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

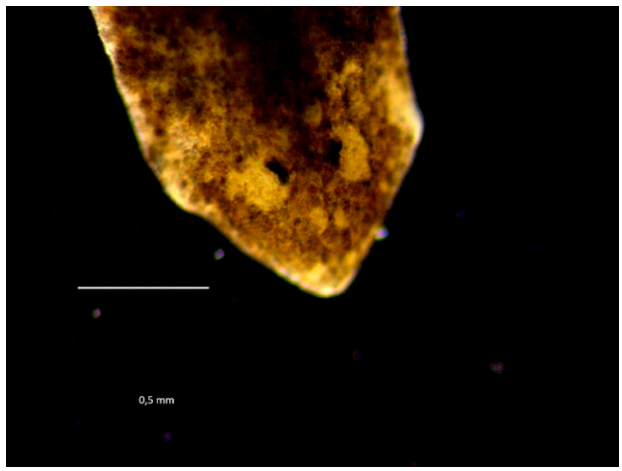

Fig. 1. *G. tigrina* from sampling site No. 3 Zabran in 2009.

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 Ćelije 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, Smith2001, 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).

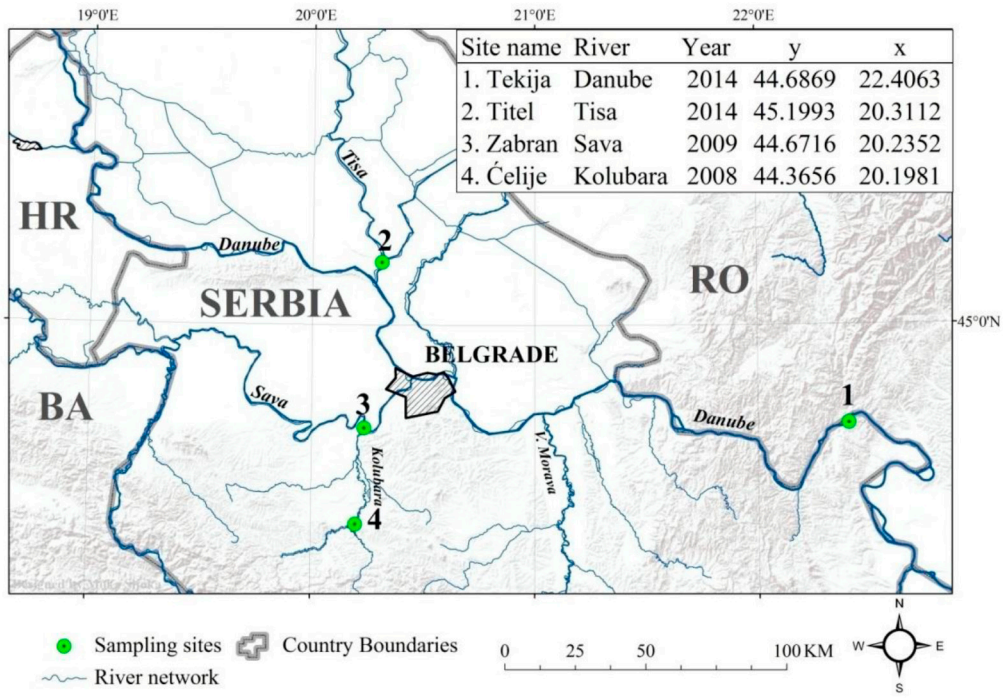

Fig. 2. The localities in Serbia where *G. tigrina* was recorded.

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 & Reynoldson 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* (Muller, 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.

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References

An der Lan H. 1962. Zur Turbellarien Fauna der Donau. Archiv für Hydrobiologiep Suppl. 27: 3-27.

Baguñà J., Carranza S., Pala M., Ribera C., Giribet G., Arnedo M., Ribas M. & Riutort M. 1999. From morphology and karyology to molecules. New methods for taxonomical identification of asexual populations of freshwater planarians. A tribute to Professor Mario Benazzi. Italian Journal of Zoology 66 (3): 207-214.

Calow P., Beveridge M. & Sibly R. 1979. Heads and tails: adaptational aspects of asexual reproduction in freshwater triclads. The American Zoologist 19: 715-727.

Charni M., Harrath A. H., Sluys R., Tekaya S. & Zghal F. 2004. The freshwater planarian *Dugesia sicula* Lepori, 1948 (Platyhelminthes, Tricladida) in Tunisia: ecology, karyology and morphology. Hydrobiologia 517 (1-3): 161-170.

Dahm A. G. 1958. Taxonomy and ecology of five species groups in the family Planariidae (Turbellaria, Tricladida, Paludicola). Malmö, 241 p.

