

New Data to the Earthworm Fauna of the Korean Peninsula with Redescription of *Eisenia koreana* (Zicsi) and Remarks on the *Eisenia nordenskioldi* Species Group (Oligochaeta, Lumbricidae)

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Yong Hong and Csaba Csuzdi (2016) The earthworm fauna of the Korean peninsula, especially the Northern part (Democratic People's Republic of Korea) is still poorly known. Altogether 148 earthworm taxa are reported from Korea of which 22, including some uncertain records, belong to the Holarctic family Lumbricidae. From these 22 lumbricid taxa only eight are thought to be autochthonous, including the widely distributed *Eisenia nordenskioldi* species group which consists of six highly diverged DNA lineages. Due to a lack of material, the phylogenetic affinities of the Korean *E. nordenskioldi* s.l. specimens have not been determined so far. Here we report on the first lumbricid records from the Northern part of the peninsula (DPRK) after the description of the endemic earthworm species *Eisenia koreana* (Zicsi, 1972) together with a redescription of its type specimens. The supposedly autochthonous *Eisenia nordenskioldi* species group is briefly reviewed and the specimens collected recently in South Korea (Republic of Korea) were DNA barcoded and compared to the published lineages from Siberia (Russia). The redescription of the type specimen of *Eiseniella koreana* confirmed its inclusion in the genus *Eisenia*. Barcoding of the unpigmented Korean *E. nordenskioldi* specimens revealed that they form an independent clade placed quite far from the previously published unpigmented *E. nordenskioldi* forms and might represent a distinct species.

Key words: North Korea, South Korea, Earthworm fauna, DNA barcode, *Eisenia nordenskioldi* species group.

BACKGROUND

The systematic earthworm research in the Korean Peninsula dates back to the work of Shinjiro Kobayashi (1934, 1936a, b) who later summarized the earthworm fauna of Korea, reporting 38 species including 10 lumbricids (Kobayashi 1938, 1941). After Kobayashi's pioneering work until the last decade, there was only one lumbricid species described from the peninsula; *Eiseniella koreana* Zicsi, 1972 from Taesong Mt., Pyongyang, North Korea. In the family Megascolecidae, Song and Paik (1969, 1970a, 1970b, 1971, 1973) presented data on the earthworm fauna of the Korean Peninsula including descriptions of four new species.

At the beginning of the new millennia Hong

(2000) reviewed the lumbricid fauna of Korea reporting seven species including some doubtful records (i.e. *Aporrectodea tuberculata* (Eisen, 1874) or *Bimastos beddardi* (Michaelsen, 1894)). Unfortunately, at that time the sole endemic lumbricid species *E. koreana* was not listed.

From the early 2000's, due to the work of Yong Hong and Sam James and later Robert Blakemore, the number of the earthworm taxa reported from the Korean Peninsula has rapidly grown to 94 (Blakemore 2006), later to 106 (Hong and James 2009) and recently reached 148 (Blakemore et al. 2012, Blakemore 2014, Blakemore et al. 2014, Blakemore et al. 2015). However, most of the recent works deal with species belonging to the family Megascolecidae and are almost exclusively

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from South Korea. From the family Lumbricidae after Zicsi's work (1972) only four taxa were described from Korea: *Eisenia gaga* Blakemore, 2012, *Eisenia sindo* Blakemore, 2012, *Eisenia japonica vaga* Blakemore, 2013 and most recently *Eisenia muuido* Blakemore, 2015, all from South Korea.

Here we report on earthworms collected on a trip by the second author to North Korea in 1988 and several other lumbricid specimens collected in different parts of South Korea by the first author. Furthermore, we have redescribed *E. koreana* (Zicsi, 1972) from an examination of the type material housed in the Hungarian Natural History Museum, Budapest. This work was undertaken as Blakemore and Park (2012) reported two new *Eisenia* species from South Korea and mentioned that *E. koreana* had not been recorded since the original description and also that the original account of Zicsi (1972) is incomplete in several ways.

The widely distributed *Eisenia nordenskioldi*

species complex has recently been analysed using DNA barcodes and ITS2 sequences (Shekhovtsov et al. 2013). In this work six highly diverged *E. nordenskioldi* lineages were identified apart from the unpigmented *E. n. pallida* Malevich, 1956 which represented also an independent lineage. The inter-lineage genetic distances (K2P) for COI gene were between 13.6% and 19.1% suggesting that some of the lineages are beyond the intraspecific distance limit determined by Chang and James (2011).

Since the analysis by Shekhovtsov et al. (2013) did not include the recently reported Mongolian specimens (Blakemore & Park 2012, Blakemore 2013) nor representatives from the Korean Peninsula, to determine the position of the Korean *E. nordenskioldi* s.l. specimens we reanalysed Shekhovtsov et al. (2013) data complemented with the newly sequenced Korean specimens and the published DNA sequences of Blakemore and Park (2012) and Blakemore (2013b) (Table 1).

Table 1. Species and COI references/GenBank accession numbers used in this study

Species	Accession/Identification Number	Reference
<i>Eisenia andrei</i>	AY874509	Pérez-Losada et al. 2005
<i>Eisenia andrei</i>	AY874510	«
<i>Eisenia andrei</i>	JN870085	Voua Omoto et al. 2013
<i>Eisenia andrei</i>	JN870087	«
<i>Eisenia andrei</i>	JN870088	«
<i>Eisenia andrei</i>	JN870086	«
<i>Eisenia cf andrei</i>	w11 Jeju	Blakemore and Lee 2013
<i>Eisenia cf japonica</i>	WO19 large NIBRIV0000250888 Korea	«
<i>Eisenia fetida</i>	FJ214228	Lund et al. 2009
<i>Eisenia fetida</i>	H6 Busan	Blakemore and Lee 2013
<i>Eisenia fetida</i>	JX531566	Shekhovtsov et al. 2013
<i>Eisenia fetida</i>	WO12 S1 from Jeju Island Korea	Blakemore and Lee 2013
<i>Eisenia fetida</i>	FJ214216	Lund et al. 2009
<i>Eisenia fetida</i>	AY874516	Pérez-Losada et al. 2005
<i>Eisenia fetida</i>	AY874517	Pérez-Losada et al. 2005
<i>Eisenia fetida cf xanthurus</i>	W018 from mainland Korea	Blakemore and Lee 2013
<i>Eisenia gaga</i>	Holotype WM1 INV 0000245509	Blakemore and Park 2012
<i>Eisenia gaga</i>	Paratype WM3 INV 0000245513	«
<i>Eisenia gaga</i>	Paratype1 WM2 INV 0000245511	«
<i>Eisenia japonica</i>	AB542698	Minamiya et al. unpublished
<i>Eisenia japonica japonica</i>	JET170 An-417 Enoshima topotype	Blakemore and Lee 2013a
<i>Eisenia japonica</i>	JET124 Tokyo An-466.1 Nogeyama	«
<i>Eisenia japonica vaga</i>	H5 holotype from Busan	«
<i>Eisenia japonica vaga</i>	WO20 paratype IV0000250889	«
<i>Eisenia japonica</i>	w18b Jeju	Blakemore 2013a
<i>Eisenia nordenskioldi</i> clade 1	JX531495	Shekhovtsov et al. 2013
<i>Eisenia nordenskioldi</i> clade 1	JX531498	«
<i>Eisenia nordenskioldi</i> clade 1	JX531503	«
<i>Eisenia nordenskioldi</i> clade 2	JX531465	«

MATERIALS AND METHODS

Earthworms were collected by the diluted formaldehyde method (Raw 1959), with digging and searching under stones, mosses, and the bark of fallen logs. The collecting sites in North Korea were scattered around three centres; the Baekdu Mts close to the Chinese border, the Myohynag Mts in the central mountainous region and Haeju, close to the South Korean border. In South Korea the collection sites are scattered all over the country and covered mainly agricultural fields.

