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# First record of the invasive land flatworm *Bipalium adventitium* (Platyhelminthes, Geoplanidae) in Canada

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## Summary

Specimens of *Bipalium adventitium* (Platyhelminthes, Geoplanidae) were found in Montréal, Québec, Canada. The specimens showed the typical colour pattern of the species and barcoding (Cytochrome Oxidase I) demonstrated near-identity with a sequence of the same species from the USA. This is the first record of the species in Canada.

**Résumé.** Des spécimens de *Bipalium adventitium* (Plathelminthes, Geoplanidae) ont été trouvés à Montréal, Québec, Canada. Les spécimens montraient le motif de couleur typique de l'espèce et le barcode (cytochrome oxydase I) était quasi-identique à une séquence de la même espèce provenant des États-Unis. Ceci est la première mention de l'espèce au Canada.

Land flatworms are transported with earth and pot plants between continents and often become invasive (Justine *et al.*, 2014, 2015; Winsor, 1983) Since land flatworms are predators, they constitute a potential danger for the local soil fauna of the areas they invade. Among these land flatworms (family Geoplanidae), the hammerhead flatworms (subfamily Bipaliinae) are spectacular, by their large size, up to one meter, their distinctive colours, and the typical shape of their head which makes them easy to identify (Winsor, 1983). Most species have their origin in Asia but have invaded the world. The two "giant" species *Bipalium kewense* Moseley, 1878 and *Diversibipalium multilineatum* (Makino & Shirasawa, 1983), which can be as long as 30 cm, have been reported in many locations worldwide (Justine *et al.*, 2018b; Mazza *et al.*, 2016; Morffe *et al.*, 2016; Winsor, 1983). *Bipalium adventitium* Hyman, 1943, which is smaller, but still a noticeable species up to about 10 cm in length, is known only from the USA (Hyman, 1943; Kawakatsu *et al.*, 2002); however, since bipaliines do not have their biogeographical origin in North America, the species is not a part of the native fauna. Rather it is an invasive species known only from its invaded land.

In this paper, we record a finding of *Bipalium adventitium* in Montréal, Canada, with identification based on both external morphological and molecular characters.

Specimens were collected by one of us (TT). One specimen, 13 May 2018, Mont Royal, Montréal, Québec, Canada, N 45.5074°, W 73.6091°, under a stone in a land covered with trees (mainly sugar maple, *Acer saccharum* Marsh., northern red oak, *Quercus rubra* L., and American basswood, *Tilia americana* L.) ; specimen photographed then lost by the post. One specimen, 27 May 2018, under a stone, Montréal, N 45.5069° W 73.6111°, deposited in Muséum National d'Histoire Naturelle, Paris, France (MNHN) as MNHN JL327, sampled for molecular analysis. One specimen, 27 May 2018, under a stone, Montréal, N 45.5069°, W 73.6087°, deposited in MNHN as MNHN JL328, sampled for molecular analysis. Two specimens, 30 September 2018, Montréal, N 45.5069°, W 73.6111°, under a stone (in the Collection Entomologique Ouellet-Robert, University of Montréal, Canada (QMOR) and in T. Théry Collection, France). Four specimens, 11 May 2019, Montréal, including two under a stone, N 45.5069°, W

