Rapid Communication

First record of the Asian freshwater snail *Sinotaia* cf. *quadrata* (Benson, 1842) from Africa

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Abstract

The occurrence of the alien invasive freshwater snail *Sinotaia* cf. *quadrata* is reported for the first time in Africa. This species is a global invader of freshwater bodies as evidenced by its very recently reported discoveries in Europe and the Americas. We use DNA barcoding and phylogenetic analysis to confirm species identity and show low genetic divergence with haplotypes from Japan. The introduction of *S.* cf. *quadrata* to South Africa is undoubtedly recent, but its introduction pathways are currently uncertain. Unintentional introduction via the aquarium or aquaculture trades are a possibility, but this snail may have been introduced for human consumption. The *S.* cf. *quadrata* population is established in the artificial impoundment known as Zoo Lake in Johannesburg. Population dynamics indicate very high fecundity and densities of 1078 ind. m^{-2} ± 236 SE can be attained in disturbed and polluted environments. It has the potential to spread over the northern parts of South Africa, into the Kruger National Park and into Mozambique. Further monitoring is recommended in the region.

Key words: DNA barcoding, alien, mollusc, high abundance, urban lake, food trade, health risk

Introduction

Invasions of non-indigenous species (NIS) are a significant ecological threat to aquatic systems (Gallardo et al. 2016; David and Janac 2018; Reid et al. 2019). Of all the species that are introduced, a few are successful in establishing populations, only some of these can spread, and fewer still become pests (Mack et al. 2000). Over the last few decades there has been a global increase in the establishment of NIS (Simberloff 2014) and freshwater ecosystems are particularly vulnerable to introductions and invasion, because of their close association with human activities and because of the high dispersal rates exhibited by most freshwater organisms (Tricarico et al. 2017). Early detection and rapid response are critical components of invasion management since prevention of further introductions is more likely to succeed than eradication or control of established NIS

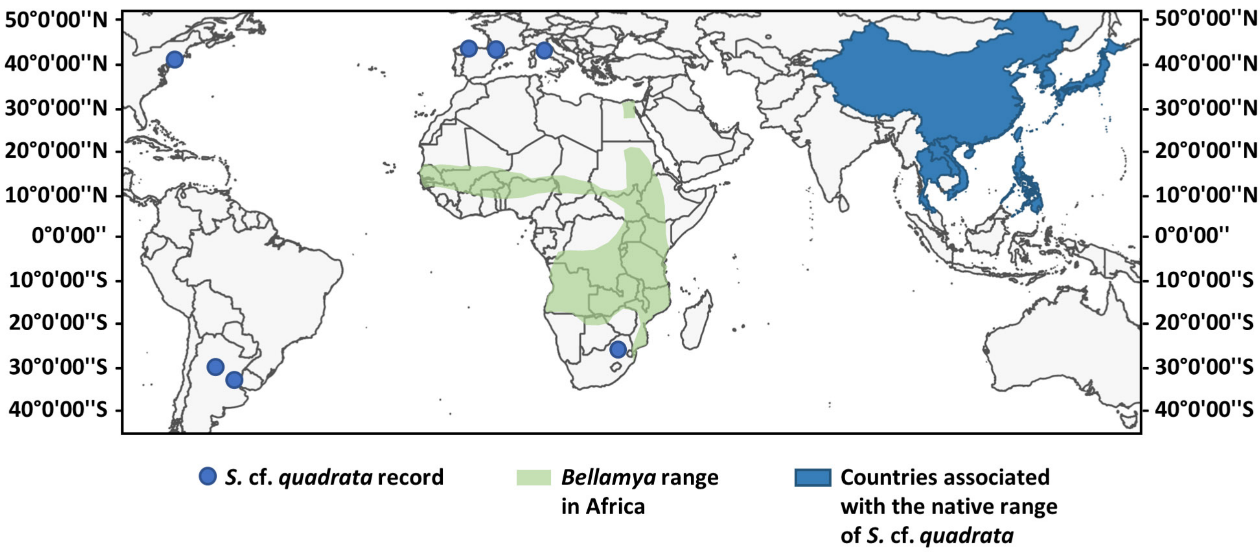

Figure 1. Map showing the global distribution of viviparid snails: *Sinotaia* cf. *quadrata* and the genus *Bellamya* in Africa (the latter adapted from Brown).

(Williams and Grosholz 2008; Tricarico et al. 2017). Ongoing scientific monitoring programmes and the use of molecular tools are proving to be very effective for identifying species and contributing to early detection of NIS (Ardura et al. 2017; Arias et al. 2020; O'Leary et al. 2021).

