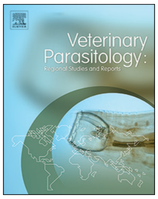
Veterinary Parasitology: Regional Studies and Reports 17 (2019) 100318

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr



Original Article

Molecular characterization of liver fluke intermediate host lymnaeids (Gastropoda: Pulmonata) snails from selected regions of Okavango Delta of Botswana, KwaZulu-Natal and Mpumalanga provinces of South Africa

Mokgadi P. Malatji*, Jennifer Lamb, Samson Mukaratirwa

School of Life Sciences, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville Campus, Durban 4001, South Africa

ARTICLE INFO

Keywords:
Lymnaeidae
ITS-2
Okavango delta (OKD)
KwaZulu-Natal (KZN) province
Mpumalanga province

ABSTRACT

Lymnaeidae snail species are known to be intermediate hosts of human and livestock helminths parasites, especially *Fasciola* species. Identification of these species and their geographical distribution is important to better understand the epidemiology of the disease. Significant diversity has been observed in the shell morphology of snails from the Lymnaeidae family and the systematics within this family is still unclear, especially when the anatomical traits among various species have been found to be homogeneous. Although there are records of lymnaeid species of southern Africa based on shell morphology and controversial anatomical traits, there is paucity of information on the molecular identification and phylogenetic relationships of the different taxa. Therefore, this study aimed at identifying populations of Lymnaeidae snails from selected sites of the Okavango Delta (OKD) in Botswana, and sites located in the KwaZulu-Natal (KZN) and Mpumalanga (MP) provinces of South Africa using molecular techniques. Lymnaeidae snails were collected from 8 locations from the Okavango delta in Botswana, 9 from KZN and one from MP provinces and were identified based on phylogenetic analysis of the internal transcribed spacer (ITS-2). Analyses based on the ITS-2 marker identified the presence of a well-supported *Radix* clade containing *Radix auricularia, R. natalensis* and *R. rubiginosa*, which were not well resolved. Experimental samples from the OKD and KZN present in this clade were referable to these species. An unidentified experimental taxon from the OKD formed a well-supported sister clade to the *Radix* clade, although it was not possible to identify it. *Galba truncatula* was well supported in a sister relationship to a well-supported *Pseudosuccinea columella* clade which included samples from MP and KZN provinces of South Africa. We observed that *P. columella* shared the same habitats with *R. natalensis* and *R. auricularia* in KZN. Our study contributes new knowledge on the Lymnaeidae species present in Southern Africa and their phylogenetic relationships. The study further identifies the species which are likely to co-exist in the same environment and this information will be of use to those designing control programs for fasciolosis. This is the first study reporting the presence of *R. auricularia* in the OKD of Botswana and KZN province of South Africa.

1. Introduction

Snails of Lymnaeidae and R family are freshwater, hermaphrodite gastropods with a global distribution (Dar et al., 2016). They act as vectors for various trematodes of major medical and veterinary importance worldwide (Correa et al., 2010). Species from this group have been reported to transmit at least 71 trematodes from 13 families, which includes some species from the Schistosomatidae (Horák and Kolárová, 2001), Echinostomatidae (Graczyk and Fried, 1998), and Paramphistomatidae, which are of veterinary importance (Degueurce et al., 1999). However, the Lymnaeidae family is mainly linked to the transmission of *Fasciola* species, the causative agents of fascioliasis in human, domestic animals and wildlife (Mas-Coma et al., 2005; Correa et al., 2010). Fascioliasis is responsible for immense economic loss in domestic and wild ruminants due to decreased animal productivity (e.g. milk and meat production), mortality and liver condemnation (Mohammed, 2015). About 17 million cases of human fascioliasis have been reported worldwide (WHO, 2007; Mas-Coma et al., 2009; Correa et al., 2010).

Approximately 20 Lymnaeidae species have been described as potential intermediate hosts for *Fasciola* species (Torgerson and Claxton, 1999), but they differ in their capacity to sustain complete developmental stages of the parasite and their distribution is determined by climatic factors (temperature and rainfall), and ecological (habitat and

---

* Corresponding author.
E-mail address: Pulanemalatji@gmail.com (M.P. Malatji).

https://doi.org/10.1016/j.vprsr.2019.100318
Received 20 September 2018; Received in revised form 6 March 2019; Accepted 24 June 2019
Available online 26 June 2019
2405-9390/ © 2019 Elsevier B.V. All rights reserved.

substratum type) factors. According to Brown (1994) and Graczyk (1999), *Galba truncatula* (Muller, 1774) and *Pseudosuccinea columella* (Say, 1817) are preferred intermediate hosts of *Fasciola hepatica*, whilst *Radix natalensis* (Krauss, 1848) and *R. auricularia* (Linnaeus, 1758) are hosts of *F. gigantica*. In South Africa, *G. truncatula* is has been found mostly in cooler areas and is responsible for the transmission of *F. hepatica* (DeKock et al., 2003), whereas *R. natalensis,* which transmit *F. gigantica* (Moema et al., 2008) and *P. columella,* which transmits both *F. gigantica* and *F. hepatica* are widely distributed (DeKock et al., 1989). The geographical distribution of lymnaeid species serve as biomarkers for the occurrence and distribution of *F. hepatica* and *F. gigantica* (Dar et al., 2016), and can thus be used to validate the mathematical modelling based on remote sensing-geographical information system (RS-GIS) tools for the control of fascioliasis (Fuentes et al., 1999, 2001). Therefore, there is a need for a reliable identification system and phylogenetic framework for Lymnaeidae snails to assist in defining areas of epidemiological risk (Correa et al., 2010; Standley et al., 2013) especially in southern Africa where fascioliasis is highly endemic.