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73.6110° and N 45.5074°, W 73.6083° (in the Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Canada (CNC), and two under a rotten log, N 45.5072°, W 73.6083° (in the Collection of the Insectarium of Montréal, Canada (IMQC), and in T. Théry Collection, France). Specimens were collected alive and photographed. They were then fixed in pure ethanol and a small portion of the specimen was taken for molecular analysis. Sequences were obtained using our routine protocol (Justine *et al.*, 2018b). Briefly, genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen). Fragments of the COI gene were amplified with the primers BarS , COIR, JB3 and JB4.5. PCR products were purified and sequenced in both directions on a 3730xl DNA Analyzer 96-capillary sequencer (Applied Biosystems). Results of both analyses were concatenated to obtain a COI sequence of 401 bp in length. The sequence was edited using CodonCode Aligner software (CodonCode Corporation, Dedham, MA, USA), compared to the GenBank database content using BLAST and deposited in GenBank under accession number MN192402 and MN192403. MEGA7 (Kumar *et al.*, 2016) was used to estimate genetic distances. A phylogenetic analysis was performed with our new sequence and from all sequences available for members of the Bipaliinae; the geographical origin of specimens was checked in publications pertaining to sequences (Álvarez-Presas *et al.*, 2008; Justine *et al.*, 2018b; Moon *et al.*, 2011) For the ML tree, the best model determined by MEGA7 was the Hasegawa-Kishino-Yano model with discrete Gamma distribution (HKY+G).

The specimens, about 60 mm in length when alive (Fig 1a), are elongate with the expanded headplate typical of bipaliines, tapering to a pointed posterior end. The dorsal ground colour was yellow-brown ("Raw Sienna") with a fine dark brown median dorsal stripe that does not extend onto the head. The headplate is fan-shaped without recurved lappets, and greyish around the anterior margin (Fig 1b). The ventral surface is a pale cream colour. The eye pattern and the presence of a gonopore were not examined. The external morphology of the specimens accords well with the original description of the species (Hyman, 1943).

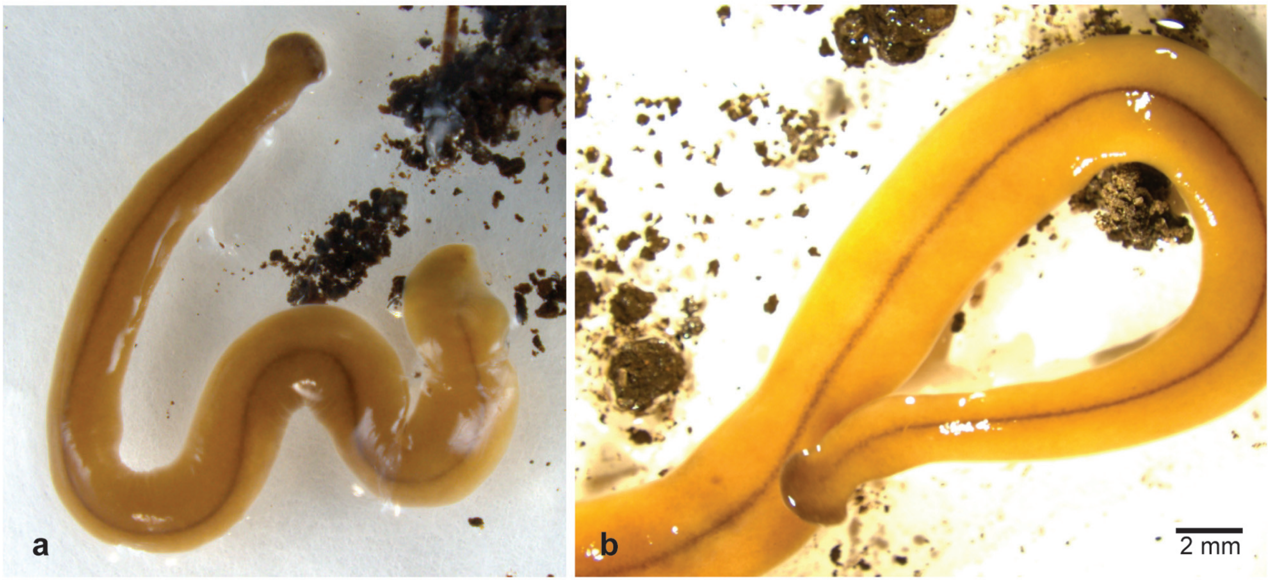

**FIGURE 1.** Living specimens. from Montréal, Québec. a. *Bipalium adventitium*, whole specimen, dorsal aspect. Figure 1 b. *Bipalium adventitium*, anterior end, dorsal aspect. Note the fan-shaped headplate with darker anterior margin, and the dark median dorsal stripe that terminates before the headplate.

