

Distribution and genetic diversity of two earthworms, *Helodrilus oculatus* and *Satchellius mammalis* (Clitellata: Lumbricidae) in Scandinavia

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We report on the Scandinavian distribution of two earthworm species, *Helodrilus oculatus* and *Satchellius mammalis*. Both appear relatively new to the Scandinavian Peninsula, as they were not included in the monographic revisions of the earthworm fauna of Sweden and Norway in the mid-1900s. We provide recent records of both species from Norway and Sweden, *H. oculatus* also from Denmark, and haplotype networks of four markers (COI, 16S, H3, and ITS2) are used to visualise the genetic diversity within each species. There is moderate genetic variation in COI for both taxa, and for *H. oculatus*, there is a West-East division between the specimens from Norway, Gothenburg (western Sweden) and Bavaria (Germany), and the ones from eastern Sweden and the Bornholm island (easternmost Denmark). This could potentially be explained by different origin and colonisation routes. In the other markers the variation is limited, and in the nuclear genes no pattern to support this split is seen. We also analyse the phylogenetic positions of *H. oculatus* and *S. mammalis* in the family Lumbricidae by combining our data (including also some 12S, 18S and 28S sequences) with a published dataset. We conclude that neither *Helodrilus* nor *Satchellius* are monophyletic. *Helodrilus oculatus* (type species of *Helodrilus*), however, forms a clade with some of its current congeners.

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INTRODUCTION

The Scandinavian earthworm fauna consists of about 35 nominal species, all in the family Lumbricidae with the exception of a few introduced species, e.g., *Amyntas corticis* (Kinberg, 1867) (Megascolecidae) (Gates 1972) and two species of *Dichogaster* Beddard, 1888 (Acanthodrilidae) (Erséus *et al.* 1994), found in greenhouses or indoors in bathrooms. Two comprehensive studies of the Scandinavian earthworms were published by Stöp-Bowitz (1969) for Norway, and Julin (1949) for Sweden. There is also a newer checklist compiled by Blakemore (2007) available online, which lists 41 species from Denmark, Finland, Norway and Sweden.

In this paper we give several distributional records of two earthworms *Helodrilus oculatus* Hoffmeister, 1845 and *Satchellius mammalis* (Savigny, 1826) (Figure 1) in southern Scandinavia, supplemented by new records also from Germany and the Netherlands. Neither of these species were reported by Stöp-Bowitz (1969) or Julin (1949). Blakemore (2007) mentions *H. oculatus* from Denmark and *S. mammalis* from Denmark and Norway, and Sims and Gerard (1985) mentions *S. mammalis* from Norway, but this appears to be second-hand information without original (published) references. There are records of both species from Denmark (Møller *et al.* 2015).

Our limited samples revealed, for both species, a considerable level of variation in the COI barcoding gene, i.e., our primary marker for species recognition. To eliminate the possibility that cryptic species are involved, we herein also describe the genetic variation of the studied populations, by studying four different gene markers. Lastly, we analyse the phylogenetic position of these two taxa by adding them to a large dataset of DNA sequences of Lumbricidae studied by Domínguez *et al.* (2015).

The genus *Helodrilus* Hoffmeister, 1845 consists of about 20 described species and subspecies found from Morocco, the Iberian Peninsula, France, and the British Isles in the west to the Levant and Caucasus regions in the east (Szederjesi *et al.* 2014). Although only three species (and none of which is the type species, i.e., *H. oculatus*) were analysed by Domínguez *et al.* (2015), the latter authors found *Helodrilus* to be polyphyletic in their study. Representatives of this genus live mainly in moist soil and can be found in riverbanks, swamps and caves (Szederjesi *et al.* 2014). This is also true for *H. oculatus*, which can be found in running water, ditches, ponds, as well as in waterlogged soil, mainly in broadleaved woodlands (Sims & Gerard 1985; Sherlock 2018).

Satchellius Gates, 1975 consists of only three species (Szederjesi

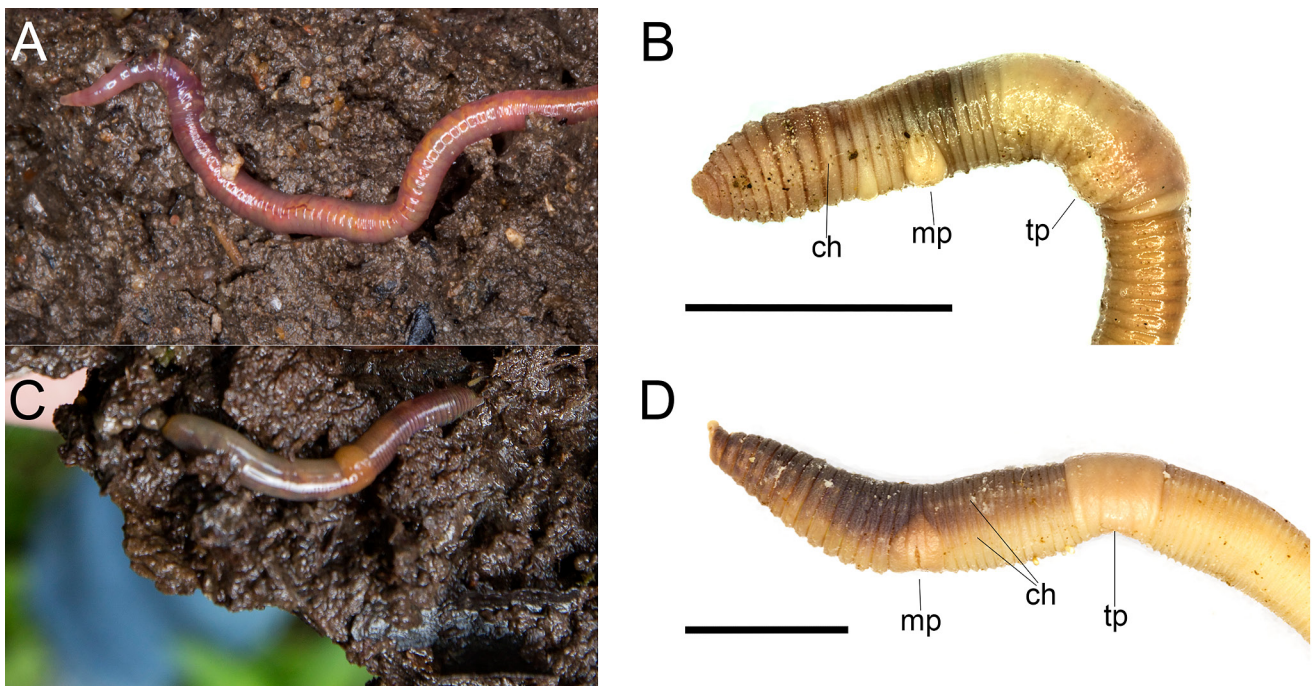


Figure 1. *Helodrilus oculatus* (A-B) and *Satchellius mammalis* (C-D). A and C are live worms (Photo: Johan Wingborg); B and D worms preserved in ethanol (Photo: Svante Martinsson). A. Specimen from Vitsippsdalen, Gothenburg, Sweden; worm’s anterior end was buried in the soil (outside the right edge of the photo). B. Specimen CE26263 from Tommarpsån River, Simrishamn, Sweden. C-D. Specimen CE26115 from Vitsippsdalen. Scale bars = 5 mm; ch = chaetae, mp = male pore, tp = tuberculum pubertatis.

