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

Svante Martinsson¹, Märten Klinth¹ and Christer Erséus¹

Martinsson S, Klinth M and Erséus C. 2021. Distribution and genetic diversity of two earthworms, *Helodrilus oculatus* and *Satchellius mammalis* (Clitellata: Lumbricidae) in Scandinavia. Fauna norvegica 41: 1–14.

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.

doi: 10.5324/fn.v41i0.3874. Received: 2020-12-14. Accepted: 2021-02-26. Published online: 2021-05-27. ISSN: 1891-5396 (electronic).

Keywords: DNA-barcoding, haplotype networks, phylogeny, Norway, Sweden

1. Systematics and Biodiversity, Department of Biological and Environmental Sciences, University of Gothenburg, Box 463, Göteborg SE-405 30, Sweden

Corresponding author: Christer Erséus
E-mail: christer.erseus@bioenv.gu.se

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.*, *Amynthas 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.

& 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 IDs, 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          | GenBank Accession # | Species          | GenBank Accession # |
|------------------|---------------------|------------------|---------------------|
| *H. oculatus*    | COI                 | *H. oculatus*    | COI                 |
| CE1230           | MW535839, MW544108  | CE1230           | MW535839, MW544108  |
| SWE Västergötland|                     | SWE Västergötland|                     |
| 57.6813          | MW535839, MW544108  | 57.6813          | MW535839, MW544108  |
| 11.9562          | MW535839, MW544108  | 11.9562          | MW535839, MW544108  |
| MW535854         | MW544098            | MW535854         | MW544098            |
| MW534291         | MW542595            | MW534291         | MW542595            |
| MW534290         | MW542596            | MW534290         | MW542596            |
| MW534295         | MW542600            | MW534295         | MW542600            |
| MW534290         | MW542596            | MW534290         | MW542596            |
| MW534294/        | MW542596/           | MW534294/        | MW542596/           |
| MW534295/        | MW542600            | MW534295/        | MW542600            |
| MW534290/        | MW542596            | MW534290/        | MW542596            |
| MW534297/        | MW542601            | MW534297/        | MW542601            |
| MW542603/        | MW542602            | MW542603/        | MW542602            |
| H. oculatus      | 12S, 18S, 28S       | H. oculatus      | 12S, 18S, 28S       |
| CE5690           | MW538118            | CE5690           | MW538118            |
| SWE Västergötland|                     | SWE Västergötland|                     |
| 57.6817          | MW538118            | 57.6817          | MW538118            |
| 11.9567          | MW538118            | 11.9567          | MW538118            |
| MW538116         | MW538113            | MW538116         | MW538113            |
| MW534300/        | MW534301            | MW534300/        | MW534301            |
| MW534302/        | MW534303            | MW534302/        | MW534303            |
| MW534304/        | MW534305            | MW534304/        | MW534305            |
| MW534301/        | MW534302            | MW534301/        | MW534302            |
| MW534303/        | MW534304            | MW534303/        | MW534304            |
| MW534305/        | MW534306            | MW534305/        | MW534306            |
| MW534306/        | MW534307            | MW534306/        | MW534307            |
| MW534299         | MW542608            | MW534299         | MW542608            |
| MW534288/        | MW542609            | MW534288/        | MW542609            |
| MW534288/        | MW542609            | MW534288/        | MW542609            |
| MW534306/        | MW534307            | MW534306/        | MW534307            |
| MW534308/        | MW534309/           | MW534308/        | MW534309/           |
| MW534309/        | MW534310/           | MW534309/        | MW534310/           |
| MW534311         | MW542616            | MW534311         | MW542616            |
| MW534312/        | MW542617            | MW534312/        | MW542617            |
| MW534313/        | MW542618            | MW534313/        | MW542618            |
| MW534314/        | MW542619            | MW534314/        | MW542619            |
| MW534315         | MW542619            | MW534315         | MW542619            |
| MW534316/        | MW542619            | MW534316/        | MW542619            |
| MW534317/        | MW542619            | MW534317/        | MW542619            |
| MW534318/        | MW542620            | MW534318/        | MW542620            |
| MW534319         | MW542620            | MW534319         | MW542620            |
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 et al. 2001) as implemented in DNAsp. The phasing was run for 100 iterations after 100 initial runs and masked all positions with two or more alleles (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 suffices _1 or _2. All new sequences produced in this study are deposited in GenBank, and all 16S and COI are also deposited on BOLD.

