates, well within the typical for species level divergence in pulmonate snails (Desouky and Busais, 2012; Elejalde et al., 2008; Lawton et al., 2015). Collado (2017) noted that different *P. acuta* populations tend to be described as distinct taxa because of environmental specific morphological plasticity in distinct snail populations. For example, *Physa nodulosa* from Chile was identified as *P. acuta* based on mitochondrial gene sequences further emphasising the essential requirement for COI DNA barcoding for *P. acuta* and related species identification in this species as with other medically important snails such as the snail vectors of schistosomiasis with in the genus *Bulinus* (Jones et al., 2001; Kane et al., 2008; Rollinson et al., 2009) and *Indoplanorbis* (Liu et al., 2010), and the lymnaeid and bithyniid snails responsible for the transmission of *Fasciola* and other food borne trematodes (Correa et al., 2011; Kulsantiwong et al., 2013; Lawton et al., 2015).

The phylogenetic analysis also illustrated four distinct linages of *P. acuta* across four clades from Africa which were also reflected in the haplotype network. Each of the Africa clades showed there to be haplotypes either shared between populations from the USA, Canada or Mexico or radiating from such New World haplotypes. None of the African clades were related with only a few haplotypes being shared between Burundi and Angola and the South African snails having a greater affinity with isolates from Europe and the Middle East. Both the phylogenetic and haplotype network analyses are illustrative of multiple invasion events into Africa from either the New World or through secondary invasion events from populations which established in Europe and then were transported to Africa. Both events are likely to be the result of the movement of aquatic plants for gardens and aquariums with several American

species of wet land plant such as fanwort, *Cabomba caroliniana*, Canadian water weed, *Elodea canadensis* and spade-leaf sword *Echinodorus cordifolius* having established in Africa and known to be habitats and food stuff for *P. acuta* (Martin and Coetzee, 2011). Surprisingly, the AMOVA analyses between continents and between the African isolates showed there to be significant molecular divergence between populations based on frequency of different haplotypes between localities. Although the haplotype network showed there to be relatively low genetic variation between *P. acuta* isolates, there were a high frequency of haplotypes based on a low number of segregating sites. Gaitán-Espitua *et al*. (2013) described the same patterns in populations of the terrestrial snail *Cornu aspersum* invading the America from North Africa and suggested that it could be the result of recent divergence events and high substitution rates, which could arise as haplotypes become 'trapped' in highly structured subpopulations. This structuring would have been caused during invasion events because of local bottle necking events resulting in isolation between subpopulations caused by heterogeneous habitats, repeated range fragmentation and re-colonisation due to multiple human mediated invasion events. This would have led to the introduction of new haplotypes and perhaps the extinction of others causing genetic diversity and differentiation within and between populations of *P. acuta* (Gaitán-Espitua *et al*., 2013). However, this process of subpopulation structuring requires an extended length of time with a long history of multiple invasion events and in most cases the occurrence of *P. acuta* in new countries has only been recorded since the 19th century, particularly in Europe and since 1954 in South Africa at least (Albrecht *et al*., 2009; De Kock and Wolmarans, 2007). Therefore, considering the recent invasion event into South Africa it could also be suggested that the diversity found in the snail populations may have been caused by the isolation of a subset of haplotypes that had arisen in the USA first and was then translocated into aquatic habitats in Africa. *Physa acuta* has high tolerances to pollution and high fast proliferation rates caused by the ability to self fertilise

providing it with a distinct advantage when colonising new habitats relative to other species (Albercht *et al*., 2009). This ability to self fertilise would have allowed the rapid propagation of the few original haplotypes from the initial snail populations that invaded Africa from the USA and has also been suggested as the key to the success of *P. acuta* in Europe (Dillon *et al*., 2002; Henry *et al*., 2005).

Physa acuta has colonised several areas of Africa (Appleton, 2003), including Nigeria and Ghana (Brown, 1994), and Southern Mozambique (Dobson, 2004), and in all cases populations of *Bulinus* and *Biomphalaria* species have almost been completely replaced. Although, a concern for local ecosystems, the introduction of *P. acuta* to water systems has been advocated as a form of biological control to hault the transmission of schistosomiasis (Dobson, 2004; Gashaw *et al*., 2008). The identification of four distinct African clades illustrates the ability of *P. acuta* to establish rapidly and to be successfully maintained within African habitats making this organism an ideal candidate as an agent of biological control. However, it is only through strict management strategies to control the invasion of *Physa* populations can ecological impact be reduced and the benefits to human health be increased. Molecular genetic approaches aid not only in identification but also in monitoring of snail populations contributing to the construction of accurate distribution maps which have helped to target control especially when resources are limited and controversial control strategies utilising biological control are being used (Rollinson *et al*., 2009).

