

Two New *Eisenia* Species from South Korea Similar to *E. koreana* and Comparable to *Eisenoides* from USA (Oligochaeta: Lumbricidae)

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ABSTRACT

New Korean lumbricids are described: *Eisenia gaga* Blakemore sp. nov. from remote Gageodo Island and *E. sindo* Blakemore, sp. nov. from an island at Incheon. Both are comparable to *Eisenia koreana* (Zicsi, 1972) from near Pyongyang, North Korea. A remarkable yet previously unrecognized similarity to American earthworm *Eisenoides carolinensis* (Michaelsen, 1910) is discussed, but synonymy is rejected on tenuous morphological grounds (form of nephridial vesicle bladders) with taxonomy supported by objective molecular data (mtDNA cytochrome c oxidase subunit 1 barcodes).

Keywords: Asian biodiversity, soil fauna, endemic invertebrates vs. invasive species

INTRODUCTION

Lumbricidae is a relatively large holarctic family of mostly terrestrial earthworms comprising approximately 670 valid taxa from a total of 1,130 names in 63 genera (Blakemore, 2008a). Natural distribution is from North America (e.g., *Bimastos lawrenceae* Fender, 1994 on Vancouver Island, Canada) throughout boreal Europe and the Middle East to far-eastern Asia (e.g., *Eisenia anzac* Blakemore, 2011 from Japan). Commonly transported lumbricids comprise just 33 species from a total of about 150 other cosmopolitan earthworms as detailed in Blakemore (2010). Of approximately 100 earthworm species currently known from Korea (Blakemore, 2008b), a minority dozen are Lumbricidae, most introduced exotics, some shared by neighbouring countries. Only *Eisenia koreana* (Zicsi, 1972) is thought wholly endemic, albeit unrecorded for the last 40 years. Here two new Korean lumbricids are described that are comparable but separable genetically, leaving *E. koreana* as yet unconfirmed.

Specimens, lodged in National Institute of Biological Resources (NIBR), are described in the author's usual style (e.g. Blakemore, 2010). Cytochrome c oxidase subunit 1 (COI barcode) sequences are appended with analyses via megaBLAST (www.blast.ncbi.nlm.nih.gov/BLAST.cgi). Abbreviations are rhs, right hand side; lhs, left hand side; TP, tuberculae pubertates.

SYSTEMATIC ACCOUNTS

Order Megadrilacea Benham, 1890

Family Lumbricidae Rafinesque-Schmaltz, 1815

Genus *Eisenia* Malm, 1877 (type-species: *Enterion fetidum* Savigny, 1826)

Eisenia gaga sp. nov. (Figs. 1, 2)

Material examined. Holotype, NIBR IV IV0000245509 (dissected and figured, providing DNA-WM1) (Fig. 1); eight paratypes, NIBR IV0000245510–IV0000245517 two dissected (specimen #2 WM2) and one photographed (#5 WM3) (Fig. 2), all fixed in 75% EtOH. Collected 26 Jan 2012 by Park TS, Seo HY from damp leaf litter on slopes of Mt. Doksil, 34° 4'32.73N, 125° 6'31.88E; summit 639 m, on Gageodo Island in Yellow Sea of South Korea. Found with a damaged *Amyntas gageodo* Blakemore, 2012 (NIBR IV0000245518) – see Blakemore et al. (2012).

Etymology. After historical name for Gageodo Island, “Gaga” meaning “beautiful.”

Description. Body square in section after segment 12 with setae at each rounded corner; colour bleached in alcohol. Lengths 80–105 mm (holotype H 100, paratype P1 80, P2 100, P3 105). Segments 124–162 (H 162, P1 124, P2 145, P3 120). Prostomium epilobic, narrow. Setae closely paired. Tu-