### Table 1. Continued.

| Species        | Specimen ID | Country Province | GenBank Accession # |
|----------------|-------------|------------------|---------------------|
| *H. oculatus*  | CE26264     | SWE Skåne        | MW535851/MW544100/MW534319/MW542621 |
| *H. oculatus*  | CE26265     | SWE Skåne        | MW535841/MW544106/MW534320/MW542622 |
| *H. oculatus*  | CE26266     | SWE Skåne        | MW535858 - - - - |
| *H. oculatus*  | CE30992     | SWE Västergötland| MW535870/MW544087/MW534322/MW542623 |
| *H. oculatus*  | CE16604     | NOR Oslo         | MW535855/MW544097/MW534323/MW542614 |
| *H. oculatus*  | CE16605     | NOR Oslo         | MW535852/MW544099/MW534325/MW542615 |
| *S. mammalis*  | CE4365      | SWE Småland      | MW535873/MW544085/MW534331/MW542633/MW542634 |
| *S. mammalis*  | CE5391      | SWE Västergötland| MW535867/MW544089/MW534333/MW542635 |
| *S. mammalis*  | CE26115     | SWE Västergötland| MW535863/MW544092/MW534327/MW542632 |
| *S. mammalis*  | CE11843     | NOR Rogaland      | MW535868/MW544088/MW534326/MW542624 |
| *S. mammalis*  | CE12753     | NOR Hordaland     | MW535844 - - - - |
| *S. mammalis*  | CE15054     | NOR Hordaland     | MW535843/MW544105/MW534332/MW542625 |
| *S. mammalis*  | CE15055     | NOR Hordaland     | MW535861 - - - - |
| *S. mammalis*  | CE15056     | NOR Hordaland     | MW535871 - - - - |
| *S. mammalis*  | CE15057     | NOR Hordaland     | MW535865 - - - - |
| *S. mammalis*  | CE15058     | NOR Hordaland     | MW535857 - - - - |
| *S. mammalis*  | CE15059     | NOR Hordaland     | MW535845/MW544104/MW534330/MW542628 |
| *S. mammalis*  | CE15060     | NOR Hordaland     | MW535864/MW544091/MW534329/MW542629 |
| *S. mammalis*  | CE18431     | NED Gelderland    | MW535856/MW544096/MW534328/MW542630 |
| *S. mammalis*  | CE21316     | NOR Rogaland      | MW535869 - - - - |

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 Dominguez 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        | 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. |
|        | HCO2198        | Folmer et al. (1994) | 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. |
|        | COI-E          | Bely and Wray (2004) | 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. |
| 16S    | 16SAR-L        | 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 90 sec, finally, 72°C for 8 min. |
| 16SBRH |                 | 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 90 sec, finally, 72°C for 8 min. |
| H3     | H3F            | 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            | Colgan et al. (1998) | 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. |
| ITS2   | 606F           | Liu and Erséus (2017) | 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. |
|        | 1082R          | Liu and Erséus (2017) | 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. |
| 12S    | 12SE1          | Jamieson et al. (2002) | 95°C for 5 min, 43 cycles each of 95°C for 40 sec, 45°C for 45 sec and 72°C for 1 min, finally, 72°C for 8 min. |
|        | 12SH           | Jamieson et al. (2002) | 95°C for 5 min, 43 cycles each of 95°C for 40 sec, 45°C for 45 sec and 72°C for 1 min, finally, 72°C for 8 min. |
| 18S    | TimA           | Norén and Jondelius (1999) | 95°C for 5 min, 35 cycles each of 95°C for 30 sec, 60°C for 30 sec, 72°C for 1 min and 30 sec, finally, 72°C for 8 min. |
|        | 1100R          | Norén and Jondelius (1999) | 95°C for 5 min, 35 cycles each of 95°C for 30 sec, 60°C for 30 sec, 72°C for 1 min and 30 sec, finally, 72°C for 8 min. |
|        | 600F           | Norén and Jondelius (1999) | 95°C for 5 min, 35 cycles each of 95°C for 30 sec, 60°C for 30 sec, 72°C for 1 min and 30 sec, finally, 72°C for 8 min. |
|        | TimB           | Norén and Jondelius (1999) | 95°C for 5 min, 35 cycles each of 95°C for 30 sec, 60°C for 30 sec, 72°C for 1 min and 30 sec, finally, 72°C for 8 min. |
| 28S    | 28SC1′         | Jamieson et al. (2002) | 95°C for 5 min, 35 cycles each of 95°C for 30 sec, 50°C for 30 sec and 72°C for 1 min, finally, 72°C for 8 min. |
|        | 28SD2′         | Jamieson et al. (2002) | 95°C for 5 min, 35 cycles each of 95°C for 30 sec, 50°C for 30 sec and 72°C for 1 min, finally, 72°C for 8 min. |

