Zootaxa 4179 (3): 495–529
http://www.mapress.com/j/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://doi.org/10.11646/zootaxa.4179.3.7
http://zoobank.org/urn:lsid:zoobank.org:pub:B0854097-2CA6-41CF-B44C-8A0F49819DA0

Asian pheretimoid earthworms in North America north of Mexico: An illustrated key to the genera *Amynthas*, *Metaphire*, *Pithemera*, and *Polypheretima* (Clitellata: Megascolecidae)

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Abstract

The invasion of the pheretimoid earthworms in North America, especially the genera *Amynthas* and *Metaphire*, has raised increasing concerns among ecologists and land managers, in turn increasing the need for proper identification. However, the commonly used keys to this group are more than 30 years old with outdated taxonomic information and are based primarily on internal morphology. The requirement of significant amount of taxonomic expertise and dissection, even from the first entry of the key, has prevented broader use of these keys. As a result, many publications in the United States have used *Amynthas* spp. to represent the group without identifying the species. We present here a new key and diagnoses for the 16 pheretimoid earthworm species recorded in North America north of Mexico, including four genera: *Amynthas* (10), *Metaphire* (4), *Pithemera* (1), and *Polypheretima* (1). The descriptions were based on published records with modifications following inspection of specimens archived at the National Museum of Natural History, Smithsonian Institution, Washington, D.C. Photos of external and internal characters, including male pores, spermathecal pores, genital markings, spermathecae, prostate glands, and intestinal caeca, are presented to help identification. A summary of current knowledge about the ecology and historical context is provided for each species. We also highlight the previously overlooked and potentially common and widespread co-occurrence of three species—*A. agrestis*, *A. tokioensis*, and *M. hilgendorfi*—and point out that many recent claims of invasion of *A. agrestis* need to be re-evaluated for potential misidentification.

Key words: *Pheretima* complex, invasive species, *Amynthas hilgendorfi*, *Metaphire hilgendorfi*, *Amynthas agrestis*, *Amynthas tokioensis*, Oligochaeta

Introduction

The pheretimoid earthworms, also variously referred to as *Pheretima auct.*, the *Pheretima* group of genera, or the *Pheretima* complex (Sims & Easton 1972; Chang *et al.* 2009a; Blakemore 2010a), form a clade of earthworms indigenous to Australia, Southeast Asia, and the eastern part of East Asia. Previously in a single genus *Pheretima sensu lato*, this group currently contains more than 1,000 species in 14 genera (Sims & Easton 1972; James 2004; Blakemore 2010b). The pheretimoids are one of the most active fields of earthworm diversity research (Chang & Chen 2004, 2005; James 2004, 2005, 2009; Chang *et al.* 2008, 2014; Tsai *et al.* 2009, 2010; Blakemore 2010a, b; 2013b; Hong & James 2011, 2013; Shen *et al.* 2013, 2014), with four new genera described in the last decade. Species from five genera, *Amynthas*, *Metaphire*, *Pheretima*, *Pithemera* and *Polypheretima*, have been reported as invasive in temperate and tropical regions worldwide (Sims & Easton 1972; Easton 1981; Gates 1982; Chang *et al.* 2009a; Blakemore 2010a). The first record of the pheretimoid earthworms in North America north of Mexico was *Metaphire californica* (Kinberg, 1867) (as *Pheretima californica*) around San Francisco, CA, in 1866 (Kinberg 1867). Since then, 16 species have been reported. These species belong to four genera, *Amynthas*, *Metaphire*, *Pithemera* and *Polypheretima*, including 10 species of *Amynthas* and four species of *Metaphire*. All of the 16

Accepted by R.M. Schmelz: 20 Sept. 2016; published: 1 Nov. 2016

species have been reported in the USA, while only two species have been reported in Canada (Reynolds 2014). In recent years, invasion of some of the species, especially *A. agrestis*, *A. corticis*, *A. gracilis,* and *M. hilgendorfi*, into deciduous forests in the northern and eastern US has raised concerns about their potential effects on plant communities, native soil fauna, and soil carbon and nitrogen cycling (Burtelow *et al.* 1998; Callaham *et al.* 2003; Snyder *et al.* 2009, 2011, 2013; Greiner *et al.* 2012; Chang *et al.* 2016a, b). One species, *A. agrestis*, has been shown to compete with native millipedes for food resources, and may reduce species richness and diversity in the latter (Snyder *et al.* 2011, 2013). Moreover, many of the forests invaded by *Amynthas* and *Metaphire* have native earthworm fauna and/or have already gone through significant changes due to invasion by European lumbricid earthworms, primarily of the genera *Lumbricus*, *Aporrectodea*, and *Octolasion* (Chang *et al.* 2016a). The impacts of Asian earthworms will depend on how *Amynthas* and *Metaphire* interact with resident native and non-native species, and whether earthworm communities dominated by Asian earthworms function differently from those primarily composed of European species. In forests already dominated by European species, interspecific competition as a result of *Amynthas* or *Metaphire* invasion may lead to changes in earthworm community structure. Competition for leaf litter has been demonstrated between *M. hilgendorfi* and *Lumbricus rubellus*, one of the most common European earthworm species, with *M. hilgendorfi* being the superior competitor (Chang *et al.* 2016a). *Lumbricus rubellus* also suffered reduced biomass in the presence of *A. agrestis* (Zhang *et al.* 2010). Although invasion by these pheterimoids is often visible and dramatic, the consequences to ecosystem function remain unclear.

Checklists of North American earthworm taxa have been provided by Gates (1982) and recently by Blakemore (2006) and Reynolds & Wetzel (2008). The latter two studies list 183 and 253 species respectively, of which 123 and 186 are native. The difference in the numbers of species is partially due to different geographical coverage in the two studies, as well as to disagreement in taxonomic decisions. Even though Gates' comprehensive review (Gates 1982) lists 15 of the 16 pheretimoids currently known in North America, our knowledge about the taxonomy of the recorded species has evolved gradually. As a result, several names listed by Gates (1982) and subsequently by Reynolds and Wetzel (2004, 2008) have been regarded as junior synonyms and their use should be discontinued: *A. hawayanus*, *A. diffringens* and *M. levis* (Blakemore 2010a).

Accurate species identification is crucial to understand how soil community structure and function are altered by pheretimoid earthworms, and how species-specific responses mediate the processes and determine the state of the system. Major challenges of species identification in this group include understanding the range of morphological variations of a species, which is usually spread across several taxonomic publications and further complicated by parthenogenetic degradations. These challenges are further exacerbated by the lack of a user-friendly key specifically designed for North American species. The comprehensive key to cosmopolitan pheretimoid earthworms by Blakemore (2010a) is a useful primary resource for identifying peregrine species in general, but it contains many species that have not been recorded in North America. The available keys for North American pheretimoid species by Reynolds (1978) and Gates (1982) are outdated and start with the presence or absence of intestinal caeca, and therefore require users to dissect the specimens from the onset of using the keys. With some experience, identification of mature specimens of the 16 species recorded in the US is possible without dissection, although internal morphology may still be required to confirm the identification.

Here we present a new key to the pheretimoid earthworms currently listed in North America north of Mexico, with photos and diagnoses. The key was designed to enable species identification without dissecting specimens. At the same time, internal morphologies were still included in the key to double-check the identification or to deal with situations in which external structures are poorly preserved due to non-optimal specimen processing, fixation, or preservation. The photos were primarily based on specimens archived at the National Museum of Natural History (USNM), Smithsonian Institution, Washington D.C., USA. Our objective is to provide a "field guide" to ecologists, managers and educators whose work requires identifying these species. This study does not aim to serve as the taxonomic authority of the species involved.

Materials and methods

Most of the specimens examined and photographed in this study are specimens archived at the National Museum of Natural History (USNM), Smithsonian Institution, Washington, D.C., USA. A total of three specimens of

Amynthas agrestis and *Metaphire hilgendorfi* were collected from Baltimore, Maryland, USA by C.-H. Chang and donated to USNM. These newly collected specimens were anesthetized in 10% ethanol and preserved in 70% ethanol (see Appendix B for a detailed protocol). Collection details, including dates, locations, and identifier of all USNM specimens photographed can be found at the USNM website (http://collections.nmnh.si.edu/search/iz/) by searching their catalog numbers. The photographed specimens of *Amynthas carnosus* were part of those reported in Carrera-Martínez & Snyder (2016) and are part of the research collection of B.A. Snyder.

Specimens archived at USNM are preserved in either 70% ethanol or 10% formalin. Most of them have apparently been treated with some fixatives, most likely 10% formalin. Specimens were examined and photographed using a stereomicroscope and a Canon T4i camera coupled with an adaptor. Photos of internal characters were taken primarily from specimens that have been previously dissected. When such specimens were not available and dissection was needed, dissection was done longitudinally along the mid-dorsal line from the third segment to ca. the 35th segment. When necessary, septa were carefully cut and the body walls were pinned on a dissecting pad using insect pins in order to expose the organs for examination and photography. As dissection itself is considered destructive, we tried to minimize the number of specimens we dissected.

In order to cover the intraspecific variations as much as possible, in most cases, several specimens per species were selected for photography. In some cases where US specimens were either unavailable or in bad conditions, we selected specimens collected from other parts of the world.

We follow the species list by Blakemore (2006) and the convention of listing synonyms from previous studies under each species. However, we restrict our lists of synonyms only to those publications that report North American records or have significant taxonomic importance. The lists are not meant to be exhaustive, but to provide enough information on the name changes and synonyms that researchers working on North American pheretimoid earthworms are most likely to encounter. Full lists of synonyms are reported in Blakemore (2010a). The majority of the morphological descriptions presented under the diagnosis of each species were compiled from published studies, which were explicitly listed under the "data sources." In most cases, we include only original morphological descriptions in our data sources and avoid publications which themselves are largely compilations of published work (e.g., Chang *et al.* 2009a; Blakemore 2010a). However, the latter were occasionally cited for, and only for, the original information reported therein.

We followed the convention of using Roman numerals for segments for both external and internal characters, and Arabic numerals for intersegmental furrows (the external boundary between adjacent segments) for external characters. For instance, 'male pores paired in XVIII' indicates one pair of male pores on the body surface of segment 18; 'spermathecae three pairs in VI–VIII' indicates one pair of spermathecae inside each of segments six, seven, and eight; and 'spermathecal pores three pairs in 5/6/7/8' indicates one pair of spermathecal pores opened to the body surface between segments five and six, six and seven, and seven and eight. Photographs of internal characters presented in this study were all taken with a dorsal (in the cases of spermathecae and prostate glands) or dorsal lateral (in the case of intestinal caeca) view. All anatomical photographs were presented with the anterior body end to the top unless the annotated segment numbers in the figures indicate otherwise.

General morphology

In North America, the pheretimoid earthworms can easily be distinguished from other earthworm species by their numerous, evenly distributed setae in all segments except the first one, and by having an annular (as opposed to saddle-shaped) clitellum in XIV–XVI. Many people use the "crazy" jumping and snake-like moving behaviors to distinguish them. However, only some of the species have these behaviors when irritated, and earthworms that do not show these behaviors can still be one of the pheretimoid species.

The description in the next paragraph applies to both amphimictic and parthenogenetic pheretimoid species, but various degrees of degradation related to spermathecal pores, spermathecae, male pores, prostate glands, and their associated genital markings can be found in the latter, ranging from complete loss of all the structures to loss of some of the spermathecae/spermathecal pores or one of the two male pores. As such, species are often found in multiple places in the key (noted as 'in part'). The descriptions are not exhaustive, and apply only to species in North America north of Mexico. Descriptions are intended to provide enough information to help understand and recognize the group. However, we recognize that species identification of this group is never easy, as there will be irregular forms not fully covered by our figures, and structures may not be found due to parthenogenetic degradation or poor preservation conditions. Readers who are not familiar with the morphology of the pheretimoid earthworms should read Appendix A (Fig. 23) for guidance on what to expect when examining specimens before using this key. More details about the morphology and taxonomy of the pheretimoid earthworms can be found in Gates (1972), Sims and Easton (1972), and Easton (1979, 1982).

FIGURE 1. Color photographs of live individuals of (**A**) *Amynthas agrestis*, (**B**) *Amynthas corticis*, (**C**) *Amynthas gracilis*, (**D**) *Amynthas hupeiensis*, (**E**) *Amynthas minimus*, (**F**) *Amynthas morrisi*, (**G**) *Amynthas tokioensis*, (**H**) *Metaphire californica*, (**I**) *Metaphire hilgendorfi*, (**J**) *Metaphire posthuma*, (**K**) *Pithemera bicincta*, and (**L**) *Polypheretima elongata*, showing interspecific variations in colorations and body shapes. Specimens are from US (A, B, D, G, I) and Taiwan (C, E, F, H, J, K, L). Photo credit: Chih-Han Chang (A, B, D, E, G, H, I) and Wen-Jay Chih (C, F, J, K, L). The photographs of *A. minimus* and *M. californica* have been previously presented in Chang *et al.* (2009a).

The general morphology of the pheretimoid earthworms in North America is as follows: Colors of live specimens variable within and among species, ranging from brown, dark green and green to dark red, light red and pink, often with some iridescence (Fig. 1). Clitellum annular in XIV–XVI, intersegmental furrows absent. Setae numerous, forming a complete equatorial circle around each segment (perichaetine), beginning on II. Spermathecal pores 1–5 pairs in 4/5–8/9, ventral or lateral, occasionally dorsal (Fig. 2). Female pores single or closely paired in XIV, mid-ventral (Fig. 3). Male pores one pair in XVIII, ventral, superficial or in copulatory pouches. Spermathecae 1–5 pairs in V–IX. Testes two pairs in X, XI (holandric). Gizzard single in VIII–IX. Prostate glands one pair in XVIII, racemose, extending anteriorly and posteriorly through several segments. Intestinal caeca present or absent; when present, one pair, simple or manicate (hand-shaped, i.e., complex with several finger-like, deep incisions), originating from XXVII or XXII, usually extending anteriorly through 2–4 segments.

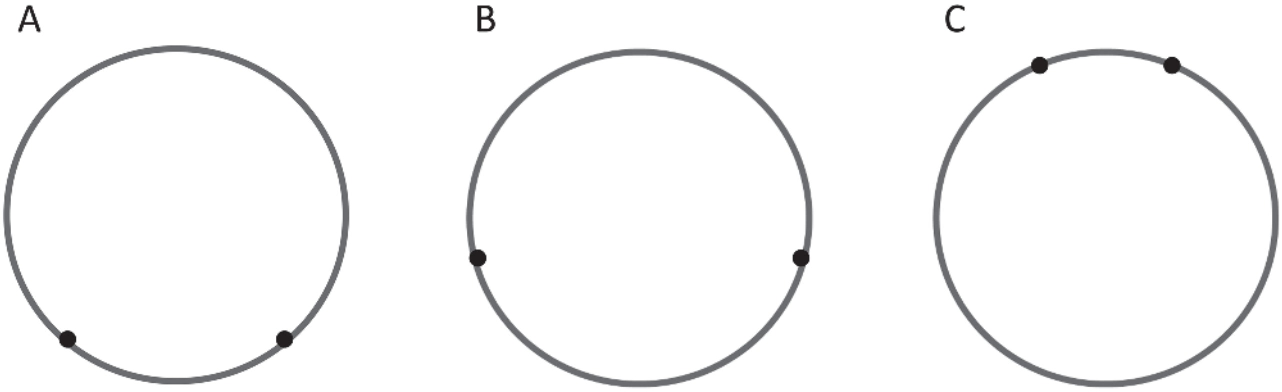

FIGURE 2. Schematic diagrams of cross sections of earthworms showing locations of spermathecal pores in intersegmental furrows. When spermathecal pores are present in a specimen, most pheretimoid earthworms in North America have spermathecal pores that are ventrally (**A**) or ventrolaterally (**B**) located. The only except is *Amynthas rodericensis*, whose spermathecal pores are located dorsally (**C**).

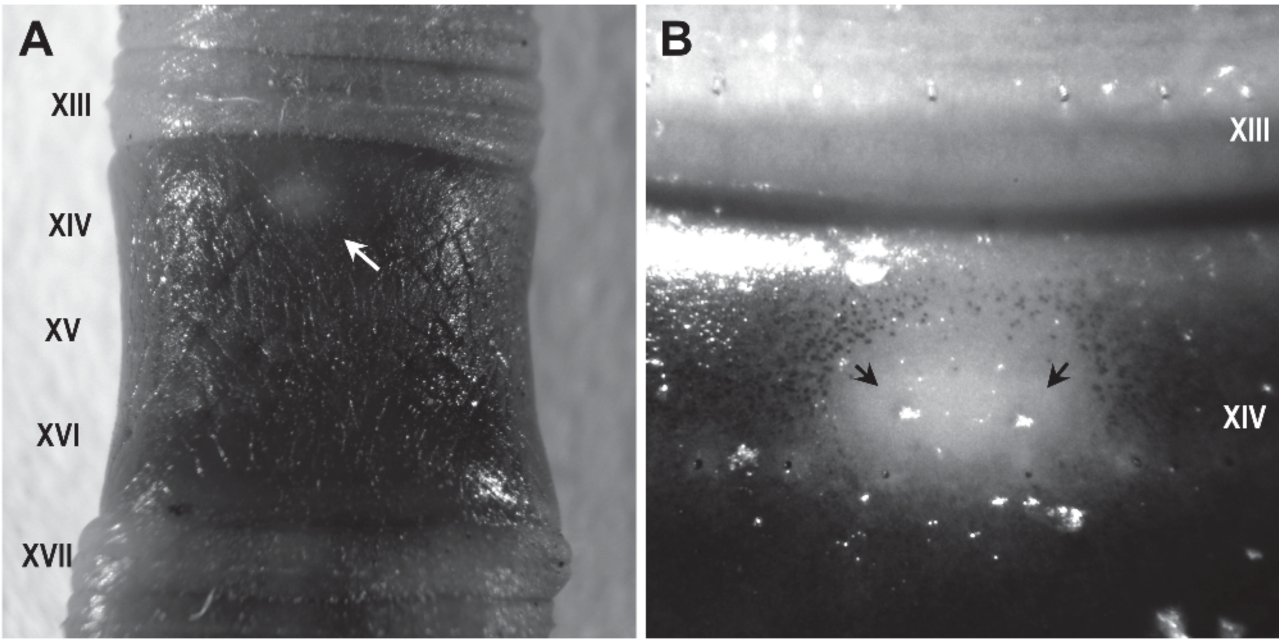

FIGURE 3. Female pores. (**A**) Female pore (arrow) on XIV on the ventral side of clitellum in the pheretimoid earthworms. (**B**) *Pithemera bicincta* is the only pheretimoid species in North America with two female pores (arrows).