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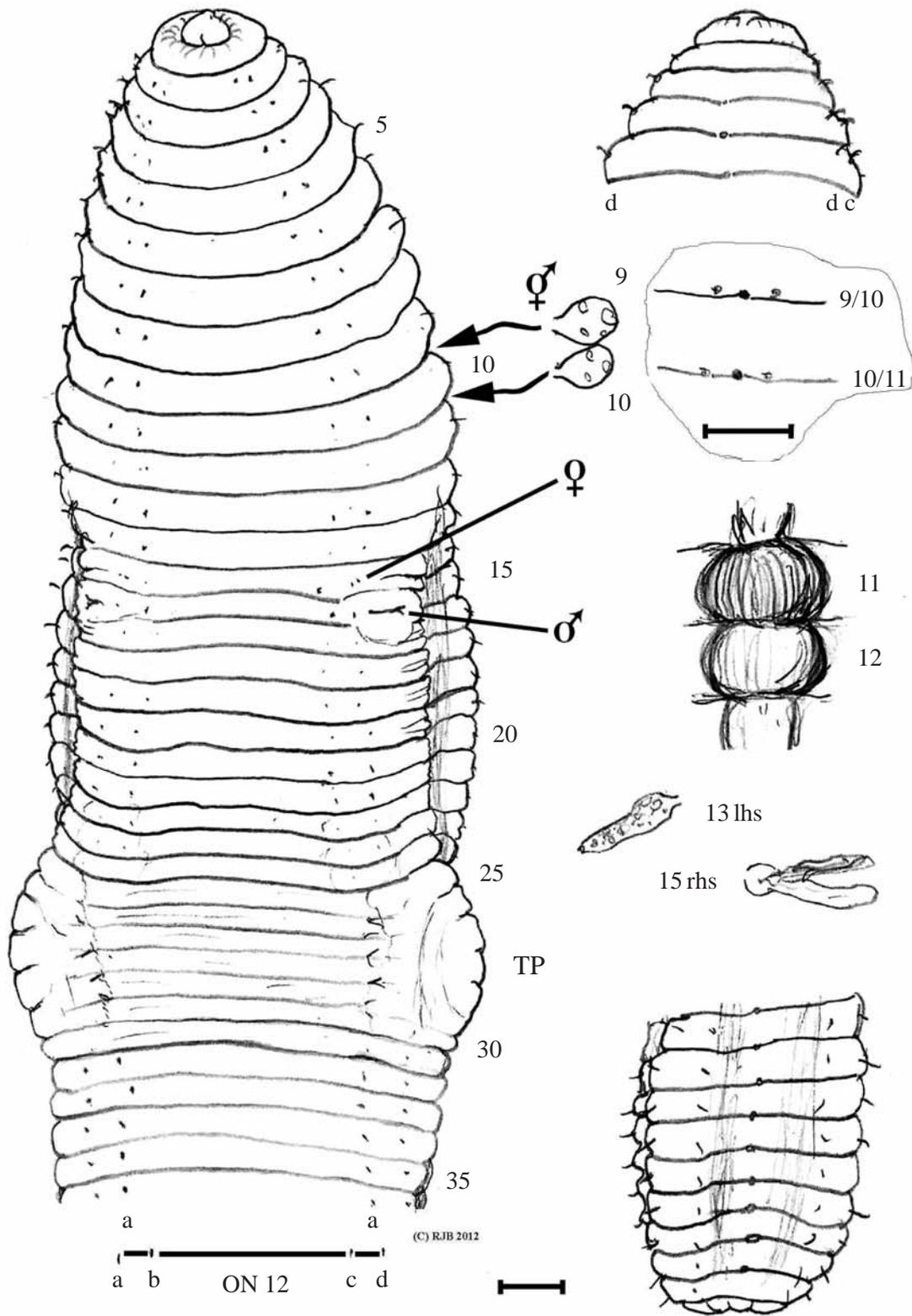


Fig. 1. *Eisenia gaga*: holotype (contracted specimen #1 IV000245509) ventral view of anterior, prostomium and dorsal pores, enlargement of spermathecal and dorsal pores; spermathecae *in situ*, sketch of calciferous glands in 11 and 12 ovaries in 13 lhs, nephridium in 15 rhs; posterior and pygidium, and actual setal ratios on segment 12. rhs, right hand side; lhs, left hand side. Scale bars=1 mm.



Fig. 2. Photo of dorsum of specimen #5 (IV000245513) (by Blakemore RJ on Hanna Bae's camera).

midity obscures ventral setae in some of 11, 13, 14, 16, 17 and 22–31 and on 10 & 11 laterally; ab on 14 & 15 are slightly displaced. Clitellum pale 24,25–30,31, typically 25–31. Tubercula pubertatis on ca. 27–29 wide of setal b line on each side. Dorsal pores from 3/4 (minute), open from 4/5. Nephropores sporadically visible above d setae. Spermathecal pores in 9/10/11 close to mid-dorsal line. Female pores 14; male pores 15 with tumid mounds impinging slightly into adjacent segments.

