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REVIEW

New insights into the systematics of *Lumbricillus* and *Marionina* (Clitellata: Enchytraeidae) inferred from Southern Hemisphere samples, including three new species

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Enchytraeid worms collected in South Africa and on the Marion, South Orkney, South Georgia and South Shetland Islands during 2008–2015 were studied using morphology and seven genetic markers. Nine species were recognized: one terrestrial (*Christensenidrilus blocki*) and all the others marine littoral (five *Lumbricillus* and three *Marionina s.s.*). An estimated phylogeny including other enchytraeids from the Northern Hemisphere, many of which are members of *Lumbricillus* and some representing *Marionina s.l.*, confirmed a non-monophyletic *Lumbricillus*, with some of its current species closely related to *Grania* or *Marionina s.s*. The phylogeny also corroborated a non-monophyletic *Marionina s.l.*, with *Marionina s.s.* closely related to *Grania* and *Lumbricillus s.l.*, but not to the remaining sequenced '*Marionina*' or to *Ch. blocki*. These results provide a long-needed starting point for a revision of both *Marionina* and *Lumbricillus*. We provide morphological descriptions of all nine species, three of which are new to science: ***Lumbricillus finisafricae* sp. nov.**, ***Lumbricillus nivalis* sp. nov.**, and ***Marionina fusca* sp. nov.** Comments on three related species of *Marionina s.s.* based on re-examined type material are also provided.

ADDITIONAL KEYWORDS: Antarctic Peninsula – *Christensenidrilus* – integrative taxonomy – multispecies coalescent – Snow Island – South Georgia.

INTRODUCTION

Enchytraeidae is a family of clitellate annelid worms with > 700 species (Schmelz & Collado, 2015) distributed worldwide in both terrestrial and aquatic habitats. The family has been studied carefully since the latter half of the 19th century, at least in the Northern Hemisphere and especially in Europe. Despite this, one and a half centuries later there are still major gaps of knowledge regarding species delimitation, phylogeny and ecology of this group (Rota & De Jong, 2015). However, these gaps are overshadowed by those for the enchytraeids of the

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Southern Hemisphere, where Africa, Australia and South America remain largely unstudied. An exception might be the Subantarctic (roughly between 46 and 60°S) and the Antarctic regions, where a number of studies were carried out in the golden days of scientific expeditions, for example, by Michaelsen (1888, 1905a, 1935) for South Georgia Island, the Kerguelen Islands and the South Atlantic, respectively, by Ude (1896) for Tierra del Fuego, by Benham (1905, 1922) for Macquarie Island and by Stephenson (1932) again for South Georgia Island and the Palmer Archipelago near the Antarctic Peninsula. More recent studies include those by Block & Christensen (1985) for the South Orkney and South Shetland Islands, Rota & Erséus (1996, 1997) and Rota (2001) for the Ross Sea and South Georgia Island, Dózsa-Farkas & Convey (1997) for the South Orkney Islands, and Wang & Liang (1997), Rodriguez & Rico (2008) and Lee *et al.* (2019) for the South Shetland Islands. However, the samples studied by these authors were from only a few selected areas, and their taxonomic and faunal study had, in all cases, a limited scope. Furthermore, there is a general shortage of molecular data for Enchytraeidae from the Southern Hemisphere, and there have been few phylogenetic studies comparing the relationship between the species of the Southern and Northern Hemispheres, with the exception of the recent work on *Grania* Southern, 1913 (De Wit *et al.*, 2011; Prantoni *et al.*, 2016).

Recently, Klinth *et al.* (2017a, b) studied the phylogeny and taxonomy of members of the enchytraeid genus *Lumbricillus* Ørsted, 1844 from northern Europe. Species of this genus are found typically in the intertidal zone of beaches, where they live in sand, gravel and/or among decaying algae or under stones. *Lumbricillus* contains some 80 described species worldwide and seems to be rich in both species and specimens in the temperate and polar areas, but it is apparently rare in tropical zones. Species of *Lumbricillus* reported and described from the Southern Hemisphere come, in particular, from Subantarctic and Antarctic islands (for a visual summary of this, see Rodriguez & Rico, 2008: fig. 1), but no molecular studies have been carried out on them.

Molecular phylogenetic studies of the northern species found *Lumbricillus* to be closely related to *Grania* (Erséus *et al.*, 2010; Klinth *et al.*, 2017a; Martinsson *et al.*, 2017), and Klinth *et al.* (2017a, b) found support for four subgroups within *Lumbricillus* (the *Lumbricillus lineatus*, *Lumbricillus pagenstecheri*, *Lumbricillus buelowi* and *Lumbricillus arenarius* groups), with the species within each group sharing certain morphological characters. Two of these groups are relevant for our present study; the *L. lineatus* group, which has testis sacs deeply divided into a fan of club-shaped lobes and spindle-shaped spermathecae, and the *L. arenarius* group, with irregularly lobed testis sacs and spermathecae in various shapes. However, the mentioned phylogenetic studies questioned the monophyly of *Lumbricillus*, because the *L. arenarius* group in some trees was found to be closer to *Grania* than to the remaining *Lumbricillus* (Erséus *et al.*, 2010; Klinth *et al.*, 2017a; Martinsson *et al.*, 2017).

Marionina Michaelsen, 1890 (in Pfeffer, 1890) is another enchytraeid genus represented in the Subantarctic and Antarctic regions. It has a particularly intricate taxonomic history, described in detail by Rota *et al.* (2008). The Subantarctic *Marionina georgiana* (Michaelsen, 1888) was assigned as the type species of the genus by Brinkhurst & Jamieson (1971), after the other six species from the original assemblage had been transferred by Nielsen & Christensen (1959) into either *Cognettia* Nielsen & Christensen, 1959 or *Lumbricillus*. The genus *Marionina* has since included many species with heterogeneous morphology, resulting in a broad diagnosis, and it is commonly considered as a non-monophyletic taxon (Xie & Rota, 2001). The type species *M. georgiana* was redescribed thoroughly by both Rota *et al.* (2008) and Schmelz & Collado (2008), and its description can be used as a starting point for revising the diagnosis of this ambiguous genus. We will refer to the current, heterogeneous, non-monophyletic assemblage of species as *Marionina s.l.* and what we consider to be the 'true' clade, to which *M. georgiana* belongs, as *Marionina s.s.*

The third and final enchytraeid genus relevant to this study is *Christensenidrilus* Dózsa-Farkas & Convey, 1998 (initially named *Christensenia* Dózsa-Farkas & Convey, 1997). *Christensenidrilus* with its only known species, *Christensenidrilus blocki* (Dózsa-Farkas & Convey, 1997), was described from Signy Island in the South Orkney Archipelago, situated between South Georgia Island (where *M. georgiana* was originally described) and the Antarctic Peninsula. Owing to the asserted similarity between the peculiar coelomocytes of *Christensenidrilus blocki* and those of *M. georgiana*, the latter was also placed within *Christensenidrilus* by Dózsa-Farkas and Convey (1997), which would instantly have made *Christensenidrilus* a junior synonym of *Marionina* (Xie & Rota, 2001). However, the re-examination of the type material of *M. georgiana* by Rota *et al.* (2008) and Schmelz & Collado (2008) discovered sufficient differences to warrant the separation of the two genera. Nevertheless, the phylogenetic placement of *Christensenidrilus* in relationship to *Marionina* and *Lumbricillus* remains unanswered.

The aim of the present study is to resolve the phylogenetic relationships and taxonomy of these three genera. For this, we were fortunate to receive properly fixed enchytraeid samples, mainly of *Lumbricillus*, from a number of locations in the Southern Hemisphere,

collected during several scientific expeditions. We also received much-needed material of *Ch. blocki*, through Rüdiger Schmelz. We used the DNA of these worms to estimate the systematic position of the southern specimens in a broader phylogeny of the genus *Lumbricillus s.l.* (i.e. including species from the previously mentioned *L. arenarius* group), by adding taxa from the Northern Hemisphere. Furthermore, the species recognized in the new material are described morphologically herein; three of them are new to science.

MATERIAL AND METHODS

Collection and preservation

The samples studied (a total of 22 specimens) originate from South Africa, the Subantarctic Marion Island, Signy Island and South Georgia Island and Snow Island (South Shetland Islands) near the Antarctic Peninsula, collected during 2008–2015 by the Shallow Marine Survey Group, the XXXIII Brazilian Antarctic Expedition or individual contributors (a full list of collectors and exact sampling locations can be found in Supporting Information, Table S1). A few immature *Lumbricillus* specimens from Marion Island and the Antarctic Peninsula (which were not sequenced successfully) plus some Naididae from Marion Island and South Africa were excluded from this study.

The collected worms were preserved in ethanol and, after examination under a dissecting microscope, we cut each individual into two parts. The posterior ends were stored in 95% ethanol for later DNA extraction, whereas the anterior ends were stained with Paracarmine and mounted in Canada Balsam on microscope slides (following Erséus, 1994), to be used for morphological study. The worms were identified using existing literature, in particular the zoological reports of the early expeditions to the areas concerned (see Introduction), and were also compared with types from those explorations, borrowed from the Natural History Museum, London (BMNH) or the Centre of Natural History, Zoological Museum, Hamburg (CeNak). The type material of *M. georgiana* was examined by temporarily mounting the specimens in clove oil. In the case of the species described by Stephenson from South Georgia (1932), we found (at BMNH) only specimens in alcohol that were labelled as type material and with specification of collecting locality and date, whereas slides with sectioned specimens from 'South Georgia Island' belonging to the 'Stephenson's collection' bore a later registration date and no 'type' designation. However, being certain that these specimens came from the same expedition and were used to describe the species, we felt authorized to treat them as syntypes (see ICZN, article 72.4.1.1). All new specimens were examined morphologically, and illustrations were drawn using a camera lucida. Some specimens and vouchers were photographed using a Nikon DXM1200 digital camera, and the figures were edited using the software GIMP v.2.8.10.

In the Taxonomy section, we provide brief lists of the cited use of taxon names (chresonymies) that we found most relevant for each species.

DNA extraction and amplification

DNA was extracted with the help of QuickExtract DNA Extraction Solution 1.0 (Epicentre, Madison, WI, USA), EZNA Tissue DNA kit (Omega Bio-Tek, Norcross, GA, USA) or DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany). All extracts were used for polymerase chain reaction (PCR) amplification of cytochrome *c* oxidase subunit I (*COI*), histone H3 (*H3*) and the second part of the internal transcribed spacer region (ITS2), used for species delimitation; *COI* is a mitochondrial marker, whereas *H3* and ITS2 are nuclear. A subset of the extracts was also used for amplification of the mitochondrial ribosomal DNA markers 12S and 16S, and the nuclear ribosomal DNA markers 18S and 28S, to be used in the species tree estimation. For primers and PCR programs, see Klinth *et al.* (2017a), except for ITS2, which was run using the primers 606F and 1082R following Liu & Erséus (2017), and some 16S sequences, which were run using Ann16SF and Ann16SR following Sjölin *et al.* (2005). The PCRs were run using Red Taq DNA Polymerase Master Mix (VWR, Haasrode, Belgium) in 25 µL reactions. Gel electrophoresis, using 1% agarose gel with GelRed, was used to determine successful reactions. The PCR products were purified with exonuclease I and FastAP thermosensitive alkaline phosphatase and were sent to MWG Eurofins Operon (Edersberg, Germany) for sequencing. Finally, GENEIOUS v.6.1.8 (created by Biomatters; available from http://www.geneious.com) was used to assemble the sequences. The microscope slides, including the type material of the new species described, were deposited as vouchers in either the Iziko South African Museum (SAMC), Cape Town, South Africa, or the Swedish Museum of Natural History (SMNH), Stockholm, Sweden, and all sequences were uploaded to GenBank (Table 1; Supporting Information, Table S1).

Genetic analyses

Gene trees were estimated for *COI*, *H3* and ITS2 using our newly generated sequences together with the dataset from the species tree estimation in the study by Klinth *et al.* (2017a), plus a few additional species of *Marionina s.l.* and *Enchytronia* Nielsen & Christensen, 1959 (Supporting Information, Table S1) used in previous molecular studies (De Wit & Erséus, 2010; Erséus *et al.*, 2010; Matamoros *et al.*, 2012; Martinsson & Erséus, 2014; Martinsson *et al.*, 2017). The following

Table 1. Specimens from the Southern Hemisphere used in this study, with specimen identification number, region of the collection site, GenBank accession numbers for the seven genetic markers, and voucher numbers (with museum acronym)

Species	Identity	Collection locality	*COI*	12S	16S	18S	28S	ITS	*H3*	Voucher no.
Christensenidrilus blocki	CE35374	S. Orkney Islands	*	**MZ412951**	**MZ412963**	*	**MZ412905**	*	**MZ394816**	SMNH198141
Ch. blocki	CE35375	S. Orkney Islands	*	**MZ412952**	**MZ412964**	**MZ412941**	**MZ412906**	**MZ412917**	**MZ394817**	SMNH198142
Ch. blocki	CE35376	S. Orkney Islands	**MZ393944**	**MZ412953**	**MZ412965**	*	**MZ412907**	**MZ412918**	**MZ394818**	SMNH198143
Lumbricillus antarcticus	CE12479	South Georgia	**MZ393945**	**MZ412954**	**MZ412966**	**MZ412942**	**MZ412908**	**MZ412919**	**MZ394819**	SMNH198144
L. antarcticus	CE12480	South Georgia	**MZ393946**	–	–	–	–	**MZ412920**	**MZ394820**	SMNH198145
L. antarcticus	CE12481	South Georgia	**MZ393947**	–	–	–	–	**MZ412921**	**MZ394821**	SMNH198146
L. antarcticus	CE12482	South Georgia	**MZ393948**	–	–	–	–	**MZ412922**	**MZ394822**	SMNH198147
L. antarcticus	CE34641	S. Shetland Islands	**MZ393949**	–	–	–	–	**MZ412923**	**MZ394823**	SMNH198148
L. antarcticus	CE34643	S. Shetland Islands	**MZ393950**	–	–	–	–	**MZ412924**	**MZ394824**	SMNH198149
Lumbricillus finisafricae	CE26170	South Africa	**MZ393951**	**MZ412955**	**MZ412967**	**MZ412943**	**MZ412909**	**MZ412925**	**MZ394825**	SAMC-A094448
Lumbricillus nivalis	CE34644	Snow Island	**MZ393952**	**MZ412956**	**MZ412968**	**MZ412944**	**MZ412910**	**MZ412926**	**MZ394826**	SMNH Type Coll. 9310
Lumbricillus sp. 'Marion Is.'	CE5477	Marion Island	**MZ393953**	**MZ412957**	**MZ412969**	**MZ412945**	**MZ412911**	**MZ412927**	**MZ394827**	SAMC-A094449
'*Lumbricillus*' cf. *macquariensis*	CE12483	South Georgia	**MZ393954**	**MZ412958**	**MZ412970**	**MZ412946**	**MZ412912**	**MZ412928**	**MZ394828**	SMNH198150
'*L.*' cf. *macquariensis*	CE12484	South Georgia	**MZ393955**	–	–	–	–	**MZ412929**	**MZ394829**	SMNH198151
'*L.*' cf. *macquariensis*	CE12485	South Georgia	**MZ393956**	–	–	–	–	**MZ412930**	**MZ394830**	SMNH198152
'*L.*' cf. *macquariensis*	CE12486	South Georgia	**MZ393957**	–	–	–	–	**MZ412931**	**MZ394831**	SMNH198153
Marionina aestuum	CE12477	South Georgia	**MZ393958**	**MZ412959**	**MZ412971**	**MZ412947**	**MZ412913**	**MZ412932**	**MZ394832**	SMNH198154
Marionina fusca	CE12475	South Georgia	*	–	–	–	–	**MZ412933**	**MZ394833**	SMNH Type Coll. 9311
M. fusca	CE12476	South Georgia	**MZ393959**	**MZ412960**	**MZ412972**	**MZ412948**	**MZ412914**	**MZ412934**	**MZ394834**	SMNH Type Coll. 9312

Table 1. Continued

Species	Identity	Collection locality	*COI*	12S	16S	18S	28S	ITS	*H3*	Voucher no.
M. fusca	CE12478	South Georgia	*	–	–	–	–	**MZ412935**	**MZ394835**	SMNH Type Coll. 9313
M. sp. 'Snow Is.'	CE34647	S. Shetland Islands	**MZ393960**	**MZ412961**	**MZ412973**	**MZ412949**	**MZ412915**	**MZ412936**	**MZ394836**	SMNH198155
M. sp. 'Snow Is.'	CE34648	S. Shetland Islands	**MZ393961**	**MZ412962**	**MZ412974**	**MZ412950**	**MZ412916**	**MZ412937**	*	SMNH198156

More detailed collection data and a full list of included specimens are given in the Supporting Information (Table S1).
*Unsuccessfully sequenced. New species in bold.

specimens were not sequenced successfully for one of the above three markers and thus excluded from the corresponding gene tree: CE664 *Lumbricillus lineatus* (Müller, 1774), CE838 *Cernosvitoviella minor* Dózsa-Farkas, 1990, CE879 *Lumbricillus tuba* Stephenson, 1911, CE986 *L. lineatus*, CE2246 *Lumbricillus* sp. G *sensu* Klinth *et al.* (2017a) and CE34648 *Marionina* sp. 'Snow Is.' all missing *H3*; *CE12475* and CE12478 *Marionina fusca* and CE35374 and CE35375 *Ch. blocki* all missing *COI*; and CE35374 *Ch. blocki* missing ITS2. Thus, in total, the analyses included 69 specimens for *COI*, 67 specimens for *H3* and 72 specimens for ITS2, including outgroups, and all genes were aligned using the Auto algorithm with a gap open penalty of 1.53 in MAFFT (Katoh *et al.*, 2002). The trees were estimated in MRBAYES v.3.2.6 (Ronquist *et al.*, 2012), where *COI* and *H3* were partitioned according to codon position, with each partition unlinked to allow for different base frequencies, shape of the gamma distribution, proportion of invariable sites and substitution rates. All trees were estimated using nst = mixed (to allow the program to estimate the substitution models), rates = invgamma and brlenspr = unconstrained:Exp(100). The Markov chain Monte Carlo (MCMC) was set to run for ten million generations, with two chains sampled every 10 000 generations, and consensus trees were summarized after discarding the initial 25% as burn-in. To ensure proper convergence, the p-files were evaluated in TRACER v.1.7 (Rambaut *et al.*, 2018).

