Rapid Communication

First record of freshwater planarian *Girardia sinensis* Chen & Wang, 2015 (Platyhelminthes, Tricladida, Continenticola) in South Africa

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Abstract

The occurrence of the alien freshwater planarian *Girardia sinensis* Chen & Wang, 2015 is reported for the first time in sub-Saharan Africa. This species is a global invader of freshwater bodies as evidenced by its very recently reported discoveries in Europe, Asia and Australia. We used DNA barcoding and phylogenetic analysis to confirm species identity, as morphological identification alone is not accurate. Unintentional introduction via the aquarium trade through contamination is considered a possibility. Taken together with other reports of recent introductions of invasive freshwater species in South Africa, possibly through aquarium trade, this raises serious concerns about the ineffective control of aquarium trade.

Key words: aquatic alien, flatworm, Korsman Bird Sanctuary, citizen science, cytochrome oxidase subunit 1

Introduction

The first comprehensive review of aquatic, free-living flatworms in Africa was done by Young (1976), who listed 80 species in 29 genera. Other scholars (Sluys 2007; Schockaert et al. 2008) built upon this foundation and subsequently listed 85 species in 28 genera from the Afrotropical region. Recently, faunastic and taxonomic works on Afrotropical planarians have resulted in several new species descriptions and increased our understanding of their diversity and distribution in the region (De Vries 1988; Sluys 2007; Stocchino et al. 2002, 2012, 2014, 2017; Harrath et al. 2019). Despite this, aquatic planarians are still poorly known in Southern Africa, and there are still no taxonomic keys to species or genera (Kawakatsu 1972; Appleton 2002). Lack of specialist knowledge and documentation results in alien species not being recognised (McGeoch et al. 2012).

Preliminary estimates of Young (1976) suggested a modest percentage of freshwater planarians in Africa as being non-native. However, recent estimates of freshwater planarian invasiveness are much higher (Sluys 2016; Stocchino et al. 2019; Fourcade et al. 2022). This high rate of invasiveness amongst free-living flatworms is surprising, as they tend to have a very limited dispersal ability (Lázaro and Riutort 2013; Sluys and Ruitort 2018). Although some taxa have the potential for natural long distance dispersal via migratory birds and wind, as they produce drought-resistant eggs (Young 1976), most free-living flatworms have instead been unwittingly transported by humans across their natural, historical boundaries (Sluys 2016). However, pin-pointing the exact mode of human-mediated introduction has been challenging, as there are several possible routes; including aquarium trade, ships' ballast waters, and soil or timber contamination (Lázaro and Riutort 2013; Sluys and Ruitort 2018; Stocchino et al. 2019). Once introduced, free-living flatworm species are often successful, as they can tolerate sub-optimal environmental conditions and reproduce via fissiparity, without the need for egg-laying (Sluys and Ruitort 2018).

Girardia Ball, 1974, is a genus originally native to South America, that spread to North America via natural dispersal routes but is now invasive in several other continents (Benítez-Álvarez et al. 2023). Invasive freshwater planarians exhibit puzzling dispersal routes (see, Solà et al. 2022) and *Girardia* is no exception. *Girardia* has since spread across the world, with a high probability of becoming invasive. The first dispersal of this genus into Europe occurred when *Girardia tigrina* (Girard, 1850) was recorded in the UK during the 1920s (Wright 1987). This species has subsequently been recorded across that continent (Ilić et al. 2018; Kanana and Ruitort 2019). Until recently, *Girardia* was not known from African waters. Benítez-Álvarez et al. (2022) reported the first record of this genus in Morocco, a country in the Palearctic region of Africa. However, there is currently no formal record of *Girardia* in sub-Saharan Africa. The current study reports for the first-time the occurrence of a feral population of *Girardia sinensis* Chen & Wang, 2015 in South Africa. Originally described from China (Chen et al. 2015), where it may be non-native, *G. sinensis* is a native of North America, and has subsequently been introduced into many countries, including Australia, Morocco, Spain, Italy, France, Germany and Netherlands (Benítez-Álvarez et al. 2022, 2023). Given the difficulties of morphological identification of fissiparous triclads lacking copulatory apparatus (Lázaro and Riutort 2013), the species level identification was confirmed using a fragment of the mitochondrial gene cytochrome oxidase I (COI).