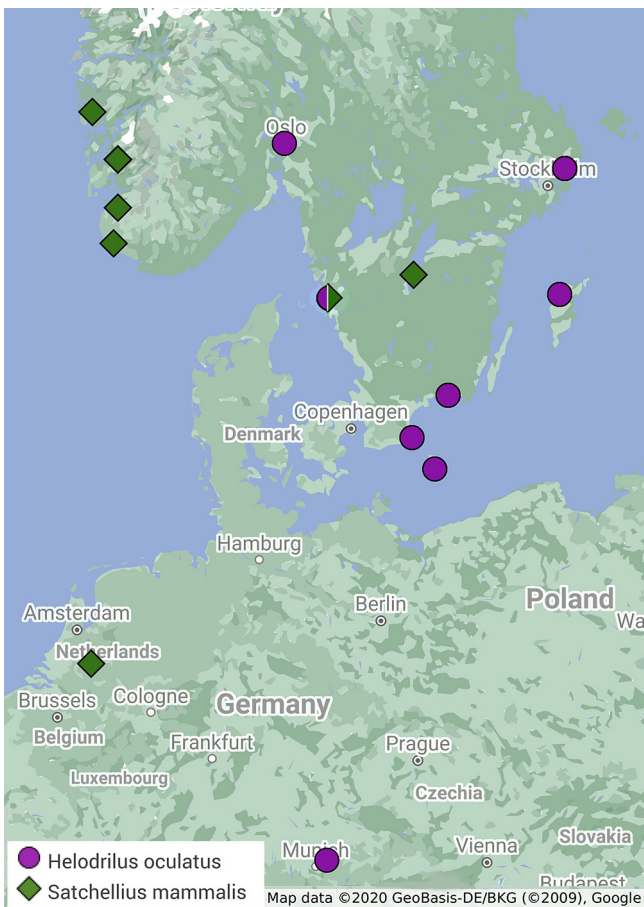


Figure 2. Distribution of Scandinavian specimens of *Helodrilus oculatus* and *Satchellius mammalis* used in this study. In Gothenburg (the locality shared between the two species) several closely situated sites are combined for clarity, see Appendix 1 for exact positions. The map is created in Google Maps and an interactive version of the map can be found at <https://www.google.com/maps/d/edit?mid=1mZbMePUxDLZZ4uQ-IXWp829-LtgYU7ot&usp=sharing>.

& Csuzdi 2016), all found on the Iberian peninsula, but the type species *S. mammalis* is found in several other W. European countries too (Sims & Gerard 1985). Only one species was included in the lumbricid phylogeny by Domínguez *et al.* (2015), *S. madeirensis* (Michaelsen, 1891) as *S. gatesi* Qiu & Bouché, 2000, the two taxa later synonymised by Szederjesi and Csuzdi (2016). *Satchellius mammalis* is mainly found in woodland in areas with high organic content, in old pastures and river banks (Sherlock 2018; Sims & Gerard 1985).

MATERIAL AND METHODS

In total 39 specimens of *Helodrilus oculatus* (2 from Norway, 34 from Sweden, 2 from Denmark, and 1 from Germany), and 14 specimens of *Satchellius mammalis* (10 from Norway, 3 from Sweden and 1 from the Netherlands), collected between 2005 and 2017 were included in the study (Table 1; Appendix 1). The distribution of included individuals are visualised using Google Maps (Figure 2).

DNA extraction and sequencing

DNA was extracted from a small sample of the body wall taken from the posterior part of each specimen using either Qiagen DNeasy Blood & Tissue Kit or Epicentre QuickExtract DNA Extraction Solution 1.0, following the manufacturer’s instructions. The worms are stored in ethanol to serve as physical vouchers, and are deposited in either the Swedish Museum of Natural History (SMNH), Stockholm, Sweden, or the University Museum of Bergen (ZMBN), Bergen, Norway (accession numbers in Appendix 1).

For all specimens the mitochondrial Cytochrome c oxidase subunit I (COI) gene was amplified, and for a subset of worms the mitochondrial ribosomal 16S gene, the complete nuclear ribosomal Internal Transcribed Spacer 2 (ITS2), and the nuclear gene Histone H3 (H3), were successfully amplified. The primers and PCR programmes used are listed in Table 2. For most specimens, all sequencing was carried out by Macrogen Inc. (Seoul, Korea) or Eurofins MWG

Table 1. Specimens of *Helodrilus oculatus* and *Satchellius mammalis* used in this study, with specimen ID:s, collection country and province, collection coordinates and GenBank Accession numbers. For more detailed collection information see Appendix 1. Note that for four specimens, three supplementary genes (12S, 18S, 28S) have been sequenced; their GenBank ## are given on inserted lines. In ITS2 and H3 some specimens are represented by two sequences as they are heterozygous.

Species	Specimen ID	Country Province	Coordinates		BOLD	GenBank Accession #			
			North	East		COI	16S	ITS2	H3
<i>H. oculatus</i>	CE1230	SWE Västergötland	57.6813	11.9562	NVLUM001-21	MW535839	MW544108	-	-
<i>H. oculatus</i>	CE5690	SWE Västergötland	57.6817	11.9567	NVLUM002-21	MW535854	MW544098	MW534290/ MW534291	MW542595
<i>H. oculatus</i>	CE5691	SWE Västergötland	57.6817	11.9567	NVLUM003-21	MW535835	-	-	-
<i>H. oculatus</i>	CE5692	SWE Västergötland	57.6817	11.9567	NVLUM004-21	MW535842	-	-	MW542596/ MW542597
<i>H. oculatus</i>	CE5693	SWE Västergötland	57.6817	11.9567	NVLUM005-21	MW535846	MW544103	MW534292	MW542610
<i>H. oculatus</i>	CE5694	SWE Västergötland	57.6817	11.9567	NVLUM006-21	MW535866	MW544090	MW534293	MW542598
<i>H. oculatus</i>	CE5695	SWE Västergötland	57.6817	11.9567	LUTCE188-10	HQ573048	-	-	-
<i>H. oculatus</i>	CE5700	SWE Västergötland	57.6817	11.9567	NVLUM007-21	MW535862	MW544093	MW534294/ MW534295	MW542598/ MW542600
<i>H. oculatus</i>	CE5705	SWE Västergötland	57.6817	11.9567	NVLUM008-21	MW535853	-	-	-
<i>H. oculatus</i>	CE7085	SWE Västergötland	57.6817	11.9567	LUTCE478-10	HQ989840	-	-	-
<i>H. oculatus</i>	CE7087	SWE Västergötland	57.6817	11.9567	LUTCE479-10	HQ989841	-	-	-
<i>H. oculatus</i>	CE6500	SWE Uppland	59.5710	18.5502	NVLUM009-21	MW535840	MW544107	MW534296/ MW534297	MW542611
<i>H. oculatus</i>	CE6501	SWE Uppland	59.5710	18.5502	LUTCE307-10	HQ573155	-	-	-
<i>H. oculatus</i>	CE6502	SWE Uppland	59.5710	18.5502	LUTCE308-10	HQ573156	-	-	-
<i>H. oculatus</i>	CE6503	SWE Uppland	59.5710	18.5502	LUTCE309-10	HQ573157	-	-	-
<i>H. oculatus</i>	CE6504	SWE Uppland	59.5710	18.5502	LUTCE310-10	HQ989697	-	-	-
<i>H. oculatus</i>	CE6505	SWE Uppland	59.5710	18.5502	NVLUM010-21	MW535838	MW544109	MW534298	MW542601/ MW542602
<i>H. oculatus</i>	CE8815	GER, Bavaria	48.2507	11.889	LSWED290-11	JN261155	-	MW534299	MW542603/ MW542604
<i>H. oculatus</i>	CE9307	SWE, Gotland	57.741	18.404	LSWED412-11	JN261264	-	MW534300/ MW534301	MW542605
<i>H. oculatus</i>	CE9308	SWE, Gotland	57.741	18.404	LSWED413-11	JN261265	-	MW534302/ MW534303	MW542607
<i>H. oculatus</i>	CE9311	SWE, Gotland	57.741	18.404	LSWED414-11	JN261266	-	MW534304/ MW534305	MW542606
<i>H. oculatus</i>	CE9732	DEN, Bornholm	55.0361	14.9092	LSWED436-11	JN261283	-	MW534288/ MW534289	XX000000/ MW542609
<i>H. oculatus</i>	CE9733	DEN, Bornholm	55.0361	14.9092	LSWED437-11	JN261284	-	-	MW542608
<i>H. oculatus</i>	CE11347	SWE Västergötland	57.6847	11.9753	NVLUM011-21	MW535837	MW544110	MW534306/ MW534307	MW542612
<i>H. oculatus</i>	CE11393	SWE Västergötland	57.6822	11.9551	NVLUM012-21	MW535836	MW544111	MW534308/ MW534309	MW542613
						12S MW538117	18S MW538113	28S MW538298	
<i>H. oculatus</i>	CE11468	SWE Västergötland	57.6822	11.9551	NVLUM013-21	MW535847	-	-	-
<i>H. oculatus</i>	CE11469	SWE Västergötland	57.6822	11.9551	NOEAR057-18	MW535874	-	-	-
<i>H. oculatus</i>	CE18776	SWE Blekinge	56.199	15.282	NVLUM014-21	MW535860	MW544094	MW534310/ MW534311	MW542616
<i>H. oculatus</i>	CE18777	SWE Blekinge	56.199	15.282	NVLUM015-21	MW535859	MW544095	MW534312/ MW534313	MW542617
						12S MW538118	18S MW538116	28S MW538299	
<i>H. oculatus</i>	CE18778	SWE Blekinge	56.199	15.282	NVLUM016-21	MW535849	MW544101	MW534314/ MW534315	MW542618
<i>H. oculatus</i>	CE18780	SWE Blekinge	56.199	15.282	NOEAR084-18	MW535850	-	-	-
<i>H. oculatus</i>	CE26114	SWE Västergötland	57.6807	11.9591	NVLUM017-21	MW535848	MW544102	MW534316/ MW534317	MW542619
<i>H. oculatus</i>	CE26263	SWE Skåne	55.5321	14.2701	NVLUM018-21	MW535872	MW544086	MW534318	MW542620

