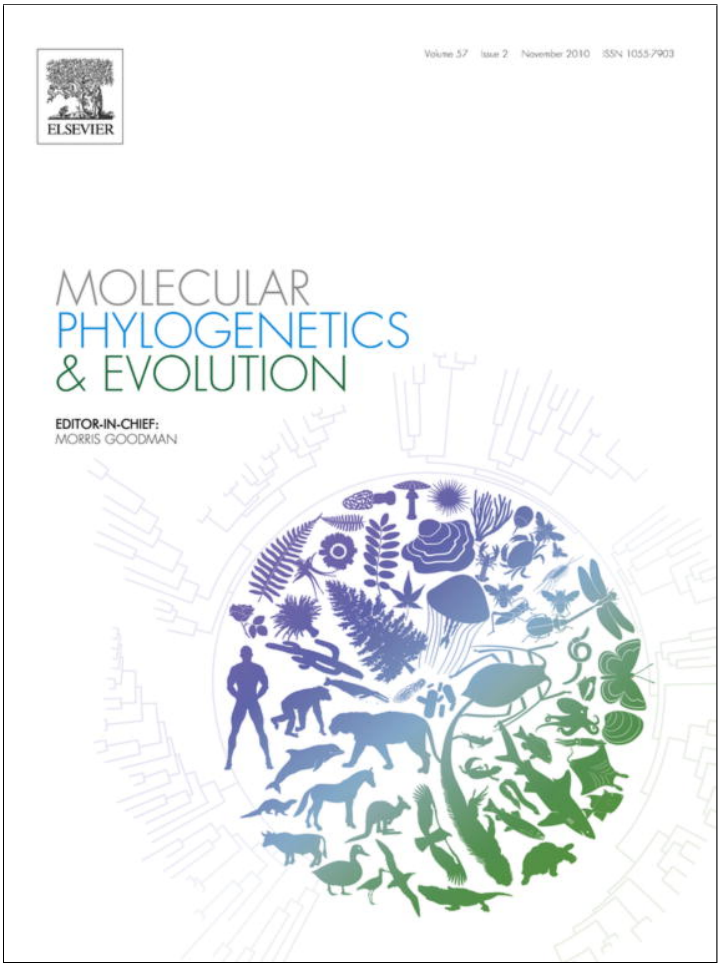
Volume 57 Issue 2 November 2010 ISSN 1055-7903
ELSEVIER
MOLECULAR
PHYLOGENETICS
& EVOLUTION
EDITOR-IN-CHIEF:
MORRIS GOODMAN

Molecular Phylogenetics and Evolution 57 (2010) 849–858

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Molecular phylogeny of Enchytraeidae (Annelida, Clitellata)

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ARTICLE INFO

Article history:
Received 20 April 2010
Revised 8 July 2010
Accepted 8 July 2010
Available online 14 July 2010

Keywords:
Oligochaetes
Molecular systematics
Bayesian inference

ABSTRACT

A multigene data set (12S, 16S, and COI mitochondrial DNA; 18S and 28S nuclear DNA) was analyzed by Bayesian inference to estimate the phylogeny of a sample of the clitellate family Enchytraeidae (86 species representing 14 nominal genera). Monophyly, as well as a basal dichotomy, of the family Enchytraeidae obtained maximum support, with one clade containing *Hemienchytraeus* and *Achaeta*, the other the remaining 12 genera analysed. The latter group is basally resolved in several well-supported clades. *Lumbricillus* and *Grania* are closely related. *Bryodrilus, Oconnorella, Henlea* and two species of *Marionina* (*M.* cf. *riparia*, and *M. communis*) form a well-supported clade. *Cognettia* is sister to *Stercutus*, and *Cernosvitoviella* sister to *Mesenchytraeus*, and the four together appear to be a monophyletic group. A large part of the taxonomically problematic *Marionina* appears to be a group not closely related to the type species (*M. georgiana*), and this group also includes *Enchytronia*. Further, this *Marionina/Enchytronia* group appears to be sister to a clade comprising the more or less littoral marine genera *Stephensoniella* and *Enchytraeus*. *Hemifridericia, Buchholzia* and *Fridericia*, the three genera characterized by two types of coelomocytes, also form a well-supported clade. The study corroborates most of the multi-species genera analysed (*Cognettia, Cernosvitoviella, Mesenchytraeus, Oconnorella, Henlea, Enchytraeus, Grania, Buchholzia* and *Fridericia*); only *Lumbricillus* and *Marionina* are non-monophyletic as currently defined.