The family Viviparidae includes highly variable species of operculate freshwater snails that commonly exhibit brooding behavior and can be found in tropical and temperate areas worldwide (Strong et al. 2008; Ovando and Cuezzo 2012; Hirano et al. 2019; Stelbrink et al. 2020). *Sinotaia quadrata* (Benson, 1842) is native to China, Taiwan and Korea and has been introduced to Japan, Thailand and the Philippines. The species has been recently reported from Europe (Cianfanelli et al. 2017; Arias et al. 2020), South America (Ovando and Cuezzo 2012) and North America (O'Leary et al. 2021) (Figure 1). In December 2019, a single specimen preliminarily identified as a viviparid species of Asian origin was found in Johannesburg Zoo Lake, South Africa. Further visits to the area in 2020 and 2021 revealed that a large population of these snails was present in the lake. This study presents DNA barcoding evidence of the first record of *Sinotaia* cf. *quadrata* from South Africa and, to our best knowledge, this record is also the first report of the species in Africa.

Materials and methods

Specimens were collected from Zoo Lake (GPS: 26°09′23.96″S; 028°01′43.64″E) (Figure 2), an artificial impoundment that was built during the first decade of the 20th century (Lötter 1992). This lake is 1621 m above sea level, has a water volume of 64 000 m^3, a depth of 1–2 m, and is 300 m long and 100 m wide, with a surface area of 4.3 ha (Wiechers et al. 1996). The lake is fed by storm water from an urban catchment made up of local residential areas and park land. The Zoo Lake catchment receives most of its rainfall between

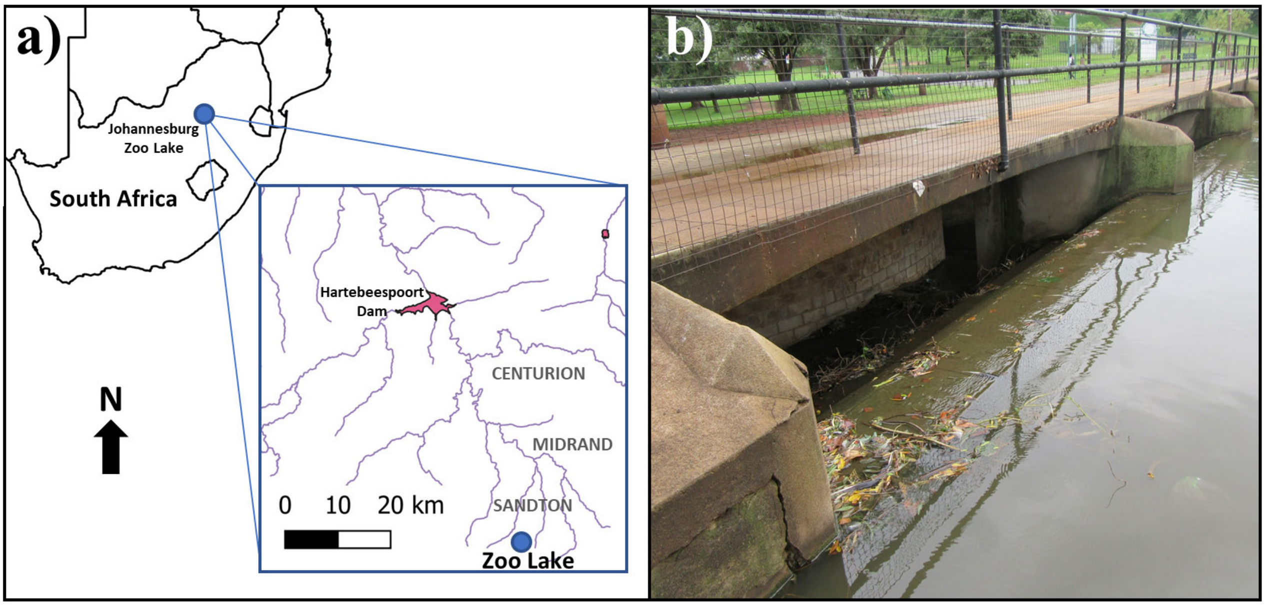

Figure 2. Map showing a) the location of Zoo Lake and b) the artificial impoundment, where *Sinotaia* cf. *quadrata* is present. Photo by SJT.

