BioInvasions Records (2023) Volume 12, Issue 1: 257–264

Rapid Communication

First record of the globally invasive planarian *Girardia tigrina* (Girard, 1850) sensu lato in Morocco

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Citation: Mabrouki Y, Taybi AF, Vila-Farré M (2023) First record of the globally invasive planarian *Girardia tigrina* (Girard, 1850) sensu lato in Morocco. *BioInvasions Records* 12(1): 257–264, https://doi.org/10.3391/bir.2023.12.1.21

Received: 25 April 2022

Accepted: 4 September 2022

Published: 28 November 2022

Handling editor: Agnese Marchini

Thematic editor: Kenneth Hayes

Abstract

The planarian *Girardia tigrina* sensu lato is the most successful freshwater invasive triclad. While Antarctica remains a continent without reports for this species, the single African record is unreliable. Therefore, the species is still unknown on this continent despite the presence of adequate water masses for its occurrence. Here, we report the finding of *G. tigrina* sensu lato in Morocco at Oued Fès, which constitutes probably its first record in the entire African continent. Despite being located in only one out of more than 100 localities sampled by us, ongoing investigations could soon reveal more occurrences of the species in the country and the rest of the continent.

Key words: Africa, aquatic invasion, triclad, alien flatworm, Sebou River basin

Introduction

Invasions of alien species are significant threats to global biodiversity and ecosystems functioning worldwide, possibly causing species extinction, habitat degradation and ecosystem alteration (Pimentel et al. 2005). Freshwater habitats are particularly vulnerable to bioinvasions due to the high specialisation of their biodiversity (Vitousek et al. 1997; Strayer 2010). That circumstance is worsened by the multiple human disturbances and the effect of climate change (Vitousek et al. 1997; Macdonald and Tonkin 2008; Gherardi et al. 2009). As a result, considerable impact, sometimes with irreversible ecological consequences, has been attributed to invasive species in freshwater environments (Strayer 2010; Havel et al. 2015).

Several invasive planarian species constitute a threat to biodiversity (Sluys 2016). *Girardia tigrina* (Girard 1850), a freshwater species native and widely distributed in North America (Kenk 1974; Thorp and Covich 1991), represents one of the best-known invasive planarians. The species comprises at least four different forms or classes (Ribas et al. 1989; Stocchino

et al. 2019), and it is morphologically and molecularly quite similar to the recently described *Girardia sinensis* (Chen et al. 2015) (Kanana and Riutort 2019). Given that it is still difficult to distinguish the different forms, we will use *Girardia tigrina* in a broad sense, i.e. *Girardia tigrina* sensu lato.

Girardia tigrina s.l. is a generalist eurythermic species, tolerant to a wide range of environmental factors, one of the traits behind its success as a worldwide invader (Wright 1987; Claussen and Walters 1982). Moreover, *G. tigrina* s.l. has a plastic diet and can exploit various food resources, causing potential strong interspecific competition for food with native triclads and other freshwater organisms (Pickavance 1971; Gee et al. 1998; Stocchino et al. 2019). Finally, asexual reproduction by fission enables planarians to colonise new water bodies, potentially starting with a single or few individuals, followed by a rapid increase in population size (Kenk 1937; Calow et al. 1979; Charni et al. 2004; Lázaro and Riutort 2013). Accordingly, most known introduced populations of *G. tigrina* s.l. reproduce by fission, while sexually reproducing populations are very scarce (Vila-Farré et al. 2004; Sluys et al. 2005; Stocchino et al. 2019).

Since its introduction in Germany in the 1920s (Meinken 1925), the species has successfully invaded several other European countries, as well as Japan and Australia (Gourbault 1969; Ball and Reynoldson 1981; Sluys et al. 1995; Ribas et al. 1989; Gee et al. 1998; Sluys et al. 2010; Vila-Farré et al. 2011; Kanana and Riutort 2019). To the best of our knowledge, Africa has a single *Girardia* record (Beltagi and Amina 2015) that is analysed in the discussion.

Here, we report the occurrence of *Girardia tigrina* s.l. in Morocco, very likely the first record in Africa. In addition, we discuss possible mechanisms of introduction to increase awareness of the impacts of invasive species on native fauna in Morocco and Africa in general.

