



A new Korean earthworm (Oligochaeta: Megadrilacea: Megascolecidae)*

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Abstract

Amyntas gageodo Blakemore, **sp. nov.** is described from small Gageo-do Island, offshore to the southwest of the Korean Peninsula in the Yellow Sea. It is an octothecal species (four pairs of spermathecae) comparable to Japanese *Amyntas carnosus* (Goto & Hatai, 1899) (synonyms: Korean *kyamikia* Kobayashi, 1934, *monstrifera* Kobayashi, 1936, *sangyeoli* Hong & James, 2001, *youngtai* Hong & James, 2001, *kimhaeiensis* Hong & James, 2001, *sinsiensis* Hong & James, 2001, *baemsagolensis* Hong & James, 2001, Taiwanese *monsoonus* James *et al.*, 2005) and to Chinese *A. pingi* (Stephenson, 1925) (synonym: *fornicata* Gates, 1935). Species associations in its forest litter habitat on the remote island included terrestrial leeches, planarian flatworm predators and other worms. MtDNA COI barcodes indisputably identify types of *A. gageodo* as a new model for future Korean earthworm species characterizations.

Key words: *Amyntas*, pheretimoid, island biodiversity, Asian endemic invertebrates.

Introduction

Surveys of invertebrates on Gageo-do Island (~9.2 km²) were conducted by the National Institute of Biological Resources in 2011. Amongst the animals collected were a manifestly new pheretimoid earthworm species as described in this paper.

Materials and Methods

Specimens were collected by digging and hand-sorting from leaf litter and humic soil. Taxonomic determinations by the senior author follow the style, systematics, methodology and conventions in Blakemore (2000, 2002, 2010b). Small tissue samples were taken from non-essential posterior segments as per Blakemore *et al.* (2010) for DNA extraction and cytochrome-c oxidase subunit 1 (COI) barcoding by MacroGen Inc., Seoul, with results presented in an Appendix.

Taxonomic Results

Amyntas gageodo Blakemore, **sp. nov.**

Diagnosis: Size 150–170 mm. Spermathecal pores lateral in 5/6/7/8/9. Dorsal pores from 12/13. Genital markings as closely paired, mid-ventral, presetal discs in 8–10, 11 and in 17, 18–20 plus more widely paired postsetal discs in 8–9 and 18–19, 20 (total numbering up to twenty six with some markings unpaired unilateral, or all more widely paired). Intestinal caeca simple from 27.

