

## Identifying the Asian earthworms *Amyntas agrestis*, *Amyntas tokioensis*, and *Metaphire hilgendorfi*

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Information contained in this guide is extracted from the following paper:

‘Chang, C.-H., Snyder, B., Szlavecz K. (2016) Asian pheretimoid earthworms in North America north of Mexico: An illustrated key to the genera *Amyntas*, *Metaphire*, *Pithemera*, and *Polypheretima* (Clitellata: Megascolecidae). *Zootaxa* 4179 (3), 495–529’.

If you need to cite information in this article, please cite the paper directly.

### Introduction

This guide is designed to help both ecologists and the general public to distinguish *Amyntas agrestis*, *Amyntas tokioensis*, and *Metaphire hilgendorfi*: three frequently co-occurring species that has been invading the Eastern US. The widespread co-occurrence is a big challenge to ecologists and land managers, and becomes a confounding factor in studies where species identification was not done based on a reliable taxonomy reference or by experienced persons.

## Species identification

### Three steps

#### 1. Preclitellar genital markings

Look for preclitellar genital markings on the ventral side anterior to the clitellum:

Fig. 1B, C → *Amyntas agrestis*

Fig. 2B → *Amyntas tokioensis*

Fig. 3D, E → *Metaphire hilgendorfi*

The ones in *M. hilgendorfi* should be very obvious, but those in *A. agrestis* could be hard to see. The ones in *A. tokioensis* are usually associated with spermathecal pores. In *A. tokioensis* specimens with no spermathecal pores, the markings are also absent. In that case, you will have to look at the number of spermathecal pores.

You should be able to unambiguously identify all specimens of *M. hilgendorfi* at this step. At least some specimens of *A. tokioensis* can also be sorted.

#### 2. Male pores

Male pores (segment 18, two segments behind the clitellum) can sometimes be found in *Amyntas tokioensis* (Fig. 2A). The other two species usually do not have male pores (Fig. 1A, 3A-C) due to parthenogenetic degradation. Male pores of the three species look very different, and can be used to distinguish them if present. However, you need to be really lucky to see one in *A. agrestis* or *M. hilgendorfi*.