Table 1. Continued.

Species	Specimen ID	Country Province	Coordinates		BOLD	GenBank Accession #			
			North	East		COI	16S	ITS2	H3
<i>H. oculatus</i>	CE26264	SWE Skåne	55.5321	14.2701	NVLUM019-21	MW535851	MW544100	MW534319	MW542621
<i>H. oculatus</i>	CE26265	SWE Skåne	55.5321	14.2701	NVLUM020-21	MW535841	MW544106	MW534320	MW542622
<i>H. oculatus</i>	CE26266	SWE Skåne	55.5321	14.2701	NOEAR121-18	MW535858	-	-	-
<i>H. oculatus</i>	CE30992	SWE Västergötland	57.6864	11.9548	NVLUM021-21	MW535870	MW544087	MW534321/ MW534322	MW542623
<i>H. oculatus</i>	CE16604	NOR Oslo	59.9281	10.7059	NVLUM022-21	MW535855	MW544097	MW534323	MW542614
<i>H. oculatus</i>	CE16605	NOR Oslo	59.9281	10.7059	NVLUM023-21	MW535852	MW544099	MW534324/ MW534325	MW542615
<i>S. mammalis</i>	CE4365	SWE Småland	58.0372	14.3127	NVLUM024-21	MW535873	MW544085	MW534331	MW542633/ MW542634
<i>S. mammalis</i>	CE5391	SWE Västergötland	57.6850	11.8997	NVLUM025-21	MW535867	MW544089	MW534333/ MW534334	MW542635
<i>S. mammalis</i>	CE26115	SWE Västergötland	57.6807	11.9591	NVLUM026-21	MW535863	MW544092	MW534327	MW542632
<i>S. mammalis</i>	CE11843	NOR Rogaland	58.5021	5.9183	NVLUM027-21	MW535868	MW544088	MW534326	MW542624
<i>S. mammalis</i>	CE12753	NOR Hordaland	60.3716	5.3325	NOREW065-11	MW535844	-	-	-
<i>S. mammalis</i>	CE15054	NOR Hordaland	59.7067	6.0477	NVLUM028-21	MW535843	MW544105	MW534332	MW542625
<i>S. mammalis</i>	CE15055	NOR Hordaland	59.7067	6.0477	NOEAR066-18	MW535861	-	-	-
<i>S. mammalis</i>	CE15056	NOR Hordaland	59.7067	6.0477	NOEAR067-18	MW535871	-	-	-
<i>S. mammalis</i>	CE15057	NOR Hordaland	59.7067	6.0477	NVLUM029-21	MW535865	-	MW534335	MW542626
<i>S. mammalis</i>	CE15058	NOR Hordaland	59.7067	6.0477	NVLUM030-21	MW535857	-	MW534336	MW542627
<i>S. mammalis</i>	CE15059	NOR Hordaland	59.7067	6.0477	NVLUM031-21	MW535845	MW544104	MW534330	MW542628
<i>S. mammalis</i>	CE15060	NOR Hordaland	59.7067	6.0477	NVLUM032-21	MW535864	MW544091	MW534329	MW542629
<i>S. mammalis</i>	CE18431	NED Gelderland	51.793	5.298	NVLUM033-21	MW535856	MW544096	MW534328	MW542630
<i>S. mammalis</i>	CE21316	NOR Rogaland	59.0163	6.0436	NVLUM034-21	MW535869	-	MW534337	MW542631

Operon (Ebersberg, Germany); 17 specimens were handled by the Canadian Centre for DNA Barcoding (CCDB) (Guelph, Canada), with COI data stored at the Barcode of Life Datasystems (BOLD). For four of these latter worms, DNA was also extracted at the University of Gothenburg, which enabled sequencing of some of the remaining markers (for details, see Table 1). For two specimens each of *H. oculatus* and *S. mammalis* the markers 12S, 18S and 28S (D1-D2 region) were also amplified and sequenced (PCR-programs and primers listed in Table 2).

Sequences were assembled in Geneious Pro v. 7.1 (Biomatters Ltd.; <http://www.geneious.com>) and aligned separately for each species and gene using MAFFT v7.017 (Katoh *et al.* 2002), as implemented in Geneious Pro v. 7.1, using the auto-algorithm and default settings. In the H3 and ITS datasets, a few individuals showed clear signs of heterozygosity, i.e., distinct double peaks at certain positions in the sequencing chromatograms. Due to this, we separated H3 and ITS alleles using the PHASE algorithm (Stephens & Donnelly 2003; Stephens *et al.* 2001) as implemented in DNAsp v. 5.10 (Librado & Rozas 2009). The phasing was run for 100 iterations after 100 initial burn-in iterations, with a thinning interval of 1 using default settings. For homozygous specimens only one of the two identical alleles were kept, for heterozygous specimen the two alleles are separated by the suffix *_1* or *_2*. All new sequences produced in this study are deposited in GenBank, and all 16S and COI are also deposited on BOLD.

Haplotype networks and genetic distances

To visualize haplotype diversity, haplotype networks were constructed for all four genes of both species in PopART v1 (Leigh & Bryant 2015) using statistical parsimony (Clement *et al.* 2002; Templeton *et al.* 1992). For all markers, sections with missing data at the ends of the alignments, and for 16S also sections with gaps, were masked and not included in the analysis. For COI we also constructed a second set of networks including additional publicly available sequences on BOLD. For *Helodrilus* four more sequences were added, and for *Satchellius* 20 more sequences were added (Appendix 2). However, although this set was based on a larger sample of specimens, the masked alignments obtained for the analyses were shorter than those used for the networks based on our own, more limited dataset; some of the BOLD sequences are only about 300 bp long.

Uncorrected genetic p-distances were calculated for the COI datasets with only our data in MEGA X (Kumar *et al.* 2018), using pairwise deletion for missing data, the distances were analysed and summarised in a histogram, using R version 3.6.3 (with the packages ggplot2, dplyr, and readr). The alignments used for the networks, the distance files, and R-script are available at https://github.com/Svante-Martinsson/Helodrilus_Satchellius.

Phylogenetic analysis

To estimate the systematic positions of the two species within Lumbricidae, sequences from Domínguez *et al.* (2015) were downloaded from Genbank, and combined with data from two of

Table 2. List of PCR-primers and programs used in the study.

