

## **A guide to identifying the Asian earthworms *Amyntas agrestis*, *Amyntas tokioensis*, and *Metaphire hilgendorfi***

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### **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 Northeast and Southeast US. Since 2015, many invaded sites previous thought to have only *A. agrestis* or *M. hilgendorfi* ended up being found to contain at least two, usually all three species. 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 with a reliable taxonomy reference provided or by experienced earthworm experts.

## Species identification

### Three simple steps

1. 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*

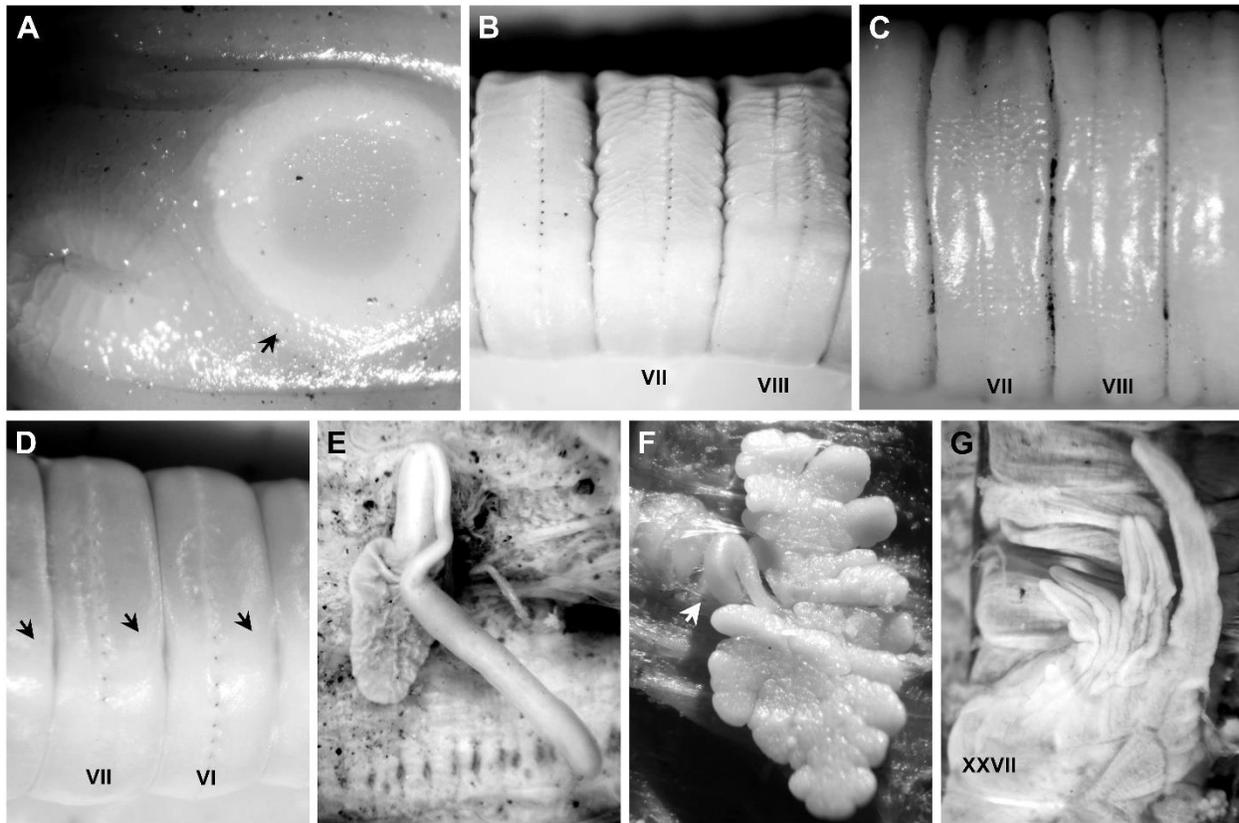
2. Male pores (segment 18, two segments behind the clitellum) can sometimes be found in *Amyntas tokioensis* (Fig 2A). The other two species usually do not have male pores (Fig 1A, 3A-C). Male pores of the three species look very different, and can be used to distinguish them if present. Again, you need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

3. If spermathecal pores are present in 5/6, it should be an *A. agrestis*. In most cases, you should see all three pores (5/6, 6/7, and 7/8) on at least one side. Keep in mind that if you do not see any pores in 5/6, it does not mean that the specimen is not an *A. agrestis*. Simple logic!