Materials and methods

To promote knowledge of the freshwater biodiversity of Morocco, we conducted several field expeditions since 2014 in the northern part of the country, focusing on the Middle Atlas and the Moulouya and Sebou River basin. We have visited more than 150 localities, in most cases three times (e.g., see Mabrouki et al. 2020 for details). The samples of benthic fauna, including triclads, were collected using kick nets, entomological forceps or brushes.

Conductivity, pH, and dissolved oxygen were measured *in situ* with a multiparametric measuring device (WTW, Multi-Line P4). The current velocity (converted to $cm.s^{-1}$) was quantified by its mean value at three locations of the same station. The other parameters (ammonium, nitrates, orthophosphates and turbidity) were measured in the laboratory. Two replicates of water samples from each station were taken in 500 ml polyethylene bottles. Water samples were preserved with 2 ml of concentrated hydrochloric

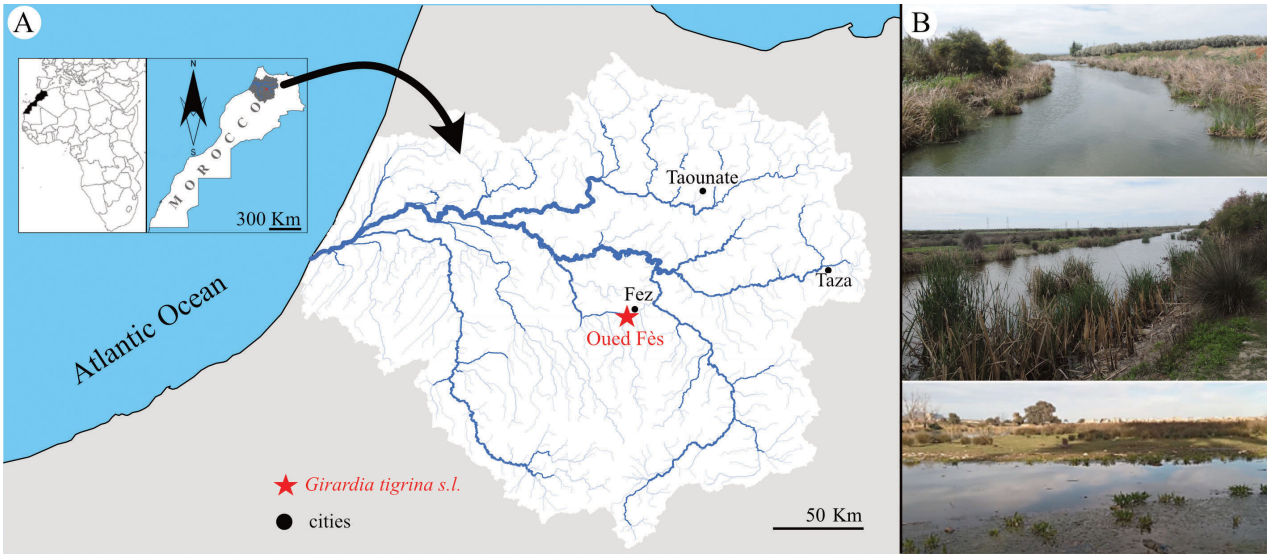

Figure 1. A. Map showing the distribution of *Girardia tigrina* s.l. in the Sebou River basin, Morocco. Insets: position of Morroco in the African continent and shape of the country. B. Views of the habitats in Oued Fès inhabited by the species. Photographs by Youness Mabrouki

acid (pH = 2). According to the ISO 5667-6 (1990), ISO 5667-2 (1991) and ISO 5667-3 (1994) standards, they were conveyed to a cooler at a low temperature (± 4 °C) to stop the metabolic activities. The variables were determined according to AFNOR (1997) standards and Rodier et al. (1996).

Results

During our visit to Oued Fès (34°03′32.4″N; 4°59′44.9″W) in March 2022, about forty individuals of *G. tigrina* s.l. were sampled (Figures 1 and 2). In the Sebou River basin near the agglomeration of Fès, the study area is strongly impacted by human activities since it receives untreated domestic and industrial waste from this city (Hayzoun 2014). As a result, the analysis of the physicochemical parameters of the water, such as turbidity and orthophosphate concentration, revealed a mediocre to poor environmental quality of the habitat (Table 1).