1. Introduction

Clitellata is a large taxon comprising about one third of all annelid species known to date (Erséus, 2005). Traditionally, it has been divided in two groups, Oligochaeta and Hirudinea, but molecular data support that the latter has evolved within the former (Martin, 2001; Siddall et al., 2001; Erséus and Källersjö, 2004; Rousset et al., 2007, 2008; Struck et al., 2007; Marotta et al., 2008), making the name Oligochaeta synonymous to Clitellata. Although there is now good evidence for hirudineans and other leech-like taxa (Branchiobdellida and Acanthobdellida) being closely related to the oligochaetous family Lumbriculidae (e.g., Marotta et al., 2008), the basal phylogeny of the clitellate groups remains largely unresolved (Erséus and Källersjö, 2004; Erséus, 2005; Marotta et al., 2008).

Enchytraeidae is a large clitellate taxon. With a total of almost 700 nominal species, distributed in all kinds of aquatic and terrestrial habitats throughout the world, it is probably the most ubiquitous of all clitellate families (Erséus, 2005). Enchytraeids are particularly numerous in intertidal sands along the seashores and in soils on land, but they are also known from fine sediments in the deep sea (Rota and Erséus, 2003; Erséus and Rota, 2003) and the ice of glaciers (e.g., Hartzell et al., 2005). Yet, their phylogenetic position and intra-familial evolutionary history are far from understood. Beddard (1895, Fig. 34) placed Enchytraeidae in a basal position within Oligochaeta/Clitellata, separate from most other oligochaete groups recognized at the time, a position also held by Michaelsen (1928), Kasprzak (1984), and Omodeo (1998). This has been modified in various ways in the evolutionary schemes presented by various 20th century workers, as reviewed, e.g., by Čekanovskaya (1962), Timm (1981) and Rota (1994a). On the basis of morphological evidence only, Enchytraeidae has later on tended to be regarded as a taxon close to other ”microdrile” families, i.e., Phreodrilidae and the large assemblage today recognized as Naididae *sensu* Erséus et al. (2008, 2010); see, e.g., Yamaguchi (1953), Brinkhurst (1984) and Jamieson (1988). Moreover, Coates (1986) removed *Propappus* from Enchytraeidae to form a separate monotypic family, Propappidae; according to Brinkhurst (1994) the two families are sister taxa.

In slight contrast to the above, the first phylogenetic assessments using molecular data (but including only a few enchytraeids) instead indicated a sister relationship between Enchytraeidae and Crassiclitellata Jamieson, 1988, i.e., the large taxon with multi-layered clitellum and including most “earthworms” (Martin et al., 2000; Siddall et al., 2001; Erséus and Källersjö, 2004; Rousset et al., 2008). Further, when Propappidae was included in the analyses (Erséus and Källersjö, 2004; Rousset et al., 2008), it did not come

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doi:10.1016/j.ympev.2010.07.005

out as sister to Enchytraeidae. However, when using a combination of molecular and morphological characters, Marotta et al. (2008) obtained strong support for Crassiclitellata being more closely related to the lumbriculid-hirudinean clade, and that these groups together are the sister to a group comprising Enchytraeidae and Propappidae.