Despite the applied interest in the Lymnaeidae family, present knowledge on the taxonomy and *Lymnaea-Fasciola* interrelationship is still insufficient (Mas-Coma et al., 2005). Lymnaeid taxonomy is controversial (Standley et al., 2013), especially at the species level due to interspecific uniformity in anatomical characters and great diversity in shell morphology, resulting in difficulties in specimen classification (Bargues et al., 2003, 2011; Bargues and Artigas, 2007; Standley et al., 2013). Furthermore, the shell characters between and within species are extremely plastic and do not always accurately reflect the evolutionary relationship of the family as they appear to be linked to environmental conditions or other external forces (Mas-Coma et al., 2005; Bargues et al., 2011).

As a result, there has been an increase in the analysis of molecular-based tools such as analysis of the mitochondrial (16S and Cox1) and nuclear (18S and ITS) DNA regions which are suitable for species identification; these have been used to clarify the taxonomic status of closely related or cryptic species (Bargues et al., 2011; Standley et al., 2013; Alda et al., 2018). Therefore, this study was carried out to identify populations of Lymnaeidae snails collected from selected areas of the Okavango delta (OKD) in Botswana, and KwaZulu-Natal (KZN) and Mpumalanga (MP) provinces of South Africa using molecular technique.

2. Materials and methods

2.1. Snail collections sites and sampling

Snails for this study were collected from 8 locations in the OKD Botswana and 9 locations in KZN and one location in MP provinces of South Africa (Fig. 1). The Okavango Delta is one of the largest inland deltas in the world, occurring within a massive shallow basin in the middle of southern Africa (Ramberg et al., 2006). The delta sources its water from the Okavango River basin, which arises from a series of headwater streams on the southern slopes of Angolan highlands and flows in the southeast direction along the border of the northern Namibia before entering Botswana and emptying into the Okavango Delta (Ashton et al., 2003). The delta is known to have a highly variable and complex aquatic ecosystem (Dallas and Mosepele, 2007). KwaZulu-Natal and MP provinces are considered to have the largest diversity of freshwater mollusc in South Africa (DeKock and Wolmarans, 1998). Snails collected from each individual site using a metal scoop were regarded as a population. Snails were euthanized and fixed in ethanol for molecular analyses.

2.2. Molecular analysis

DNA was extracted using the Genomic DNA™ Tissue MiniPrep kit (Zymo Research Corporation) according to the manufacturer's instructions. PCR was performed based on ITS-2 primers (News 5′-TGTGTCGATGAAGAACGCAG-3′ and Rixo2 5′-TTCTATGCTTAAATTCAGGGG-3′) (Correa et al., 2010). This marker is commonly used in the systematics and taxonomy of Lymnaeidae (Mas-Coma et al., 2005). Amplification was performed in a 25 μl reaction volume, containing 12.5 μl PCR Master Mix (2×) (Thermo Scientific), 2 μl of each primer (10 μM), 6 μl sterile water and 2.5 μl genomic DNA. Thermal cycling conditions were set at: 95 °C for 10 min; followed by 40 cycles of (60s at 95 °C, 60s at 53 °C, 60s at 72 °C) and lastly final extension for 7 min at 72 °C. Fragments were separated by electrophoresis in 2% agarose gels (in 0.5× TBE buffer) at 80 V for one hour, stained with ethidium bromide. Sequencing was done at Inqaba biotechnical industries (Pty) Ltd. (Pretoria, South Africa) with ABI 3500XL sequencer using the Big Dye technology.

Sequences were assembled, manually edited and aligned using the Clustal W (Thompson et al., 1997) option of the BioEdit program (Hall, 1999). Aligned sequences were trimmed to a common length of 270 nucleotides for the ITS-2 region. jModeltest (Posada, 2008) was used to select the most appropriate model of nucleotide substitution for use in neighbour-joining and Bayesian Inference analyses. The HKY + 1 model (Hasegawa et al., 1985) was selected under the AIC information criterion. Maximum parsimony (MP) and neighbour-joining (NJ) trees were generated using PAUP* 4.0 (Swofford, 1998). For parsimony analysis, starting trees were obtained by stepwise addition. The addition sequence was random, with one tree held at each step and ten replicates. For both methods, nodal support was estimated using 1000 bootstrap pseudo-replicates. Bayesian analysis was executed in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). Four Markov chains were run for 5 million generations, until the standard deviation of the split frequencies was less than 0.01. The first 500,000 trees were discarded as burnin. The phylograms were 50% majority-rule consensus trees with nodal support indicated as posterior probabilities. A statistical parsimony haplotype network was created in TCS v1.2.1 (Clement et al., 2000). Gaps were included in the analysis.