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sector. The authors would like to thank all members of the Water Research Group (Ecology), North-West University, South Africa, who were involved in the

collection of snail material and the biodiversity survey of the Vaal River, as well as transporting the equipment and personnel in order to perform the study. The authors would also like to the thank Dr M. Brito and Dr J. C. Sousa-Figueiredo and their staff at from CISA (Camões - Instituto da Cooperação e da Língua, I.P, Fundação Calouste Gulbenkian, Governo Provincial do Bengo and Ministério da Saúde de Angola), for providing snail samples from Angola. This material was collected as part of a collaborative project with the Natural History Museum, London, involving Prof. D. Rollinson, which was funded by the Gulbenkian foundation (GCF). We would like to thank the Ministry of Health Burundi malacology team and Dr P. Jourdan and A. Zivieri (SCI) for snail collections in Burundi. Funding for collections in Burundi was through BMGF administered by SCORE secretariat UGA. In addition, thanks also go to the Molecular Sequencing Facility at the Natural History Museum, London, for performing and running the Sanger sequencing reactions needed for this project. Dr F. Allan was financially supported by the Wellcome Trust (SCAN Project WT104958MA). Finally, the authors would like to thank the late Prof. A. Davies who was originally involved in this project but passed away unexpectedly before its completion.

References

Albrecht, C., Kroll, O., Moreno Terrazas, E., Wilke, T., 2009. Invasion of ancient Lake Titicaca by the globally invasive *Physa acuta* (Gastropoda: Pulmonata: Hygrophila). Biol. Invasions 11, 1821-1826.

Al-Bdairi, A. B. M., Mohammad, M. K., Al-Miali, H. M., 2014. Freshwater snail diversity in the middle and south regions of Iraq. Adv. Biores. 5, 166-171.

Allan, F., Sousa-Figueiredo, J. C., Emery, A. M., Paulo, R., Mirante, C., Sebastião, A., Brito, M., Rollinson, D., 2017. Mapping freshwater snails in north-western Angola: distribution, identity and molecular diversity of medically important taxa. Parasit. Vectors. 10, 460. doi: 10.1186/s13071-017-2395-y

Appleton C.C., 2003. Alien and invasive fresh water Gastropoda in South Africa. Afr. J. Aquat. Sci. 28, 69-81.

Brown, D.S., 1994. Freshwater Snails of Africa and their Medical Importance (2nd edn.). Taylor & Francis, London, 609 pp.

Collado, A.C., 2017. Unravelling cryptic invasion of a freshwater snail in Chile based on molecular and morphological data. Biodivers. Conserv. 26, 567-578.

Correa A.C., Escobar, J.S., Durand, P., Renaud F., David P., Jarne, P., Pointer J–P., Huetrez-Bousses, S., 2010. Bridging gaps in the molecular phylogeny of the Lymnaeidae (Gastropoda: Pulmonate), vectors of fascioliasis. BMC Evol. Biol. 10, 381. DOI: 10.1186/1471-2148-10-381

De Kock, K.N., and Walmarans, C.T., 2007. Distribution and habitats of the alien invader freshwater snail *Physa acuta* in South Africa. Water S.A. 33, 717-722.

Desouky, M.M.A., Busais, S., 2012. Phylogenetic relationships of the land snail; Eobania vermiculata (Müller, 1774) from Egypt and Saudi Arabia. A combined morphological and analysis. J. Basic. Appl. Zool. 65, 141–151.

Dillon, R.T., Wethington, A. R., Rhett, J.M., Smith, T.P., 2002. Populations of the European freshwater pulmonate *Physa acuta* are not reproductively isolated from American *Physa heterostropha* or *Physa integra*. Invertebr. Biol. 121, 226-234.

Dillion, R.T., 2009. Empirical estimates of reproductive isolation among the *Physa* species of South Carolina (Gastropoda: Pulmonata: Basommatophora). The Nautilus 123, 276-281.

Dobson, M., 2004. Replacement of native freshwater snails by exotic *Physa acuta* (Gastropoda: Physidae) in southern Mozambique; a possible control mechanism for schistosomiasis. Ann. Trop. Med. Parasitol. 98, 543-543.

Dreyfuss, G., Vignoles, P., Abrous, M., Rondelaud, D., 2002. Unusual snail species involved in the transmission of *Fasciola hepatica* in watercress beds in central France. Parasite 9, 113-20.

El-Bahy, M.M., 1997. Fascioliasis among animal, snail and human hosts in Kafr El-Sheikh governorate with special reference to species infecting humans. Vet. Med. J. Giz. 45, 187-209.