A species tree was estimated under the multispecies coalescent model as implemented in *BEAST (Heled & Drummond, 2010), using a subset of the specimens from the Southern Hemisphere together with the dataset for the species tree in the study by Klinth *et al.* (2017a); and with a few additional species of *Marionina s.l.* and *Enchytronia* (Supporting Information, Table S1). The seven genetic markers used, owing to their linked inheritance, were grouped into three effective datasets, the first two with a linked tree model: *COI*, 12S and 16S from the mitochondrial genome, 18S, 28S and ITS2 from the nuclear ribosomal gene complex, and *H3* as an independent nuclear gene. The following specimens lacking one of the seven markers were included by replacing the missing sequence with a sequence entirely made up of Ns (representing completely polymorphic nucleotide positions): CE2549 *Lumbricillus rubidus* Finogenova & Streltsov, 1978 and CE3502 *Lumbricillus rutilus* Welch, 1914 both missing 12S; CE35374 and CE35376 *Ch. blocki* both missing 18S; CE35374 and CE35375 *Ch. blocki* both missing *COI*; CE35374 *Ch. blocki* missing ITS2; and CE664 *L. lineatus*, CE838 *Ce. minor*, CE879 *L. tuba*, CE986 *L. lineatus*, CE2246 *L.* sp. G and CE34648 *Marionina* sp. 'Snow Is.' all missing *H3*. All genes were aligned using MAFFT. An XML file was generated in BEAUti v.1.8.4 (Drummond *et al.*, 2012), where the 63 specimens were grouped into 48

species. Each genetic marker was set as unlinked for site and clock models but, as previously mentioned, not for tree models. Each marker was given its own HKY+G+I substitution model, with empirical base frequencies. The model was selected as a compromise between the number of parameters that needed to be estimated (thereby reducing computation time) and the fit to the data. The clocks were set as lognormal relaxed uncorrelated, with the evolutionary rate for *COI* set as one and all other rates estimated in relationship to that. The tree priors used the Yule process, with piecewise linear and constant root for population sizes. The mitochondrial partition was given the corresponding ploidy type in BEAUti, but the ploidy size had to be doubled manually in the XML file to account for the fact that the worms are hermaphrodites, thus having two potential mothers from which the mitochondrion can be inherited in each mating. The clock priors (which were arranged in relationship to the fixed value of one for *COI*) were set as uniform, ranging from zero to two with an initial value of one for 12S, 16S and ITS2, and from zero to one with an initial value of 0.5 for 18S, 28S and *H3*. These priors were based on previous knowledge of relative substitution rates between genes, combined with information about the genetic distances within the markers. The species.popMean and species.yule.birthRate priors were given lognormal priors with default values. The species tree was run for 500 million generations in BEAST v.1.8 (Drummond *et al.*, 2012), with sampling every 50 000 generations. The result was evaluated in TRACER v.1.7. TREEANNOTATOR v.1.8 was used to remove the first 10% as burn-in, and a maximum clade credibility tree was generated using median node heights.

Finally, we also estimated a concatenated tree in MRBAYES, in order to compare our results with the results of the concatenated analyses in the study by Klinth *et al.* (2017a). This tree was estimated using exactly the same dataset as in the *BEAST species tree, but treating missing data as '–' instead of Ns. The dataset was partitioned according to the seven markers, with *COI* and *H3* partitioned further according to codon position. The other settings were the same as for the gene trees above, with the exception that the MCMC was set to run for 50 million generations, sampling every 50 000 generations, and the consensus tree was summarized after discarding the initial 25% as burn-in. All trees were illustrated using FIGTREE v.1.4.2 (Rambaut, 2014) and edited further in ADOBE ILLUSTRATOR.

RESULTS

GENETIC ANALYSES

The *COI* gene tree (Fig. 1) revealed a number of distinct clusters of specimens, which we used as species hypotheses and evaluated by comparisons with the results of the *H3* (Supporting Information, Fig. S1) and ITS2 (Supporting Information, Fig. S2) gene trees. We found support for a total of nine species from the Southern Hemisphere, all forming reciprocally monophyletic clusters in each gene tree, and all of which were clearly separated from the species from the Northern Hemisphere considered in previous studies (Klinth *et al.*, 2017a, b). In the shown tree, the nine southern species are labelled as: *Christensenidrilus blocki*, *Lumbricillus antarcticus* Stephenson, 1932, *L. finisafricae* sp. nov., *L. nivalis* sp. nov., '*L.*' cf. *macquariensis* Benham, 1905, *L.* sp. 'Marion Is.', *Marionina aestuum* Stephenson, 1932, *M. fusca* sp. nov. and *M.* sp. 'Snow Is.'. A single specimen from Snow Island (South Shetland Is.; CE34644) was, in all gene trees, closely related to the group of specimens we later identified as *L. antarcticus*, but it remained distinctly separated in all three gene trees; therefore, we consider it as a new, possible sister species to *L. antarcticus*, which we name *Lumbricillus nivalis* (described below). Likewise, a single specimen from South Georgia Is. (CE12483) was slightly different in all gene trees from a group of three specimens from the same locality, identified as '*Lumbricillus*' cf. *macquariensis* (regarding the uncertain identification, see Taxonomy below). In this case, the short genetic distance and the lack of morphological distinction lead us to treat all four specimens as conspecific.

The *BEAST species tree (Fig. 2) revealed a well-supported [posterior probability (PP) = 1] superclade containing all sampled species from the Southern Hemisphere, except *Ch. blocki*, together with the *Lumbricillus* and *Grania* species from the Northern Hemisphere. The superclade is split into two well-supported clades, with the first (PP = 1) containing the majority of the *Lumbricillus* species and the second (PP = 0.91) containing *Grania*, the *Lumbricillus* species forming the *L. arenarius* group *sensu* Klinth *et al.* (2017a) [*Lumbricillus arenarius* (Michaelsen, 1889a), *Lumbricillus dubius* (Stephenson, 1911) and the Norwegian *Lumbricillus* sp. H *sensu* Klinth *et al.* (2017a)] and four of the southern enchytraeid species investigated herein. This second clade is divided into four subclades, which we believe could be used to represent separate genera, all with maximum support (PP = 1); *Grania* remains a well-supported genus and is supported (PP = 1) as sister to '*L.*' cf. *macquariensis*. The remaining two subclades are the *L. arenarius* group and a group of three southern species that we believe to represent *Marionina s.s.* The species tree confirmed *Marionina s.l.* to be polyphyletic, with the species not included in *Marionina s.s.* falling in three separate clusters.

As for *Lumbricillus s.s.*, if the *L. arenarius* group and '*L.*' cf. *macquariensis* are excluded, monophyly of the remaining species has maximum

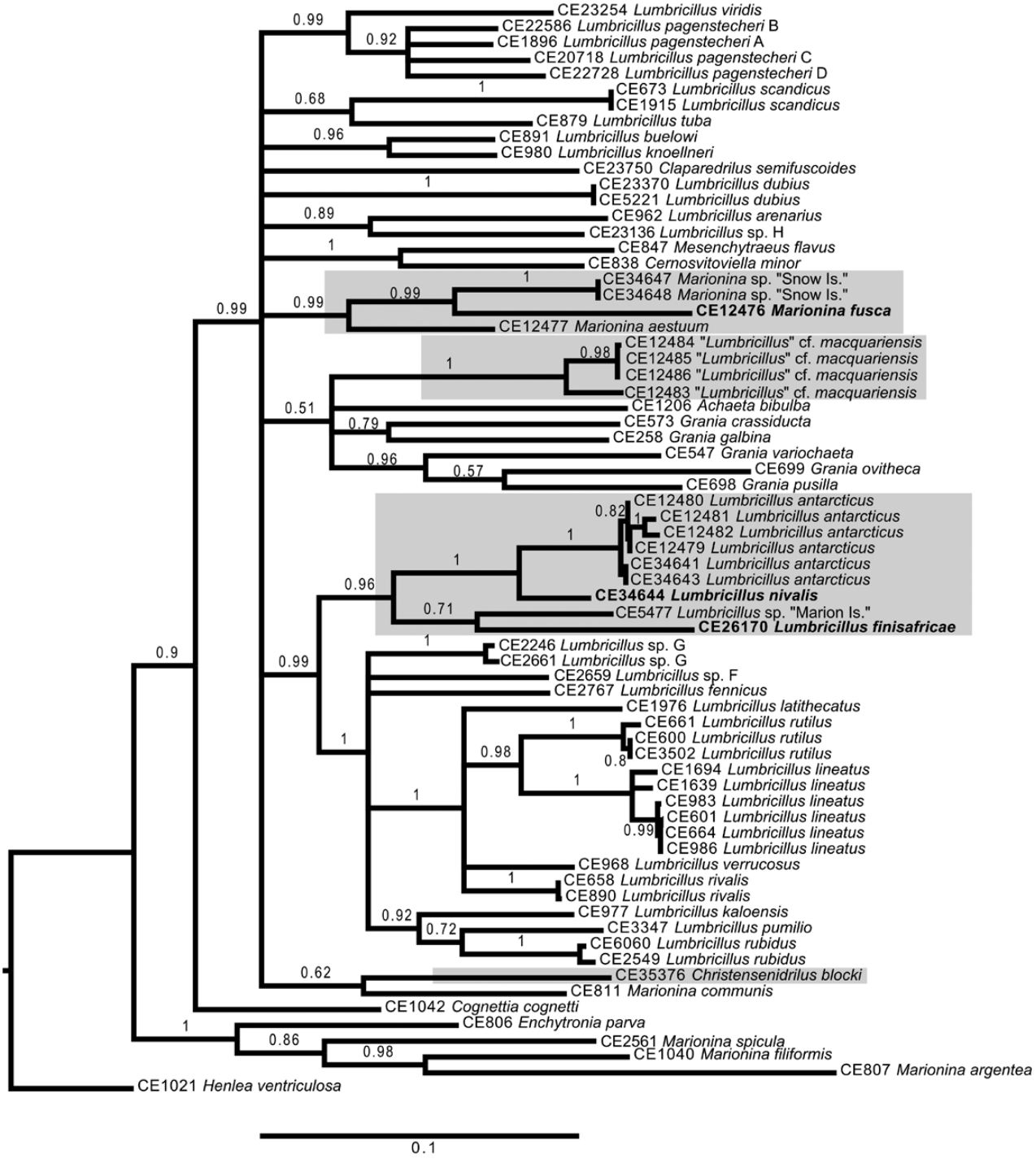

Figure 1. Majority-rule consensus tree for *COI* estimated with Bayesian inference. Specimens shaded in grey are from the Southern Hemisphere. Specimens in bold represent new species. Support values are posterior probabilities. Scale bar represents the estimated number of substitutions per site.

support, and *Lumbricillus buelowi* Nielsen & Christensen, 1959 and *Lumbricillus knoellneri* Nielsen & Christensen, 1959 make up the sister group to the rest of the species. The four southern species diagnosed as belonging to *Lumbricillus* (i.e. excluding '*L.*' cf. *macquariensis* mentioned above) fell in the well-supported (PP = 1) *L. lineatus* group (so named for containing *L. lineatus*, the

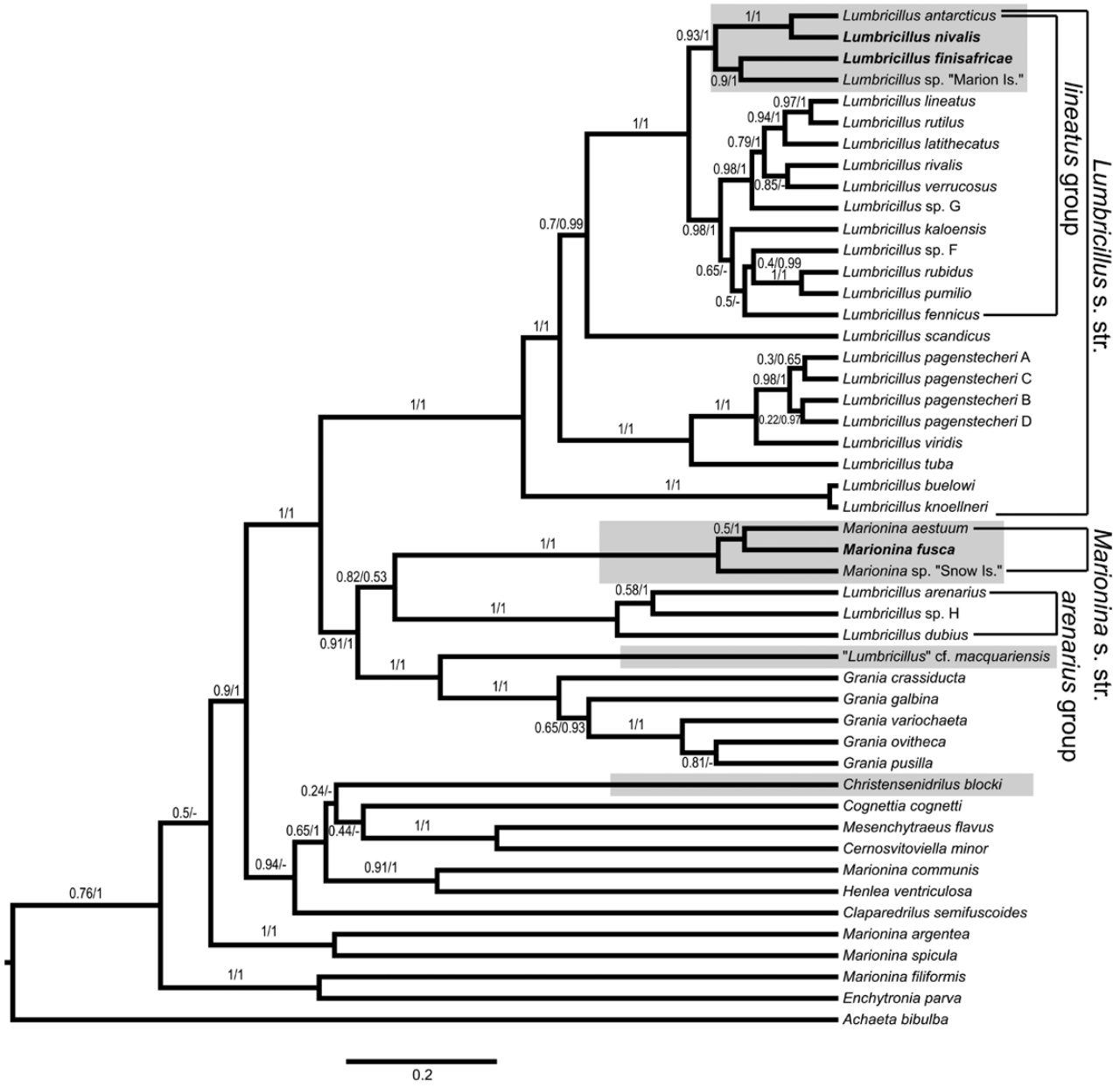

Figure 2. Species tree estimated using the multispecies coalescent model in *BEAST. Specimens shaded in grey are from the Southern Hemisphere. Specimens in bold represent new species. Two support values are presented: first, the posterior probabilities from *BEAST; second, the posterior probabilities from the concatenated MrBayes tree (see Supporting Information, Fig. S3), where '–' indicates the absence of the node in question. Scale bar shows the expected number of changes per site scaled in accordance with the substitution rate of *COI*.

type species of the genus). Genetically, the support is somewhat modest (PP = 0.93), but the four taxa comprise a morphologically coherent lineage, which shares the spindle-shaped spermathecae and other morphological characters with their northern sister clade (PP = 0.98) (see Klinth *et al.*, 2017b, where *L. antarcticus* was predicted correctly to belong to this group). It should be noted that *M. aestuum* is clearly not a member of *Lumbricillus* nor of the *L. lineatus* group, as was predicted erroneously by Klinth *et al.* (2017b); likewise, there are several other species that we believe were misplaced in that study (see *Marionina* discussion below).

Christensenidrilus blocki was not supported as a member of the superclade containing *Lumbricillus*, *Grania* and *Marionina s.s.* Instead, it was found together

with a number of enchytraeid outgroups, but with no support for any close phylogenetic relationships (Fig. 2).

The concatenated tree (Supporting Information, Fig. S3) had a largely identical topology to the *BEAST species tree, and its support values have been incorporated into Figure 2. There were some differences in the placement of the outgroups, where the concatenated tree found support for a group of *Marionina s.l.* (excluding what we consider as *Marionina s.s.* and *Marionina communis* Nielsen & Christensen, 1959) together with *Enchytronia*. The concatenated tree also found *Ch. blocki* sister to *Cognettia cognetti* (Issel, 1905) (PP = 0.9).

TAXONOMY

Here follow morphological descriptions of our new material and some shorter amended descriptions from the original material of Michaelsen (1888) and Stephenson (1932) from South Georgia and the Palmer Archipelago. *Lumbricillus antarcticus* and *M. aestuum* are redescribed because, although their original descriptions are by no means bad, they were scarcely illustrated and, for the first time, we have genetic data from these species connecting the morphological data to molecular sequences, provided our specimens are conspecific with Stephenson's species. The morphology of *M. georgiana*, *Marionina colpites* (Stephenson, 1932) and *Marionina grisea* Stephenson, 1932 is also reinvestigated and discussed for comparison, using type material. The description of '*L.*' cf. *macquariensis* is not formally a redescription, because we are not sure whether our specimens belong to the species originally described by Benham. We also provide comments on *Ch. blocki*, as a complement to its original description.

With regard to the terminology, the male apparatus can be difficult to compare, given the various synonyms used to describe similar structures (see Schmelz, 2003: 48–53). We have opted for the following. We use 'penial bulb' to describe the distinct, usually large, glandular mass surrounding the invaginate male pores in *Lumbricillus* species, and 'penial body' to distinguish the much smaller cushion of gland cells surrounding the superficial male pores in what we consider as *Marionina s.s.* (more on this in the general discussion of *Marionina* below). In *Marionina s.s.* there are one or more accessory glandular masses associated with the male pore, either connected to the 'penial body' or discharging independently through the epidermis, which we refer to as 'prostate glands', following Stephenson (1932). In some species of *Lumbricillus* (and other enchytraeid genera), the 'penial bulbs' are subdivided into two or more parts or lobes; in this case, we still refer to the whole gland as a bilobed 'penial bulb'. Knowing which of these specialized gland masses are homologous remains difficult.

Regarding the nerve system, the construction of the nerve cord is described following Rota *et al.* (2008) and Rota (2013); that is, a 'scattered' condition refers to a cord in which the neuron perikarya are distributed continuously along the ventral side of cord length, and a 'ganglionic' condition as a nerve cord where perikarya are aggregated into well-defined segmental ganglia, separated by nuclei-free interganglionic connectives (except in the foremost and hindmost segments). We reject the term 'medullar' introduced by Schmelz & Collado (2008, 2010) for the first condition, because it is lexically inappropriate: 'medullar' indicates an inner location as opposed to 'cortical'.