The evolutionary history within Enchytraeidae has been little studied in the past. In this family, Michaelsen (1929) saw two opposite poles: on one side, *Propappus* and *Mesenchytraeus* showed affinities with aeolosomatids and naidids; on the other side *Fridericia* and *Achaeta* seemed to share characters typical of Phreoryctidae (= Haplotaxidae). The Enchytraeidae could thus represent a possible evolutionary step between "primitive" and derived oligochaete taxa. However, since the position of aeolosomatids within the oligochaetes was ambiguous ("primitive" vs. secondarily simplified; see Stephenson, 1930), the direction of the "filiation" had to be left open. Černosvitov (1937) reviewed all the enchytraeid genera of his time, and presented a familial division into five subfamilies (plus Parergodrilinae, no longer considered clitellates). It is questionable, however, whether his system was built on strictly phylogenetic principles. Furthermore, subfamily level taxa have seldom been used in enchytraeid taxonomy since Černosvitov (e.g., Bell, 1962). Coates (1989) was the first to make a formal cladistic assessment of enchytraeid relationships using morphological characters, but due to considerable homoplasy there was lack of stability and corroboration of the relationships found. For instance, her analyses supported monophyly in only five of the eleven genera investigated.

Recently, Christensen and Glenner (2010) analyzed a molecular data set, a combined alignment (4977 bp total) of five mitochondrial and three nuclear loci, from specimens representing nine enchytraeid genera. They found *Enchytraeus* and *Lumbricillus* to form a paraphyletic assemblage of species, largely adapted to marine littoral conditions, and a larger monophyletic group (sister to *Lumbricillus*) containing seven more typically terrestrial genera. In the latter group, the tree topology largely follows morphological patterns in nephridial morphology, whereas other taxonomically much used features (e.g., chaetal shape, origin of dorsal blood vessel, and intestinal modifications) appear to have arisen convergently in some lineages. Their study is thus a good start for a further reconstruction of the evolutionary history of the family.

In this study, we use three mitochondrial (12S rDNA; 16S rDNA; cytochrome c oxidase subunit 1, COI) and two nuclear loci (18S rDNA; D1 region of 28S rDNA) for a larger sample of taxa: 103 species, 86 of which are regarded as the ingroup, representing 14 enchytraeid genera. This covers about half of the genera currently recognized in Enchytraeidae. The aims are to generate a well-supported hypothesis of the phylogeny of the family as a whole, and to test as far as possible, whether the currently recognized genera are monophyletic. In particular, we wish to scrutinize representatives of the genus *Marionina*, which repeatedly has been pointed out as an artificial taxon (e.g., Coates, 1989; Xie and Rota, 2001; Rota et al., 2008; Schmelz and Collado, 2008).

2. Material and methods

The great majority of data used in this study are new DNA sequences of specimens collected during 1995–2006, mostly in Sweden but also in other countries. These specimens are listed in Table 1; locality data and names of those responsible for species identifications are specified in Supplementary Table 1. The extracted DNA of 18 of the individuals have been used before, for already published sequences (i.e., those with GenBank nos. not set in bold face in Table 1), but in all these cases, one or more new sequences/loci are added here. The whole collection of worms represents 103 species, of which 17 are outgroup taxa belonging to clitellate families outside Enchytraeidae. As specified in Table 1, vouchers (normally the anterior ends) of some sequenced individuals have been deposited, as microscope slides, in the Swedish Museum of Natural History, Stockholm, or (in one case) the Australian Museum, Sydney.

Worms were processed over a period of several years and in different labs (Swedish Museum of Natural History, and University of Gothenburg). The procedures of DNA extraction, PCR and sequencing were thus not exactly the same throughout, but the work involved standard products and followed protocols recommended by the manufactures at all times.

