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FIRST OCCURRENCE OF THE EUROPEAN TERRESTRIAL FLATWORM, *MICROPLANA TERRESTRIS* (MÜLLER OF, 1773) (PLATYHELMINTHES: GEOPLANIDAE), IN MARITIME CANADA

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ABSTRACT

The first Canadian Maritime occurrence of the European terrestrial flatworm, *Microplana terrestris* (Müller OF, 1773), is confirmed via genetic barcoding. *Microplana terrestris* was observed on the soil surface in a rural garden in Tantramar, Westmorland County, New Brunswick, in November 2021, 2022 and 2023. In addition to confirming identity, barcoding also confirmed *M. terrestris* diet at the site included earthworms, *Aporrectodea* sp., and the Common Amber Snail, *Succinea putris* Linnaeus, 1758. Future barcoding efforts should prove to be a useful approach to providing a more detailed understanding of diet in *M. terrestris* and other terrestrial flatworms, and their impact on associated soil fauna. Further reporting of non-native terrestrial flatworms in Canada could perhaps be enhanced through citizen-based science monitoring.

Key Words: *Aporrectodea* sp., citizen science, diet, flatworm, invasive species, *Microplana,* New Brunswick, *Succinea putris*

INTRODUCTION

The introduction and establishment of non-native terrestrial flatworms, usually via the horticultural industry, has raised concerns in many parts of the world (Sluys 2016, Murchie and Justine 2021, Thunnissen *et al.* 2022, Mori *et al.* 2023), including Canada (Justine *et al.* 2019). However, to date there have been few such introductions to eastern Canada. Judd (1957) reported a specimen of *Bipalium kewense* Moseley, 1878 from Ontario (London). Ogren (1991) documented

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Rhynchodemus sylvaticus (Leidy, 1851) from Ontario (Toronto and Belfountain, northwest of Toronto) and *Microplana terrestris* (Müller OF, 1773) from sites in Quebec (Meech Lake) and Ontario (Toronto). Justine *et al.* (2019) reported *Bipalium adventitium* Hyman, 1943 from Canada (Montreal, Quebec) for the first time, with subsequent media reports suggesting the species is now established in both Montreal and Toronto (Landau 2022, Blais 2023). Adl *et al.* (2006) reported on a possibly undescribed *Geocentrophora* sp. collected from forest soils in mostly public parks, near Halifax, Nova Scotia. They also encountered a second species (one individual) that was morphologically identical to *G. baltica*, a species known from northern Europe. Currently, the native versus non-native status of these flatworms in Nova Scotia is unclear.

Many species of terrestrial flatworms feed on earthworms, snails, and other soft-bodied soil invertebrates (Winsor *et al.* 2004) and there is evidence that some species can reduce earthworm populations to the extent this can impact agricultural productivity (Murchie *et al.* 2003). While this is perhaps of less concern in Canada, where most earthworms are of non-native origin (Reynolds 2022), non-native terrestrial flatworms may still have the potential to effect native soil fauna and soil processes in Canada. Where terrestrial flatworms are invasive, lack of information about natural history has made it difficult to assess risk (Thunnissen *et al.* 2022), although *M. terrestris* is apparently mainly a scavenger (Jones, 2005, McDonald and Jones 2013). Winsor *et al.* (2004) notes that the impact of adventive populations of *M. terrestris* are unknown. Here we report the first occurrence of the non-native terrestrial flatworm *M. terrestris* in Maritime Canada.

METHODS

Unidentified flatworms were first noted on the exposed soil surface of a vegetable garden and under pieces of adjacent discarded carpeting at 55 Water Street, Tantramar (former village of Dorchester), Westmorland County, New Brunswick, Canada (45.88865°N 64.51709°W; Fig 1A) in early November 2021. Specimens were collected 14 November 2021, 7 November 2022, and 18 November 2023, killed in 5% ethyl alcohol or very hot water, and stored in 70% ethyl alcohol (voucher [New Brunswick Museum] NBM-GI-11577) or 95% ethyl alcohol (NBM-GI-11726; for barcoding).

Fig 1A **Habitat for *Microplana terrestris* at Tantramar, New Brunswick, Canada. Arrow marks area where flatworms were particularly abundant on the soil surface in November 2021 and 2022.**

Fig 1B ***Microplana terrestris* collected November 2023, Tantramar, New Brunswick, Canada.**