The specimens collected were killed in 75% ethanol and fixed in 4% formaldehyde solution, then transferred into 75% ethanol and deposited in the earthworm collection of the Hungarian Natural History Museum (HNHM) and National Institute of Biological Resources, Korea (NIBR). For molecular studies, some specimens from the newer samples were placed into 96% ethanol. Partial sequences of COI gene were obtained from three specimens representing the *Eisenia nordenskioldi* species

complex following the protocol described in Szederjesi and Csuzdi (2015). Sequences were compared with the GenBank nucleotide database using blastn 2.2.14 algorithm (Altschul et al. 1997). Additional DNA sequences representing the different *E. nordenskioldi* lineages (Shekhovtsov et al. 2013), *Eisenia japonica*, *Eisenia fetida* and *Eisenia andrei* were downloaded from the GenBank (Table 1).

The DNA sequences were aligned with ClustalW (Thompson et al. 1994) implemented in MEGA 6.06 (Tamura et al. 2013) using the default settings. Maximum Likelihood analysis was carried out with MEGA 6.06 (Kumar et al. 2008) using GTR + G + I model selected by the Akaike Information Criteria (Akaike 1974) implemented in MEGA 6.06. Bootstrap support was estimated from 1000 replicates. Bayesian analysis was carried out with BEAST 1.8.0 (Drummond and Rambaut 2007) using Metropolis coupled Markov chain Monte Carlo simulations for 10 million generations, sampling a tree in every 1000 generations. After

Table 1. (continued)

Species	Accession/Identification Number	Reference
<i>Eisenia nordenskioldi</i> clade 2	JX531475	Shekhovtsov et al. 2013
<i>Eisenia nordenskioldi</i> clade 2	JX531483	«
<i>Eisenia nordenskioldi</i> clade 2	JX531485	«
<i>Eisenia nordenskioldi</i> clade 3	JX531542	«
<i>Eisenia nordenskioldi</i> clade 3	JX531550	«
<i>Eisenia nordenskioldi</i> clade 3	JX531553	«
<i>Eisenia nordenskioldi</i> clade 3	JX531564	«
<i>Eisenia nordenskioldi</i> clade 3	JX531565	«
<i>Eisenia nordenskioldi</i> clade 4	JX531487	«
<i>Eisenia nordenskioldi</i> clade 4	JX531488	«
<i>Eisenia nordenskioldi</i> clade 4	JX531489	«
<i>Eisenia nordenskioldi</i> clade 5	JX531515	«
<i>Eisenia nordenskioldi</i> clade 5	JX531518	«
<i>Eisenia nordenskioldi</i> clade 5	JX531519	«
<i>Eisenia nordenskioldi</i> clade 5	JX531521	«
<i>Eisenia nordenskioldi</i> clade 6	JX531526	«
<i>Eisenia nordenskioldi</i> clade 6	JX531535	«
<i>Eisenia nordenskioldi</i> clade 6	JX531536	«
<i>Eisenia nordenskioldi</i> Korea 1	KM593696	New
<i>Eisenia nordenskioldi</i> Korea 2	KM593697	New
<i>Eisenia nordenskioldi</i> Korea 3	KM593698	New
<i>Eisenia nordenskioldi</i> mongol	Holotype wo63	Blakemore 2013b
<i>Eisenia nordenskioldi</i> mongol	Paratype wo64	«
<i>Eisenia nordenskioldi</i> onon	Holotype wo65	«
<i>Eisenia nordenskioldi</i> pallida	JX531522	Shekhovtsov et al. 2013
<i>Eisenia nordenskioldi</i> pallida	JX531523	«
<i>Eisenia sindo</i>	WO25 Holotype IV0000246435	Blakemore 2013b
<i>Eisenia sindo</i>	WO26 Paratype P1 IV0000246436	«
<i>Enchytraeus albidus</i>	GU902047	Erseus et al. 2010

removing the first 2,000 trees as burn-in, the remaining 8,000 sampled trees were analyzed with TreeAnnotator v1.8.0 and visualized by FigTree 1.4.0 (Rambaut 2012). Pairwise genetic distances were calculated in MEGA 6.0.6 using the Kimura 2-Parameter model.

RESULTS

Class Oligochaeta

Family Lumbricidae Rafinesque-Schmaltz, 1815

Aporrectodea cf. trapezoides (Dugès, 1928)

Lumbricus trapezoides Dugès, 1828: 289.

Allolobophora caliginosa f. *trapezoides*: Kobayashi 1941: 150.

Aporrectodea trapezoides: Blakemore 2008: 534 (for complete synonymy).

Aporrectodea trapezoides: Blakemore 2013a: 39.

Aporrectodea trapezoides: Blakemore and Lee 2013: 134.

Aporrectodea trapezoides: Blakemore 2014: 11.

Material examined: South Korea. Jeollanam-do, Sinan-gun, Huksando Island, 10.08.2009, 1 ex. Leg. Y. Hong., Jeollabuk-do, Gunsan-si, Sunyudo Island, 01.10.2009, 9 ex. Leg. Y. Hong., Chungcheongnam-do, Boryeong-si, Ungcheon, 11.06.2008, 5 ex. Leg. Y. Hong., Jeju-do, Jeju-si, 30.05.2008, 1 ex. Leg. Y. Hong., Jeollabuk-do, Gunsan-si, Muyedo Island, 06.10.2009, 5 juv. ex. Leg. Y. Hong., Gangwon-do, Jeongsungan, Jeongsun, 04.07.2009, 1 ex. Leg. Y. Hong., Jeollanam-do, Jangheung-gun, Jangheung, 23.09.2009. 2 ex. Leg. Y. Hong., Incheon-si, Ongjin-gun, Baekryeong-do Island, 28.08.2008, 2 ex. Leg. Y. Hong., Chungcheongbuk-do, Okcheon-gun, Okcheon, 23.08.2007, 3 ex. Leg. Y. Hong., Jeollabuk-do, Gunsan-si, Sunyudo Island, 26.04.2013, 34 ex. Leg. Y. Hong., Jeollabuk-do, Buan-gun, Dongjin, 23.05.2013, 1 ex. Leg. Y. Hong (NIBRIV0000323392)., Jeollabuk-do, Buan-gun, Dongjin, 12.10.2013, 16 ex. Leg. Y. Hong., Incheon-si, Gangwha-gun, Ganghwado, 03.05.2013, 8 ex. Leg. Y. Hong.

Remarks: All of the specimens examined have dark-brown colour on dorsum that is typical for *Ap. trapezoides* but neither of them has clearly band-shaped tubercles (Fig. 1).

Bimastos parvus (Eisen, 1874)

Allolobophora parva Eisen, 1874: 46.

Bimastos parvus: Kobayashi 1941: 155.

Bimastos parvus: Blakemore 2008: 537 (for complete synonymy).

Bimastos parvus: Blakemore 2014: 11.

Material examined: North Korea. Before Haeju, 17.07.1988, 10 ex. Leg. Cs. Csuzdi., Haeju, Su-Yong waterfall, 16.07.1988, 12 ex. Leg. Cs. Csuzdi., South Korea. Incheon-si, Ongjin-gun, Baekryeong-do Island, 28.08.2008, 3 ex. Leg. Y. Hong., Jeollabuk-do, Gunsan-si, Sunyudo Island, 17.10.2013, 3 ex. Leg. Y. Hong (NIBRIV0000323393)., Jeollabuk-do, Buan-gun, Dongjin, 23.05.2013, 1 ex. Leg. Y. Hong., Jeollabuk-do, Buan-gun, Dongjin, 12.10.2013, 3 ex. Leg. Leg. Y. Hong., Jeollabuk-do, Muju-gun, Muju, 26.10.2013, 7 ex. Leg. Leg. Y. Hong.

Remarks: *B. parvus* is perhaps of North American origin (Gates 1972) but it has been widely introduced in Europe and also in Asia (Gates 1972, Blakemore 2008, Szederjesi et al. 2013a, 2013b).

Eisenia japonica japonica (Michaelsen, 1892)

Allolobophora japonica Michaelsen, 1892: 230.

Allolobophora japonica: Kobayashi 1936b: 183.

Allolobophora japonica f. *typica*: Kobayashi 1941: 151.

