

First Report on the Non-Indigenous Triclad *Girardia tigrina* (Girard, 1850) (Tricladida, Dugesiidae) in Serbia, with Notes on its Ecology and Distribution

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Abstract: *Girardia tigrina* (Girard, 1850) (Turbellaria, Tricladida, Dugesiidae), a native freshwater triclad of North America, is a widespread species, which has been introduced by human activities into various parts of the world, including Europe. The aim of this paper is to contribute to the knowledge of this species by presenting its first record and recent distribution in Serbia. *Girardia tigrina* was recorded in Serbia in 2008 in the lower stretch of the Kolubara River. After this initial finding, the species was identified only a few more times, but with low abundance. Its presence only in the northern part of Serbia, in the Danube River and in its proximity, points to the Danube River and its larger tributaries (the South invasive corridor) as the main route of its spread in the country. Our data suggest that *G. tigrina* should not be considered an invasive species but as an alien species with a minor impact on native communities.

Key words: flatworm, alien taxa, Sava River Basin, Danube, Eastern Europe

Introduction

The triclad turbellarian *Girardia tigrina* (Girard, 1850) (syn. *Dugesia tigrina*), first described by GIRARD (1850) and subsequently by KAWAKATSU et al. (1981), is a small to middle-sized organism, up to 12 mm in length and 1.5 mm in width. It has a markedly triangular head, with a pair of bluntly pointed auricles with two eyes, each surrounded by a clear, non-pigmented area. The dorsal colour is usually mottled grey and the ventral surface is paler (REYNOLDSON 1978). Although in its native area (North America) *G. tigrina* exhibits variable external morphology, possessing either striped or spotted pigment patterns (HYMAN 1939), all European populations are more or less uniformly spotted and with a pigmented pharynx (RIBAS et al. 1989). Of particular importance in planarian taxonomy and identification is the morphology of the reproductive system. This triclad may reproduce both sexually and asexually

by fission (HYMAN 1941, KENK 1937). Thus, determination of asexual individuals is difficult (BAGUÑA et al. 1999, CHARNI et al. 2004), despite the fact that these types differ morphologically, with reproducing animals being much longer (GRASSO & BENAZZI 1973). Asexual reproduction is considered an advantage in less favourable habitats, since it allows for a more rapid increase in population size (CALOW et al. 1979, CHARNI et al. 2004). Known European populations of *G. tigrina* are almost exclusively asexual (RIBAS et al. 1989).

Although streams are typical habitats of *G. tigrina* (see HYMAN 1951), especially lentic zones where it can be found attached to aquatic plants (KNAKIEVICZ et al. 2007), specimens have also been found in sluggish waters such as lakes and ponds (STOKELY et al. 1965). This worm has a generally broad range of tolerance, preferring temperatures

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ranging between 9–25°C (DAHM 1958, STOKELY et al. 1965). *Girardia tigrina*, as most freshwater planarians, is a nocturnal carnivorous animal, which feeds on small invertebrates such as oligochaetes, isopods, chironomids, snails, caddisflies and mayflies (GEE & YOUNG 1993, LOMBARDO et al. 2011).

With only a few extant records, this apparently widespread species is still rarely observed in Eastern Europe. In Hungary, *G. tigrina* was found in alluvial floodplains in the protected area of the Szigetköz in the Ráckevei-Soroksári Danube, and in Gemenc and Béda Karapanca between 1995–2008 (FÜLEP & NOSEK 2010). In Romania, this triclad was recorded in the western Pontic Danube River (943–375 rkm; WFD ROOF REPORT 2004). In Bulgaria, *G. tigrina* was found in the south-western part of the country, in the Rilska River in 2007 (MOSKOVA & UZUNOV 2011). The presence of *G. tigrina* in the part of the Sava River in Croatia was reported in 2011 (PAUNOVIĆ et al. 2012). Fish and plant trade for aquaria and ships' ballast waters are probably the main vectors of its introduction and spread across Europe (VAN DER VELDE 1975, KNAKIEVICZ et al. 2007).

Despite the documented presence of *G. tigrina* in neighbouring countries, there is no data on the presence of this species in Serbia. This paper aims to fill the gaps in our knowledge regarding this species. Herein, we provide the first report on its presence in Serbia, as well as data of its recent distribution and main ecological preferences.