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, COI, 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 where 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 as eye-spots, hence the name *Helodrilus oculatus* (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, tp), 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 480 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 ITS 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 ITS most sequences are the same haplotype, and the rare haplotypes are separated from it by only 1-2 substitutions.
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* species are found.
**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 (Erös és co-workers, largely unpublished; but see also James et al. 2010; Martinsson & Erös 2017; Martinsson & Erös 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 & Erös 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 & Erös 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. cernosvitovianus* is sister to them, forming a clade that can be considered *Helodrilus s.str*. In both trees *H. cordezi* is instead found together with *Allobophora cheatophora*, despite being listed as a synonym to *H. oculatus* in Szeredjesi 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 2015). On the other hand, in the BI tree, *S. mammalis* is found together with *Dendrobaena sp.* 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. gati* by Dominguez et al. (2015), a species that was synonymised with *Allobophora madeirensis* Michaelsen, 1891 by Szeredjesi and Csuzdi (2016), while still regarding it as a member of *Satchellius*. In the latter paper a new species, *Zophoscolex zicius* Szeredjesi & Csuzdi, 2016, was described based on material that was considered conspecific to *A. madeirensis* by Zicsi (1969), but *Z. zicius* is to our knowledge so far only found on the island of Madeira. The specimen used by Dominguez 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.

**ACKNOWLEDGEMENTS**

We are grateful to Ainara Achurra, Anna Ansebo, Seana Davidson, Gerhard Falkner, Yingkui Li, Sam James, Ted von Proschwitz, Emilia Rota, Joachim Sturve, Christiane Tödt, Ton van Haaren, and Bronwyn Williams for providing specimens and/or assisting in field work; and to Anna Ansebo, Angelica Ardehed, Emelie Lindström, and Peter Samsson for lab assistance. The study was funded by the Swedish Taxonomy Initiative, Swedish Species Information Centre (SLU ArtDatabanken); the Norwegian Taxonomy Initiative, Norwegian Biodiversity Information Centre (Artsdatabanken); the Swedish EPA’s Environmental Research Fund in collaboration with the Swedish Agency for Marine and Water Management; the Adlerbert Research Foundation; and the Royal Society of Arts and Sciences in Gothenburg (KVVS).
REFERENCES

Anisimova M and Gascuel O. 2006. Approximate likelihood-ratio test for branches: A fast, accurate, and powerful systematic, Biological Systematics. 55(4): 539–552. doi: 10.1086/403516.

Beddard FE. 1888. On certain points in the structure of Urochaeta, E. P., and Dichogaster, nov. gen., with further remarks on the nephridia of earthworms. Quarterly Journal of microscopical Science (n.s.) 29: 235–282.

Bely AE and Wray GA. 2004. Molecular phylogeny of naidid worms (Annelida: Clitellata) based on cytochrome oxidase I. Molecular Phylogenetics and Evolution 30(1): 50–63. doi: 10.1016/j.ympev.2004.01.003.

Blakemore RJ. 2007. Scandinavian earthworms - a checklist of species, Accessed 2020-11-17. http://www.annelida.net/earthworm/Scandinavian.pdf.

Bouché MB. 1970. Remarques sur quelques Lumbricina de France et conséquences de la découverte des nouveaux taxons Vignysinae (Subfam. nov) et Diporodrilidae (Fam. nov.). Pedobiologia 10: 246–256.