The following genes (using the following primers) were amplified by standard PCR: COI (various combinations of primers LCO1490/ HCO2198, Folmer et al., 1994; COI-E^{-}, Bely and Wray, 2004; and 5′-tgattctactcaactaatcacaaagatattgg-3′, Bodil Cronholm, pers. comm.), 12S rDNA (12SE1/12SH, Jamieson et al., 2002), 16S rDNA (16SarL/16SbrH, Palumbi et al., 1991; and 16SAnnF/ 16SAnnR, Sjölin et al., 2005), and 28S rDNA (28SC1/28SC2, Dayrat et al., 2001). Fragments for 18S were first PCR amplified with the primers TimA and TimB (Norén and Jondelius, 1999); the resulting product was then used to seed another PCR with the primer combinations of TimA/1100R (Norén and Jondelius, 1999) and 660F/ TimB (660F, Erséus et al., 2002). In a few cases, the primers 600F and 1806R (Norén and Jondelius, 1999) replaced 660F and TimB, respectively. Additional internal sequencing primers were later used for 18S (4FBK, 4FB, 5f, 7fk, Norén and Jondelius, 1999). Sequencing reactions were run either on an ABI 377 Automated DNA Sequencer, a Beckman Coulter CEQ8000, or the PCR products were sent to Macrogen, Inc., South Korea for sequencing. Sequences were assembled and checked using the Staden package (Staden et al., 1998), Lasergene + (DNASTAR Inc.), or Geneious (Biomatters Ltd.).

For a majority of the species, sequences of all five genes were obtained, but for eleven ingroup and seven outgroup taxa only a total of four of them were sequenced (see Table 1). Moreover, in four cases, different genes from two individuals of the same species were combined in the analyses (Table 1). In the latter cases, a gene from the mitochondrial genome (12S, 16S or COI) was first used to verify that the two individuals actually were of the same species; they were used only when the sequences of this mitochondrial gene were identical in both specimens.

For each gene, alignment was carried out using the MUSCLE web server (available at http://www.ebi.ac.uk/Tools/muscle/index.html) (Edgar, 2004). The resulting alignments consisted of: 462 positions (of which 309 parsimony-informative) for 12S, 535 (320 informative) for 16S, 1855 (313 informative) for 18S, 340 (108 informative) for 28S, and finally, 658 (338 informative) for COI. In cases of one gene missing (see above), this gene was added as "missing data" in the alignment: 1 taxon for 12S, 4 taxa for 18S, 2 for 28S, and 11 for the COI alignment. The five alignments were then fused into one large alignment with 3850 positions. The combined alignment was partitioned according to gene, and the COI partition was further partitioned according to codon position, which created a total of seven partitions.

Each of these partitions was tested using MrModeltest 2.2 (Nylander, 2004) within PAUP*4.0b (Swofford, 2002) for the nucleotide substitution model of best fit, and the model shown by the Akaike Information Criterion (AIC) as the best-fitting one was chosen for each partition. For most partitions, the model chosen was GTR + I + G. The exceptions were the first codon position of COI, for which SYM + I + G was used, and the third codon position of COI, for which GTR + G was used.

The alignment was first split into two parts in order to test for congruence between the different loci; one with mtDNA (12S, 16S and COI) and one with nDNA (18S and 28S). Both of these were

Table 1
Specimens and DNA sequences (with GenBank accession numbers) analyzed in this study. GenBank numbers in bold indicate new sequences. Vouchers (when applicable) are deposited in the Swedish Museum of Natural History (SMNH), Stockholm, and the Australian Museum, Sydney (AMS). Each voucher marked with an asterisk (∗) is topotypic with the sequenced worm, and stored in alcohol; all other vouchers are slide-mounted anterior ends of sequenced worms. For information about specimen collection sites, see Supplementary Table 1.