Internally, septa 5/6–14/15 slightly thickened. Spermathecae round in 9 and 10. Testis and funnels free, iridescent in 10 and 11. Seminal vesicles in 9–12 those in (9 and) 10 smaller and filled with brown bodies. Ovaries small, flattened and tapering (like a folded hand) in 13. Ovisacs small anteriorly in 14 on 13/14. Hearts in 7–11. Nephridial bladders simple, sausage-shaped (in all segments inspected). Calciferous glands large and moniliform in 11 (vascularized) and in 12. Crop in 15 and, less dilated, in 16; muscular gizzard in 17–18. Low but broad intestinal typhlosole develops from around 20–22. Gut contents, fine silty soil and organic matter and gizzard had a few largish 'crop stone' grits. Apart from 'brown bodies', no evidence of parasites was observed in the coelom, blood vessels or other organs.

Remarks. *Eisenia gaga* differs slightly from Zicsi's account (1972) of *E. koreana* where he gives shorter biometry and pro-epilobous prostomium. Dorsal pore onset is perhaps just

in advance. Setal tumescences are apparently not as extensive in current specimens (different preservation methods?) although setae ab were here partly occluded by tumidity. Zicsi (1972) did not note a typhlosole. DNA barcodes in Appendix 1 define species.

***Eisenia koreana* (Zicsi, 1972)**

Eiseniella koreana Zicsi, 1972: 129.

Eisenia koreana: Easton, 1983; Blakemore, 2004: 98; 2008a: 39.

Type material. Budapest University, holotype Ei-8 and eleven paratypes registered as Nr. 7000, from a brook bed behind Pyongyang Zoo (Mt. Taesong), North Korea.

Diagnosis. Size 45–52 mm by ca. 3 mm wide with 87–123 segments. Grey green in life, pale laterally in 10–11. Pro-epilobic. Posterior squarish. Dorsal pores from 4/5. Setal distances stated as "ab=bc" in Zicsi (1972: 129) was likely a typing error for ab=cd, with aa > bc and dd < 1/2U. Setae ab tumid on 10, 16, 17, 23, 26–30 plus on cd in 10 and 11. Spermathecal pores in 9/10/11 dorsally but spermatophores also present. Clitellum 25–31, TP 27–29. Holandric with seminal vesicles in 9–12 (smaller in 10). Calciferous glands in 11 & 12. Crop in 15–16, gizzard in 17–18.

Remarks. The stated reason Zicsi (1972: 131) attributed his species to the semi-aquatic genus *Eiseniella* Michaelsen, 1900 was for its inferred amphibian habitat, although it was later transferred (e.g., Easton, 1983) to *Eisenia*. And whereas Zicsi had primarily compared his taxon to "meroandrisch" *Eisenia kucenkoi* (Michaelsen, 1902) from "Ost-Turkestan" (=Xinjiang), a remarkable similarity to *Eisenoides carolinensis* from Appalachian southeastern USA was hitherto unrecognized. Both are squarish, with clitella and TPs having same range, the spermathecae are dorsal in 9/10/11 and calciferous glands are in 11 & 12. Similarity extends to a report (Gates 1956: 19) of spermatophores on an *Ei. carolinensis* worm attached at 25/26 and on 27, as also found by Zicsi (1972) in *E. koreana* at 22/23. Exact relationship of the taxa remains unclear.

Nearby at De-sang San, Zicsi (1972: 130) reported *Eisenia nordenskiöldi* (Eisen, 1879) [corr. Blakemore (2008a: 39) ex *nordenskiöldi* non "*nordenskiöldi*" as per Blakemore (2004: 98)] also *Aporrectodea caliginosa* (Savigny, 1826).