Materials and methods

Freshwater planarians were collected from the Korsman Bird Sanctuary (−26.195°; 28.2911°) a natural depression pan that is 1627 m above sea level. The pan was 1.8 m deep with a surface area of 37 hectares at the time of collection. The pan (see Figure 1A) is fed mostly by storm water from an urban catchment of an upmarket residential area. The catchment receives most of its rainfall between November and March, with a long-term average of 744 mm and a record rainfall of 1242 mm in the 2022 hydrological year, measured locally. The pan has no natural outlet but during periods of high water level, water is pumped to Benoni Middle Lake in the Blesbokspruit river system.

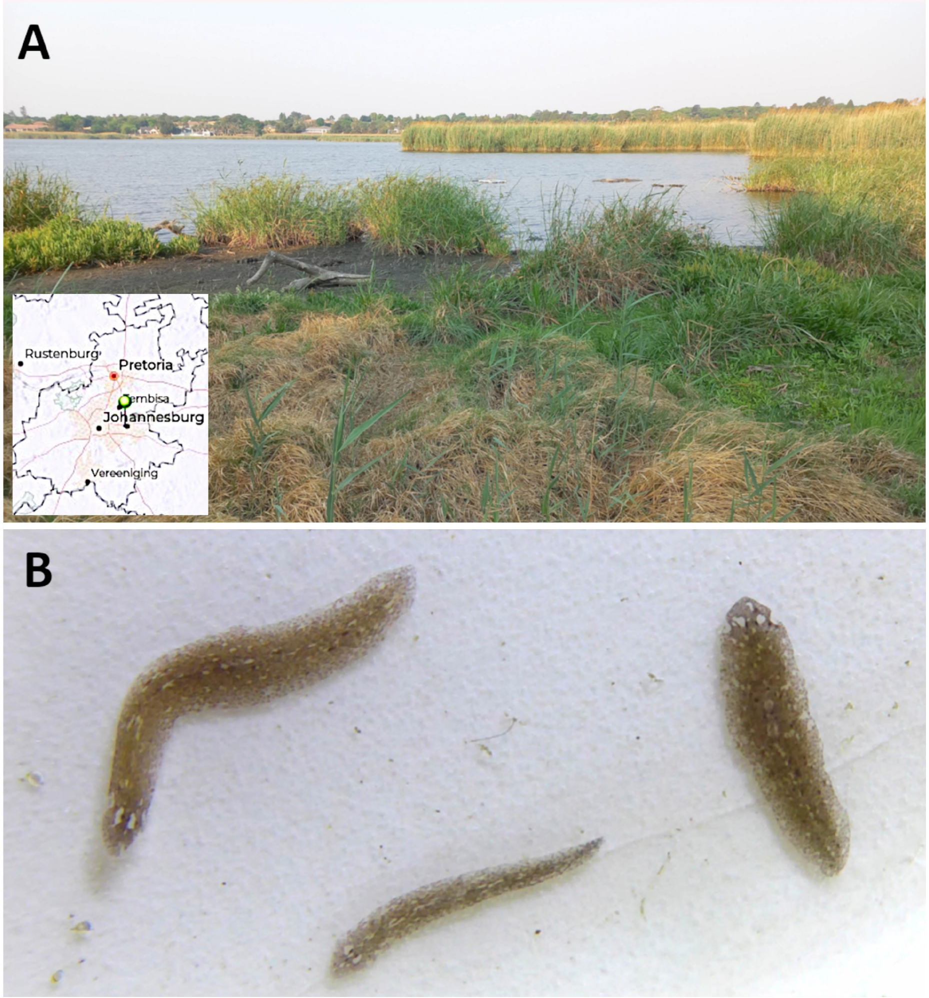

Figure 1. Photographs showing A) the study location at Korsman Bird Sanctuary, B) *Girardia sinensis* specimens found onsite. Photographs by Jane Trembath.