Taxon	Individual	12S	16S	18S	28S	COI	Voucher
Achaeta aberrans Nielsen & Christensen, 1961	CE875	**GU901670**	–	–	**GU901936**	**GU902030**	–
Achaeta aberrans Nielsen & Christensen, 1961	CE1033	–	**GU901765**	–	–	–	–
A. affinis Nielsen & Christensen, 1959	CE715	**GU901671**	**GU901766**	**GU901853**	**GU901937**	–	–
A. bibulba Graefe, 1989	CE1206	**GU901672**	**GU901767**	**GU901854**	**GU901938**	**GU902031**	–
A. bifollicula Chalupsky, 1992	CE1035	**GU901673**	**GU901768**	**GU901855**	**GU901939**	**GU902032**	–
A. cf. *bohemica* (Vejdovsky, 1879)	CE1766	**GU901674**	**GU901769**	–	**GU901940**	**GU902033**	–
A. cf. *brevivasa* Graefe, 1980	CE1234	**GU901675**	**GU901770**	**GU901857**	**GU901941**	**GU902034**	–
A. camerani (Cognetti, 1899)	CE790	**GU901676**	**GU901771**	**GU901856**	**GU901942**	**GU902035**	–
A. iberica Graefe, 1989	CE1051	**GU901677**	**GU901772**	–	**GU901943**	**GU902036**	–
A. unibulba Graefe, Dozsa-Farkas & Christensen, 2005	CE812	**GU901678**	**GU901773**	**GU901858**	**GU901944**	**GU902037**	–
Bryodrilus ehlersi Ude, 1892	CE718	**GU901680**	**GU901774**	**GU901859**	**GU901945**	–	–
Buchholzia appendiculata Buchholz, 1862	CE1204	**GU901681**	**GU901775**	**GU901860**	**GU901946**	**GU902038**	SMNH 108407
B. fallax Michaelsen, 1887	CE719	**GU901682**	**GU901776**	**GU901861**	**GU901947**	**GU902039**	–
Cernosvitoviella aggtelekiensis Dozsa-Farkas, 1970	CE839	**GU901684**	**GU901777**	**GU901862**	**GU901948**	**GU902040**	–
C. cf. *atrata* (Bretscher, 1903)	CE1014	**GU901685**	–	**GU901863**	**GU901949**	**GU902041**	SMNH 108408
C. cf. *atrata* (Bretscher, 1903)	CE1003	–	**GU901778**	–	–	–	SMNH 108409
C. immota (Knöllner, 1935)	CE895	**GU901686**	**GU901779**	**GU901864**	**GU901950**	**GU902042**	–
C. minor Dozsa-Farkas, 1990	CE838	**GU901687**	**GU901780**	**GU901865**	**GU901951**	**GU902043**	–
Cognettia cognetti (Issel, 1905)	CE1042	**GU901688**	**GU901781**	**GU901866**	**GU901952**	**GU902044**	SMNH 108410
C. sphagnetorum (Vejdovsky, 1878)	CE832	**GU901689**	**GU901782**	**GU901867**	**GU901953**	**GU902045**	–
Enchytraeus albidus Henle, 1837	CE521	**GU901693**	**GU901785**	**GU901870**	**GU901956**	**GU902047**	–
E. buchholzi Vejdovsky, 1878	CE724	**GU901694**	**GU901786**	**GU901871**	**GU901957**	**GU902048**	–
E. bulbosus Nielsen & Christensen, 1963	CE798	**GU901695**	**GU901787**	**GU901872**	**GU901958**	**GU902049**	–
E. christenseni Dozsa-Farkas, 1992	CE805	**GU901696**	**GU901788**	**GU901873**	**GU901959**	**GU902050**	–
E. crypticus Westheide & Graefe, 1992	CE2183	**GU901697**	**GU901789**	**GU901874**	**GU901960**	**GU902055**	SMNH 108411
E. japonensis Nakamura, 1993	CE881	**GU901698**	**GU901790**	**GU901875**	**GU901961**	**GU902051**	–