Materials and Methods

Extensive sampling, which encompassed the entire territory of Serbia, was conducted by the Institute for Biological Research “Siniša Stanković” in the period 2008–2015. A total of 80 rivers, canals, accumulations, lakes and ponds were examined during this survey. The standard samples of benthic macroinvertebrates were collected by a benthic hand net (mesh size 500 µm) in shallower bank regions (up to a depth of 1.5 m), and by the Van Veen grab (270 cm² grab area) in waters up to 10 m depth. Samples were collected from all available types of substrate according to EN 27828 standard. After taking into consideration the relative contribution of each microhabitat, the number of samples collected from a particular microhabitat within each reach corresponded to the relative contribution of this microhabitat to the substrate of the assessed river reach (10% = 1 sample), as described in the multi-habitat sampling procedure in HERING et al. (2004). The approximate length of the assessed reach at each sampling site was 100 m of the bank. The coordinates (latitude and longitude) of the

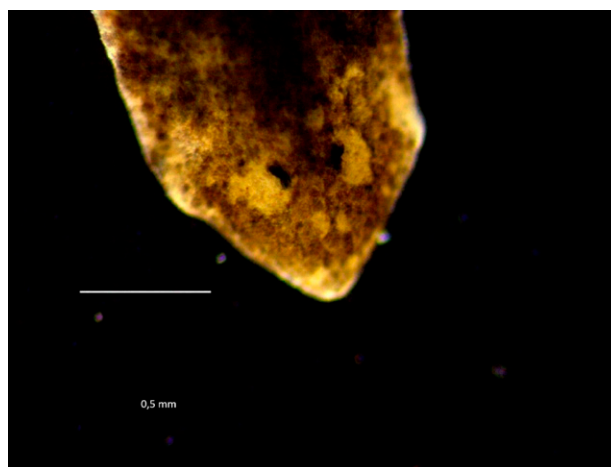


Fig. 1. *G. tigrina* from sampling site No. 3 Zabran in 2009.

sampling sites were measured by “Garmin eTrex” and GPS, and charted using ArcView 9.1 software (map 1:300,000, system WGS_1984).

Part of the sampled material was examined on site and the rest was preserved in either 4% formaldehyde or 70% ethanol and processed in the laboratory. Specimens were observed using the Carl Zeiss, Stemi 2000-C binocular magnifier, and photographed with the AxioCam ERc 5s digital camera. Collected individuals were identified using the identification key of REYNOLDSON (1978). All samples are stored in the Department for Hydroecology and Water Protection of the Institute for Biological Research “Siniša Stanković”, University of Belgrade.

Results

Our study presents the first report of the flatworm *Girardia tigrina* in Serbian inland waters. *Girardia tigrina* was found for the first time in the Kolubara River in June 2008 at the Čelije sampling site. The second finding was in the Sava River in September 2009 at the Zabran sampling site (Fig. 1). After a few years, this species was detected in the Danube River at the site Tekija in the Đerdap I accumulation and in the Tisa River at the site Titel in September 2014. Findings of *G. tigrina* in Serbia with site coordinates are presented in Fig. 2. The number of specimens was low (up to 5 specimens) in the majority of the samples, except in the Kolubara River where 20 individuals were counted.

Discussion

It has been documented that *G. tigrina* inhabits ponds, lakes, rivers and streams (KENK 1944, HYMAN 1951). In Serbia, it was predominantly found in large lowland rivers with a predominance of siliceous fine

sediments at sites with both hard and soft substrates (pebbles, sand and silt).

While the morphology, karyology and taxonomy (KAWAKATSU et al. 1981, 1982, 1985, 1993, VREYS et al. 2002), reproduction and life-cycle (KNAKIEVICZ et al. 2007) and ecotoxicology (GUECHEVA et al. 2001, PREZA, SMITH 2001, PRÁ et al. 2005) of *G. tigrina* and other planarians have been extensively studied, much less is known of their ecology and biogeography.

By late 20th century and the beginning of the 21st century, *G. tigrina* has been recorded from inland waters in Germany, Austria, Hungary and Romania (TITTIZER et al. 2000, DE YONG 2013, FÜLEP & NOSEK 2010, AN DER LAN 1962). As the Danube River is a main invasion route (PANOV et al. 2009), we suggest that it represents the main corridor of spread of this species into the region as well. The absence of data regarding this species in the Serbian stretch of the Danube River could be because the species is present in low numbers. In addition, the accelerated spread of Ponto-Caspian invaders, such as *Dendrocoelum romanodanubiale* (Codreanu, 1949), may have suppressed the growth of *G. tigrina* populations (VAN DER VELDE 2002).

The data presented here points to another possibility, namely a single introduction and subsequent spread of the species in the region. The irregularity in the dates of the reports of *G. tigrina* in Serbian water courses suggests that humans contributed to its

dispersal. WRIGHT (1987) concluded that accidental introductions during fish stocking and plant introduction contributed to the spread of *G. tigrina*, and that further dispersal can be achieved via canals and rivers, a view also shared by VAN DER VELDE (1975). A low spreading potential, probably due to the absence of a larval stage and low mobility, can be the reason for the sporadic findings of this species in Serbia, but also the morphological similarity between *G. tigrina* and *Dugesia lugubris* (Schmidt, 1861) and the resulting taxonomic misidentification. Therefore, further studies on *G. tigrina* should include examination of the organisms' genetic material.