3. Results

Phylogenetic analysis revealed the ingroup formed a well-supported monophyletic clade (A) with respect to the outgroup samples (Fig. 2). Clade B, which was well-supported and included 43 experimental isolates, was further divided into sister clades E and F. Clade E included 4 *Radix* species from GenBank; by implication all experimental samples within this clade were referred to *Radix*. This included 7 populations from the Okavango delta (OKD) and 8 populations from KwaZulu-Natal (KZN) province. Clade E formed two well supported subclades. One of these consisted of 19 isolates from 7 OKD populations and 10 isolates from KZN as well as a GenBank *R. auricularia* (KC700027.1). In the other subclade comprising of 13 isolates from KZN and two GenBank isolates (*R. natalensis* HQ283170.1 and *R. rubiginosa* KF042385.1). Thus, the phylogeny is not well resolved with respect to the *Radix* species represented, due in part to the necessity to trim alignment to the shortest common length. The phylogeny is consistent with the identification of these samples as either *L. (R.) natalensis* or *R. rubiginosa*; however, a BLAST search with our longer nucleotide sequences of these isolates supported their identity as *L. (R.) natalensis*. From here on these isolates will be referred as to *L. (R.) natalensis*. Clade F consists of one isolate from location X-OKD in Botswana. Although it is sister to the Radix clade, a lack of conforming GenBank samples does not allow identification of this sample. In well supported clade C, *G. truncatula* formed a sister clade to a well-supported clade D, which consist of our isolates from MP and 4 locations in KZN and *Pseudosuccinea columella* isolates from GenBank.

Thus, phylogenetic analysis of the ITS-2 DNA region revealed the presence of four lymnaeid species within our samples, namely *Radix auricularia*, *R. natalensis*, *P. columella*, and an unidentified taxon. Out of 49 isolates analysed, 29 (59.2%) were identified as *R. auricularia*, 13

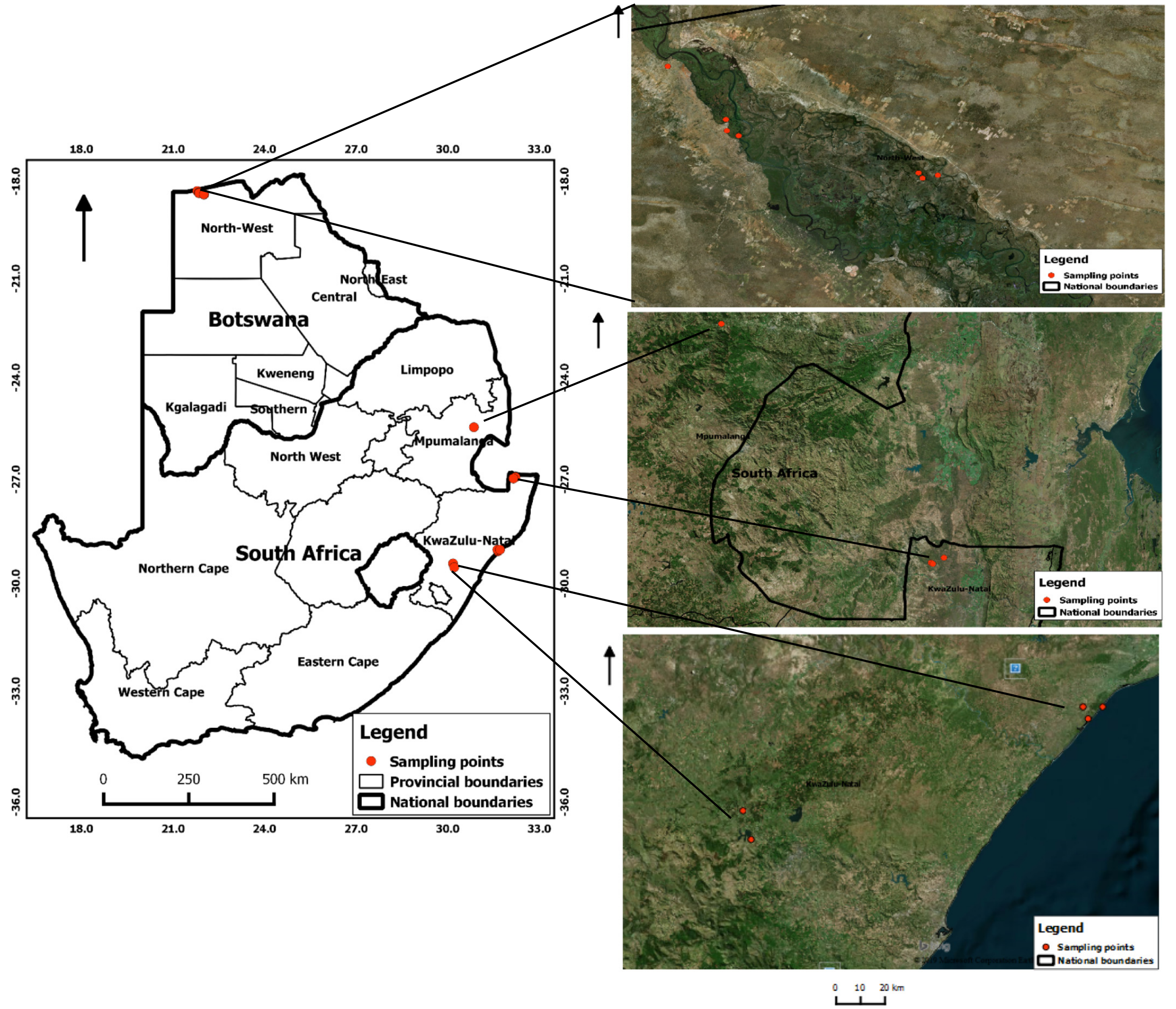


Fig. 1. Map showing sample collection sites from KwaZulu-Natal province and Okavango Delta.