De Jong Y. S. D. M. 2013. Fauna Europaea. Database: faunaeur [Internet]. Accesses: March, 18th 2016. http://www.faunaeur.org/full_results.php?id=454343

EN 27828: 1994. Water quality – Methods for biological sampling – Guidance on hand-net sampling of benthic macroinvertebrates.

Fülep T. & Nosek J. N. 2010. Contribution to the macroinvertebrate fauna of the Hungarian Danube VI. Triclads (Platyhelminthes: Tricladida). Folia Historico Naturalia Musei Matraensis 34: 5-9.

Gee H. & Young J. O. 1993. The food niches of the invasive *Dugesia tigrina* (Girard) and indigenous *Polycelis tennis* Ijima and *P. nigra* (Müller) (Turbellaria; Tricladida) in a Welsh lake. Hydrobiologia 254 (2): 99.

Girard C. 1850. A brief account of the freshwater Planariae of the United States. Proceedings of the Boston Society of Natural History 3: 264-265.

Grasso M. & Benazzi M. 1973. Genetic and physiologic control of fissioning and sexuality in planarians. Journal of Embryology and Experimental Morphology 30: 317-328.

Guecheva T., Henriques J. A. P. & Erdtmann B. 2001. Genotoxic effects of copper sulphate in freshwater planarian in vivo, studied with the single-cell gel test (comet assay). Mutation Research 497: 19-27.

Hering D., Moog O., Sandin L. & Verdonschot P. F. M. 2004. Overview and application of the AQEM assessment system. Hydrobiologia 516: 1-21.

Hyman L. H. 1939. New species of flatworms from North, Central, and South America. Proceedings of the United States National Museum 86 (3055): 419-439.

Hyman L. H. 1941. Environmental control of sexual reproduction in a flatworm. Anatomical Record 81: suppl. 108.

Hyman L. H. 1951. The invertebrates: Platyhelmintes and Rhynochocoela, the acoelomate bilateria. Vol II. New York: McGraw-Hill.

Kawakatsu M., Hauser J., Friedrich S. M. G. & De Souza Lima O. 1982. Morphological, karyological and taxonomic studies of freshwater planarians from South Brazil. III. *Dugesia tigrina* (Girard, 1850) and *Dugesia schubarti* (Marcus, 1946) from the vicinities of São Carlos, Estado de São Paulo (Turbellaria, Tricladida, Paludicola). The Bulletin of Fuji Women's College 20 (2): 73-90.

Kawakatsu M., Oki I., Tamura S., Yamayoshi T., Hauser J. & Friedrich S. M. G. 1981. Morphological, karyological and taxonomic studies of freshwater planarians from south Brazil. II. *Dugesia tigrina* (Girard, 1850) (Turbellaria, Tricladida, Paludicola). The Bulletin of Fuji Women's College 19: 113-136.

Kawakatsu M., Oki I., Tamura S. & Yamayoshi T. 1985. Reexamination of freshwater planarians found in tanks of tropical fishes in Japan, with a description of a new species, *Dugesia austroasiatica* sp. nov. (Turbellaria; Tricladida; Paludicola). *Bulletin of the Biogeographic Society of Japan* 40: 1-19.

Kawakatsu M., Tamura S., Takai M., Yamamoto K., Ueno R. & Oki I. 1993. The first record of occurrence of a naturalized population of *Dugesia tigrina* (Girard, 1850) at Nagasaki, Kyûshû, Japan (Turbellaria: Tricladida: Paludicola). Bulletin of the Biogeographic Society of Japan 48: 28-34.

Kenk R. 1937. Sexual and asexual reproduction in *Euplanaria tigrina* (Girard). Biological Bulletin 73: 280-284.

Kenk R. 1944. The freshwater triclads of Michigan. Miscellaneous Publications of the Museum of the Zoology, University of Michigan 60: 1-44.

Knakieivcz T., Lau A. H., Prá D. & Erdtmann B. 2007. Biogeography and karyotypes of Freshwater Planarians (Platyhelminthes, Tricladida, Paludicola) in Southern Brazil. Zoological Science 24: 123-129.

Liška L., Wagner F., Sengl M., Deutsch K. & Slobodnik J. 2015. Joint Danube Survey – Final Report ICPDR. Vienna: International Commission for the Protection of the Danube River, 235 p.

Lombardo P., Giustini M., Miccoli F. P. & Cicolani B. 2011. Fine-scale differences in duel activity among nocturnal freshwater planarias (Platyhelminthes: Tricladida). Journal of Circadian Rhythms 9 (1): 2.

Moskova G. D. & Uzunov Y. 2011. The macrozoobenthos of the Rilska River, Southwest Bulgaria. Annual of University of Sofia, Book 1, Zoology 99: 55-73.