Korsman Bird Sanctuary water environmental parameter data (pH, electrical conductivity (EC), nitrates, ammonia (NH_4), sulphates ($SO_{4,}$ phosphates (PO_4), chemical oxygen demand (COD) and faecal coliform counts) were sourced from monthly water quality data obtained from the City of Ekurhuleni. Water physico-chemistry recorded at the time of specimens collections were as follows: Temperature (°C) 14–19, pH 7.8–8.4, Conductivity (mS cm^{-2}) 71–78, Nitrate (mg/l) 0.1, Ammonia (mg/l) 0.2–1.2, Sulphate (mg/l) 34–46, Phosphate (mg/l) 0.1, Sodium (mg/l) 61–77, COD (mg/l) 22–52, F coli (counts/100 ml) 12–37000. The eutrophic pan is subject to regular sewerage spills from an ageing sewer network and pumping stations within the perimeter of the Sanctuary. A sewer manhole within a few metres of the collection site overflows when the pump station is inoperative.

In May 2022, the lead author, a citizen scientist, found suspected *Girardia* sp. planarians in a water sample taken from flooded grassland in the vicinity of the sewer manhole. Three home-made planarian traps were placed in the vicinity of the find. The traps were made from small plastic containers drilled with 2 mm holes and provisioned with freshly killed *Cornu aspersum* (Müller, 1774) snails and buried in soft mud. The traps were removed after

36 hours and collectively contained 16 *Girardia* specimens. A previous attempt, provisioning the traps with pieces of cooked red meat, was unsuccessful in attracting planarians. However, on both attempts the traps attracted numerous leeches (*Helobdella* sp. and *Alboglossiphonia* sp.). The specimens were collected on the 6th of August 2022.

The trapped specimens were not examined to check if they were asexual or not. However, one of the specimens collected earlier in May showed signs of recent fission. The specimens were measured before killing and the approximate extended body length ranged from 4 mm to 9 mm. Larger specimens were generally more highly coloured. Each specimen had a different spot pattern (Figure 1B). They were not starved before fixation and killed shortly after retrieval in 99% ethanol. 11 specimens were submitted to Inqaba Biotech (Pretoria, South Africa), where the DNA extractions and sequencing was conducted, using standard procedures. An approximately 640-bp section of the COI gene was amplified using LCO1490 (50- GGTC AACAATCATAAAGATATTGG) and HCO2198 (50- TAAACTTCGGGT GACCAAAAAATCA) primers (Folmer et al. 1994), however the final length varied as ambiguous ends were visualized and trimmed using Chromas (Version 2.6.6).

In total, 54 COI sequences were used to infer the phylogenetic relationships of *Girardia* by using Maximum Likelihood and Bayesian Inference approaches. Four new sequences were obtained from the collected specimens (Table 1), with the remaining 50 sequences obtained from Genbank (Figure 2). The downloaded sequences were taken from Benítez-Álvarez et al (2022, 2023), and represented six species of *Girardia* from across the world, including *G. schubarti* Marcus, 1946, *G. sinensis*, *G. dorotocephala* Woodworth, 1897, *G. sanchezi* Hyman, 1955, *G. clandestina* Sluys & Benítez-Álvarez, 2022, and *G. tigrina*. The sequences were aligned using the online version of MAFFT 7, using the default parameters (Katoh et al. 2019). The subsequent alignment was visualized and trimmed in AliView 1.27 (Larsson 2014). A phylogenetic tree was estimated using a Maximum Likelihood approach conducted in W-IQ-Tree (Trifinopoulos et al. 2016) with 1000 bootstrap replicates using the Ultrafast algorithm, using a partition scheme by codon (Table 2). Additionally, a Bayesian Inference approach was conducted in Mr Bayes 3.2.7a (Ronquist et al. 2012), using a partitioned scheme by codon and the models identified by the model selection tool in W-IQ-Tree (Table 2). The MrBayes test was conducted using the CIPRES science gateway (Miller et al. 2010; accessible at https://www.phylo.org/index.php/). The final alignment used for molecular analysis was trimmed to 827 base pairs, with 16% of the data ambiguous, or missing.