Clement M, Yoder M, Stadler Q, Wilke P, Posada D and Crandall, K. 2002. TCS: estimating gene genealogies. Proceedings of the 16th International Parallell & Distributed Processing Symposium 2: 184. doi: 10.1109/IPDPS.2002.1061585.

Colgan DJ, McLauchlan A, Wilson GDF, Livingston SP, Edgecombe GD, Macarans D, Cassis G and Gray MR. 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Australian Journal of Zoology 46(5): 419–437. doi: 10.1071/Zo98048.

Csuzdi C, Chang CH, Pavlícek T, Szederjesi T, Esopi D and Szlávecz A. 2010. DNA barcoding reveals cryptic diversity in earthworm genus Dichogaster (Oligochaeta: Octochaetidae). Molecular Phylogenetics and Evolution 53(5): 179–187. doi: 10.1016/j.ympev.2010.03.005.

Dichogaster saliens (Oligochaeta: Octochaetidae) for branches: A fast, accurate, and powerful alternative, Systematic Biology 54(9): 1242–1244. doi: 10.1002/molb.10019.

Eisen G. 1874. Om Skandinaviens lumbricider. Öfversigt af Kongliga Vetenskaps-Akademiens Förhandlingar 30(8): 43–56.

Erséus C, Omodeo P and Rota E. 1994. First records of the allotogenous species Dichogaster saliens and D. boleai (Oligochaeta: Octochaetae) from Sweden. Megadriligologia 6(2): 17–20.

Folmer O, Black M, Hoch W, Lutz R and Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294–299.

Gates GE. 1972. Contributions to North American earthworms (Annelida). No. 5. On variation in another anthropochrous species of the oriental earthworm genus Pheretima Kinberg 1866 (Megascoleididae). Bulletin of Tall Timbers Research Station 13: 38–44.

Gates GE. 1975. Contributions to a revision of the earthworm family Lumbricidae XII. Entieron mammale Savygin, 1826 and its position in the family Megadriligologia 2(1): 1–5.

Guindon S, Dufayard JF, Lefort V, Anisimova M and Gascuel O. 2009. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59(3): 370–372. doi: 10.1093/sysbio/sys010.

Hoffmeister W. 1884. Die bis jetzt bekannten Arten aus der Familie der Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W and Gates GE. 1972. Contributions to North American earthworms (Annelida) 45: 23–30. doi: 10.1016/j.sysbio.2011.09.018.

Kumar S, Steeger G, Li M, Knyaz C and Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35(6): 1547–1549. doi: 10.1093/molbev/msy096.

Lefort V, Longueville J-E and Gascuel O. 2017. SMS: Smart Model Selection in PhyML. Molecular Biology and Evolution 34(9): 2422–2424. doi: 10.1093/molbev/msx149.

Leigh JW and Bryant D. 2015. POPART: full-feature software for haplotype network construction. Methods in Ecology and Evolution 6(9): 1110–1116. doi: 10.1111/2041-210X.12410.

Librado P and Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25(11): 1451–1452. doi: 10.1093/bioinformatics/btp187.

Lund MB, Fritz M, Holmstrup M and Schramm A. 2006. Co-speciation of earthworms and their nephridial symbionts, Acidovorax spp. In: The Hidden Powers - Microbial Communities in Action. Proceedings of the 11th International Symposium on Microbial Ecology (ISME-11), International Society for Microbial Ecology, Vienna, Austria. 182pp.

Marchán DF, Fernández R, de Sosa I, Sánchez N, Cosín DJD and Novo M. 2018. Integrative systematic revision of a Mediterranean earthworm family: Hormogastridae (Annelida, Oligochaeta). Invertebrate Systematics 33(3): 652–671. doi: 10.1071/is17048.

Marchán DF, Hedde M, Lapied E, Maggia M-E, Novo M, Domínguez J and Decaëns T. 2020. Contrasting phylogeographic patterns of earthworms (Crassiclitellata, Lumbricidae) on near-shore Mediterranean islands. European Journal of Soil Biology 101: 103242. doi: 10.1016/j.ejsobi.2020.103242.

Martinsson S and Erséus C. 2017. Cryptic speciation and limited hybridization within Lumbricus earthworms (Clitellata: Lumbricidae). Molecular Phylogenetics and Evolution 106: 18–27. doi: 10.1016/j.ympev.2016.09.011.

