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Redescription of *Amynthas hupeiensis* (Michaelsen, 1895) with DNA Barcoding Data

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Amynthas hupeiensis 재기재와 DNA barcoding 데이터

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ABSTRACT

Amynthas hupeiensis with 6/7/8/9 intersegmental spermathecal pores keys to the *sieboldi*-group in Sims and Easton (1972). Two pairs of round genital papillae are always between segments 17/18 and 18/19, and male pore regions are positioned in between the genital papillae. Korean *Amynthas hupeiensis* is usually collected from various agro-ecosystems. Description of the *Amynthas hupeiensis* is provided, including illustrations of ventral view and spermathecae, DNA barcoding data, and photo.

Key words : Earthworms, *Amynthas hupeiensis*, DNA barcoding, Korea

INTRODUCTION

Kobayashi reported *Amynthas hupeiensis* at the several locations in Korea including North Korea (1938). Song and Paik's (1969) recorded from Ulleungdo Island on the basis on specimens in 1966 (collected by Y.K. Kim) and 1968 (Y. Heo). In 2005, Hong and Kim also listed this species from Ulleungdo Island. Korean *Amynthas hupeiensis* has habitats in agro-ecosystems characterically, but not so abundant compared with other species, i.e., *A. agrestis, A. koreanus*, and *A. heteropodus* (Hong and Kim, In submit). This species is widely distributed owing to increasing commercial transportation to and from Asian countries, now recorded in Korea, China, Taiwan, and Japan, but further introduced into North America and New Zealand. It occurs in Japan from Hokkaido to Okinawa (Easton 1981, Blakemore 2003). The Chinese species, *Amynthas hupeiensis*, has been mistaken for *Metaphire posthuma* (Gates 1972). According to Blakemore (2003), Ishizuka's two names also are synonyms; *Pheretima hypogaea* (Ishizuka 1999) and *P. edoensis* (Ishizuka 2000).

The diagnostic characteristics of genus *Amynthas* are number and location of spermathecal pores, shape of male pores, shape of spermathecae, and genital markings. We redescribed external and internal characters of *A. hupeiensis* comparing with those of adjacent country (Table 1). Specimens were collected by digging and hand sorting from May to October, 2006 in agro-ecosystem in Korea. Taxonomy in this paper follows Sims and Easton (1972), and Easton (1981). Illustrations are of anatomical views containing important features, prepared with a camera lucida. Descriptions are based on the external examination and dorsal dissection under the stereomicroscope.

DNA barcoding is a technique for identifying and discovering species using a short DNA sequence from a standardized position in the genome. DNA barcode sequences are short and they can be obtained reasonably quickly. The cytochrome c oxidase subunit 1 mitochondrial region (COI) is emerging as the standard barcode region. DNA barcodes vary among individuals of the same species, but only to very minor degree. The DNA was then used as template for PCR amplification of COI using two primers, forward primer, 5′- GGT

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CAA CAA ATC ATA AAG ATA TTG G-3′ and reverse primer 5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′.

The amplification regime consisted of 2 min at 94°C followed by 35 cycles of 30 seconds at 94°C, 45 seconds at 56°C, and 1 min at 72°C, and a final 10 min at 72°C. PCR products were visualized in a 1.0% agarose gel. All PCR reactions that generated a single, circa 711-bp, product were then cycle sequenced, while gel purification was used to recover the target gene product in cases where more than one band was present. Sequencing reactions were analyzed on an 3730xl DNA analyzer.

DESCRIPTION

Family Megascolecidae Rosa, 1891

Genus *Amynthas* Kinberg, 1867

***Amynthas hupeiensis* (Michaelsen, 1895)**

Perichaeta hupeiensis Michaelsen, 1895, p. 35.

Pheretima hupeiensis: Michaelsen, 1900, p. 273; Chen, 1933, p. 251; Chen, 1936, p. 271; Song and Paik, 1969, p. 16.

Amynthas hupeiensis: Easton, 1981, p. 53; Hong and Kim, 2005, p. 131.