Eisenia japonica: Blakemore and Grygier 2011: 270 (for complete synonymy).

Eisenia japonica: Blakemore 2013a: 42.

Material examined: South Korea. Jeju-do, 01.06.2008, 5 ex. Leg. Y. Hong.

Remarks: Our specimens are unpigmented, greyish. The biometry varies between 46 × 3 mm, segment No. 98 and 72 × 4 mm segment No. 120. Clitellum on 1/n 23-31, tubercles two distinct maize kernel-shaped protuberances on 27, 29. Genital papillae completely missing.

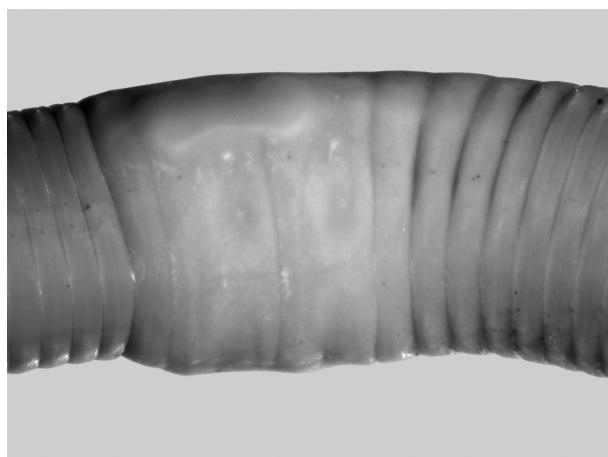


Fig. 1. *Aporrectodea trapezoides* (Dugès, 1828); ventrolateral view of the clitellar region.

***Eisenia vaga* Blakemore, 2013 stat. nov.**

Eisenia japonica vaga Blakemore, 2013: 132.
Eisenia japonica vaga: Blakemore 2014: 11.

Material examined: South Korea. Incheon-si, Ongjin-gun, Baekryeong-do Island, 28.08.2008, 2 ex. Leg. Y. Hong., Chungcheongnam-do, Cheongyang-gun, Cheongyang, 17.06.2008, 1 ex. Leg. Y. Hong., Jeju-do, Jeju-si, 30.05.2008, 3 ex. Leg. Y. Hong.

Remarks: This is the first record of *E. vaga* after its original description. Our specimens agree well with the original description except the



Fig. 2. *Eisenia vaga* Blakemore, 2013b; ventrolateral view of the clitellar region.

somewhat shorter clitellum, presence of slight red pigmentation on dorsum and the somewhat smaller biometry. Length 32–35 × 2 mm, segment No. 110–122. Clitellum ½ 24–31, tubercles two dorsally connected maize kernel-shaped protuberances on 27, 29. Two pairs of prominent genital papillae around ab on 25, 26 (Fig. 2). Setal arrangement after clitellum aa:ab:bc:cd:dd = 7:1.25:5:1:23.

Vesicles four pairs in 9–12, but those in 9 and 10 are much smaller. Spermathecae 9/10–10/11 roundish clearly stalked and filled with sperms, open laterally in cd. Calciferous glands small in 11–12, typhlosole moderate T-shaped, nephridial bladders elongated sausage-shaped with slightly curved ectal part.

The morphological difference between *E. japonica* and *E. vaga* are quite scanty (biometry and the distribution of genital papillae) however the genetic distance between *japonica* and *vaga* (Fig. 13, 14, Table 2) clearly indicates that they represent independent species.

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

Eiseniella koreana Zicsi, 1972: 129.
Eisenia koreana: Perel 1979: 82.
Eisenia koreana: Easton 1983: 480.
Eisenia koreana: Blakemore and Park: 2012: 299.

Material examined: Holotype. HNHM/12715 with label data: *Eiseniella koreana* sp. n. typus, Phenjan, 22.V.1970. Leg. S. Mahunka u. H.

Table 2. Pairwise nucleotide K2P differences (in %) between the investigated earthworm species/lineages

species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. <i>E. sindo</i>	0.01															
2. <i>E. n. cf. pallida</i>	0.225	0														
3. <i>E. n. onon</i>	0.238	0.165	-													
4. <i>E. n. mongol</i>	0.249	0.179	0.082	0												
5. <i>E. nordenskioeldi_Korea</i>	0.24	0.231	0.19	0.217	0.008											
6. <i>E. nordenskioeldi_6</i>	0.235	0.215	0.207	0.207	0.219	0.005										
7. <i>E. nordenskioeldi_5</i>	0.23	0.213	0.176	0.203	0.229	0.219	0.068									
8. <i>E. nordenskioeldi_4</i>	0.228	0.199	0.216	0.219	0.2	0.215	0.214	0.027								
9. <i>E. nordenskioeldi_3</i>	0.226	0.224	0.196	0.203	0.178	0.212	0.204	0.212	0.046							
10. <i>E. nordenskioeldi_2</i>	0.248	0.222	0.21	0.222	0.223	0.209	0.207	0.145	0.201	0.052						
11. <i>E. nordenskioeldi_1</i>	0.236	0.187	0.129	0.151	0.222	0.205	0.163	0.204	0.206	0.195	0.065					
12. <i>E. j. vaga</i>	0.244	0.21	0.204	0.206	0.215	0.236	0.226	0.237	0.222	0.248	0.219	0.057				
13. <i>E. j. japonica</i>	0.23	0.228	0.205	0.204	0.235	0.224	0.234	0.239	0.224	0.247	0.223	0.208	0.123			
14. <i>E. gaga</i>	0.206	0.25	0.241	0.253	0.226	0.228	0.229	0.237	0.209	0.247	0.238	0.235	0.239	0.005		
15. <i>E. andrei</i>	0.24	0.234	0.23	0.225	0.262	0.195	0.235	0.253	0.249	0.237	0.219	0.213	0.217	0.258	0.007	
16. <i>E. fetida</i>	0.241	0.22	0.237	0.229	0.266	0.224	0.218	0.239	0.249	0.238	0.217	0.202	0.222	0.249	0.16 0	

In the diagonal (bold) the within lineage differences are shown. K2P values shaded in light grey refer to subspecific differences, in dark-grey to infra-subspecific differences according to Szederjesi et al. (2011).

Steinmann., Paratypes. HNHM/7000 3 adult + 2 preadult complete specimen, 2 adult specimen with missing tail, 1 juvenile and 1 tail part with label: *Eiseniella koreana* paratype, Phenjan 22.V.70. Leg. S. Mahunka u. H. Steinmann.

New material: North Korea. Haeju, Su-Yong waterfall, 16.07.1988. Leg. Cs. Csuzdi (HNHM/16919 1 juv. ex).

Redescription: External characters. Holotype 44 mm long and 2.8 mm wide. Number of segments 100. Paratypes 41-52 mm long and 2.8-3 mm wide. The tail is prominently quadrangular. Number of segments 125-130. Colour pale with slight hints of previous pigmentation. Prostomium epilobous $\frac{1}{2}$ closed. First dorsal pore at intersegmental furrow 4/5. Setae closely paired. Setal arrangement behind clitellum: aa:ab:bc:cd:dd = 5.7:1.2:5.2:1:8.6 (Fig. 3). Male pores on segment 15, surrounded by glandular crescents, slightly protruding into segments 14 and 16. Female pores as small slits on 14, just above the setae b. Nephridial pores irregularly alternate between setal line b and above d. Clitellum on segments 25-31 but segment 24 also modified slightly (Fig. 4). Tuberula pubertatis as glandular trench on segments 27- $\frac{2}{3}$ 29, 29 (Fig. 5). Genital papillae on Holotype; 10 cd, 10 ab Rhs, 11 cd, 16, 26-28, 30