(26.5%) as *L. (R.) natalensis*, 6 (12.1%) as *P. columella* and 1 (20%) was unidentified. *R. auricularia* and the unknown taxon were identified in OKD, with the former being the predominant in OKD occurring in 7 of 8 sampled sites. Furthermore, *R. auricularia*, *R. natalensis* and *P. columella* were found to occur in KZN, with *R. natalensis* and *P. columella* predominant; occurring in 50% of the sites. *Pseudosuccinea columella* shared the same habitat with *R. auricularia* and *R. natalensis* in sites S_4-KZN and S_{13}-KNZ, and A-KZN respectively and only *P. columella* was identified in MP site.

Table 1 shows the haplotype data and status of the GenBank derived Lymnaeidae isolates and studied isolates from OKD, KZN and MP. The statistical parsimony haplotype network (Fig. 3) yielded 7 distinct networks when set at 95% connection limit. When set at a connection limit of 150 steps, it yielded a single network. The structure of the network corresponded substantially to the structure of the phylogenetic tree (Fig. 2), yielding what are likely to be two *Radix* clades (Clade 1 and 2), and a what is likely to be a *Pseudosuccinea columella* clade (Clade 3).

4. Discussion

The study identified two Lymnaeidae species, *Radix auricularia* and an unknown taxon in the Okavango Delta, Botswana. As the unknown taxon formed a well-supported clade, sister to the *Radix* clade, it might be allied to *Radix*. According to the genetic species concept (Bradley and Baker, 2001), it is unlikely that it can be referred to *Radix*, as it is separated from the *Radix* species by a large mean genetic distance of 32.4%; this is more similar to the mean intergeneric distance between *Radix*, *Pseudosuccinea* and *Galba* of 44.0% than it is between the intrageneric distance between *Radix* clades 1 and 2 (5.9%, Fig. 2). Thus, it is likely that the unknown taxon is representative of a different, yet unidentified genus within the families Racidinae or Lymnaeidae. *Radix auricularia* was identified in 87.5% of the studied sites compared to 1.25% of the unknown taxon. Previous reports of freshwater mollusc occurring in the Okavango delta documented the existence of only *R. natalensis* in the delta and the Okavango river basin which flows into the delta (Alonso and Nordin, 2004). Hence, this is the first record of *R. auricularia* in the Okavango delta of Botswana, and its epidemiological role in the transmission of *Fasciola* spp. in South Africa is not known.

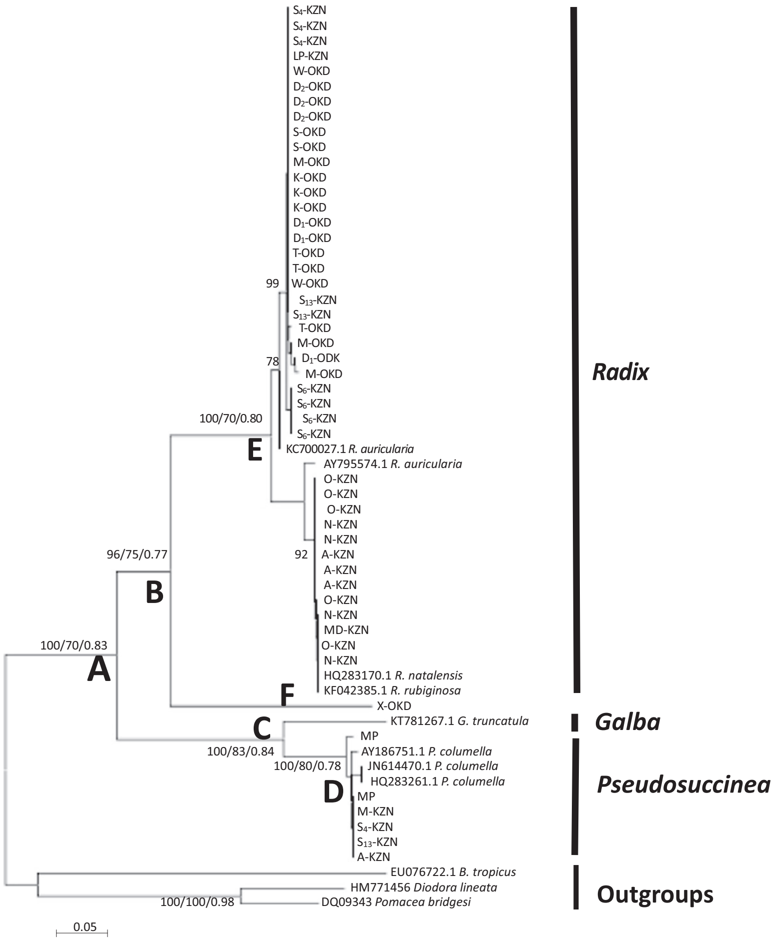


Fig. 2. Tree based on the nuclear ribosomal ITS-2 region illustrating relationships between experimental samples, close matches downloaded from the NCBI Genbank and outgroups.