Key to species in North America north of Mexico

This key is designed for species and specific morphs recorded in North America north of Mexico. While our goal is to make it possible to use only external morphology for species identification, confirming the identity of specimens will require a dissection, especially when the numbers and positions of spermathecal pores cannot be unambiguously determined. We strongly recommend that readers confirm their species identifications by dissecting several specimens of each species they identify and by comparing both the external and internal morphologies with the diagnoses and photographs of the respective species listed after the key. A closer look at genital markings, the shape and positions of spermathecae, and the shape of intestinal caeca may be especially useful.

1a. Size of mature (clitellate) specimens 20–50 mm by 1.5–2.0 mm; both male pores present, large, simple, superficial; color of live specimens light red to reddish white .... ***Amynthas minimus***

1b. Size of mature (clitellate) specimens >50 mm, or <50 mm but wider than 2.0 mm 2

2a. Female pores closely paired (Fig. 3B); spermathecal pores five pairs in 4/5/6/7/8/9; intestinal caeca in XXII .... ***Pithemera bicincta***

2b. Female pore single; spermathecal pores four pairs or fewer; intestinal caeca in XXVII or absent 3

3a. Male pores absent or a single pore present 4

3b. Both male pores present with large, widely paired genital markings on XVII and XIX; 17/18 and/or 18/19; or XIX−XX, XXI, XXII, XXIII, XIV, slightly median to male pores (Fig. 4) 5

3c. Both male pores present in invaginations/copulatory pouches with no apparent genital markings (Fig. 5) 6

3d. Both male pores present, superficial; genital markings present or absent, when present, not arranged as in 3b (Fig. 6) 7

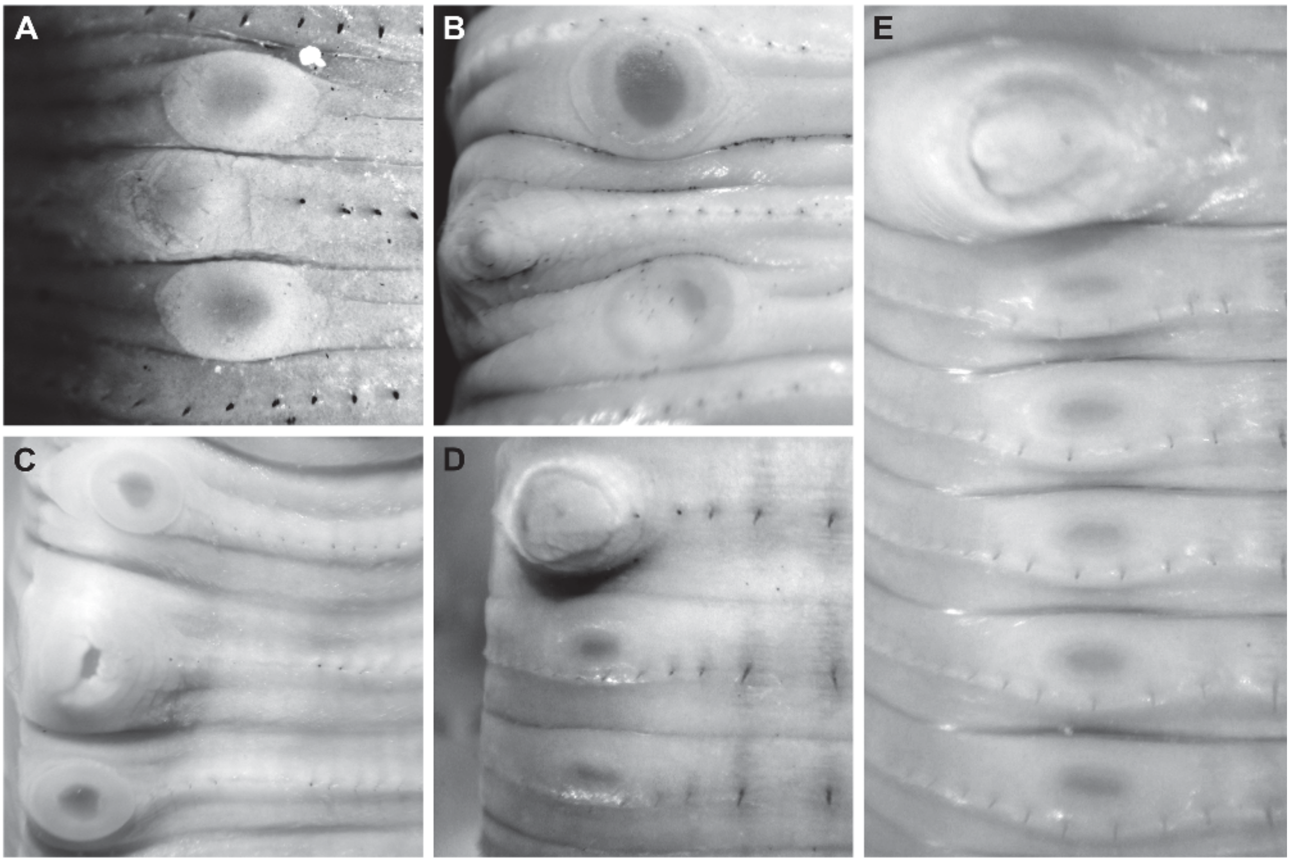

FIGURE 4. Male pores with large genital markings in some or all of XVII–XXIV. (**A**) *Amynthas hupeiensis*. (**B**) *Amynthas rodericensis*. (**C**) *Metaphire posthuma*. (**D**, **E**) *Polypheretima elongata*.

4a. Spermathecal pores three pairs with or without finely wrinkled or crosshatched epidermal modification on VII and/or VIII (Fig. 7B–D) .... ***Amynthas agrestis*** (in part)

4b. Spermathecal pores two pairs or fewer with mid-ventral, pre-setal clusters of small tubercles in some or all of VIII–IX (Fig. 18D, E) .... ***Metaphire hilgendorfi*** (in part)

4c. Spermathecal pores two pairs or fewer with small discs adjacent to the pores (Fig. 16B) .... ***Amynthas tokioensis*** (in part)

4d. Spermathecal pores two pairs or fewer with no genital markings .... ***Amynthas* sp.**, not further identifiable, possibly *A. agrestis*, *A. tokioensis* or *M. hilgendorfi*

5a. Paired genital markings on 17/18 and 18/19; color green (Figs. 1D, 11A); spermathecal pores three pairs on the anterior margins of VII–IX .... ***Amynthas hupeiensis***

5b. Paired genital markings on 17/18 and/or 18/19; spermathecal pores dorsal, four pairs in 5/6/7/8/9 (Figs. 2C, 15A, B, D) .... ***Amynthas rodericensis*** (in part)

5c. Paired genital markings on setal line on XVII and XIX; male pores in invaginations (Fig. 20A); color brown .... ***Metaphire posthuma***

5d. Paired pre-setal genital markings on some or all of XIX–XXI (Fig. 22A, B); color light grey with pink or red anterior .... ***Polypheretima elongata***

6a. Spermathecal pores two pairs in 7/8/9 with no pre-clitellar genital markings; first dorsal pores in 11/12 or 12/13; opening of copulatory pouches transversely slit-like with notched edges (Fig. 17A–C); color red .... ***Metaphire californica***

6b. Spermathecal pores three pairs or fewer in 6/7/8/9, sometimes with genital markings next to the pores; first dorsal pore in 9/10 or 10/11; opening of copulatory pouches C-shaped (Fig. 19A, B) .... ***Metaphire houlleti***

7a. Spermathecal pores dorsal, four pairs in 5/6/7/8/9 (Figs. 2C, 15D) .... ***Amynthas rodericensis*** (in part)

7b. Spermathecal pores four pairs in 5/6/7/8/9; post-clitellar genital markings 0–3 around each male porophore, confined within concentric circular folds; prostate gland vestigial or absent (Fig. 9A–D, F) .... ***Amynthas corticis***

7c. Spermathecal pores four pairs in 5/6/7/8/9; post-clitellar genital markings 0–3 on each side, not confined by concentric circular folds around the male pore, paired or unilateral, pre-setal and post-setal on XVIII, mid-ventral or slightly median to male pores; prostate gland well-developed .... ***Amynthas carnosus*** (in part)

7d. Spermathecal pores three pairs or fewer **8**

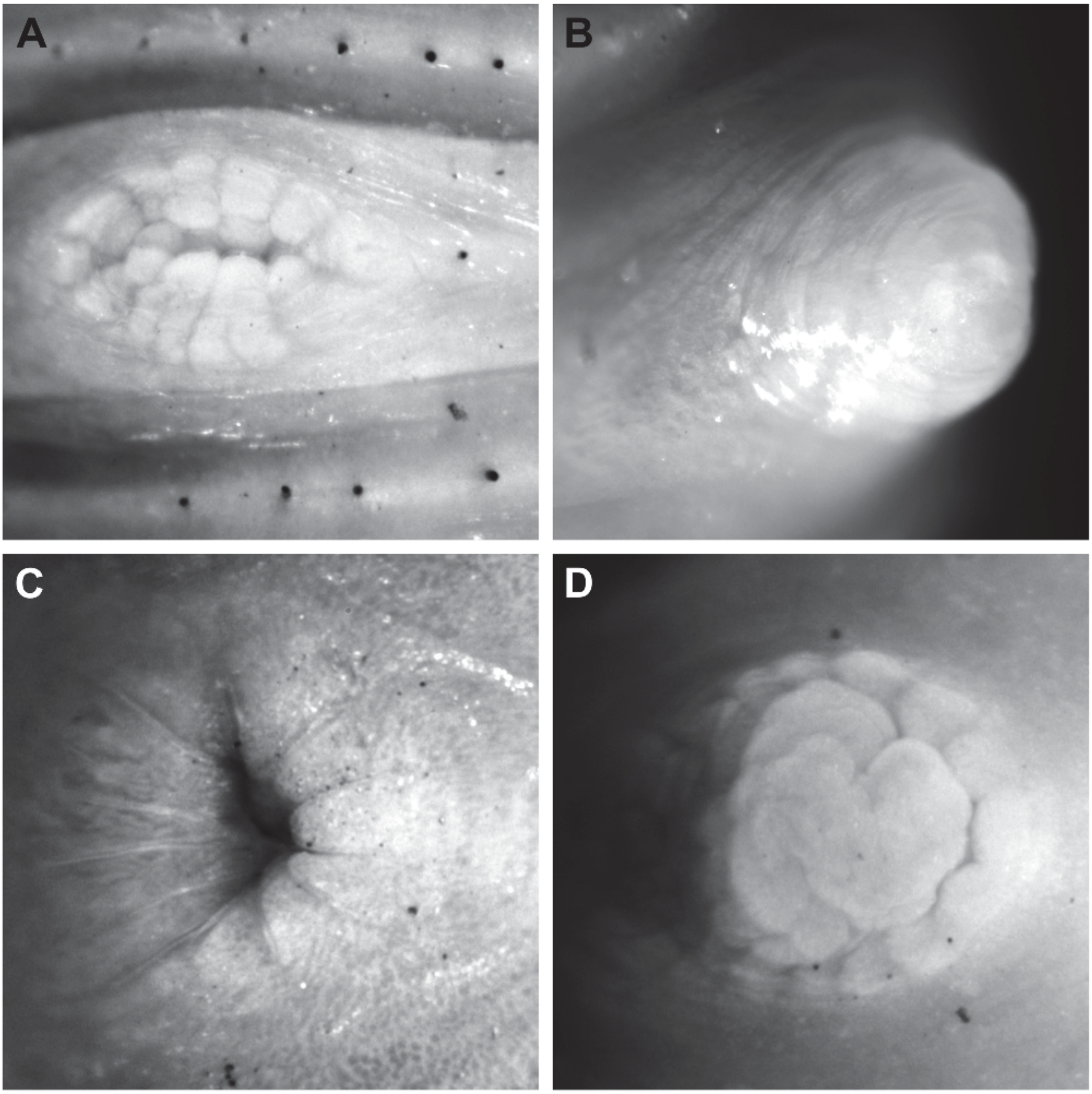

FIGURE 5. Male pores in invaginations/copulatory pouches with no apparent genital markings. (**A**, **B**) *Metaphire californica*, showing copulatory pouches not everted (A) or everted (B). (**C**, **D**) *Metaphire houlleti*, showing copulatory pouches not everted (C) or everted (D).

8a. Spermathecal pores three pairs in 5/6/7/8; post-clitellar genital markings present in XVIII, paired post-setal small discs median to male pores in tight clusters of 1–11 (Fig. 10A, B); color red or light red . . . . ***Amynthas gracilis***

8b. Spermathecal pores three pairs in 6/7/8/9; post-clitellar genital markings 0–3 on each side, not confined by concentric circular folds around the male pore, paired or unilateral, pre-setal and post-setal on XVIII, mid-ventral or slightly median to male pores (Fig. 8A, B) . . . . ***Amynthas carnosus*** (in part)

8c. Spermathecal pores two pairs or fewer in 6/7/8; male pore with 1–3 papillae, with one of them pre-setal median to the pore; intestinal caeca manicate (Fig. 16A, E) . . . . ***Amynthas tokioensis*** (in part)

8d. Spermathecal pores two pairs in 5/6/7; intestinal caeca simple **9**

9a. Each spermathecal pore associated with two papillae, one immediately anterior to and one immediately posterior to each spermathecal pore, with the posterior papillae more median to the pore; post-clitellar genital markings absent outside the male pore area surrounded by circular folds (usually two papillae present within the area; Fig. 12A–C) . . . . ***Amynthas loveridgei***

9b. Pre-clitellar genital markings present, highly variable but never like those in 9a; post-ciltellar genital markings absent or present outside the male pore area surrounded by circular folds (usually two papillae present within the area), when present, pre-setal on XVIII and/or XIX (Fig. 14A–H) . . . . ***Amynthas morrisi***

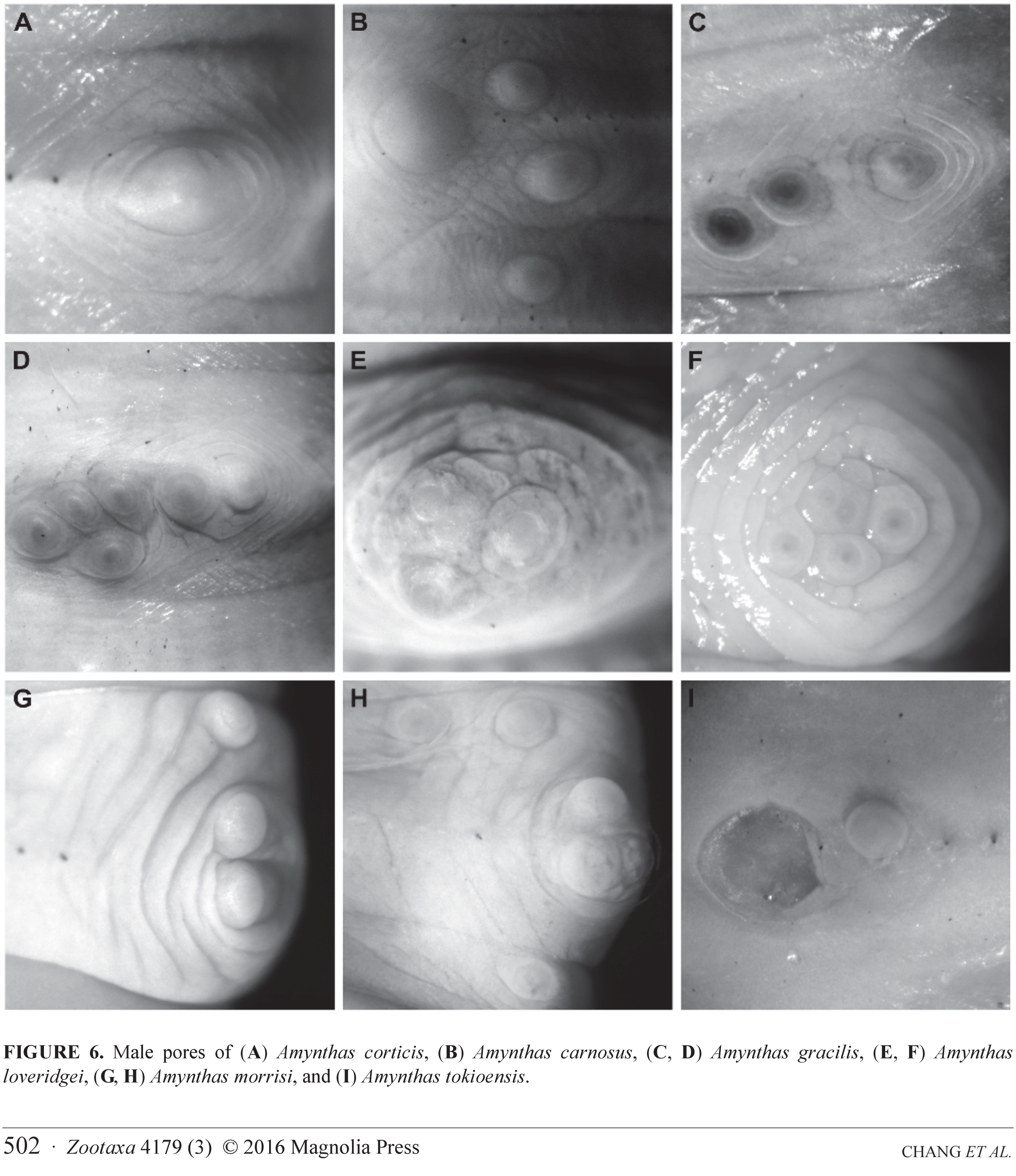

FIGURE 6. Male pores of (**A**) *Amynthas corticis*, (**B**) *Amynthas carnosus*, (**C**, **D**) *Amynthas gracilis*, (**E**, **F**) *Amynthas loveridgei*, (**G**, **H**) *Amynthas morrisi*, and (**I**) *Amynthas tokioensis*.

Species diagnoses and remarks

1. *Amynthas agrestis* (Goto & Hatai, 1899)

(Figures 1A, 7; Table 1)

Perichaeta agrestis Goto & Hatai, 1899: 17, 24.
Pheretima agrestis—Howell 1939: 231. Gates 1953: 5; 1954: 224; 1958: 1, 31; 1963: 11; 1982: 38.
Amynthas agrestis—Sims & Easton 1972: 235. Reynolds 1978: 119, 127; 2010: 143; 2011: 269. Reynolds & Wetzel 2004: 88; 2008: 179. Blakemore 2010a: 429; 2013b: 56, 57.
Metaphire agrestis—Blakemore 2003: 7, 28.

Data sources. Goto & Hatai (1899); Gates (1953, 1954, 1982); Blakemore (2010a, 2013b); this study (USNM 1421431).