ABBREVIATIONS IN THE FIGURES

as, anteseptale of nephridum; b, brain; dv, dorsal blood vessel; e, egg; ed, ectal duct of spermatheca; eg, ectal gland of spermatheca; ep, epithelial plate; mu, musculature; n, nephridium; nd, nephridial duct; oe, oesophagus; ov, ovary; pb, penial bulb; pbo, penial body; pc, prostomial cluster of perikarya; pg, pharyngeal glands; ph, pharynx; ppb, postpharyngeal bulb; pr, prostate gland; ps, postseptale of nephridium; sa, spermathecal ampulla; sb, sperm bundle; sc, subbuccal cluster of perikarya; sf, sperm funnel; sm, sperm mass; sp, spermathecal pore; sub, subbuccal bulb; t, testis; ts, testis sac; vd, vas deferens; vn, ventral nerve cord.

LUMBRICILLUS ØRSTED, 1844

Type species: *Lumbricus lineatus* Müller, 1774.

LUMBRICILLUS ANTARCTICUS STEPHENSON, 1932 (FIGS 3, 4A–C)

Lumbricillus antarcticus Stephenson, 1932: 256–257, fig. 8.

Lumbricillus sp. 2 – Prantoni *et al.*, 2018 (confirmed by comparison of *COI* data).

Lumbricillus cf. *antarcticus* – Lee *et al.*, 2019: 4–5, fig. 4.

Type material: BMNH 1933.2.23.899–900, two mature sectioned specimens (studied), BMNH 1931.06.23.89/90 (in alcohol, not studied). Syntypes. Loc. Wilson Harbour, South Georgia. Leg. 'Discovery' 1925–1927 (Stephenson, 1932), see Boros & Sherlock (2010).

Type locality: Wilson Harbour, South Georgia Island. According to Stephenson (1932), the sample was labelled as 'moss dwellers', but the sampling station was listed as 'hauled' from 15–45 and 26–83 m using a beam trawl.

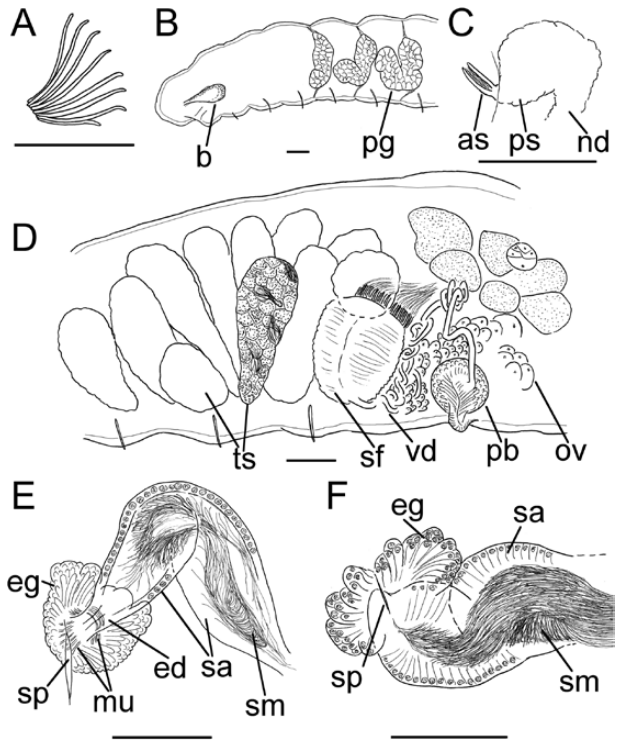

Figure 3. *Lumbricillus antarcticus*. A, chaetal bundle (CE12480). B, anterior part of body (CE12479). C, nephridium (CE12480). D, genitalia (CE12479). E, F, spermathecae (CE12479 and CE34641). Abbreviations are defined under 'Taxonomy'. Scale bars: 100 μm.

New material examined: SMNH198144–198147 (CE12479–CE12482), four mature specimens collected intertidally in 2010 from South Georgia Island, and SMNH198148 (CE34641) and SMNH198149 (CE34643), two mature specimens collected in 2015 from algae on intertidal rocks at Snow Island (South Shetland Islands, Antarctica). All new specimens are mounted on slides and serve as vouchers of genetic data. For details on collection and GenBank accession numbers for *COI* barcodes (and other genes for some specimens), see Table 1 and the Supporting Information (Table S1).

Diagnosis: This species, now differentiated by a unique *COI* barcode, is a member of the *L. lineatus* group, meaning that it has testis sacs forming club-shaped lobes arranged in a fan shape, and spindle-shaped spermathecae. It can be distinguished from other *L. lineatus* group members by combining: (1) short sperm funnel, 1.0–1.5 times longer than wide; (2) spermatheca that widens gradually from the ectal pore, to be widest midway or two-thirds in and gradually tapers towards the oesophagus, where it connects to the latter; and (3) the geographical range, in that it is known only from the Subantarctic Islands and Antarctic Peninsula.

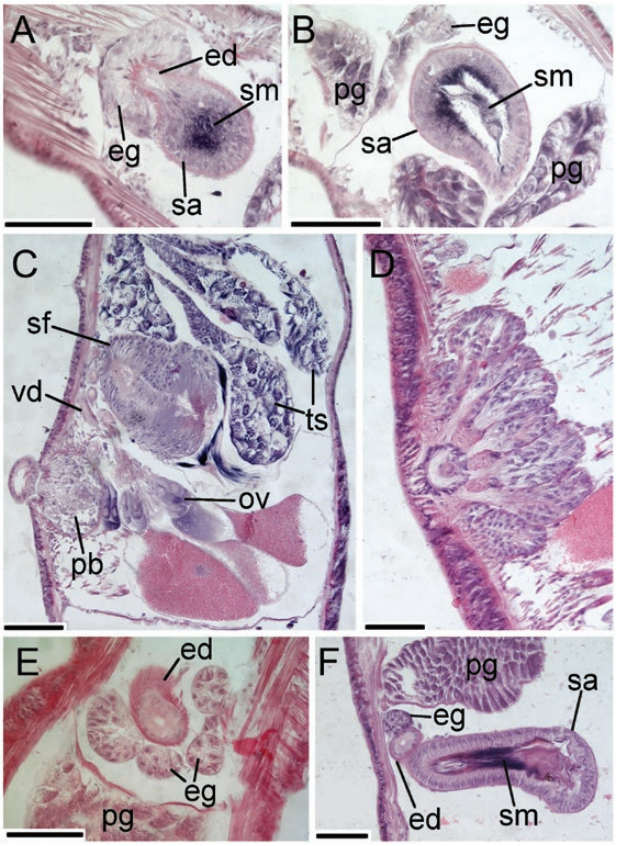

Figure 4. *Lumbricillus antarcticus* and *Marionina colpites*. A–C, *L. antarcticus*, sectioned specimen BMNH 1933.2.23.899. A, B, spermatheca. C, genitalia. D–F, *M. colpites*. D, male pore and associated glands (BMNH 1933.2.23.888). E, F, spermatheca (BMNH 1933.2.23.886 and BMNH 1933.2.23.888). Abbreviations are defined under 'Taxonomy'. Scale bars: 100 μm.

Description: Length of first 20–26 segments is 3.1–7.1 mm (fixed, amputated specimens); first 15 segments 2.0–4.3 mm long; width at clitellum is 0.45–0.67 mm. Chaetae sigmoid (Fig. 3A). Upper bundles dorsolateral (above the lateral line but closer to it than the ventral bundles), with (three) four to six (eight) chaetae anterior to clitellum, and three to six (eight) chaetae in postclitellar segments, at least to XXVI. Ventral bundles with (four) six to eight (ten) chaetae anterior to clitellum, and (four) five to seven (nine) chaetae posteriorly. Longest measured chaetae of each worm 70–85 μm long, ~3–5 μm wide. Epidermis loosely covered with rows of pale gland cells. Clitellum with reticulate pattern of gland cells, extending over XII–XIII, absent ventrally.

Coelomocytes numerous, 15–20 μm long; round, oval or spindle shaped; granulated with distinct nucleus. Paired pharyngeal glands (Fig. 3B) in IV, V and VI; each pair converging dorsally, but connections not discernible. Dorsal vessel originating in XIII, with

peristomial bifurcation. Nephridia (Fig. 3C) ~85–110 µm long, observed in 7/8–9/10 and postclitellar segments. Anteseptale small, consisting of funnel only. Postseptale oval, tapering into posteroventral efferent duct. Brain with posterior incision.

Male genitalia paired (Figs 3D, 4C). Testes originating in anterior of XI, with testis sacs forming regular club-shaped lobes extending forwards into IX, sometimes VIII. Sperm funnels in XI, 175–250 µm long, 150–235 µm wide, making them about as long as wide or 1.5 times longer than wide; funnels tapering towards vasa deferentia. Most of vasa irregularly coiled in and confined to XII, 15–20 µm wide. Penial bulbs round, 100–140 µm in diameter. Ovaries in XII. One to three mature eggs present at a time.

Spermathecae (Figs 3E, F, 4A, B) in V, spindle shaped, with short ectal duct covered in musculature and rapidly widening into ampulla. Ampulla with midway bend dividing it into two sections; ectal part slightly narrower than ental part; ental part connecting with oesophagus. Sperm in lumen of entire ampulla; heads of spermatozoa embedded in wall of ampulla; more concentrated in the ental part. Spermathecae 270–390 µm long, 25–35 µm wide at the ectal duct, 75–115 µm wide at widest part of ampulla. Gland cells surrounding ectal duct, forming compact mass 100–145 µm in diameter at its widest part. Midventral subneural glands observed in XIII–XIV(XV), 75–130, 85–130 and 55–95 µm long, respectively.

Geographical distribution and habitat: Stephenson (1932) originally described this species from South Georgia Island, which is where most material comes from, in addition to two specimens that came from Snow Island (South Shetland Islands) near the Antarctic Peninsula, not far from King George Island, from where it was reported from a tidal pool by Lee *et al.* (2019). It should be noted that Stephenson's material was labelled as 'moss dwellers' and was sampled ('hauled') from 15–45 and 26–83 m using a beam trawl. The species is also reported in the literature from Heard Island, the Kerguelen Islands, Macquarie Island and the South Orkney Islands, where the samples from Macquarie Island were from freshwater (Dartnall *et al.*, 2005), but we have not been able to confirm these reports by comparing morphology or genetic data.

Remarks: Our specimens, sampled from the intertidal zone on South Georgia Island and Snow Island, agree with the original description, particularly in the short sperm funnels, but they have on average more chaetae per bundle, the penial bulbs are smaller, and the spermathecal ampullae of our specimens are widest in their ental portion rather than ectally as in the illustration by Stephenson (1932: fig. 8). In these respects, our specimens are similar to those of Lee *et al.* (2019), who motivated the uncertain identification *L.* cf. *antarcticus* owing to these differences. However, in Stephenson's sectioned specimens, the penial bulbs are of the same size as in our material, and the proportions of the spermathecae also agree, making us confident that our specimens and those of Lee and Stephenson are conspecific. In his publication, Stephenson also recorded *L. lineatus* from South Georgia and considered it close to *L. antarcticus*, but distinguished by the much longer sperm funnels; five to ten times as long as wide for *L. lineatus* against 1.25 times as long as wide for *L. antarcticus* (matching the sperm funnels of our specimens). The short sperm funnels seem to distinguish *L. antarcticus* from most other species in the *L. lineatus* group. There are a few species from the Northern Hemisphere with sperm funnels only 1.5 times longer than wide: *Lumbricillus alaricus* Shurova, 1974 from the Kurile Islands, *Lumbricillus fennicus* Nurminen, 1964 from northern Europe, *Lumbricillus parabolus* Shurova, 1978 from the eastern coast of Kamchatka and *L. rubidus* from east Murmansk (Barents Sea). *Lumbricillus antarcticus* does not have the lobed sperm funnels of *L. fennicus* or *L. parabolus* and lacks the 'muscular bulb' surrounding the spermathecal ectal duct in *L. rubidus*. The brief description of *L. alaricus* is similar to that of *L. antarcticus*, but with fewer chaetae on average and a smaller ectal gland on the spermathecae. All four mentioned species are separated from *L. antarcticus* by huge geographical distances, and *L. fennicus* and *L. rubidus* were both included in the phylogeny and were not found to be related closely to *L. antarcticus*.

The following three *Lumbricillus* species, all described from the South Shetland Islands in the Subantarctic, are similar to *L. antarcticus* and have only slightly longer sperm funnels (two to three times longer than wide). The first, *Lumbricillus sejongensis* Lee *et al.*, 2019, has spermathecae with less distinct transition from ectal duct to ampulla, and a more inflated ampulla than *L. antarcticus*. The two other candidates, *Lumbricillus incisus* Wang & Liang, 1997 and *Lumbricillus healyae* Rodriguez & Rico, 2008, have both been found in freshwater and are morphologically similar to one another. *Lumbricillus incisus* has fewer chaetae per bundle and has penial bulbs with a midway constriction, separating it from *L. antarcticus*. *Lumbricillus healyae* is described as having sac-shaped spermathecal ampullae attaching directly to the oesophagus without tapering into an ental duct, whereas we consider those of *L. antarcticus* to be more spindle shaped and to taper into an ental duct. However, this shape can be influenced by the extension or contraction of the worm [compare our

Fig. 3E, F, in addition to Rodriguez & Rico's (2008) figs 3c and 4e, f for *L. healyae*; our Fig. 3F is similar to their fig. 4f]. Nevertheless, *L. antarcticus* can be distinguished from *L. healyae* by not having strong dorsoventral muscle strands surrounding the penial bulbs (described as possibly unique for that species) and shorter sperm funnels, as mentioned earlier.

Finally, the closest species to *L. antarcticus*, both morphologically and genetically, is the new species *L. nivalis*, which we describe below. A comparison between these two species can be found under the Remarks section of this new species.

LUMBRICILLUS NIVALIS KLINTH, ROTA & ERSÉUS **SP. NOV.** (FIG. 5)

Zoobank registration: urn:lsid:zoobank.org:act:D62E6765-ED4D-498D-A300-50D5232BDD14

Holotype: SMNH Type Coll. 9310 (CE34644), a mature amputated specimen stained in Paracarmine and mounted on a slide. Leg. Karla Paresque, 15 January 2015. *COI* barcode, GenBank MZ393952; accession numbers for additional genetic data are given in Table 1 and the Supporting Information (Table S1).

Type locality: Snow Island, South Shetland Islands, Antarctica, from algae on rocks in intertidal zone, 62.7753 S, 61.2858 W.

Etymology: The Latin adjective *nivalis* means snowy, alluding to the name of the type locality, Snow Island.

Diagnosis: This species, now differentiated by a unique *COI* barcode, is a member of the *L. lineatus* group, meaning that it has testis sacs forming club-shaped lobes arranged in a fan shape and spindle-shaped spermathecae. It can be distinguished from other *L. lineatus* group members by: (1) its short sperm funnels, 1.5–2.0 times longer than wide; (2) vasa deferentia extending posterior to XII; (3) spermathecae with short ectal duct, rapidly widening into ampulla, with ampulla widest ectally, gradually tapering towards ental duct, which connects to oesophagus; and (4) clitellum ventrally absent in XII but present ventrally in (first half of) XIII.

Description: Length of first 25 segments 6 mm (fixed, amputated specimen); first 15 segments 3.7 mm long; width at clitellum 0.50 mm. Chaetae sigmoid (Fig. 5A). Upper bundles dorsolateral (higher than the lateral line but closer to it than the ventral bundles), with three to five chaetae anterior and posterior of clitellum, at least to XXV. Ventral bundles with (three)

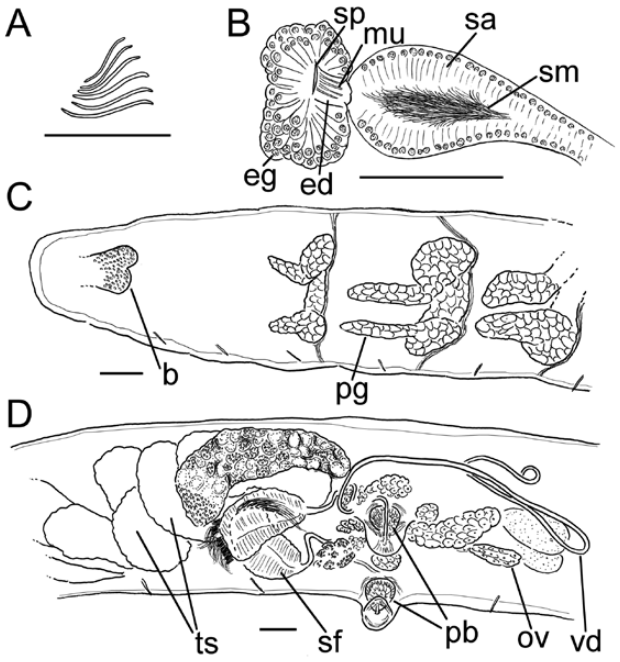

Figure 5. ***Lumbricillus nivalis***, holotype. A, chaetal bundle. B, spermatheca. C, anterior part of body. D, genitalia. Abbreviations are defined under 'Taxonomy'. Scale bars: 100 µm.

four to six throughout to XXV. The longest measured chaetae 60 µm long, ~3–5 µm wide. Epidermis loosely covered with rows of pale gland cells. Clitellum with reticulate pattern of gland cells, extending over XII–1/2XIII, interrupted ventrally in XII but present ventrally in XIII.

Coelomocytes numerous, ~10–15 µm long; round, oval or spindle shaped; granulated with distinct nucleus. Paired pharyngeal glands (Fig. 5C) in IV, V and VI; first two pairs narrowly connected dorsally, a trait not discernible in third pair owing to damaged specimen; ventral lobes elongated, possibly attributable to fixation. Dorsal vessel seemingly originating in XIII, with peristomial bifurcation. Nephridia ~130–150 µm long, observed in 7/8, 8/9 and postclitellar segments. Anteseptale small, consisting of funnel only. Postseptale oval, tapering into posteroventral efferent duct. Brain with posterior incision.

Male genitalia paired (Fig. 5D). Testes originating in anterior of XI, with testis sacs forming regular club-shaped lobes extending forwards into IX. Sperm funnels in XI, 240 µm long, 140 µm wide, making them 1.5–2.0 times longer than wide; funnels tapering towards vasa deferentia. Vasa deferentia irregularly coiled in XII–XIII, possibly reaching XIV, 15 µm wide. Penial bulbs round, 85 µm in diameter. Ovaries in XII. No mature eggs observed.