Primer	Sequence 5'-3'	Reference	PCR-program
COI			
LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)	
HCO2198	TAAACTTCAGGGTGACCAAAAATCA	Folmer et al. (1994)	95°C for 5 min, 35 cycles each of 95°C for 40 sec, 45°C for 45 sec and 72°C for 60 sec, finally, 72°C for 8 min.
COI-E	TATACTTCTGGGTGTCCGAAGAATCA	Bely and Wray (2004)	
16S			
16SAR-L	CGCCTGTTTATCAAAAACAT	Palumbi et al. (1991)	95°C for 5 min, 35 cycles each of 95°C for 30 sec, 50°C for 30 sec and 72°C for 60 sec, finally, 72°C for 8 min.
16SBRH	CCGGTCTGAACTCAGATCACGT	Palumbi et al. (1991)	
H3			
H3F	ATGGCTCGTACCAAGCAGACVGC	Colgan et al. (1998)	95°C for 5 min, 35 cycles each of 95°C for 30 sec, 50°C for 30 sec and 72°C for 90 sec, finally, 72°C for 8 min.
H3R	ATATCCTTRGGCATRATRGTGAC	Colgan et al. (1998)	
ITS2			
606F	GTCGATGAAGAGCGCAGCCA	Liu and Erséus (2017)	95°C for 5 min, 35 cycles each of 95°C for 45 sec, 55°C for 60 sec and 72°C for 90 sec, finally, 72°C for 8 min
1082R	TTAGTTTCTTTCTCCGCTT	Liu and Erséus (2017)	
12S			
12SE1	AAAACATGGATTAGATACCCRYCTAT	Jamieson et al. (2002)	95°C for 5min, 43 cycles each of 95°C for 40 sec, 45°C for 45 sec and 72°C for 1min, finally 72°C for 8min
12SH	ACCTACTTTGTTACGACTTATCT	Jamieson et al. (2002)	
18S			
TimA	AMCTGGTTGATCCTGCCAG	Norén and Jondelius (1999)	
1100R	GATCGTCTTCGAACCTCTG	Norén and Jondelius (1999)	
600F	GGTGCCAGCMGCCGCGGT	Norén and Jondelius (1999)	95°C for 5min, 30 cycles each of 95°C for 30sec, 60°C for 30sec, 72°C for 1min and 30 sec, finally 72°C for 8min.
TimB	TGATCCATCTGCAGGTTACCT	Norén and Jondelius (1999)	
28S			
28SC1'	ACCCGCTGAATTTAAGCAT	Jamieson et al. (2002)	95°C for 5min, 35 cycles each of 95°C for 30 sec, 50°C for 30 sec and 72°C for 1min, finally, 72°C for 8min.
28SD2'	TCCGTGTTTCAAGACGG	Jamieson et al. (2002)	

our *H. oculatus* worms (CE11393 and 18777) and two *S. mammalis* (CE15060 and 18431). Six gene regions were used: 12S (including tRNA-Val), 16S (including tRNA-Leu, tRNA-Ala, and tRNA-Ser), 18S, 28S, COII, and NADH, but the latter two were missing for our specimens, and for 12S and 16S we amplified a shorter fragment, not including any tRNAs. Each region was aligned using MAFFT v7.017 (Katoh *et al.* 2002), as implemented in Geneious Pro v. 7.1, using the auto-algorithm and default settings, and trimmed at the ends to reduce the amount of missing data, especially for 18S and 28S, where the sequences from Domínguez *et al.* (2015) and our newly produced sequences were only partly overlapping. The alignments were then concatenated, and the dataset was trimmed to remove specimens from species with more than two specimens and/or specimens with too much missing data. The final dataset included 110 specimens and was 5,188 bp long. Trees were estimated with Maximum Likelihood (ML) using phyML 3.0 (Guindon *et al.* 2010), and Bayesian Inference (BI) using MrBayes 3.2.7 (Ronquist *et al.* 2012). For the ML analysis, Smart Model Selection (Lefort *et al.* 2017) with Bayesian Information criterion was used for automatic model selection; and Nearest Neighbour Interchange was used for tree improvement. Branch support was calculated with the SH-like (Shimodaira-Hasegawa test-like) approximative Likelihood Ratio Test (aLRT) (Anisimova & Gascuel 2006). For the BI analysis the dataset was partitioned into genes, partitions were unlinked. Rate variation across sites was set to gamma distribution with a proportion of invariable sites; model jumping was implemented to integrate over substitution model space. The analyses were run for 50 million generations sampling every 10 000 generations, the first 25% were discarded as burn-in,

and a majority-rule consensus tree was constructed. The trees were drawn in FigTree 1.4.2 (Rambaut 2014) and further edited in Adobe Illustrator and Inkscape. Taxon names are updated in accordance with Szederjesi and Csuzdi (2016), Csuzdi *et al.* (2017), and Marchán *et al.* (2018). The alignments and output files from the analysis are available at https://github.com/Svante-Martinsson/Helodrilus_Satchellius.

RESULTS

Identification of specimens

Sexually mature worms (Figs. 1B, D) were identified using Sims & Gerard (1985), while identification of immature individuals was based on clustering of the COI barcodes. For some specimens of *Helodrilus oculatus*, we observed the characteristic lateral dark spots in some segments, large male pores (Figure 1C, mp), ridge-shaped tubercula pubertatis on XXIX-XXX (Figure 1C, tp), and closely paired chaetae that often have black tips (Figure 1C, ch). The segmental spots were visible also immediately after preservation in ethanol, but they appeared to fade with time in the fluid. The original author (Hoffmeister 1845: 39) claimed that the lateral spots (by him regarded as eye-spots, hence the name *oculatus*) and the black chaetae only occur in large and assumedly old worms.

For the sexually mature *Satchellius mammalis*, we noted, in particular, the conspicuous bulbous area surrounding each male pore in segment XV (Figure 1D, mp), small tubercula pubertatis on XXXIII-XXXIV (Figure 1D, tp), and widely paired chaetae (Figure 1D, ch).

Sequences

For *Helodrilus oculatus* COI was successfully sequenced for all 39 specimens, and the alignment is 658 bp long, of which 566 bp were used to construct a haplotype network; 16S was sequenced for 19 specimens, and the alignment is 485 bp long, of which 482 bp were used to construct the haplotype network; ITS2 for 23 specimens, and after phasing with 38 sequences, 547 bp, of which 527 bp were used to construct the haplotype network; and H3 for 25 specimens, after phasing with 29 sequences, 328 bp, of which 286 bp were used to construct the haplotype network. Sequences of 12S, 18S and 28S were also obtained from specimens CE11393 and CE18777.

For *Satchellius mammalis* COI was successfully sequenced for all 14 specimens, and the alignment is 658 bp long, of which 619 bp were used to construct a haplotype network; 16S was sequenced for eight specimens, and the alignment is 483 bp long, of which 480 bp were used to construct the haplotype network; ITS2 for 11 specimens, and after phasing with 12 sequences, 564 bp, of which 531 bp were used to construct the haplotype network; and H3 for 11 specimens, after phasing with 12 sequences, 328 bp, of which 297 bp were used to construct the haplotype network. Sequences of 12S, 18S and 28S were also obtained from specimens CE15060 and 18431.

New north-west European records

Specimens of *Helodrilus oculatus* were found at one locality in southeastern Norway, the Vigeland Park in Oslo, and ten localities in Sweden; six sites all in close proximity to each other in Gothenburg (province Västergötland), and one in each of the provinces Uppland, Gotland, Blekinge, and (eastern) Skåne (Figure 2). We also include two specimens from a locality in eastern Denmark (Bornholm) and one from southern Germany (Bavaria). All localities represent wet habitats, and include riverbanks, ditches, ponds, and groundwater seepages.

Satchellius mammalis was found at four localities in southwestern Norway (two in Hordaland, and two in Rogaland) and three in Sweden (two in Gothenburg, and one in province Småland), we also obtained one specimen from the Netherlands (Gelderland) (Figure 2). The localities include composts and deciduous woodland.

The two species were found together at one locality in Gothenburg, a stream bank in a deciduous woodland.

Haplotype networks and genetic distances

For *Helodrilus oculatus* (Figure 3 A-D) five haplotypes were found in COI, divided in two well-separated groups (clusters), one with an eastern distribution, with specimens from the Swedish east coast (Uppland, Gotland, Blekinge and Skåne) and easternmost Denmark (Bornholm), and the other with a western distribution, i.e., the worms from Norway and western Sweden; the German individual is included in this latter group too. Four 16S haplotypes, five H3 haplotypes and four ITS2 haplotypes were found. In the latter three markers, no clear haplotype groups are observed; for each of H3 and ITS2 most sequences are the same haplotype, and the rare haplotypes are separated from it by only 1-2 substitutions.