However, since species of the genus *Radix* are known to act as intermediate hosts of *F. gigantica* (Mas-Coma, 2005), it is possible that this *Radix* species may be transmitting *F. gigantica* in Botswana.

In this study, *P. columella*, *R. natalensis* and *R. auricularia* were found to occur in KZN, whilst only *P. columella* was found in MP province, South Africa, based on the extent of our sampling. Results also showed that *R. natalensis* and *P. columella* are more predominant in KZN, than *R. auricularia* based on the frequency they were found in the selected locations. The difference in distribution of these species, and the apparently more limited distribution of *R. auricularia* may be due to the difference in ecological requirements of the species. *Radix natalensis* and *P. columella* can adapt to a variety of water bodies or habitats which results in their wider geographical distribution as compared to *R. auricularia*. They are often found to share the same habitat, mainly in permanent water bodies, which includes streams, reservoirs and drains, and impoundments such as small dams (Brown, 1994). They also inhabit shallow but constantly seeping water but are rarely found in seasonally filled pools (Brown, 1994). *Radix natalensis* is native in Africa, and widely distributed in South Africa (DeKock et al., 2003; Moema et al., 2008). According to Brown (1994), this species belongs to the *R. auricularia* super species of Hubendick (1951) and is the most prevalent intermediate host of *F. gigantica* in many sub-Saharan African countries (DeKock et al., 2003; DeKock and Wolmarans, 2008). The presence of both Radix species in KwaZulu-Natal may potentially increase the distribution of *F. gigantica* as this is the first report of *R. auricularia* in KZN province, South Africa.

According to DeKock et al. (1989), *P. columella* has been one of the most successful colonist freshwater species in South Africa. This species is invasive and widely distributed in the country and has been previously documented in KZN and MP (DeKock et al., 1989; DeKock and Wolmarans, 1998; Appleton, 2003; DeKock and Wolmarans, 2008; Perissinotto et al., 2014). It has been reported to act as an intermediate host for both *F. gigantica* and *F. hepatica* (Mas-Coma et al., 2005; Grabner et al., 2014) in many countries. Although this species has been reported to transmit both *F. hepatica* and *F. gigantica* in South Africa, *P. columella* has only been recently proven to transmit *F. gigantica* in the country (Malatji and Mukaratirwa, 2019). Therefore, the presence of this invasive species may potentially increase the geographical distribution of both *Fasciola* species.

The study also showed that *P. columella* shared the same habitat with *R. auricularia* in two sites and *R. natalensis* in one site in KZN province, South Africa. Co-habitation between *P. columella* and *R. natalensis* has also been observed in Nelspruit in MP province (results not shown). Although this is the first report of co-existence of *P. columella* and *R. auricularia* in the same water body, the co-habitation of *P. columella* and *R. natalensis* has been previously observed and reported along the Crocodile and Marico river and catchment, South Africa (Kemp et al., 2015). Wolmarans and DeKock (2006) also documented this co-habitation in the Lower Sabie (Mpondo dam) and Berg-en-Dal (Matjulu Spruit) areas in Kruger National park, MP province, South Africa. Co-habitations of different Lymnaeidae species consequently may lead to the overlap in the distribution of *F. gigantica* and *F. hepatica* which has been reported in both KZN and MP provinces of South Africa.

In summary, our phylogenetic analysis supported the presence of three genera of Lymnaeidae among our experimental samples. This phylogenetic structure corresponded with the haplotype network

Table 1
Haplotype table showing the haplotype status of the GenBank and studied isolates from Okavango Delta in Botswana, KwaZulu-Natal and Mpumalanga provinces, South Africa, based on ITS-2.

Haplotype	No. of isolates in a haplotype	Studied isolates	Genbank isolates
1	3	A-KZN, A-KZN, N-KZN, N-KZN	–
2	4	A-KZN, MP, S_{13}-KZN, S_4-KZN	–
3	16	D_1-OKD, D_1-OKD, K-OKD, K-OKD, K-OKD, LP-KZN, M-KZN, D_2-KZN, D_2-KZN, D_2-KZN, S-OKD, S-OKD, S_4-KZN, S_4-KZN, T-OKD, W-OKD	–
4	1	D_1-OKD	–
5	1	M-OKD	–
6	1	M-OKD	–
7	1	MD-KZN	–
8	1	MP	–
9	4	N-KZN, O-KZN	HQ2832701 *Radix natalensis*, KF0423851 *Radix rubiginosa*
10	1	N-KZN	–
11	1	O-KZN	–
12	2	O-KZN, O-KZN	–
13	3	S_{13}-KZN, S_{13}-KZN, W-OKD	–
14	1	S_4-KZN	–
15	4	S_6-KZN, S_6-KZN, S_6-KZN, S_6-KZN	–
16	1	T-OKD	–
17	1	T-OKD	–
18	1	–	AY186751.1 *Pseudosuccinea columella*
19	1	–	AY795574.1 *Radix auricularia*
20	1	–	EU076722.1 *Bulinus tropicus*
21	2	–	HQ283261.1 *Pseudosuccinea columella*, JN614470.1 *Pseudosuccinea columella*
22	1	–	KC7000271 *Radix auricularia*
23	1	–	KT7812671 *Galba truncatula*
24	1	X-OKD	–