E. lacteus Nielsen & Christensen, 1961	CE813	**GU901699**	**GU901791**	**GU901876**	**GU901962**	**GU902052**	–
E. luxuriosus Schmelz & Collado, 1999	CE2175	**GU901700**	**GU901792**	**GU901877**	**GU901963**	**GU902053**	SMNH 108412
E. norvegicus Abrahamsen, 1968	CE804	**GU901701**	**GU901793**	**GU901878**	**GU901964**	–	–
E. norvegicus Abrahamsen, 1968	CE1225	–	–	–	–	**GU902054**	–
Enchytronia parva Nielsen & Christensen, 1959	CE806	**GU901702**	**GU901794**	**GU901879**	**GU901965**	**GU902056**	–
Fridericia bisetosa (Levinsen, 1884)	CE783	**GU901703**	**GU901795**	**GU901880**	**GU901966**	**GU902057**	–
F. bulboides Nielsen & Christensen, 1959	CE797	**GU901704**	**GU901796**	**GU901881**	**GU901967**	**GU902058**	SMNH 108413
F. christeri Rota & Healy, 1999	CE816	**GU901706**	**GU901798**	**GU901883**	**GU901969**	**GU902060**	–
F. connata Bretscher, 1902	CE728	**GU901707**	**GU901799**	**GU901884**	**GU901970**	**GU902061**	SMNH 108414*
F. digitata Cognetti, 1901	CE729	**GU901708**	**GU901800**	**GU901885**	**GU901971**	**GU902062**	SMNH 108415*
F. galba (Hoffmeister, 1843)	CE730	**GU901709**	**GU901801**	**GU901886**	**GU901972**	**GU902063**	–
F. heliota Zalesskaja, 1990	CE324	**GU901710**	**GU901802**	**GU901887**	**GU901973**	**GU902064**	–
F. isseli Rota, 1994b	CE792	**GU901711**	**GU901803**	**GU901888**	**GU901974**	**GU902065**	–
F. magna Friend, 1899	CE803	**GU901712**	**GU901804**	**GU901889**	**GU901975**	**GU902066**	–
F. nemoralis Nurminen, 1970	CE1226	**GU901713**	**GU901805**	**GU901890**	**GU901976**	**GU902067**	–
F. parathalassia Schmelz, 2002	CE1029	**GU901714**	**GU901806**	**GU901891**	**GU901977**	**GU902068**	–
F. paroniana Issel, 1904	CE733	**GU901715**	**GU901807**	**GU901892**	**GU901978**	–	–
F. perrieri (Vejdovsky, 1878)	CE734	**GU901716**	**GU901808**	**GU901893**	**GU901979**	**GU902069**	SMNH 108416*
F. ratzeli (Eisen, 1872)	CE782	**GU901717**	**GU901809**	**GU901894**	**GU901980**	**GU902070**	–
F. cf. *renatae* Möller, 1971	CE800	**GU901705**	**GU901797**	**GU901882**	**GU901968**	**GU902059**	–
F. sardorum Cognetti, 1901	CE735	**GU901718**	**GU901810**	**GU901895**	**GU901981**	**GU902071**	SMNH 108417*
F. sohlenii Rota, Healy & Erséus, 1998	CE736	**GU901719**	**GU901811**	**GU901896**	**GU901982**	–	SMNH 108418*
F. sohlenii Rota, Healy & Erséus, 1998	CE835	–	–	–	–	**GU902072**	–
F. striata (Levinsen, 1884)	CE893	**GU901720**	**GU901812**	**GU901897**	**GU901983**	**GU902073**	–
F. sylvatica Healy, 1979	CE801	**GU901721**	**GU901813**	**GU901898**	**GU901984**	**GU902074**	–
F. tuberosa Rota, 1995	CE23	DQ459884	AY340457	AF209453	AY340394	**GU902075**	–
F. waldenstroemi Rota & Healy, 1999	CE897	**GU901722**	**GU901814**	**GU901899**	**GU901985**	**GU902076**	–