***Eisenia sindo* sp. nov. (Fig. 3)**

Material examined. Holotype, H IV0000246435 (sample #3 mature, dissected; DNA WO25) (Fig. 3); paratype P1 IV0000246436 (sample #4 WO26) and paratypes IV0000246437 (12 matures, 5 juveniles, 5 immatures, and two hatchlings plus three fragments), all fixed in 75% EtOH. Collected 4

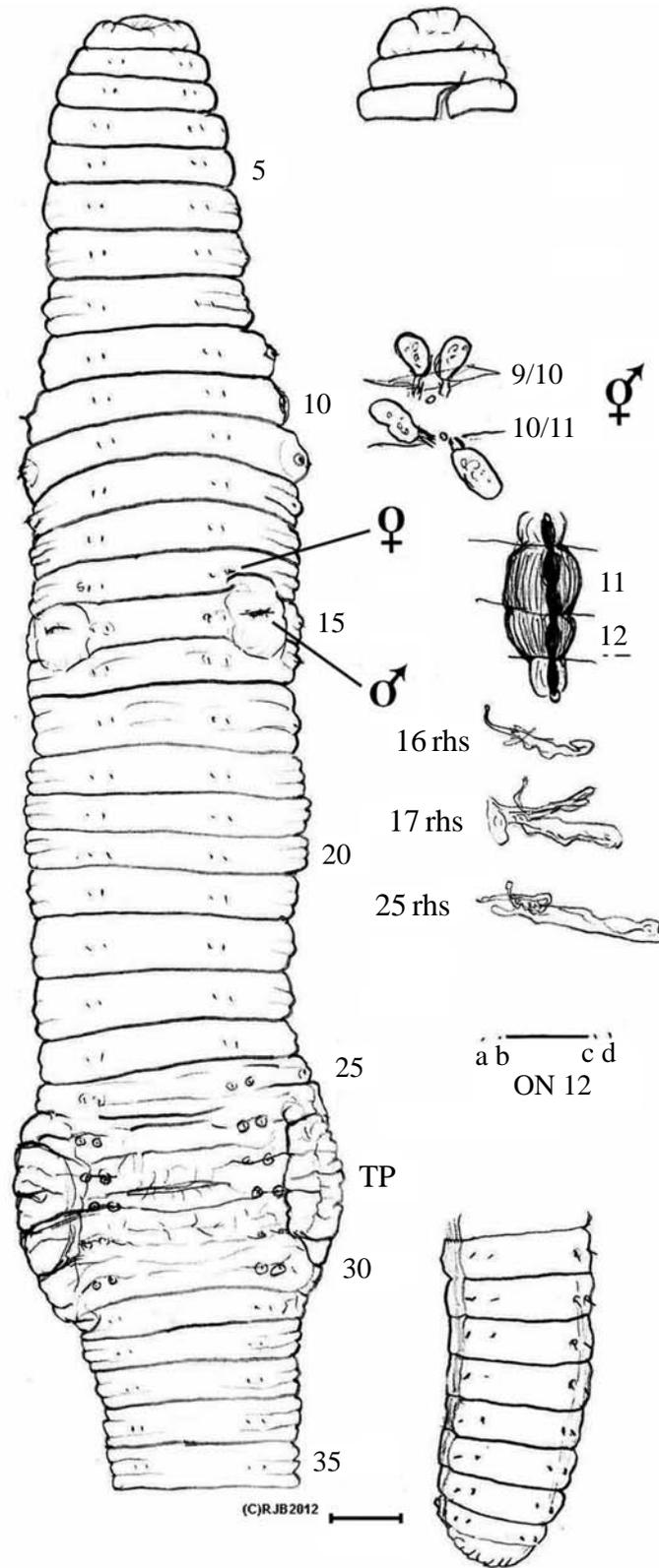


Fig. 3. *Eisenia sindo*: holotype (relaxed specimen IV0000246435) ventral view of anterior and prostomium; spermathecae *in situ*, sketch of calciferous glands in 11 & 12, vesiculate nephridia in 16, 17 & 25 rhs; posterior and actual setal ratios on 12. rhs, right hand side; TP, tuberculae pubertates. Scale bar=1 mm.

May 2012 by Blakemore RJ from mud beside creek between paddy fields just 200 m North of Sido bridge on Sido Island, 37° 53'045"N, 126° 43'974"E, Incheon.

Description. Body tapering, flattened in posterior (somewhat rectangular); palid colour with light brown pigment. Lengths 100–120 mm (H and P1). Segments 148–152 (P1 and H). Prostomium epilobic wide. Setae closely paired, obscured by tumidity ventrally on 16 and around clitellum; cd setae tumid on 10 and 11 laterally. Clitellum pale 25–31. TP lateral on ca. 26,27–29. Dorsal pores from 4/5. Spermathecal pores paired in 9/10/11 near mid-dorsal line. Female pores on 14, male pores on mounds in 15.