Results and discussion

Both the Maximum Likelihood (Supplementary material Figure S1) and Bayesian Inference (Figure 2) methodologies recovered phylogenetic trees with similar topologies. Unfortunately, the Maximum Likelihood tree provided

Table 1. Genbank Accession Numbers of all specimens used in the Maximum Likelihood and Bayesian Inference approaches.

GenBank Accession Details			
Voucher Number	Species I.D.	Country	Genbank Accession
3BARS	*Girardia sinensis*	South Africa	
4BARS	*Girardia sinensis*	South Africa	
5BARS	*Girardia sinensis*	South Africa	
6BARS	*Girardia sinensis*	South Africa	
	Girardia sinensis	Morocco	OM480692
	Girardia sinensis	Morocco	OM480693
	Girardia sinensis	Morocco	OM480694
	Girardia sp. (*sinensis*)	China	KP091895
	Girardia tigrina	Spain	OM307091
	Girardia sinensis	Spain	OM307098
	Girardia sinensis	Italy	OM307099
	Girardia sinensis	Netherlands	OM307155
	Girardia sinensis	Australia	OM307161
	Girardia sinensis	China	OM307169
	Girardia sinensis	Cuba	OM307174
	Girardia sinensis	Spain	OM307089
	Girardia sinensis	Australia	OM307162
	Girardia sinensis	Spain	OM307159
	Girardia sinensis	Spain	OM307158
	Girardia sinensis	Spain	OM307157
	Girardia sinensis	Germany	OM307153
	Girardia sinensis	France	OM307152
	Girardia sinensis	Australia	OM307146
	Girardia sinensis	Spain	OM307128
	Girardia sinensis	France	OM307122
	Girardia sinensis	France	OM30712
	Girardia sinensis	Spain	OM307106
	Girardia sinensis	Spain	OM307102
	Girardia sinensis	Italy	OM307100
	Girardia sinensis	Spain	OM307097
	Girardia sinensis	Spain	OM307096
	Girardia sinensis	Spain	OM307090
	Girardia dorotocephala	Spain	OM307103
	Girardia dorotocephala	Brazil	OM307111
	Girardia dorotocephala	Hawaii	OM307129
	Girardia dorotocephala	USA	OM307124
	Girardia dorotocephala	Hawaii	OM307130
	Girardia dorotocephala	Japan	OM307164
	Girardia dorotocephala	Japan	OM307165
	Girardia dorotocephala	Japan	OM307166
	Girardia tigrina	Spain	OM307127
	Girardia tigrina	France	OM307105
	Girardia tigrina	Italy	OM307101
	Girardia tigrina	Spain	OM307091
	Girardia tigrina	France	OM307088
	Girardia tigrina	France	OM307087
	Girardia tigrina	Spain	OM307078
	Girardia tigrina	Spain	OM307077
	Girardia clandestina	Brazil	OM307081
	Girardia clandestina	Brazil	OM307085
	Girardia sanchezi	Chile	OM307167
	Girardia schubarti	Brazil	OM307131
	Girardia schubarti	Brazil	OM307080
	Girardia schubarti	Brazil	OM307079