Martinsson S and Erséus C. 2018. Hybridisation and species delimitation of Scandinavian Eisenia spp. (Clitellata: Lumbricidae). European Journal of Soil Biology 88: 41–47. doi: 10.1016/j.ejsobi.2018.06.003.

Martinsson S, Rhodén C and Erséus C. 2017. Barcoding gap, but no support for cryptic speciation in the earthworm Apoerectoda longa (Clitellata: Lumbricidae). Mitochondrial DNA 26(2): 147–155. doi: 10.1080/19401736.2015.1115487.

Michaelis W. 1891a. Oligochaeten des Naturhistorischen Museums in Hamburg. Part iv. Jahrbuch der Hamburgischen Wissenschaftlichen Anstalten 8: 299–340.

Michaelis W. 1891b. Terricolen der Berliner Zoologischen Sammlung. I. Mammalia, nov. gen., with further remarks on the nephridia of earthworms. Bayerische Gesellschaft für Naturkunde. Abhandlungen XX: 235–282.

Michaelis W. 1900. Oligochaeten. Friedländer & Sohn, Berlin.

Møller P, Lund MB and Schramm A. 2015. Evolution of the tripartite symbiosis between earthworms, Verminephrobacter and Flexibacter-like bacteria. Frontiers in Microbiology 6: 529. doi: 10.3389/fmicb.2015.00529.

Nöör M and Jondelius U. 1999. Phylogeny of the Prolecithophora (Platyhelminthes) inferred from 18S rDNA sequences. Cladistics,15(2): 103–112. doi: 10.1111/j.1041-0361.1999.tb00252.x.

Omodeo P. 1956. Contributo alla revisione dei Lumbricidae. Archivio di Zoologia 46(5): 419–437. doi: 10.1071/Zo98048.

Martinsson, Klinth and Erséus: H. ocellatus and S. mammalis in Scandinavia
Omodeo P and Rota E. 1989. Earthworms of Turkey. Bolletino di Zoolgia 56(2): 167–198. doi: 10.1080/11250008909355639.

Palumby SR, Martin A, Romano S, Mc-Millian WO, Stice L and Grabawski G. 1991. The simple fool's guide to PCR, version 2.0. Privately published, compiled by S. Palumby, University of Hawaii, Honolulu.

Pérez-Losada M, Bloch R, Breinholt JW, Pfenninger M and Dominguez J. 2012. Taxonomic assessment of Lumbricidae (Oligochaeta) earthworm genera using DNA barcodes. European Journal of Soil Biology 48: 41–47. doi: 10.1016/j.ejsobi.2011.10.003.

Qiu JP and Bouché MB. 2000a. Une nouvelle évaluation du genre Helodrilus (sensu Zici, 1985) (Oligochaeta: Lumbricidae). Documents Pedozoologiques et Intégrologiques 4: 8–16.

Qiu JP and Bouché MB. 2000b. Contribution à la taxonomie des Dendrobaenini trib. Nov. (Oligochaeta: Lumbricidae): Iberoscolex gen. nov. et nouveaux taxons de Dendrobaena Eisen, 1874 et Satchellius Gates, 1975. Documents Pedozoologiques et Intégrologiques 4(14): 153–163.

Rambaut A. 2014. FigTree v1.4.2. http://tree.bio.ed.ac.uk/software/figtree/

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA and Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. doi: 10.1093/sysbio/sys029.

Rosa D. 1893. Viaggio del Dr. E. Festa in Palestina, nel Libano e regioni vicine.–II. Lumbricidi. Bollettino dei Musei di Zoolgia ed Anatomia comparata della R. Université di Torino 8(160): 1–14.

Savigny JC. 1826. Analyses de travaux de l’Académie Royale des Sciences pendant l’année 1821, partie physique. Zoologie. Mémoires de l'Acadéémie des sciences de l'Institut de France (Histoire) 5: 176–184.

Sherlock E. 2018. Key to the earthworms of the UK and Ireland. FSC, Telford.

Sims RW and Gerard BM. 1985. Earthworms: Keys and notes for the identification and study of the species. Brill, London.