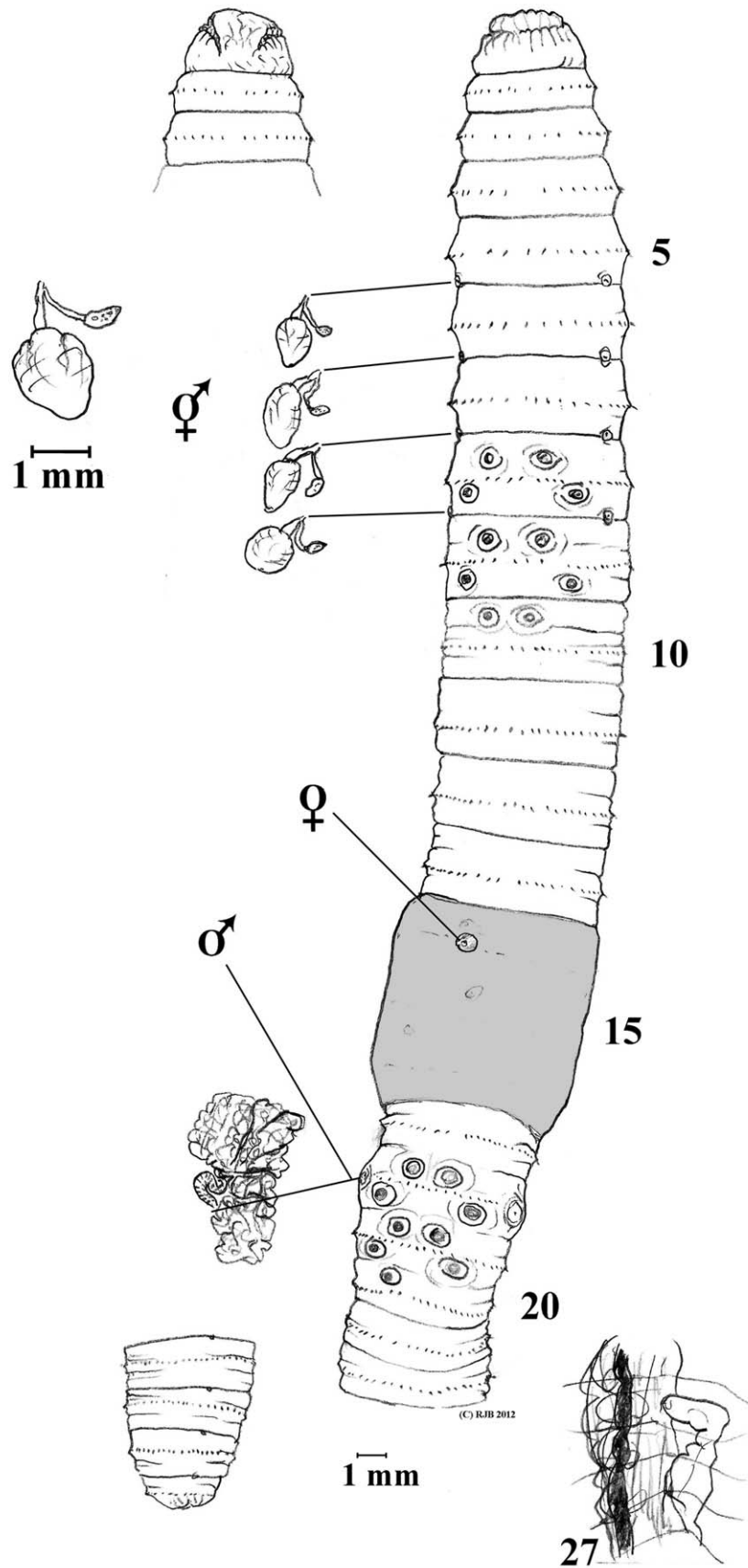


FIGURE 1. *Amynthus gageodo* sp. nov., holotype showing prostomium, ventral view of body with spermathecae and prostates *in situ* (genital markings nearby), posterior pygidium, and intestinal caecum in 27; plus an enlargement of 6lhs (left-hand-side) spermatheca.

Distribution: South Korea, Gageo-do Island, on slopes of Mt Doksil (34° 4' 32.73 N, 125° 6' 31.88 E; summit 639m).

Material inspected: Holotype (H), NIBR IV0000245037, mature specimen fixed in formalin and stored in 70% ethanol (EtOH), here dissected and figured (Fig. 1), collected 27.VII.2011 by Dr H.-Y. Seo and T.S. Park; Paratype (P1), IV0000245038, undissected mature, highly contracted after fixation in 100% EtOH, with a small tissue sample taken for DNA amplification and COI barcoding analysis, collection details as for H; P2, IV0000245039, ditto P1; P3, IV0000245040, ditto P1; P4, IV0000245041, undissected mature specimen with irregular annuli in segments 19 and 20, in same batch as holotype (H); P5–P11, IV0000245042, seven mature specimens fixed in formalin in a separate jar with same collection information except that collection date was 26.IX.2011.

Etymology: Noun in apposition after the Island's current name (previously Gaga-do meaning "Beautiful Island" also called Soheuksan-do or Little Heuksan Island from the Japanese colonial period; since 1896 it is known as Gageo-do or "Liveable Island").

Description: Body length 155 mm (holotype H), ca. 150–170 mm paratypes, segments 91 (H). In life, a dark greyish-brown; preserved, dorsum dark grey to ca. 23 then brown with lighter grey clitellum and paler ventrum. First dorsal pore 12/13. Setae 66–74 per segment behind segment 12 (H, P1). Spermathecal pores ca. 0.3 circumference apart in 5/6/7/8/9. Genital markings closely paired mid-ventral and presetal disks in 8–9, 10, 11 and in 17, 18–19, 20 plus more widely paired and postsetal in 8–9 and 18–19, 20; thus usually eight or more in both spermathecal and male fields, with some markings unpaired, unilateral; occasionally all presetal markings in 8–10 paired as widely as the postsetal ones in 8–9 e.g. in P2 and one of paratypes P5–11. Maximum markings per worm were twenty-four in one of the latter paratypes (150 mm long posterior-amputee) that had two unilateral markings (in 8 and 20), thus indicating potentially thirteen pairs or twenty-six markings in total.