For *Satchellius mammalis* (Figure 3 E-H) six COI haplotypes were found in three well separated groups, one with most of the Norwegian specimens and all individuals from Sweden, another with one specimen from Norway and one from the Netherlands, and the third with only a single specimen from Norway. Four 16S haplotypes, two H3 haplotypes, and two ITS2 haplotypes were found. In the latter three markers no clear groups are observed; for each of H3 and ITS2 most sequences are the same haplotype, and the rare haplotypes are separated from it by only 1-2 substitutions.

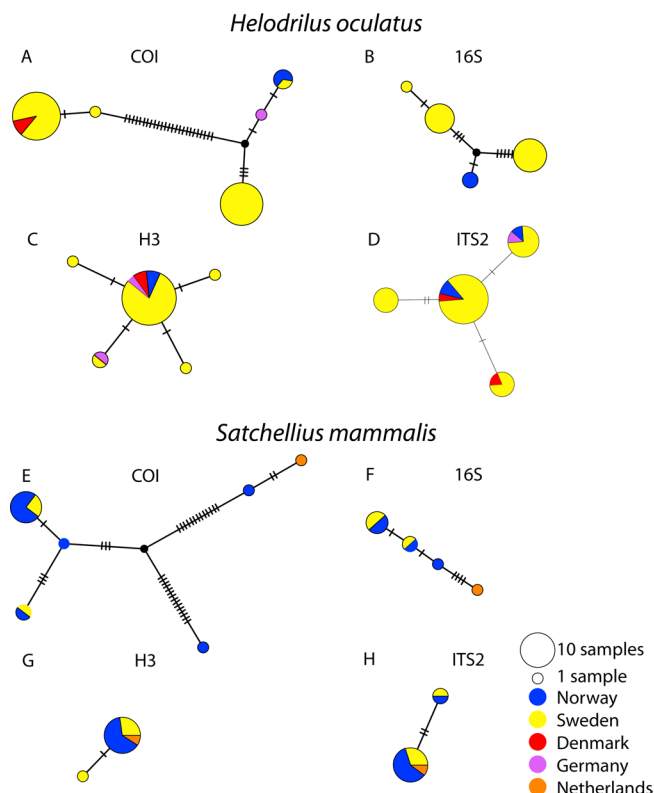


Figure 3. Statistical parsimony haplotype networks for *Helodrilus oculatus* (A-D) and *Satchellius mammalis* (E-H). A, E. COI networks, data from newly sequenced specimens only; B, F. 16S networks; C, G. H3 network; D, H. ITS2 networks. The size of the circles is relative to the number of sequences sharing that haplotype, the colours correspond to country of origin, and the hatch marks denote the number of substitutions between haplotypes.

The COI networks including publicly available data (Figure 4) are similar to the networks based on our data only. In the *H. oculatus* network, based on 285 bp, records from Geneva, Switzerland (Vivien *et al.* 2017; Vivien *et al.* 2015), Aarhus, Denmark (Møller *et al.* 2015; Lund *et al.* 2006), and Germany match our “western” group. In the *S. mammalis* network, based on 308 bp, the majority of our data is close to a record from Normandie, France, one of our records from Norway is close to records from Aarhus, Denmark (Møller *et al.* 2015), and one of our Norwegian records and the one from the Netherlands cluster together with records from Ireland (Klarica *et al.* 2012), and Aarhus, Denmark (Møller *et al.* 2015).

The uncorrected COI p-distances for *Helodrilus oculatus* vary between 0.000 and 0.055, with a gap between 0.008 and 0.044, and for *Satchellius mammalis* the distances vary between 0.000 and 0.049, with a gap between 0.006 and 0.027 (Figure 5).

Phylogenetic analysis

The tree resulting from the ML analysis (Figure 6) is largely well supported and congruent with the trees in Dominguez *et al.* (2015). However, we do not recover Lumbricidae as monophyletic; *Criodrilus lacuum* Hoffmeister, 1845 (Criodrilidae) is found as sister to *Diporodrilus pilosus* Bouché, 1970, but without support. *Helodrilus oculatus* is found in a clade also containing *H. patriarchalis* (Rosa, 1893) and *H. cernovitovianus* (Zicsi, 1967), whereas the last member of *Helodrilus* included in the tree, *H. cortezi* Qui & Bouché, 2000, is well separated from the rest and being the sister to *Allolobophora chaetophora* Bouché, 1972, despite being listed as a junior synonym to *H. oculatus* (Szederjesi *et al.* 2014). *Satchellius* is not monophyletic

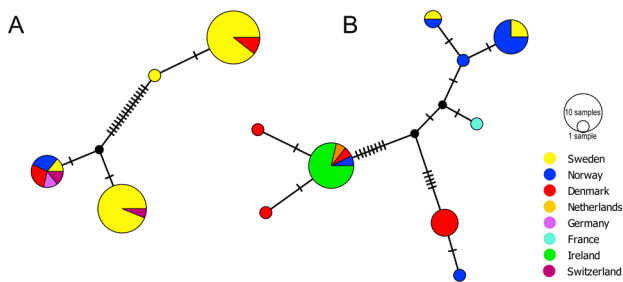


Figure 4. Statistical parsimony haplotype networks for *Helodrilus oculatus* (A) and *Satchellius mammalis* (B). The networks are constructed with a combination of our data and publically available data from BOLD. The size of the circles is relative to the number of sequences sharing that haplotype, the colours correspond with country of origin, and the hatch marks correspond to the number of substitutions between haplotypes.

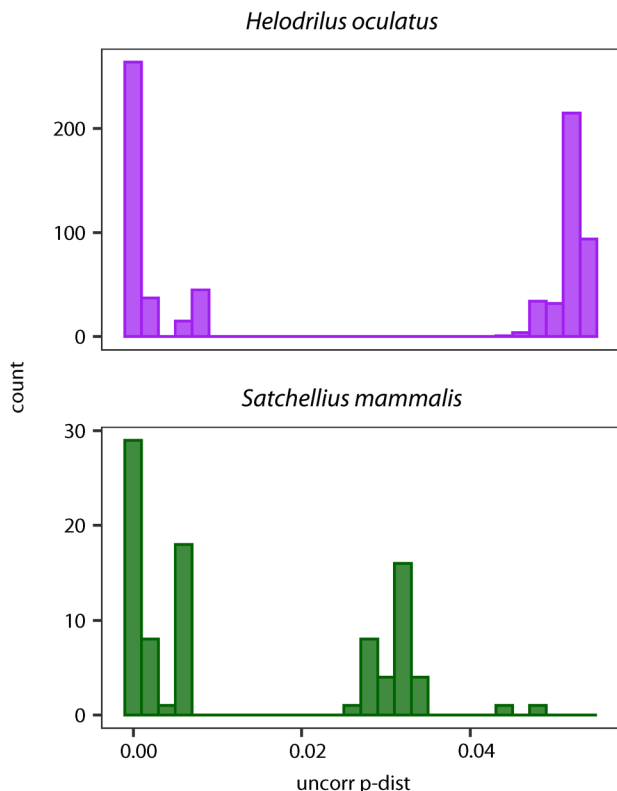


Figure 5. Histograms of uncorrected pairwise genetic distances in COI for our specimens of *Helodrilus oculatus* and *Satchellius mammalis*.

either, *S. mammalis* is found as sister to a *Healyella* sp., in a clade also including *Eiseniella* Michaelsen, 1900, *Eiseniona* Omodeo, 1956, and part of *Dendrobaena* Eisen, 1874. The other species of *Satchellius*, *S. madeirensis* is instead found in a clade including *Proctodrilus antipai* (Michaelsen, 1891) and an unidentified Lumbricidae; this clade is the sister to the *Helodrilus* clade.

The Bayesian analysis had problem with convergence, and the resulting tree (Appendix 3) is generally less resolved than the ML tree. Lumbricidae is not recovered as monophyletic, instead, there is a trichotomy consisting of *Criodrilus lacuum*, *Diporodrilus pilosus*, and remaining Lumbricidae. Neither *Helodrilus* nor *Satchellius* are recovered as monophyletic. *Helodrilus oculatus* is recovered as sister to *H. patriarchalis*, but without support, these two species are found in a large polytomy, where also *H. cernosvitovianus* is found. The last species of *Helodrilus*, *H. cortezi* is found together with *Allolobophora cheatophora* as sister to the large polytomy where the other *Helodrilus*



Figure 6. Lumbricidae phylogeny based on data from Domínguez et al. (2015) and newly produced sequences from *Helodrilus oculatus* and *Satchellius mammalis*. The phylogeny is based on a concatenated data matrix consisting of 110 specimens and it is 5,188 bp long. The phylogeny is estimated using ML in phyML. Numbers at branches are SH-like aLRT support values, only values >0.75 are shown. Scale shows expected number of changes per site.

species are found. *Satchellius mammalis* is recovered together with a *Dendrobaena* sp. but without support, these two are found in the large polytomy mentioned above; *S. madeirensis* is found in this polytomy too.