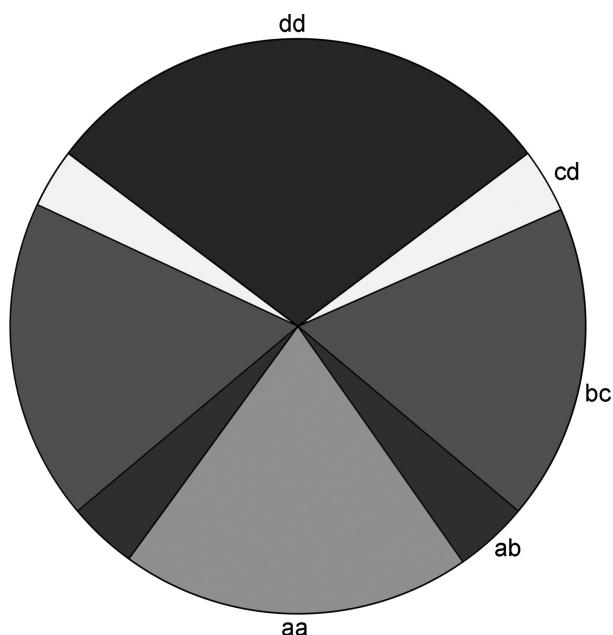


Fig. 3. *Eisenia koreana* (Zicsi, 1972) setal arrangement. aa = distance between the ventral setal lines, ab = distance between the ventrolateral setal lines, cd = distance between the dorsolateral setal lines, dd = distance between the dorsal setal lines.

ab. Paratype 1; 11 cd, 9, 10 ab Rhs, 11 cd Lhs, 16, 25, 30. Paratype 2; 23 ab Rhs, 17 ab Lhs, 11 cd, 16, 26-28, 30 ab. Spermatophore at 22/23 on Holotype.

Internal characters: Septa 7/8-8/9 thickened, 6/7, 9/10-10/11 slightly strengthened, and also 12/13-14/15 somewhat stout. Testes and funnels paired in segments 10-11, free. The entalmost part of the sperm ducts somewhat coiled and slightly thickened. Seminal vesicles in 9-10 small, 11-12 larger. Spermathecae drop-shaped in 9/10-10/11 with external openings close to the mid-dorsal line. Ovarium in 13, elongated and slightly flattened. Ovarian funnel large, with undulated rim, ovisac small, pending from 13/14 just above

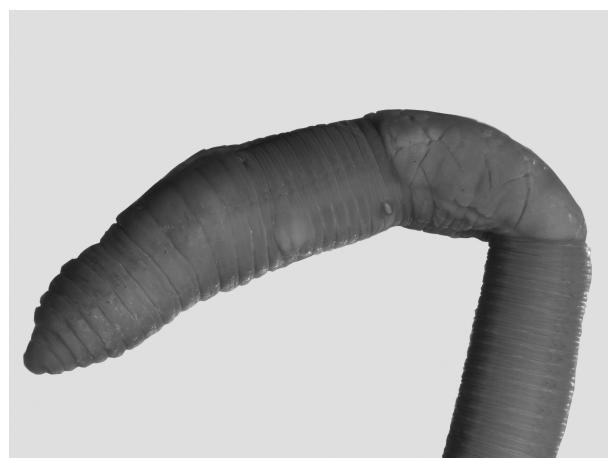


Fig. 4. *Eisenia koreana* (Zicsi, 1972) ventrolateral view of the fore-body.



Fig. 5. *Eisenia koreana* (Zicsi, 1972) ventrolateral view of the clitellar region.

the ovarian duct. Calciferous glands in 11-12 with slightly more developed diverticula in segment 12. Paired hearts appear in segments 7-11, with a pair of small extraoesophageal vessel in 12. Nephridial bladders simple, slightly elongated sac-shaped (Fig. 6). Crop in segments 15-16, and gizzard in segments 17-18. Typhlosole bilobed, moderate (Fig. 7). Longitudinal muscle layer is of pinnate type.

Remarks: On the basis of the gizzard (in 17-18) and the position of the female pores (above setae b) *koreana* is surely fits into the genus *Eisenia* (e.g. Perel 1979, Easton 1983) and seems to be very close to *E. gaga* Blakemore, 2012 (in Blakemore & Park 2012) described from Gageodo Island, S. Korea. The only substantial differences found from this species are the size and segment number ($41\text{-}52 \times 2.8\text{-}3$ mm vs. $80\text{-}105$ (diameter not noted in *gaga*) and $100\text{-}130$ vs. $124\text{-}162$). *E. koreana* is close also to *E. sindo* Blakemore, 2012 from Sindo Island, Incheon, S. Korea but differs from it in the smaller biometry and presence of a typhlosole (lacking in *sindo*). As the two recently described species were mainly defined by their DNA barcodes, adequately preserved North

Korean specimens would needed to clear the relationship between *E. koreana*, *E. sindo* and *E. gaga*.

Eisenia nordenskioldi cf. *pallida* Malevic, 1956

Eisenia nordenskioldi f. *typica*: Kobayashi 1941: 148 (misidentification).

Eisenia nordenskioldi species complex: Blakemore 2008: 580.

Eisenia nordenskioldi *pallida*: Blakemore 2014: 12.

Notes: *E. nordenskioldi* represents a quite heterogeneous species complex with different polyploid races (Perel 1997) and several, genetically quite diverged lineages (Shekhovtsov 2013). According to Perel (1997) in the Korean Peninsula the slightly pigmented form *E. nordenskioldi* *pallida* Malevic, 1956 occurs which, as stated by Blakemore (2013b), might be a synonym of *Helodrilus* (*Allolobophora*) *acystis* Michaelsen, 1903 from Turkestan.

Material examined: North Korea. Myohyang Mts. Kuchung, 1000 m, 06.1985, 1 ex. Leg. Z. Tóth (HNHM/16902)., Baekdu Mts. near Pochon, 13.07.1988, 1 ex. Leg. Cs. Csuzdi (HNHM/16904)., Baekdu Mts. near Samjiyon, 12.07.1988, 3 ex. Leg. Cs. Csuzdi (HNHM/16905)., between Nam-Chon and Haeju, 17.07.1988, 3 ex. Leg. Cs. Csuzdi (HNHM/16920)., Haeju, Su-Yong waterfall, 16.07.1988, 1 ex. Leg. Cs. Csuzdi (HNHM/16921)., Myohyang Mts., near Hyangsan, 07.07.1988, 3 ex. Leg. Cs. Csuzdi (HNHM/16922). South Korea. Jeju-do, Jeju-si, 30.05.2008, 2 ex., 31.05.2008, 1 ex. Leg. Y. Hong., Incheon-si, Ongjin-gun, Baekryeong-do Island, 28.08.2008, 5 ex. Leg. Y. Hong., Incheon-si, Gangwha-gun, Gangwhado, 03.05.2013, 5 ex. Leg. Y. Hong., Jeollabuk-do, Gunsan-si, Sunyudo Island, 26.04.2013, 2 ex. Leg. Y. Hong., Jeollabuk-do, Gunsan-si, Muyeodo Island, 18.10.2013, 1 ex. Leg. Y. Hong., Daegu-si, 03.08.1965, 1 ex. Leg. M.J. Song, Gyeongsangbuk-do, Gimcheon, 16.07.1966, 1 ex. Leg. M.J. Song.

Remarks: None of our specimens possessed red pigmentation even alive. Their colour is greyish, more dark on the dorsum at the head. Setae closely paired (Figs. 8, 9), the biometry varies between $44\text{-}74 \times 3\text{-}4.5$ mm, segment No. 80-141. Clitellum 25, 26-33, 1/n 34, tubercles $\frac{1}{2}$ 29, $29\text{-}\frac{1}{2}$ 31, $\frac{1}{2}$ 32. Number of papillae before clitellum varied, but they are constant on the clitellum around ab 27-33, (34). (Table 3). Four pairs of vesicles in 9-12 but the first two pairs in 9-10 are rather small. Spermathecae in 9/10-10/11 drop-shaped open just near to the dorsal pores.

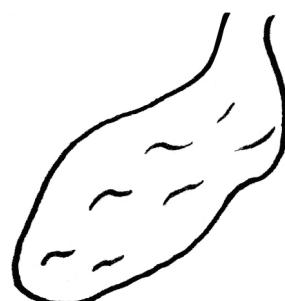


Fig. 6. *Eisenia koreana* (Zicsi, 1972) nephridial bladder.