Spermathecae (Fig. 5B) in V, pear or spindle shaped, with short ectal duct covered in musculature and rapidly widening into ampulla. Ampulla widest ectally and gradually tapering towards ental duct, which connects to oesophagus. Sperm in lumen of ectal part of ampulla; heads of spermatozoa embedded in wall of ampulla. Spermathecae 255 µm long, 20 µm wide at the ectal duct, expanding to 80 µm at widest part of ampulla. Gland cells surrounding ectal duct as a compact mass, 100 µm in diameter at its widest part. No midventral subneural glands observed.

Geographical distribution: Known from the South Shetland Islands only.

Remarks: Genetically and morphologically, this species is most closely related to the specimens we identified as *L. antarcticus*, which were found at the same locality on Snow Island. As such, this new species can be separated from other members of the *L. lineatus* group in the same way as described in the remarks for *L. antarcticus* above, where primarily the short sperm funnels help to distinguish this species from many somewhat similar *Lumbricillus* species from the Subantarctic. Owing to their likeness, we had to consider which of our two species was the true *L. antarcticus*. The clitellum is saddle-shaped in *L. antarcticus*, but in our single specimen of *L. nivalis* it is also present ventrally in the anterior of XIII. Moreover, *L. nivalis* has smaller penial bulbs and lacks subneural glands (possibly attributed to it not being fully mature; no mature eggs were observed). Finally, *L. nivalis* can be separated by its vasa deferentia extending all the way back to segment XIII or possibly XIV, whereas the vasa are consistently confined to XII in our and Stephenson's material of *L. antarcticus*.

LUMBRICILLUS FINISAFRICAE KLINTH, ROTA & ERSÉUS **SP. NOV.**
(FIG. 6)

Zoobank registration: urn:lsid:zoobank.org:act:E7F65CC6-083F-4889-B03B-7DA9137B2665

Holotype: SAMC Type Coll. SAMC-A094448 (CE26170), a mature amputated specimen stained in Paracarmine and mounted on a slide. Leg. Gavin Rishworth, 20 April 2015. *COI* barcode, GenBank MZ393951; accession numbers for additional genetic data are given in Table 1 and the Supporting Information (Table S1).

Type locality: Cape Recife Natural Reserve, Port Elizabeth, South Africa, on stromatolites. 34.0450 S, 25.5689 E.

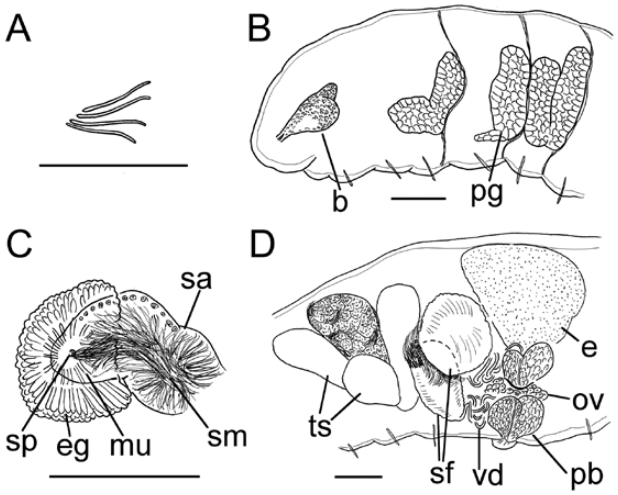

Figure 6. ***Lumbricillus finisafricae***, holotype. A, chaetal bundle. B, anterior part of body. C, spermatheca. D, genitalia. Abbreviations are defined under 'Taxonomy'. Scale bars: 100 µm.

Etymology: The new species epithet derives from the Latin *finis*, boundary or end, and the continent Africa, referring to this species being found at the end of Africa.

Diagnosis: This species is a member of the *L. lineatus* group, meaning that it has testis sacs forming club-shaped lobes arranged in a fan shape, and spindle-shaped spermathecae. It can be separated from other species in this group by having penial bulbs bulging into an anterior and a posterior lobe. *Lumbricillus sadovskyi* Marcus, 1965 has penial bulbs lobed in a similar manner, but which are much smaller, five to six times the diameter of vasa, compared with 12 times for *L. finisafricae*.

Description: Length of first 28 segments 2.4 mm (fixed, amputated specimen); first 15 segments 1.2 mm long; width at clitellum 0.45 mm. Chaetae sigmoid (Fig. 6A). Upper bundles dorsolateral (closer to lateral line than the ventral bundles), with three to four chaetae anterior to clitellum, two to four (five) chaetae in postclitellar segments, at least to XXVIII. Ventral bundles with four to five chaetae anterior to clitellum, three to four chaetae posteriorly. The longest measured chaetae 55 µm long, ~3 µm wide. Epidermis loosely covered with rows of pale gland cells. Clitellum with reticulate pattern of gland cells, extending over XII–1/2XIII, absent ventrally.

Coelomocytes numerous, ~15 µm long; round, oval or spindle shaped; granulated, with distinct nucleus. Paired pharyngeal glands (Fig. 6B) present in IV, V and VI; each pair converging dorsally, but connections

not discernible. Dorsal vessel originating in XIII, with peristomial bifurcation. Nephridia ~75–90 µm long, not observed in preclitellar segments but in 14/15, 19/20 and further postclitellar segments. Anteseptale small, consisting of funnel only. Postseptale oval, tapering into posteroventral efferent duct. Brain with posterior incision.

Male genitalia paired (Fig. 6D). Testes originating in anterior of XI, with testis sacs forming regular club-shaped lobes extending forwards into X and IX. Sperm funnels in XI, 165 µm long, 95 µm wide, making them almost twice as long as wide; funnels tapering towards vasa deferentia. Most of vasa irregularly coiled in XII, 10 µm wide. Penial bulbs bilobed with anterior and posterior lobes, combined into a heart-shaped structure; vasa penetrating through the anterior lobe; whole structure 125 µm in diameter. Ovaries in XII. One mature egg observed.

Spermathecae (Fig. 6C) in V, spindle shaped, with ectal duct indistinguishable from the ampulla. Ampulla with sperm filling the lumen and heads of spermatozoa embedded in the walls. Ental part of ampulla seemingly connected with oesophagus. Spermathecae 130 µm long, 65 µm wide at widest part of ampulla. Gland cells surrounding ectal duct, forming compact mass, glandular body 95 µm in diameter at its widest part. No midventral subneural glands observed.

Geographical distribution: Known from the type locality in southernmost South Africa only.

Remarks: We only have one specimen of this species, but found it to be morphologically (and genetically) distinct from other *Lumbricillus* species. Therefore, it is described as a new species here. Genetically, *L. finisafricae* is placed in the *L. lineatus* group (*sensu* Klinth *et al.*, 2017b), with which it shares morphological characters such as a spindle-shaped spermatheca with ectal gland (but without glands along the ectal duct), regularly lobed testis sacs and three or more chaetae per bundle. However, unlike the other species genetically shown to be part of the *L. lineatus* group, *L. finisafricae* has a bilobed penial bulb. There are a few other species of *Lumbricillus* reported to have bilobed penial bulbs, the one most similar to our South African form being *L. sadovskyi*. The latter was described from two sites (a beach and an artificial mangrove swamp) in Brazil, and it also has an anterior and a posterior lobe in its penial bulbs. However, *L. sadovskyi* has a much smaller penial bulb [illustrated by Marcus (1965) as five to six times larger than the diameter of the vasa, whereas our specimen has a bulb 12 times larger than the diameter of the vasa], its chaetae vary from two to eight per bundle, and its spermathecae differ greatly in appearance between the two Brazilian localities, possibly indicating a mixture of two species under the same name. Regardless, although *L. sadovskyi* is possibly the closest species to our South African specimen, we find it too distinct morphologically and geographically to consider it as conspecific to *L. finisafricae.* A bilobed penial bulb was reported for '*Pachydrilus lineatus*' *sensu* Backlund (1947), but the two lobes were described as dorsal and ventral, whereas *L. finisafricae* has one anterior and one posterior lobe. Also, in *L. incisus* (from King George Island) the bilobed bulb is transversely oriented, not longitudinally oriented. There are species in the *L. arenarius* group with bilobed penial bulbs, but they have three or fewer chaetae per bundle, irregularly lobed testis sacs, and are not closely related genetically to the South African species. The *Lumbricillus* of Africa are poorly studied, and the few species reported include *Lumbricillus mangeri* (Michaelsen, 1914) from Cameroon, which is probably not a *Lumbricillus* but some form of *Marionina s.l.*, and a record of *Lumbricillus verrucosus* (Claparède, 1861) from Namibia (Michaelsen, 1914), although we cannot say whether this is the same as the *L. verrucosus* we know from the Northern Hemisphere (see Klinth *et al.*, 2017a, b). None of the aforementioned species is morphologically similar to *L. finisafricae*.

LUMBRICILLUS SP. 'MARION IS.'

(FIG. 7)

Lumbricillus sp. – Dartnall & Smith, 2012.

Material examined: SAMC-A094449 (CE5447), one semi-mature specimen from Marion Island, south-western Indian Ocean, from eutrophic freshwater in a coastal pool, possibly a wallow (a shallow depression enriched by seal and penguin excrements), April–May 2008, leg. Herbert Dartnall. GenBank accession numbers are given in Table 1 and the Supporting Information (Table S1).

Description: Length of first 26 segments 4.1 mm (fixed, amputated specimen); first 15 segments 2.1 mm long; width at clitellum 0.45 mm. Chaetae sigmoid (Fig. 7A). Upper bundles dorsolateral (closer to lateral line than the ventral bundles), with three to five chaetae anterior to clitellum, and three to four chaetae in postclitellar segments, at least to XXVI. Ventral bundles with four to six chaetae anterior to clitellum, and four to five chaetae posteriorly. The longest measured chaetae of the worm are 70 µm long, ~5 µm wide. Epidermis loosely covered with rows of pale gland cells. Clitellum not fully developed.

Coelomocytes numerous, ~10–20 µm long; round, oval or spindle shaped; granulated, with distinct nucleus. Paired pharyngeal glands (Fig. 7B) present

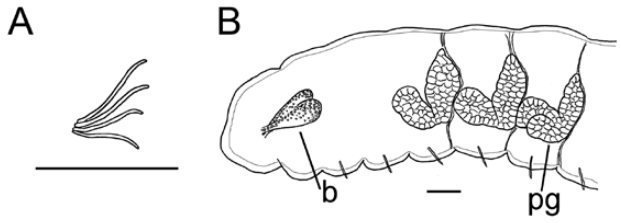

Figure 7. *Lumbricillus* sp. 'Marion Is.'. A, chaetal bundle. B, anterior part of body. Abbreviations are defined under 'Taxonomy'. Scale bars: 100 µm.

in IV, V and VI; each pair converging dorsally, but connections not discernible. Dorsal vessel originating in XIV, with peristomial bifurcation. Nephridia ~150–180 µm long, observed in 13/14–15/16, 17/18, 18/19, 21/22 and 22/23. Anteseptale small, consisting of funnel only. Postseptale oval, tapering into posteroventral efferent duct. Brain with posterior incision.

Male genitalia paired, not fully developed. Testes in anterior of XI, with testis sacs forming regular club-shaped lobes. Sperm funnels not fully formed, without sperm on collars. Vasa deferentia irregularly coiled in XII, only 3 µm wide. Penial bulbs round, 40 µm in diameter. Spermathecae in V, not fully developed. Midventral subneural glands observed in XIII, XIV and possibly in XV; first two glands 70 and 65 µm long, respectively.

Geographical distribution: Known from Marion Island only.

Remarks: Unfortunately, this specimen was not fully mature and could not be identified or described, but we could observe developing regularly lobed testis sacs, which matches with the phylogenetic placement in the *L. lineatus* group.

'LUMBRICILLUS' CF. *MACQUARIENSIS* BENHAM, 1905

(FIG. 8)

?*Lumbricillus macquariensis* Benham, 1905: 295–297, pl. XIV, figs 8, 11–13; Benham, 1915: 189–191; Benham, 1922: 6; Stephenson, 1932: 254–255, fig. 7.
?*Lumbricillus intermedius* Benham, 1909: 261–262, pl. X, figs 8–11.
?*Pachydrilus intermedius* – Michaelsen, 1924: 197–199.

Type material: No information (Reynolds & Wetzel, 2019). The material is probably located in the Otago Museum, New Zealand, because Benham mentions

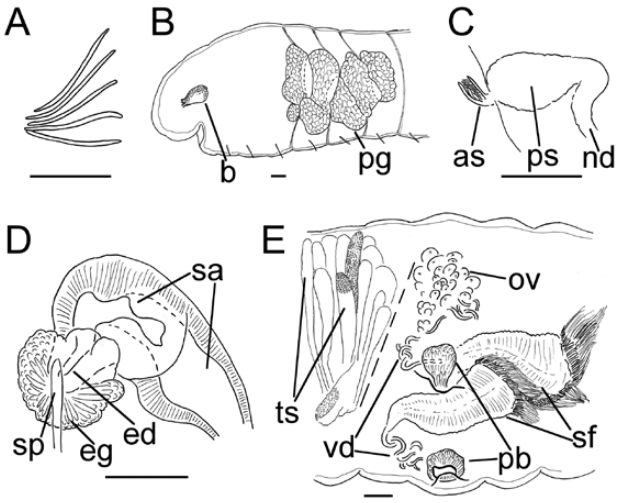

Figure 8. *'Lumbricillus'* cf. *macquariensis*. A, chaetal bundle (CE12486). B, anterior part of body (CE12485). C, nephridium (CE12484). D, spermatheca (CE12483). E, genitalia (CE12483); the testis sacs are illustrated from another specimen (CE12486) but with the same scale. Abbreviations are defined under 'Taxonomy'. Scale bars: 100 µm.

material being deposited and registered in the Museum by a Professor Parker, who was curator of the Otago museum collections at the time. Our inquiry did not receive any response.

Type locality: Macquarie Island, brackish pools, with planarians and *Siphonaria* limpets.

Material examined: SMNH198150 (CE12483), one mature specimen, and SMNH198151–198153 (CE12484–CE12486), three immature or semi-mature specimens, all collected in 2010 from South Georgia. For details of collection and GenBank accession numbers for *COI* barcodes and other gene sequences, see Table 1 and the Supporting Information (Table S1).

Description: Length of first 15–48 segments 2.5–7.1 mm (fixed, amputated specimens); first 15 segments 2.5–3.0 mm long; width at clitellum 0.85–1.10 mm. Chaetae slightly sigmoid (Fig. 8A). Upper bundles dorsolateral (closer to lateral line than the ventral bundles), with four to six chaetae anterior to clitellum, and three to four chaetae in postclitellar segments, at least to segment XLVIII. Ventral bundles with (four) five to seven chaetae anterior to clitellum, and four to six chaetae posteriorly. The longest measured chaetae of each worm 135–145 µm long, ~6–7 µm wide. Epidermis loosely covered with rows of pale gland cells. Clitellum not fully developed. Head pore not observed.

Coelomocytes numerous, 15–30 µm long; round, oval or spindle shaped; granulated with distinct nucleus. Paired pharyngeal glands (Fig. 8B) present in IV, V and VI, with third pair extending back into VII; each pair converging dorsally, but connections not discernible; dorsal lobes of about equal size, ventral lobes in IV–VI, increasing in size from IV to VI, absent in VII. Dorsal vessel originating in XVI–XVII, with peristomial bifurcation. Nephridia (Fig. 8C) ~110–190 µm long, observed in 7/8–9/10 and postclitellar segments. Anteseptale small, consisting of funnel only. Postseptale oval, tapering into posteroventral efferent duct. Brain with posterior incision.

Male genitalia paired (Fig. 8E). Testes originating in anterior of XI, with testis sacs forming regular lobes extending forwards into X, but these lobes are finger-like, thats is, much thinner than those of most species of *Lumbricillus*. Sperm funnels in XI, in one specimen extending backwards into XII (see Fig. 8E), 425 µm long, 155 µm wide, making them about three times as long as wide; funnels tapering towards vasa deferentia. Most of vasa irregularly coiled in XII, 25 µm wide. Penial bulbs round or pear shaped, 125 µm in diameter, discharging into deep invagination of body wall (Fig. 8E). Ovaries in XII. No mature eggs observed.

Spermathecae (Fig. 8D) in V, pouch shaped, with short ectal duct gradually widening into ampulla. Ampulla with thicker epithelium in the ectal parts, which transitions to thinner epithelium in the more ental parts. Ental part seemingly connected with oesophagus. No sperm observed. Spermathecae 340 µm long, 55 µm wide at the ectal duct, 120 µm wide at widest part of ampulla. Gland cells surrounding ectal duct, forming compact mass, glandular body 125 µm in diameter at its widest part. In the mature specimen, we observed only one midventral subneural gland, 190 µm long, in XIV, but said specimen was amputated and ended in this segment.

Geographical distribution: Our specimens were collected from South Georgia Island, where *L. macquariensis* was recorded by Stephenson (1932). However, the species was originally described from specimens that came from Macquarie Island (Benham, 1905) and since then has also been reported from the Campbell and Auckland Islands (Benham, 1922), Heard and McDonald Islands (Lee, 1968) and Bishop Island (Davies *et al.*, 1997).

Remarks: Our specimens are similar to those identified as *L. macquariensis* by Stephenson (1932) from South Georgia Island. In the original description from Macquarie Island (which lies south of Australia and New Zealand), Benham (1905) illustrated a spermatheca with a narrow ental duct connecting the sac-like ampulla with the oesophagus. Benham (1909) described *L. intermedius* (from Auckland Island), which he considered as intermediate between *Lumbricillus maximus* (Michaelsen, 1888) and *L. verrucosus*, and which has a spermatheca with a small pore connecting the ampulla to the oesophagus without any narrow ental duct. However, Benham (1915) revisited his two species (*L. macquariensis* and *L. intermedius*) and concluded that they were, in fact, the same, making *L. intermedius* a junior synonym of *L. macquariensis*. He also concluded that in both samples the spermatheca did not have a narrow ental duct, but a pore connecting it directly to the oesophagus. The spermatheca illustrated by Benham (1909) is similar to that described by Stephenson (1932); both authors showed a thicker epithelium in the ectal part, much like in our specimens. This change in height of the duct epithelium and its expansion to merge with the ampulla are also reminiscent of the spermathecae of the re-examined types of *L. maximus* (Rota, 2001: fig. 1f). Furthermore, Stephenson and Benham noted pharyngeal glands as far back as VII, which is similar to what we observe and which distinguishes *L. macquariensis* from most other *Lumbricillus* species, except *L. maximus* (Rota, 2001). Such posteriad extension of the third pair of glands represents a distinct situation from the development of an extra pair of glands in VII, a character distributed erratically also in other genera otherwise characterized by pharyngeal glands in IV–VI, for example, *Fridericia* Michaelsen, 1889a (Rota, 2001, 2015; Schmelz & Collado, 2010).