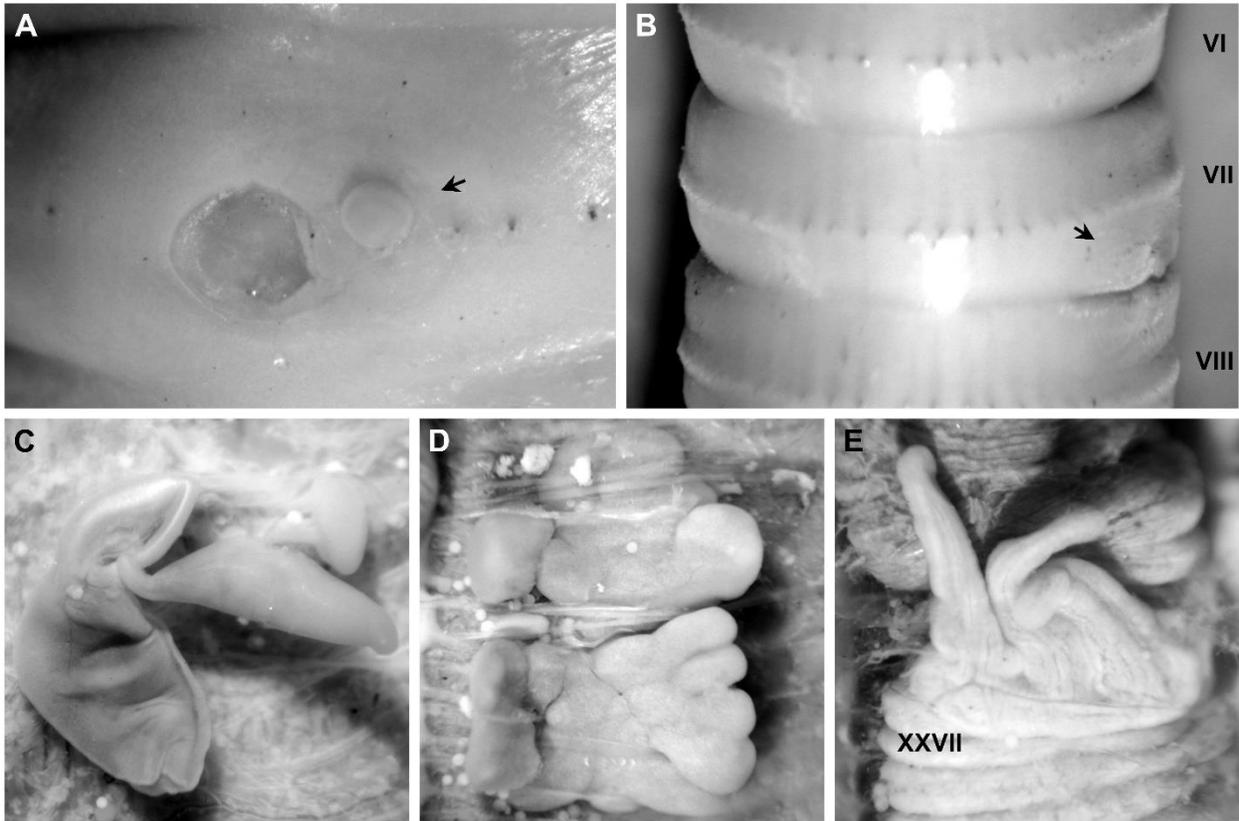
### Other characters to look at

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.

