







Research article

# A silent predator *ad portas* – *Dolichoplana striata* Moseley, 1877 (Platyhelminthes: Tricladida: Geoplanidae) recorded for the first time in Bulgaria

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**Abstract:** The first record of alien terrestrial planaria *Dolichoplana striata* Moseley, 1877 (Platyhelminthes: Tricladida: Geoplanidae) in Bulgaria and on the Balkan Peninsula is reported. It was found in a greenhouse with exotic plants near Plovdiv City (Southern Bulgaria). The distribution of the invasive planarians has to be regularly monitored as they are responsible for a negative impact on the soil biodiversity.

**Keywords:** Balkan Peninsula, invasive alien species, terrestrial flatworms

## Introduction

Land flatworms, a group of about 910 species, typically live in tropical jungles or temperate wooded areas and can survive only in moist soil environments (Sluys, 2016).

The data on terrestrial flatworms in Bulgaria is very scarce. There are only three records 70 years ago with three species: *Microplana terrestris* (Müller OF, 1773) (as *Rhynchodemus terrestris* (Müller OF, 1773)) and *Rhynchodemus sylvaticus* (Leidy, 1851) (as *Rhynchodemus bilineatus* (Metschnikoff, 1865)) and one undescribed *Rhynchodemus* sp. are published (Valkanov, 1956). In the last two decades of the 21 century the interest in land planarians as invasive alien animals significantly grew in Europe (Thunnissen et al., 2022). The situation in Bulgaria is the same in the last years, especially with the start of some scientific projects related to the invasive species and their control and monitoring in the country (Trichkova et al., 2016).

In this paper, we present data about the first record of the non-native land flatworm in Bulgaria and Balkan Peninsula.

## Material and methods

The terrestrial flatworms were searched according to the methodology by Winsor (1991) and Thunnissen et al. (2022) modified for the project's purpose. Because of the fact that many places in the world are being invaded by land flatworms that have stowed away in the soil of imported ornamental plants (Sluys, 2016; Thunnissen et al., 2022) we focused our studies on different botanical gardens, nursery gardens for exotic ornamental plants and exotic flowers, green houses, flower's stocks and markets. The Botanical Garden of the Bulgarian Academy of Sciences in Sofia, two private botanical gardens in the region of Plovdiv, and 10 nursery gardens for exotic ornamental plants and flowers with green houses, flower's stocks and markets in regions of Sofia, Burgas, Yambol and Plovdiv were visited and extensively investigated by hand searching beneath leaf litter, moss, fallen logs and wooden parts, stones in damp, but generally not wet, sites, under plastic and ceramic pots, large polyethylene sheets or other debris.

One live specimen was kept in a cool place for two days until laboratory processing. The fixation

procedures of the studied specimen followed Winsor (1998) and Winsor & Sluys (2018) with a few modifications: narcotising in 5% of ethanol for 8–10 min; after relaxation removing mucus and particles of detritus; killing procedure with near boiling water; transfer in clean Petri dish; a small sample (about 4 mm) from the posterior end of the specimen was cut off and fixed in 95–100% ethanol for molecular studies; the specimen was transferred in fixative (Formol-Calcium) for 24 h. Later it was washed two times with distilled water and transferred in 70% ethanol for subsequent storage and histological processing. The 8 minutes for narcosis was not enough for our specimen. It was still slightly active when touched after this period.

The material is deposited in the National Museum of Natural History, Sofia (SOFM) collections.

For the basic morphological determination, the keys by Jones (2005) and de Luna & Boll (2023) were used.

#### Molecular procedures and identification

Total DNA was extracted from the collected tissue sample using ‘DNeasy Blood & Tissue Kit’ (Qiagen) following the instructions of the manufacturer. For the purpose of molecular identification we amplified two partially overlapping fragments of the mitochondrial cytochrome oxidase I gene (COI) using primer pairs: 1) FlatwormCOIF and FlatwormCOIR (Sunnucks et al. 2006); 2) BarS and COIR (Lázaro et al. 2009; Alvarez-Presas et al. 2011). Thermal cycling followed Carbayo et al. (2013). PCR products were sequenced as an external service (Macrogen Europe). Chromatograms were trimmed, aligned and assembled to obtain a single COI fragment using CodonCode Aligner v. 8.0.2 (Dedham, USA). The DNA sequence was checked for stop codons using DAMBE7 (Xia 2018) and was used to query the NCBI core nucleotide database via the BLAST algorithm (Altschul et al. 1990).

#### Results and discussion

During a trip and visits to different botanical gardens, green houses, flowers stocks and markets in Thracian lowland (Plovdiv Region) in October 2024 a specimen of terrestrial flatworm with stripes drew our

attention. The locality and material examined are as follows:

Phylum Platyhelminthes Gegenbaur, 1859

Class Trepaxonemata Ehlers, 1984

Order Tricladida Lang, 1884

Family Geoplanidae Stimpson, 1858

Genus *Dolichoplana* Moseley, 1877

*Dolichoplana striata* Moseley, 1877

Material examined: BG-NMNHS-ENT-000000011717; Bulgaria, Thracian lowland, near town of Asenovgrad, 175 metres a.s.l., (N 42.0635°, E 024.8196°), 8.x.2024, 1 specimen observed and collected by N. Simov and V. Antonova (Fig. 1), morphologically identified by N. Simov. The habitat is a greenhouse with many different evergreen exotic plants grown inside in pots (Fig. 2). The specimen was collected under a plastic pot half filled with soil and compost.