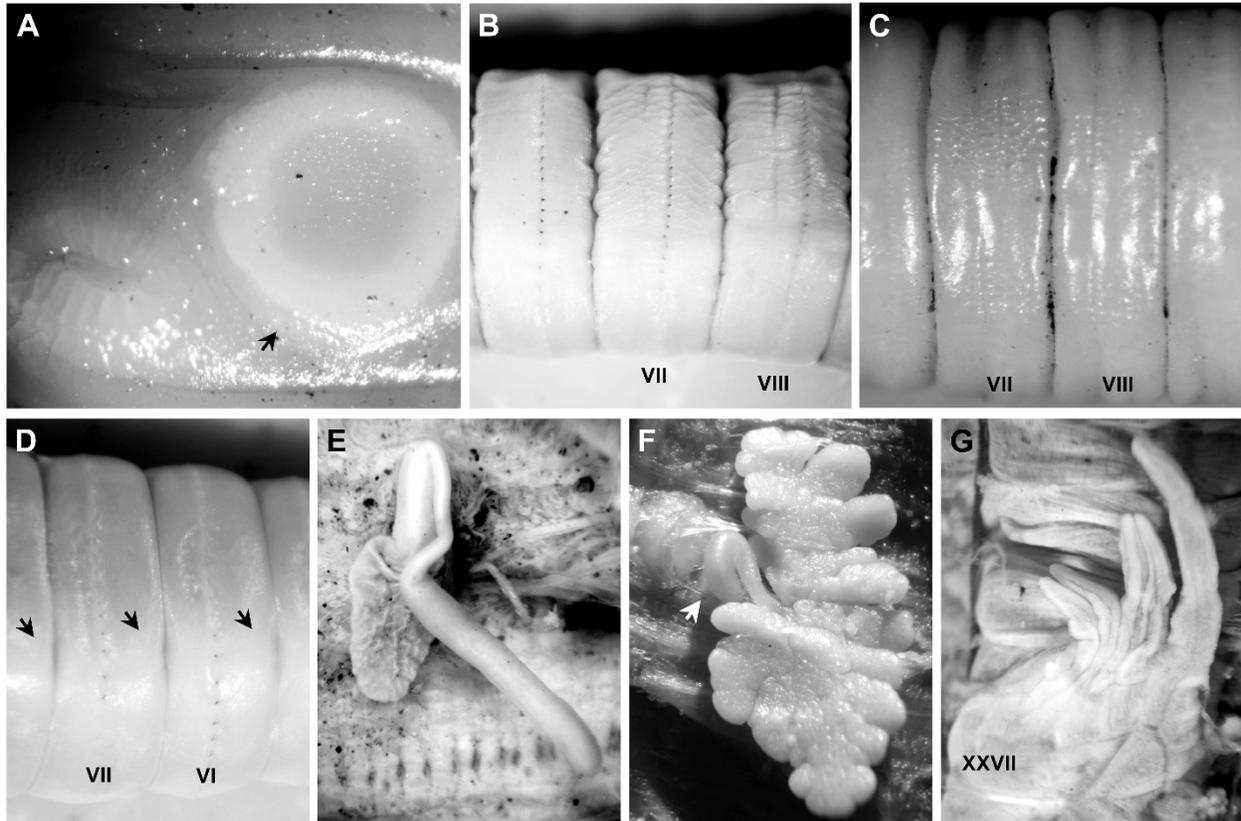
#### 3. Spermathecal pores

*M. hilgendorfi* always has two pairs of spermathecal pores in 6/7 and 7/8, while *A. agrestis* usually has three pairs in 5/6, 6/7, and 7/8. So, if spermathecal pores are present in 5/6, the specimen is definitely an *A. agrestis*. In most cases, you should see all three pores (5/6, 6/7, and 7/8) on both sides. The spermathecal pores of *A. tokioensis* are in 6/7 and 7/8. However, specimens with fewer or even no spermathecal pores are common. If a specimen has no male pores, no spermathecal pores, no genital markings, it is an *A. tokioensis*. When the pores are present in an *A. tokioensis*, they usually come with genital markings (Fig. 2B).