Internally, small sessile glands correspond to the external genital markings. Other accessory glands found neither in spermathecal nor male fields. The pharyngeal mass extends to 4 and tufted meroic nephridia are in forests on anterior of 5/6 and 6/7. Septa none especially thickened, 8/9/10 are aborted; 10/11/12/13 are slightly stronger, thereafter membranous. Spermathecae in 6–9 with slender, clavate diverticula (terminal bulbs inseminated) each about half the length of the duct plus saccular ampulla combined. Dorsal vessel single; hearts in 10–13. Testis in small sacs, paired anteriorly in 10 and 11; seminal vesicles moderately large in 11 and 12; pseudovesicles on posterior of 12/13 and 13/14 (the latter possibly vestigial ovisacs). Ovaries and funnels in 13. Intestine origin in 16 with simple caeca from 27 extending forward to 24; typhlosole not found. Gut contains organic debris suggesting a detritivorous diet.

Remarks: The current species appears particularly close to *A. carnosus* (Goto & Hatai, 1899) from Japan, Korea and probably China which is itself comparable to Chinese *A. pingi* (Stephenson, 1925) that is provisionally maintained separately as per Blakemore (2002, 2003a, 2003b, 2008, 2010b, 2012). Kobayashi (1936a) studied two Japanese specimens of *A. carnosus* (sent with tacit agreement of S. Hatai, the original author) plus 204 Korean specimens having variations of up to eight markings in some of 7, 8–9 and six or fewer in 18–19. Kobayashi (1936a) also placed his *Pheretima kyamikia* Kobayashi, 1934 in synonymy as soon as this became apparent – as any good scientist would – and he considered the genital marking variations he encountered encompassed those in *A. pingi*. Indeed, Chen (1933: 231) had allowed papillae as rarely absent or one to three pairs (occasionally up to five pairs in total) near male and spermathecal pores in his concept of *A. pingi*, while having fourteen or fewer markings seems permissible for *A. carnosus* proper (Blakemore 2012). In contrast, *A. gageodo* markings may total up to twenty-six, with eight to twelve preclitellar markings and a similar number and arrangement in the corresponding postclitellar male pore region. On this feature alone, it is considered a species new to science with unambiguous objective confirmation provided via DNA COI barcoding of its types (Appendix).

Habitat and Species Associations: Humid litter layer of dense silver magnolia (*Magnolia* sp.) and sloumi (*Daphniphyllum macropodum*) evergreen forest. Terrestrial leeches were identified as *Orobdella* sp. while unidentified earthworm-feeding *Bipalium* sp. planaria were also present (specimens in NIBR). Lizards were common on site and the island has an abundant avifauna (Anonymous 2010), both groups likely predators. An *Eisenia* sp. lumbricid, possibly an introduced species that is to be described elsewhere, was found sympatrically on the island.

Behaviour: Rapid 'snaking' escape movement when its litter habitat is disturbed. Evidence from a later NIBR survey in 2012 indicates that this worm enters some form of diapause during the coldest winter period.

