

Co-invasion of three Asian earthworms, *Metaphire hilgendorfi*, *Amyntas agrestis* and *Amyntas tokioensis* in the USA

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Abstract Earthworm invasions are one of the most serious causes of ecological deterioration in the temperate deciduous forests of North America. Non-native earthworms impact understory vegetation, leaf litter layer, carbon dynamics, nutrient availability, and the associated food webs. Here we report a significant status change and confirm expansion of known range of *Amyntas agrestis*, one of the most serious invasive species in North America, and two of its close relatives, *A. tokioensis* and *Metaphire hilgendorfi*. The three species have never been confirmed to co-occur in North American ecosystems. We examined 1760 earthworms collected from 30 sites across northeastern USA, and identified them using a new morphological key. Our data show that sympatric occurrence of at least two, and often all three, species

is more common than having only one species. In addition, *A. tokioensis* was dominant in many of these earthworm communities. The status change in species composition from only one species to two or three and the shift in dominance are most likely caused by previous incorrect species identification. Our results support expansion of known range of *A. tokioensis* and *M. hilgendorfi* northward and westward into states with colder winters. This range expansion may have taken place alongside that of *A. agrestis* in the last 10–20 years, but has long been overlooked. Altogether, results highlight an urgent need for correct species identification. The recognition of an expanding multi-species system represents a unique opportunity to further evaluate complex interactions among

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co-invading and resident species, and to investigate whether interspecific interactions have unexpected non-additive impacts on ecological processes.

Keywords Earthworm invasion · *Amyntas agrestis* · *Amyntas tokioensis* · *Metaphire hilgendorfi* · Co-invasion

Introduction

Invasive earthworms are one of the most important ecological drivers currently changing forest ecosystems in North America (Bohlen et al. 2004; Dávalos et al. 2015; Craven et al. 2017). Of particular concern are three Asian species in the genera *Amyntas* and *Metaphire* due to their impacts on forest floor, soil structure, carbon dynamics, nutrient concentration, and other soil organisms (Zhang et al. 2010; Snyder et al. 2011, 2013; Greiner et al. 2012; Qiu and Turner 2017). In the scientific and popular literature these species are frequently referred to as “*Amyntas* spp.” (e.g. Dávalos et al. 2015) and nicknamed “crazy worm” or “jumping worm”. They invade remote forests in the United States (Callaham et al. 2003), and one species in particular, *Amyntas agrestis* (Goto & Hatai, 1899), was recently discovered spreading north and west from the Mid-Atlantic into New England and the Midwest (Görres and Melnichuk 2012; Reynolds et al. 2015; Schult et al. 2016).

So far most of the *Amyntas* and *Metaphire* sightings in the northeastern United States involved only *A. agrestis* (Callaham et al. 2003; Snyder et al. 2011; Görres and Melnichuk 2012; Görres et al. 2014; Reynolds et al. 2015; Schult et al. 2016), with occasional reports of *Metaphire hilgendorfi* (Michaelsen, 1892), often as *Amyntas hilgendorfi* (e.g. Chang et al. 2016a; see Chang et al. 2016b for details), a species closely related to *A. agrestis*. The two species have never been reported at the same locality. However, recent genetic analysis indicated sympatric occurrence of three species-level cryptic lineages within invading *Amyntas* in upstate New York (Schult et al. 2016). Meanwhile, at several locations where *A. agrestis* or *M. hilgendorfi* was reported, we also found a previously overlooked species, *Amyntas tokioensis* (Beddard, 1892) (Chang et al. 2016b). These findings led us to re-examine archived and new

specimens to understand the extent of co-occurrence of the three species.

We examined 1224 specimens previously reported from 15 sites as *Amyntas* spp. (Dávalos et al. 2015; Schult et al. 2016; Table 1) and 536 newly collected specimens from another 15 sites where Asian earthworms have never been reported (Wilmette, IL) or only one of *A. agrestis* or *M. hilgendorfi* was previously reported or observed (Szlavec et al. 2006, 2014; Görres and Melnichuk 2012; Chang et al. 2016a). All samples were collected from 2002 to 2016 with a variety of techniques, including hand sorting, digging, and extraction by formalin or mustard solutions. Species identification followed Chang et al. (2016b).