Diagnosis. Size 70–160 mm by 5–8 mm. Segment numbers 63–110. Color of live specimens red. Male pores usually absent; when present, small, transversely slit-like. Post-clitellar genital markings usually absent; when present, single, large circular pad, pre-setal on XVIII, just median to male pores, with a concave center surrounded by a narrow but distinct, raised rim, reaching posteriorly slightly behind the setal line on XVIII and anteriorly to the setal line on XVII. Spermathecal pores three pairs in 5/6/7/8 or variously missing. Pre-clitellar genital markings present or absent; when present, ventral, areas of slight epidermal modification on VII and/or VIII, occasionally on VI and IX, unpaired and median or symmetrically paired, forming setal gaps, epidermis finely wrinkled or crosshatched, sometimes darker in color in live specimens. Female pore single in XIV. First dorsal pore 12/13. Spermathecae present or absent; when fully present, three pairs in VI–VIII, duct shorter than ampulla; diverticulum longer than duct and ampulla combined. Prostate glands present or absent; when present, extending through some or all of XVI–XXIII, ducts in XVIII. Intestinal caeca paired in XXVII, manicate.

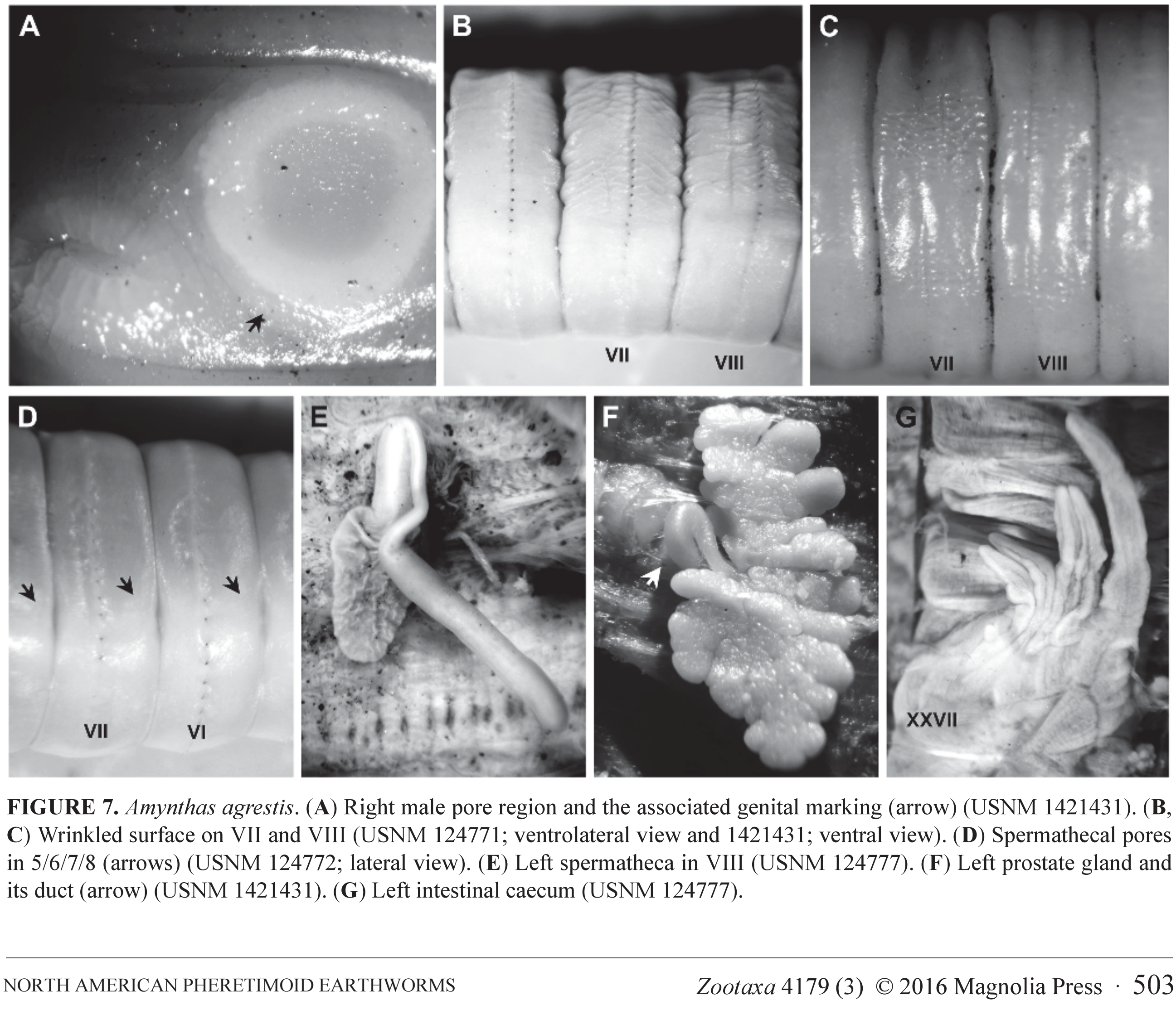

FIGURE 7. *Amynthas agrestis*. (**A**) Right male pore region and the associated genital marking (arrow) (USNM 1421431). (**B**, **C**) Wrinkled surface on VII and VIII (USNM 124771; ventrolateral view and 1421431; ventral view). (**D**) Spermathecal pores in 5/6/7/8 (arrows) (USNM 124772; lateral view). (**E**) Left spermatheca in VIII (USNM 124777). (**F**) Left prostate gland and its duct (arrow) (USNM 1421431). (**G**) Left intestinal caecum (USNM 124777).

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

	Amynthas agrestis	***Amynthas tokioensis***	***Metaphire hilgendorfi***
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 symmatrically 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
Intestinal caeca	Manicate	Manicate	Manicate

* 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.

Remarks. *Amynthas agrestis* has been frequently reported in the continental US, and is one of the two pheretimoid species recorded in Canada. The first record of this species in the continental US was in 1939 from the Homewood campus of the Johns Hopkins University, Baltimore, Maryland (Howell 1939; Gates 1954; 1982). That record was the second of *A. agrestis* outside Japan, where the species is native. It has recently been confirmed to be abundant in Baltimore and has been observed to co-occur frequently with *M. hilgendorfi* and/or *A. tokioensis* in Maryland, Connecticut, Vermont, New Hampshire, and Wisconsin (C.-H. Chang, personal observation), two species morphologically similar to *A. agrestis* (Table 1). This latter observation suggests the high possibility of misidentification. Reproduction of *A. agrestis* in North America is parthenogenetic. In field conditions, *A. agrestis*, an annual species, overwinters only as cocoons and the adults reproduce in summer and die by the end of fall (Callaham *et al.* 2003; Richardson *et al.* 2009; Görres *et al.* 2014). However, in the laboratory, its adults can survive through November–February (Snyder *et al.* 2013; Ikeda *et al.* 2015). *Amynthas agrestis* is epi-endogeic, and its successful invasion in US forests has been attributed to dietary flexibility (Zhang *et al.* 2010). It has been known to compete with native millipedes in southeastern US for food resources, particularly the fragmented, partially decomposed leaf litter (Snyder *et al.* 2011; 2013). Current practice of using commercial mulch for horticulture and landscaping may help spreading of this invasive species (Belliturk *et al.* 2015).

For years, what the male pores and the associated genital markings of *A. agrestis* look like has remained an unanswered question until our study. Male pores are almost always absent in *A. agrestis*. They were rarely described, and have never been illustrated before. Blakemore (2010a) illustrated the male pores of a Japanese specimen that was believed to be *A. agrestis*. However, later he questioned his earlier conclusion and noted that the previously reported specimen may belong to a different species (Blakemore 2014). Gates (1982) apparently saw one *A. agrestis* specimen with a male pore on one side, as his detailed description of the male pore and genital marking matches our Figure 5A. However, his observation was confounded by having two specimens with different male pores and he even questioned whether he had two species.

2. *Amynthas carnosus* (Goto & Hatai, 1899)

(Figure 8)

Perichaeta carnosa Goto & Haitai, 1899: 15.
Pheretima carnosa—Kobayashi 1936: 115.
Amynthas carnosus—Sims & Easton 1972: 235. Blakemore 2012a: 36; 2013b: 58; 2013c: 101. Carrera-Martínez & Snyder 2016: 297.
Amynthas pingi (Stephenson, 1925)—Sims & Easton 1972: 235. Blakemore 2013c: 112.

Data sources. Blakemore (2012a, 2013b, c); Carrera-Martínez & Snyder (2016).

Diagnosis. Size 110–340 mm by 4–9 mm. Segment numbers 106–179. Color of live specimens dark brown or purple. Male pores paired in XVIII, simple, on round or elliptical porophores. Post-clitellar genital markings paired, pre-setal on XVIII and XIX, mid-ventral or widely spaced; post-setal on XVIII, slightly median to male pores; markings variously lacking or completely absent. Spermathecal pores four pairs in 5/6/7/8/9 or three pairs in 6/7/8/9. Pre-clitellar genital markings paired on VIII and IX, pre-setal, mid-ventral or widely spaced, variously lacking. Female pore single in XIV. First dorsal pore 12/13. Spermathecae four pairs in VI–IX or three pairs in VII–IX, ampulla oval or pear-shaped, duct equal to or slightly shorter than ampulla, diverticulum reaching one-third to half of ampulla with a slender stalk and a wider seminal chamber. Prostate glands well-developed, extending through XVII–XIX. Intestinal caeca paired in XXVII, simple, extending anteriorly to XXIII or XXIV.

Remarks. This species is newly recorded in North America in 2015 near Manhattan in Kansas (Carrera-Martínez & Snyder 2016), and therefore has never been included in any previous US pheretimoid earthworm keys (Reynolds 1978; Gates 1982). *Amynthas carnosus* has only been recorded from Japan, Korea and China, and a previous report from Taiwan has been confirmed to belong to a different species (Blakemore 2012a; 2013c). Reproduction of *A. carnosus* is presumably amphimictic.

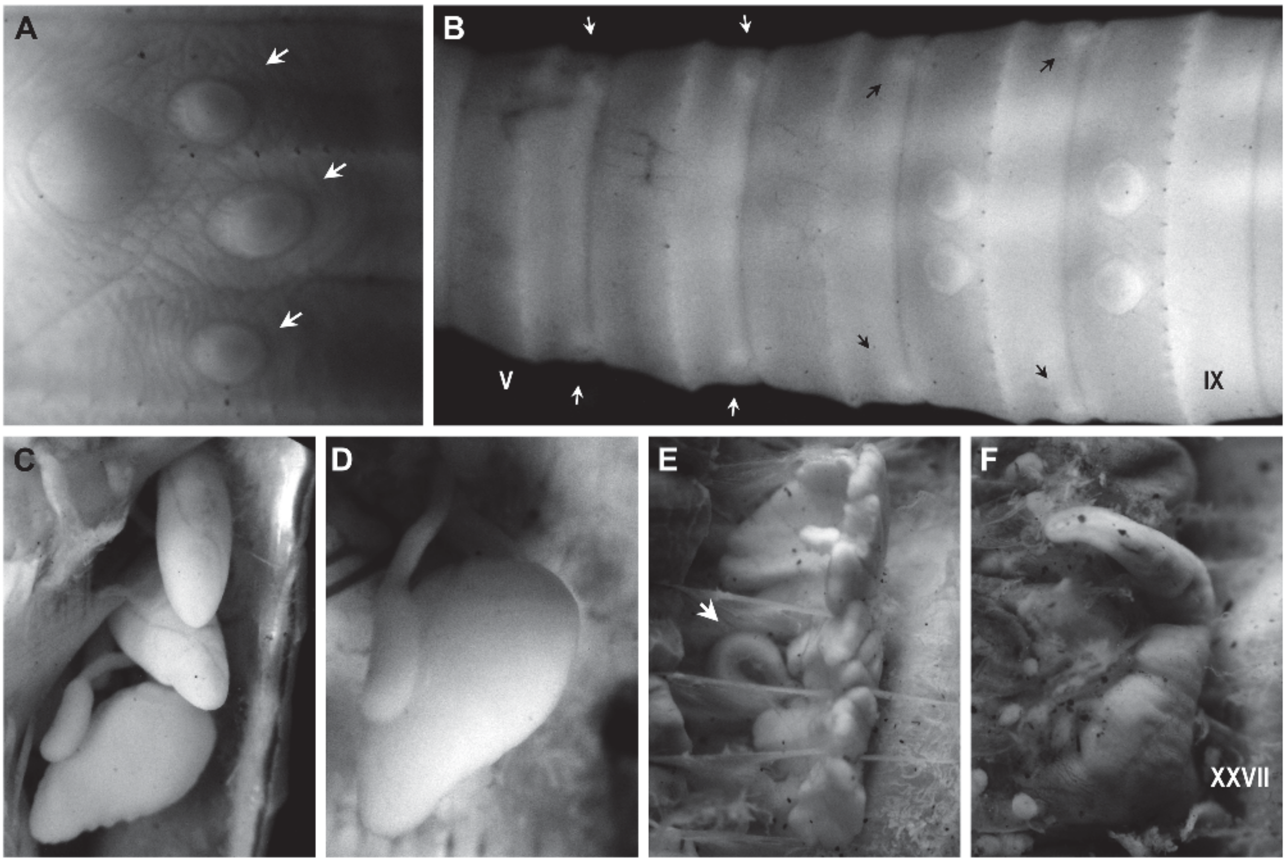

FIGURE 8. *Amynthas carnosus*. (**A**) Right male pore region and the associated genital markings (arrows). (**B**) Pre-clitellar genital markings paired mid-ventral on VIII and IX and eight spermathecal pores right behind eight genital markings (arrows). (**C**) Right spermathecae in VII–IX. Only the spermathecal diverticulum in IX is visible. (**D**) Right spermatheca in IX. (**E**) Right prostate gland and its duct (arrow). (**F**) Right intestinal caecum.

3. *Amynthas corticis* (Kinberg, 1867)
(Figures 1B, 9)

Perichaeta corticis Kinberg, 1867: 102.
Pheretima diffringens (Baird, 1869)—Gates 1937: 350; 1954: 227; 1958: 31; 1963: 12; 1982: 44.
Amynthas corticus—Sims & Easton 1972: 235. Easton 1981: 49.
Amynthas diffringens—Sims & Easton 1972: 235. Reynolds 1978: 120, 127; 2010: 144; 2011: 270. Reynolds & Wetzel 2004: 88; 2008: 179.

Data sources. Gates (1937, 1972, 1982); Blakemore (2013a).

Diagnosis. Size 45–170 mm by 3–6 mm. Segment numbers 79–121. Color of live specimens greenish brown. Male pores paired in XVIII, simple, on circular to oval porophores. Post-clitellar genital markings present or absent, when present, 1–3, small, around each male porophore, confined within concentric circular folds. Spermathecal pores four pairs in 5/6/7/8/9. Pre-clitellar genital markings variously present, pre-setal and post-setal; the pre-setal ones widely paired or unpaired on VII–X, behind and median to spermathecal pores; the post-setal ones just in front of each spermathecal pore. Female pore single in XIV. First dorsal pore 10/11, 11/12, or 12/13. Spermathecae four pairs in VI–IX, duct shorter than ampulla, diverticulum with an oval seminar chamber and a longer, slender stalk; stalked glands associated with external genital markings. Prostate glands absent, present, or rudimentary, ducts usually present. Intestinal caeca paired in XXVII, simple, extending anteriorly to XXII.

Remarks. Historically referred to as *Pheretima diffringens* or *Amynthas diffringens*, *A. corticis* has been frequently reported in the US. The name *A. diffringens* has been regarded as a junior synonym of *A. corticis* for a long time (Easton 1981; Blakemore 2003; 2010a; Chang *et al.* 2009a); recent resurrection of *A. diffringens* by

Blakemore (2013c) based on morphology of syntype specimens is still pending confirmation or rejection by molecular studies. The first record of *A. corticis* in the continental US was in 1866 in San Francisco, California, although the specimen was originally misidentified as part of "*Pheretima californica*" (Gates 1937, 1954, 1982). Reproduction of *A. corticis* is usually parthenogenetic. *Amynthas corticis*, an epi-endogeic species, competes directly with native millipedes for food resources in the southern Appalachian Mountains (Snyder *et al.* 2009).

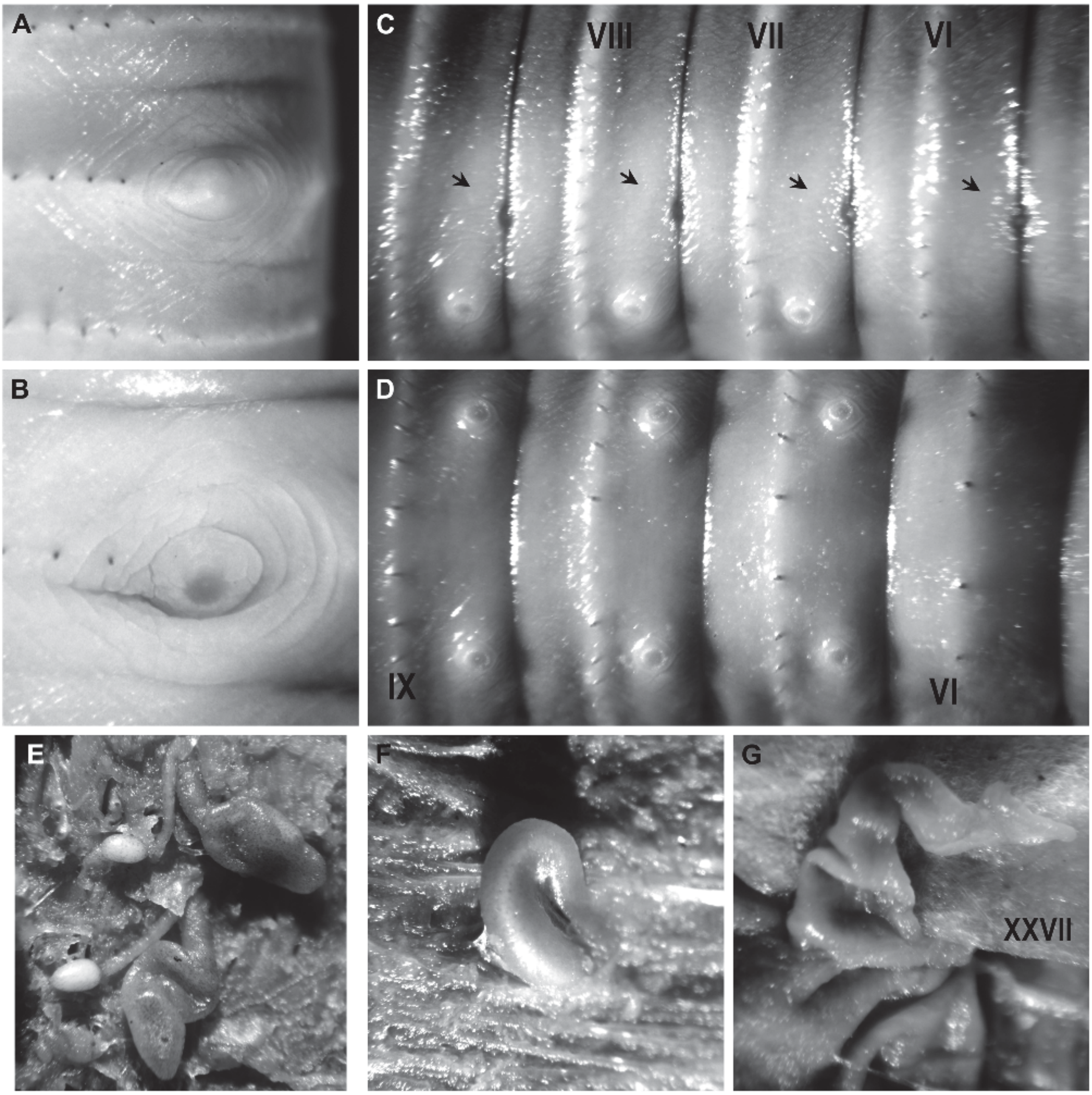

FIGURE 9. *Amynthas corticis*. (**A**, **B**) Left male pores (USNM 124810 and 124880). (**C**) Four spermathecal pores in 5/6/7/8/9 (arrows) and genital markings in VII–IX; lateral view. (**D**) Paired genital markings on VII–IX; ventral view. (**E**) Right spermathecae in VI and VII. (**F**) Right prostate duct, showing absence of prostate glands. (**G**) Right intestinal caecum. C–G from USNM 124947.