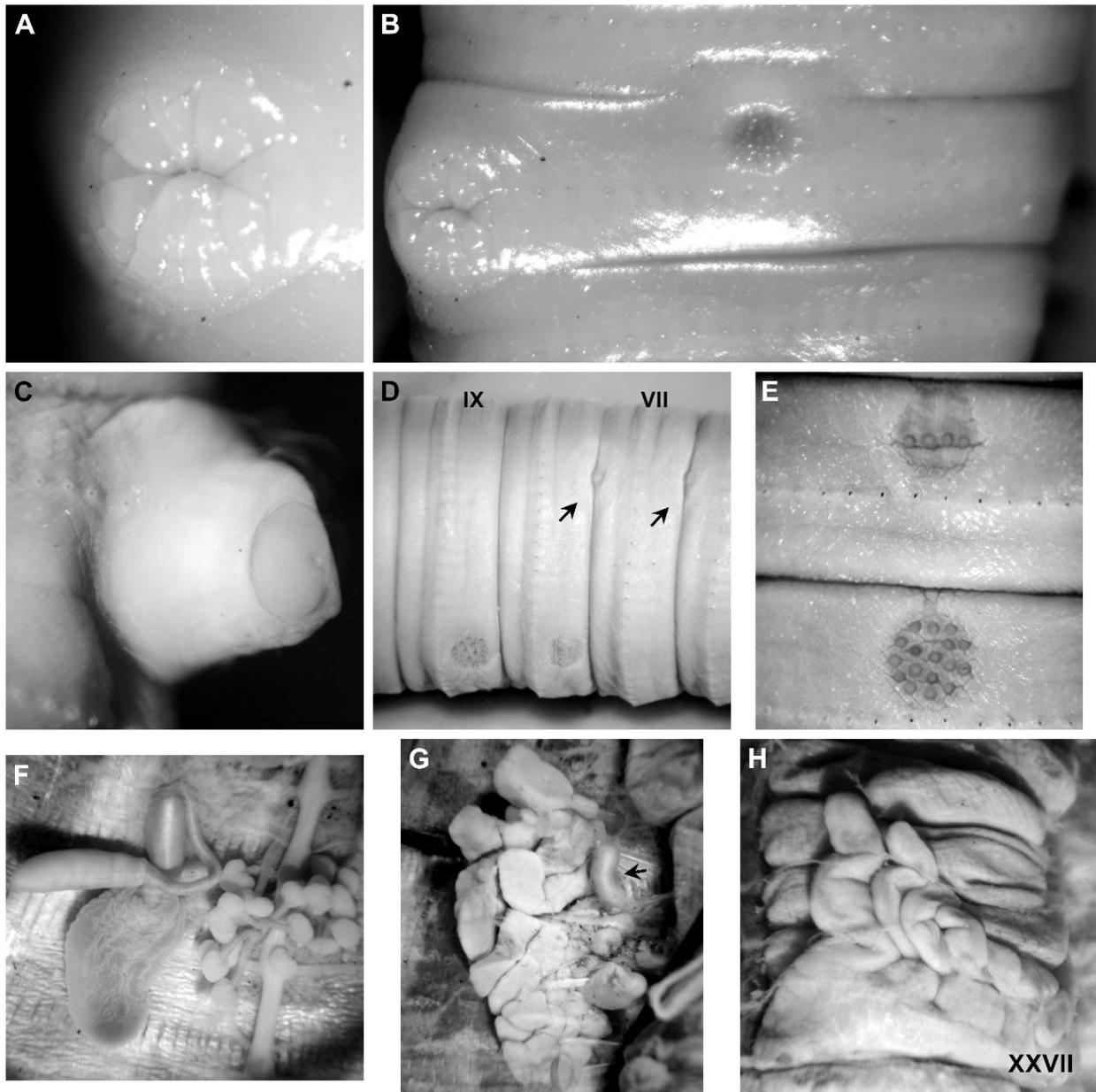
Internally, the three species are in general not distinguishable. However, if you cannot figure out what species you get, or even not sure if your specimens belong to any of the three species, you may want to dissect the specimens and look at the number and location of their spermathecae. That can help 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 or fewer in 5/6/7/8	Two pairs or fewer in 6/7/8	Two pairs or fewer 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.