DISCUSSION

This study confirms that *Helodrilus oculatus* and *Satchellius mammalis* are two well established species in the southern Scandinavia. However, on the basis of their relative scarcity in a very large collection of Lumbricidae assembled and DNA-barcoded in Sweden and Norway during the last 10–15 years (Erséus and co-workers, largely unpublished; but see also James *et al.* 2010; Martinsson & Erséus 2017; Martinsson & Erséus 2018; Martinsson *et al.* 2017), we see no evidence of them spreading rapidly in the Nordic countries. For *S. mammalis*, this is in line with the attribute given to this taxon of being “uncommon” on the British Isles (Sherlock 2018), and it is noteworthy that the currently known Norwegian distribution of this species (Figure 2) is restricted to the southwestern coastline of the country. In Sweden, the species is truly rare, with only a total of three individuals being found in two southern provinces (Västergötland and Småland). *Helodrilus oculatus* is largely an aquatic worm, in Sweden and Norway associated with muddy substrates near some streams and small rivers, which is in accordance with what is known about the habitat preferences of the species (Sherlock 2018; Sims & Gerard 1985; Szederjesi *et al.* 2014). On the British Isles *H. oculatus* is “very rare” (Sherlock 2018), whereas it seems quite well spread in southern Scandinavia (Figure 2), but so far it is only known from one locality in Norway (a park in Oslo). *Helodrilus oculatus* also has a wider European distribution compared with that of *S. mammalis*, which appears restricted to Western Europe (Sherlock 2018; Sims & Gerard 1985; Szederjesi *et al.* 2014; Szederjesi & Csuzdi 2016).

Despite the existence of clear haplotype clusters in COI for both species, nothing else suggests that there are cryptic species in our material. The lack of structure in H3 and ITS2, with most sequences being identical, in particular, support that we only have one species of *Helodrilus* and *Satchellius* respectively in our dataset. Further, the distances between the COI clusters are smaller than what is commonly seen between different, but closely related lumbricid species, where distances as a rule are well above 10% (e.g., Martinsson & Erséus 2017; Pérez-Losada *et al.* 2012), whereas in our dataset of *H. oculatus* and *S. mammalis* the maximal intra-specific distances are about 5%.

The high genetic variation, in COI, in both species does not seem to support single introductions, but rather that each species has arrived to the Scandinavian Peninsula from more than one source population, and thus at more than one occasion. In *H. oculatus* the two COI haplogroups match the distribution, with one eastern group from Blekinge, Gotland, Uppland, southeastern Skåne and Bornholm, and one western group from Gothenburg and Oslo that also is found in Bavaria, southern Germany. This could indicate two distinct colonisation routes into Scandinavia, from two separate populations. In *S. mammalis* no such pattern can be seen, but with its more limited general (West European) distribution, and fewer specimens included in the present study, this is not surprising. However, in all three groups, there are matching records from outside the Scandinavian Peninsula, but without any apparent pattern. In a phylogeographical study of the earthworms of the French Mediterranean Hyères archipelago Marchán *et al.* (2020) discovered two different patterns, one of local *in situ* divergence during the Plio-Pleistocene (~5 mya to 12 kya), with clades restricted to the archipelago, and one characterised by the lack of geographical structure, and with the clades represented in the archipelago having a wide distribution. The latter pattern is consistent with human-mediated introduction. Lack of geographical structure is commonly seen in the Scandinavian earthworm fauna (e.g., Martinsson & Erséus 2017), and *H. oculatus* is one of the few species with a clear geographical structure so far observed. This is, however,

not surprising as Scandinavia until recently was ice-covered, and earthworms and other fauna are recent colonisers.

Both phylogenetic analyses showed that, as currently defined, neither *Helodrilus* nor *Satchellius* are monophyletic. Unfortunately, the Bayesian analysis had problems with convergence, which probably, at least partly explains the poor resolution in the resulting tree. Both trees recover *H. oculatus*, the type species of *Helodrilus*, as sister to *H. patriarchalis*, and in the ML tree *H. cernovitovianus* is sister to them, forming a clade that can be considered *Helodrilus s.str.* In both trees *H. cortezi* is instead found together with *Allolobophora cheatophora*, despite being listed as a synonym to *H. oculatus* in Szederjesi *et al.* (2014). In the ML tree *Satchellius mammalis* is found as the sister to a *Healyella sp.* and together found within a clade consisting of some *Dendrobaena* species, and *Eiseniona* and *Eiseniella*, which is interesting as both *Satchellius* and some species of *Healyella* have been regarded as members of *Dendrobaena* (Gates 1975; Omodeo & Rota 1989), and both have calciferous glands with diverticula in X (Csuzdi *et al.* 2017; Gates 1975). On the other hand, in the BI tree, *S. mammalis* is found together with *Dendrobaena sp.* but without support. The second included species, *S. madeirensis* is not recovered close to *S. mammalis*. In the ML tree it is found in the sister clade to *Helodrilus s.str.*, whereas in the BI tree it is found in a large polytomy. The taxonomy of this species is complicated. The sequences used here are from a specimen identified as *S. gatesi* by Domínguez *et al.* (2015), a species that was synonymised with *Allolobophora madeirensis* Michaelsen, 1891 by Szederjesi and Csuzdi (2016), while still regarding it as a member of *Satchellius*. In the latter paper a new species, *Zophoscolex zicsianus* Szederjesi & Csuzdi, 2016, was described based on material that was considered conspecific to *A. madeirensis* by Zicsi (1969), but *Z. zicsianus* is to our knowledge so far only found on the island of Madeira. The specimen used by Domínguez *et al.* (2015) is from northwestern Spain and it seems likely that it is conspecific with *S. madeirensis*.

Our new records of *H. oculatus* and *S. mammalis* are all from within the last 15 years. As Julin (1949) and Stöp-Bowitz (1969) gave no evidence of their existence on the Scandinavian Peninsula ca. 55–75 years earlier, it seems likely that the species have been, and still are, in a process of range extension towards the north. Our data are too limited to enable conclusions about the speed of the spread or whether these new taxa have any negative effect on other earthworms or the Scandinavian ecosystems as such. However, the scarce and patchy distribution of our records, as well as other findings in Europe, suggest that *H. oculatus* and *S. mammalis* probably should not be regarded as invasive earthworms.

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Appendix 1. Locality and collection information for specimens of *Helodrilus oculatus* and *Satchellius mammalis* included in this study. See Table 1 for GenBank Accession numbers of the gene sequences obtained from these specimens.