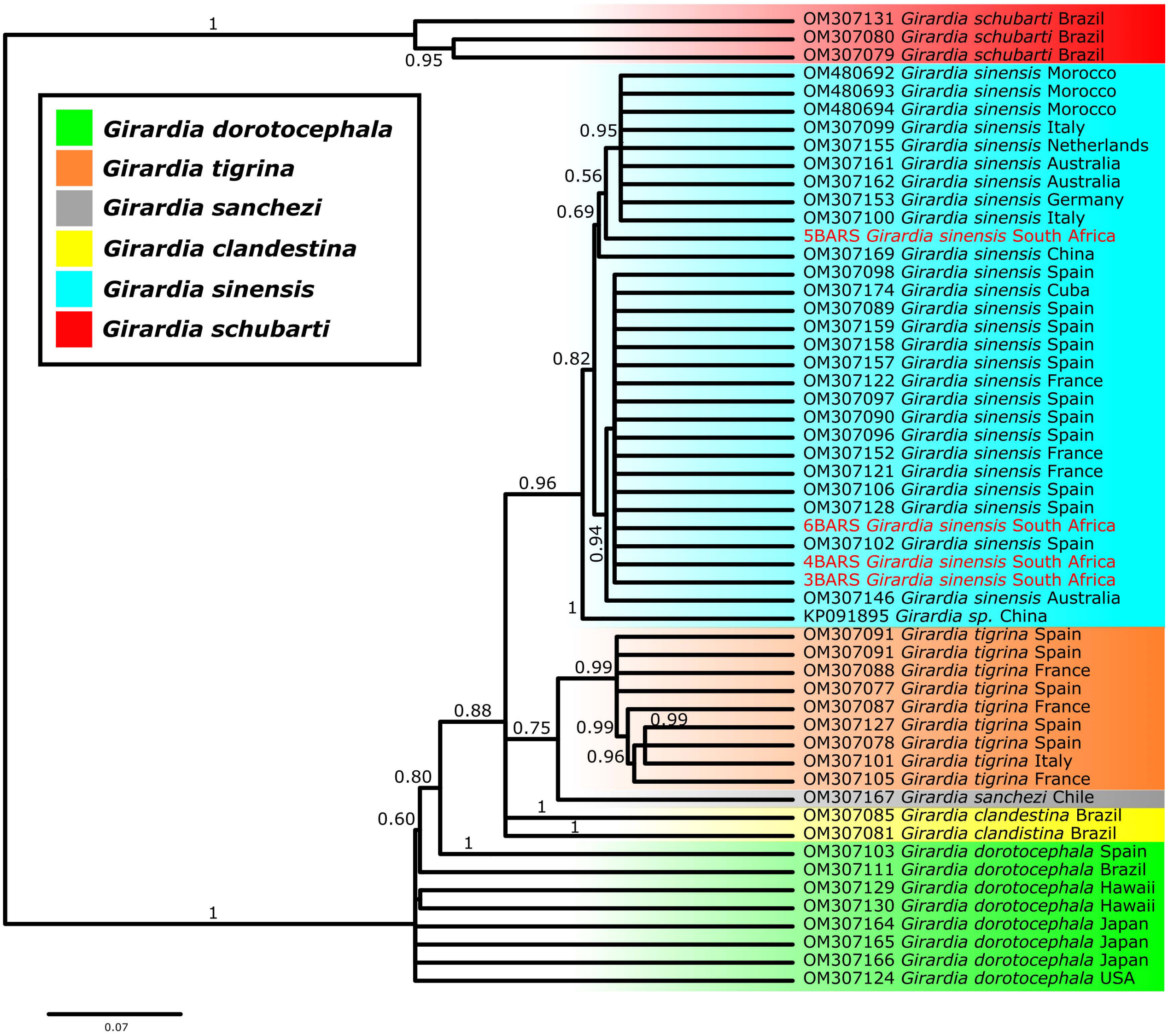

Figure 2. Bayesian Inference phylogeny of *Girardia sinensis* and related species constructed using cytochrome oxidase subunit 1 (COI). Numbers given on each branch represent posterior probabilities.