Material examined. 8 clitellates, 6 semiclitellates, 4 aclitellates: Litter layers in soils, Woosan-ri (N 34° 48′40″ E 126° 55′29″), Jangpyeong-myon, Jangheung-gun, Jeollanam-do, 25 May 2006 (Y. Hong coll.); 1 clitellate: Litter layers in soils, Yangsan-ri, Dongjin-myon, Buan-gun, Jeollabuk-do, 20 May 2006 (Y. Hong coll.); 1 clitellate: Litter layers in soils, Aesan1-ri (N 37° 23′05.5″ E 128° 40′28.0″), Jeonsun-gun, Gangwon-do, 28 June 2006 (Y. Hong, Y.P. Kim colls.); 3 clitellates, 3 aclitellates: Cheongam-myon (N 35° 14′17.9″ E 127° 42′47.3″), Hadong-gun, Jeollanam-do, 23 August, 2006.

External characters. Dimensions 72-90 mm, by 3.8-4.5 mm at segment X, 4.0-4.4 mm at segment XXX, 3.6-4.2 mm at clitellum; body small, segments 92-117. Setae crowded, evenly distributed around segmental equators, numbering 107-111 at VII, 67-80 at XX, 6-13 between male pore, setal formula AA : AB : ZZ : YZ=1 : 1 : 1 : 1 at XIII and XXX.

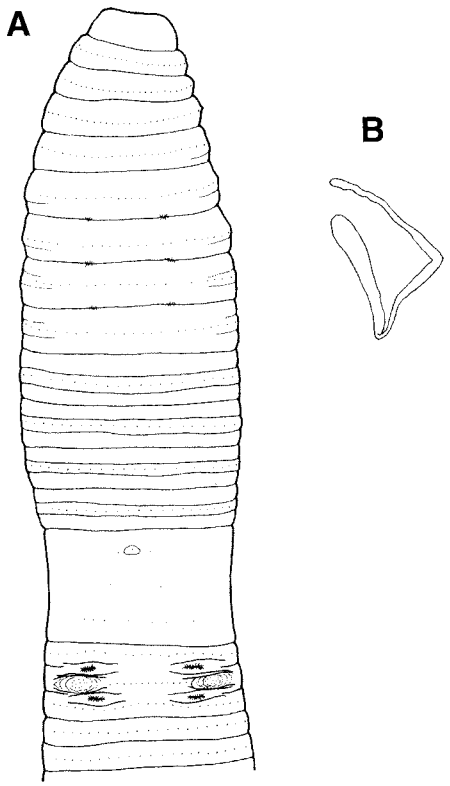

Fig. 1. *Amynthas hupeiensis*. A: ventral view; B: spermathecae.

Table 1. Comparison of characters of *Amynthas hupeiensis* recorded from China, Taiwan, and Korea

Character	Korea		Taiwan	China	
	Hong et Kim	Kobayashi, 1938	Tsai, 1964	Michaelsen, 1900	Chen, 1931
Body length (mm)	72-90	61-150	147-170	40-55	70-130
Segment number	92-117	97-132	128-130	119-132	128
Setal number	107-111 (VII)	10-16 (VII)	100-121 (VIII)	95 (X)	84-100 (VIII)
	67-80 (XX)	10-16 (XVI)	79-88 (XX)	81 (XIII)	80-88 (XXV)
				72 (XXVI)	
Between male pores	6-13	10-16	14		12-15
Spermathecal pores	6/7/8/9	6/7/8/9	6/7/8/9	6/7/8/9	6/7/8/9
Genital papillae					
Shape	round		round (oval)	17/18-18/19	oval
Location	17/18-18/19		17/18-18/19		17/18-18/19
Diverticulum	thin, long		slender, very long		

Female pore single in XIV, on 0.4-0.5 mm oval. Prostomium epilobic with tongue open, clitellum coffee color, formalin preservation. First dorsal pores at 11/12. Clitellum annular XIV-XVI.

Male pore simple only elevated with white areas, approximately oval-shaped forward to ventrally, at segment XVIII, 2.0-2.5 mm distance between male pores setal line. Two pairs of round genital papillae always between segments 17/18, 18/19 closes to male pore regions and some individuals found with genital papillae at 19/20. Spermathecal pore three pairs in 6/7-8/9 close together on ventrally around 1.4-1.5 mm distance between spermathecal pores. Genital markings lacking.

Internal characters. Septa 5/6-11/12 thickened, 12/13, 13/14 thin, septum 8/9/10 present. Gizzard globular in VIII. Intestine begins in XVI, lymph glands small pairs from segment XXVII along the dorsal vessel. Typhlosole big, sized from XXVI. Intestinal caeca simple, originating in XXVII, extending anteriorly about to XXI, finger-shaped. Esophageal hearts four pairs in X-XIII. Male sexual system holandric, testes and funnels in ventrally joined sacs in X-XI. Seminal vesicles paired in XI-XII small sized. Prostates in XVIII; extending between XVIII-XIX glandular parts consist of 2 main lobes each divided again into 3-4 small lobes. Genital papilla glands not found.