4. *Amynthas gracilis* (Kinberg, 1867)

(Figures 1C, 10)

Nitocris gracilis Kinberg, 1867: 102.
Pheretima hawayana (Rosa, 1891)—Gates 1937: 354; 1954: 229; 1958: 31; 1963: 13; 1982: 47.

Amynthas gracilis—Sims & Easton 1972: 235.

Amynthas hawayanus—Sims & Easton 1972: 235. Reynolds 1978: 121, 127; 2010: 145; 2011: 271. Reynolds & Wetzel 2004: 88; 2008: 179.

Data sources. Gates (1937, 1972, 1982); Blakemore (2013a).

Diagnosis. Size 56–156 mm by 3–6 mm. Segment numbers 70–101. Color of live specimens red. Male pores paired in XVIII, small, superficial, on a small porophore. Post-clitellar genital markings present or absent; when present, paired post-setal small discs median to male pores, in tight clusters of 1–11. Spermathecal pores three pairs in 5/6/7/8. Pre-clitellar genital markings present or absent; when present, paired post-setal small discs median to spermathecal pores on some of VI–IX. Female pore single in XIV. First dorsal pore 10/11 or 11/12. Spermathecae three pairs in VI–VIII, duct slender, as long as or shorter than ampulla, with a tubular diverticulum shorter than the main axis. Prostate glands well developed, extending through some or all of XVI–XXIV. Intestinal caeca paired in XXVII, simple, extending anteriorly to XXIV.

Remarks. Historically referred to as *Pheretima hawayana* or *Amynthas hawayanus*, *A. gracilis* has been frequently reported in the US. The first record of this species in the continental US was in 1905 in Illinois (Gates 1937; 1954; 1982). Reproduction of *A. gracilis* is amphimictic. *A. gracilis*, an epi-endogeic species, has been suggested to increase C and N flux in forest soils in northeastern US and may have potential long-term impacts on nutrient cycling (Burtelow *et al.* 1998).

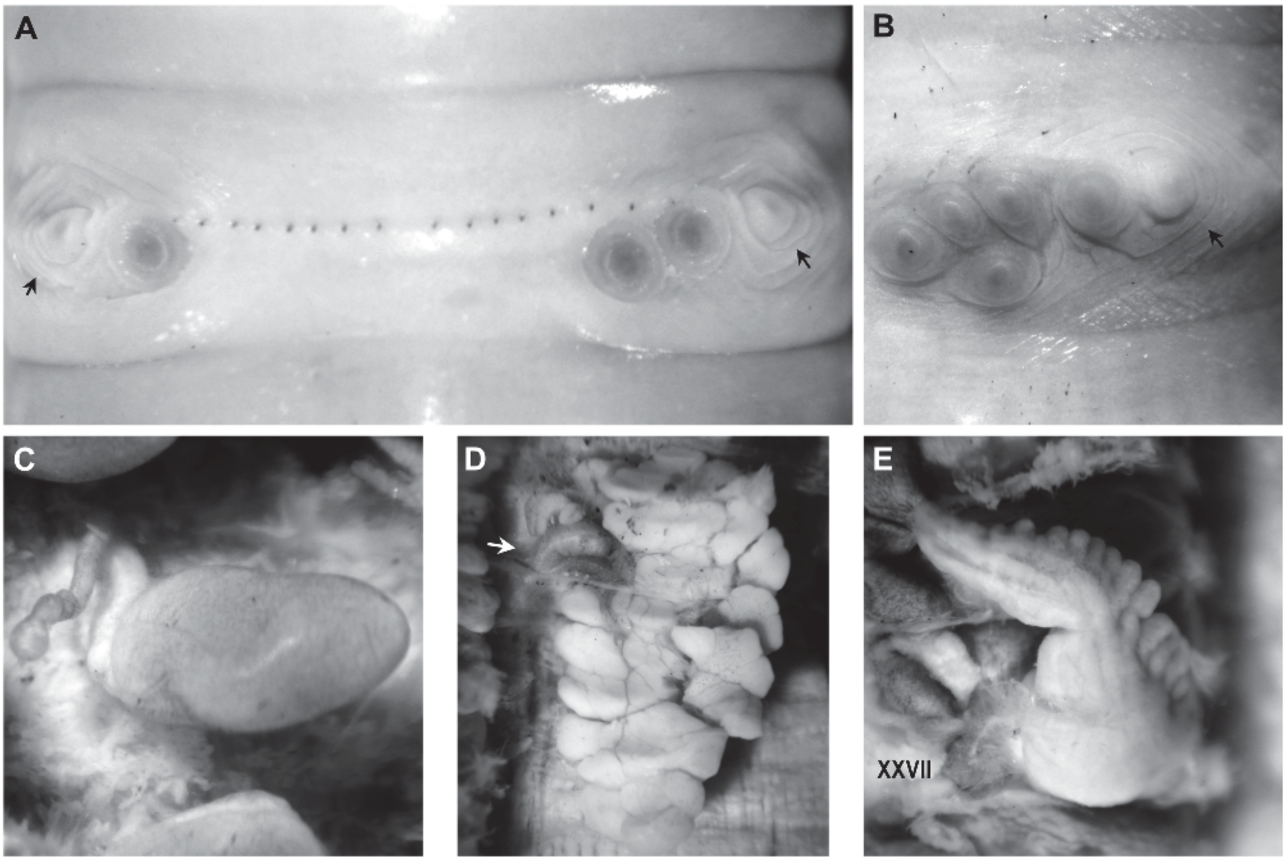

FIGURE 10. *Amynthas gracilis*. (**A**) Both male pores (arrows) and the associated genital markings (USNM 125011). (**B**) Left male pore (arrow) and the associated genital markings (USNM 125019). (**C**) Right spermatheca in VII. (**D**) Right prostate gland and its duct (arrow). (**E**) Right intestinal caecum. C–E from USNM 125021.

5. *Amynthas hupeiensis* (Michaelsen, 1895)

(Figures 1D, 11)

Perichaeta hupeiensis Michaelsen, 1895: 35.

Pheretima hupeiensis—Gates 1937: 356; 1954: 234; 1958: 17, 31; 1963: 13; 1982: 52.

Amynthas hupeiensis—Sims & Easton 1972: 237. Reynolds 1978: 123, 127; 2010: 147; 2011: 273. Reynolds & Wetzel 2004: 88; 2008: 179.

Data sources. Gates (1937, 1954, 1958, 1982).

Diagnosis. Size 40–220 mm by 3–6 mm. Segment numbers 97–138. Color of live specimens green. Male pores paired in XVIII, on a small circular porophore. Post-clitellar genital markings two pairs, one on 17/18 and one on 18/19, slightly median to the male pores. Spermathecal pores three pairs on the anterior margin of VII–IX. Pre-clitellar genital markings absent. Female pore single in XIV. First dorsal pore 11/12 or 12/13. Spermathecae three pairs in VII–IX, duct shorter than ampulla; diverticulum longer than main axis, with a stalk shorter than the elongate tubular seminal chamber. Prostate glands paired, extending anteriorly to XVI, XVII and posteriorly to XIX, XX. Intestinal caeca paired in XVII, simple, extending anteriorly to XXIV.

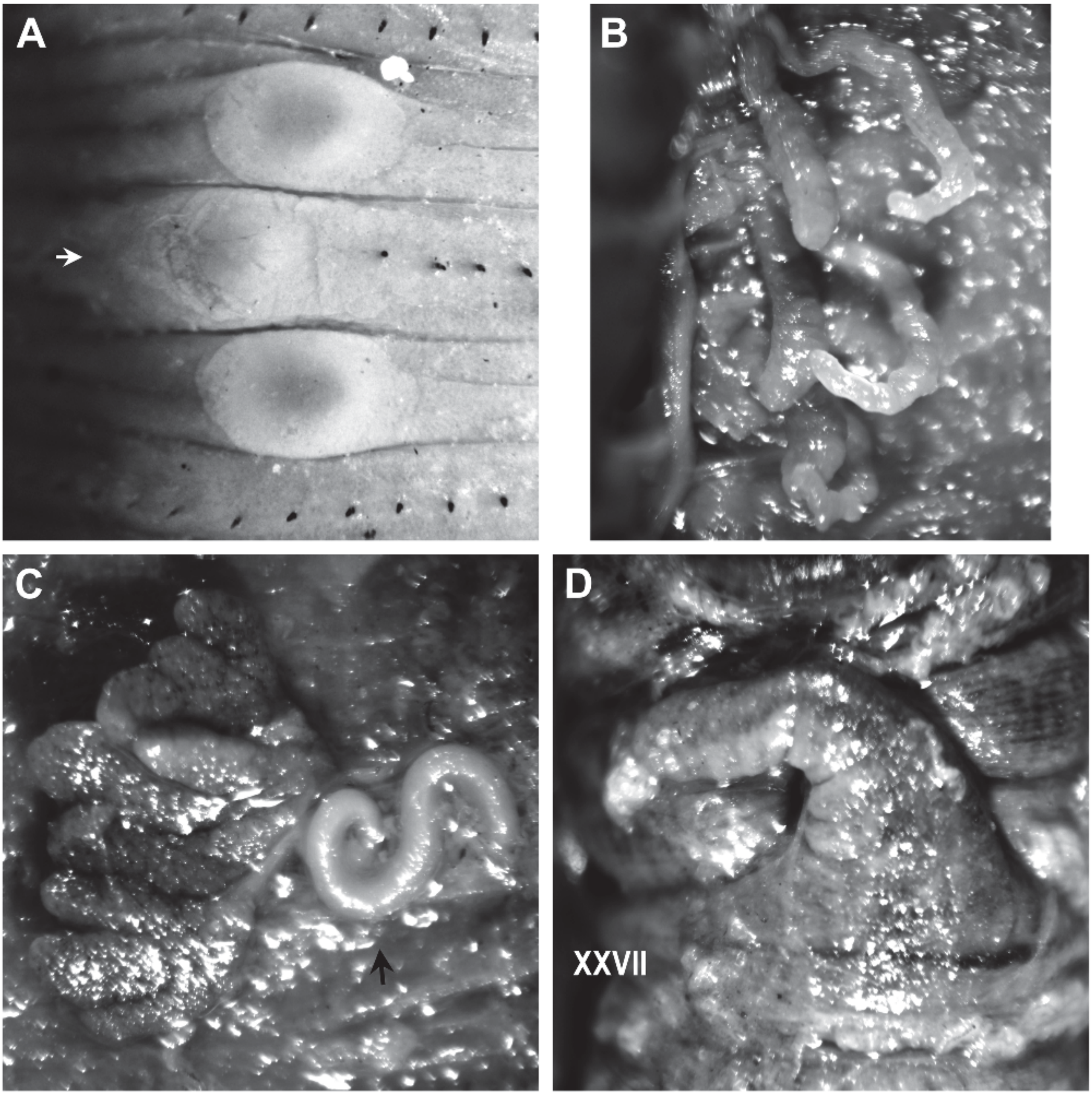

FIGURE 11. *Amynthas hupeiensis*. (**A**) Right male pores (arrow) and the associated genital markings. (**B**) Right spermathecae in VII–IX. (**C**) Left prostate gland and its duct (arrow). (**D**) Left intestinal caecum. All from USNM 125079.

Remarks. Frequently reported in North America, *A. hupeiensis* was first recorded from specimens collected in 1910 in Washington, D.C. While this species probably originated from China, Gates (1982) believed that it came to the US from Japan with the flowering cherry trees currently featured in the "Cherry Blossom Festival" in the nation's capital. All American specimens reported so far are parthenogenetic, but some specimens from other parts of the world were claimed to be amphimictic (Gates 1982). *Amynthas hupeiensis* prefers sandy soils and can be found along sandy riverbanks (Szlavecz *et al.* 2014). The density of this species can reach 110 individuals/m^2, and its casts have caused problems in golf courses (Gates 1982; Redmond *et al.* 2014), where the species is considered a pest.

6. *Amynthas loveridgei* (Gates, 1968)

(Figure 12)

Pheretima loveridgei Gates, 1968: 257; 1982: 57.

Amynthas loveridgei—Sims & Easton 1972: 236. Reynolds 1978: 127; 2011: 274. Reynolds & Wetzel 2004: 88; 2008: 180. Blakemore 2014: 129, 130.

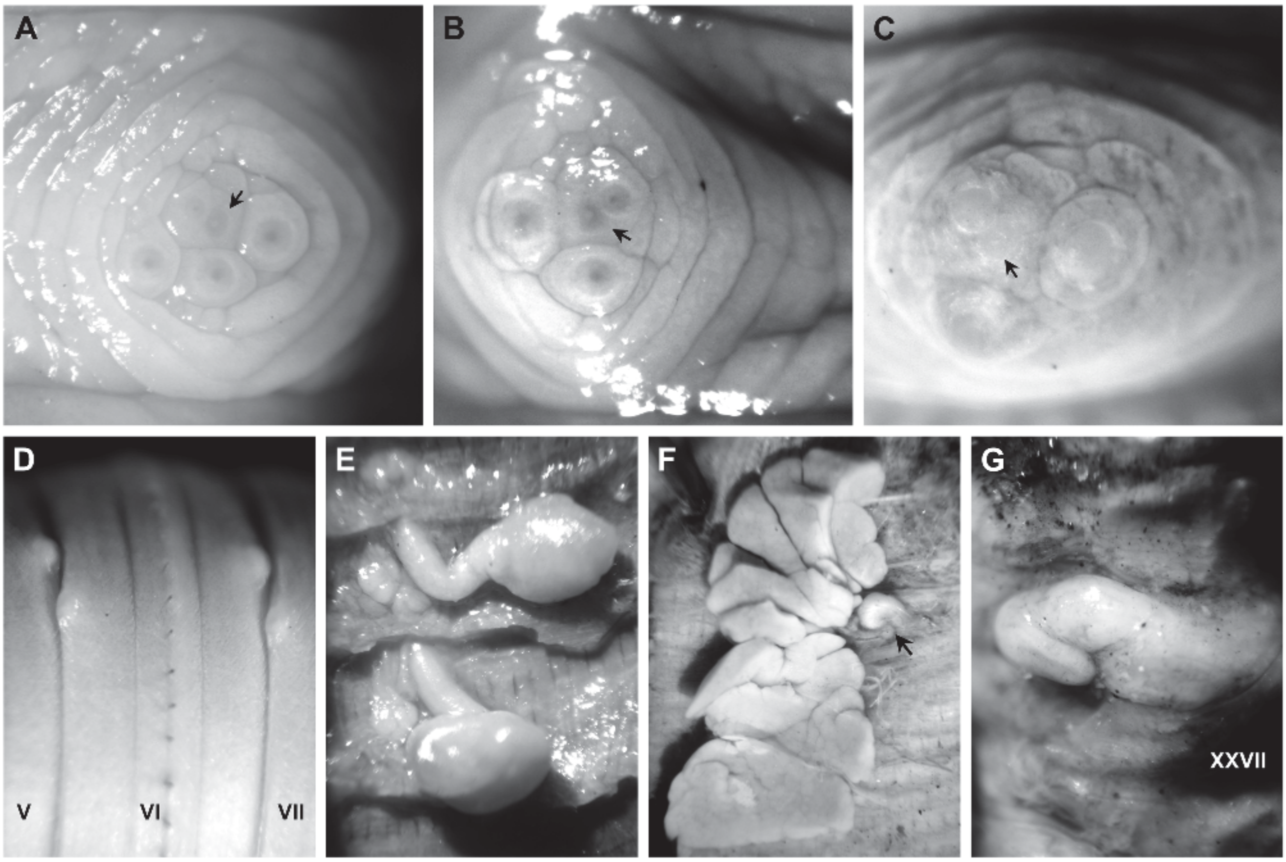

FIGURE 12. *Amynthas loveridgei*. (**A**) Left male pore and the associated genital markings, showing four papillae and the primary male pore (arrow) at the middle (USNM 125056). (**B**) Right male pore and the associated genital markings, showing three papillae and the primary male pore (arrow) at the middle (USNM 125056, a different specimen from that in A). (**C**) Left male pore and the associated genital markings. The primary male pore (arrow) can barely be seen at the middle of three papillae. (**D**) Two sets of genital markings associated with the two left spermathecal pores (not seen); ventrolateral view. (**E**) Right spermathecae in VI and VII. (**F**) Left prostate gland and its duct (arrow). (**G**) Left intestinal caecum. C–G from one of the syntypes (USNM 136910).

Data sources. Gates (1968, 1982); Blakemore (2014); this study (USNM 136910 (syntypes), 125052, 125056).

Diagnosis. Size 90–113 mm by 4–6 mm. Segment numbers 118–169. Color of live specimens unknown. Male pores paired in XVIII, sitting near the lower edge on a pre-setal genital marking. Post-clitellar genital markings 3–6 papillae or tubercles on each side on XVIII; typically three, arranged in a triangle (one lateral, one pre-setal, and one post-setal), often the anterior-most marking extends posteriorly to contain the primary male pore. All markings surrounded by deep, concentric furrows. Spermathecal pores two pairs in 5/6/7. Pre-clitellar genital markings present, one in front of and one behind each spermathecal pore, the latter more median to the pore. Female pore single in XIV. First dorsal pore 10/11 or 11/12. Spermathecae two pairs in VI–VII, variable in size, duct slender, as long as ampulla; diverticulum small or degenerated, usually shorter than the respective spermatheca, with a slender stalk and an ellipsoidal to pear-shaped seminal chamber. Prostate glands absent or well developed; when present, large, extending from XVI, XVII to XXIII, XXIV. Intestinal caeca paired in XXVII, simple, variable in size, extending anteriorly as far as XXIII.