Elejalde, M.A., Madeira, M.J., Arrébola, J.R., Muñoz, B., Gómez-Moliner, B.J., 2008. Molecular phylogeny, taxonomy and evolution of the land snail genus *Iberus* (Pulmonata: Helicidae). J. Zool. Syst. Evol. Res. 46, 193–202.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 291-299.

Gaitán-Espitia, J.D., Belén Arias, M., Lardies, M.A., Nespolo, R.F., 2013. Variation in thermal sensitivity and thermal tolerances in an invasive species across a climatic gradient: lessons from the land snail *Cornu aspersum*. PLoS One, 8 :e70662. doi: 10.1371/journal.pone.0070662.

Gashaw, F., Erko, B., Teklehaymanot, T., Habtesellasie, R., 2008. Assessment of the potential of competitor snails and African catfish (*Clarias gariepinus*) as biocontrol agents snail hosts transmitting schistosomiasis. Trans. R. Soc. Trop. Med. Hyg 102, 774-779.

Gustafson, K.D., Kensinger, B.J., Bolek, M.G., Luttbeg, B., 2014. Distinct snail (*Physa*) morphotypes from different habitats converge in shell shape and size under common garden conditions. Evol. Eco. Res. 16, 77–89.

Hall, T.A., 1999. BioEdit: a user friendly biological sequence alignment program editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95-98.

Henry, P., Bousset, L., Sourrouille, P., Jarne, P., 2005. Partial selfing, ecological disturbance and reproductive assurance in an invasive fresh water snail. Heredity 95, 428-436.

Jones, C.S., Rollinson, D., Mimpfoundi, J., Ouma, J., Kariuki, H.C., Noble, L.R., 2001. Molecular evolution of freshwater snail intermediate hosts within the *Bulinus forskalii* group. Parasitology 123, 277-292.

Kane, R.A., Stothard, J.R., Emery, A.M., Rollinson, D., 2008. Molecular characterization of freshwater snails in the genus *Bulinus*: a role for barcodes? Parasit. Vectors 1, doi: 10.1186/1756-3305-1-15.

Kanev, I., 1994. Life-cycle, delimitation and description of *Echinostoma revolutum* (Froelich, 1802) (Trematoda: Echinostomatidae). Syst. Parasitol. 28, 125-144.

Kulsantiwong, J., Prasopdee, S., Ruangsittichai, J., Ruangjirachuporn, W., Boomars, T. Viyanant, V., Pierossi, P., Herbert, P.D.N., Tesana, S., 2013. DNA barcode identification of freshwater snails in the family Bithyniidae from Thailand. PLoS One 8, e79144.

Lawton, S. P., Lim, R. M., Dukes, J. P., Kett, S. M., Cook, R. T., Walker, A. J., Kirk, R. S., 2015. Unravelling the riddle of *Radix*: DNA barcoding for species identification of freshwater snail intermediate hosts of zoonotic digeneans and estimating their inter-population evolutionary relationships. Infect. Genet. Evo. 36, 63 – 74.

Leigh, J.W., Bryant, D., 2015. PopART: Full-feature software for haplotype network construction. Methods Ecol. Evol. 6, 1110–1116.

Librado, P., Rozas, J., 2009. DnaSPv5: software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451-1452.

Liu, L., Mondal, M.M.H., Idris, M.A., Lokman, H.S., Jeyanthe Rajapakse, P.R.V., Satrija, F., Diaz, J.L., Upatham, E.S., Attwood, S.W., 2010. The phylogeography of *Indoplanorbis exustus* (Gastropoda: Planorbidae) in Asia. Parasit. Vectors 3, 57.

Madsen, H., Frandsen, F., 1989. The spread of fresh water snails including those of medical and veterinary importance. Acta Trop. 46, 139 – 149.

Martin, G.D., Coetzee, J.A., 2011. Pet store, aquarists and the internet trade as modes of introduction and spread of invasive macrophytes in Southern Africa. Water S. A. 37, 371-380.

Rollinson, D., Webster, J.P., Webster, B., Nyakaana, S., Jørgensen, A., Stothard, J.R., 2009. Genetic diversity of schistosomes and snails: implications for control. Parasitology 136, 1801-1811.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol. Biol. Evol. 28, 2731-2739.

Wethington A.R., Lydeard C.A., 2007. Molecular phylogeny of Physidae (Gastropoda: Basommatophora) based on mitochondrial DNA sequences. J. Molluscan Stud. 73, 241–257.