Species	Museum voucher	Specimen ID	Country	Province	Locality	Habitat	Coordinates		Collection date	Collector
							North	East		
<i>H. oculatus</i>	SMNH 191198	CE1230	SWE	Västergötland	Gothenburg, Änggården. Vitsippsdalen at Botanical Garden	Wet dark muddy soil on stream bank	57.6813	11.9562	08 Nov 2005	E. Rota, C. Erséus
<i>H. oculatus</i>	SMNH 191199	CE5690	SWE	Västergötland	Gothenburg, Änggården. Vitsippsdalen at Botanical Garden	Forest with maple and elm trees, wet dark soil and wet grey fine sand	57.6817	11.9567	08 Apr 2009	C. Erséus
<i>H. oculatus</i>	SMNH 191200	CE5691	SWE	Västergötland	Gothenburg, Änggården. Vitsippsdalen at Botanical Garden	Forest with maple and elm trees, wet dark soil and wet grey fine sand	57.6817	11.9567	08 Apr 2009	C. Erséus
<i>H. oculatus</i>	SMNH 191201	CE5692	SWE	Västergötland	Gothenburg, Änggården. Vitsippsdalen at Botanical Garden	Forest with maple and elm trees, wet dark soil and wet grey fine sand	57.6817	11.9567	08 Apr 2009	C. Erséus
<i>H. oculatus</i>	SMNH 191202	CE5693	SWE	Västergötland	Gothenburg, Änggården. Vitsippsdalen at Botanical Garden	Forest with maple and elm trees, wet dark soil and wet grey fine sand	57.6817	11.9567	08 Apr 2009	C. Erséus
<i>H. oculatus</i>	SMNH 191203	CE5694	SWE	Västergötland	Gothenburg, Änggården. Vitsippsdalen at Botanical Garden	Forest with maple and elm trees, wet dark soil and wet grey fine sand	57.6817	11.9567	08 Apr 2009	C. Erséus
<i>H. oculatus</i>	SMNH 191204	CE5695	SWE	Västergötland	Gothenburg, Änggården. Vitsippsdalen at Botanical Garden	Forest with maple and elm trees, wet dark soil and wet grey fine sand	57.6817	11.9567	08 Apr 2009	C. Erséus
<i>H. oculatus</i>	SMNH 191205	CE5700	SWE	Västergötland	Gothenburg, Änggården. Vitsippsdalen at Botanical Garden	Forest with maple and elm trees, wet dark soil and wet grey fine sand	57.6817	11.9567	08 Apr 2009	C. Erséus
<i>H. oculatus</i>	SMNH 191206	CE5705	SWE	Västergötland	Gothenburg, Änggården. Vitsippsdalen at Botanical Garden	Forest with maple and elm trees, wet dark soil and wet grey fine sand	57.6817	11.9567	08 Apr 2009	C. Erséus
<i>H. oculatus</i>	SMNH 191207	CE7085	SWE	Västergötland	Gothenburg, Änggården. Vitsippsdalen at Botanical Garden	Forest with maple and elm trees, wet dark soil and wet grey fine sand	57.6817	11.9567	08 Apr 2009	C. Erséus
<i>H. oculatus</i>	SMNH 191208	CE7087	SWE	Västergötland	Gothenburg, Änggården. Vitsippsdalen at Botanical Garden	Forest with maple and elm trees, wet dark soil and wet grey fine sand	57.6817	11.9567	08 Apr 2009	C. Erséus
<i>H. oculatus</i>	SMNH 191209	CE6500	SWE	Uppland	Österåker, Vira Bruk, Viraån River	River bank with wet sand	59.5710	18.5502	06 Jun 2009	C. Erséus
<i>H. oculatus</i>	SMNH 191210	CE6501	SWE	Uppland	Österåker, Vira Bruk, Viraån River	River bank with wet sand	59.5710	18.5502	06 Jun 2009	C. Erséus
<i>H. oculatus</i>	SMNH 191211	CE6502	SWE	Uppland	Österåker, Vira Bruk, Viraån River	River bank with wet sand	59.5710	18.5502	06 Jun 2009	C. Erséus
<i>H. oculatus</i>	SMNH 191212	CE6503	SWE	Uppland	Österåker, Vira Bruk, Viraån River	River bank with wet sand	59.5710	18.5502	06 Jun 2009	C. Erséus
<i>H. oculatus</i>	SMNH 191213	CE6504	SWE	Uppland	Österåker, Vira Bruk, Viraån River	River bank with wet sand	59.5710	18.5502	06 Jun 2009	C. Erséus
<i>H. oculatus</i>	SMNH 191214	CE6505	SWE	Uppland	Österåker, Vira Bruk, Viraån River	River bank with wet sand	59.5710	18.5502	06 Jun 2009	C. Erséus
<i>H. oculatus</i>	SMNH 191215	CE8815	GER	Bavaria	Erding, Alter Erdinger bog, 1 km NE of St Koloman	Drainage ditch	48.2507	011.889	29 Jul 2006	T. von Proschwitz, G. Falkner
<i>H. oculatus</i>	SMNH 191216	CE9307	SWE	Gotland	Gotland, Lummelunda, Lummelunda River	Under stones on river bank	57.741	18.404	07 Aug 2010	C. Erséus
<i>H. oculatus</i>	SMNH 191217	CE9308	SWE	Gotland	Gotland, Lummelunda, Lummelunda River	Under stones on river bank	57.741	18.404	07 Aug 2010	C. Erséus
<i>H. oculatus</i>	SMNH 191218	CE9311	SWE	Gotland	Gotland, Lummelunda, Lummelunda River	Under stones on river bank	57.741	18.404	07 Aug 2010	C. Erséus
<i>H. oculatus</i>	SMNH 191219	CE9732	DEN	Bornholm	Laeså River, under bridge of Søndre Landevej Road	River bank with grass and blackberries, wet sandy soil	55.0361	14.9092	01 Sep 2010	A. Ansebo
<i>H. oculatus</i>	SMNH 191220	CE9733	DEN	Bornholm	Laeså River, under bridge of Søndre Landevej Road	River bank with grass and blackberries, wet sandy soil	55.0361	14.9092	01 Sep 2010	A. Ansebo

Appendix I. Continued.

Species	Museum voucher	Specimen ID	Country	Province	Locality	Habitat	Coordinates		Collection date	Collector
							North	East		
<i>H. oculatus</i>	SMNH 191221	CEI1347	SWE	Västergötland	Gothenburg, Södra Guldheden, SW of Dr. Fries Torg	Soil in seepage of water on forest floor	57.6847	11.9753	11 Apr 2011	A. Achurra, C. Erséus
<i>H. oculatus</i>	SMNH 191222	CEI1393	SWE	Västergötland	Gothenburg, Änggården. Vitsippsdalen at Botanical Garden	Deciduous forest floor, soil in ground-water seeps	57.6822	11.9551	29 Mar 2011	A. Achurra, C. Erséus
<i>H. oculatus</i>	SMNH 191223	CEI1468	SWE	Västergötland	Gothenburg, Änggården. Vitsippsdalen at Botanical Garden	Deciduous forest floor, soil in ground-water seeps	57.6822	11.9551	29 Mar 2011	A. Achurra, C. Erséus
<i>H. oculatus</i>	SMNH 191224	CEI1469	SWE	Västergötland	Gothenburg, Änggården. Vitsippsdalen at Botanical Garden	Deciduous forest floor, soil in ground-water seeps	57.6822	11.9551	29 Mar 2011	A. Achurra, C. Erséus
<i>H. oculatus</i>	SMNH 191225	CEI8776	SWE	Blekinge	Ronneby, Ronneby Brunnspark	Wet soil near duck pond and stream	56.199	15.282	30 May 2013	C. Erséus, B. Williams, S. Martinsson
<i>H. oculatus</i>	SMNH 191226	CEI8777	SWE	Blekinge	Ronneby, Ronneby Brunnspark	Wet soil near duck pond and stream	56.199	15.282	30 May 2013	C. Erséus, B. Williams, S. Martinsson
<i>H. oculatus</i>	SMNH 191227	CEI8778	SWE	Blekinge	Ronneby, Ronneby Brunnspark	Wet soil near duck pond and stream	56.199	15.282	30 May 2013	C. Erséus, B. Williams, S. Martinsson
<i>H. oculatus</i>	SMNH 191228	CEI8780	SWE	Blekinge	Ronneby, Ronneby Brunnspark	Wet soil near duck pond and stream	56.199	15.282	30 May 2013	C. Erséus, B. Williams, S. Martinsson
<i>H. oculatus</i>	SMNH 191229	CE26114	SWE	Västergötland	Gothenburg, Änggården. Vitsippsdalen at Botanical Garden	Stream bank, soil under log	57.6807	11.9591	27 May 2015	C. Erséus
<i>H. oculatus</i>	SMNH 191230	CE26263	SWE	Skåne	Simrishamn, Tommarpsån River between Tommarp and Järrestad	River bank, wet clayey soil	55.5321	14.2701	19 Jul 2015	C. Erséus
<i>H. oculatus</i>	SMNH 191231	CE26264	SWE	Skåne	Simrishamn, Tommarpsån River between Tommarp and Järrestad	River bank, wet clayey soil	55.5321	14.2701	19 Jul 2015	C. Erséus
<i>H. oculatus</i>	SMNH 191232	CE26265	SWE	Skåne	Simrishamn, Tommarpsån River between Tommarp and Järrestad	River bank, wet clayey soil	55.5321	14.2701	19 Jul 2015	C. Erséus
<i>H. oculatus</i>	SMNH 191233	CE26266	SWE	Skåne	Simrishamn, Tommarpsån River between Tommarp and Järrestad	River bank, wet clayey soil	55.5321	14.2701	19 Jul 2015	C. Erséus
<i>H. oculatus</i>	SMNH 191234	CE30992	SWE	Västergötland	Gothenburg, Medicinareberget, bottom of large stone at Arvid Wallgrens Backe Street	Freshwater seep, gravel and sand	57.6864	11.9548	22 Mar 2017	C. Erséus
<i>H. oculatus</i>	ZMBN 109442	CEI6604	NOR	Oslo	Oslo, Majorstua, Vigeland Park at Vigelandsbadet (swimming pools)	Bank of small river flowing into duck pond, hard clayey soil	59.9281	10.7059	10 Oct 2012	C. Erséus, S. Martinsson, Y. Liu
<i>H. oculatus</i>	ZMBN 109443	CEI6605	NOR	Oslo	Oslo, Majorstua, Vigeland Park at Vigelandsbadet (swimming pools)	Bank of small river flowing into duck pond, hard clayey soil	59.9281	10.7059	10 Oct 2012	C. Erséus, S. Martinsson, Y. Liu
<i>S. mammalis</i>	SMNH 191235	CE4365	SWE	Småland	Jönköping, Visingsö, Rökings, Rökingsö, Haraldsgård Farm	Garden compost	58.0372	14.3127	04 Jul 2008	C. Erséus
<i>S. mammalis</i>	SMNH 191236	CE5391	SWE	Västergötland	Gothenburg, Kungsten, Sjöbergen Cultivation Association (gardens)	Deciduous tree stand, rich soil under log	57.6850	11.8997	22 Nov 2008	J. Sturve
<i>S. mammalis</i>	SMNH 191237	CE26115	SWE	Västergötland	Gothenburg, Änggården. Vitsippsdalen at Botanical Garden	Soil on stream bank	57.6807	11.9591	27 May 2015	C. Erséus
<i>S. mammalis</i>	ZMBN 109862	CEI1843	NOR	Rogaland	Eigersund, Heigrestad, Rd 44 at limestone quarry (bus stop)	Stream bank with oak trees, thin soil layer on limestone bedrock	58.5021	5.9183	15 Jun 2011	C. Erséus