The distribution of this triclad species and other non-native aquatic invertebrate taxa such as *Hypania invalida* (Grube, 1860), *Branchiura sowerbyi* (Beddard, 1892), Chinese pond mussel *Sinanodonta woodiana* (Lea, 1834), Spiny cheek crayfish *Orconectes limosus* (Rafinesque, 1817), Asian clam *Corbicula fluminea* (O. F. Müller, 1774) as well as Guaga mussel *Dreissena rostriformis bugensis* (Andrusov, 1897), confirms that heavily modified waterways are suitable recipient areas for species introduction and adaptation (PAUNOVIĆ et al. 2007, ZORIĆ et al. 2011, RAKOVIĆ et al. 2013).

Information on the interaction between *G. tigrina* and other macroinvertebrates is limited. It has been documented that *G. tigrina* coexists with other Turbellaria species due to its ability to adjust its diet to the predominant prey (VAN DER VELDE 1975).

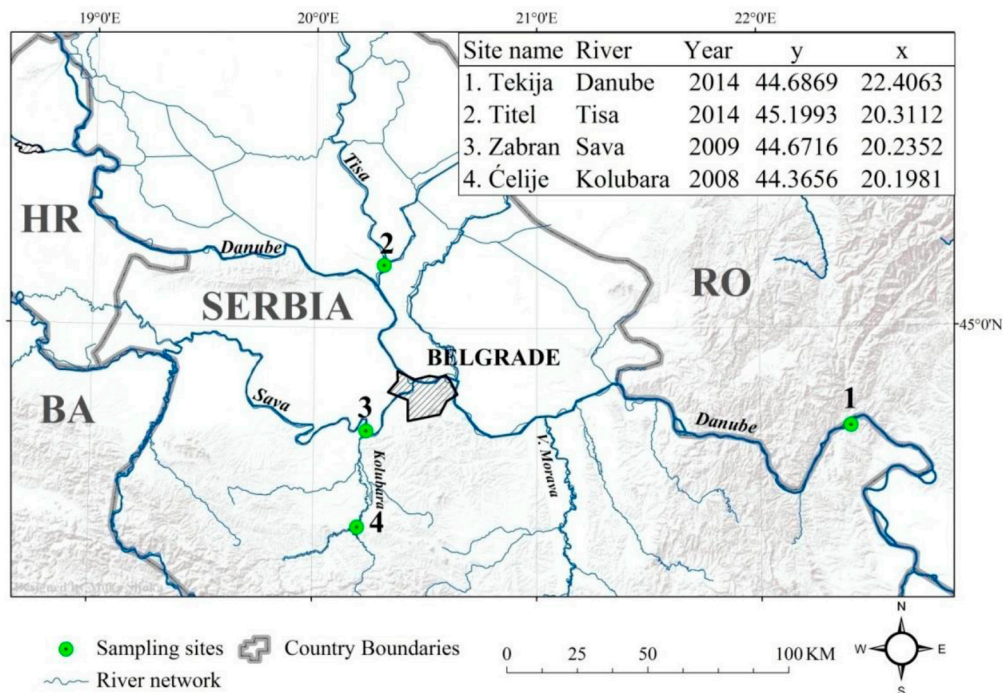


Fig. 2. The localities in Serbia where *G. tigrina* was recorded.

We established the coexistence of *G. tigrina* with *Planaria torva* (O. F. Müller, 1773) and *D. lugubris*, with predominance of *D. lugubris* in the Kolubara samples. According to VAN DER VELDE et al. (2002), in the Dutch Rhine delta the initial colonizer *G. tigrina* was completely replaced by the triclad predator *D. romanodanubiale*. The spread of *D. romanodanubiale* recorded during the Joint Danube Survey 3 in 2013 (authors' unpublished data, LIŠKA et al. 2015) suggests that *G. tigrina* may be replaced by this more aggressive planarian competitor also in Serbia.

The rapid invasion of running waters by *G. tigrina* is well documented (WRIGHT 1987). The dispersal phase most likely involves the adult animal, although transport and dissemination of cocoons is also possible (YOUNG & REYNOLDS 1999). GEE & YOUNG (1993) stated that the invasion of northern Wales by *G. tigrina* resulted in the almost complete

displacement of the native *Policelis tenuis* (Ijima, 1884) and *Policelis nigra* (Müller, 1774), presumably as a result of inter-specific food competition.

In conclusion, the presence of *G. tigrina* in low abundance indicates that the species cannot be considered as an important member of benthic macroinvertebrate communities. Its impact on native species in Serbian waters is limited. Future studies of this alien species should be carried out in order to trace its spreading.

Acknowledgement: This work was supported and financed by the Ministry of Education, Science and Technological Development of Republic of Serbia, Grants TR 037009, III 043002 and OI 173025, as well as by the European Union's Seventh Programme for research, technological development and demonstration under grant agreement No. 603629 „GLOBAQUA“ – „Managing the effects of multiple stressors on aquatic ecosystems under water scarcity“.

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Received: 03.12.2016
Accepted: 30.04.2017