Discussion

Of the 950 known Asiatic pheretimoid species (Blakemore unpublished), approximately 130 share spermathecae in 5/6/7/8/9 and simple, non-maniculate intestinal caeca. Korea has about 100 named earthworms, and a dozen octothecal *Amyntas* Kinberg, 1867 are from Japan and/or Korea with ten or so names claimed from just the Korean Peninsula, not all valid (Blakemore 2003a, 2003b, 2008, 2012). These ten are: *Pheretima kyamikia* that was soon shown by Kobayashi (1936a) to be a synonym of prior *Amyntas carnosus*, *A. monstiferus* (Kobayashi 1936b: 168) which seems to be another synonym (Blakemore 2012), then *A. morii* Kobayashi, 1938 and *A. murayamai* Kobayashi, 1938. Hong & James (2001) added five “new” names [viz. *sangyeoli*, *youngtai* (with its segments miscounted), *kimhaeiensis*, *sinsiensis* and its synonym *baemsagolensis*] which were mainly mutually compared, with none differing sufficiently from variations permitted in Kobayashi’s (1936, 1937) or Ohfuchi’s (1937) earlier redescriptions of *A. carnosus*, thus they too enter its synonymy ambit. This conclusion is surprisingly still unrecognized in Korea despite being determined by Blakemore (2003a, 2003b, 2008, 2012) as independently confirmed by Shen *et al.* (2003, 2005). Finally, Korean *A. sangumburi* Hong & Kim, 2002 (currently *incertae sedis* as its segments are also miscounted in these authors’ fig. 4) which, in its smaller size and absence of markings (indicating delayed development?), especially resembles both *A. toriii* (Ohfuchi, 1941) as well as *Pheretima fornicata* Gates, 1935, an erstwhile synonym of *A. pingi* also redescribed by Shen *et al.* (2003). Taiwanese *Amyntas monsoonus* James *et al.*, 2005 is possibly related to *A. carnosus* as it has the same classical markings in 7–9, but its spermathecae are said to be in 6/7/8/9 – as permitted in two of the 204 of Kobayashi’s (1936a) Korean specimens, leading him, and current revisions, to accept this as a rare interspecific variation. (Note: this was pointed out to the authors’ in a referee review but was seemingly ignored – see Blakemore 2010a Appendix II). *A. monsoonus* is, nevertheless, most often regarded as a junior synonym of *A. tungpuensis* Tsai *et al.*, 1999, e.g. by Tsai *et al.* (2006, 2009).

The relationships and synonymies of all of these names remain to be fully worked out from first principles of priority and typification, although *Pheretima morii* Kobayashi, 1938 is generally considered a junior synonym of *Amyntas corticis* (Kinberg, 1867) along with *Perichaeta heteropoda* Goto & Hatai, 1898 and *Megascolex diffringens* Baird, 1869—which appear both to be yet retained by some contemporary Korean workers. Nevertheless, none of these prior taxa possess the extensive size or range of genital markings display as in *A. gageodo*. Neither was a close molecular match yet found from COI barcode comparisons (Appendix).

Whereas combined morphological and molecular characterization is not a mandatory requirement of the current ICZN (1999) code, it is a most helpful and powerful tool for unambiguous, objective and rapid identification, especially when based on a species’ types and then preferably the holotype (Blakemore & Kupriyanova 2010; Blakemore *et al.* 2010). Formalin prevented holotype use in this case.

The presence on Gageo-do of a closely associated and large earthworm-predatory leech genus also found in Japan, suggests some reciprocal exchange between these islands and raises the possibility that either one, or both, are introduced rather than endemic. The presence of an *Eisenia* sp. lumbricid, possibly introduced, may provide further evidence of soil fauna transportation.

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Appendix. Cytochrome c oxidase subunit 1 (COI) 'barcode' data

COI-G1 (= *A. gageodo* P1) Length=658

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AACTTTATATTTTATCTTAGGAATTTGGGCCGGCATGGTGGGTGCCGGGATAAGATTACTTATTCCGATT
GAGCTAAGGCAGCCCGGGTCCTTTATAGGAAGCGATCAACTATAACAACAATTGTTACTGCACACGC
ATTTTAAATAATCTTTTTCTTAGTTATAACCAGTATTTATTGGGGGGTTTGGAAATTGGCTACTACCTCTTA
TATTGGGGACACCAGACATAGCATTCCCACGACTAAATAACATAAGATTCTGACTACTACCGCCGTCTC
TAATTTTACTAGTGTCTCAGCAGCAGTAGAGAAGGGGGCAGGAACCGGGTGAACGGTATATCCACC
CCTAGCAAGAAATATTGCACATGCTGGACCTTCTGTGGATCTAGCAATTTTCTCACTCCACTTAGCCGG
AGCATCCTCAATTCTAGGGGCTATCAACTTCATCACCACAGTAATTAATATACGCTGATCGGGACTACG
ATTAGAACGAATTCCCCTATTTGTATGAGCAGTAGTTATTACCGTAGTACTTCTATTGCTCTCATTGCC
GTACTGGCTGGGGCTATTACAATACTACTAACAGACCGAAACCTAAATACATCATTCTTCGATCCTGCA
GGCGGGGGGACCCTATTCTATATCAGCACCTATTT
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COI-G2 (= *A. gageodo* P2)