Stephens M and Donnelly P. 2003. A comparison of bayesian methods for haplotype reconstruction from population genotype data. American Journal of Human Genetics 73(5): 1162–1169. doi: 10.1086/379378.

Stephens M, Smith NJ and Donnelly P. 2001. A new statistical method for haplotype reconstruction from population data. American Journal of Human Genetics 68(4): 978–989. doi: 10.1086/319501.

Stöp-Bowitz C. 1969. Beiträge zur Kenntnis der ungarischen Lumbricidenfauna, IV. Opuscula Zoologica 6: 187–190. 

Szederjesi T and Csuzdi C. 2016. Review of the species allocated to the genus Satchellius (Oligochaeta: Lumbricidae) with description of a new species. Biologia 71(2): 169–175. doi: 10.1515/biolog-2016-0023.

Templeton AR, Crandall KA and Sing CF. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132(2): 619–633.

Vivien R, Wyler S, Lafont M and Ferrari BJ. 2015. Molecular barcoding of aquatic oligochaetes: implications for biomonitoring. PLoS ONE 10(4): e0125485. doi: 10.1371/journal.pone.0125485.

Zicsi A. 1969. Regenwürmer (Lumbricidae) aus Madeira und von den Kanarischen Inseln. Acta Zoologica Hungarica 15: 243–246.
Appendix 1. Locality and collection information for specimens of *Helodrilus oculatus* and *Satchellius mammalus* 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          |
|-------------------|----------------|-------------|------------------|-----------------------------------------------|----------------------------------------------|-------------|-----------------|--------------------|
| *H. oculatus*     | SMNH 191198    | CE1230      | SWE Västergötland| Gothenburg, Änggården. Vitsippudalen at Botanical Garden | Wet dark muddy soil on stream bank            | 57.6813     | 11.9562         | 08 Nov 2005       | E. Rota, C. Erséus |
|                   | SMNH 191199    | CE5690      | SWE Västergötland| Gothenburg, Änggården. Vitsippudalen 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          |
|                   | SMNH 191200    | CE5691      | SWE Västergötland| Gothenburg, Änggården. Vitsippudalen 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          |
|                   | SMNH 191201    | CE5692      | SWE Västergötland| Gothenburg, Änggården. Vitsippudalen 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          |
|                   | SMNH 191202    | CE5693      | SWE Västergötland| Gothenburg, Änggården. Vitsippudalen 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          |
|                   | SMNH 191203    | CE5694      | SWE Västergötland| Gothenburg, Änggården. Vitsippudalen 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          |
|                   | SMNH 191204    | CE5695      | SWE Västergötland| Gothenburg, Änggården. Vitsippudalen 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          |
|                   | SMNH 191205    | CE5700      | SWE Västergötland| Gothenburg, Änggården. Vitsippudalen 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          |
|                   | SMNH 191206    | CE5705      | SWE Västergötland| Gothenburg, Änggården. Vitsippudalen 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          |
|                   | SMNH 191207    | CE7085      | SWE Västergötland| Gothenburg, Änggården. Vitsippudalen 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          |
|                   | SMNH 191208    | CE7087      | SWE Västergötland| Gothenburg, Änggården. Vitsippudalen 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          |
|                   | SMNH 191209    | CE6500      | SWE Uppland      | Österåker, Vira Bruk, Vinäån River            | River bank with wet sand                     | 59.5710     | 18.5502         | 06 Jun 2009        | C. Erséus          |
|                   | SMNH 191210    | CE6501      | SWE Uppland      | Österåker, Vira Bruk, Vinäån River            | River bank with wet sand                     | 59.5710     | 18.5502         | 06 Jun 2009        | C. Erséus          |