Girardia tigrina s.l. can be distinguished from other Moroccan triclads by its markedly triangular head provided with two large, close-set eyes anterior to prominent auricular slits; a blotchy and grey dorsal surface; and a pale ventral surface that contrasts with the dorsal colouration pattern (Figure 2). In Morocco, only representatives of the genus *Dugesia* have this characteristic head shape. Nevertheless, the blotchy pigment pattern of *G. tigrina* s.l. is very distinctive and different from that observed in *Dugesia*. In addition, the specimens had a pigmented pharynx. Regarding the reproductive strategy of the animals, while gonopores were absent from collected specimens, some showed blastemas in the posterior region of the body, thus suggesting active reproduction by fission. The external and pharyngeal pigmentation patterns and the existence of signs of asexual reproduction render Moroccon individuals similar to those of the morphological class A of *G. tigrina* described in Ribas et al. (1989).

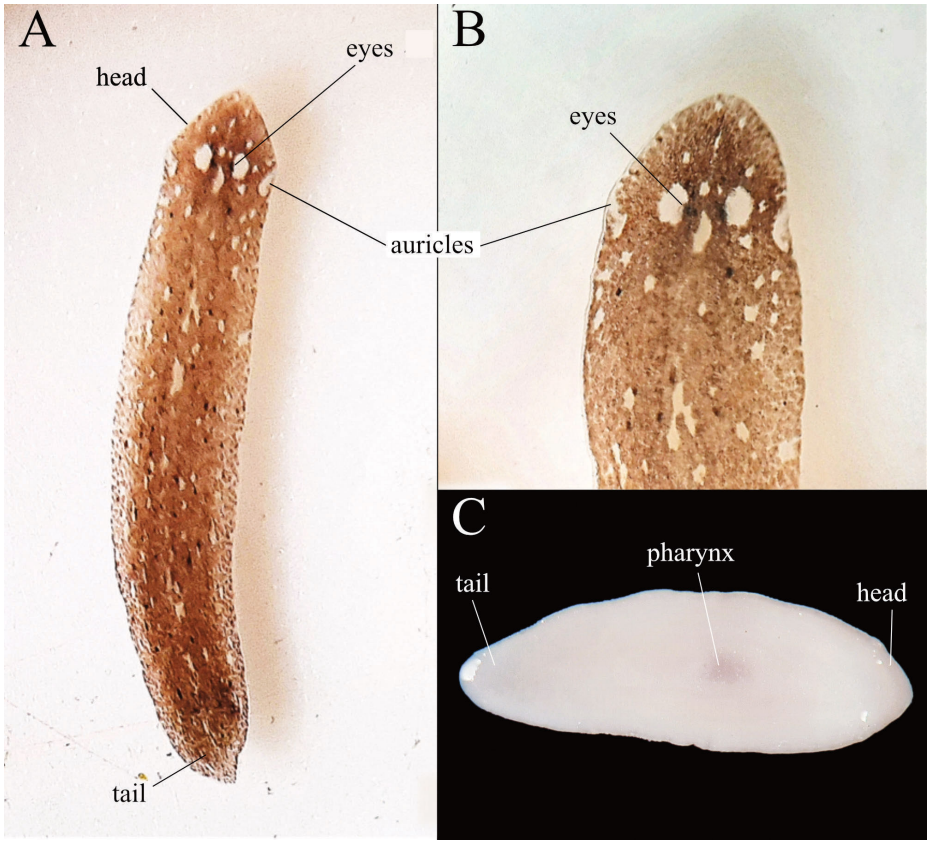

Figure 2. External anatomy of *Girardia tigrina* s.l. from Oued Fès, Morocco. A. Dorsal view of a living specimen in movement. B. Detail of the cephalic region of a living specimen. C. Ventral view of a fixed specimen. Photographs by Youness Mabrouki.

Table 1. Mean for the physical and chemical water parameters measured at the sampling locality (Sebou River, Oued Fès, Morocco).