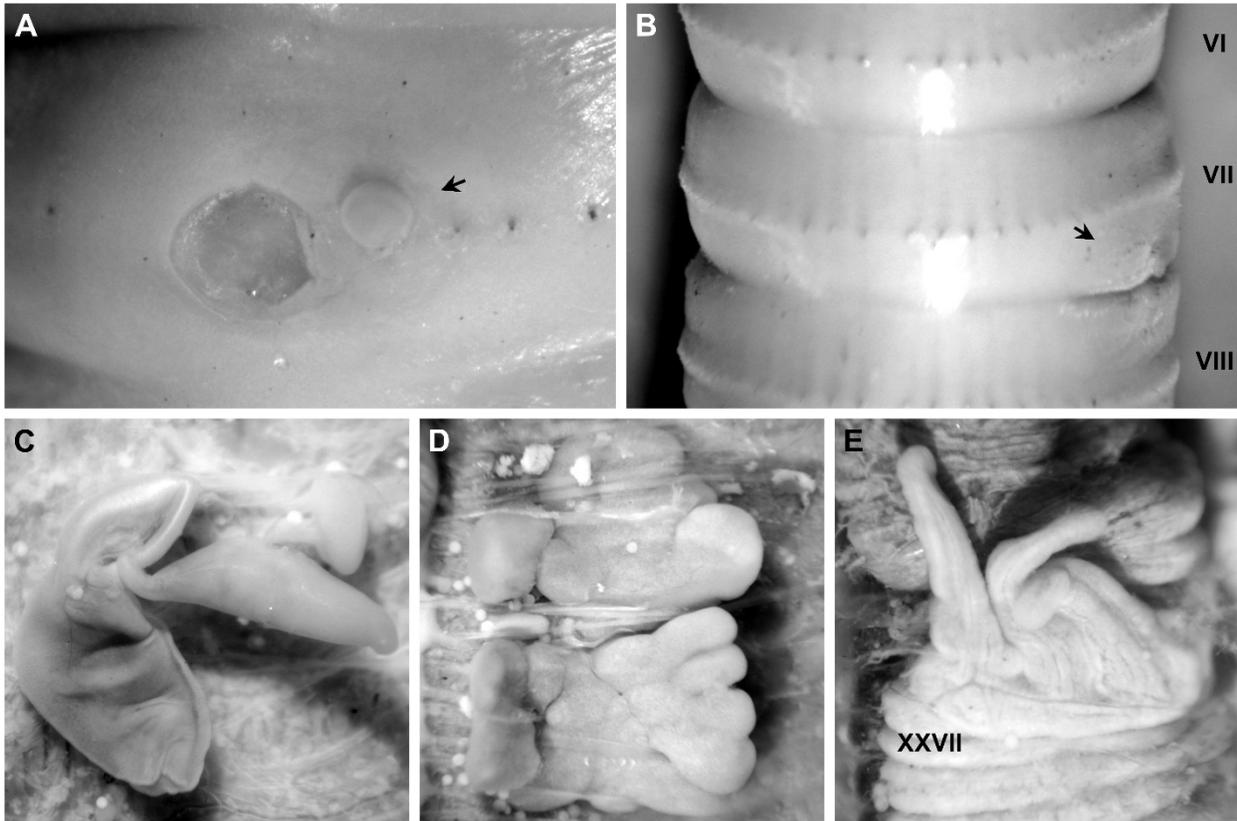
Other things to consider

Size-wise, on average, *M. hilgendorfi* is larger than *A. agrestis*, which is larger than *A. tokioensis*. However, there is significant overlap, even between *M. hilgendorfi* and *A. agrestis*. Do not use size-along to ID any species. You will end up having a lot of mis-identified specimens.

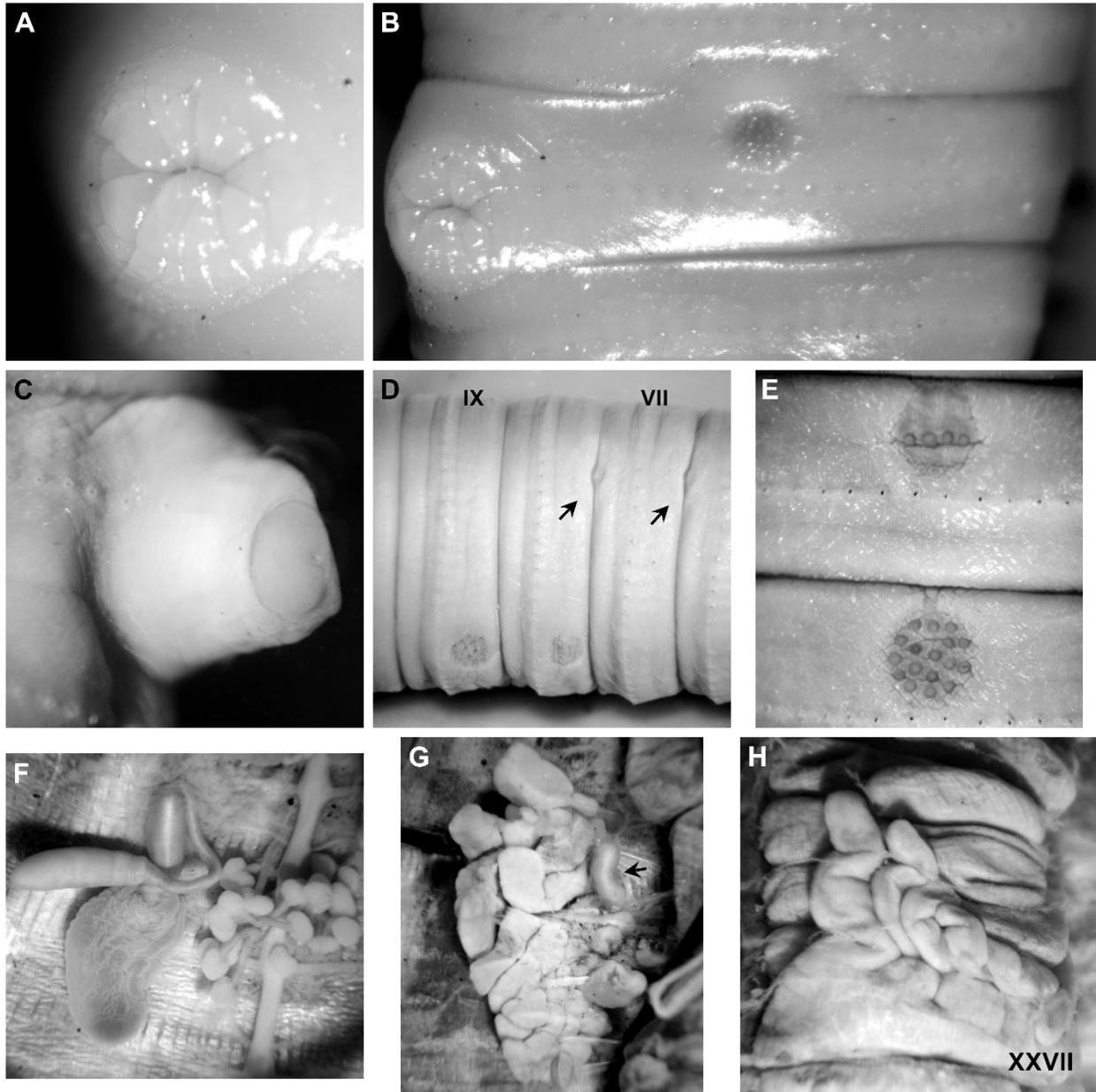
Internally, the three species are in general not distinguishable. However, if you cannot figure out what species you get, or even not sure if your specimens belong to any of the three species, you may want to dissect the specimens and look at the number and location of their spermathecae. That can help you determine where the spermathecal pore is if you cannot see them from outside. If you see spermathecae in segments 6-8, that means you have spermathecal pores in 5/6-7/8, and you get an *A. agrestis*.