November and March, with a long-term annual average rainfall of 800 mm. Zoo Lake water flows into Braamfontein Spruit via an underground drainage system. Braamfontein Spruit joins the Jukskei River north of Johannesburg and eventually flows into Hartebeespoort Dam (Figure 2a) and then into the east-flowing Crocodile River system.

Zoo Lake water environmental parameter data (temperature, pH, conductivity, nitrates, ammonia, sulphates, phosphates, chloride, chemical oxygen demand (COD) and *Escherichia coli* counts) of the water were sourced from Quarterly Water Quality status reports of the Jukskei River catchment compiled by the City of Johannesburg.

A 20 × 20 cm quadrat was placed haphazardly in the non-vegetated shore for snail counts, this action was replicated twice. A Vernier caliper (error margin: 0.05 mm) was used to determine shell length (SL) of all the individuals within the quadrat. Size classes were selected according to Ferreira et al. (2017). A few specimens were collected and preserved in 99% ethanol and stored in a minus 20 °C freezer prior to molecular analyses. Voucher specimens were deposited in the KwaZulu-Natal Museum (accession code: NMSA-Mol 0P1893). Shells were cracked and ~ 2 mg of tissue just above the operculum was dissected and dried at 56 °C in a heating block. DNA was extracted and isolated from foot tissue with the E.Z.N.A.® Tissue DNA extraction kit using the solid tissue protocol. Fragments of the cytochrome *c* oxidase subunit I (COI) mitochondrial DNA were amplified by polymerase chain reaction (PCR) in a 25 µl mixture and sequenced using the forward primer LCO1490; reverse primer HCO2198 and protocols outlined by Folmer et al. (1994). PCR conditions were: 1 min at 95 °C, then 35 cycles at 95 °C for 30 s, 52 °C for 30 s, 72 °C for 30 s, followed by 72 °C for 3 min for COI. Amplified PCR products were visualized in 2% agarose gel electrophoresis stained with ethidium bromide. Amplicons were cut and purified using the Bioflux gel extraction kit. Purified PCR products were Sequenced at the Central Analytical Facilities (CAF) – Stellenbosch University with an ABI 3730 Capillary Sequencer utilizing the forward primer and Big Dye technology. Three sequences were deposited into the GenBank database (accession codes: OL692444–OL692446). GenBank's BLAST tool and Species Level Barcode option from the Barcode of Life Database (BoLD v4) yielded a 99.69% and 100% similarity index with *Sinotaia quadrata*. A COI dataset was assembled based on preliminary identification using the sequence dataset of O'Leary et al. 2021, which includes the datasets of Arias et al. (2020) and Stelbrink et al. (2020) and comprises two prominent clades: (1) a clade of exclusive Asian origin, including invasive viviparids reported from America and Europe, and (2) a viviparid clade of exclusive African origin. Sequences were aligned and edited using the Clustal W alignment tool in BioEdit ver.7.2.5 (Hall 1999). After editing, 637 bp fragments remained for analysis. Phylogenetic analysis was conducted using 68 COI sequences. A maximum-likelihood tree was constructed in MEGA X (Kumar et al. 2018) using the Tamura-Nei model nucleotide substitution model, as determined by Akaike Information Criterion calculation for best fit model, and 1000 bootstrap replicates. Pairwise uncorrected genetic distances (p-distance) were also calculated in MEGA X.

Results and discussion

This is the first report of *Sinotaia* cf. *quadrata* on the African continent and the fourth freshwater alien snail species of Asian origin recorded in South Africa since the 1990s. The recent introduction of these species to South Africa coincides with general recent increase in imports from Asia and has also been associated with the activities of the aquarium trade (Appleton and Miranda 2015). The aquarium trade is a common introduction pathway as many freshwater molluscs, including *Sinotaia*, have been unintentionally introduced all over the world together with plants and fishes (Appleton and Miranda 2015; Ng et al. 2016). Accidental introductions of these snails along with fish are also possible in aquaculture (Ferreira et al. 2017). However, intentional introduction for human consumption is also a possibility as these snails are a common food source throughout Asia (Qian et al. 2014; Arias et al. 2020). Although the introduction of *S.* cf. *quadrata* to South Africa is most likely very recent, its pathways are currently uncertain.