|                   | SMNH 191211    | CE6502      | SWE Uppland      | Österåker, Vira Bruk, Vinäån River            | River bank with wet sand                     | 59.5710     | 18.5502         | 06 Jun 2009        | C. Erséus          |
|                   | SMNH 191212    | CE6503      | SWE Uppland      | Österåker, Vira Bruk, Vinäån River            | River bank with wet sand                     | 59.5710     | 18.5502         | 06 Jun 2009        | C. Erséus          |
|                   | SMNH 191213    | CE6504      | SWE Uppland      | Österåker, Vira Bruk, Vinäån River            | River bank with wet sand                     | 59.5710     | 18.5502         | 06 Jun 2009        | C. Erséus          |
|                   | SMNH 191214    | CE6505      | SWE Uppland      | Österåker, Vira Bruk, Vinäån River            | River bank with wet sand                     | 59.5710     | 18.5502         | 06 Jun 2009        | C. Erséus          |
|                   | SMNH 191215    | CE8815      | GER, Bavaria     | Erding, Alter Erdingerbogi, 1 km NE of St Koloman | Drainage ditch                               | 48.2507     | 01.1889         | 29 Jul 2006        | T. von Proschwitz, G. Falkner |
| *H. oculatus*     | SMNH 191216    | CE9307      | SWE Gotland      | Gotland, Lummelanda, Lummelanda River         | Under stones on river bank                   | 57.741      | 18.404          | 07 Aug 2010        | C. Erséus          |
|                   | SMNH 191217    | CE9308      | SWE Gotland      | Gotland, Lummelanda, Lummelanda River         | Under stones on river bank                   | 57.741      | 18.404          | 07 Aug 2010        | C. Erséus          |
|                   | SMNH 191218    | CE9311      | SWE Gotland      | Gotland, Lummelanda, Lummelanda River         | Under stones on river bank                   | 57.741      | 18.404          | 07 Aug 2010        | C. Erséus          |
| *H. oculatus*     | SMNH 191219    | CE9732      | DEN, Bornholm    | Laeså River, under bridge of Söndre Landevej Road | River bank with grass and blackberries, wet sandy soil | 55.0361     | 14.9092         | 01 Sep 2010        | A. Ambeo           |
|                   | SMNH 191220    | CE9733      | DEN, Bornholm    | Laeså River, under bridge of Söndre Landevej Road | River bank with grass and blackberries, wet sandy soil | 55.0361     | 14.9092         | 01 Sep 2010        | A. Ambeo           |
| Species | Museum voucher | Specimen ID | Country Province | Locality | Habitat | Coordinates | Collection date | Collector |
|---------|---------------|-------------|------------------|----------|---------|-------------|----------------|----------|
| H. oculatus | SMNH 91221 | CE11347 | 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 |
| H. oculatus | SMNH 91222 | CE11393 | SWE Västergötland | Gothenburg, Ångården. Vitsippsdalensan Botanical Garden | Deciduous forest floor, soil in ground-water seeps | 57.6822 | 11.9551 | 29 Mar 2011 | A. Achurra, C. Erséus |
| H. oculatus | SMNH 91223 | CE11468 | SWE Västergötland | Gothenburg, Ångården. Vitsippsdalensan Botanical Garden | Deciduous forest floor, soil in ground-water seeps | 57.6822 | 11.9551 | 29 Mar 2011 | A. Achurra, C. Erséus |
| H. oculatus | SMNH 91224 | CE11469 | SWE Västergötland | Gothenburg, Ångården. Vitsippsdalensan Botanical Garden | Deciduous forest floor, soil in ground-water seeps | 57.6822 | 11.9551 | 29 Mar 2011 | A. Achurra, C. Erséus |
| H. oculatus | SMNH 91225 | CE18776 | 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 |
| H. oculatus | SMNH 91226 | CE18777 | 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 |
| H. oculatus | SMNH 91227 | CE26114 | SWE Västergötland | Gothenburg, Änggården. Vitsippsdalensan Botanical Garden | Stream bank, soil under log | 57.6807 | 11.9591 | 27 May 2015 | C. Erséus |