Results

A total of 1760 specimens (1124 identifiable) belonging to three species, *M. hilgendorfi*, *A. agrestis* and *A. tokioensis*, were collected from 30 sites across eight states in the Midwest and northeastern USA (Fig. 1; Table 1). *Amyntas tokioensis*, *M. hilgendorfi* and *A. agrestis* were found in 25, 23 and 22 sites, respectively. Of the 30 sites, 27 had at least two species, and 13 had all three. In addition to being the most common species, *A. tokioensis* was also dominant in 11 of the 25 sites based on relative abundance, whereas *A. agrestis* was only dominant in nine sites. Among 51 specimens that were analyzed genetically in Schult et al. (2016) and re-examined morphologically per Chang et al. (2016b), 22 were unidentifiable, but 29 were identified as *A. tokioensis* (8), *A. agrestis* (12), or *M. hilgendorfi* (9), and all species identifications corresponded unequivocally to the three species-level genetic lineages, A, B and C reported in Schult et al. (2016), respectively.

Discussion

Our data highlight widespread species co-occurrence in the current range of *Amyntas* and *Metaphire* in northeastern USA, and provide the first confirmed reports of *A. tokioensis* in Maryland, Delaware, Vermont, Illinois, and Wisconsin and *M. hilgendorfi* in New Hampshire. We herein emphasize that co-occurrence of two or three species is what should be

Table 1 Sampling locations in northeastern USA and numbers of *Metaphire hilgendorfi*, *Amyntas agrestis* and *Amyntas tokioensis* recorded

Location	State	Label	GPS-N	GPS-W	Collection date	Total	NID	<i>Metaphire hilgendorfi</i> *	<i>Amyntas agrestis</i>	<i>Amyntas tokioensis</i> *	References
Flintwood Preserve Blowdown	DE	DE19	39°49'31.0"N	75°36'18.9"W	7/2013	81	32	33	–	16	a
Flintwood Preserve Diversity	DE	DE20	39°49'26.0"N	75°36'13.2"W	7/2013	63	38	9	–	16	a
Flintwood Preserve Former Field	DE	DE21	39°48'46.0"N	75°36'16.6"W	7/2013	51	12	18	–	21	a
Wilmette	IL	WMT	42°04'38"N	87°43'25"W	10/2016	112	–	42	2	68	–
Cub Hill, Parkville	MD	CUH	39°24'45.2"N	76°31'14.9"W	7/2002, 10/2003	36	8	13	5	10	b
Johns Hopkins Univ., Baltimore	MD	JHU	39°19'45.9"N	76°37'28.6"W	8/2015	61	–	8	16	37	b
Stony Run, Baltimore	MD	STR	39°21'18.0"N	76°37'47.8"W	7/2015	31	13	2	12	4	b
Oregon Ridge Park, Cockeysville	MD	ORP	39°28'43.0"N	76°41'21.4"W	9/2015	32	–	6	7	19	b
Plummers Island, Cabin John	MD	PIS	38°58'13.2"N	77°10'35.1"W	9/2012	11	–	7	–	4	b
Treefall, SERC, Edgewater	MD	SERC2	38°53'30.7"N	76°33'56.4"W	10/2016	9	–	9	–	–	b
Triangle, SERC, Edgewater	MD	SERC1	38°53'28.2"N	76°33'43.9"W	9/2015	18	4	–	14	–	b
Hanover	NH	HNV	43°41'49.5"N	72°17'22.1"W	10/2015	11	–	9	2	–	b
Clinton	NY	BCA8/ 24	43°02'57.2"N	75°24'00.7"W	9/2014, 10/2015	30	17	–	7	6	c
Hamilton	NY	BCA22	42°47'49.0"N	75°30'20.0"W	10/2015	10	3	–	5	2	c
Millbrook	NY	BCA21	41°52'34.0"N	73°40'33.0"W	11/2015	9	–	9	–	–	c
Mundy Wildflower Preserve	NY	NY3	42°27'00.7"N	76°28'08.2"W	7/2013	159	125	9	24	1	a
West Point	NY	WP5	41°23'26.7"N	74°00'36.3"W	7/2011	160	78	30	13	39	a
West Point	NY	WP7	41°22'15.2"N	74°02'20.5"W	7/2011	180	41	62	45	32	a
West Point	NY	WP9	41°20'18.9"N	74°04'40.5"W	7/2011	162	67	39	42	14	a
Kendal-Crosslands Communities	PA	PA16	39°52'38.4"N	75°39'37.9"W	7/2013	84	48	9	7	20	a
Longwood For.	PA	PA17	39°52'34.3"N	75°40'38.5"W	7/2013	84	36	18	28	2	a
Longwood Garden	PA	PA18	39°52'25.1"N	75°40'29.8"W	7/2013	50	33	6	3	8	a
Waterloo Mills Preserve	PA	PA15	40°01'32.7"N	75°25'25.1"W	7/2013	14	–	9	–	5	a
Welkinweir Old	PA	PA14	40°09'08.5"N	75°41'06.9"W	7/2013	87	44	40	–	3	a
Audubon, Huntington	VT	ADB	44°20'49.6"N	72°59'46.5"W	10/2015	49	25	–	18	6	b