Factor	Ph	Conductiviy	Disolved oxygen	Turbidity	Nitrate	Orthophosphate	Ammonium
Mean	7,8	1070 mg/l	4,5 mg/l	16,6 NTU	3,15 mg/l	0,75 mg/l	1,5 mg/l

Discussion

The only known record of *Girardia tigrina* s.l. in Africa, Egypt (Beltagi and Amina 2015) lacks sufficient detail, particularly graphic documentation. Regarding the internal anatomy, no photomicrographs or original reconstruction drawings are available. No information is provided on the presence of pigment granules in the pharyngeal wall, characteristic of several *Girardia* strains. Regarding the external appearance, despite the author's mention that "the body has yellow and white spots", thus resembling the mottled pattern of living *Girardia tigrina* s.l., living animals in Figure 2a in Beltagi and Amina (2015) are uniformly brown, not blotchy, except for the tail tip. This aspect is even more apparent when comparing those animals with our material from Morocco. The external pattern of animals pictured in Beltagi and Amina 2015, with a triangular shape of the head and uniform colour pattern, resembles that of several *Dugesia* species. Whether these animals are *G. tigrina* or a different species can only be confirmed or disregarded with additional anatomical or

barcoding data obtained from the same specimens. Therefore, the Egyptian record for *G. tigrina* s.l. should be treated cautiously without more solid evidence. In summary, our report for *G. tigrina* s.l. in Morroco is the first reliable report for the species in Africa.

Morocco represents a contact area between Europe and Africa and a compulsory passage for much of the fauna between the Palaearctic and Afrotropical regions and between the Mediterranean Sea and the Atlantic Ocean. Additionally, Morocco has multiple geographical barriers both around and within the country, such as the Moulouya River Basin, the Sahara, the Rif Mountains and the Atlas Massif. The latter divides this country into two bioclimatic regions, causing high levels of endemism. (Taybi et al. 2019; Mabrouki et al. 2020). This resulted in biological communities being vulnerable to the arrival of invasive species that lack a shared evolutionary history with them (Ellender et al. 2015). Alien and invasive species are continuously recorded, at an exponential rate, in the aquatic ecosystems of Morocco, including fish, annelids, molluscs and arthropods (Mabrouki et al. 2019a, b; Mabrouki et al. 2020; Taybi and Mabrouki 2020; Taybi et al. 2020; Mabrouki et al. 2021; Taybi et al. 2021). *Girardia tigrina* s.l. is a new addition to the list of alien species in Morocco and the first alien freshwater triclad flatworm reported in the country. Since it is also the first reliable observation of the species in the African continent, it constitutes a warning signal of the undesired environmental changes that the continent is currently suffering.

Girardia tigrina s.l. is one of the first noted invasive planarian species. Among the several hypotheses explaining the introduction mechanisms of the species, the pet aquarium trade stands as the most likely vector of its introduction (Van der Velde 1975; Ilić et al. 2018). Similarly, Oued Fès is infested by the ornamental aquatic plant *Pistia stratiotes* L., 1753 (Khabbach et al. 2019). Thus, *Girardia tigrina* s.l. could have been introduced into the country associated with this or other exotic plants, a mechanism suggested before for other invasive triclads (Sluys et al. 2014). Additionally, the Oued Fès River's poor water quality could also favour this generalist species' expansion.

The introduced populations of *G. tigrina* s.l. are mostly fissiparous (Vila-Farré et al. 2004; Sluys et al. 2005; Stocchino et al. 2019). This agrees with our observation that Moroccan specimens show no external trace of gonopore, while there is evidence of asexual reproduction by fission. Extended monitoring of this population may confirm the species' reproductive strategy in Oued Fès. In summary, the continuous deterioration of Africa's freshwater environments and the great asexual reproduction ability of the species suggest a possible future expansion of *G. tigrina* s.l. in this continent, similar to that in Europe. Therefore, monitoring the presence and expansion of the species within invaded areas and protecting freshwater environments are crucial to mitigate the possible impact on native communities.

Acknowledgements

We thank our reviewers for their helpful comments on the manuscript.

Authors' contribution

Y.M.: research conceptualization, sample design and methodology, data analysis and interpretation; original draft; A.F.T.: research conceptualization, sample design and methodology; M.V-F: research conceptualization, data analysis and interpretation, review and editing.

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