OKD- Okavango delta, KZN- KwaZulu-Natal, MP- Mpumalanga province, A- Amatikulu, N- Nyazane, D_1- Diseta site 1, D_2- Diseta site 2, M- Mehembo (OKD), LP- Lynnfield park, K- Kadimbe, S- Shamatota, W- Wenela, S4- Site 4, S6- Site 6, S13, Site 13, T- Tjoroga, O– Obanjeni, M- Mackenzie dam (KZN), X- Xakanaka.

structure, which yielded two *Radix* clades and a *Pseudosuccinea* clade (Fig. 3). Results also showed that the species of the genus *Radix* included in this analysis are closely related to each other and are separated by mean genetic distances of 5.9%. Dung et al. (2013) also presented a phylogenetic tree showing a deep clade formed by the *Radix* species, as was observed in this study. The species of this genus transmit *F. gigantica* in Africa (Mas-Coma, 2005), Asia and USA (Dung et al., 2013). Results further showed that *Galba truncatula* and

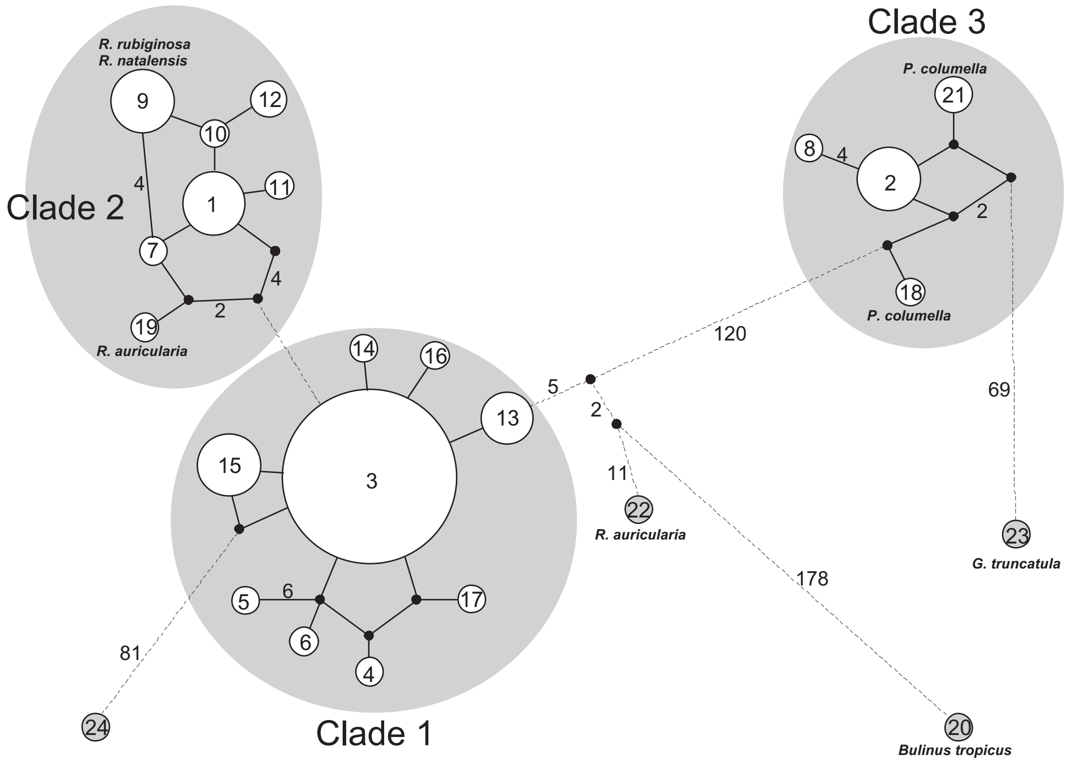


Fig. 3. Statistical parsimony haplotype network depicting mutational relationships between study samples and sequences downloaded from the NCBI Genbank.

Pseudosuccinea columella are members of a strongly supported clade and are thus descended from a common ancestor, consistent with their shared ability to transmit *F. hepatica* (Mas-Coma, 2005; Mas-Coma et al., 2005).

Declaration of Competing Interest

None.

Acknowledgement

The authors acknowledge staff from the Malaria and Bilharziasis in Southern Africa project who assisted in the collection of some of the snail samples. Financial assistance was from the National Research Foundation of South Africa.

Ethical statement

Experimental protocols of this study were reviewed and approved by the Animals Ethics Committee of the University of KwaZulu-Natal (Ref: AREC/044/016D) in accordance with the South African national guidelines on animal care, handling and use for biomedical research.