extremely poor resolution, as it recovered several polytomies and generally low bootstrap values. Additionally, it recovered a polyphyletic *G. clandestina*, and relationships between species were not resolved. Despite this, it recovered a strongly supported (97%) monophyletic clade of *G. sinensis*, which included the newly collected specimens from South Africa. Comparatively, with the exception of *G. dorotocephala*, species level clades were monophyletic and well supported in the Bayesian Inference analysis (> 0.90), although relationships between the species were not resolved. As with the Maximum Likelihood approach, the newly collected South African species were clustered together within *G. sinensis* in a monophyletic clade. This provides strong support for the assignment of the South African species to *G. sinensis*. Thus, the current manuscript reports the second record of this cosmopolitan species in Africa (Benítez-Álvarez et al. 2022), and a first from Sub-Saharan Africa. The discovery of this feral population shows the power of citizen and formal science working together (Daniels et al. 2022), as we were alerted to the presence of this population by a citizen scientist.

Table 2. Results of jModelTest2, and the relevant primers selected for the Bayesian Inference Test of phylogeny conducted in MrBayes

Maximum Likelihood	
AIC Model, Partition 1	TN+F+I
AIC Model, Partition 2	F81+F+I
AIC Model, Partition 3	K3Pu+F+G4
Bayesian Inference	
AIC Model	HKY+I+G
p-inv	0.517
Gamma Shape	2.6801

In the case of the discovery at Korsman Bird Sanctuary, circumstantial evidence supports the theory of *Girardia* being introduced into the pan via the sewer network, possibly originating onwards from the aquarium trade. Freshwater invertebrates have been shown to have a high propensity of being accidentally transported as contaminants in the aquarium trade (Patoka et al. 2017). The initial discovery and subsequent successful trapping occurred within a few metres of a frequently overflowing sewer manhole. A nearby residence, connected to this sewer system, makes regular use of an aquarium service company. The company owner recognised photographs of the *Girardia* specimens as an aquarium pest. They were unaware of *Girardia*'s environmental tolerance and believed that they would not survive in sewerage. As they were found within an area which had been drenched by waste water, this could suggest tolerance of severely degraded water quality.

Recreational lakes in and around Johannesburg surburbia appear to be on the receiving end of invasive species, largely coming off aquarium and pet trade (van Wilgen et al. 2010; Martin and Coetzee 2011). For example, Miranda et al. (2022) recently reported discovery of a new invasive snail, *Sinotaia* cf. *quadrata* (Benson, 1842), at Zoo lake. This site is about 30 km from where are reporting the new record of *G. sinensis* in South Africa. The full extent of the distributional occurrence of this species is unknown, however given the high connectivity of urban waters, it is highly unlikely that it is restricted to the reported site. These new records of invasive species in these lakes are alarming, given the already long list of invasive freshwater invertebrates in the country, some with massive environmental and socio-economic impacts (Weyl et al. 2020). The next iteration of the National Invasive Species Report (e.g. Zengeya and Wilson 2020) should include these new species. However, to assess the invasive status of these species and their potential impacts (Zengeya and Wilson 2020), more information on their distributional occurrence is needed. As such, large scale assessment of the occurrence and ecological impact of these species in waters around Johannesburg is warranted.

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Authors contribution

JT, MCM: research conceptualization; JT, MCM: sample design and methodology; JT: Investigation and data collection; APK: data analysis and interpretation; JT, PN, MM: roles/writing – original draft; JT, PN, APK, MM: roles/writing – review and editing.

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Supplementary material

The following supplementary material is available for this article:

Figure S1. Maximum likelihood phylogeny of *Girardia sinensis* and related species constructed using cytochrome oxidase subunit 1 (COI).

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2023/Supplements/BIR_2023_Trembath_etal_SupplementaryFigure.pdf