Another peculiar trait of our species is the structure of the testis sacs, which seemed at first sight to fall in the regularly lobed arrangement seen in most *Lumbricillus* species. However, the lobed sacs here are much thinner. This could perhaps be an indication that the sacs were not fully developed, but the developing testis sacs of, for example, *L.* sp. 'Marion Is.', from Marion Island (reported above) look completely different. It is possible that both Stephenson and Benham saw this structure but did not consider it deviant and therefore made no remarks upon it.

Our specimens might well belong to the same species as those studied by Stephenson from South Georgia and, like Stephenson, we found a good correspondence with Benham's descriptions from Macquarie Island, particularly in the deep invagination of the body wall where the penial bulb discharges. A circumpolar distribution of the species would not be implausible, because a corresponding pattern has been observed for *Lumbricillus* species in the Northern Hemisphere. The other possibility is that these are two or three separate but closely related species.

Genetically, our '*Lumbricillus*' cf. *macquariensis* is sister to *Grania* in our phylogeny, but although its morphology is dissimilar in many ways to that of most

Lumbricillus (mentioned above), it is by no means closer to that of *Grania*. Species of *Grania* have a much more slender body, only one chaeta per 'bundle' and most often lack chaetae in several segments, and have masses of developing sperm cells and oocytes enveloped in septal distensions (seminal vesicles and ovisacs, respectively) extending backwards. In order to retain the well-defined genera *Grania* and *Lumbricillus s.s.*, '*Lumbricillus*' cf. *macquariensis* needs to be transferred to another, probably new, genus. However, owing to the unresolved taxonomy and limited number of mature specimens we leave this to future studies.

MARIONINA MICHAELSEN, 1890

Type species: Pachydrilus georgianus Michaelsen, 1888.

In addition to the *Lumbricillus* specimens described above, we received six other enchytraeid specimens from the recent southern expeditions. Two of them were small, semi-mature and unpigmented individuals from Snow Island, and four were large, mature worms with dark pigmentation from South Georgia. Although the six specimens differed greatly in appearance, the molecular data strongly supported them as a monophyletic group of three species. The two unpigmented specimens belonged to the same, but yet unidentified, species, whereas the four pigmented specimens (constituting two additional species) were all highly reminiscent of *M. aestuum*, a taxon placed in *Lumbricillus* by Nielsen & Christensen (1959), but here regarded as a member of *Marionina s.s.*

As mentioned in the Introduction, *Marionina s.l.* has grown to become a large genus, with a wide definition allowing for the inclusion of many unrelated taxa. A formal revision of this genus is beyond the scope of our study, but we aim here to provide enough information to allow for such a revision in the near future. The definition of *Marionina s.s.* can only originate from the type species *M. georgiana*, which fortunately has been given two extensive redescriptions (Rota *et al.*, 2008; Schmelz & Collado, 2008). *Marionina georgiana* is marine and has the following characters: epidermis densely glandular; sigmoid chaetae, with upper bundles dorsolateral (closer to lateral line than the ventral bundles); ventral nerve cord with continuous, scattered occurrence of perikarya (and not concentrated in separate ganglia like that of Achaetinae; Rota *et al.*, 2008); brain with posterior incision; three pairs of pharyngeal glands; gradual transition between oesophagus and intestine; oesophageal appendages absent; dorsal vessel arising in XIII and bifurcating anteriorly at front of peristomium (= lumbricilline pattern); nephridia with anteseptale consisting of funnel only and nucleated coelomocytes. Unfortunately, all these somatic characters also fit most species of *Lumbricillus* (Rota *et al.*, 2008; Schmelz & Collado, 2008).

However, the reproductive organs of *M. georgiana* are not very similar to those of *Lumbricillus*. The sperm funnels and vasa deferentia are smaller than in most *Lumbricillus* species, although the general shape is the same. Most *Lumbricillus* species have testes divided into multiple lobes, each surrounded by a peritoneal sheath, and the maturing male cells are thus enclosed (with the exception of the *L. arenarius* group) in a fan-like cluster of testis sacs. The male gonads of *M. georgiana* are not divided into multiple lobes, but our re-examination of the type material has revealed a peritoneal sheath surrounding these organs, except on their distal frayed edge, from which free-floating maturing sperm cysts are released. As the mass of male cells grows and bulges into adjoining segments it becomes enveloped in a seminal vesicle. The spermatheca of *M. georgiana* is simple, more or less a straight tube from the ectal pore to the oesophagus, with which it is connected. The tube is slightly wider at the ampulla, making it somewhat club shaped. The ectal pore is not surrounded by a rosette of glands, commonly found in *Lumbricillus*, but instead associated with two pairs of separate glands (Rota *et al.*, 2008: fig. 6C, D; Schmelz & Collado, 2008: fig. 9).

A most striking feature of *M. georgiana*, and which might be a potential apomorphy of the genus, is the penial body, a rudimental glandular cushion centrally pierced by the vas deferens and lacking a bursa, that is, an epidermal invagination, such that the pore is on the surface. The male opening is accompanied anteriorly and posteriorly by two large pedunculate glands whose stalks join the body wall close to the male pore. These accessory glands [by us referred to as prostate glands, following Stephenson (1932)] were overlooked by Rota *et al.* (2008) but illustrated by Schmelz & Collado (2008). An architecture of the copulatory organ such as that just mentioned has never been reported among terrestrial enchytraeids assigned to *Marionina* before. The 'extra glandular bulb' occurring submedially in front of the paired penial bulbs in *Marionina vesiculata* Nielsen & Christensen, 1959 is not connected to the male pores. Instead, a number of marine enchytraeids from the Southern Hemisphere, some of which were placed in *Lumbricillus* by Nielsen & Christensen (1959), but which we now transfer back into *Marionina s.s.*, have the simple penial body, devoid of bursa and accompanied by prostate glands described for *M. georgiana*: *Marionina werthi* Michaelsen, 1905a from Kerguelen Island ('vasa deferentia open out through a tiny, onion-shaped bulb that is completely hidden in the body wall. On this

bulb, which sometimes protrudes somewhat as a tiny outer papilla, sits a weakly lobed prostate protruding into the body cavity'; Michaelsen, 1905a: 15), *Marionina antipodum* Benham, 1905 from Antipodes Island ['the penial apparatus (Plate XIV, fig. 9) is comparatively small and it scarcely exceeds the thickness of the longitudinal muscles of the body wall. However, opening into it is a conspicuous prostate gland'; Benham 1905: 294], *Marionina benhami* Stephenson, 1932 (= *M. werthi sensu* Benham, 1922) from Macquarie Island ('there is, so far as I can make out from my sections, no "bulb" in the sense in which the term is used in *Lumbricillus*, &c. The sperm duct passes nearly vertically into the body wall, between groups of gland cells constituting the prostate gland; it rims down on the mesial side of one of these groups, to perforate the body wall simply; there is neither glandular investment nor muscular covering. The prostate glands, some in front of, and others behind the sperm pore, rise up inside the body-wall to the level of the intestine and are separated from the body cavity by a sheet of obliquely vertical muscles fibres; a few fibres also pass between the groups of gland cells. The glands open through the body-wall independently of the duct'; Benham, 1922: 13–14), *Marionina grisea* Stephenson, 1932 from the Antarctic Peninsula ('the vas deferens … forms a loose coil, which pierces through a rudimentary penial body to end on a rather indefinite male papilla. […] The penial body … is pierced by the end of the vas deferens and also by the stalks of two large glands, the "prostates"'; Stephenson, 1932: 244–245) and *Marionina aestuum* Stephenson, 1932 from South Georgia ('The vas deferens reaches the surface through a cleft in the muscular mass which represents the penial body, then runs between the stalks of the two "prostates" …, and finally penetrates the junction of the two stalks and the mass of cells which mingles with them where they abut on the surface. The "prostate" glands, in segment xii, are large, and lie, one anterior and the other posterior in position'; Stephenson, 1932: 247–248). We also consider *Marionina colpites* (Stephenson, 1932) from South Georgia to be part of this group ('The penial body is of the enchytraeine rather than of the lumbricilline type. It consists of a number of pear-shaped masses of gland cells, about eight such masses being visible in a single longitudinal section, and the total number on each side being perhaps in the neighbourhood of two dozen. These gland masses are closely compacted together, but separated from each other by, and each individually pear-shaped mass more or less enveloped in, muscular strands; there is no common capsule binding the whole together, and the upper (dorsal) ends of the masses are without covering. The glands are composed of cells derived from the surface epithelium, and discharge on the surface around the small aperture of the vas deferens, which comes to the surface after passing between the glandular masses'; Stephenson, 1932: 262). *Marionina antipodum* and *M. colpites* were already placed in *Marionina* by Nielsen & Christensen (1959), but all the species mentioned except *M. antipodum* were erroneously treated as *Lumbricillus* by Klinth *et al.* (2017b), and all but *M. colpites* were placed in the *L. lineatus* group.

All these species have high numbers of sigmoid chaetae per bundle, prostate glands (one, two or more) associated with the male pore, and spindle- or tube-shaped spermathecae lacking rosettes of glands around the ectal pore (Table 2), and many of them have been discussed as similar to *M. georgiana* (Schmelz & Collado, 2008). If one were to focus on only the condition of the male apparatus, one might ask whether the intertidal marine *Marionina appendiculata* Nielsen & Christensen, 1959 of the Northern Hemisphere is also part of this group ('the penial bulb consists of one or two separate compact structures attached to the body wall immediately in front of and behind the male pore; if only one is present it is the posterior one'; Nielsen & Christensen, 1959: 122, fig. 174; see also Coates & Ellis, 1981: 2137), but in the absence of genetic data we are more inclined to consider it a result of convergent evolution, because this species has a marionine branching of the dorsal vessel and a different morphology of the spermathecae. Prostate-like glands associated with the male pore are also described for the marine genus *Randidrilus* Coates & Erséus, 1985, known from the north-western Atlantic, and for the terrestrial/freshwater Holarctic genus *Mesenchytraeus* Eisen, 1878. However, in both these taxa the male pore opens into an epidermal invagination surrounded by an eversible glandular bulb.

Unlike *M. georgiana*, most of the southern species we consider part of *Marionina s.s.* are pigmented and have testes and masses of developing male cells said to be lobed and somewhat reminiscent of those of *Lumbricillus* (Michaelsen, 1905a; Stephenson, 1932). However, the shape of such structures is hard to compare between species, because the descriptions are often poor and do not distinguish between what is the testis and the free-floating maturing sperm cysts, the testis sacs or the seminal vesicles (Table 2). In our two pigmented species, we found the mass of developing male cells closest to the testes and the testes themselves appearing slightly lobed, somewhat similar to the arrangement typical of *Lumbricillus*. Distally, however, the testes break off into free-floating maturing sperm cysts. This seems to indicate the presence of testis sacs with thin membranes enveloping the early stages of developing male cells but interrupted at the most apical, frayed edge of the gonad; something speculated by Michaelsen (1905a)

Table 2. Morphological characters for species that we consider as part of *Marionina s.s.*

Species/type locality	Pigmented body wall	Length:width (mm)	No. of chaetae	Clitellum	Structure of testes	Male apparatus	Spermathecal ectal glands
Marionina aestuum Stephenson, 1932 S. Georgia Is.	Yes	11–12:0.6	7–17	XII–XIII saddle shaped	Basally lobed in possible testis sacs; ends break up into free-floating cells	Minute penial body; one anterior and one posterior prostate	2
Marionina antipodum Benham, 1905 Antipodes Is.	No	11–15:0.75	4–7	XII–XIII girdle shaped	Small, loose structure, no lobes but slightly frayed edge; free-floating male cells in X–XI	Small penial body; two or three anterior and one or two posterior prostates	2
Marionina benhami Stephenson, 1932 = *Marionina werthi sensu* Benham (1922) Macquarie Is.	Yes	10:1	7–13	No information; assumed to be the same as for *M. werthi*	No information; assumed to be the same as for *M. werthi*	No penial body; group of prostate glands discharging independently of vasa	2
Marionina colpites (Stephenson, 1932) S. Georgia Is.	No	15–16:0.8–1	2–7	XII–XIII saddle shaped	Small basal lobes in testis sacs; free-floating male cells in XI	No penial body; group of prostate glands discharging independently of vasa	3–4
Marionina fusca Klinth, Rota & Erséus S. Georgia Is.	Yes	> 4.3:0.45–0.5	3–12	(1/2XI)XII–XIII saddle shaped	Basally lobed in possible testis sacs; ends break up into free-floating cells	Small penial body; one anterior and one posterior prostate	2
Marionina georgiana (Michaelsen, 1888) S. Georgia Is.	No	4–5:0.18–0.42	2–7	XII–1/2XIII saddle shaped	Basal compact club-shaped mass; free-floating male cells in XI	Small penial body; one anterior and one posterior prostate	3–4*
Marionina grisea Stephenson, 1932 Antarctic Peninsula	Yes	11–17:0.68	4–8	XII–XIII saddle shaped	Triangular basal mass lobed in possible testis sacs; ends break up into free-floating cells	Small penial body; one anterior and one posterior prostate	2–3
Marionina werthi Michaelsen, 1905 Kerguelen Is.	Yes	8:0.65	2–10	XII–XIII saddle shaped	Basal bunch of thin, thread-like lobes; ends break up into free-floating cells	Small penial body; one prostate	0

*One or two pedunculate glands and two or more sessile spherical glands anterior to the ectal pore (Rota *et al.*, 2008: fig. 6C, D; Schmelz & Collado, 2008: fig. 9). New species in bold.

and Stephenson (1932). Although we did not observe any basal separation into lobes in *M. georgiana*, a much smaller species than our pigmented ones, we did observe the same membrane enveloping the developing cells but distally breaking up.

Further morphological characters of potential importance, concerning *Marionina s.s.*, are to be found in the epidermal gland cells and the nerve system. Rota *et al.* (2008) and Schmelz & Collado (2008) illustrated the epidermis of *M. georgiana* as intensely glandular, that is, covered with densely packed rows of pale cells. We found similar densely glandular epidermis in both the newly collected specimens and the borrowed type material of the species we consider to be *Marionina s.s*. However, the epidermis of the southern *Lumbricillus* species included in this study was also densely glandular; hence, this character might be more related to habitat than to genus (see Rota, 2001). Schmelz & Collado (2008) reported clusters of perikarya ('prostomial ganglia') on the prostomial nerves of *M. georgiana* (see their fig. 1) similar to those discovered by Rota *et al.* (1999: fig. 3b, f) in *Achaeta* and *Henlea*. We observed similar clusters on the prostomial nerves of *M. aestuum* and *M.* sp. 'Snow Is.' (Fig. 9A). We also found similar aggregations at the ventral ends of the circumoesophageal connectives, close to where they merge with the ventral nerve cord, in all of *M. aestuum*, *M.* sp. 'Snow Is.' and *M. georgiana*. Furthermore, we found a globular mass of deeply staining cells apparently connected with the anterior end of the ventral nerve cord, and also anchored to (discharging in?) the ventral groove of the body wall between I and II (Fig. 9A–D). This unprecedented 'subbuccal bulb' (seemingly glandular, perhaps similar to the subneural glands found in the proximity of the clitellum in many enchytraeids) was observed by us in *M. aestuum* (Fig. 9B, C), *M. colpites*, *M. fusca*, *M. grisea*, *M.* sp. 'Snow Is.' (Fig. 9A) and *M. georgiana* (Fig. 9D). It was not observed in all specimens and was particularly hard to find in *M. georgiana*, only clearly visible in two of 15 studied specimens, both of which were unstained. It is possible that this organ varies in size with development, in a similar manner to the subneural glands. We did not observe a subbuccal bulb in any of the studied species of *Lumbricillus* or in *Ch. blocki*.

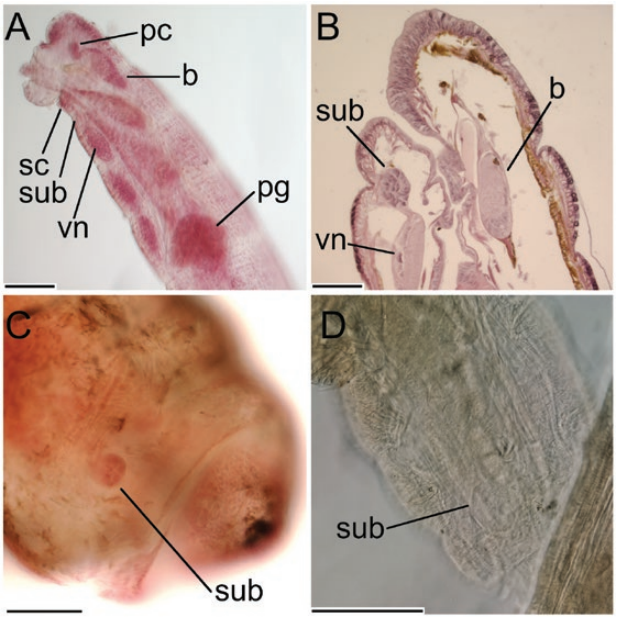

Figure 9. A, *Marionina* sp. 'Snow Is.' (CE34647), anterior body (lateral view). B, *Marionina aestuum*, sectioned specimen BMNH 1933.2.23.895, cephalic region (lateral view). C, *M. aestuum* (CE12477), cephalic region (ventral view). D, *Marionina georgiana* (paralectotype 13), cephalic region (ventral view). Abbreviations are defined under 'Taxonomy'. Scale bars: 100 μm.

MARIONINA AESTUUM STEPHENSON, 1932

(FIGS 9B, C, 10, 11)

Marionina aestuum Stephenson, 1932: 246–251.

Lumbricillus aestuum – Nielsen & Christensen, 1959: 96.

Type material: BMNH 1931:6:23:42–43 (in alcohol, not studied), BMNH 1933.2.23.893–896, four mature sectioned specimens (studied). Syntypes. Loc. Shore of Bay of Isles, South Georgia. Leg. 'Discovery' 1925–1927 (Stephenson, 1932) (Boros & Sherlock, 2010).

Type locality: Shore of Bay of Isles, South Georgia Island.