Remarks. One of the two pheretimoid species originally described from the US, *A. loveridgei* was first recorded in 1966 (Gates 1968) in Greenville, Madison County, Florida. In the US this species has been reported so far only in Georgia, Florida, and Minnesota. Reproduction of *A. loveridgei* is parthenogenetic. It can be easily confused with *A. morrisi*. Gates separated the two species mainly by their pre-clitellar genital markings and segment numbers. In fact, the specimens of *A. loveridgei* almost always look more slender than those of *A. morrisi*, and the two species can further be distinguished by the locations of male pores with respect to the adjacent genital markings. The syntypes in the Smithsonian Institution National Museum of Natural History (USNM 136910) and the British Museum of Natural History have the same collection data (27 May, 1966, Honey Lake, 5 miles west of Greenville, Madison County, Florida, USA). The syntypes at USNM have 22 adults and 40 subadults, including individuals with three or four genital markings in the male pore area. The specimens are in formalin and well-preserved, but the morphology of the male pore area in the syntypes is not as clear as that in USNM 125052 (from Georgia, USA) and USNM 125056 (from Minnesota, USA). From the latter two samples, it is now clear that the primary male pore is on the lower edge of the pre-setal genital marking, on top of the presumed setal line. While the primary male pores are generally not visible in the USNM syntype specimens, in the syntype specimen dissected (Figure 10C), the primary male pore can barely be seen on the left-hand-side male pore.

7. *Amynthas minimus* (Horst, 1893)

(Figures 1E, 13)

Perichaeta minima Horst, 1893: 66.
Pheretima minima—Gates 1982: 57.
Amynthas minimus—Sims & Easton 1972: 236. Reynolds 1978: 127; 2011: 275. Reynolds & Wetzel 2004: 88; 2008: 180.

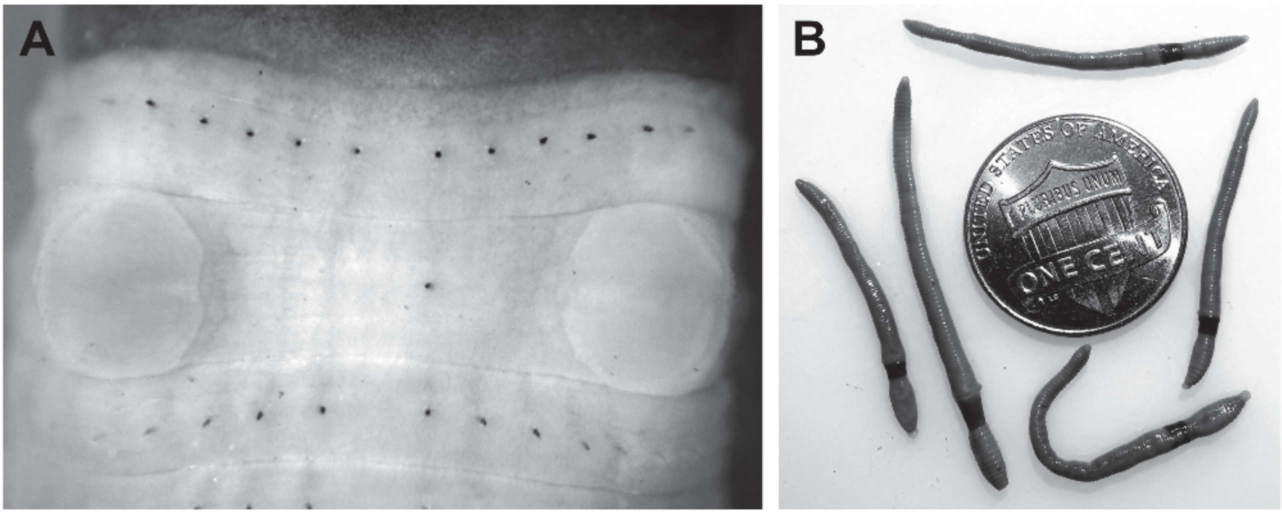

FIGURE 13. *Amynthas minimus*. (**A**) Both male pore regions. (**B**) Sizes compared to a one cent coin (19.05 mm diameter). USNM 125106.

Data sources. Gates (1972, 1982); Chang *et al.* (2009a; for color of live specimens).

Diagnosis. Size 20–56 mm by 1.5–2 mm. Segment numbers 77–115. Color of live specimens light red to reddish white. Male pores paired in XVIII, on a relatively large porophore. Post-clitellar genital markings unpaired, small mid-ventral tubercles, pre-setal on some of XVII, XIX–XXI. Spermathecal pores paired in 5/6 or absent. Pre-clitellar genital markings paired small pre-setal tubercles on some of V–VIII. Female pore single in XIV. First dorsal pore 11/12 or 12/13. Spermathecae present or absent; when present, one pair or single in VI, large. Prostate glands present or absent; when present, large, extending through some or all of XVI–XXIII. Intestinal caeca simple, extending anteriorly to XXIII.

Remarks. Easily distinguished from most pheretimoid species by its body size, *A. minimus* is one of the smallest pheretimoid earthworms in the world. It was first recorded in the continental US in 1969 in Louisiana (Gates 1982). Reproduction of this species is parthenogenetic.

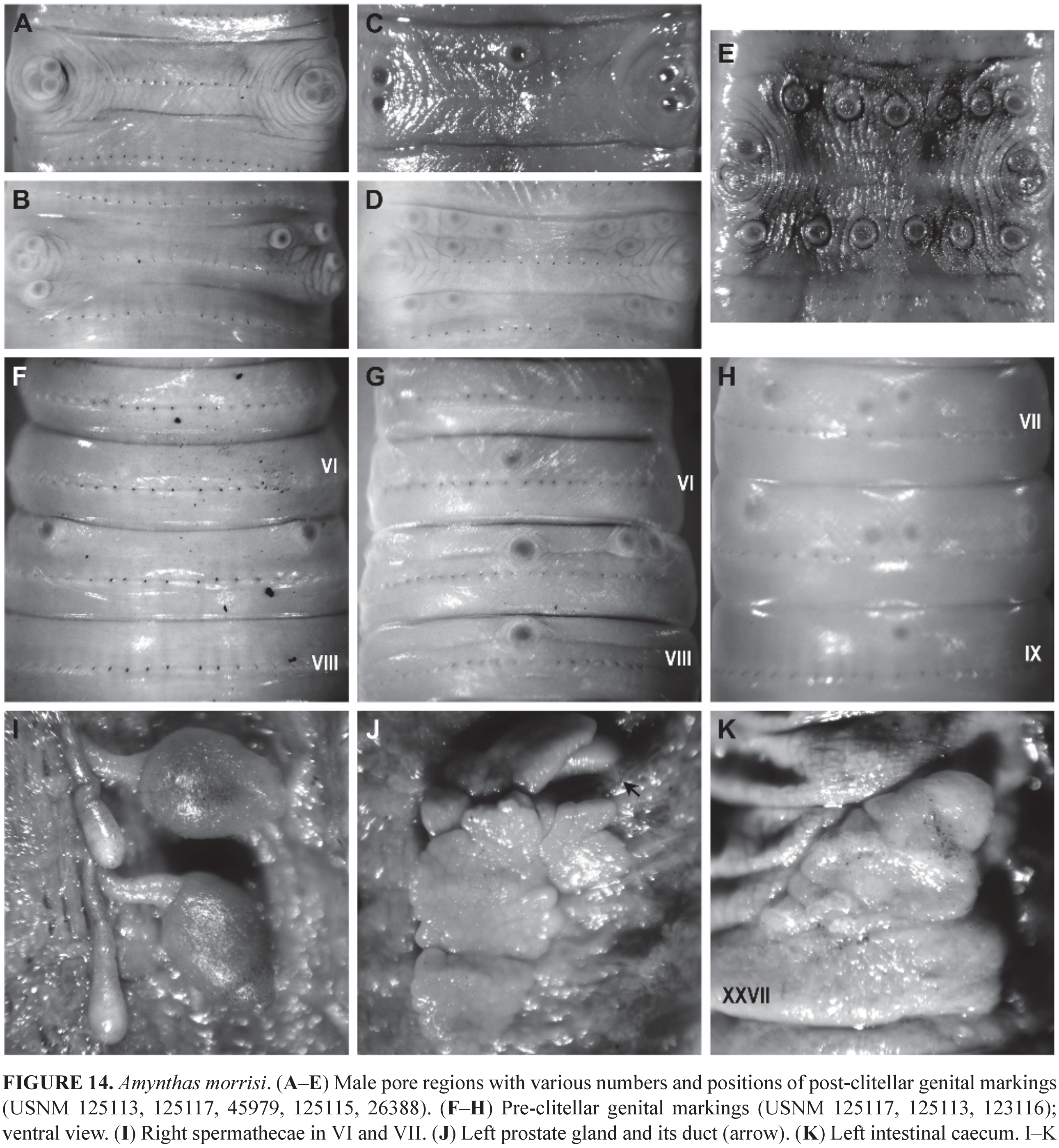

FIGURE 14. *Amynthas morrisi*. (**A**–**E**) Male pore regions with various numbers and positions of post-clitellar genital markings (USNM 125113, 125117, 45979, 125115, 26388). (**F**–**H**) Pre-clitellar genital markings (USNM 125117, 125113, 123116); ventral view. (**I**) Right spermathecae in VI and VII. (**J**) Left prostate gland and its duct (arrow). (**K**) Left intestinal caecum. I–K from USNM 125114.

8. *Amynthas morrisi* (Beddard, 1892)

(Figures 1F, 14)

Perichaeta morrisi Beddard, 1892a: 166.
Pheretima morrisi—Gates 1937: 361; 1954: 238; 1958: 31; 1963: 14; 1968: 253; 1982: 60.
Amynthas morrisi—Sims & Easton 1972: 236. Reynolds 1978: 127; 2010: 148; 2011: 276. Reynolds & Wetzel 2004: 88; 2008: 180. Blakemore 2014: 131.

Data sources. Gates (1937, 1968, 1972, 1982); Blakemore (2014).

Diagnosis. Size 40–150 mm by 2–6 mm. Segment numbers 75–102. Color of live specimens unknown. Male pores paired in XVIII, on small, circular porophores. Post-clitellar genital markings present or absent; when present, two immediately median to each male porophore, one pre-setal and one post-setal, one of the two sometimes absent, slightly larger than the porophore, surrounded by several concentric furrows. Additional pre-setal markings on XVIII and XIX in some specimens, mid-ventral or median to male pores, numbers variable. Spermathecal pores two pairs in 5/6/7. Pre-clitellar genital markings paired and/or unpaired small discs varying in numbers and positions; paired on some of VI–IX, median to spermathecal pore; unpaired on some of V–IX, mid-ventral, pre-setal. Female pore single in XIV. First dorsal pore 10/11 or 11/12. Spermathecae two pairs in VI–VII. Prostate glands paired, extending through some or all of XVII–XXIII. Intestinal caeca paired in XXVII, simple, extending anteriorly to XIV.

Remarks. *Amynthas morrisi* was first recorded in the continental US in 1916 in Waxahachie, Texas (Gates 1937). Reproduction of this species is amphimictic. The identity of what have been commonly recognized as "*A. morrisi*" in Asia and North America was recently questioned by Blakemore (2014), who believes the name *A. morrisi* should be restricted to specimens with no genital markings around male pores. Regardless of whether the US specimens should be called *A. morrisi* or not, all US specimens registered as *A. morrisi* in the USNM collection appear to belong to a single species (C.-H. Chang, personal observation). As the type specimen of *A. morrisi* is missing and the original description by Beddard lacks enough details, addressing this issue is unattainable at this moment.

9. *Amynthas rodericensis* (Grube, 1879)

(Figure 15)

Perichaeta rodericensis Grube 1879: 554.
Pheretima rodericensis—Gates 1954: 239; 1958: 31; 1963: 14; 1982: 63.
Amynthas rodericensis—Sims & Easton 1972: 235. Reynolds 1978: 127; 2010: 149; 2011: 277. Reynolds & Wetzel 2004: 88; 2008: 180.

Data sources. Gates (1937, 1954, 1972, 1982).

Diagnosis. Size 55–150 mm by 3–10 mm. Segment numbers 80–100. Color of live specimens unknown. Male pores paired in XVIII, small, superficial, on small, circular porophores. Post-clitellar genital markings paired, oval, large, median to male pores, across 17/18 and/or 18/19. Spermathecal pores four pairs in 5/6/7/8/9, dorsal. Pre-clitellar genital markings absent. Female pore single in XIV. First dorsal pore 11/12 or 12/13. Spermathecae four pairs in VI–IX, duct shorter than ampulla; diverticulum stalk longer than the duct, seminal chamber elongate, ellipsoidal, or moniliform. Prostate glands paired, extending from XVI, XVII to XXI, XXII. Intestinal caeca paired in XXVII, simple, extending anteriorly to XXIV–XXV.

Remarks. The first record of *A. rodericensis* in the continental US was in 1950 in Lutz, Florida (Gates 1954). This species is the only US pheretimoid earthworm with dorsally positioned spermathecal pores. Identification of this species can easily be achieved by combining this unique spermathecal pore position and the genital markings in the male pore area. Reproduction of *A. rodericensis* is amphimictic.

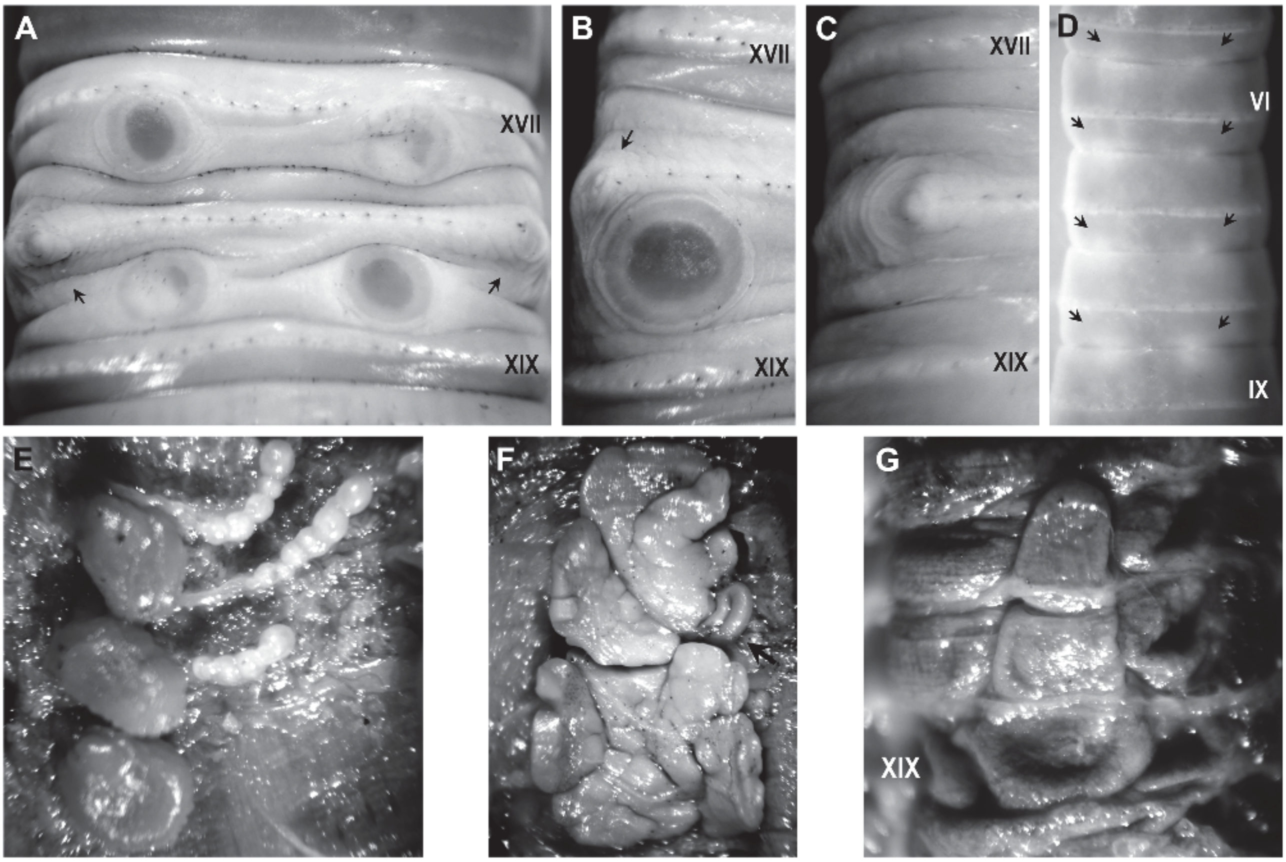

FIGURE 15. *Amynthas rodericensis*. (**A**) Both male pores (arrows) and the associated genital markings, showing two on each side. (**B**) Right male pore (arrow) with only the post-setal genital marking. (**C**) Right male pore with no genital marking. A–C all from USNM 125143, different specimens. (**D**) Paired dorsal spermathecal pores in 5/6/7/8/9 (arrows); dorsal view. The relatively light-colored pores can usually be easily seen due to the relatively dark dorsal pigmentation (USNM 125161). (**E**) Left spermathecae in VII–IX. (**F**) Left prostate gland and its duct (arrow). (**G**) Left intestinal caecum. E–G from the same specimen in USNM 125143.

10. *Amynthas tokioensis* (Beddard, 1892)

(Figures 1G, 16; Table 1)

Perichaeta tokioensis Beddard, 1892b: 762.
Perichaeta levis Goto & Hatai, 1899: 20.
Pheretima levis—Gates 1954: 234; 1958: 21, 31; 1963: 14; 1982: 54.
Metaphire levis—Sims & Easton 1972: 238. Reynolds 1978: 124, 127; 2010: 151; 2011: 280. Reynolds & Wetzel 2004: 88; 2008: 181.
Amynthas tokioensis—Sims & Easton 1972: 237. Blakemore 2010a: 422.
Amynthas levis—Easton 1981: 51; Blakemore 2012b: 111.

Data sources. Gates (1954, 1958, 1982); Blakemore (2010a, 2012b); this study (for color of live specimens).

Diagnosis. Size 30–125 mm by 3–7 mm. Segment numbers 84–102. Color of live specimens red or brown. Male pores present or absent; when present, unilaterally or paired in XVIII, on a small porophore surrounded by a deep furrow. Post-clitellar genital markings small, circular discs, 1–3 on each side of XVIII, one sometimes on the porophore, two median or lateral to the male porophore. Spermathecal pores two pairs in 6/7/8, variously lacking. Pre-clitellar genital markings 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. Female pore single in XIV. First dorsal pore 12/13. Spermathecae present or absent; when fully present, two pairs in VII–VIII, duct as long as or shorter than ampulla, diverticulum longer than the respective spermatheca, stalk as long as seminal chamber. Two to three stalked accessory glands associated with each spermatheca. Prostate glands paired, extending through some or all of XV–XXIII. Two to three accessory glands associated with each male pore. Intestinal caeca paired in XXVII, manicate.