**FIGURE 1.** *Amynthus agrestis*. (A) Right male pore region and the associated genital marking (arrow). (B, C) Wrinkled surface on VII and VIII (ventrolateral view and ventral view, respectively). (D) Spermathecal pores in 5/6/7/8 (arrows) (lateral view). (E) Left spermatheca in VIII. (F) Left prostate gland and its duct (arrow). (G) Left intestinal caecum. [Source: Chang et al. 2016]



**FIGURE 2.** *Amynthes tokioensis*. (A) Right male pore and the associated genital marking (arrow). (B) Single spermathecal pore in 7/8 on the lower right (arrow), compared to its absence in 6/7 on the right and in 6/7/8 on the left; ventral view. (C) Left spermatheca in VIII. (D) Right prostate gland. (E) Right intestinal caecum. [Source: Chang et al. 2016]



**FIGURE 3.** *Metaphire hilgendorfi*. (A, B) Right male pore region and the associated genital markings, showing presence of a male pore only on one side and absence on the other. (C) Left male pore region with an everted copulatory pouch. (D) Right spermathecal pores in 6/7/8 and pre-setal genital markings on VIII and IX; ventrolateral view. (E) Pre-setal genital markings mid-ventral on VIII and IX. (F) Left spermatheca and accessory glands in VIII. (G) Left prostate gland and its duct (arrow). (H) Right intestinal caecum. [Source: Chang et al. 2016]

TABLE 1. Comparisons of *Amyntas agrestis*, *A. tokioensis*, and *Metaphire hilgendorfi*.

	<i>Amyntas agrestis</i>	<i>Amyntas tokioensis</i>	<i>Metaphire hilgendorfi</i>
Origin	Japan	Japan	Japan
Reproduction	Parthenogenetic	Parthenogenetic	Parthenogenetic
Life cycle	Annual	Annual	Annual
Functional group	Epi-endogeic	Epi-endogeic	Epi-endogeic
Size*	70-160 mm by 5-8 mm	75-125 mm by 5-7 mm	109-170 mm by 6-8 mm
Segment numbers	63-110	84-102	98-118
Spermathecal pores	Three pairs in 5/6/7/8, sometime only on one side	Two pairs or fewer in 6/7/8	Two pairs in 6/7/8
Pre-clitellar genital markings	Areas of epidermal modification on VII and/or VIII, occasionally on VI and IX, ventral, unpaired and median or symmetrically paired, forming setal gaps, epidermis finely wrinkled or crosshatched	Present or absent, when present, small, circular discs, paired in front of the setal line on VII and VIII, median to the spermathecal pores, some specimens with an additional disc right in front of the pore on VI and/or VII.	Unpaired, mid-ventral, pre-setal clusters of numerous small tubercles on VIII-IX, occasionally on VII, X, XI
Male pores	Usually absent; when present, small, transversely slit-like.	Present or absent; when present, on a small porophore surrounded by a deep furrow	Usually absent; when present, in a copulatory pouch
Post-clitellar genital markings	Usually absent; when present, single large pad with a concaved center, pre-setal, median to male pores	Present or absent; when present, small, circular discs, 1-3 on each side of XVIII, one sometimes on the porophore, two median or lateral to the male porophore	Usually absent; when present, on XVII or XVIII, similar to those in the pre-clitellar region
Prostate glands	Usually absent	Present or absent	Usually absent
Caeca	Maniccate	Maniccate	Maniccate

\* Length by width. While the ranges of size are similar among the three species, *A. tokioensis* is generally considered a small species and is smaller than the other two species.