References

Alda, P., Lounnas, M., Vázquez, A.A., Ayaqui, R., Calvopiña, M., Celi-Erazo, M., Dillon, R.T., Jarne, P., Loker, E.S., Muñiz, F.C., Muzzio-Aroca, J., Narváez, A.O., Noya, O., Martini, L., Rodríguez-Hidalgo, R., Uribe, N., David, P., Pointier, J.P., Hurtrez-Boussès, S., 2018. A new multiplex PCR assay to distinguish among three cryptic *Galba* species, intermediate hosts of *Fasciola hepatica*. Vet. Parasitol. 251, 101–105. https://doi.org/10.1016/j.vetpar.2018.01.006.

Alonso, L.E., Nordin, L.A., 2004. A Rapid Biological Assessment of the Aquatic Ecosystems of the Okavango Delta. High Water Survey, Conservation International, Botswana.

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

Ashton, P.J., Nordin, L., Alonso, L.E., 2003. Chapter 1: Introduction to the Okavango Delta and the AquaRap expedition. In: Alonso, L.E., Nordin, L. (Eds.), A Rapid Biological Assessment of the Aquatic Ecosystems of the Okavango Delta, High Water Survey, Botswana. RAP Bulletin of Biological Assessment No. 27. Conservation International, Washington, USA, pp. 29–37.

Bargues, M., Artigas, P., 2007. Mera y Sierra R.L., Pointier J.P. and Mas-Coma S., Characterisation of *Lymnaea cubensis*, *L. viatrix* and *L. neotropica* n. sp., the main vectors of *Fasciola hepatica* in Latin America, by analysis of their ribosomal and mitochondrial DNA. Ann. Trop. Med. Parasitol. 101, 621–641.

Bargues, M.D., Horak, P., Patzner, R.A., Pointier, J.P., Jackiewicz, M., Meier-Brook, C., Mas-Coma, S., 2003. Insights onto the relationships of Paleatctic and Nearctic lymnaeids (Molliuscia: Gastropoda) by rDNA ITS-1 sequencing phylogeny of stagnicoline intermediate host species of Fasciola hepatica. Parasit. 10, 243–255.

Bargues, M.D., Artigas, P., Khoubbane, M., Flores, R., Glöer, P., Rojas-García, R., Ashrafi, K., Falkner, G., Mas-Coma, S., 2011. *Lymnaea schirazensis*, an overlooked snail distorting fascioliasis data: genotype, phenotype, ecology, worldwide spread, susceptibility, applicability. PLoS One 6, e24567. https://doi.org/10.1371/journal.pone.0024567.

Bradley, R.D., Baker, R.J., 2001. A test of the genetic species concept: cytochrome-b sequences and mammals. J. Mammal. 82, 960–973.

Brown, D., 1994. Freshwater Snails of Africa and Their Medical Importance. Taylor & Francis Ltd, London.

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9, 1657–1660.

Correa, A.C., Escobar, J.S., Durand, P., Renaud, F., David, P., Jarne, P., Pointier, J.P., Hurtrez-Boussès, S., 2010. Bridging gaps in the molecular phylogeny of the Lymnaeidae (Gastropoda: Pulmonata), vectors of Fascioliasis. BMC Evol. Biol. 10, 381.

Dallas, H.F., Mosepele, B., 2007. A preliminary survey and analysis of the spatial distribution of aquatic invertebrates in the Okavango Delta, Botswana. Afr. J. Aquat. Sci. 32, 1–11.

Dar, Y., Amer, S., Zein, E.R., Dreyfuss, G., 2016. Characterisation of *Pseudosuccinea columella* and *Radix natalensis* (Gastropoda: Lymnaeidae) in Egypt using shell and molecular data. Molluscan Res. 36, 22–28.

Degueurce, F., Abrous, M., Dreyfuss, G., Rondelaud, D., Genrey, J., 1999. Paramphistomum daubneyi and *Fasciola hepatica*: the prevalence of natural or experimental infections in four species of freshwater snails in eastern France. J. Helminthol. 73, 197–202.

DeKock, K.N., Wolmarans, C.T., 1998. A re-evaluation of the occurrence of freshwater molluscs in the Kruger National Park. Koedoe 14, 1–8.

DeKock, K.N., Wolmarans, C.T., 2008. Invasive Alien Freshwater Snail Species in the Kruger National Park, South Africa. African Protected Area Conservation and Science. 40. pp. 49–53.

DeKock, K., Joubert, P.H., Pretorius, S.J., 1989. Geographical distribution and habitat preferences of the invader freshwater snail species *Lymnaea columella* (Mollusca: Gastropoda) in South Africa. Onderstepoort J. Vet. Res. 56, 271–275.

DeKock, K., Wolmarans, C.T., Bornmas, M., 2003. Distribution and habitats of the snail *Lymnaea truncatula*, intermediate host of the liver fluke *Fasciola hepatica*, in South Africa. J. S. Afr. Vet. Assoc. 74, 117–122.

Dung, B.T., Doanh, P.N., The, D.T., Loan, H.T., Losson, B., Caron, Y., 2013. Morphological and molecular characterization of lymnaeid snails and their potential role in transmission of *Fasciola* spp. in Vietnam. Korean J. Parasitol. 51, 657.