Remarks. Historically referred to as *Pheretima levis* or *Metaphire levis* in North American publications, *A. tokioensis* has been frequently reported in the US. The first record of this species in the continental US was in 1947 in New York City (Gates 1954). Gates (1982) believed that it was probably brought to the US directly from Japan. The original designation of *M. levis* as a junior synonym of *A. tokioensis* by Blakemore (2006) was later supported by morphological data of lectotype of *A. tokioensis* (Blakemore 2010a) and syntypes of *M. levis* (Blakemore 2012b). *A. tokioensis* frequently co-occurs with *A. agrestis* and/or *M. hilgendorfi*, and is probably often overlooked partially due to its smallest size among the three species and difficulties in separating *A. tokioensis* from the other two species (Table 1). Reproduction of *A. tokioensis* is parthenogenetic.

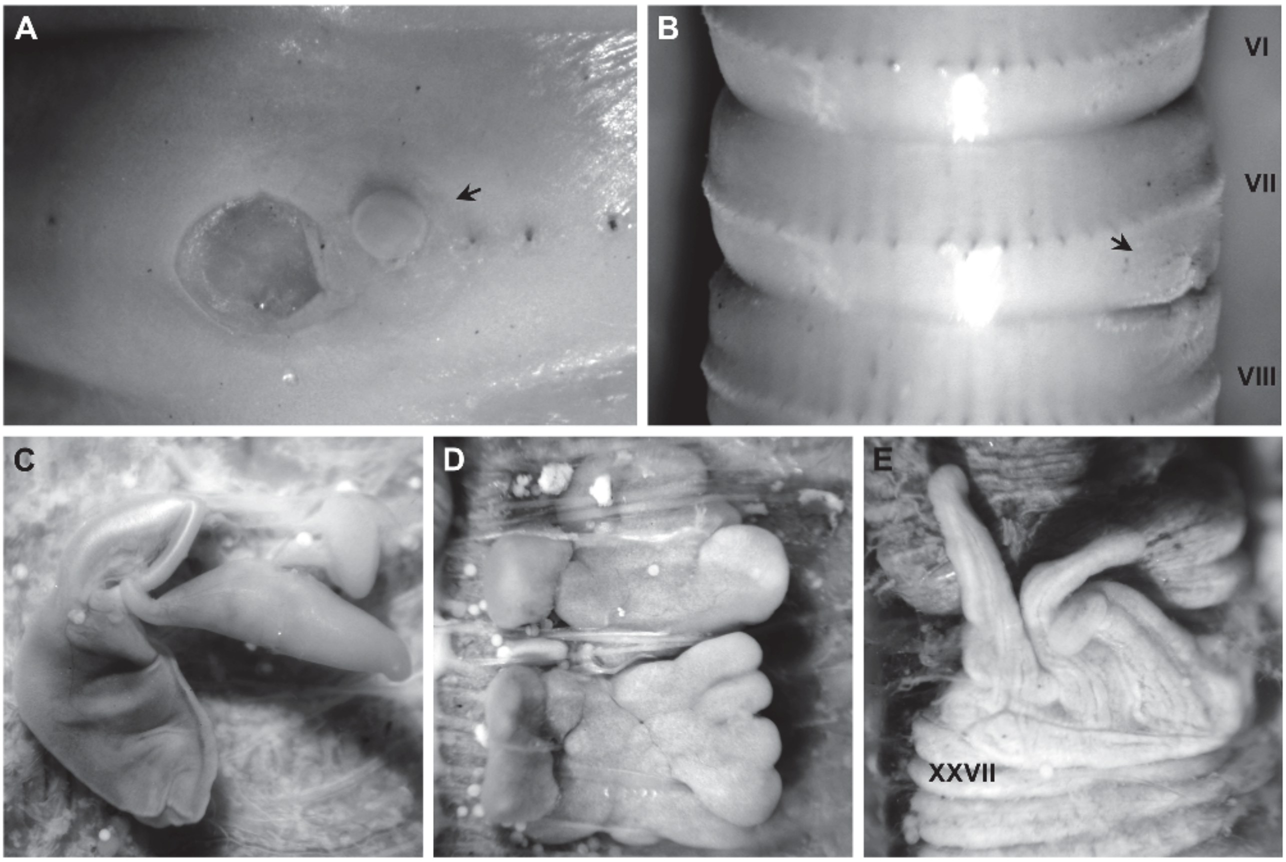

FIGURE 16. *Amynthas tokioensis*. (**A**) Right male pore and the associated genital marking (arrow). (**B**) Single spermathecal pore in 7/8 on the lower right (arrow), compared to its absence in 6/7 on the right and in 6/7/8 on the left; ventral view. (**C**) Left spermatheca in VIII. (**D**) Right prostate gland. (**E**) Right intestinal caecum. All photos from USNM 125070, three different specimens: A, specimen 1; B, C, specimen 2; D, E, specimen 3.

11. *Metaphire californica* (Kinberg, 1867)

(Figures 1H, 17)

Pheretima californica Kinberg, 1867: 102. Gates 1937: 348; 1954: 226; 1958: 31; 1963: 12; 1982: 42.
Metaphire californica—Sims & Easton 1972: 238. Reynolds 1978: 127; 2010: 150; 2011: 278. Reynolds & Wetzel 2004: 88; 2008: 180.

Data sources. Gates (1937, 1972, 1982).

Diagnosis. Size 50–132 mm by 3–5 mm. Segment numbers 82–115. Color of live specimens red. Male pores paired in XVIII, each in an invagination with a transverse, slit-like opening. Post-clitellar genital markings absent. Spermathecal pores two pairs in 7/8/9. Pre-clitellar genital markings absent. Female pore single in XIV. First dorsal pore 11/12 or 12/13. Spermathecae two pairs in VIII–IX, small to medium, ducts shorter than ampulla;

diverticulum coiled or looped. Prostate glands present, extending through some or all of XVI–XXII. Intestinal caeca paired in XXVII, simple, extending anteriorly to XXII.

Remarks. One of the two pheretimoid species originally described from the US, *M. californica* was first recorded in 1866 near San Francisco, California (Kinberg 1867), and is also the first pheretimoid earthworm recorded in the continental US. Reproduction of this species is amphimictic.

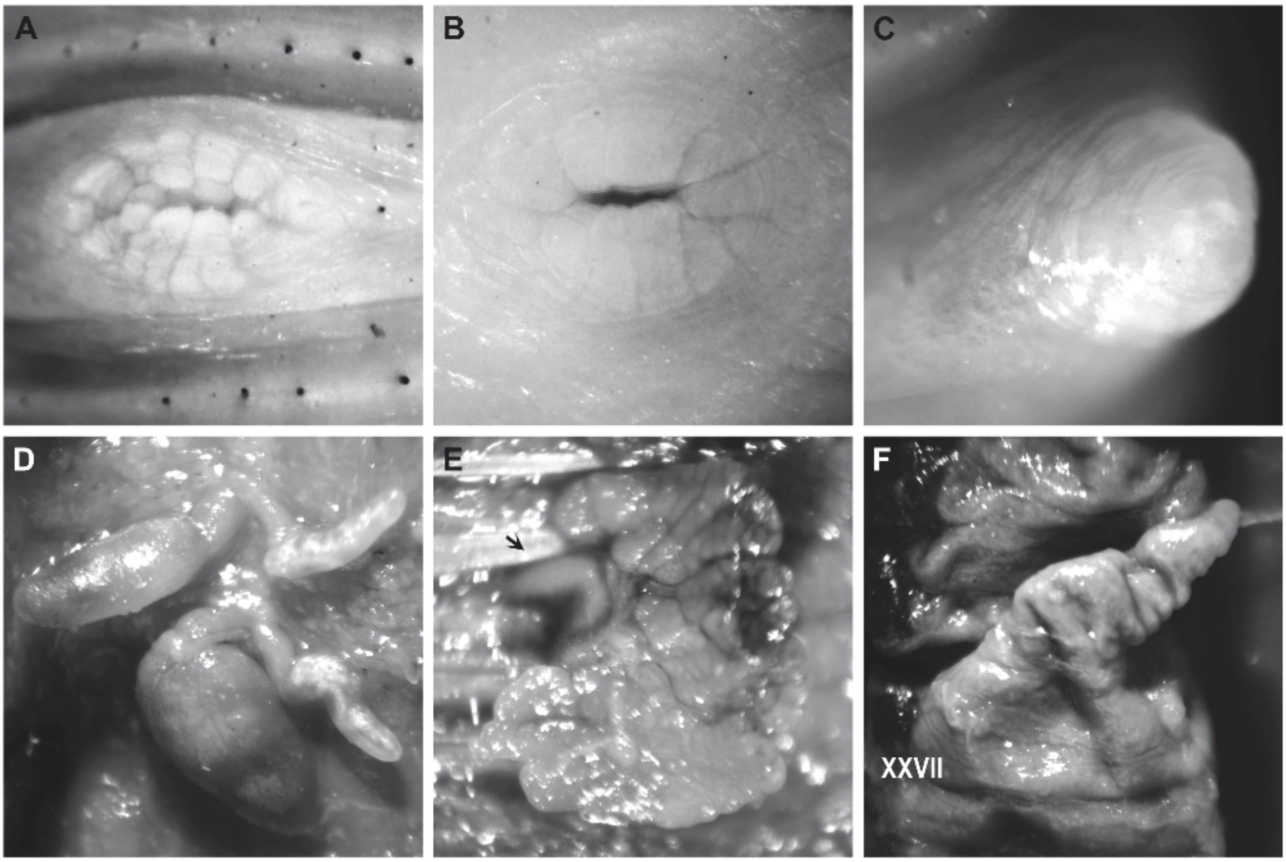

FIGURE 17. *Metaphire californica*. (**A**, **B**) Right and left male pore regions from two different specimens (USNM 124785). (**C**) Left male pore region with the copulatory pouch everted. (**D**) Left spermathecae in VIII and IX. (**E**) Right prostate gland and its duct (arrow). (**F**) Right intestinal caecum. C–F from USNM 124784.

12. *Metaphire hilgendorfi* (Michaelsen, 1892)

(Figures 1I, 18; Table 1)

Perichaeta hilgendorfi Michaelsen, 1892: 235.

Pheretima hilgendorfi—Gates 1954: 230; 1958: 11, 31; 1982: 49.

Amynthas hilgendorfi—Sims & Easton 1972: 235, 237. Reynolds 1978: 122, 127; 2010: 146; 2011: 272. Reynolds & Wetzel 2004: 88; 2008: 179.

Metaphire hilgendorfi—Blakemore 2010a: 416; 2012b: 106, 108; 2013b: 61.

Data sources. Gates (1954, 1982); Blakemore (2010a, 2013b); this study (USNM 125091, 1421430, 1421432).

Diagnosis. Size 109–170 mm by 6–8 mm. Segment numbers 98–118. Color of live specimens red or reddish brown. Male pores usually absent; when present, unilateral in XVIII, in a large, transversely slit-like copulatory pouch. Primary male pore minute, on the center of a genital pad at the bottom of the copulatory pouch, usually not visible from outside unless everted. Other post-clitellar genital markings present or absent; when present, unpaired, mid-ventral, pre-setal clusters of numerous small tubercles on XVII–XVIII, occasionally on XIX–XXII. Spermathecal pores two pairs in 6/7/8. Pre-clitellar genital markings unpaired, mid-ventral, pre-setal clusters of numerous small tubercles on VIII–IX, occasionally on VII, X, XI, similar to those in the post-clitellar region. Female pore single in XIV. First dorsal pore 11/12 or 12/13. Spermathecae two pairs in VII–VIII, large, duct shorter than ampulla, with a diverticulum longer than the main axis; genital marking glands present with long coelomic stalks. Prostate glands present or absent; when present, large in XV–XXI. Intestinal caeca paired in XXVII, manicate, extending anteriorly to XXII, XXIII, or XXIV.

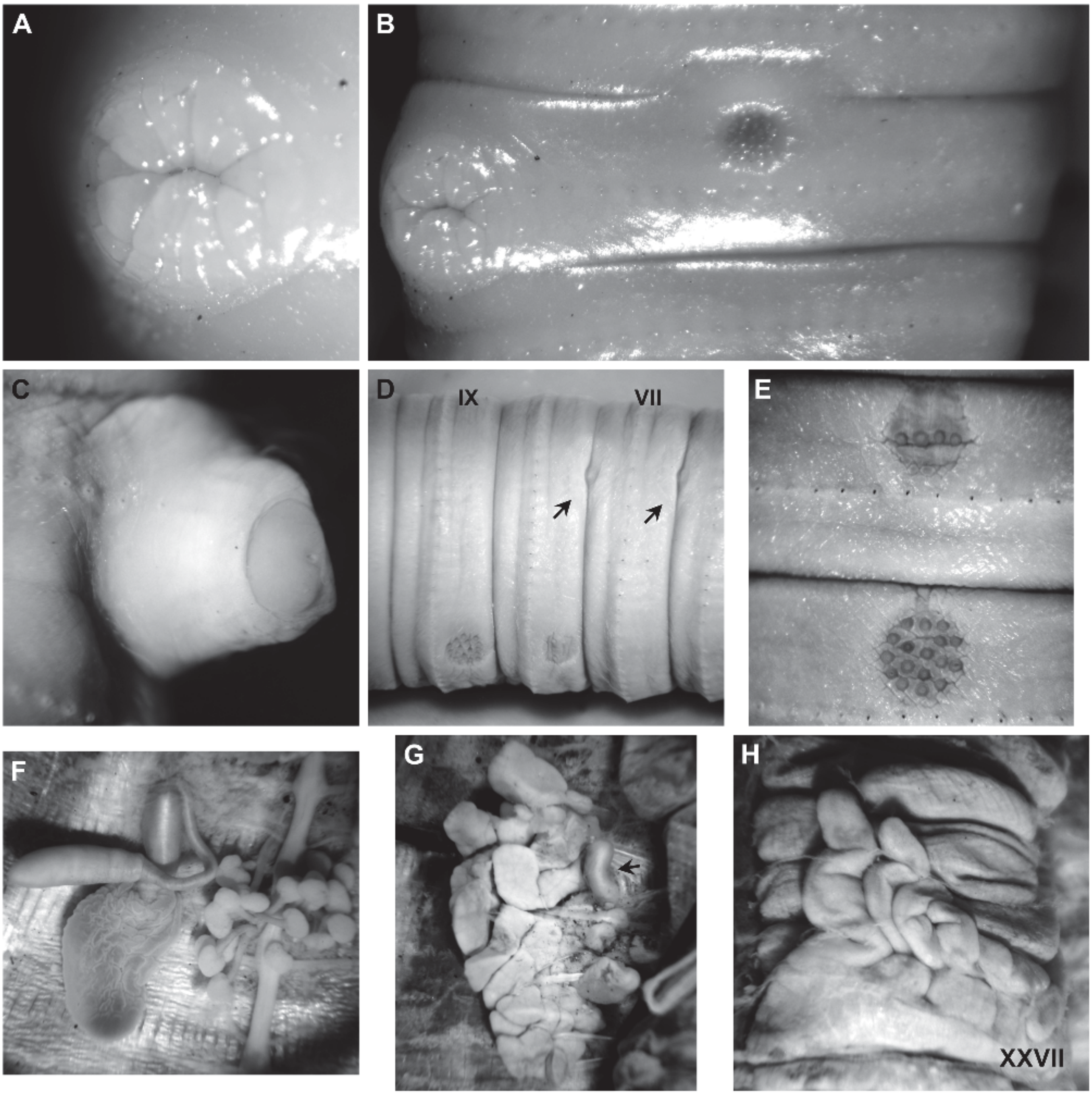

FIGURE 18. *Metaphire hilgendorfi*. (**A**, **B**) Right male pore region and the associated genital markings, showing presence of a male pore only on one side and absence on the other (USNM 1421430). (**C**) Left male pore region with an everted copulatory pouch. (**D**) Right spermathecal pores in 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. C, E–H from one specimen in USNM 125091; D from another specimen in USNM 125091.

Remarks. Frequently reported in the continental US and one of the two pheretimoid species recorded in Canada, *M. hilgendorfi* has been known as *Amynthas hilgendorfi* for a long time in North America. It was first recorded in 1948 in Kingston, New York (Gates 1954). Reproduction of this species is obligately parthenogenetic. *Metaphire hilgendorfi* was recently transferred from *Amynthas* to *Metaphire* by Blakemore (2010a; 2013b), but this change has not been reflected in North American literature, partially because all US specimens reported so far lack male pores. However, an USNM specimen identified by G.E. Gates (USNM125091, collected from South Carolina in 1969) has a male pore in the form of an everted copulatory pouch on one side (Figure 16C). Moreover, two recently collected specimens from Baltimore, MD (USNM 1421430, 1421432) apparently show a male pore on one side inside a transversely slit-like copulatory pouch opening (Figure 16A, B), unambiguously supporting transferring the species to *Metaphire*. *Metaphire hilgendorfi* is morphologically similar to *A. agrestis* and *A. tokioensis*, with the male pores usually lacking in all three species (Table 1). Co-occurrence of two or all three of the species may be quite common. *Metaphire hilgendorfi* is an epi-endogeic species. At high abundance it transforms the top several centimeters of soil into granular casts. It is common in urban and suburban areas and is also invading deciduous forests in eastern US (C.-H. Chang and K. Szlavecz, personal observation). Its spread is probably facilitated by commercial mulches, as is in *A. agrestis* (Belliturk *et al.* 2015). It has been shown to be the superior competitor when interacting with *Lumbricus rubellus*, an epi-endogeic European earthworm common in the US, for leaf litter. It does not appear to affect *Eisenoides lonnbergi*, a large native lumbricid common in riparian areas, wetlands, and some upland habitats in the eastern US (Chang *et al.* 2016a).