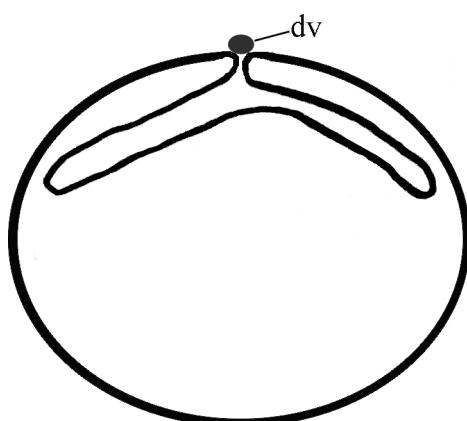


Fig. 7. *Eisenia koreana* (Zicsi, 1972) typhlosole. dv = dorsal vessel.

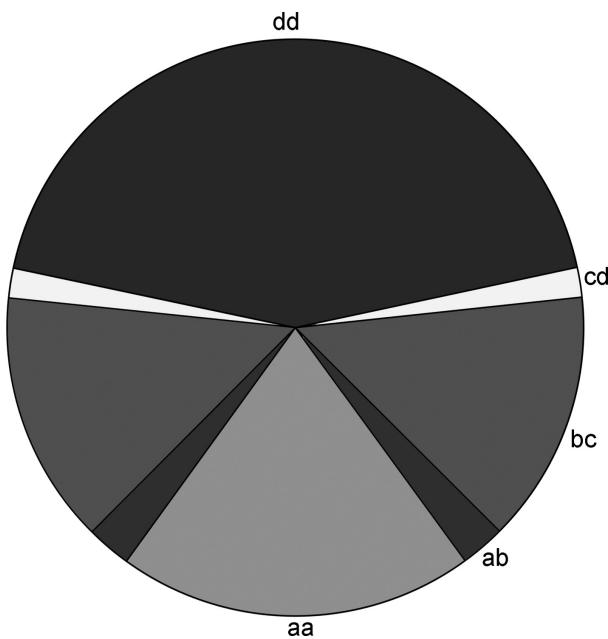


Fig. 8. *Eisenia nordenskioldi* cf. *pallida* Malevic, 1956; setal arrangement of the Korean specimens. aa = distance between the ventral setal lines, ab = distance between the ventrolateral setal lines, cd = distance between the dorsolateral setal lines, dd = distance between the dorsal setal lines.

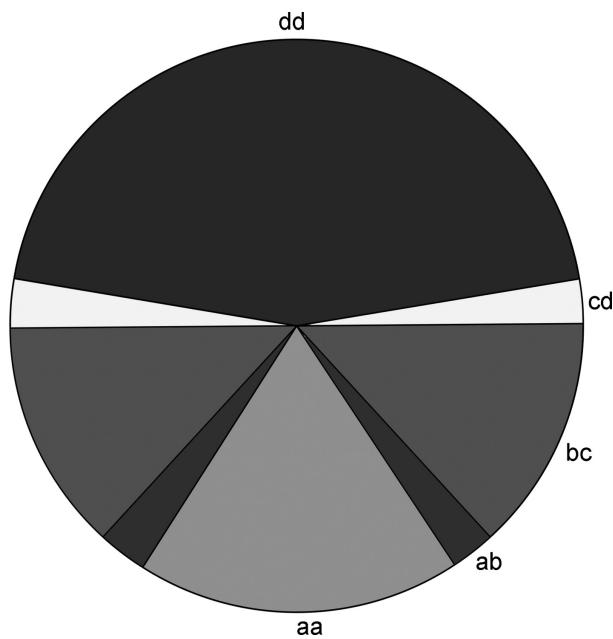


Fig. 9. *Eisenia nordenskioldi* cf. *pallida* Malevic, 1956 setal arrangement of the type specimen. aa = distance between the ventral setal lines, ab = distance between the ventrolateral setal lines, cd = distance between the dorsolateral setal lines, dd = distance between the dorsal setal lines.

Table 3. Main morphological characters of the *Eisenia nordenskioldi* subspecies

Species Reg. No.	Biometry mm, segment No.	Clitellum	Tubercles	Papillae
<i>E. n. pallida</i> Korea 16905	74 × 4 132	(25)26-1/n34	29-1/n32	ab 10-12,14,16,22-24,27-34
16902	55 × 3 113	(26)27-33	29-31	ab 11,12,16,23-24,27-33
16904	58 × 4 141	27-33	½29-½32	ab 9-12,16-17,24-34
16922	42 × 3 123	(26)27-33	29-31	ab 25-33
16903	57 × 4.5 80	(24)25-1/n34	29-31	ab 9-13,16,25-34
16921	47 × 3 136	27-33	29-31	ab 27-32 (not fully adult)
Gangwon-do	50 × 3.5 115	26-33	29-31	ab 16,23-24,27-32,34
<i>E. n. pallida</i> Malevic, 1956	?	(25)26,27-33	29-31	ab 24-25,27-34
<i>E. n. acystis</i> (Michaelsen, 1903)	90 × 4 126	26-33	29-30, 1/n31	abcd 9-12
<i>E. n. polypalpilla</i> Perel, 1969	55-80 × 4-5 102-137	26,27-32	½ 28,29-½31,31	ab 16-18,21-23,26,32-34
<i>E. tracta</i> Perel, 1985	47-138 × 5-12 111-168	26-33	29-½32	ab 8-10,16,17,23-36,37 cd 11,12
<i>E. ventripapillata</i> Perel, 1985	40-90 × 3-4 111-139	26-33	½28,28-30,½31	ab 10,11,26-32 cd 10,(11)
<i>E. n. mongol</i> Blakemore, 2013	80-110 × ? 131-142	(25)26-33	29-31	ab 7, 11, 26, 27-32,33 cd 8-11
<i>E. n. onon</i> Blakemore, 2013	100 × ? 170	(24)25-33	28-31	ab 11-12

Typhlosole moderate, bilobed or T-shaped.

The above description agrees well with the original description of *E. n. papillida*. Especially the shared papillar segments are characteristic; therefore we regard our Korean specimens to be identical to *E. n. pallida* described from environs of Vladivostok (Russia), just ca. 150 km NE from the North Korean border.

Helodrilus (Allolobophora) acystis Michaelsen, 1903 is also a similar species however, *E. n. pallida* clearly differs from it in the setal arrangement (in *pallida* *dd* is always less than $\frac{1}{2}$ circumference, in *acystis* *dd* = $\frac{7}{13}$ circumference) and in the distribution of the genital papillae (in *acystis* they are confined to segments 9-12, in *pallida* they are more widespread). Therefore we agree with the opinion of Perel (1997: 70) that *H. (A) acystis* Michaelsen, 1903 represents a different (sub) species. To clear the positions of the different species/subspecies in the *Eisenia nordenskioldi* species complex a thorough morphological/molecular analysis of the species involved are needed (cf. Blakemore 2013b).

Eisenia nordenskioldi nordenskioldi (Eisen, 1879)

Allolobophora nordenskioldi Eisen, 1879: 6.

Eisenia nordenskioldi nordenskioldi: Perel 1997: 69.

Eisenia nordenskioldi nordenskioldi: Blakemore 2013b: 2.

Notes: To compare with the Korean *E. nordenskioldi* material we have obtained several specimens from Siberia (Russia) belonging to the nominal subspecies.

Material examined: Russia, South Siberia, Tyumen, 18.07.2013, 4 ex. Leg. V. Chernov.

Remarks: All the four specimens are dark red especially on dorsum, with paler spots on both side around 9-11 *cd*. Setae closely paired, size of the single complete specimen 100×7 mm, segment No. 143. Clitellum $27\frac{1}{2}-33$, tubercles 29-31. Genital papillae around setae *ab* on segments 8, 27-31. (Table 2). Four pairs of vesicles in 9-12, the first two pairs in 9-10 are somewhat smaller than the last two. Spermathecae in 9/10-10/11 elongated, irregular sac-shaped open near to the dorsal pores. Typhlosole small, tubular protuberance of the intestine.