The two specimens JL327 and JL328 had identical COI sequences. A tree was built from all sequences available for members of the Bipaliinae; for *Bipalium kewense*, *Bipalium vagum* and *Diversibipalium multilineatum*, since the sequences from various localities are identical (Justine *et al.*, 2018b), only one sequence was used. The Maximum Likelihood tree is shown in Figure 2. The two sequences of *Bipalium adventitium* from Montréal, Canada formed a monophylum with *B. adventitium* from Kingston, Pennsylvania, USA; indeed, for the part of the COI sequence included in the matrix, the three sequences are identical. The tree provides a clear indication that the species found in Montréal and Kingston is the same; however, the very low value of most branches precludes any attempts as using the tree to understand phylogenetic relationships between species. The distance (p-distance) from *B. adventitium* to the other bipaliine species in the analysis ranged from 12.6% (*B. kewense*) to 18.2% (*Diversibipalium* sp. "blue" from Mayotte). We noted that two sequences had aberrant positions in the tree: HM346597, allegedly *B. adventitium* and HM346600, allegedly *D. multilineatum*; these sequences formed a clade with no relation with either of the two species mentioned. This may indicate that the original specimens were misidentified.

The morphological characters, colour pattern and the similarity of COI sequence clearly indicate that the specimens found in Montréal, Québec, are *B. adventitium*. *Bipalium adventitium* is a predator of earthworms (Ducey & Noce, 1998; Zaborski, 2002) The species actively tracks its prey, follows trails of earthworm mucus and can capture earthworms in their tunnels (Fiore *et al.*, 2004). It can prey on earthworms 55 times larger than itself in mass (Zaborski, 2002). The species reproduces by sexual reproduction, laying egg capsules that take about 23 days to hatch and contain 1-8 offspring (Ducey *et al.*, 2005). As it often occurs with other invasive species, it has no known predator and experiments with salamanders and snakes have demonstrated that potential native predators do not consume it (Ducey *et al.*, 1999). *Bipalium adventitium* is one of the two species of terrestrial animals, with *B. kewense*, in which the presence of tetrodotoxin, a powerful neurotoxin, has been identified (Stokes *et al.*, 2014). An analysis of interactions of invasive and native plants and animals identified *Bipalium adventitium* as one of the many species involved in complex relationships of what the authors named an "extensive invasional meltdown in North America" (Heimpel *et al.*, 2010).