Septa 5/6–8/9 slightly thick. Spermathecae inseminated, elongate in 9 and 10. Testis and funnels free, iridescent in 10 and 11. Seminal vesicles in 9–12 those in 10 small. Ovaries small in 13, ovisacs vestigial in 14. Hearts in 7–11. Nephridial bladders undeveloped or simple, mostly elongate sausage-shaped. Calciferous glands moniliform in 11 & 12. Crop in 16, muscular gizzard in 17–18. No typhlosole. Gut contains silty mud.

Remarks. Superficially similar to *E. gaga*, the current species is separated on small qualitative morphological differences (e.g., biometry, width of prostomium, shape of spermathecae, fewer nephridial bladders and lack of typhlosole) but mainly on their objective DNA barcode profiles (Appendix 1). *Eisenia sindo* differs from *E. koreana* in much the same way that *E. gaga* does whilst also complying superficially with *Eisenoides carolinensis*. However, due to proximity, it is more likely to be genetically closer to *E. koreana* than *E. gaga* is.

DISCUSSION

The North American genus *Eisenoides* Gates, 1969 [type-species *Allolobophora lönnbergi* Michaelsen, 1894 corr. Blakemore (2008a: 42) to *lönnerbergi* non “lönnbergi”] was defined by Gates on its supposedly distinctive U-shaped nephridial bladders and, although these are somewhat variable, even intraspecifically (Blakemore, 2010), this is the only substantial morphological difference between similar genera since both *Eisenia* and *Eiseniella* are usually characterized by simple, sausage-shaped nephridial bladders.

Gates (1955: 10) said that seminal vesicles in segment 10 in one (or more?) of *Eisenia pearsei* Stephenson, 1933 types was all that distinguished it from *Ei. carolinensis* and that this did not warrant taxonomic recognition. Dr. Csaba Csuzdi (personal communication 21st–24th Feb 2012) informs that the single type of *Ei. carolinensis* in Hamburg, and the *Ei. pearsei* paratypes in London (its holotype being desiccated) have nephridial bladders that are indeed proclined, U-shaped

(this information currently unpublished). Nephridial bladders were not explicitly described by Zicsi (1972), yet in the other two *Eisenia* species newly described herein they appear elongate or sausage-shaped, presumably in *Eisenia koreana* too, and solely on this feature they differ from *Eisenoides carolinensis* (Michaelsen, 1910). In other respects the present specimens and *Eisenia koreana* tolerably match earlier descriptions of *Eisenoides carolinensis* which is a remarkable morphological convergence co-incidence for taxa so widely separated geographically.

Support for their divergent separation is decided by DNA barcode comparison of types of *E. gaga* and of *E. sindo* with a voucher of *Eisenoides carolinensis* (Appendix 1). Dr. Csuzdi, a colleague of original author Dr. András Zicsi, also mentioned that primary types of *Eisenia koreana* from Mt. Taesong that he inspected in Budapest, due to their age and formal preservation, are unlikely to yield meaningful DNA data. Possibly *E. sindo* is closer with *E. koreana*, nevertheless, conspecificity can only be determined by DNA from its topotypes which are not currently accessible. In view of the DNA results for *E. sindo* and *E. gaga*, it is highly likely the three taxa form an *Eisenia koreana* species-complex with each speciating in and seeming to prefer localized moist or semi-aquatic habitats.

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Appendix 1. DNA cytochrome c oxidase subunit 1 data and BLAST analysis.

> WM1 *Eisenia gaga* holotype (INV 0000245509) (Fig. 1)

TAAGTGTGGATAGAGGATTGGGTCCCCCTCCCCCGCTGGATCAAAAAATGAAGTATTAAGATTTTCGATCTGTTAGGAGTATGGTAATTGCCCTGCT
AAAAGTGGTAAAGAAAGGAGGAGAAGAACTACTGTGATTACTACAGCTCAAACAAATAGGGGAATTCGTTCAAGTCGTAATCCTTTTCATCGTATAT
TAATAACAGTCGTAATGAAGTTGATGGCACCTAAGATTGAGGATGCACCTGCTAAGTGAAGAGAGAAAAATGGCCAGATCTACTGAGGGGCCAGAG
TGTGCGAGATTTCTAGATAGGGGGGATAGACAGTTCATCCTGTTCCAGCTCCTTTTTCTACAGCAGCCGAGGATACTAATAAAATAAGAGATGGT
GGAAGTAATCAAAATCTTATATTATTTAATCGGGGGAATGCCATGTCAGGGGCACCAAGTATTAGTGGGAGAAGCCAGTTTCCAATCCTCCGATA
AATACAGGTATCACAAGAAAAAATTATTACAAATGCATGCGCTGTAACGATAGTGTATATAGTTGATCGCTACCTAAAAATGCTCCAGGTTGGC
TAACTCAATTCGGATTAGGAGGCTTATTCCTGCACCCACTATACCAGCTCAAACCTCTAGAATAAAATATA