Shells were yellowish-green or brown, slightly convex, whorls ranged from 5 to 7, sutures were well marked and deep, the spire was conic obtuse. Juveniles exhibited spiral line with triangular lamella, the protoconch of the adults was whitish, the outer margin of the aperture was blackish. Individual SL ranged from 4.18 to 34 mm (Figure 3a, b), mean SL = 15.1 mm ± 0.58 SE (N = 276) (Figure 4). Radular and conchological examinations were not adequate for species identification because the morphology of these

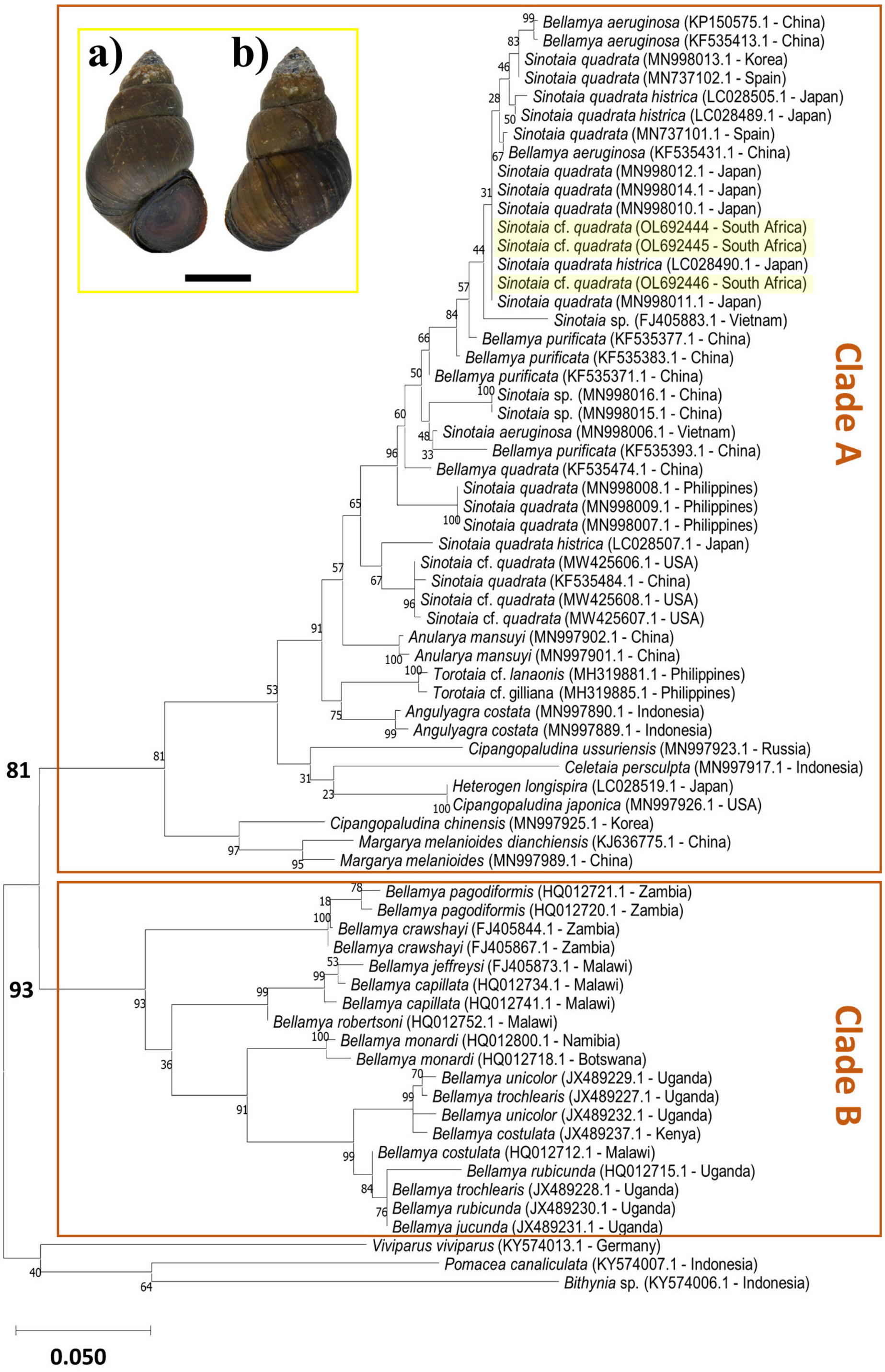

Figure 3. *Sinotaia* cf. *quadrata*: a) ventral and b) dorsal views of a specimen collected from Zoo Lake (scale bar: 10 mm) and Maximum-Likelihood phylogenetic tree of selected viviparid taka, including GenBank numbers and locality, based on 1000 bootstrap replicates. Taxa sequenced in this study are highlighted. Photo insert by NAFM.

snails is highly variable and is affected by the environment, as observed in previous studies (Chiu et al. 2002; Ferreira et al. 2017; Kagawa et al. 2019; Arias et al. 2020; Ye et al. 2021).