Appendix 1. Continued.

Species	Museum voucher	Specimen ID	Country	Province	Locality	Habitat	Coordinates		Collection date	Collector
							North	East		
<i>S. mammalis</i>	ZMBN 109863	CEI2753	NOR	Hordaland	Bergen, Lövstakken, garden at Lövstakktien 6	Grass compost	60.3716	5.3325	22 Aug 2011	C. Todt, C. Erséus
<i>S. mammalis</i>	ZMBN 109864	CEI5054	NOR	Hordaland	Etne, at Rd. 134, N of Stordalsvatnet Lake, forest slope near Noreimsstranda	Mountain Ash forest, clayey soil and compost dumped along road	59.7067	6.0477	11 Jun 2012	C. Erséus
<i>S. mammalis</i>	ZMBN 139015	CEI5055	NOR	Hordaland	Etne, at Rd. 134, N of Stordalsvatnet Lake, forest slope near Noreimsstranda	Mountain Ash forest, clayey soil and compost dumped along road	59.7067	6.0477	11 Jun 2012	C. Erséus
<i>S. mammalis</i>	ZMBN 139016	CEI5056	NOR	Hordaland	Etne, at Rd. 134, N of Stordalsvatnet Lake, forest slope near Noreimsstranda	Mountain Ash forest, clayey soil and compost dumped along road	59.7067	6.0477	11 Jun 2012	C. Erséus
<i>S. mammalis</i>	ZMBN 139017	CEI5057	NOR	Hordaland	Etne, at Rd. 134, N of Stordalsvatnet Lake, forest slope near Noreimsstranda	Mountain Ash forest, clayey soil and compost dumped along road	59.7067	6.0477	11 Jun 2012	C. Erséus
<i>S. mammalis</i>	ZMBN 139018	CEI5058	NOR	Hordaland	Etne, at Rd. 134, N of Stordalsvatnet Lake, forest slope near Noreimsstranda	Mountain Ash forest, clayey soil and compost dumped along road	59.7067	6.0477	11 Jun 2012	C. Erséus
<i>S. mammalis</i>	ZMBN 109865	CEI5059	NOR	Hordaland	Etne, at Rd. 134, N of Stordalsvatnet Lake, forest slope near Noreimsstranda	Mountain Ash forest, clayey soil and compost dumped along road	59.7067	6.0477	11 Jun 2012	C. Erséus
<i>S. mammalis</i>	ZMBN 109866	CEI5060	NOR	Hordaland	Etne, at Rd. 134, N of Stordalsvatnet Lake, forest slope near Noreimsstranda	Mountain Ash forest, clayey soil and compost dumped along road	59.7067	6.0477	11 Jun 2012	C. Erséus
<i>S. mammalis</i>	SMNH 191238	CEI8431	NED	Gelderland	Rossum	Unknown	51.793	5.298	28 Apr 2013	T. van Haaren
<i>S. mammalis</i>	ZMBN 139019	CEI21316	NOR	Rogaland	Strand, Jørpeland, harbour near mouth of Jørpelandåna River	Small stand of trees, sandy soil with decaying wood	59.0163	6.0436	13 May 2014	C. Erséus, M. Klinth

Appendix 2. Publicly available COI sequences of *Helodrilus oculatus* and *Satchellius mammalis* included in this study.

Species	Country	BOLD	GenBank Accession #	Species	Country	BOLD	GenBank Accession #
<i>H. oculatus</i>	Denmark	GBAN10811-19	KP420586	<i>S. mammalis</i>	Denmark	GAHAP3310-15	KP420605
<i>H. oculatus</i>	Switzerland	GBAN15212-19	LT904774	<i>S. mammalis</i>	Ireland	GBMINI695-12	JN869968
<i>H. oculatus</i>	Denmark	GBAN2210-09	FJ374775	<i>S. mammalis</i>	Ireland	GBMINI696-12	JN869969
<i>H. oculatus</i>	Switzerland	GAHAP2949-15	LN810242	<i>S. mammalis</i>	Ireland	GBMINI697-12	JN869970
<i>S. mammalis</i>	France	ACHL0092-09	HM417950	<i>S. mammalis</i>	Ireland	GBMINI698-12	JN869971
<i>S. mammalis</i>	Denmark	GAHAP3303-15	KP420598	<i>S. mammalis</i>	Ireland	GBMINI699-12	JN869972
<i>S. mammalis</i>	Denmark	GAHAP3304-15	KP420599	<i>S. mammalis</i>	Ireland	GBMINI700-12	JN869973
<i>S. mammalis</i>	Denmark	GAHAP3305-15	KP420600	<i>S. mammalis</i>	Ireland	GBMINI701-12	JN869974
<i>S. mammalis</i>	Denmark	GAHAP3306-15	KP420601	<i>S. mammalis</i>	Ireland	GBMINI702-12	JN869975
<i>S. mammalis</i>	Denmark	GAHAP3307-15	KP420602	<i>S. mammalis</i>	Ireland	GBMINI703-12	JN869976
<i>S. mammalis</i>	Denmark	GAHAP3308-15	KP420603	<i>S. mammalis</i>	Ireland	GBMINI704-12	JN869977
<i>S. mammalis</i>	Denmark	GAHAP3309-15	KP420604	<i>S. mammalis</i>	Ireland	GBMINI705-12	JN869978

Appendix 2. Continued.



Appendix 3. Lumbricidae phylogeny based on data from Dominguez et al. (2015) and newly produced sequences from *Helodrilus oculatus* and *Satchellius mammalis*. The phylogeny is based on a concatenated data matrix consisting of 110 specimens and it is 5,188 bp long. The phylogeny is estimated using BI in MrBayes. Numbers at branches are posterior probabilities, only values >0.75 are shown. Scale shows expected number of changes per site.