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AACTTTATATTTTATCTTAGGAATTTGGGCCGGCATGGTGGGTGCCGGGATAAGATTACTTATTCCGATT
GAGCTAAGGCAGCCCGGGTCCTTTATAGGGAGCGATCAACTATAACAACAATTGTTACTGCACACGC
ATTTCTAATAATCTTTTTCTTAGTTATAACCAGTATTTATTGGGGGCTTTGGAAATTGGCTACTACCTCTTA
TATTGGGGACACCAGACATAGCATTCCCACGACTAAATAACATAAGATTCTGACTACTACCGCCGTCTC
TAATTTTACTAGTATCCTCAGCAGCAGTAGAGAAGGGGGCAGGAACCGGGTGAACGGTATATCCACCC
CTAGCAAGAAATATTGCACATGCTGGACCTTCTGTGGATCTAGCAATTTTCTCACTCCACTTAGCCGGA
GCATCCTCAATTCTAGGGGCTATCAACTTCATCACCACAGTAATTAATATACGCTGATCGGGACTACGAT
TAGAACGAATTCCCCTATTTGTATGAGCAGTGGTTATTACCGTAGTACTTCTATTGCTCTCATTGCCCGT
ACTGGCTGGGGCTATTACAATACTTCTAACAGACCGAAACCTAAATACATCATTCTTCGATCCTGCAGG
CGGGGGAGACCCTATTCTATATCAGCACCTATTT
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COI-G3 (= *A. gageodo* P3)

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AACTTTATATTTTATCTTAGGAATTTGGGCCGGCATGGTGGGTGCCGGGATAAGATTACTTATTCCGATT
GAGCTAAGGCAGCCCGGGTCCTTTATAGGAAGCGATCAACTATAACAACAATTGTTACTGCACACGC
ATTTCTAATAATCTTTTTTTTAGTTATAACCAGTATTTATTGGGGGGTTTGGAAATTGGCTACTACCTCTTA
TATTGGGGACACCAGACATAGCATTCCCACGACTAAATAACATAAGATTCTGACTACTGCCGCCGTCTC
TAATTTTACTAGTATCCTCAGCAGCAGTAGAGAAGGGGGCAGGAACCGGGTGAACGGTATATCCACCC
CTAGCAAGAAATATTGCACATGCTGGACCTTCTGTGGATCTAGCAATTTTCTCACTCCACTTAGCCGGA
GCATCCTCAATTCTAGGGGCTATTAACTTCATCACCACAGTAATTAATATACGCTGATCGGGACTACGAT
TAGAACGAATCCCCTATTTGTATGAGCAGTGGTTATTACCGTAGTACTTCTATTGCTCTCATTGCCCGT
ACTGGCTGGGGCTATTACAATACTACTAACAGACCGAAACCTAAATACATCATTCTTCGATCCTGCAGG
CGGGGGAGACCCTATTCTATATCAGCACCTATTT
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BLAST sequence alignment results (8th March, 2012)

Program BLASTN 2.2.26+ (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>)

P1 vs. P2 - Identities = 651/658 (99%), Gaps = 0/658 (0%)

P1 vs. P3 - Identities = 650/658 (99%), Gaps = 0/658 (0%)

MegaBLAST nucleotide analysis results for COI-G1 (= *A. gageodo* P1):

DQ835672.1 *Metaphire tschiliensis tschiliensis* from China:Sichuan Province, cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial. Length=640

Score = 649 bits (351), Expect = 0.0

Identities = 533/622 (86%), Gaps = 8/622 (1%)

AY960809.1 *Metaphire feijani* from southern Taiwan, cytochrome c oxidase subunit 1 (COI) gene, partial cds; mitochondrial. Length=1056

Score = 630 bits (341), Expect = 3e-177
Identities = 549/650 (84%), Gaps = 12/650 (2%)

AB543184.1 *Amyntas carnosus* from Kansai Japan, mitochondrial COI gene for cytochrome oxidase subunit 1, partial cds. Length=872

Score = 627 bits (339), Expect = 4e-176
Identities = 544/644 (84%), Gaps = 10/644 (2%)

Summary: Paratypes P1-3 alignment was >99%. No barcode match closer than 86% was found by megaBLAST comparison of GenBank; the nearest, above, are assumed to be correctly identified despite none being based on types of their respective species.