New material examined: SMNH198154 (CE12477) one mature specimen collected in 2010 from South Georgia. For information on collection localities and GenBank accession numbers for *COI* barcodes, see Table 1 and the Supporting Information (Table S1).

Description of new, mounted, material with comparative notes to Stephenson's sectioned material: Dark grey worm (at least when preserved), with subepithelial black pigmentation, densest dorsally, decreasing in intensity ventrally. Length of first 20 segments 2.1 mm (fixed, amputated specimen); first 15 segments 1.9 mm long; width at clitellum 0.65 mm. Chaetae sigmoid (Fig. 10A). Upper bundles dorsolateral (closer to lateral line than the ventral bundles), with five to eight (possibly more) chaetae anterior to clitellum; numbers not discernable in postclitellar segments. Ventral bundles with 11–15 chaetae anterior to clitellum, nine to 11 chaetae posteriorly, at least to XX. The longest

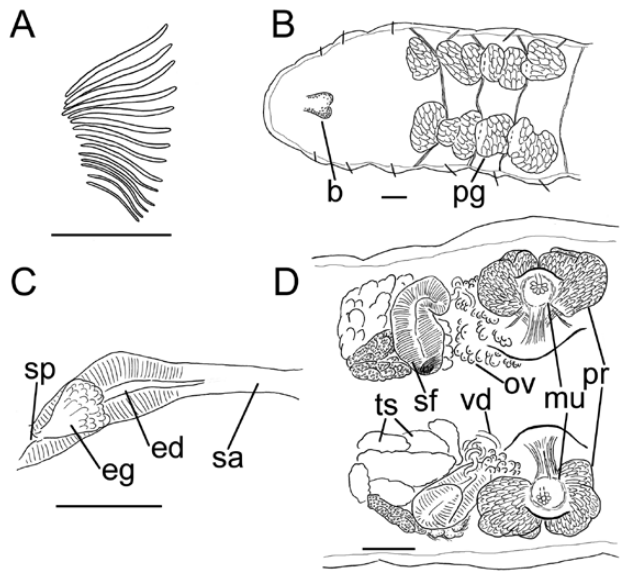

Figure 10. *Marionina aestuum* (CE12477, ventral view). A, chaetal bundle. B, anterior part of body. C, spermatheca; note that there is another hidden ectal gland behind the spermatheca. D, genitalia. Abbreviations are defined under 'Taxonomy'. Scale bars: 100 µm.

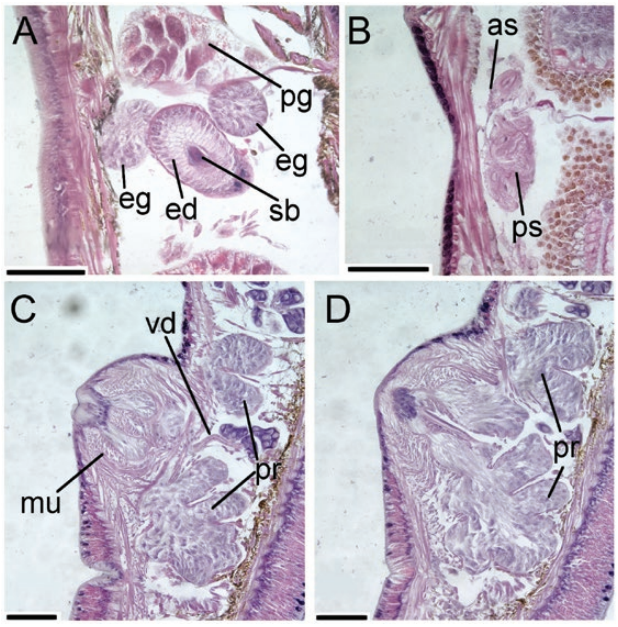

Figure 11. *Marionina aestuum*. A, B, BMNH 1933.2.23.896. A, spermatheca. B, nephridium. C, D, BMNH 1933.2.23.895, penial body and associated prostate glands (same specimen). Abbreviations are defined under 'Taxonomy'. Scale bars: 100 µm.

measured chaetae 95 µm long, ~5 µm wide. Epidermis densely covered with rows of pale gland cells; sectioned material also showed dense, deeply staining granular gland cells. Clitellum with reticulate pattern of gland cells, extending over XII–XIII, absent ventrally. Head pore not observed.

Coelomocytes numerous, ~15 µm long; round, oval or spindle shaped; granulated, with distinct nucleus. Paired pharyngeal glands (Fig. 10B) present in IV, V and VI, with third pair extending back into VII; each pair dorsally separate. Origin of dorsal vessel not discernable, with peristomial bifurcation. Nephridia ~95 µm long (longer in sectioned material: Fig. 11D), observed in 7/8–9/10. Anteseptale narrower than, and about half as long as, postseptale, consisting of distinct funnel and part of the nephridial body, seen in sectioned material (Fig. 11B). Postseptale oval, tapering into posteroventral efferent duct. Brain with posterior incision, with clusters of perikarya on prostomial nerves and at ventral ends of circumoesophageal connectives. Ventral nerve cord frontally bearing a large subbuccal bulb (Fig. 9B, C); see *Marionina* discussion above.

Male genitalia paired (Fig. 10D). Testes originating in anterior of XI, with maturing male cells enveloped in testis sacs, cleft lengthwise into irregular lobes, distally breaking up into free-floating cysts. Sperm funnels in XI, 240 µm long, 110 µm wide, that is, about two times longer than wide; funnels tapering towards vasa deferentia. Most of vasa irregularly coiled in XII, 15 µm wide. Penial bodies indistinct; each male pore surrounded by a small ring of gland cells embedded in a muscular framework, bearing two prostate glands, one anterior and one posterior, 125 and 160 µm long, respectively; each prostate with thread-like ventral connections to the male pore. The sectioned material (Fig. 11C, D) shows these structures in more detail, with a few gland cells surrounding the pore through which the vasa discharge; the gland cells connected via two stalks to two prostate glands that widen gradually and end in several dorsal lobes, and the muscular framework that surrounds all but the dorsal part of the prostates. The muscles cause the body wall to protrude around the male pore. Ovaries in XII. No mature eggs observed.

Spermathecae (Figs 10C, 11A) in V, spindle or tube shaped; in our new material 315 µm long, 65 µm wide at the ectal duct and 30 µm wide at widest part of ampulla, and in the sectioned material 85 µm wide at the ectal duct and 60 µm wide at widest part of ampulla. Ectal duct difficult to distinguish from ampulla, but according to Stephenson (1932) the ectal duct is the wider part with tall epithelial cells, whereas the ampulla has a thinner epithelium, and this is supported in his sectioned material. In our mounted specimen (Fig. 10C), the (thick-walled) duct is about as long as the (thin, apparently still developing) ampulla; in the sectioned material, the ampulla is 1.5–2.0 times longer than the duct. Ampulla connected to oesophagus. No sperm observed in the new material. Two separate, pedunculate glands flanking (one dorsal and the other

ventral) each spermathecal pore (both glands shown in Fig. 11A); these glands ≤ 55 µm in diameter in our worm and ≤ 100 µm in the sectioned material. No midventral subneural glands were observed in the new material.

Geographical distribution and habitat: Morphologically identified only from South Georgia. The etymology of the epithet *aestuum* (Latin, 'of the tides') refers to the position of the type locality 'between tide marks', which coincides with the habitat of our specimen. The BMNH collection has specimens collected by E. M. van Zinderen Bakker at five localities on Marion Island in 1976, determined as *L. aestuum* by E. G. Easton (Boros & Sherlock, 2010), but we have not been able to validate this find.

Remarks: Our single specimen is similar to Stephenson's (1932) in most characters, but we did not observed the two small subneural glands midventral in segments XIII and XIV, sperm filling the spermathecae or developing male cells bulging forward into X reported by Stephenson, which indicates that our specimen was not fully mature. Sections in Stephenson's slides clearly show sperm filling the spermathecal lumen, mostly in the ectal, thick-walled portion, that is, the portion referred to as the ectal duct by Stephenson (1932).

Marionina aestuum can be distinguished from other subantarctic pigmented *Marionina* in the following characters: *M. werthi* has only a single prostate gland and spermathecae without glands; *M. aestuum* has two prostate glands and spermathecae with glands. *Marionina benhami* has minute penial bodies, similar to those of *M. aestuum*, but instead of two large prostates, *M. benhami* has a number of associated glandular masses (prostates), some in front and some behind the male pores. *Marionina aestuum* is more similar to *M. grisea* (sectioned material of which we studied too; Fig. 12; for a full description, see Stephenson, 1932), but the former: (1) has more chaetae per bundle (*M. grisea* has up to eight in ventral bundles, five in laterals); (2) spermathecae of about even width (*M. grisea* has a shorter spermathecal duct that is twice as thick as the ampullae; in the fully matured sectioned material of *M. aestuum* it is 1.5 times thicker); and (3) lacks the thickened ventral epithelial plate in X (Fig. 12B; described for mature specimens by Stephenson, 1932). Nevertheless, our species is still morphologically closest to the new species *M. fusca*, which will be discussed below.

MARIONINA COLPITES (STEPHENSON, 1932)

(FIG. 4D–F)

Enchytraeus colpites Stephenson, 1932: 260–263, figs 12–13.

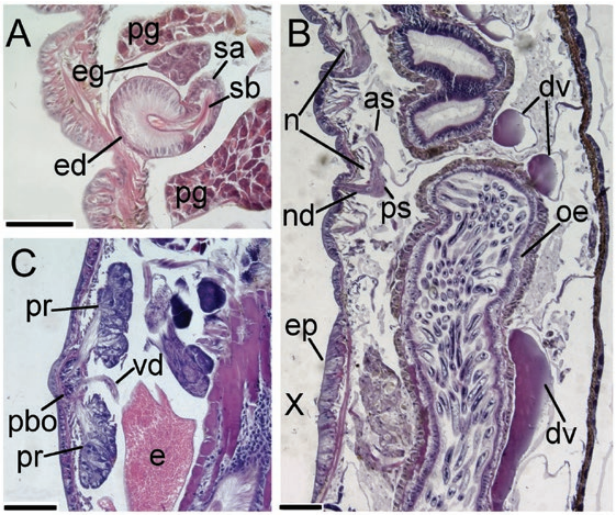

Figure 12. *Marionina grisea*. A, BMNH 1933.2.23.902, spermatheca. B, BMNH 1933.2.23.901, segments VIII–X. C, BMNH 1933.2.23.902, male apparatus. Abbreviations are defined under 'Taxonomy'. Scale bars: 100 µm.

Lumbricillus colpites – Nielsen & Christensen, 1959: 96.

Type material: BMNH 1933.2.23.885–892, eight mature sectioned specimens (studied). Syntypes. Loc. Shore of Bay of Isles, South Georgia Leg. 'Discovery' 1925–1927 (Stephenson, 1932).

Type locality: Shore of Bay of Isles, South Georgia Island.

Additions to Stephenson's original description: Chaetae ~110 µm long. Epidermis covered with rows of pale gland cells, and with a few rows of deeply staining granular gland cells, latter rows aggregated anterior and posterior to chaetae (see *M. fusca* below). Dorsal vessel with peristomial bifurcation. Nephridia in 7/8–10/11 and postclitellar segments. Ventral nerve cord with large subbuccal bulb; see *Marionina* discussion above. Sperm funnels ≥ 900 µm long, 230 µm wide. Spermathecae (Fig. 4E, F) ≥ 600 µm long, 60 µm wide at duct, 180 µm wide at ampulla, with glands 55–100 µm wide surrounding pore.

Remarks: Stephenson was uncertain whether to place this species in *Lumbricillus* or *Enchytraeus* Henle, 1837 (and apparently did not consider *Marionina*), and decided pro *Enchytraeus* because of the lack of lobed testis sacs and absence of defined penial bulb. The testes are indeed small but do appear partly lobed; we could not make out whether the mass of developing cells was fully enveloped in testis sacs or distally free, but the mass is

enveloped in a seminal vesicle as it bulges into anterior segments. The male apparatus, with its separate glands that discharge independently around the male pore, is similar to that described for *M. benhami*.

MARIONINA FUSCA KLINTH, ROTA & ERSÉUS SP. NOV.

(FIG. 13)

Zoobank registration: urn:lsid:zoobank.org:act:720EDF21-78D8-4169-BE8F-EC4860A9B909

Holotype: SMNH Type Coll. 9311 (CE12475), a mature amputated specimen stained in Paracarmine and mounted on a slide. Leg. Paul Brewin & Alison Massey (Shallow Marine Survey Group), 7 December 2010. No *COI* barcode, accession numbers for other genetic data in Tables 1 and S1. Holotype illustrated in Fig. 13D and 13F.

Type locality: South Georgia, Corral Bay, Cumberland East Bay, intertidal zone, 54.3023 S, 36.3768 W.

Paratypes: SMNH Type Coll. 9312 (CE12476) and SMNH Type Coll. 9313 (CE12478), one mature and one semi-mature specimen from type locality, amputated and mounted on slides. *COI* barcode (CE12476), GenBank MZ393959; accession numbers for additional genetic data are given in Table 1 and the Supporting Information (Table S1).

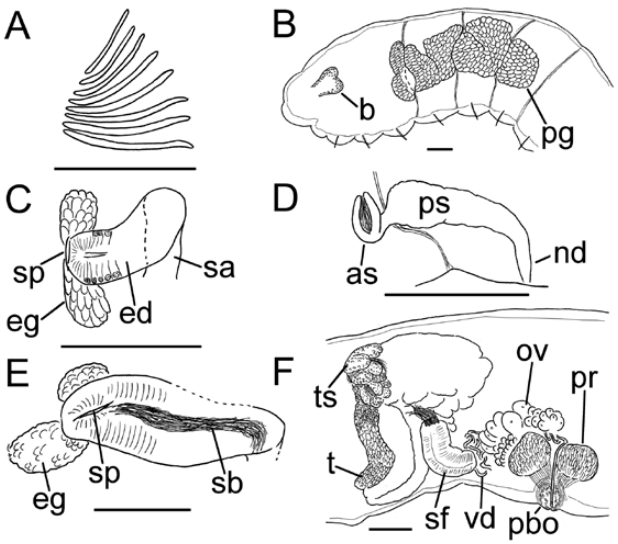

Figure 13. ***Marionina fusca***. A, chaetal bundle (CE12478). B, anterior part of body (CE12478). C, spermatheca (CE12478). D, nephridia (holotype). E, spermatheca (CE12476). F, genitalia (holotype). Abbreviations are defined under 'Taxonomy'. Scale bars: 100 µm.

Etymology: From the Latin adjective *fuscus*, dark or swarthy.

Diagnosis: This species can be separated from other darkly pigmented, southern marine enchytraeid species in the following characters: (1) the presence of penial bodies, connected to one anterior and one posterior prostate gland; and (2) the absence of a thickened ventral epithelial plate in X.

Description: Dark grey worms (at least when preserved), with subepithelial black pigmentation, densest dorsally, decreasing in intensity ventrally. Length of first 19–27 segments 2.9–4.3 mm (fixed, amputated specimens); first 15 segments 2.1–3.0 mm long; width at clitellum 0.45–0.50 mm. Chaetae sigmoid (Fig. 13A). Upper bundles dorsolateral (closer to lateral line than the ventral bundles), with four to eight chaetae anterior to clitellum, three to five in postclitellar segments, at least to XXVII. Ventral bundles with six to 12 chaetae anterior to clitellum, and six to ten chaetae posteriorly, at least to XXVII. Longest measured chaetae of each worm 95–105 µm long, ~5 µm wide. Epidermis covered with rows of pale gland cells, and with a few rows of deeply staining granular gland cells, with the latter rows aggregated anterior and posterior of chaetae. Clitellum with reticulate pattern of gland cells, extending over (1/2XI) XII–XIII, absent ventrally. Head pore at 0/I.

Coelomocytes numerous, 10–15 µm long; round, oval or spindle shaped; granulated, with distinct nucleus. Paired pharyngeal glands (Fig. 13B) present in IV, V and VI, with third pair extending back into VII; dorsal connection indiscernible, dorsal and ventral lobes smallest in IV, ventral lobes absent in VII. Dorsal vessel originating in XII–XIII, with peristomial bifurcation. Nephridia (Fig. 13D) ~120 µm long, observed in 7/8–9/10. Anteseptale consisting of a distinct funnel and a small part of nephridial body. Postseptale oval, tapering into posteroventral efferent duct. Brain with posterior incision. Ventral nerve cord with small subbuccal bulb; see *Marionina* discussion above.

Male genitalia paired (Fig. 13E). Testes originating in anterior of XI, with maturing male cells enveloped in testis sacs, cleft lengthwise into irregular lobes, distally breaking up into a mass of free-floating cysts. Sperm funnels in XI, ≥ 170–270 µm long, 65–130 µm wide, that is, about two to three times longer than wide; funnels tapering towards vasa deferentia. Most of vasa irregularly coiled in XII, 15 µm wide. The male pores are surrounded by distinct, small, spherical, penial bodies (35) 60–95 µm in diameter, with cells

mostly embedded in the epidermis, encapsulated by musculature. Each penial body bearing two prostate glands, one anterior and one posterior, 95–160 and 95–100 µm long, respectively; each prostate with thread-like ventral connections to the penial bodies. Ovaries in XII. One mature egg observed.

Spermathecae (Fig. 13C, E) in V, spindle or tube shaped; ectal duct difficult to distinguish from ampulla, and exact shape of ental part of ampulla difficult to discern owing to heavy pigmentation. Bundled sperm observed in lumen of ectal duct and entally towards ampulla. Spermathecae 120–180 µm long, 40–65 µm wide at the ectal duct and 30 µm wide at widest part of ampulla. Two separate glands at ectal end of each spermatheca, one dorsal and one ventral to spermathecal pore; glandular bodies 45–60 µm in diameter at widest part. One midventral subneural gland, 105 µm long, observed in XV of one specimen.

Geographical distribution: Known only from South Georgia.

Remarks: We found two darkly pigmented species from South Georgia, both reminiscent of *M. aestuum*. Although genetically distinct, these two species are morphologically similar. Both have two pairs of large prostate glands (one pair anterior and one posterior to the male pores), sperm funnels between two and three times longer than wide, and spermathecae shaped as long tubes, slightly wider in the ectal duct than the ental (ampullar) part, and with two glands at each of the pores. In the specimen attributed to *M. aestuum* (see above), we did not observe any midventral subneural glands, whereas *M. fusca* has one such gland in XV, neither condition of which matches the original description of *M. aestuum* that reported two glands, one in XIII and one in XIV. The development of said glands might vary with sexual maturity, and slight variation does occur in some enchytraeid species (Schmelz, 2003). There are two principal differences between our two darkly pigmented species. First, *M. aestuum* lacks distinct penial bodies, whereas *M. fusca* has small but distinct penial bodies (more like those of *M. grisea*; Fig. 12C). Second, the average number of chaetae per bundle is larger in *M. aestuum*, with as many as 15 in a bundle, compared with *M. fusca*, with a maximum of 12 chaetae in a bundle and usually no more than ten (*M. aestuum* was originally described as having as many as 17 chaetae per bundle).