Fuentes, M.V., Valero, M.A., Bargues, M.D., Esteban, J.G., Angles, R., Mas-Coma, S., 1999. Analysis of climatic data and forecast indices for human fascioliasis at very high altitude. Ann. Trop. Med. Parasitol. 93, 835–850.

Fuentes, M.V., Malone, J.B., Mas-Coma, S., 2001. Validation of a mapping and predicting model for human fasciolosis transmission in Andean very high altitude endemic areas using remote sensing data. Acta Trop. 79, 87–95.

Grabner, D.S., Mohamed, F.A.M.M., Nachev, M., Meabed, E.M.H., Sabry, A.H.A., Sures, B., 2014. Invasion biology meets parasitology: a case study of parasite spill-back with Egyptian *Fasciola gigantica* in the invasive snail *Pseudosuccinea columella*. PLoS One 9, e88537.

Graczyk, T.K., Dalton, J.P., 1999. Development of *Fasciola hepatica* in the intermediate host. In: Fasciolosis. CABI Publishing, CAB International, Wallingford Oxon OX10 8DE, UK, pp. 1–544.

Graczyk, T., Fried, B., 1998. Echinostomiasis: a common but forgotten food-borne disease. Am. J. Trop. Med. Hyg. 58, 501–504.

Hall, T.A., 1999. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symposium Series. Information Retrieval Ltd, London, pp. c1979–c2000.

Hasegawa, M., Kishino, H., Yano, T., 1985. Dating the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22, 160–174.

Horák, P., Kolárová, L., 2001. Birds schistosomes: do they die in mammalian skin? Trends in Parasitology. 17, 66–69.

Hubendick, F., 1951. Recent Lymnaeidae. Their variation, morphology, taxonomy, nomenclature, and distribution. 3. Küngliga Svenska Vetenskapsakademiens Handlingar, pp. 1–223.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics. 17, 754–755.

Kemp, M., DeKock, K.N., Zaayman, J.L., Wolmarans, C.T., 2015. A comparison of mollusc diversity between the relatively pristine Marico River and the impacted Crocodile River, two major tributaries of the Limpopo River, South Africa. Water SA 42, 2.

Malatji, M.P., Mukaratirwa, S., 2019. Molecular detection of natural infection of *Lymnaea* (*Pseudosuccinea*) *columella* (Gastroposa: Lymnaeaidae) with *Fasciola gigantica* (Digenea: Fasciolidae) from two provinces of South Africa. J. Helminthol. 1–6.

Mas-Coma, S., 2005. Epidemiology of fascioliasis in human endemic areas. J. Helminthol. 79, 207–216.

Mas-Coma, S., Bargues, M.D., Valero, M.A., 2005. Fascioliasis and other plant-borne trematode zoonoses. Int. J. Parasitol. 35, 1255–1278.

Mas-Coma, S., Valero, M.A., Bargues, M.D., 2009. *Fasciola*, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. Adv. Parasitol. 69, 41–146.

Moema, E.B.E., King, P.H., Baker, C., 2008. Cercariae developing in *Lymnaea natalensis* Krauss, 1848 collected in the vicinity of Pretoria, Gauteng Province, South Africa. Onderstepoort J. Vet. Res. 173, 215–223.

Mohammed, B.R., 2015. The impact of fasciolosis on food security in Nigeria: a review. Int. J. Multidiscip. Curr. Res. 3 (July/Aug).

Perissinotto, R., Miranda, N.A.F., Raw, J.L., Peer, N., 2014. Biodiversity census of lake St Lucia, iSimangaliso wetland park (South Africa): gastropod molluscs. ZooKeys 440, 1.

Posada, D., 2008. jModelTest: Phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.

Ramberg, L., Hancock, P., Lindholm, M., Meyer, T., Ringrose, S., Sliva, J., Van As, J., VanderPost, C., 2006. Species diversity of the Okavango delta, Botswana. Aquatic Sci. 68, 310–337.

Standley, C.J., Prepelitchi, L., Pietrokovsky, S.M., Issia, L., Stothard, J.R., Wisnivesky-Colli, C., 2013. Molecular characterization of cryptic and sympatric lymnaeid species from the *Galba/Fossaria* group in Mendoza Province, Northern Patagonia, Argentina. Parasit. Vectors 6, 304.

Swofford, D., 1998. Phylogenetic Analysis Using Parsimony (Paup), Version 4. Sinauer Associates, Sunderland, MA.

Thompson, J.D., Gibson, F., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24, 4876–4882.

Torgerson, P., Claxton, J., Dalton, J.P., 1999. Epidemiology and control. In: Fasciolosis. CAB International, Oxon, pp. 113–149.

WHO, 2007. Report of the WHO Informal Meeting on Use of Triclabendazole in Fascioliasis Control, Held at WHO Headquarters, Geneva, Switzerland, October 2006.

Wolmarans, C.T., DeKock, K.N., 2006. The Current Status of Freshwater Molluscs in the Kruger National Park. Koedoe. 49. pp. 39–44 (Pretoria. ISSN 0075-6458).