> WM2 *E. gaga* paratype1 (INV 0000245511 2nd dissected specimen)

AAGTGTGGATAGAGGATTGGGTCCCCCTCCCCCGCTGGATCAAAAAATGAAGTATTAAGATTTTCGATCTGTTAGGAGTATGGTAATTGCCCTGCT
AAAAGTGGTAAAGAAAGGAGGAGAAGAACTACTGTGATTACTACAGCTCAAACAAATAGGGGAATTCGTTCAAGTCGTAATCCTTTTCATCGTATAT
TAATAACAGTCGTAATGAAGTTGATGGCACCTAAGATTGAGGATGCACCTGCTAAGTGAAGAGAGAAAAATGGCCAGATCTACTGAGGGGCCAGAG
TGTGCGAGATTTCTAGATAGGGGGGATAGACAGTTCATCCTGTTCCAGCTCCTTTTTCTACAGCAGCTGAGGATACTAATAAAATAAGAGATGGT
GGAAGTAATCAAAATCTTATATTATTTAATCGGGGGAATGCCATGTCAGGGGCACCAAGTATTAGTGGGAGAAGCCAGTTTCCAATCCTCCGATA
AATACAGGTATCACAAGAAAAAATTATTACAAATGCATGCGCTGTAACGATAGTGTATATAGTTGATCGCTACCTAAAAATGCTCCAGGTTGGC
TAACTCAATTCGGATTAGGAGGCTTATTCCTGCACCCACTATACCAGCTCAAACCTCTAGAATAAAATA

> WM3 *E. gaga* paratype (INV 0000245513 photographed specimen) (Fig. 2)

AATAAGTGTGGATAGAGGATTGGGTCCCCCTCCCCCGCTGGATCAAAAAATGAAGTATTAAGATTTTCGATCTGTTAGGAGTATGGTAATTGCCCT
GCTAAAAGTGGTAGAGAAAGGAGGAGAAGAACTACTGTGATTACTACAGCTCAAACAAATAGGGGAATTCGTTCAAGTCGTAATCCTTTTCATCGT
ATATTAATAACAGTCGTAATGAAGTTGATGGCACCTAAGATTGAGGATGCACCTGCTAAGTGAAGAGAGAAAAATGGCCAGATCTACTGAGGGGCCA
GAGTGTGCGAGATTTCTAGATAGGGGGGATAGACAGTTCATCCTGTTCCAGCTCCTTTTTCTACAGCAGCCGAGGATACTAATAAAATAAGAGAT
GGTGAAGTAATCAAAATCTTATATTATTTAATCGGGGGAATGCCATGTCAGGGGCACCAAGTATTAGTGGGAGAAGCCAGTTTCCAATCCTCCG
ATAAATACAGGTATCACAAGAAAAAATTATTACAAATGCATGCGCTGTAACGATAGTGTATATAGTTGATCGCTACCTAAAAATGCTCCAGGTT
GGCTAACTCAATTCGGATTAGGAGGCTTATTCCTGCACCCACTATACCAGCTCAAACCTCTAGAATAAAATAT

> WO25 *Eisenia sindo* holotype (IV0000246435) (Fig. 3)