Marionina georgiana (Michaelsen, 1888)

(Fig. 9D)

Pachydrilus georgianus Michaelsen, 1888: 65–66, pl. II, fig. 7a, b.

Marionia georgiana – Michaelsen, 1889b: 29.

Marionina georgiana – Michaelsen in Pfeffer, 1890: 511; Beddard, 1895: 332; Michaelsen, 1900: 76; Nielsen & Christensen, 1959: 109; Brinkhurst & Jamieson, 1971: 662; Rota *et al.*, 2008: 425–433, figs 2–6; Schmelz & Collado, 2008: 12–18, figs 1–13.

Pachydrilus (*Marionina*) *georgiana* – Černosvitov, 1937: 293.

Christensenia georgiana – Dózsa-Farkas & Convey, 1997: 483–484, fig. 9; *partim*.

Christensenidrilus georgiana [sic] – Dózsa-Farkas & Convey, 1998: 292; *partim*.

Non *Marionina georgiana* – Michaelsen, 1905a: 15–17, pl. I, fig. 2; Michaelsen, 1905b: 5; Stephenson, 1932: 241.

Type material studied: Lectotype CeNak V428 and paralectotypes CeNak V428 2, 5–17, in alcohol. Loc. South Georgia Island.

Type locality: South Georgia Island, near the German station 1882–1883, 54.533 S, 36.017 W, at low water mark, from slate debris, algal weeds and sponge canals, Febuary 1883.

Additions to Michaelsen's original description and recent redescriptions of types: Paired clusters of perikarya on prostomial nerves and at ventral ends of circumoesophageal connectives. Ventral nerve cord with small subbuccal bulb (Fig. 9D); see *Marionina* discussion above. Developing male cells enveloped in testis sac, distally frayed, releasing free-floating cysts, parts of which extend into X and XII in seminal vesicle.

Remarks: This species was thoroughly redescribed by Rota *et al.* (2008) and Schmelz & Collado (2008), but neither described the subbuccal bulb (which we could not make out in most specimens) and neither considered the male cells enveloped in a testis sac but both mention the large seminal vesicle.

Marionina grisea Stephenson, 1932

(Fig. 12)

Marionina grisea Stephenson, 1932: 243–246, figs 4, 5.

Lumbricillus griseus – Nielsen & Christensen, 1959: 96.

Type material: BMNH 1933.2.23.901–903, three sectioned specimens (studied). Syntypes. Loc. Wiencke Island, Palmer Archipelago. 'Discovery' 1925–1927 (Stephenson, 1932).

Type locality: Shore of Port Lockroy, Wiencke Island, Palmer Archipelago.

Additions to Stephenson's original description: Chaetae ~75 µm long. Epidermis covered with rows of pale gland cells and deeply staining granular gland cells. Dorsal vessel with peristomial bifurcation. Nephridia (Fig. 12B) in 7/8–10/11 and postclitellar segments. Ventral nerve cord with small anterior subbuccal bulb; see *Marionina* discussion above. Sperm funnels ≥ 450 µm long, 160 µm wide. Spermathecae (Fig. 12A) ≥ 300 µm long, 110 µm wide at duct and 70 µm wide at ampulla, with glands ~75 µm wide surrounding pore.

Remarks: This species is discussed together with *M. aestuum* and *M. fusca*.

MARIONINA SP. 'SNOW IS.'

(FIGS 9A, 14)

Material examined: SMNH198155 (CE34647) and SMNH198156 (CE34648), two semi-mature specimens from Snow Island (South Shetland Islands), Antarctica. For information on collection localities and GenBank accession numbers for *COI* barcodes, see Table 1 and the Supporting Information (Table S1).

Description: Live colour unknown; and specimens apparently without pigmentation. Length of first 16–19 segments 2.4–2.9 mm (fixed, amputated specimens); first 15 segments 2.2–2.3 mm long; width at XII 0.27 mm. Chaetae slightly sigmoid (Fig. 14A). Upper bundles dorsolateral (closer to lateral line than ventral bundles), with three to four chaetae anterior to clitellum, (three) four in postclitellar segments, at least to XIX. Ventral bundles with four chaetae throughout to XIX. The longest measured chaetae of each worm 55 µm long, ~3 µm wide. Epidermis densely covered with rows of pale gland cells. Clitellum not developed. Head pore at 0/I.

Coelomocytes numerous, 10–15 µm long; round, oval or spindle shaped; granulated, with distinct nucleus. Paired pharyngeal glands (Fig. 14B) present in IV, V and VI; only third pair with ventral lobes and dorsally converging but connection not discernible. Origin of dorsal vessel indiscernible, but with peristomial bifurcation. Nephridia observed in 7/8–8/9, anteseptale with funnel only, postseptale elongated oval with terminal efferent duct. Brain with posterior incision, with clusters of perikarya on prostomial nerves and at ventral ends of circumoesophageal connectives (Fig. 9A). Ventral nerve cord with small subbuccal bulb (Fig. 9A); see *Marionina* discussion above.

Male genitalia not fully developed. Developing male cells free floating in XI. Developing sperm funnels in XI, 60–65 µm long, 40 µm wide. Thin line of cells extending backwards from sperm funnels to what will become the male opening. Developing spermathecae in V, shape uncertain. No midventral subneural glands observed.

Geographical distribution: Known only from Snow Island (South Shetland Islands).

Remarks: The two small specimens of this species were first believed to be juvenile *Lumbricillus*, but they were placed genetically within a separate clade together with the two larger, darkly pigmented species, *M. aestuum* and *M. fusca*. Upon closer examination, despite their submature condition, we found our specimens to have free-floating developing male cells, more akin to *Marionina s.s.* than to *Lumbricillus*. Our *Marionina* sp. 'Snow Is.' is close to *M. georgiana* in lacking pigmentation, having a densely glandular epidermis, about four chaetae per bundle, similar pharyngeal glands, presence of perikarya on the prostomial nerves and at ventral ends of circumoesophageal connectives, and in the presence of the subbuccal bulb. However, fully mature specimens from Snow Island and/or DNA from *M. georgiana* will be needed to establish whether the two are the same or separate species.

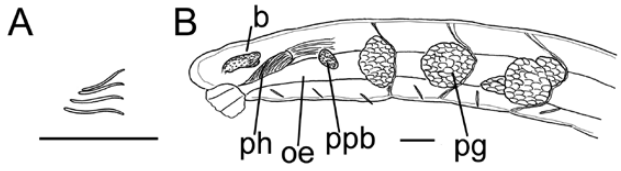

Figure 14. *Marionina* sp. 'Snow Is.' (CE34647). A, chaetal bundle. B, anterior part of body. Abbreviations are defined under 'Taxonomy'. Scale bars: 100 µm.

CHRISTENSENIDRILUS BLOCKI DÓZSA-FARKAS & CONVEY, 1998

Christensenia blocki Dózsa-Farkas & Convey, 1997: 483–486, figs 1–8.

Christensenidrilus blocki – Dózsa-Farkas & Convey, 1998: 292.

Material examined: SMNH198141–198143 (CE35374–35376), three mature specimens from the type locality on Signy Island (South Orkney Islands). For information on collection localities and GenBank accession numbers for *COI* barcodes, see Table 1 and the Supporting Information (Table S1).

Remarks: Our specimens perfectly resemble those originally described by Dózsa-Farkas & Convey;

therefore, we decided not to give complete descriptions and illustrations, but instead a few additional comments about the morphology. Schmelz & Collado (2008), in their comparison between *Ch.blocki* and *M. georgiana*, were left with a few questions that we will try to answer. First, we did not observe neuron perikarya in the prostomium of *Ch. blocki*, similar to those described by Schmelz & Collado (2008) in *M. georgiana* and observed by us also in *M. aestuum* and *M.* sp. 'Snow Is.'. Second, the dorsal blood vessel bifurcates in both species under the brain in a lumbricilline (peristomial) pattern. Third, the penial bulbs of *Ch. blocki*, if they can be considered as such, which were described originally as compact and medium sized, were hard to distinguish, composed of a small aggregation of cells (< 50 µm in diameter) surrounding the superficial (non-invaginate) male pores, at least apparently similar to the penial bodies of *M. georgiana*, but lacking any prostate glands. The developing male gametes, although numerous and completely occupying segments X–XII, do not show any sign of being contained in lobed testis sacs. Based on these new elements and the presence of anucleate coelomic corpuscles in place of nucleated coelomocytes in *Ch. blocki*, we cannot but agree with Rota *et al.* (2008) and Schmelz & Collado (2008), in that *Ch. blocki* does not share enough morphological characters with *M. georgiana* to assume it to be closely related to *Marionina s.s.*, which has also been supported by our molecular data. Unfortunately, we found no support for any specific placement of *Christensenidrilus* within the family Enchytraeidae as a whole, probably because of the lack of suitable outgroups with which to make comparison.

DISCUSSION

The monophyly of *Lumbricillus* has been questioned before, because some molecular analyses have found a few species to be closer to *Grania* than to the type species, *L. lineatus* (Erséus *et al.*, 2010; Martinsson *et al.*, 2017; see also the species tree based on concatenated data in the study by Klinth *et al.*, 2017a). *Lumbricillus* was found as monophyletic in a multispecies coalescent species tree by Klinth *et al.* (2017a), who analysed the dataset on which we based the tree in the present study. The fact that our new species tree (Fig. 2) instead finds *Lumbricillus* non-monophyletic might be explained by the inclusion of molecular data from *Marionina s.s.* and by linking the tree models for, respectively, the mitochondrial and nuclear ribosomal markers. In order to retain *Lumbricillus* and *Grania* as two well-defined genera, a taxonomic revision is clearly needed. The species in the *L. arenarius* group (see Klinth *et al.*, 2017b) need to be transferred to a separate genus. Klinth *et al.* (2017b) already discussed whether to establish a separate genus for these species or whether they could be placed within *Enchytraeoides*. The type species of *Enchytraeoides*, *Pachydrilus enchytraeoides* Saint-Loup, 1885 [or possibly *Enchytraeoides marioni* Roule, 1888; see Rota *et al.* (2008) for the complicated taxonomic and nomenclatural history of these two nominal species], shares some morphological characters with the *L. arenarius* group, such as irregularly lobed testis sacs and sac-shaped spermathecae, but the relationship between these taxa remains unknown.

In this study, we are confident that we have obtained representatives of *Marionina s.s.* and we have been able to place them phylogenetically using molecular data. Schmelz & Collado (2008) had contemplated the possibility to extend *Marionina s.s.* to part of this assemblage, but expressed many perplexities regarding the taxonomic delimitation of such a group, for example, how it should relate to *Ch. blocki*, to *Randidrilus* and to members of *Lumbricillus* and *Marionina* with apparently similar features. We started from a more advantageous position, because we benefitted from the articulated vision of *Lumbricillus* obtained by us on a molecular and morphological basis (Klinth *et al.*, 2017a, b), which has been a fundamental support to obtain the present results. Nevertheless, the recognition of the true *Marionina* will be proved indisputably only when the type species, *M. georgiana*, has been sampled and sequenced.

Our well-supported clade of three species, two of which are from South Georgia where *M. georgiana* was originally found, all share morphological characters with the type species (see Taxonomy for *Marionina* above), particularly in the lack of regularly lobed testis sacs and the simple penial cushion and associated prostate glands (although the male apparatus was observed only in the two species with mature specimens). Neither the *BEAST species tree nor the concatenated tree supported close relationship between *Marionina s.s.* and the included species traditionally placed in *Marionina*, that is, the species-rich groups here represented by *Marionina argentea* (Michaelsen, 1889a), *Marionina spicula* (Leuckart, 1847) and *Marionina filiformis* Nielsen & Christensen, 1959 (where *M. filiformis* is likely to be a member of *Enchytronia*), in addition to the lone species *M. communis*, which appears to represent a lineage of its own that might deserve the status of a separate genus. Thus, our molecular findings confirm *Marionina s.l.* as an artificial assemblage of species, as previously suggested based on morphology (Xie & Rota, 2001; Rota *et al.*, 2008).

The close phylogenetic relationship between *Marionina s.s.* and *Lumbricillus* suggested by the present study is an old notion (see Rota *et al.*, 2008).

Černosvitov (1937) revised the taxonomy of *Marionina* and regarded it as a subgenus to *Pachydrilus* (*Lumbricillus*), and Erséus *et al.* (2010) predicted that *M. georgiana* would probably be found close to *Lumbricillus* and *Grania* in the phylogeny of Enchytraeidae. Genetically, *Marionina* is closer to the *L. arenarius* group, which shares some morphological traits of the developing testes (see below). The phylogenetic placement of *Marionina s.s.* provides a much-needed starting point for the revision of the genus (which is beyond the scope of this paper), whereby most of the species currently placed under this genus name will probably have to be excluded.

The species herein identified as '*Lumbricillus*' cf. *macquariensis* does not belong genetically in *Lumbricillus*, nor in the *L. arenarius* group or *Marionina s.s*. Its sister-group relationship with *Grania* is well supported, but its morphology is too divergent for it to be included (conventionally) in that genus; *Grania* is a group of marine interstitial worms, somewhat nematode like, with a reduced number of usually large and straight chaetae. We have insufficient material sampled to be able to identify this *L. macquariensis* look-alike confidently, and it is possible that increased future samplings from the Southern Hemisphere will reveal additional species related to this taxon, which might represent a lineage that will deserve the status of yet another new genus.

The new phylogeny allows us to draw some conclusions about the evolution of morphological characters within the superclade containing *Lumbricillus*, *Grania*, the *L. arenarius* group and *Marionina s.s.* (and possibly *Randidrilus*). In *Lumbricillus*, the *L. arenarius* group and *Marionina s.s.*, the testes and the maturing sperm cells are covered by a thin membrane, creating so-called testis sacs. This is also present in *Enchytraeus*, *Stephensoniella* Černosvitov, 1934 and *Claparedrilus* (none of which is closely related to these groups), but absent in *Grania* and probably *Randidrilus*. In the majority of *Lumbricillus* species groups, each testis is divided down to the base into a regular bunch of lobes. The small species in the *L. buelowi* group seem to have only a single lobe, and this group has been shown to be the sister to the remaining *Lumbricillus* (Fig. 2). In the *L. arenarius* group, the testes are also lobed, at least distally, but the lobes themselves do not seem to be arranged in any regular pattern, and in some species the lobes can break off, releasing free-floating fragments. The species assigned by us to *Marionina s.s.* have testes which (similar to the *L. arenarius* group) tend to cleave irregularly lengthwise, appearing lobed at least apically, but sometimes being cleft down to the base (*M. aestuum*). The surrounding peritoneal membrane (testis sac) tends to be frayed distally and to release free maturing sperm cysts. When the latter overcrowd segment XI, their mass starts to bulge into adjacent (mostly anterior) segments, enveloped by a dorsal diverticulum of septa, named the 'seminal vesicle', which is also seen in other enchytraeids that lack testis sacs. *Grania* lacks testis sacs but has a remarkable posterior extension of the seminal vesicle, with maturing sperm within, enclosed in the egg sac. A similar posterior extension can be found in the genus *Randidrilus*, which is similar morphologically and is probably related closely to *Grania*. *Grania* is in many ways morphologically distinct from the other groups in this superclade, which is probably a result of its highly adapted interstitial life among grains of sand. Lastly, the species herein provisionally referred to as '*Lumbricillus*' cf. *macquariensis*, and supported as the sister of *Grania* in the phylogeny, has testes, just like the ones in *Lumbricillus*, arranged as fan-shaped lobes. However, the lobes themselves seem to be much thinner, almost finger-like, and their way of developing (i.e. the free or lobe-enclosed maturation of sperm) remains to be studied by histological sections or additional material.

The phylogeny (Fig. 2) also provides some insights into the phylogeography of *Lumbricillus*. Out of the specimens studied, those belonging to the true *Lumbricillus*, despite the large geographical distance between them (South Africa and the Antarctic Peninsula), all fall in the same morphogroup and in a clade sister to the species of the *L. lineatus* group from the Northern Hemisphere (see Klinth *et al.*, 2017b). The fact that we have not found any of the other morphogroups (Klinth *et al.*, 2017b) could be attributed to the small sample size. This clade in the species tree, however, was not fully supported; hence, they might instead make up a paraphyletic part within the *L. lineatus* group, which would be interesting because it could indicate a southern origin for this group, but once again the sample size is far too small to draw such conclusions. Furthermore, the species we identified as belonging to *Marionina s.s.* are all, like the type species *M. georgiana*, from the Subantarctic or Antarctic and are not genetically close to any other species that have been labelled as '*Marionina*' from the Northern Hemisphere (Erséus *et al.*, 2010; Matamoros *et al.*, 2012; C. Erséus, unpublished data). This sparks the question whether *Marionina s.s.* is, in fact, distributed only in the Southern Hemisphere, possibly even in the very southern part of it.

Conclusion

The inclusion of genetic data from species of the Southern Hemisphere shows *Lumbricillus* to be non-monophyletic and suggests that species in the *L. arenarius* group should be transferred to a separate genus. The four species of *Lumbricillus* described in this study were nested within *Lumbricillus s.s.*, thus

not supporting a basal split between the species of the Southern and Northern Hemispheres. We found three species with strong morphological similarities to *M. georgiana*, the type species of *Marionina s.s.*, and in our phylogeny they were not closely related to any other included '*Marionina*' species. This phylogenetic placement might provide an important step towards the revision of the ambiguous genus *Marionina*, whereby most species will have to be transferred to other genera. If the southern enchytraeids mentioned are to be included in *Marionina s.s.*, the definition needs to be modified to include pigmented species, allow for pharyngeal glands occupying four segments, allow for nephridial anteseptale with slightly more than just the funnel, and permit male apparatus lacking an eversible glandular bulb but with surface male pore and one, two or more prostate glands. It is possible that a revised *Marionina s.s.* would contain only marine species, all of which are from the temperate and Antarctic areas of the Southern Hemisphere. Finally, we found *Ch. blocki* to be close to neither *Marionina s.s.* nor *Lumbricillus*, but its precise placement within Enchytraeidae remains unknown.