Ovaries in XIII. Spermathecae pore three pairs in VII-IX small pouches lanceolate-shaped with segmented on surfaces, stout stalked shorter than ampullae, diverticulum character with thin and long throughout the body longer than ampullae some coiling, no nephridia on spermathecae duct. Genital marking gland not found.

DNA data. The sequence was revealed *Amynthas hupeiensis* voucher Cytochrome c oxidase subunit I (COI) has 711 bp, partial cds, mitochondrial region. GGTCAACAATCATAAA GATATTGGAACACTATACTTCATCCTTGGCGTTTGAG CAGGGTCAACAATCATAAAGATATTGGAACACTA TACTTCATCCTTGGCGTTTGAGCAGGAATGGTTGGAG CTGGTATAAGACTCCTTATTCGAATTGAATTAAGCCA ACCTGGAGCATTTTTAGGAAGAGACCAACTATATAA CACATATTGTAACAGCACATGCATTCTTAATAATTTT CTTTTTAGTAATACCAGTATTTATTGGGGGATTTGGA AATTGACTACTCCCACTTATACTAGGTGCACCTGATA TAGCATTCCCACGATTAAATAATATAAGGTTCTGACT ACTACCCCCATCACTCATTCTATTAGTTTCTTCCGCG GCCGTAGAAAAAGGCGCGGGAACAGGATGAACAGT TTATCCTCCTCTAGCAAGAAATACTGCTCACGCCGGA CCATCAGTAGATTTAGCTATTTTCTCTCTACACTTAG

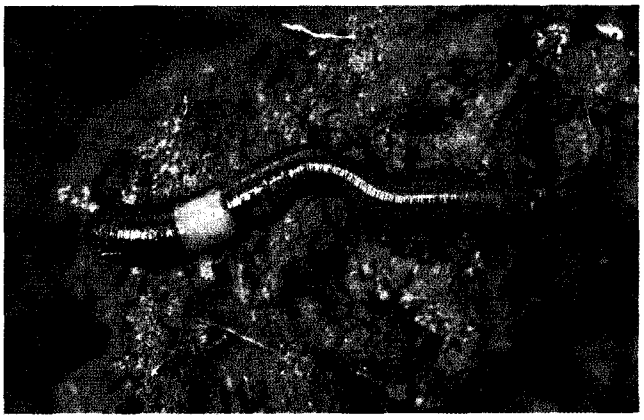

Photo 1. *Amynthas hupeiensis* (Michaelsen, 1895).

CCGGTGCATCATCAATTCTAGGTGCCATTAACTTTAT TACTACAGTGATTAATATACGATGATCAGGCCTACG ATCTAGAAACGAATTCCTTTATTCGTGTGAGCAGTCG TTATTACCGTAGTACTGCTTCTTCTCTCTTTACCAGTA CTAGCAGGCGCAATTACCATGCTACTAACAGACCGA AATCTTAATACATCATTTTTTGACCCTGCAGGAGGTG GGGACCCCATTTTATATCAACATCTATTCTTGATTTT TTGGTCACCCTGAAGTTTA

Distribution. Korea, China, Taiwan, Japan, and America.

Remark. *Amynthas hupeiensis* with 6/7, 7/8, 8/9 intersegmental spermathecal pores keys to the *sieboldi*-group in Sims and Easton (1972). Sims and Easton made *sieboldi*-group by 49 species. After 1972, some species added to this group from Taiwan and Korea. Simple intestinal caeca type group is *A. tungpuensis* Tsai *et al.* 1999, *A. tayalis* Tsai *et al.* 1999, *A. sexpectatus* Tsai *et al.* 1999, *A. binoculatus* Tsai *et al.* 1999, *A. tantulus* Shen *et al.* 2003, *A. fenestrus* Shen *et al.* 2003, *A. monsoonus* James *et al.* 2005, *A. huangi* James *et al.* 2005. *A. moakensis* was recorded from Korea (Hong and Kim 2002). These all new members of *sieboldi*-group have simple intestinal caeca type and habitats in mountains. This species is distinguished from *Metaphire bahli* and *M. peguana* by its superficial male pores (Blakemore 2003).

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