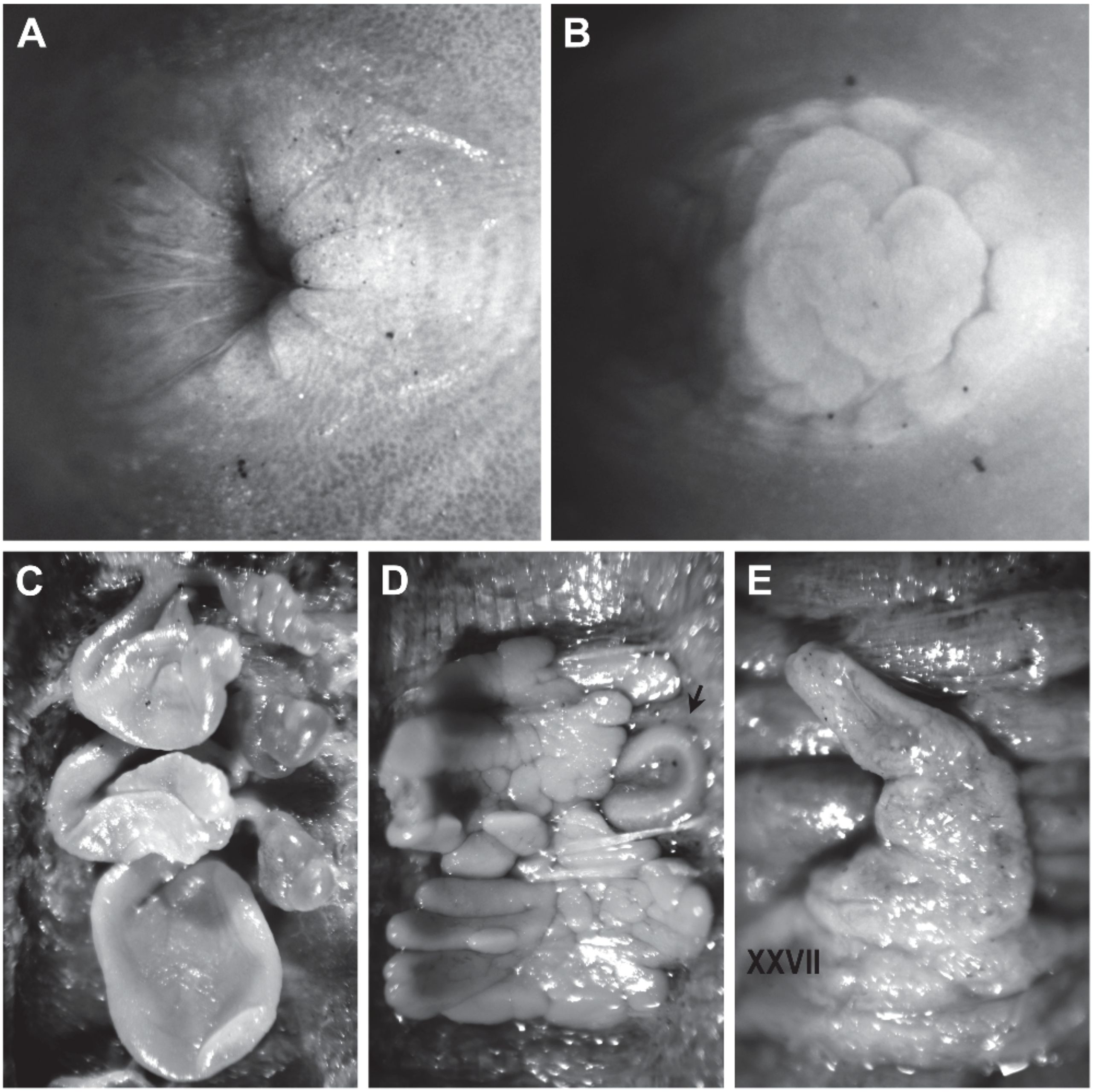

FIGURE 19. *Metaphire houlleti*. (**A**) Right male pore region (USNM 27574). (**B**) Right male pore region with an everted copulatory pouch. (**C**) Left spermathecae in VII–IX. (**D**) Left prostate gland and its duct (arrow). (**E**) Left intestinal caecum. B–E from USNM 125080.

13. *Metaphire houlleti* (Perrier, 1872)

(Figure 19)

Perichaeta houlleti Perrier, 1872: 99.
Pheretima houlleti—Gates 1958: 31; 1982: 52.
Metaphire houlleti—Sims & Easton 1972: 238. Reynolds 1978: 127; 2011: 279. Reynolds & Wetzel 2004: 88; 2008: 180.

Data sources. Gates (1937, 1972, 1982); Shen *et al.* (2005).

Diagnosis. Size 107–200 mm by 3–8 mm. Segment numbers 86–131. Color of live specimens unknown. Male pores paired in XVIII, each in a copulatory pouch with a C-shaped opening. Post-clitellar genital markings two in each copulatory pouch. Spermathecal pores three pairs in 6/7/8/9, some or all missing in parthenogenetic morphs. Pre-clitellar genital markings absent or present; when present, within the intersegmental furrow, next to each spermathecal pore, one anterior, one posterior. Female pore single in XIV. First dorsal pore 9/10 or 10/11. Spermathecae three pairs in VII–IX, some or all lacking in parthenogenetic morphs, duct shorter than ampulla, diverticulum with short, slender stalk and wider, elongate, coiled seminal chamber; accessory gland stalked. Prostate glands paired, extending through some or all of XVI–XXIII. Intestinal caeca paired in XXVII, simple, extending anteriorly to XXII–XXIV.

Remarks. *Metaphire houlleti* was first recorded in the continental US in 1969 in Georgia and Florida (Gates 1982). Both amphimictic and parthenogenetic morphs have been reported in the species.

14. *Metaphire posthuma* (Vaillant, 1868)

(Figures 1J, 20)

Perichaeta posthuma Vaillant, 1868: 146.
Pheretima posthuma—Gates 1958: 31; 1982: 61.
Metaphire posthuma—Sims & Easton 1972: 239. Reynolds & Wetzel 2004: 88; 2008: 181. Reynolds 2011: 281.

Data sources. Gates (1937, 1972, 1982); Tsai 1964.

Diagnosis. Size 60–210 mm by 3–8 mm. Segment numbers 91–140. Color of live specimens brown. Male pores paired in XVIII, in an invagination. Post-clitellar genital markings paired on setal circle on XVII, XIX, slightly median to male pores. Spermathecal pores four pairs, on the posterior margin of segments, just in front of 5/6/7/8/9. Pre-clitellar genital markings absent. Female pore single in XIV. First dorsal pore 11/12 or 12/13. Spermathecae four pairs in VI–IX, each with a short, stout duct and oval or heart-shaped ampulla; diverticulum stalk short and slender, seminal chambers usually longer than stalks. Prostate glands paired, extending through some or all of XV–XXI; accessory glands in XVII and XIX, corresponding to external genital markings. Intestinal caeca paired in XXVII, simple, extending anteriorly to XIV.

Remarks. The first record of *M. posthuma* in the continental US was in 1967 in Homestead, Florida (Gates 1982). Reproduction of this species is amphimictic.

15. *Pithemera bicincta* (Perrier, 1875)

(Figures 1K, 21)

Perichaeta bicincta Perrier, 1875: 1044.
Pheretima bicincta—Gates 1958: 31; 1963: 12; 1982: 40.
Pithemera bicincta—Sims & Easton 1972: 202, 232. Reynolds & Wetzel 2004: 88; 2008: 181. Reynolds 2010: 152.
Pithemira bicincta—Reynolds 2011: 283.

Data sources. Gates (1937, 1972, 1982).

Diagnosis. Size 40–80 mm by 2–3 mm. Segment numbers 77–101. Color of live specimens red. Male pores paired in XVIII, small, on a porophore with variable size and shape. Post-clitellar genital markings present or absent; when present, paired on XVIII or on 18/19. Spermathecal pores five pairs in 4/5/6/7/8/9. Pre-clitellar genital markings absent. Female pores closely paired in XIV. First dorsal pore 12/13. Spermathecae present or absent; when present, five pairs in V–IX, with a duct as long as the ampulla; diverticulum usually shorter than duct and ampulla combined. Prostate glands present or absent; when present, medium to large, extending through some or all of XVI–XX. Intestinal caeca paired in XXII, simple, sometimes extending to XXI.

Remarks. *Pithemera bicincta* was first recorded in the continental US in 1963 in a greenhouse in Maine (Gates 1963; 1982). Outside greenhouses, it has only been reported in Georgia and Florida. Reproduction of *Pithemera bicincta* is likely to be parthenogenetic.

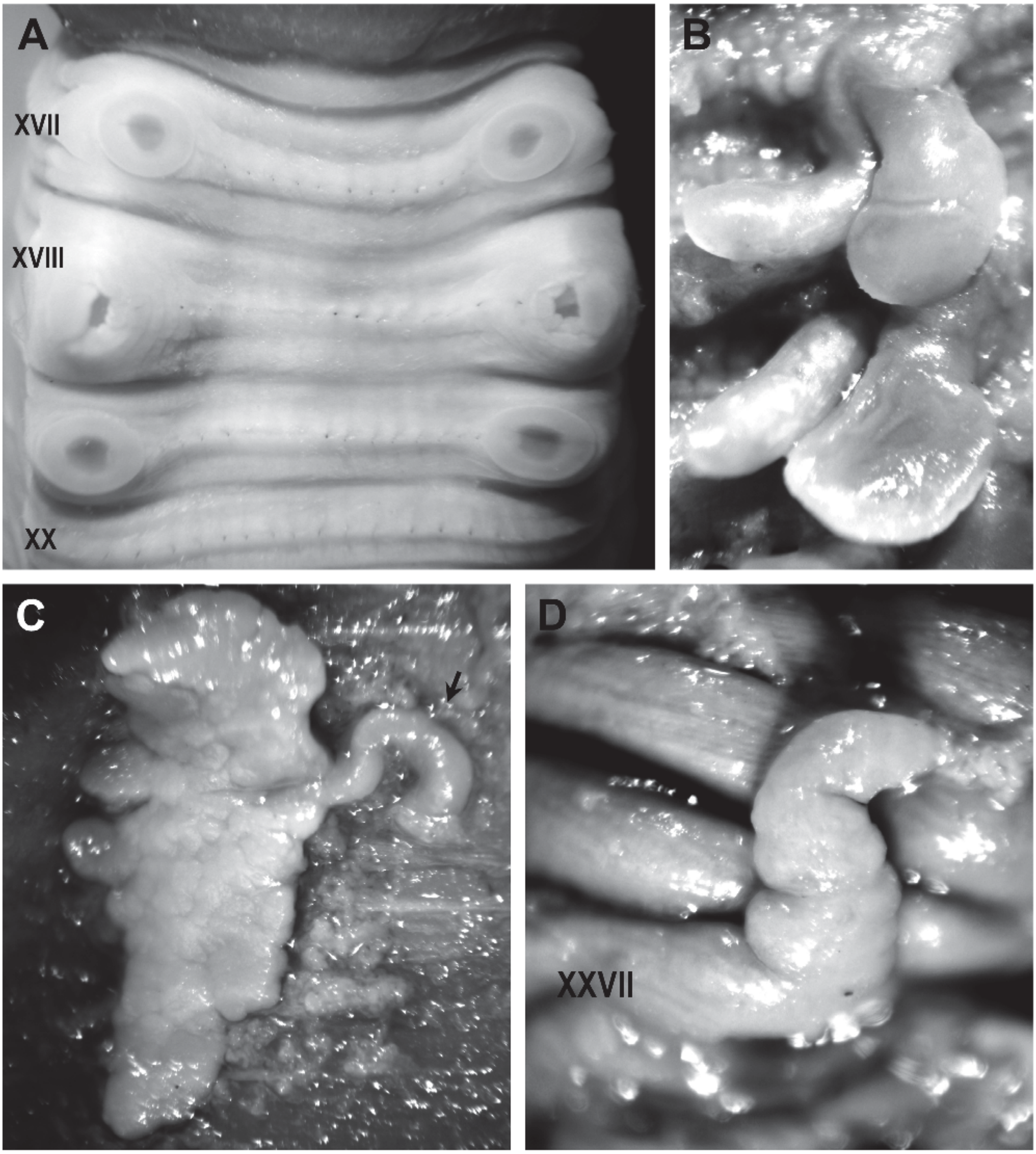

FIGURE 20. *Metaphire posthuma*. (**A**) Both male pore regions and the associated genital markings on XVII and XIX. (**B**) Right spermathecae in VI and VII. (**C**) Left prostate gland and its duct (arrow). (**D**) Left intestinal caecum. All from USNM 125139.

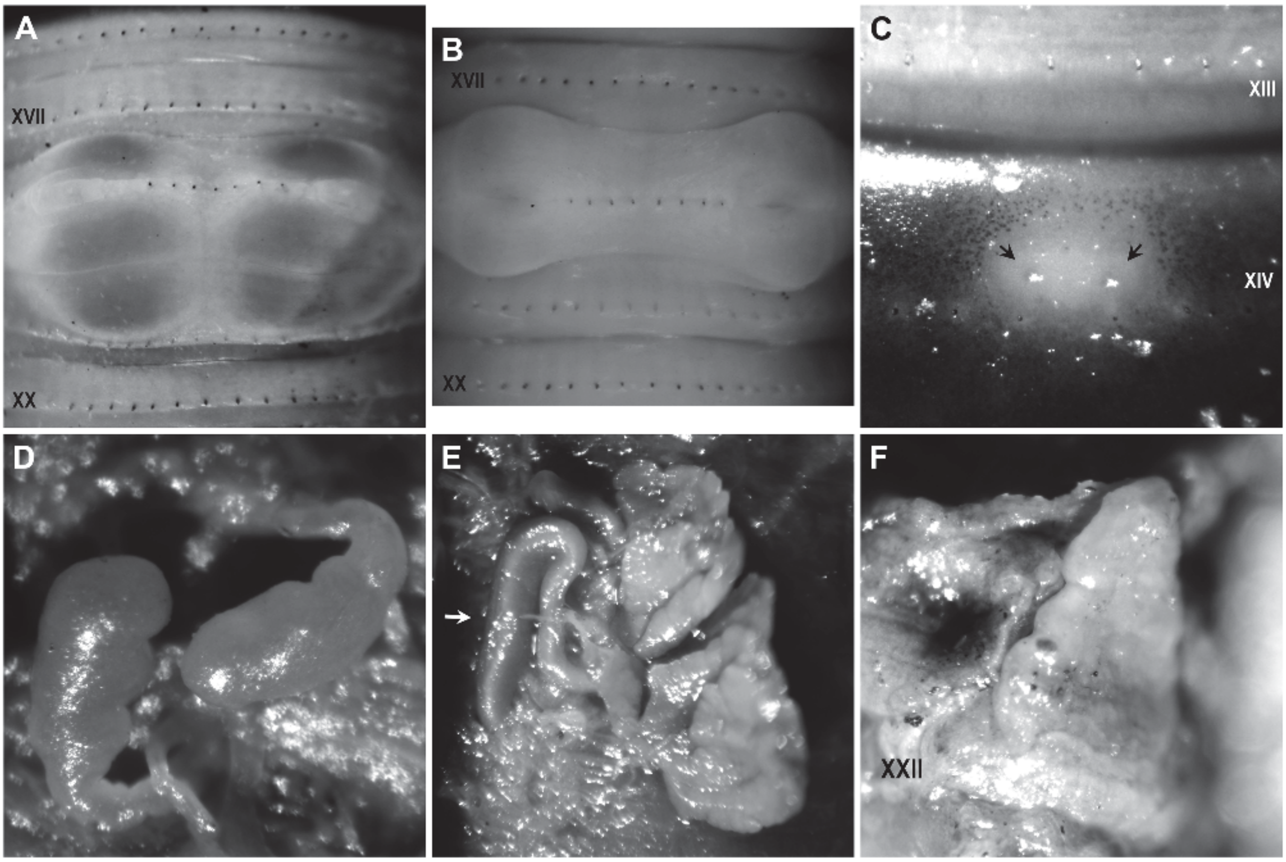

FIGURE 21. *Pithemera bicincta*. (**A**, **B**) Male pore regions and the associated genital markings of two specimens (USNM 124780 and 124781). (**C**) Two female pores in XIV (arrows); note the lighter color surrounding the female pores compared to that of the clitellum in the background. (**D**) Left spermathecae in V and VI. (**E**) Right prostate gland and its duct (arrow). (**F**) Right intestinal caecum in XXII, as opposed to in XXVII as in *Amynthas* and *Metaphire*. C–F from USNM 124780.

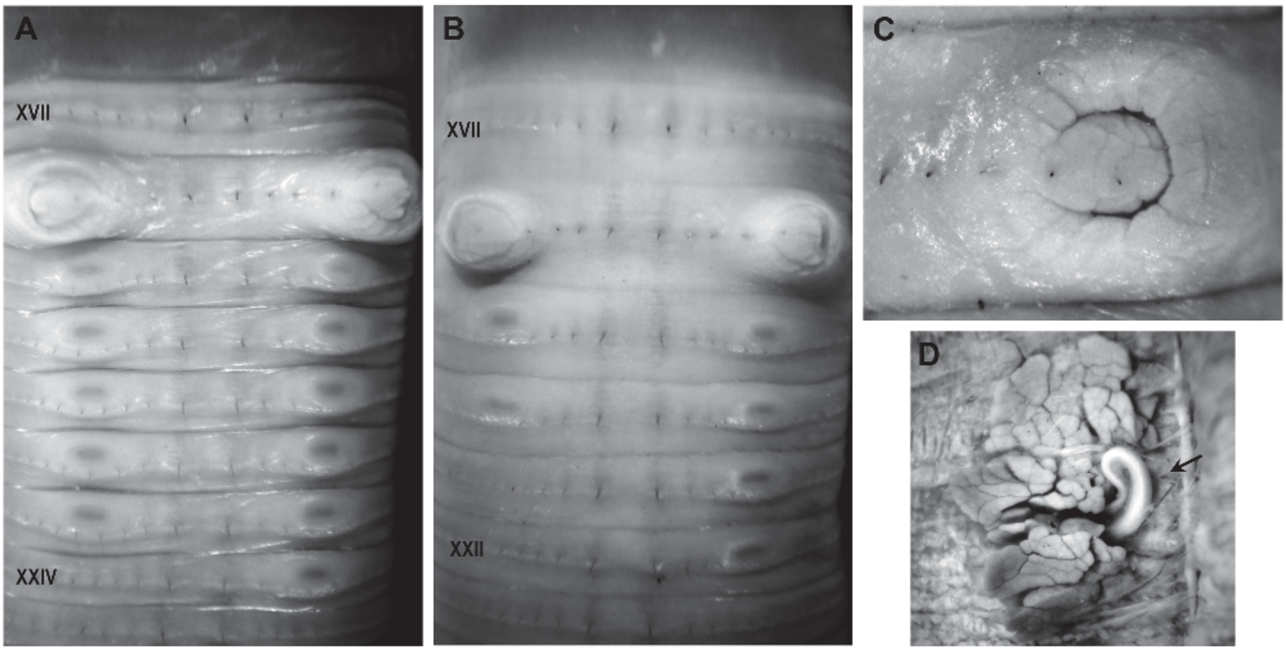

FIGURE 22. *Polypheretima elongata*. (**A**, **B**) Male pore regions and post-clitellar genital markings (two specimens from USNM 124962). (**C**) Left male pore region (USNM 124961). (**D**) Left prostate gland and its duct (arrow) (USNM 124962).