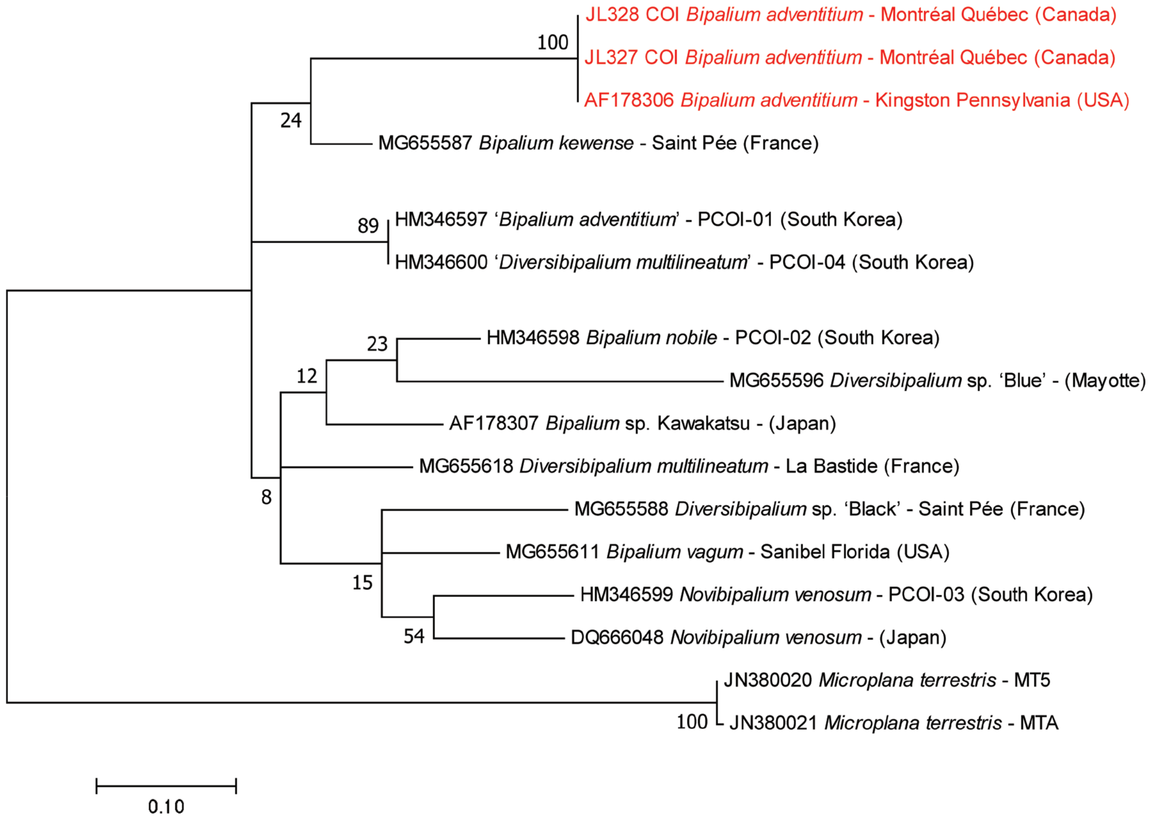

**FIGURE 2.** Maximum-likelihood tree of bipaliines, based on COI sequences. Localities are indicated for specimens. For sequence AF178306, the actual locality is Kingston, Pennsylvania, USA (erroneously indicated as "Leingston, USA") in the paper (Marta Álvarez-Presas, personal communication, 18 April 2019). Red: *Bipalium adventitium*.

After its description based on specimens from California (Hyman, 1943), the species has been recorded from a number of US states, including California, Illinois, Massachusetts, Michigan, Ohio, New York, Pennsylvania, Tennessee and West Virginia (Álvarez-Presas *et al.*, 2008; Ducey & Noce, 1998; Ducey *et al.*, 2005; Fiore *et al.*, 2004; Kawakatsu *et al.*, 2002; Stokes *et al.*, 2014; Zaborski, 2002). A search in GBIF ( https://www.gbif.org/species/5892130) yielded about 80 occurrences (April 2019), mostly from citizen science, in various states, mainly along the Eastern and Western coasts of the USA. The finding in Montréal is probably the Northernmost record of *B. adventitium* in North America, and our observations over two years show that the species can survive winter in Québec. *Bipalium adventitium* is a potential threat to the soil fauna and the presence of invasive land flatworms in an additional country should be recorded, especially with molecular information (Breugelmans *et al.*, 2012; Chaisiri *et al.*, 2018; Justine *et al.*, 2018a; Mazza *et al.*, 2016; Morffe *et al.*, 2016).

The COI sequences of bipaliine species differed by more than 10%, suggesting that COI barcode can be reliably used for the identification of species of bipaliines. Longer sequences, such as complete mitogenomes, will be useful in the future (Gastineau *et al.*, 2019) In our analysis, we remarked that two COI sequences did not group with the species corresponding to the names entered in GenBank when the sequences were deposited; these are HM346597, allegedly *B. adventitium* and HM346600, allegedly *D. multilineatum*, both from South Korea and both from the same paper (Moon *et al.*, 2011). These sequences should not be used for future identification of bipaliine species.

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