RESULTS

Live flatworms were 15-25 mm in total length and 1-3 mm in width when extended during forward movement over the substrate (Fig 1B). Dorsally, the worms were a uniform dark black in colour and very light grey-white ventrally. A full-length DNA barcode of 549 base-pairs (bp) was generated from sample NBM-GI-11726 using primer cocktail C-LepFoIF_C-LepFoIR. The sequence recovered was a 98.82% match to NCBI (National Centre for Biotechnology Information (NCBI) https://www.ncbi.nlm.nih.gov/) reference records representing *Microplana terrestris*. Additional sequences generated from *M. terrestris* samples collected at Tantramar provide some insight into the diet of *M. terrestris* at the site. A full-length DNA barcode of 658 base-pairs (bp) generated from the sample using primer cocktail C_VF1LFt1/C_VR1LRt1 was a 99.39% match to BOLD (Barcode of Life Data System at www.boldsystems.org/) reference

records representing *Aporrectodea* sp. (Oligochaeta: Lumbricidae), while a full-length DNA barcode of 655 base-pairs (bp) using primer cocktail C-LepFoIF_C-LepFoIR was a 100% match to BOLD reference records representing the Common Amber Snail (*Succinea putris* Linnaeus, 1758).

DISCUSSION

External morphology of flatworms matched the description of *M. terrestris* provided by Ogren (1984). However, Mateos *et al.* (2017) found that a series of samples initially assigned to *M. terrestris* morpho-types fell into different genetic clades. Although *M. terrestris* appears to have a wide distribution in western Europe and Great Britain (Jones 2005, Álvarez-Presas *et al.* 2012), recent investigations (Álvarez-Presas, *et al.* 2022) suggest European populations of *M. terrestris* are a complex of "pseudo-cryptic" species. While our barcode results have identified New Brunswick material as *M. terrestris*, the results can only be as accurate as the reference records available. To ensure accurate identification, and in light of recent phylogenetic work (Sluys *et al.* 2016, Mateos *et al.* 2017, Álvarez-Presas *et al.* 2022), future barcode analyses of North American *Microplana* sp. will need access to a wider range of genetic reference records than may currently be archived.

Microplana terrestris, like most terrestrial flatworms, is reported to be a generalist predator of gastropods, earthworms and small arthropods (Winsor *et al.* 2004). Jennings (1959) lists small earthworms and slugs, injured collembolla, isopods, insect larvae, and myriapods among prey. Our barcode results, a match for *Aporrectodea* sp., and *S. putris*, are clearly among the known prey categories for *M. terrestris* (Winsor et al 2004). However, there seems to be little species-specific data on *M. terrestris* prey. Roy *et al.* (2021) seem to have been the only previous investigators to make use of barcoding to better understand terrestrial flatworm diets. In a proof-of-concept approach for *Obama nungara,* Carbayo, Álverez-Presas, Jones & Riutort, 2016, a South American native, they were able to confirm that where this species is invasive in France it is likely to be a threat to native soil biota. Future barcoding efforts should prove to be a useful approach to providing a more detailed understanding of diet

in *M. terrestris* and other terrestrial flatworms, and their impact on associated soil fauna.

There are, as of yet, few Canadian reports of terrestrial flatworms posted to iNaturalist (see iNaturalist.org) or similar platforms, and in some cases images alone will not be sufficient to confirm species identification (i.e. *Microplana* spp.) Regardless, our report from a single locality may not be a true reflection of the occurrence of *M. terrestris* occurrence in Maritime Canada. Álverez-Presas *et al.* (2022) have noted that *Microplana* sp. are easily overlooked due to small size, occurrence at low density, dull colouration, nocturnal habits, and proclivity to hide under leaf litter and other forest floor debris. Furthermore, they are rarely collected using the extraction methods followed for more widely studied groups of soil organisms. Following the experience of Mori *et al.* (2021) in Italy and elsewhere (Mori *et al.* 2023), further reporting and monitoring of non-native terrestrial flatworms in Canada could perhaps be enhanced through citizen-based science projects.

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LITERATURE CITED

Adl, S.M. Ruiz-Trillo, M. & Wilson, L. (2006). Observations on a *Geocentrophora* sp; (Lecithoepithoeliata) flatworm from forest soils in Nova Scotia. *Journal of Natural History* 40: 1381-1387.

Álvarez-Presas, M., Mateos, E., Vila-Farré, M., Sluys, R. & Riutort, M. (2012). Evidence for the persistence of the land planarian species *Microplana terrestris* (Müller, 1774) (Platyhelminthes, Tricladida) in microrefugia during the Last Glacial Maximum in the northern section of the Iberian Peninsula. *Molecular Phylogenetics and Evolution* 64: 491-499. doi: 10.1016/j.ympev.2012.05.001.

Álvarez-Presas, M., Mateos, E., Sluys, R. & Riutort, M. (2022). Cryptic diversity in European terrestrial flatworms of the genus *Microplana* (Platyhelminthes, Tricladida, Geoplanidae). *In* A.K. Monro and S.J. Mayo (eds.) Cryptic species, morphological stasis, circumscription, and hidden diversity. Cambridge University Press, pp. 281-293. doi.org/10.1017/9781009070553.011.