Phylogenetic analysis using COI sequences from a wide range of viviparid taxa show similar topology and clades reported in previous studies (Figure 3) (Stelbrink et al. 2020; Arias et al. 2020; O'Leary et al. 2021). South African

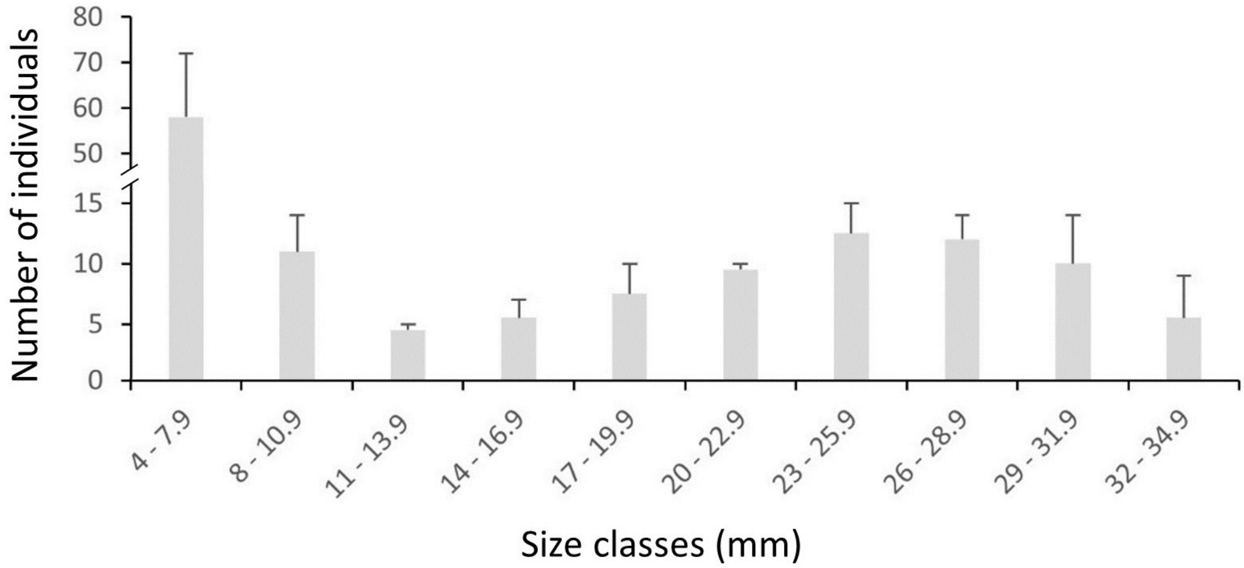

Figure 4. Average (+ SE) size class distribution of *Sinotaia* cf. *quadrata* (N = 276) recorded at Zoo Lake in November 2020.

Table 1. General environmental parameter ranges measured at Zoo Lake, based on quarterly samples.

	2019
Temperature (°C)	19–27
pH	7.3–8.3
Conductivity (μS cm^{-2})	30–33
Nitrate (mg l^{-1})	0.5–1.2
Ammonia (mg l^{-1})	0.5–2.0
Sulphate (mg l^{-1})	23–28
Phosphate (mg l^{-1})	0.5–1.6
Chloride (mg l^{-1})	24–28
COD (mg l^{-1})	31–91
E. coli (counts/100 ml)	3300–6700

specimens are part of Clade A which includes all viviparids of Asian origin and coincided with *Sinotaia quadrata* haplotypes found in Japan, all of which exhibit very low genetic divergence (p-distance < 0.0001) (Figure 3). The taxonomic status of *Sinotaia* is unclear and recent efforts to revise the taxon show evidence for synonymizing many previously recognized species within the genus with *S. quadrata* (Ye et al. 2021) There is a wide range of genetic distances across all *S. quadrata* which indicates this is a species complex as shown and discussed by previous studies (Ferreira et al. 2017; Stelbrink et al. 2020; Arias et al. 2020; O'Leary et al. 2021; Ye et al. 2021). We therefore also refer to South African specimens provisionally as *Sinotaia* cf. *quadrata*.