The combination of the terrestrial habits, elongate form, head narrow and inconspicuous, the presence of a single pair of relatively large eyes near the anterior end (Fig. 3) shows that studied land flatworm undoubtedly belongs to family Geoplanidae: Rhynchoderminae (Jones, 2005; Sluys et al., 2009; de Luna & Boll, 2023). The relatively big size (29 mm fixed), the very elongated and flattened body with creeping sole of moderate width, presence of well visible four dark stripes on dorsal side (Fig. 1) suggest it could be placed in the genus *Dolichoplana* Moseley, 1877 (Jones, 2005; BopCo 2020). In general habitus (with body light olive-brown, with four dark stripes, the two median stripes are narrower than the rest, and the lateral stripes are more conspicuously marked than the others, the ventral surface is cream coloured with a pale grey creeping sole with a slightly darker grey fine midline (see de Luna & Boll (2023)) our specimen resembles much more representatives of *Dolichoplana striata* Moseley, 1877. *Dolichoplana striata* could be identified by its characteristic external appearance (Álvarez-Presas et al. 2014; de Luna & Boll, 2023).

The final length of the COI fragment was 893 bp. The newly obtained DNA sequence from Bulgaria (GenBank accession number: PQ895679) showed no stop codons and is identical to two sequences of *D.*





Fig. 1. *Dolichoplana striata* Moseley, 1877 – a specimen observed and collected by N. Simov and V. Antonova near Plovdiv City.



← Fig. 2. The habitat where the specimen was recorded – a greenhouse with many different evergreen exotic plants grown inside in pots.

*striata* (KJ659683-4) previously reported from Spain (Álvarez-Presas et al. 2014), thus confirming the morphological identification of the species.

In spite of an earlier extensive literature search, the species has remained unrecorded for the Bulgarian fauna (BopCo, 2020). Our record is evidently the first for the country and Balkan Peninsula as a whole. The closest published locality of the species could be Trento, Italy (Mori et al. 2022) or Austria (BopCo, 2020), more than 1000 km in both cases.

*Dolichoplana striata* is of Indo-Malayan origin (Jones, 2005; Sluys, 2016). In Europe the first record is from Ireland (Anderson, 1986). Later the species is discovered in Belgium, Jersey, Germany, United Kingdom, Spain, Italy (Jones, 2005; Álvarez-Presas et al., 2014; Mori et al., 2022, Thunnissen et al., 2022), Austria, Czech Republic, Finland, Norway, Poland and Portugal (BopCo, 2020). In the last five years the number of records of *D. striata* rapidly increased in different regions of the World: Italy,





Fig. 3. Bulgarian specimen of *Dolichoplana striata* Moseley, 1877 – head and eyes.

Mexico, Bermuda, Jamaica, Brazil, Panama (Brown et al., 2022; Mori et al., 2022, de Luna & Boll, 2023; Lago-Barcia et al., 2023; Collantes et al., 2024). The reasons are the rapid spreading of the species due to globalisation of the world's trade of exotic plants (Sluys, 2016), the increasing of the efforts based on citizen science (Mori et al., 2022, de Luna & Boll, 2023) and invasive alien species researches (Álvarez-Presas et al., 2014; Jones & Sluys, 2016; Justine et al., 2014a; Justine et al., 2018; Mori et al., 2021; Thunnissen et al., 2022; present study).

Bulgarian record as all previously published reports of *D. striata* in European countries (Jones, 2005; Álvarez-Presas et al., 2014; BopCo, 2020; Thunnissen et al., 2022) is found in a greenhouse. The species is not recorded in natural habitats.

*Dolichoplana striata* like other terrestrial flatworms is top-level predator of soil organisms, and its presence can change nutrient cycling, endanger native species of flatworms, and alter an ecosystem's plant community, resulting in a reduction of ecological functions and additionally the planarian invasion might cause significant economic losses in agriculture and damage to the ecosystems (Sluys, 2016; Álvarez-Presas et al., 2014; Justine et al., 2014b; Justine et al., 2015, Justine et al., 2018). With only one record in Bulgaria at the moment the risk assessment of *D. striata* for the country is practically not possible. On the other hand the climate of the country (with warm, but in some years dry summer and cold winters) does not allow the development of this land planarian outside greenhouses or in natural habitats. The last years' tendency to milder winters and rapid spread of species (also invasive) with southern European range to the

North (Grozeva et al. 2013; Simov et al., 2012, Langourov et al., 2023) led us to suppose that more attention on this species in the future is needed. The invasive planarians have to be regularly monitored as they may be spread very fast by the trade of exotic plants.

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