Table 1 continued

Location	State	Label	GPS-N	GPS-W	Collection date	Total	NID	<i>Metaphire hilgendorfi</i> *	<i>Amyynthas agrestis</i>	<i>Amyynthas tokioensis</i> *	References
Duxbury	VT	DXB	44°22'30.7"N	72°57'17.2"W	10/2015	6	1	–	4	1	b
Centennial Woods, South Burlington	VT	CTW	44°28'33.2"N	73°11'16.4"W	9/2015	18	–	2	16	–	b
HRC, South Burlington	VT	HRC	44°25'52.5"N	73°11'58.9"W	9/2015	103	–	–	15	88	b
UVM Nursery, South Burlington	VT	UVM	44°25'45.3"N	73°12'09.1"W	9/2010	23	11	1	3	8	b
UW Arboretum, Madison	WI	UWA	43°02'36.6"N	89°25'30.8"W	9/2015	16	–	–	5	11	b
Total						1760	636	390	293	441	

Total total number of specimens examined, NID number of individuals that are not identifiable due to parthenogenesis or immaturity

*First state records of *M. hilgendorfi* for IL and NH and *A. tokioensis* for IL, WI, VT, MD

^aIdentified in Dávalos et al. (2015) as *Amyynthas* spp.

^bMentioned in Chang et al. (2016b) as unpublished data or personal observation

^cIdentified in Schult et al. (2016) as *Amyynthas* spp. and grouped into three genetic lineages

expected, whereas the situation with a single species presence is rare. Sympatric occurrence between *A. agrestis* and *A. tokioensis* has been confirmed in compost (Gates 1958), but never before from natural habitats such as forest soils. Moreover, the universal presence or even dominance of *A. tokioensis* in forested habitats has been overlooked for a long time. This lack of reporting might be due to difficulties with specimen identification, rather than absence of the species from a locality. Recent scientific literature documented the invasion as only by *A. agrestis*. In some cases, species identification of *A. agrestis* was achieved by examining only a small number of individuals, or was based on the color and shape of clitellum and the jumping and snake-like behavior. Unfortunately, all of these characteristics are also shared by *A. tokioensis* and *M. hilgendorfi* and thus should not be used as a basis for species diagnosis. While we recognize the difficulty of identifying pheretimoid earthworms, we urge researchers and managers to re-examine their collections and confirm species identities by taxonomically reliable morphological traits (e.g., number and location of genital pores). A key to identify *Amyynthas* and *Metaphire* species in North America is now available for this purpose (Chang et al. 2016b). Identification should preferably include at least 20 adult specimens to adequately address potential co-occurrence of more than one species.

Amyynthas tokioensis has been previously reported living in natural habitats in only six states, all of which are in the Southeast or Mid-Atlantic (Reynolds and Wetzel 2008). Confirmed records of *A. tokioensis* in Vermont, Illinois and Wisconsin clearly show that the known distribution of this species has expanded further north and northwest into New England and the Midwest and the species is able to survive cold winters (presumably as cocoons; Görres et al. 2016). Similarly, *M. hilgendorfi* had not been recorded outside of greenhouse or compost habitats in any of the New England or Midwest states (Reynolds and Wetzel 2008) until it was found recently in Vermont (Reynolds et al. 2015) and now in New Hampshire and Illinois. Altogether, our findings of *M. hilgendorfi* and *A. tokioensis* and recent reports of *A. agrestis* from Wisconsin, Illinois, Vermont and New Hampshire (Görres and Melnichuk 2012; Reynolds et al. 2015; Schult et al. 2016; Qiu and Turner 2017) clearly show a northward and westward range expansion of the