The population size class distribution (Figure 4) suggests *S.* cf. *quadrata* is well established and exhibits a high fecundity rate. It is also able to thrive in waters with high nutrient and bacterial concentrations (Table 1). The average density of *S.* cf. *quadrata* in Zoo Lake in November 2020 was 1078 ind. m^{-2} ± 236 SE. This is the highest population density on record for this species, greatly surpassing densities recorded in other areas in the world (Table 2). These patterns are similar to those exhibited by *Tarebia granifera*

Table 2. Summary of. *Sinotaia* cf. *quadrata* records outside of its native distribution range ca 2022.

Country	Earliest year of record	Habitats	Highest densities	Plausible introduction and spread vectors	References
Argentina	2009	Rivers, streams, reservoirs, artificial ponds	55.7 ind. m^{-2}	Aquarium trade; accidental introduction associated with aquaculture	Ovando and Cuezzo 2012; Ferreira et al. 2017
Italy	2013	Riverine	90 ind. m^{-2}	Food trade	Cianfanelli et al. 2017
Spain	2017	Rivers and ponds	Only 1–7 specimens collected	Aquarium trade?	Arias et al. 2020
France	2017	Lake	Only 1 specimen collected	Aquarium trade?	Arias et al. 2020
South Africa	2019	Artificial impoundment	1078 ind. m^{-2}	Aquarium trade? / Food trade?	Current paper
USA	2020	Riverine, boat jetty	Only 3 specimens collected	Aquarium plant trade	O'Leary et al. 2021

(Lamarck, 1822), another recently introduced alien invasive gastropod of Asian origin that has spread in South Africa and also attains very high densities dominating the benthic invertebrate community (Appleton et al. 2009; Appleton and Miranda 2015). *Sinotaia* cf. *quadrata* may interact with native and alien species and have significant ecological effects in its invaded range. It is known to feed on eggs and larval stages of fishes such as the bluegill *Lepomis macrochirus* Rafinesque, 1819 (Nakao et al. 2006) which is a widespread alien invasive in South Africa (Ellender and Weyl 2014). *Sinotaia* cf. *quadrata* occurs in habitats together with native and alien snails (Ovando and Cuezzo 2012). *Sinotaia* may spread unnoticed and either displace or co-exist with other native viviparids in Africa, such as *Bellamya* (Figure 1), since these genera can easily be mistaken for each other given their overlapping morphologies and habitats. *Physella acuta* (Draparnaud, 1805), another globally invasive freshwater alien snail (De Kock and Wolmarans 2007; Ebbs et al. 2018) was found together with *S.* cf. *quadrata* in Zoo Lake. Commonly known as the "sewage snail", *P. acuta* has a wide distribution in South Africa and favors eutrophic, polluted and anthropogenically disturbed habitats (Brown 1994; De Kock and Wolmarans 2007).

There are health risks as *Sinotaia* cf. *quadrata* is susceptible to parasites that can infect humans. All new occurrences therefore represent a new bio-sanitary risk to the receiving location. Populations of *S.* cf. *quadrata* may host the nematode *Angiostrongylus cantonensis* (Chen, 1935) which can cause eosinophilic meningitis and was recently reported from South Africa (Archer et al. 2011; Lu et al. 2018). Echinostomatid flukes are also known to infect these snails, however, there are no reported diagnosed cases of echinostomiasis or angiostrongyliasis in the country, which does not necessarily mean that these diseases are not prevalent. It can also become unsanitary to consider *S.* cf. *quadrata* for human consumption due to its reported ability to accumulate an extremely high bacterial content (Arias et al. 2020 and references therein) (Table 1).

Given the known invasive range and wide environmental tolerance of *S.* cf. *quadrata*, including its apparent ability to spread across neighboring streams and even polluted water bodies (Tables 1, 2; Ferreira et al. 2017), its high fecundity (Keller et al. 2007) and brooding behavior, its potential predatory release as a newly established alien species (see Dudgeon and Cheung 1990), and its current geographic position in Africa in connection to the Crocodile River system, we suggest that it has the potential to spread, much as *P. acuta* and *T. granifera* have, over the northern parts of South Africa, into the Kruger National Park and into Mozambique. Further field monitoring on an ongoing basis and investigations of possible invasion pathways, particularly through the food trade, are recommended in the region.

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Authors' contribution

Nelson A.F. Miranda: research conceptualization, sample design and methodology, investigation and data collection, data analysis and interpretation, funding acquisition, writing – original draft, review and editing; Sue J. Taylor: research conceptualization, investigation and data collection, writing – original draft; Yandisa Cwewe: data analysis and interpretation, writing – original draft; Christopher C. Appleton: research conceptualization, data analysis and interpretation, writing – original draft.

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