16. *Polypheretima elongata* (Perrier, 1872)

(Figures 1L, 22)

Perichaeta elongata Perrier, 1872: 124.
Pheretima elongata—Gates 1958: 31; 1982: 45.
Metapheretima elongata—Reynolds & Wetzel 2004: 88; 2008: 181.
Polypheretima elongata—Easton 1979: 53.

Data sources. Gates (1937, 1972, 1982); Chang *et al.* (2009a).

Diagnosis. Size 75–355 mm by 3–6 mm. Segment numbers 136–297. Color of live specimens light grey to red with pink to red anterior. Male pores paired in XVIII, on small disc. Post-clitellar genital markings widely paired on some or all of XIX–XXIV, sometimes becoming unilateral posteriorly, pre-setal, median to male pores. Spermathecal pores present or absent; when present, in some or all of 5/6/7, paired groups of 2–5. Pre-clitellar genital markings absent. Female pore single in XIV. First dorsal pore 12/13. Spermathecae present or absent; when present, polythecal, in some or all of VI–VII, with a duct shorter than ampulla, diverticulum present, genital marking glands sessile. Prostate glands XVI–XXI. Intestinal caeca absent.

Remarks. The only record of *Polypheretima elongata* in the continental US was in a PhD thesis in 1969 from Louisiana (Gates 1958; 1982). Whether this species actually occurs in the US is questionable. *Polypheretima elongata* was listed in the checklists by Gates (1982) and by Reynolds & Wetzel (2008). However, it was not reported in the Louisiana earthworm study by Reynolds (2008), and was not listed in the checklists by Reynolds & Wetzel (2004) and by Reynolds (2011), either. Both amphimictic and parthenogenetic reproduction has been reported in *Polypheretima elongata*.

Discussion

Invasion of Asian earthworms in temperate deciduous forests in the continental US has spanned from northern to southeastern states, including, but not limited to, Minnesota, Wisconsin, Illinois, Ohio, Michigan, New York, Vermont, New Hampshire, Massachusetts, Connecticut, New Jersey, Maryland, North Carolina, South Carolina, Tennessee, Georgia, and Florida. The so-called *Amynthas* invasion actually comprises at least five common species belonging to two genera, *Amynthas* and *Metaphire*. These species are *A. agrestis*, *A. corticis*, *A. gracilis*, *A. tokioensis,* and *M. hilgendorfi*. The last four species have been frequently referred to in North American literature as *A. diffringens*, *A. hawayanus*, *M. levis,* and *A. hilgendorfi*, respectively. These latter names are either junior synonyms or old combinations, and their use should be strongly discouraged to avoid confusion.

While in most cases a single species was reported invading into the forests, such as *M. hilgendorfi* (Greiner *et al.* 2012), *A. agrestis* (Görres *et al.* 2014), or *A. gracilis* (Burtelow *et al.* 1998), co-occurrence of two or three species may be more common than previously understood. In addition to the documented co-occurrence of *A. agrestis* and *A. corticis* in the Smoky Mountains (Snyder *et al.* 2011), recent re-examination of samples collected at six locations in northern and central Maryland all revealed co-occurrence of some combinations of *A. agrestis*, *A. tokioensis*, and *M. hilgendorfi* (Chang and Szlavecz, unpublished data), and samples from Wisconsin, Vermont, New Hampshire, and Connecticut also show the same pattern (C.-H. Chang, personal observation). This finding implies that co-occurrence may have been widely overlooked until now. The potential co-occurrence poses a challenge to North American ecologists who have been assuming that the "*Amynthas*" currently invading their field plots are *A. agrestis*, a single species, without morphologically confirming the identity of their species and excluding the possibility of co-occurrence of multiple *Amynthas* and/or *Metaphire*. To correctly assess pheretimoid assemblages in a locality we recommend taking a large number of specimens (e.g., at least 10 but preferably 20+) whenever possible for species identification. The key and diagnosis presented in this study will be helpful for ecologists to address this challenge.

Parthenogenesis poses another challenge to species identification. As earthworms evolve to be parthenogenetic, they may start to lose parts of the male sexual system (Gates 1956). *Amynthas agrestis*, *A. tokioensis,* and *M. hilgendorfi*, the three most common pheretimoid invaders, are all parthenogenetic, with different degrees of degradation in their male pores, spermathecal pores, and genital markings. As the three species all have manicate intestinal caeca and, due to parthenogenetic degradation, may bear two pairs or fewer spermathecal pores, species identification based entirely on morphology becomes impossible when male pores and genital markings are both absent. In this extreme case, DNA barcoding (Chang *et al.* 2009b; Rougerie *et al.* 2009) may become necessary. Fortunately, to our knowledge, this extreme situation has rarely been the case for US specimens, among which the majority of *A. agrestis* have three spermathecal pores on one or both sides of the body, and the majority of *M. hilgendorfi* have two pairs of spermathecal pores and distinct patches of pre-clitellar genital markings.

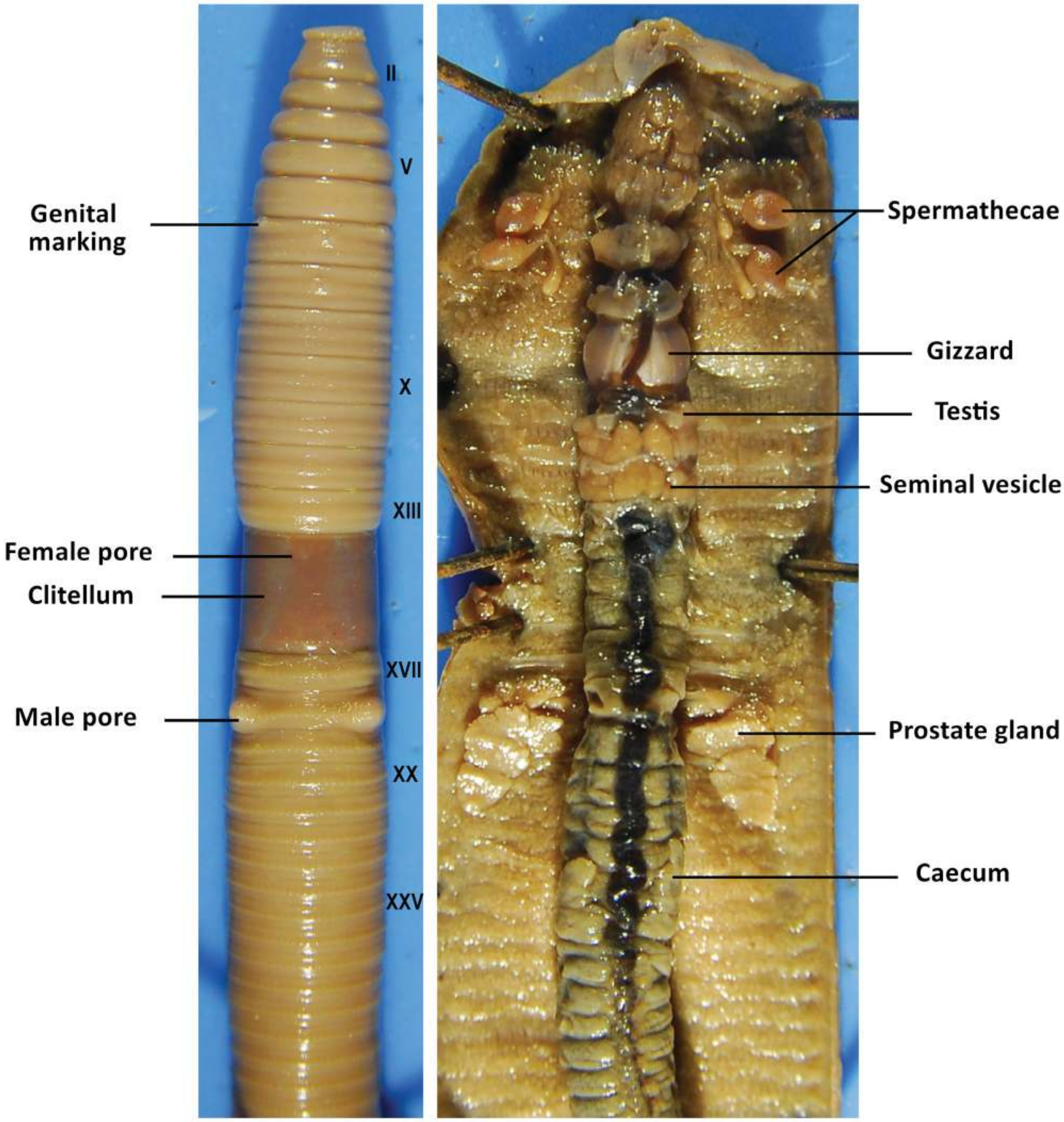

FIGURE 23. External (left, ventral view) and internal (right, dorsal view) morphology of a pheretimoid earthworm.

Co-occurrence of the three species raises many interesting ecological questions regarding *Amynthas* and *Metaphire* invasion. For example, (1) do these species facilitate each other and increase the chance of successful invasion or do they compete with each other, (2) are their cumulative effects on ecosystem functions non-additive under co-occurrence, and, (3) are earthworm communities dominated by Asian *Amynthas* and *Metaphire* in North America different from those dominated by European species in their impact on ecological processes and properties? Some recent studies showed that the effects of Asian earthworms, including *A. agrestis*, *A. corticis*, *A.*

gracilis, and *M. hilgendorfi*, on several measurements of ecosystem function are similar and comparable to those induced by European species (Burtelow *et al.* 1998; Snyder *et al.* 2009; 2011; Greiner *et al.* 2012; Zhang *et al.* 2013). However, there is no evidence suggesting that the mechanisms and/or pathways leading to these effects are the same between *Amynthas*/*Metaphire* and European lumbricid species. In fact, other studies have highlighted that compared to common European species, some common Asian invaders in North America have a stronger negative effect on the soil microbial community (Zhang *et al.* 2010; Chang *et al.* 2016a, b), and the two groups have distinct effects on the mechanisms through which litter-derived fresh soil organic matter is protected or mineralized (Chang *et al.* 2016a, b). As microbial transformation of leaf litter is one of the most important contributors to stable soil organic matter (Cotrufo *et al.* 2015), the collective effects of invading *Amynthas* and *Metaphire* on the two processes documented in Zhang *et al.* (2010) and Chang *et al.* (2016a, b) may be an altered trajectory of leaf litter carbon mineralization and soil organic matter formation. As these impacts appear to be species-specific, the questions outlined above can only be addressed by proper and correct identification of the species involved.

Acknowledgements

We are grateful to the Smithsonian Institution National Museum of Natural History, Washington, D.C. and the associated Museum Support Center, Suitland, MD for access to its earthworm collection, and the hospitality and help from William Moser and Anna Phillips. We thank an anonymous reviewer for his/her constructive and insightful comments on an earlier version of the manuscript. Chih-Han Chang appreciates Wen-Jay Chih for permission to use his photos, and Huei-Ping Shen, Samuel James, and Robert Blakemore for sharing information and their insights on pheretimoid taxonomy. Funding to this work is partially supported by National Science Foundation (EF-1550795, ACI-1244820, DEB-1227188).

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APPENDIX A

Morphological characters of the pheretimoid earthworms in North America north of Mexico

As of 2016, sixteen species of pheretimoid earthworms have been recorded in North America north of Mexico. They belong to four genera, *Amynthas*, *Metaphire*, *Pithemera* and *Polypheretima*, with only one species for each of the last two genera. All morphological descriptions in this document apply only to the 16 species, and should not be regarded as a general description for the pheretimoid earthworms as a whole. The descriptions are not exhaustive. Only characters used for diagnosis in the study are included (Fig. 23).

Body size. The body length of these species range from around 30 cm in *Amynthas carnosus* to only 2 cm in *Amynthas minimus*. Many of the species, such as *Amynthas agrestis* and *Amynthas corticis*, have large intraspecific variations in their size. Altogether, body size in most cases is a useful but not reliable character in species identification. In addition, an earthworm specimen may be relaxed and stretched, or contracted and shortened, making it even harder to use the measurement of body length for species identification. The same goes for body width, which is frequently measured at the clitellum.

Body shape. Some species are relatively slender, such as *Amynthas corticis* and *Polypheretima elongata*; some species are relatively robust, such as *Metaphire hilgendorfi*. However, just as body size, shapes of earthworms change dramatically in an individual and the measurement is influenced by specimen anesthetization and/or fixation. The cross section is generally circular.

Color. Colorations and the associated patterns can come from pigments in the body wall, as well as colors of internal organs, tissue, coelomic fluid, and gut contents. The latter is due to semi-transparency of the body wall in many species. The general overall colorations are brown, dark red, pink, green, and greenish brown. Some species have very consistent colors; for instance, *Amynthas hupeiensis* is almost always green or dark green. Others can be more variable. For instance, *Amynthas gracilis* can be dark red, red, or light red; *Amynthas corticis* can be brown or green. Similarly to body size, coloration is a useful but not very reliable character for species identification. Moreover, colors change dramatically in formalin and ethanol, the two most common chemicals for fixation and preservation. For preserved specimens, colors also change through time, as pigments are gradually degraded in the preservatives, especially ethanol. There is no good way to preserve the color except taking photos.

Clitellum. The most apparent structure on a mature pheretimoid earthworm is its clitellum. Clitellum is always in XIV-XVI, annular, unsegmented, and usually without setae (*A. gracilis* and *A. morrisi* are two exceptions; they have setae on XVI). However, segments may still be visible on young adults. Compared to common European non-native species, which have a saddle-shaped clitellum composed of more than three segments, pheretimoid earthworms have an annular (ring-like), shorter clitellum that is also closer to the anterior end of their bodies. In the US, where most common earthworms are non-native, these differences are one of the primary diagnostic characters for pheretimoids.

Segment numbers. Numbers of segments can vary greatly even within a species. Conventionally segments are indicated using Roman numerals (e.g., VII-IX); intersegmental furrows (the visible external boundary between adjacent segments) are indicated using slashes between Arabic numerals (e.g., 6/7/8/9). The first segment with setae is segment II, and clitellum is on XIV-XVI. One can count the position of organs or pores by using the mouth or clitellum as the reference starting points.

Setae. The pheretimoid earthworms have numerous setae (as many as 150) per segment (perichaetine). These setae arrange in a ring around all segments (around the equator of all segments), except the first and last ones. The numbers of setae vary slightly from one segment to another and from one specimen to another. Within-specimen variations are usually largest in the pre-clitellar region. This type of setal arrangement is quite different from that in the earthworm family Lumbricidae, which has only eight setae per segment in four pairs (lumbricine), and from native North American species which also have a lumbricine setal arrangement.

Dorsal pores. Dorsal pores are situated in the intersegmental furrows along the mid-dorsal line. The first dorsal pore may occur in any furrow between 9/10 and 12/13, depending on the species.

Male pores. Male pores are always widely paired, ventral in XVIII in amphimictic specimens and are still present in some parthenogenetic specimens. They could be superficial (the pore is located at the exterior of the body) or inside a copulatory pouch. There are usually genital markings associated with male pores on XVIII and/or the surrounding segments.

Copulatory pouches. Copulatory pouches are invaginations of the ventral body wall surrounding the male pores. A copulatory pouch contains the primary male pore and its opening is the secondary male pore (frequently referred to as the "male pore"). A copulatory pouch can be quite shallow and confined within the body wall or large and penetrating into the coelom. Sometimes, recognition of a copulatory pouch may be more difficult than expected. A superficial male pore may look like it is within an invagination due to contraction of muscles around the male pore during specimen preservation. In contrast, strong muscle contraction may cause a copulatory pouch to be fully everted and look like a cone-shaped superficial male pore. These deformations of the male pore area make the recognition of copulatory pouches sometimes challenging.

Female pores. Female pores are usually single but occasionally closely paired, mid-ventral in XIV (the first segment of clitellum). Detection is not difficult, as the color surrounding the pores is usually lighter than that of the clitellum.

Spermathecal pores. There are one to five pairs in amphimictic individuals, usually at or close to the intersegmental furrows (the visible external boundary between adjacent segments). During copulation, sperm is passed from one individual's male pores into the spermathecal pores of a second individual. These are eventually stored in the latter's spermathecae. The numbers and positions of the pores are important species identification characters. The pores are usually ventral, 0.2–0.4 body circumferences apart, but can be lateral or dorsal in some species. They are sometimes miniature or could be buried deep in the intersegmental furrows in contracted specimens and may not be visible externally.

Genital markings. Genital markings are pre-clitellar (anterior to the clitellum) or post-clitellar (posterior to the clitellum). Pre-clitellar genital markings are usually associated with spermathecal pores; post-clitellar genital markings are usually associated with male pores. Depending on the size and shape, genital markings are sometimes referred to as tubercles, papillae or pads. Some genital markings are connected internally with accessory glands.

Spermathecae. Spermathecae are the organs that store sperm received from the "donor" individual during copulation. There are one to five pairs in amphimictic individuals. Spermathecae open to the body wall through spermathecal pores near the intersegmental furrows anterior to each segment. For instance, a spermatheca in IX usually has its spermathecal pore in 8/9.

Prostate glands. Amphimictic individuals have one pair of large, racemose prostate glands in XVIII, usually extending anteriorly and posteriorly for several segments. The ducts of prostate glands are always in XVIII, where they meet with sperm ducts (vas deferens) and connect to the male pores. The shape of the glands and the size of the glands relative to the body are important species identification characters. The relative size is usually indicated using the numbers of segments the prostate gland covers (e.g. XVII–XIX). This range, as well as the shape, varies to some degree within a species.

Caeca (Intestinal caeca). Caeca, or intestinal caeca, are a pair of pouches protruded from the gut. They originate from either XXVII or XXII and extend anteriorly for several segments. Their anterior extension is usually reported in taxonomic literatures, but intraspecific variation occurs. Caeca may be simple (finger-like), with shallow incisions, or manicate (hand-shaped, i.e., complex with several finger-like, deep incisions). These shape differences are important for species identification.

Accessory glands. Some species or individuals have accessory glands, extending from the body wall into the coelom. When present, these glands are usually connected to corresponding genital markings externally.

APPENDIX B

Earthworm specimen preparation protocol

1. Place living specimens in clean water to remove soil particles attached to the body surface.
2. Transfer specimens to 10–15% ethanol. Wait until the earthworms stop moving. It usually takes about 10 min. If the earthworms are still moving after 10 min, add more ethanol.
3. Once the earthworms stop moving, transfer them into a jar or vial filled with 70–80% ethanol. The volume of ethanol *vs.* earthworm should exceed the ratio 4:1. It is important to have enough ethanol in the container. Body fluids from earthworms will dilute the solution, and a resulting ethanol concentration lower than 70% will cause maceration of specimens.
4. Move closed vial upside-down and back after 12–36 hours for even distribution of ethanol.
5. Replace ethanol with new solution once between day 3 and 5.
6. Replace ethanol roughly at the end of week 2.
7. Replace ethanol again roughly at the end of week 4. At this point, the specimens are usually ready for shipment and long-term preservation.