E. n. nordenskioldi clearly differs from *E. n. pallida* apart from the pigmentation also in the distribution of genital the papillae, the shape of the typhlosole and setal arrangement (Fig. 10). However the real differences among the closely related dark-red pigmented species in the *E.*

nordenskioldi species group need still to be worked out (Table 4).

Eisenia lagodechiensis (Michaelsen, 1910)

Helodrilus nordenskioldi lagodechiensis Michaelsen, 1910: 18.

Eisenia lagodechiensis: Kvavadze 1985: 169.

Eisenia lagodechiensis: Perel 1997: 72.

E. nordenskioldi lagodechiensis: species incertae sedis Blakemore 2013b: 21.

Material examined: Russia, Dagestan, 05.07.1989, 45 ex. Leg. A. Zicsi, Cs. Csuzdi (HNHM/11047), Dagestan, 09.07.1989, 42 ex. Leg. A. Zicsi, Cs. Csuzdi (HNHM/11057), Dagestan, 10.07.1989, 11 ex. Leg. A. Zicsi, Cs. Csuzdi (HNHM/11063).

Remarks: Our material completely agrees with that of Kvavadze (1985) from around Lagodechi. All the specimens were red violet alive however, in the preserved material most of the pigmentation bleached out. Setae moderately paired, biometry of the worms varies between $66-115 \times 3.5-5$ mm, segment No. 117-135. Clitellum 25, 26-34, tubercles $\frac{1}{2}28-\frac{1}{2}32$, 32. Genital papillae around setae *cd* on 9-11, *ab* on segments 25-37, 38 (Table 2). Four pairs of vesicles in 9-12, the first two pairs in 9-10 are smaller than the last two pairs. Spermathecae in 9/10-10/11 elongated sac-

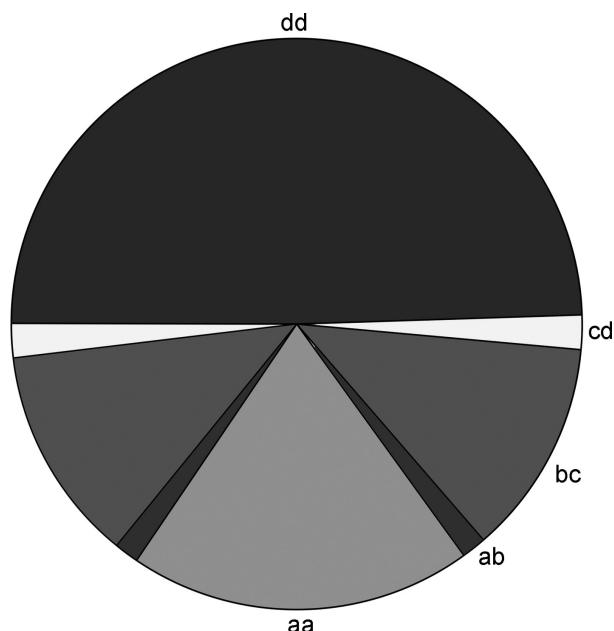


Fig. 10. *Eisenia nordenskioldi nordenskioldi* (Eisen, 1879) setal arrangement. *aa* = distance between the ventral setal lines, *ab* = distance between the ventrolateral setal lines, *cd* = distance between the dorsolateral setal lines, *dd* = distance between the dorsal setal lines.

shaped open near to the dorsal pores. Typhlosole large, T-shaped.

E. lagodechiensis is clearly close to *E. nordenskioldi*, however differs from it by the larger *ab* and *cd* setal intervals (Fig. 11), the longer clitellum and its very special papillar arrangements. Apart from having papillae around 9–11 *cd*, it has large papillae far behind the clitellum which is a

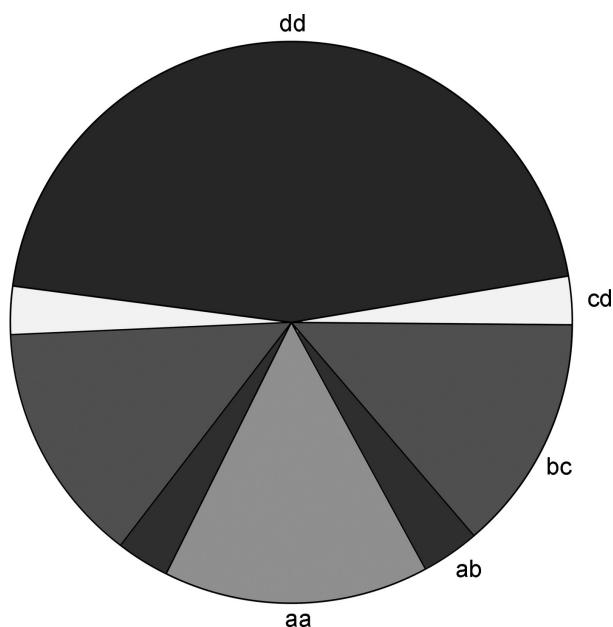


Fig. 11. *Eisenia lagodechiensis* (Michaelsen, 1910) setal arrangement. *aa* = distance between the ventral setal lines, *ab* = distance between the ventrolateral setal lines, *cd* = distance between the dorsolateral setal lines, *dd* = distance between the dorsal setal lines.

quite distinctive character. Therefore, we agree with Kvavadze (1985) and Perel (1997) that *E. lagodechiensis* is an independent species.

Eisenia sp.

Material examined: North Korea, Myohyang Mt. Wonhan Peak ca. 1500 m.s.l. 06.1985, 1 ex. leg. Z. Tóth (HNHM/16901)

Description: External characters. Length 71 mm, diameter 6 mm. Number of segments 104. Colour red-violet alive, bleaching out during preservation. Prostomium epilobous. First dorsal pore at intersegmental furrow 4/5. Setae closely paired. Seta arrangement behind clitellum: *aa:ab:bc:cd:dd* = 15:1.8:9.5:1:30 (Fig. 12). Male pores on segment 15, surrounded by small glandular crescents, confined into its own segment. Nephridial pores irregularly alternate between setal line *b* and above *d*. Clitellum on segments 25–½32 but segment 23 and 24 also slightly modified. Tubercula pubertatis on segments ½27–30. Genital papillae on 10 *cd*, 12, 17, 25–31 *ab*.

Internal characters: No septa notably thickened. Testes and funnels paired in segments 10–11, free. Seminal vesicles in 9, 11, 12, those in 9 somewhat smaller than the others. Spermathecae drop-shaped in 9/10–10/11 with external openings close to the mid-dorsal line. Ovarium in 13, elongated. Ovarian funnel with undulated rim, ovisac small, pending from 13/14 just above the ovarian duct. Calciferous glands in 11–12 with moderate diverticula. Paired hearts appear in segments 7–11, with a pair of small

Table 4. Main morphological characters in the *Eisenia nordenskioldi* species-group

Species	Biometry mm, segment No.	Clitellum	Tubercles	Papillae
<i>E. n. nordenskioldi</i> (Eisen, 1879) HNHM16722	100 × 7 143	27-½33	29-31	<i>ab</i> 8,27-31
<i>E. n. manshurica</i> Kobayasi, 1940	111-144 × 6.5 154-175	26,27-34	29-32	<i>ab</i> 22-35
<i>E. lagodechiensis</i> (Michaelsen, 1910) HNHM11047	115 × 5 135	25-34	½28-½32	<i>cd</i> 9-11, <i>ab</i> 25-38
<i>Eisenia atlavinitae</i> Perel & Graphodatsky, 1984	65-110 × 4-6 105-149	26(27)-33	28, ½28-31	<i>ab</i> 7,8,11-13, 26-33,34 <i>cd</i> 9-11
<i>Eisenia sibirica</i> Perel & Graphodatsky, 1984	55-82 × 3.5-4 87-124	27-32, 1/n33	29-30	<i>ab</i> 8, 28-31, <i>cd</i> 9-11
<i>Eisenia angusta</i> Perel, 1994	56-81 × 2.5-3 98-118	27-½ 33	½29-½31	<i>ab</i> 11, 12, 16, 26-32
<i>Eisenia nana</i> Perel, 1985	40-58 × 2.5-3 95-118	27, ½ 27-33	29-32	<i>ab</i> 8-12, <i>cd</i> 10,11

extraoesophageal vessel in 12. Nephridial bladders simple, sausage-shaped. Crop in segments 15–16, and gizzard in segments 17–18. Typhlosole bilobed, moderate. Longitudinal muscle layer is of pinnate type.