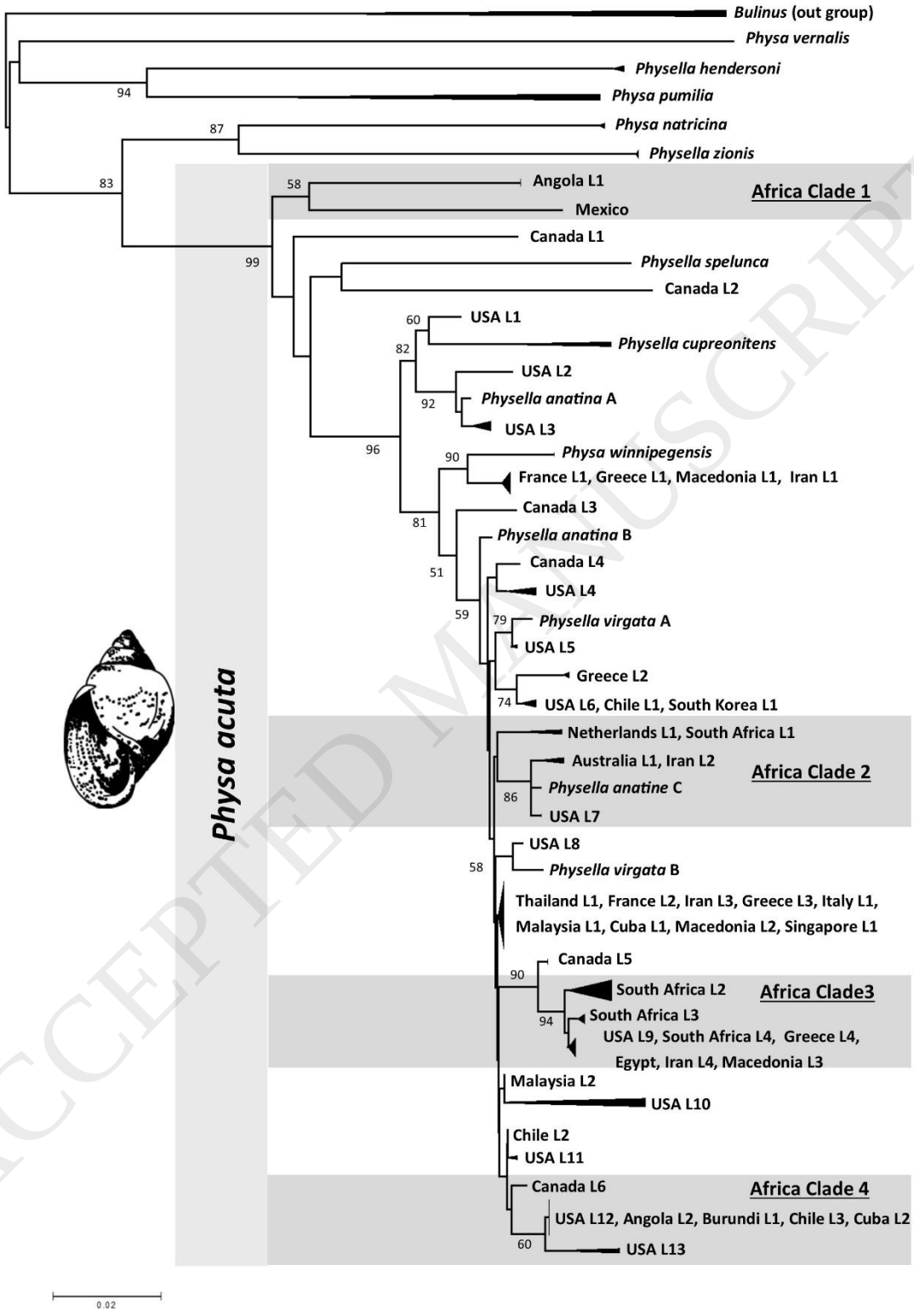
Yousif, F., El-Emam, M., El-Sayed, K., 1998. Effect of six non-target snails on *Schistosoma mansoni* miracidial host finding and infection of *Biomphalaria alexandrina* under laboratory Conditions. J. Egypt. Soc. Parasitol. 28, 559-68.

Figure Captions

Figure 1: Neigbour joining phylogeny of COI barcodes of *Physa acuta* and related species. The tree was constructed using the Kimura's 2-parameter evolutionary model, typically used for DNA bar coding. Four distinct clades were identified as being African specific as shown with dark grey shading. Distinct lineages from all countries are denoted by L

Figure 2: Evolutionary relationships and diversity of *Physa acuta* haplotypes. Where A) haplotype network analyses illustrates four distinct African lineages ; B) frequency of haplotypes based on segregating sites when all *P. acuta* are compared from across the world (i) and when only those from Africa a compared (ii) both illustrating a high number of haplotypes with few mutations between them.

Figr-1



Figr-2