Panov V. E., Alexandrov B., Arbaciauskas K., Binimelis R., Copp G. H., Grabowski M., Lucy F., Leuven R. S. E. W., Nehring S., Paunovic M., Semenchenko V. & Son M. O. 2009. Assessing the Risks of Aquatic Species Invasions via European Inland Waterways: From Concepts to Environmental Indicators. Integrated Environmental Assessment and Management 5 (1): 110-126.

Paunović M., Csányi B., Knežević S., Simić V., Nenadić D., Jakovčev–Todorović D., Stojanović B. & Cakić P. 2007. Distribution of Asian clams Corbicula fluminea (Muller, 1774) and C. fluminalis (Muller, 1774) in Serbia. Aquatic Invasions 2 (2): 105-112.

Paunović M., Tomović J., Kovačević S., Zorić K., Žganec K., Simić V., Atanacković A., Marković V., Kračun M., Hudina S., Lajtner J., Gottstein S. & Lucić A. 2012. Macroinvertebrates of the Natural Substrate of the Sava River – Preliminary Results. Water Research and Management 2 (4): 33-39.

Prá D., Lau A. H., Knakievicz T., Carneiro F. R. & Erdtmann B. 2005. Environmental genotoxicity assessment of an urban stream using freshwater planarians. Mutation Research 585: 79-85.

Preza D. L. C. & Smith D. H. 2001. Use of newborn *Girardia tigrina* (Girard, 1950) in acute toxicity tests. Ecotoxicology and Environmental Safety 50: 1-3.

Raković M., Popović N., Kalafatić V. & Martinović-Vitanović V. 2013. Spreading of *Dreissena rostriformis bugensis* (Andrusov 1897) in the Danube River (Serbia). Acta Zoologica Bulgarica 65: 349-357.

Reynoldson T. B. 1978. A key to British species of Freshwater Triclades. Freshwater Biological Association Scientific Publication 23: 32.

Ribas M., Riutort M. & Baguña J. 1989. Morphological and biochemical variation in populations of *Dugesia* (G.) *tigrina* (Turbellaria, Tricladida, Paludicola) from the western Mediterranean: biogeographical and taxonomical implications. Journal of Zoology 218 (4): 609-626.

Sluys R., Joffe B. & Cannon L. 1995. An alien flatworm in Australian waters. Memoirs of the Queensland Museum 38: 642.

Stokely P. S., Brown T. S., Kuchan F. & Slaga T. J. 1965. The distribution of Fresh-Water Triclad Planarians in Jefferson County, Ohio. Ohio Journal of Science 65 (6): 305-318.

Tittizer T., Schöll F., Bannlng M., Haybach A. & Schleuter M. 2000. Aquatische Neozoen im Makrozoobenthos der Binnenwasserstraßen Deutschlands. Lauterbonia 39: 1-72.

Van der Velde G. 1975. The immigrant triclad flatworm *Dugesia tigrina* (Girard) (Plathelminthes, Turbellaria). Range extension and ecological position in the Netherlands. Hydrobiological Bulletin 9: 123-130.

Van der Velde G., Nagelkerken I., Rajagopal S. & Bij de Vaate S. 2002. Invasions by Alien Species in Inland Freshwater Bodies in Western Europe: The Rhine Delta. In: Leppäkoski E., Gollasch S. & Olenin S. (Eds.): Invasive aquatic species of Europe – distribution, impact and management. Dordrecht et al.: Kluwer Academic Publishers, pp. 360-372.

Vreys C., Crain J., Hamilton S., Williamson S. & Steffanie N. 2002. Evidence for unconditional sperm transfer and sperm-dependent parthenogenesis in a hermaphroditic flatworm (*Girardia tigrina*) with fissipary. Journal of Zoology 257 (1): 43-52.

WFD Roof Report 2004. Danube Basin Analysis. Annexes The Danube River Basin District River basin characteristics, impact of human activities and economic analysis required under Article 5, Annex II and Annex III, and inventory of protected areas required under Article 6, Annex IV of the EU Water Framework Directive.

Wright J. F. 1987. Colonization of rivers and canals in Great Britain by *Dugesia tigrina* (Girard) (Platyhelminthes: Tricladida). *Freshwater Biology* 17: 69-78.

Young J. O. & Reynoldson T. B. 1999. Continuing dispersal of freshwater triclads (Platyhelminthes: Turbellaria) in Britain with particular reference to lakes. Freshwater Biology 42: 247-262.

Zorić K., Jakovčev-Todorović D., Đikanović V., Vasiljević B., Tomović J., Atanacković A., Simić V. & Paunović M. 2011. Distribution of the Ponto-Caspian polychaete *Hypania invalida* (Grube, 1860) in inland waters of Serbia. Aquatic Invasions 6 (1): 33-38.

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