Remarks: Unfortunately the only specimen at hand shows sign of regeneration before the male pore. However as all the inner organs are in the correct position we assume that the clitellar data is correct. This species is similar to *E. kucenkoi* Michaelsen, 1903 in the position of the clitellum and the distribution of genital papillae. However, *E. kucenkoi* is proandric (1 pair of testis in 10) and the present specimen possesses longer tubercles. It is slightly similar to *E. koreana* and the two recently described species *E. gaga* Blakemore & Park, 2012 and *E. sindo* Blakemore & Park, 2012 however differs from all the three species in the position of the tubercles (27–29 vs. ½27–30) and the number of seminal vesicles (9–12 vs. 9, 11, 12). Another difference is that the present specimen lacks the characteristic quadrangular shape which is typical for all the three species mentioned. Most probably this specimen represents a new still undescribed species however, for the formal description additional material is needed.

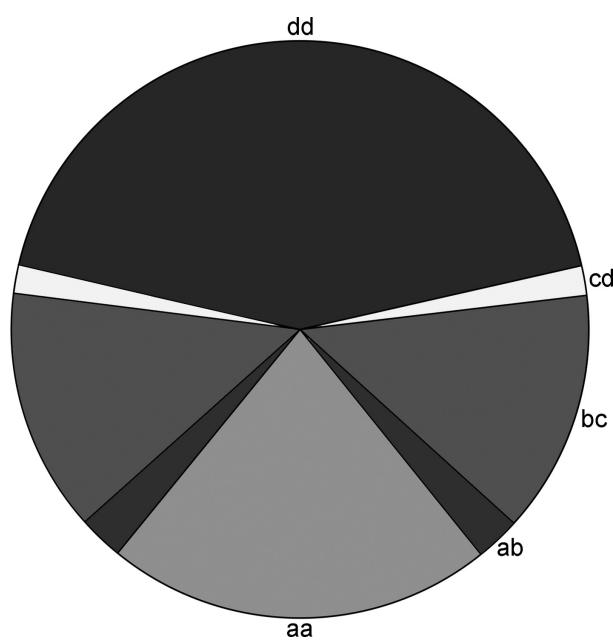


Fig. 12. *Eisenia* sp. setal arrangement. aa = distance between the ventral setal lines, ab = distance between the ventrolateral setal lines, cd = distance between the dorsolateral setal lines, dd = distance between the dorsal setal lines.

DISCUSSION

The Korean Peninsula represents a transitory region between the Eurasian (characterised by Lumbricidae) and Indo-Malayan (characterised by Megascolecidae and Moniligastridae) earthworm realms. However, its earthworm fauna is dominated by the Indo-Malayan elements (Blakemore 2014). Only 22 lumbricid species are reported so far for Korea including several unconfirmed records (e.g. *Lumbricus terrestris* Linnaeus, 1758 or *Bimastos beddardi* (Michaelsen, 1894)). In the lumbricid fauna the widely distributed peregrine species prevail. Up until now only eight taxa; *E. gaga* Blakemore & Park, 2012, *E. j. japonica* (Michaelsen, 1892), *E. j. vaga* Blakemore, 2013, *E. koreana* (Zicsi, 1972), *Eisenia muuido* Blakemore, 2015, *E. n. nordenskioldi* (Eisen, 1879), *E. n. pallida* Malevic, 1956 and *E. sindo* Blakemore & Park, 2012 can be regarded as autochthonous. Not surprisingly, all the eight taxa belong to the genus *Eisenia* which is indicative of the Turanian-Far-Eastern Lumbricid Domain (Csuzdi et al. 2011).

The most widely distributed autochthonous species is a small unpigmented form of the *E. nordenskioldi* species complex. This species complex contains a number of closely related deeply pigmented species (Table 3) and also several unpigmented taxa (Table 2).

A recent molecular analysis of the red pigmented forms referred as *E. nordenskioldi* subsp. *nordenskioldi* revealed that even the pigmented forms consist of several deeply diverged molecular lineages that might represent cryptic species (Shekhovtsov et al. 2013). Unfortunately the authors failed to provide detailed morphological descriptions of the six lineages recognized, therefore it is difficult to determine whether any (or all?) of the lineages correspond to a taxonomically recognized member of the *E. nordenskioldi* species complex (Table 3).

Shekhovtsov et al. (2013) hypothesized, that lineage 1 might represent the nominal subspecies *E. n. nordenskioldi* (Eisen, 1879). Taking into account that the 3 syntypes of *E. nordenskioldi* housed in Hamburg (No. V5319) possess a label stating: "S. am Troitzkoj, Jenissej, 65°45'N Br" this is possibly right.

The three *E. nordenskioldi* specimens analyzed from Korea represent an independent clade (Fig. 13, 14) which is most closely related to lineage 3 of Shekhovtsov et al. (2013). However the bootstrap supports of this placement especially in the Bayesian tree is rather low (0.58 and 0.86

in ML). The two *E. nordenskioldi* subsp. *pallida* specimens analysed by Shekhovtsov et al. (2013) are placed quite far from the Korean unpigmented *nordenskioldi* forms and both in the original analysis and in our trees they are close to lineage 1 and lineage 5. To this group joins the two newly described *E. nordenskioldi* subspecies *E. n. mongol* and *E. n. onon* as well.

It is interesting, that lineage 6 which represent

a distant clade also in the original analysis possesses quite different positions in the ML and the Bayesian tree which might indicate that these specimens do not belong to the *nordenskioldi* complex.

The two Korean endemic species *E. sindo* and *E. gaga* are always basal to the *E. nordenskioldi* complex (excl. lineage 6). The two *E. japonica* subspecies (*japonica* and *vaga*) either