| H. oculatus | SMNH 91228 | CE26263 | SWE Skåne | Simrishamn, Tommarpsån River between Tommarp and Järrestad | River bank, wet clayey soil | 55.5321 | 14.2701 | 19 Jul 2015 | C. Erséus |
| H. oculatus | SMNH 91229 | CE26264 | SWE Skåne | Simrishamn, Tommarpsån River between Tommarp and Järrestad | River bank, wet clayey soil | 55.5321 | 14.2701 | 19 Jul 2015 | C. Erséus |
| H. oculatus | SMNH 91230 | CE26265 | SWE Skåne | Simrishamn, Tommarpsån River between Tommarp and Järrestad | River bank, wet clayey soil | 55.5321 | 14.2701 | 19 Jul 2015 | C. Erséus |
| H. oculatus | SMNH 91231 | CE26266 | SWE Skåne | Simrishamn, Tommarpsån River between Tommarp and Järrestad | River bank, wet clayey soil | 55.5321 | 14.2701 | 19 Jul 2015 | C. Erséus |
| H. oculatus | SMNH 91232 | CE26267 | SWE Skåne | Gothenburg, Medicinareberget, bottom of large stone at Arvid Wällrens Backe Street | Freshwater seep, gravel and sand | 57.6864 | 11.9548 | 22 Mar 2017 | C. Erséus |
| H. oculatus | ZMBN 109442 | CE16604 | 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 |
| H. oculatus | ZMBN 109443 | CE16605 | 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 |
| S. mammalis | SMNH 91235 | CE4365 | SWE Småland | Jönköping, Visingsö, Rökinge, Rökinge-Haraldsgård Farm | Garden compost | 58.0372 | 14.3127 | 04 Jul 2008 | C. Erséus |
| S. mammalis | SMNH 91236 | CE5391 | SWE Västergötland | Gothenburg, Kungssten, Sjöbäcken Cultivation Association (gardens) | Deciduous tree stand, rich soil under log | 57.6850 | 11.8997 | 22 Nov 2008 | J. Starve |
| S. mammalis | SMNH 91237 | CE26115 | SWE Västergötland | Gothenburg, Ångården. Vitsippsdalensan Botanical Garden | Soil on stream bank | 57.6807 | 11.9951 | 27 May 2015 | C. Erséus |
| S. mammalis | ZMBN 109862 | CE11843 | 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 | Country     | Museum voucher | Collector | Coordinates | Specimen ID | Locality | Habitat                                                                 |
|---------|-------------|----------------|-----------|-------------|-------------|----------------|--------------------------------------------------------------------------|
| S. mammalis | NOR Hordaland | ZMBN 109863 | C. Todt, C. Erséus | 60.3716, 5.3325 | CE12753 | Bergen, Lovatnet, garden at Lovatnet | Grass compost |
| S. mammalis | NOR Hordaland | ZMBN 109864 | C. Todt, C. Erséus | 59.7067, 6.0477 | CE15054 | Etne, at Rd. 134, N of Stordalsvatnet Lake | Mountain Ash forest, clayey soil and compost dumped along road |
| S. mammalis | NOR Hordaland | ZMBN 109865 | C. Todt, C. Erséus | 59.7067, 6.0477 | CE15055 | Etne, at Rd. 134, N of Stordalsvatnet Lake | Mountain Ash forest, clayey soil and compost dumped along road |
| S. mammalis | NOR Hordaland | ZMBN 109866 | C. Todt, C. Erséus | 59.7067, 6.0477 | CE15056 | Etne, at Rd. 134, N of Stordalsvatnet Lake | Mountain Ash forest, clayey soil and compost dumped along road |
| S. mammalis | NOR Hordaland | SMNH 109867 | C. Todt, C. Erséus | 59.7067, 6.0477 | CE15057 | Etne, at Rd. 134, N of Stordalsvatnet Lake | Mountain Ash forest, clayey soil and compost dumped along road |
| S. mammalis | NED Gelderland | ZMBN 109868 | T. van Haaren | 51.793, 5.298 | CE15058 | Rossum | Unknown |

### Appendix 2.
Continued.

| Species | Country     | Accession # |
|---------|-------------|-------------|
| H. oculatus | Denmark    | KP208058 |
| S. mammalis | Ireland    | JN869990 |
| H. oculatus | Switzerland | JN869991 |
| S. mammalis | Ireland    | JN869992 |
| H. oculatus | Switzerland | JN869993 |
| S. mammalis | Ireland    | JN869994 |
| H. oculatus | Switzerland | JN869995 |
| S. mammalis | Ireland    | JN869996 |
| H. oculatus | Switzerland | JN869997 |
| S. mammalis | Ireland    | JN869998 |
| H. oculatus | Switzerland | JN869999 |
Appendix 3. 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 BI in MrBayes. Numbers at branches are posterior probabilities, only values >0.75 are shown. Scale shows expected number of changes per site.