ACCTTATACTTTATTCTTGGGGTTTGAGCCGGAATAGTAGGGCGTGAATAAGCCTCTTAATCCGAATCGAGCTAAGACAGCCTGGAGCATTCTG
GGAAGAGACCAGCTATATAATACCATTGTTACAGCTCATGCGTTTCGTAATAATCTTTTTCTTGTAACTGATTTATTGGGGGTTTCGGCAACTG
ACTTCTCCCATTAATATTGGGGGCTCCGACATAGCATTCCCTCGTTAAATAATATAAGATTCTGGCTACTTCCCTTCCCTTATTCTACTAGTCT
CATCAGCAGCGTTGAGAAAGGGCGGGAACAGGTTGAACTGTGTACCCGCCCTATCTAGAAATCTGCACACGCTGGGCCATCAGTAGACCTG
GCTATTTCTCCCTTCATTTAGCGGGTGCCTGCTATTCTAGGAGCCATCAATTTTACTACTACAGTTATCAATATACGATGAAGAGGGTTACGTCT
TGAACGAATTCCTACTTTGTGTGAGCTGTAGTAATTACTGTTGTTCTTCTTCTCTCCCTACCAGTTCTAGCAGGAGCAATTACCATACTTCTAA
CAGATCGAAACTTAAACACTTATTCTTTGACCCCGCAGGAGGTGGAGATCCTATTCTTTATCAACATCTATT

> WO26 *E. sindo* paratype P1 (IV0000246436)

CCTTATACTTTATTCTTGGGGTTTGAGCCGGAATAGTAGGGCGTGAATAAGCCTCTTAATCCGAATCGAGCTAAGACAGCCTGGAGCATTCTGG
GAAGAGACCAGCTATATAATACCATTGTTACAGCTCATGCGTTTCGTAATAATCTTTTTCTTGTAACTGATTTATTGGGGGTTTCGGCAACTGA
CTTCTCCCATTAATATTGGGGGCTCCGACATAGCATTCCCTCGTTAAATAATATAAGATTCTGGCTACTTCCCTTCCCTTATTCTACTAGTCT
ATCAGCAGCGTTGAGAAAGGAGCGGGAACAGGTTGAACTGTGTACCCGCCCTATCTAGAAATCTGCACACGCTGGGCCATCAGTAGACCTGG
CTATTTCTCCCTTCATTTAGCGGGTGCCTGCTATTCTAGGAGCCATCAATTTTACTACTACAGTTATCAATATACGATGAAGAGGGTTACGTCT
GAACGAGTTCCACTATTTGTGTGAGCTGTAGTAATTACTGTTGTTCTTCTTCTCTCCCTACCAGTTCTAGCAGGAGCAATTACCATACTTCTAAC
AGATCGAAACTTAAACACTTATTCTTTGACCCCGCAGGAGGTGGGGATCCTATTCTTTATCAACATCTATT

Barcode results and BLAST conclusions

1. BLASTn alignments: > 99% WM1=WM2=WM3, i.e., samples of same species.
2. megaBLAST of WM1 *E. gaga*: - no match better than 83% for various worm species on GenBank [GU013824 *A. caliginosa* and GU014206 "Acanthodrilidae sp." (sic!) by S. James], i.e., no similar species uploaded (therefore this presumably the first COI barcode report for *Eisenia koreana* or its ilk). GenBank upload imminent.
3. BLASTn alignment WM1 *E. gaga* vs. FJ214226 "*Eisenia carolinensis*" [sic] from Stateline, Louisiana partial COI data mined from GenBank: - no better than 79% agreement, i.e., not same species - assuming correct identification of FJ214226.
4. megaBLAST of FJ214226 *E. carolinensis*: - best match 94% with an unidentified Romanian lumbricid (!), or < 85% similar to specimens identified as "*Eisenia fetida*," i.e., no named specimen closely similar to *E. carolinensis* yet uploaded on GenBank. [Note: one website ([http://bold.ala.org.au/index.php/display/item/GBAN2219-09/July, 2012](http://bold.ala.org.au/index.php/display/item/GBAN2219-09/July,2012)) has this code and data identified as "*Eisenia carolinensis*" from Sao Paulo by Claudio Oliveira, but also recoded as "female" and "immature", so is highly dubious].
5. BLASTn alignment WM1 *E. gaga* vs. WO25 *E. sindo*: - Identities=530/646 (83%), therefore the objective conclusion is these specimens are different species.
6. BLASTn alignment WO25 vs. WO26 *E. sindo*: - Identities=649/655 (99%), i.e., tolerably same species.
7. megaBLAST WO25 *E. sindo*: - best match 83% of some Lumbricidae from GenBank, i.e., no similar species yet identified.
8. BLASTn alignment WO25 *E. sindo* vs. FJ214226 "*Eisenia carolinensis*": - Identities=516/652 (79%), i.e., these are different species with above provisos.