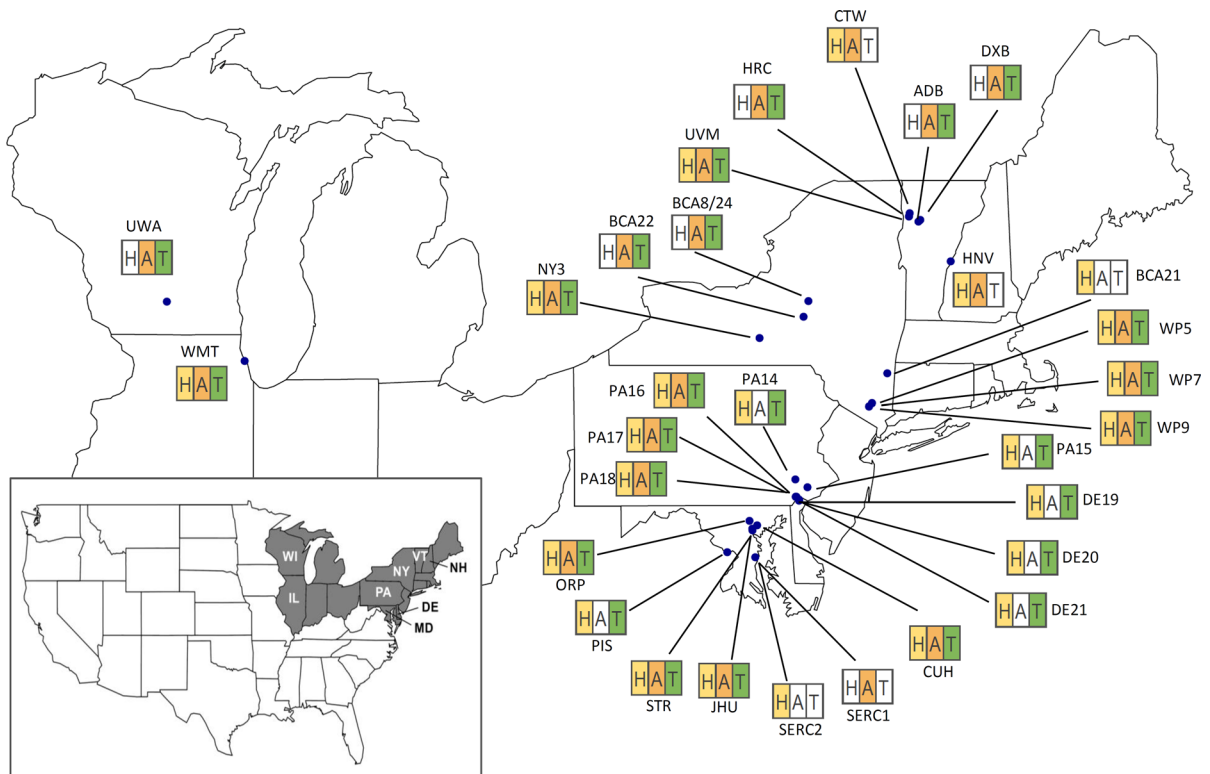


Fig. 1 Presence of *Metaphire hilgendorfi* (H, yellow), *Amyntas agrestis* (A, orange) and *A. tokioensis* (T, green) at sampling locations in northeastern United States (the Mid-Atlantic, New England, and the Midwest) based upon

morphological examination of 1760 specimens; absence of a species at a sampling location is labeled white. Labels for each sampling location match those in Table 1

three species into colder regions, presumably in the last 10–20 years. The expansion of *A. tokioensis* may have taken place alongside *A. agrestis* and/or *M. hilgendorfi* into the four states, but was overlooked in previous surveys (Chang personal observation).

Correct species identification and assessment of each species' abundance reaches beyond the simple descriptive characterization of earthworm assemblages. While functional groups help describe how one earthworm versus another may profoundly alter soil characteristics via litter consumption and soil vertical mixing (Bohlen et al. 2004), species identity more fully explains how non-native earthworms affect soil organic matter translocation and aggregate formation in interaction with native earthworms, other soil fauna, and the microbial community (e.g. Chang et al. 2016a, c, 2017). To date, *A. tokioensis*, *A. agrestis*, and *M. hilgendorfi* are only loosely categorized as epi-endogeic, and data are scarce with regard to their life history, diet, and activity in different soils

and to which depths. More importantly, we have essentially no knowledge on how these species differ ecologically. The widespread species co-occurrence implies niche differentiation and possible co-invasion. Autecological studies targeted toward each species are needed to understand how co-occurrence affects invasion success and competition with resident species, as documented between *Lumbricus rubellus* and the ecologically similar *A. agrestis* (Zhang et al. 2010) or *M. hilgendorfi* (Chang et al. 2016a). Phoretoids are frequently the sole species in invaded forests in Vermont and at West Point New York (Görres personal observation; Dávalos et al. 2015). The recognition of an expanding and multi-species system reported herein provides unique opportunities to study earthworm invasions and their impacts on forest soil properties. This assemblage information, while raising serious concerns, also allows a new set of questions to be asked regarding the complex interactions among co-invading species and the potential

non-additive impacts they may have on ecological processes and ecosystem functions.

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