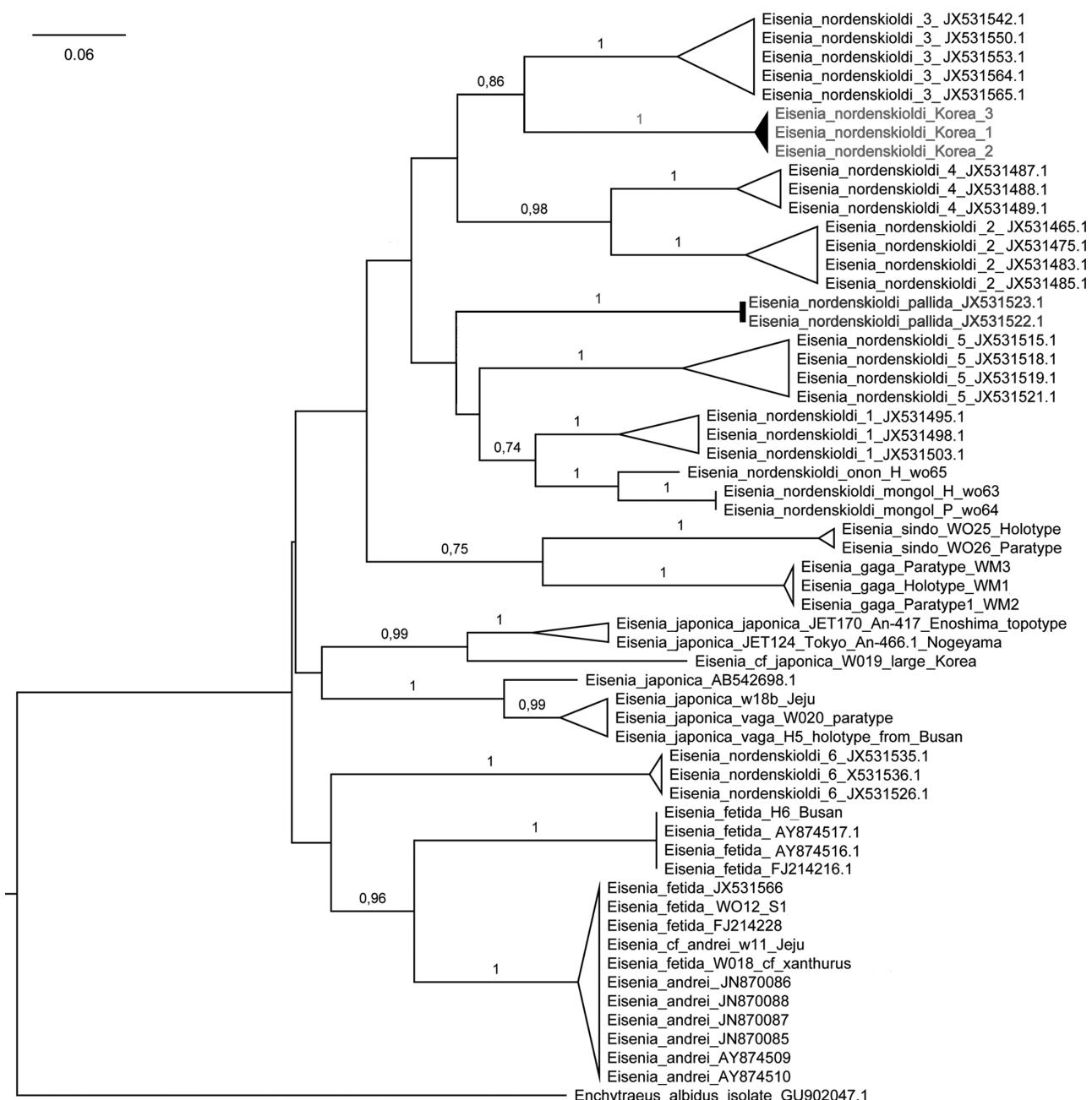


Fig. 13. Maximum Likelihood tree of the Asian *Eisenia* species using *COI/1* sequences. Numbers above branches indicate ML bootstrap support.

join to the *gaga-sindo-nordenskioldi* clade (ML) or to the clade formed by the *E. fetida* complex.

To draw a taxonomic conclusion on these molecular results is rather difficult. In general, DNA barcoding would provide a good estimation of species delimitations if the interspecific genetic distances are much higher and do not overlap with that of the intraspecific distances (Barrett and Herbert 2005). However, the applicable genetic distances are highly clade specific and should be determined for each individual group of taxa (Novo et al. 2012) independently. Chang and James (2011) combining all the available earthworm taxa determined a 9% threshold value of K2P genetic distance for conspecificity and 15% for the different species. Between these two values there is an ambiguous range where no clear taxonomic conclusion can be drawn.

However, for lumbricid earthworms Szederjesi et al. (2011) found much higher (16.5% intraspecific

and 19.5 % inter-species) genetic distances in the Alpine-Carpathian earthworm species pair *Eisenia lucens* (Waga, 1857) and *Eisenia spelaea* (Rosa, 1901). Taking into account these latter values we can assume that *E. nordenskioldi* cf. *pallida* of Shekhovtsov et al. (2013), *E. nordenskioldi mongol*, *E. nordenskioldi onon* and *E. nordenskioldi* lineage 5 might represent different subspecies of *E. nordenskioldi nordenskioldi* (Table 4). The separate subspecific statuses of *E. n. mongol* and *E. n. onon* (8.2% K2P difference) require further considerations. The Korean *E. nordenskioldi* specimens might represent an independent species (the true *Eisenia pallida* Malevic, 1956?) together with lineage 3 of Shekhovtsov et al. (2013). Also the other *nordenskioldi* lineages (2 & 4 and 6) might represent independent species however; their relationships with the described members of the *E. nordenskioldi* species group need to be determined. The two subspecies of

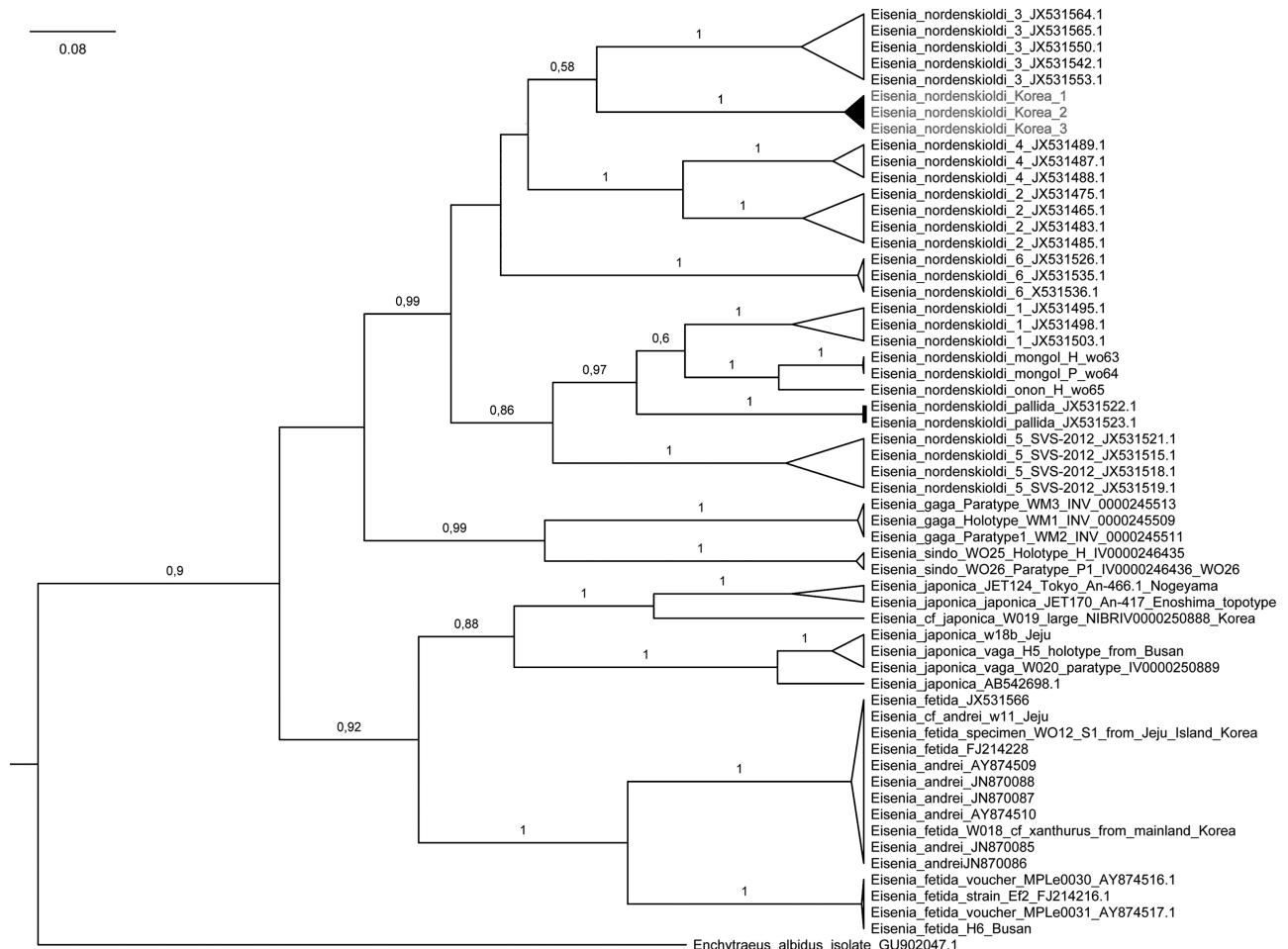


Fig. 14. Bayesian inference tree of the Asian *Eisenia* species using COI/1 sequences. Numbers above branches indicate Bayesian posterior probabilities.

Eisenia japonica seem to be representing two independent species however, this does not hold for the *E. fetida* - *E. andrei* species complex.

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