Blais, S. (2023). Worms that secrete a dangerous paralyzing toxin spreading in Montreal. National Post. nationalpost.com/pmn/news-pmn/canada-news-pmn/worms-that-secrete-a-dangerous-paralyzing-toxin-spreading-in-montreal

Jennings, J.B. (1959). Observations on the nutrition of the land planarian *Orthodemus terrestris* (O.F. Müller). *Biological Bulletin, Woods Hole* 117: 119-12.

Jones, H.D. (2005). British land flatworms. *British Wildlife* 17: 189-194.

Judd, W.W. (1957). A terrestrial flatworm collected at London, Ontario. *Canadian Field-Naturalist* 71: 154.

Justine, J.-L, Thery, T., Gey, D. & Winsor, L. (2019). First record of the invasive flatworm *Bipalium adventitium* (Platyhelminthes: Geoplanidae) in Canada. *Zootaxa* 4656(3): 591-595. doi.org/10.11646/zootaxa.4656.3.13.

Landou, J. (2022). Neurotoxic predatory worms invade Ontario and you should kill them on sight. BlogTO. www.blogto.com/city/2022/10/worms-invade-ontario/

Mateos, E., Sluys, R., Riutort, M. & Álveraz-Presas, M. (2017). Species richness in the genus *Microplana* (Platyhelminthes: Tricladida: Microplaninae): as yet no asymptote in sight. *Invertebrate Systematics* 31: 269-301. dx.doi.org/10.1071/IS16038.

McDonald, J.C. & Jones, H.D. (2013). Feeding, maintenance and reproduction of *Microplana terrestris* (Platyhelminthes: Tricladida: Continenticola: Geoplanidae: Microplaninae) under laboratory conditions. *Journal of Natural History* 48: 1-34. doi.org/10.1080/00222933.2013.809169.

Mori, E., Magoga, G., Panella, M., Montagna, M., Winsor, L. Justine, J.-L., Menchetti, M., Schifani, E., Melone, B. & Mazza, G. (2021). Discovering the Pandora's box: the invasion of alien flatworms in Italy. *Biological Invasions* 24: 205-216. doi.org/10.1007/s10530-021-02638-w.

Mori, E., Touloupakis, E., Viviano, A. & Mazza. G., (2023). Opening a gate to shade some light; alien land planarians in the eastern Mediterranean and northern Africa. *Zootaxa* 5319 (2): 295-300. doi.org/10.11646/zootaxa.5319.2.12.

Murchie A.K. & Justine, J.-L. (2021). The threat posed by invasive alien flatworms to EU agriculture and the potential for phytosanitary measures to prevent importation. Technical Report, UICN, ffhal-03547527.

Murchie, K., Moore J.P., Walters K.F.A., & Blackshaw R.P. (2003). Invasion of agricultural land by the earthworm predator, *Arthurdendyus triangulatus* (Dendy). *Pedobiologia* 47: 920-923. doi.org/10.1078/0031-4056-00281.

Ogren, R.E. (1984).The land planarian *Microplana terrestris* (Platyhelminthes:Turbellaria) from the United States. *Transactions of the American Microscopical Society* 103: 130-136.

Ogren, R.E. (1991). Land planarians from Ontario and Quebec. *Transactions of the American Microscopical Society* 110: 27-36.

Reynolds, J.W. (2022). The earthworms (Lumbricidae, Megascolecidae and Sparganophilidae) in Canada. Canadian Food Inspection Agency, Ottawa. 179 pp.

Roy, V. Ventura, M., Fourcade, Y., Justine, J-L., Gignon, A. & Dupont, L. (2022). Gut content metabarcoding and citizen science reveal the earthworm prey of the exotic terrestrial flatworm, *Obama nungara*.*European Journal of Soil Biology* 113: 103499. doi.org/10.1016/j.ejsobi.2022.103449.

Sluys, R. (2016). Invasion of the flatworms. *American Scientist* 104: 288-295. doi:10.1511/2016.122.288.

Sluys, R., Mateos, E., Riutort, M. & Álverez-Presas, M. (2016). Towards a comprehensive, integrative, analysis of the diversity of European microplanid land flatworms (Playhelminthes Tricladida, Microplaninae) with the description of two peculiar new species. *Systematics and Biodiversity* 14: 9-31. doi.org/10.1080/14772000.2015.1103323.

Thunnissen, N.W., de Waart, S.A., Collas, F.P.L., Jongejans, E., Jan Hendriks, A., van der Velde G. & Leuven, R.S.E.W. (2022). Risk screening and management of alien terrestrial planarians in the Netherlands. *Management of Biological Invasions* 13(1): 81-100. doi.org/10.3391/mbi.2022.13.1.05.

Winsor, L., Johns, P.M. & Barker, G.M. (2004). Terrestrial planarians (Platyhelminthes: Triclada: Terricola) predaceous on terrestrial gastropods. *In* Barker, G.M. (ed.) Natural Enemies of Terrestrial Molluscs. CAB International, UK, pp. 227-278.