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REFERENCES

Backlund HO. 1947. Swedish Enchytraeida II. *Lunds Universitets Årsskrift N.F. Avd. 2* **43:** 1–30.

Beddard FE. 1895. *A monograph of the order of Oligochaeta*. Oxford: Clarendon Press.

Benham WB. 1905. On the Oligochaeta from the southern islands of the New Zealand region. *Transactions and Proceedings of the New Zealand Institute* **37:** 285–297.

Benham WB. 1909. Report on Oligochaeta of the subantarctic islands of New Zealand. *The Subantarctic Islands of New Zealand* **1:** 251–294.

Benham WB. 1915. On *Lumbricillus macquariensis* Benham. *Transactions and Proceedings of the New Zealand Institute, Wellington* **47:** 189–191.

Benham WB. 1922. Oligochaeta of Macquarie Island. Australasian Antarctic expedition 1911–14. *Scientific Reports C* **6:** 1–38.

Block W, Christensen B. 1985. Terrestrial enchytraeidae from South Georgia and the maritime Antarctic. *British Antarctic Survey Bulletin* **69:** 65–70.

Boros G, Sherlock E. 2010. Catalogue of the enchytraeid worm collection (Oligochaeta: Enchytraeidae) of the Natural History Museum in London. I. Spirit collection. *Opuscula Zoologica Instituti Zoosystematici et Oecologici Universitatis Budapestinensis* **41:** 19–27.

Brinkhurst RO, Jamieson BGM. 1971. *Aquatic Oligochaeta of the world*. Edinburgh: Oliver & Boyd.

Černosvitov L. 1934. Zur Kenntnis der Enchytraeiden. I. *Zoologischer Anzeiger* **105:** 233–247.

Černosvitov L. 1937. System der Enchytraeiden. *Bulletin de l'Association Russe pour les Recherches Scientifiques à Prague* **5:** 263–295.

Claparède E. 1861. Études anatomiques sur les Annélides, Turbellariés, Opalines et Grégarines observés dans les Hébrides. *Mémoires de la Société de Physique et d'Histoire Naturelle de Genève* **16:** 71–164.

Coates KA, Ellis DV. 1981. Taxonomy and distribution of marine Enchytraeidae (Oligochaeta) in British Columbia. *Canadian Journal of Zoology* **59:** 2129–2150.

Coates KA, Erséus C. 1985. Marine enchytraeids (Oligochaeta) of the coastal northwest Atlantic (northern and mid USA). *Zoologica Scripta* **14:** 103–116.

Dartnall HJ, Hollwedel W, De Paggi JC. 2005. The freshwater fauna of Macquarie Island, including a redescription of the endemic water-flea *Daphnia gelida* (Brady) (Anomopoda: Crustacea). *Polar Biology* **28:** 922–939.

Dartnall HJ, Smith VR. 2012. Freshwater invertebrates of sub-Antarctic Marion Island. *African Zoology* **47:** 203–215.

Davies KF, Greenslade P, Melbourne BA. 1997. The invertebrates of sub-antarctic Bishop Island. *Polar Biology* **17:** 455–458.

De Wit P, Erséus C. 2010. Genetic variation and phylogeny of Scandinavian species of *Grania* (Annelida: Clitellata: Enchytraeidae), with the discovery of a cryptic species. *Journal of Zoological Systematics and Evolutionary Research* **48:** 285–293.

De Wit P, Rota E, Erséus C. 2011. Phylogeny and character evolution in *Grania* (Annelida, Clitellata). *Zoologica Scripta* **40:** 509–519.

Dózsa-Farkas K. 1990. New enchytraeid species from sphagnum-bogs in Hungary (Oligochaeta: Enchytraeidae). *Acta Zoologica Hungarica* **36:** 265–274.

Dózsa-Farkas K, Convey P. 1997. *Christensenia*, a new terrestrial enchytraeid genus from Antarctica. *Polar Biology* **17:** 482–486.

Dózsa-Farkas K, Convey P. 1998. Erratum. *Christensenia*, a new terrestrial enchytraeid genus from Antarctica. *Polar Biology* **20:** 292.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29:** 1969–1973.

Eisen G. 1878. Redogörelse för Oligochaeter samlade under de Svenska expeditionerna till Arktiska trakter. *Öfversigt af Kongliga Vetenskaps-Akademiens Förhandlingar* **3:** 63–79.

Erséus C. 1994. The Oligochaeta. In: Blake JA, Hilbig B, eds. *Taxonomic atlas of the bentic fauna of the Santa Maria Basin and western Santa Barbara Channel volume 4 Oligochaeta to Polychaeta: Phyllodocida (Phyllodocidae to Paralacydoniidae)*. Santa Barbara: Santa Barbara Museum of Natural History, 5–38.

Erséus C, Rota E, Matamoros L, De Wit P. 2010. Molecular phylogeny of Enchytraeidae (Annelida, Clitellata). *Molecular Phylogenetics and Evolution* **57:** 849–858.

Finogenova NP, Streltsov VE. 1978. Two new species of oligochaetes of the genus *Lumbricillus* (Oligochaeta: Enchytraeidae) from the East Murman Intertidal zone. *Biologiya Morya* **1:** 17–23.

Heled J, Drummond AJ. 2010. Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution* **27:** 570–580.

Henle FGJ. 1837. Ueber Enchytraeus, eine neue Anneliden-Gattung. *Müllers Archiv für Anatomie, Physiologie und Wissenschaftliche Medizin, Berlin* **1837:** 74–90.

Issel R. 1905. Oligocheti inferiori della fauna italica. Zoologische Jahrbücher. *Abteilung für Anatomie und Ontogenie der Tiere, Jena* **22:** 451–476.

Katoh K, Misawa K, Kuma KI, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30:** 3059–3066.

Klinth MJ, Martinsson S, Erséus C. 2017a. Phylogeny and species delimitation of North European *Lumbricillus* (Clitellata, Enchytraeidae). *Zoologica Scripta* **46:** 96–110.

Klinth MJ, Rota E, Erséus C. 2017b. Taxonomy of north European *Lumbricillus* (Clitellata, Enchytraeidae). *ZooKeys* **2017:** 15–96.

Lee J, Klinth MJ, Jung J. 2019. Two species of *Lumbricillus* (Enchytraeidae, Annelida) new to Antarctica. *Polar Research* **38:** 1–7.

Lee KE. 1968. Oligochaeta from subantarctic islands. *British, Australian and New Zealand Antarctic Research Expedition 1929–1931 Reports Series B (Zoology and Botany)* **8:** 149–165.

Leuckart R. 1847. Verzeichniss der zur Fauna Helgoland's gehörenden wirbellosen Seethiere. In: Frey H, Leuckart R, eds. *Beiträge zur Kenntniss wirbelloser Thiere*. Braunschweig [Brunswick]: Friedrich Vieweg und Sohn, 136–168.

Liu Y, Erséus C. 2017. New specific primers for amplification of the Internal Transcribed Spacer region in Clitellata (Annelida). *Ecology and Evolution* **7:** 10421–10439. doi:10.1002/ece3.3212

Marcus E. 1965. Naidomorpha aus brasilianischem Brackwasser. *Studies on Neotropical Fauna and Environment* **4:** 61–83.

Martinsson S, Dózsa-Farkas K, Rota E, Erséus C. 2017. Placing the forgotten: on the positions of *Euenchytraeus* and *Chamaedrilus* in an updated enchytraeid phylogeny (Clitellata: Enchytraeidae). *Invertebrate Systematics* **31:** 85–90.

Martinsson S, Erséus C. 2014. Cryptic diversity in the well-studied terrestrial worm *Cognettia sphagnetorum* (Clitellata: Enchytraeidae). *Pedobiologia* **57:** 27–35.

Matamoros L, Rota E, Erséus C. 2012. Cryptic diversity among the achaetous *Marionina* (Annelida, Clitellata, Enchytraeidae). *Systematics and Biodiversity* **10:** 509–525.

Michaelsen W. 1888. Die Oligochaeten von Süd-Georgien nach der Ausbeute der Deutschen Station von 1882–1883. *Jahrbuch der Hamburgischen Wissenschaftlichen Anstalten* **5:** 53–73.

Michaelsen W. 1889a. Oligochaeten des Naturhistorischen Museums in Hamburg. *Jahrbuch der Hamburgischen Wissenschaftlichen Anstalten* **6:** 1–17.

Michaelsen W. 1889b. Synopsis der Enchytraeiden. *Abhandlungen aus dem Gebiete der Naturwissenschaften herausgegeben vom Naturwissenschaftlichen Verein in Hamburg* **11:** 1–61.

Michaelsen W. 1900. Oligochaeta. *Das Tierreich* **10:** 1–575.

Michaelsen W. 1905a. Die Oligochaeten der Deutschen Südpolar-Expedition 1901–1903 nebst Erörterung der Hypothese über einen früheren groβ en die Südspitzen der Kontinente verbindenden antarktischen Kontinent. *Deutsche Südpolar-Expedition IX, Zoologie* **1:** 1–58.

Michaelsen W. 1905b. Die Oligochaeten der Schwedischen Südpolar-Expedition. *Schwedischen Südpolar-Expedition 1901–1903, Stockholm* **5:** 1–12.

Michaelsen W. 1914. Oligochaeta. In: Beiträge zur Kenntnis der Land- und Süβwasserfauna Deutsch- Südwestafrikas. *Ergebnisse der Hamburger deutsch-südwestafrikanischen Studienreise* **1911:** 137–182.

Michaelsen W. 1924. Papers from Dr. Th. Mortensen's Pacific Expedition 1914–16. XVII. Oligochäten von Neuseeland und den Auckland-Campbell-Inseln, Nebst einigen anderen Pacifischen Formen. *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening i København* **75:** 197–240.

Michaelsen W. 1935. Meeresstrand-Enchyträiden des südlichen Atlantischen Ozeans. *Scientific Results of the Norwegian Antarctic Expeditions 1927–1928 et sqq., instituted and financed by consul Lars Christensen* **14:** 2–7.

Müller OF. 1774. *Vermium terrestrium et fluviatilum, seu animalium infusoriorum, helminthicorum, et testaceorum, non marinorum, succincta historia. Vol. 1, Part 2*. Copenhagen and Leipzig: Heineck and Faber.

Nielsen CO, Christensen B. 1959. The Enchytraeidae: critical revision and taxonomy of European species. *Natura Jutlandica* **8–9:** 1–160.

Nurminen M. 1964. *Lumbricillus fennicus* sp. n. and some other enchytraeids (Oligochaeta) from Finland. *Annales Zoologici Fennici* **1:** 48–51.

Ørsted AS. 1844. *De regionibus marinis*. Copenhagen: J. C. Scharling, 1–89.

Pfeffer G. 1890. Die niedere Thierwelt des antarktischen Ufergebietes. In: Neumayer G, ed. *Die internationale Polarforschung 1882–1883. Die Deutschen Expeditionen und ihre Ergebnisse. Band II. Beschreibende Naturwissenschaften in einzelnen Abhandlungen*. Berlin: A. Asher & Co., 455–574.

Prantoni AL, Belmonte-Lopes R, Lana PC, Erséus C. 2018. Genetic diversity of marine oligochaetous clitellates in selected areas of the South Atlantic as revealed by DNA barcoding. *Invertebrate Systematics* **32:** 524–532.

Prantoni AL, De Wit P, Erséus C. 2016. First reports of *Grania* (Clitellata: Enchytraeidae) from Africa and South America: molecular phylogeny and descriptions of nine new species. *Zoological Journal of the Linnean Society* **176:** 485–510.

Rambaut A. 2014. *Figtree, a graphical viewer of phylogenetic trees*. Available at: http://tree.bio.ed.ac.uk/software/figtree

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018. *Tracer v1.7*. Available at: http://beast.community/tracer

Reynolds JW, Wetzel MJ. 2019. *Nomenclatura Oligochaetologica – A catalogue of names, descriptions and type specimens. Editio Secunda*. Available at: https://www.inhs.illinois.edu/people/mjwetzel/nomenoligo

Rodriguez P, Rico E. 2008. A new freshwater oligochaete species (Clitellata: Enchytraeidae) from Livingston Island, Antarctica. *Polar Biology* **31:** 1267–1279.

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

Rota E. 2001. Oversized enchytraeids (Annelida, Clitellata): a comparative study, with a revised description of *Lumbricillus maximus* (Michaelsen). *Organisms Diversity & Evolution* **1:** 225–238.

Rota E. 2013. How many lookalikes has *Marionina argentea* (Michaelsen, 1889) (Annelida: Clitellata: Enchytraeidae)? Three new species described from morphological evidence. *Zoologischer Anzeiger* **252:** 123–137.

Rota E. 2015. Five new species of Enchytraeidae (Annelida: Clitellata) from Mediterranean woodlands of Italy and reaffirmed validity of *Achaeta etrusca*, *Fridericia bulbosa* and *F. miraflores*. *Journal of Natural History* **49:** 1987–2020.

Rota E, De Eguileor M, Grimaldi A. 1999. Ultrastructure of the head organ: a putative compound georeceptor in *Grania* (Annelida, Clitellata, Enchytraeidae). *Italian Journal of Zoology* **66:** 11–21.

Rota E, Erséus C. 1996. Six new species of *Grania* (Oligochaeta, Enchytraeidae) from the Ross Sea, Antarctica. *Antarctic Science* **8:** 169–183.

Rota E, Erséus C. 1997. A re-examination of *Grania monochaeta* (Michaelsen) (Oligochaeta: Enchytraeidae), with descriptions of two new species from Subantarctic South Georgia. *Journal of Natural History* **31:** 27–42.

Rota E, de Jong Y. 2015. Fauna Europaea: Annelida – terrestrial Oligochaeta (Enchytraeidae and Megadrili), Aphanoneura and Polychaeta. *Biodiversity Data Journal* **3:** e5737.

Rota E, Matamoros L, Erséus C. 2008. In search of *Marionina* (Clitellata, Enchytraeidae): a taxonomic history of the genus and redescription of the type species *Pachydrilus georgianus* Michaelsen, 1888. *Italian Journal of Zoology* **75:** 417–436.

Roule L. 1888. Sur la structure histologique d'un oligochaete marin appartenant à un genre nouveau. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences* **116:** 308–310.

Saint-Loup R. 1885. Sur l'organisation du *Pachydrilus enchytraeoides*. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences* **101:** 482–485.

Schmelz RM. 2003. Taxonomy of *Fridericia* (Oligochaeta, Enchytraeidae). Revision of species with morphological and biochemical methods. *Abhandlungen des Naturwissenschaftlichen Vereins in Hamburg (Neue Folge)* **38:** 1–415.

Schmelz RM, Collado R. 2008. A type-based redescription of *Pachydrilus georgianus* Michaelsen, 1888, the type species of *Marionina* Michaelsen, 1890, with comments on *Christensenidrilus* Dózsa-Farkas & Convey, 1998 (Enchytraeidae, "Oligochaeta", Annelida). *Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg* **44:** 7–22.

Schmelz RM, Collado R. 2010. A guide to European terrestrial and freshwater species of Enchytraeidae (Oligochaeta). *Soil Organisms* **82:** 1–176.

Schmelz RM, Collado R. 2015. Checklist of taxa of Enchytraeidae (Oligochaeta): an update. *Soil Organisms* **87:** 149–152.

Shurova NM. 1974. Enchytraeidae of the genus *Lumbricillus* (Oligochaeta) from the intertidal zone of the Kurile Islands. In: Kusakin OG, ed. *Plant and animal world of the litoral zone of the Kurile Islands*. Novosibirsk: Far-Eastern Scientific Centre, 128–136.

Shurova NM. 1978. The intertidal oligochaetes from Eastern Coast of Kamchatka. In: Kusakin OG, ed. *Litoral Beringova morya i yugo-vostochnoj Kamchatki [Littoral of the Bering Sea and the southeastern Kamchatka]*. Moscow: Nauka, 98–105.

Sjölin E, Erséus C, Källersjö M. 2005. Phylogeny of Tubificidae (Annelida, Clitellata) based on mitochondrial and nuclear sequence data. *Molecular Phylogenetics and Evolution* **35:** 431–441.

Southern R. 1913. Clare Island survey part 48: Oligochaeta. *Proceedings of the Royal Irish Academy* **31:** 1–48.

Stephenson J. 1911. On some littoral Oligochaeta of the Clyde. *Transactions of the Royal Society of Edinburgh* **48:** 31–65.

Stephenson J. 1932. Oligochaeta. Part. I. Microdrili. *Discovery Reports* **4:** 233–264.

Ude H. 1896. Enchytraeiden. *Hamburger Magalhaensische Sammelreise* **3:** 1–43.

Wang H, Liang Y. 1997. Two new species of Oligochaeta (Annelida) from King George Island, Antarctica. *Oceanologia et Limnologia Sinica* **28:** 177–184.

Welch PS. 1914. Studies on the Enchytraeidae of North America. *Bulletin of the Illinois State Laboratory of Natural History, Urbana* **10:** 123–211.

Xie Z, Rota E. 2001. Four new terrestrial species of *Marionina* (Clitellata, Enchytraeidae) from China and re-examination of *M. hoffbaueri* Möller. *Journal of Natural History* **35:** 1417–1431.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Majority-rule consensus tree for *H3* estimated with Bayesian inference. Specimens shaded in grey are from the Southern Hemisphere. Specimens in bold represent new species. Support values are posterior probabilities. Scale bar represents the estimated number of substitutions per site.
Figure S2. Majority-rule consensus tree for ITS2 estimated with Bayesian inference. Specimens shaded in grey are from the Southern Hemisphere. Specimens in bold represent new species. Support values are posterior probabilities. Scale bar represents estimated number of substitutions per site.
Figure S3. Majority-rule consensus tree, for a concatenated matrix of 12S, 16S, *COI*, 18S, 28S, ITS2 and *H3*, estimated using Bayesian inference. Specimens shaded in grey are from the Southern Hemisphere. Specimens in bold represent new species. Support values are posterior probabilities. Scale bar shows expected number of changes per site.
Table S1. List of specimens used in this study, with specimen identification number, collection data, GPS coordinates (in decimal degrees), GenBank accession numbers for seven different markers (bold numbers are new sequences generated in this study) and voucher numbers. Letters for *Lumbricillus pagenstecheri* refer to barcoding clusters (see Klinth *et al.*, 2017a). Country codes: AU, Australia; ES, Spain; FR, France; GL, Greenland; NL, The Netherlands; NO, Norway; SE, Sweden; UK, United Kingdom. *Unsuccessfully sequenced marker.