Grania ersei Coates, 1990	CE565	**GU901723**	**GU901815**	**GU901900**	**GU901986**	**GU902077**	SMNH 90236
G. galbina De Wit & Erséus, 2007	CE258	**GU901724**	**GU901816**	**GU901901**	**GU901987**	**GU902078**	SMNH 108218*
G. maricola Southern, 1913	PDW40	**GU901725**	**GU901817**	**GU901902**	**GU901988**	GU473633	SMNH 107704
G. monospermatheca Erséus & Lasserre, 1976	PDW1	**GU901726**	**GU901818**	**GU901903**	**GU901989**	GU473628	SMNH 107808
G. trichaeta Jamieson, 1977	PDW34	**GU901727**	**GU901819**	**GU901904**	**GU901990**	GQ247645	AMS W35558
Hemienchytraeus sp. Lizard Island	CE1578	**GU901729**	**GU901820**	**GU901905**	**GU901991**	**GU902080**	SMNH 108419
Hemifridericia parva Nielsen & Christensen, 1959	CE794	**GU901730**	**GU901821**	**GU901906**	**GU901992**	**GU902081**	–
Henlea cf. *andreae* Rodriguez & Giani, 1986	CE814	**GU901731**	**GU901822**	**GU901907**	**GU901993**	**GU902082**	SMNH 108421
H. nasuta (Eisen, 1878)	CE824	**GU901732**	**GU901823**	**GU901908**	**GU901994**	**GU902083**	–
H. perpusilla Friend, 1911	CE853	**GU901733**	**GU901824**	**GU901909**	**GU901995**	**GU902084**	–
H. ventriculosa (Udekem, 1854)	CE1021	**GU901734**	**GU901825**	**GU901910**	**GU901996**	**GU902085**	SMNH 108422
Lumbricillus arenarius (Michaelsen, 1889)	CE962	**GU901736**	**GU901826**	**GU901911**	**GU901998**	**GU902086**	SMNH 108423
L. buelowi Nielsen & Christensen, 1959	CE891	**GU901735**	**GU901827**	**GU901912**	**GU901999**	**GU902087**	–
L. kaloensis Nielsen & Christensen, 1959	CE977	**GU901737**	**GU901828**	**GU901913**	**GU902000**	**GU902088**	SMNH 108424
L. lineatus (Müller, 1774)	CE983	**GU901738**	**GU901829**	**GU901914**	**GU902001**	**GU902089**	SMNH 108425
L. rivalis Levinsen, 1883	CE658	**GU901739**	**GU901830**	**GU901915**	**GU902002**	**GU902090**	–
L. tuba Stephenson, 1911	CE879	**GU901740**	**GU901831**	**GU901916**	**GU902003**	**GU902091**	–
Marionina argentea (Michaelsen, 1889)	CE807	**GU901741**	**GU901832**	**GU901917**	**GU902004**	**GU902092**	–

(*continued on next page*)

Table 1 (*continued*)

Taxon	Individual	12S	16S	18S	28S	COI	Voucher
M. clavata Nielsen & Christensen, 1961	CE849	**GU901746**	**GU901837**	**GU901921**	**GU902009**	**GU902097**	–
M. coatesae Erséus, 1990	CE136	**GU901747**	**GU901838**	**GU901922**	**GU902010**	–	–
M. communis Nielsen & Christensen, 1959	CE811	**GU901748**	**GU901839**	**GU901923**	**GU902011**	**GU902098**	–
M. filiformis Nielsen & Christensen, 1959	CE1040	**GU901749**	**GU901800**	**GU901885**	**GU901971**	**GU902062**	SMNH 108415*
M. cf. *levitheca* Erséus, 1990	CE1339	**GU901742**	**GU901801**	**GU901886**	**GU901972**	**GU902063**	–
M. cf. *minutissima* Healy, 1975	CE843	**GU901743**	**GU901802**	**GU901887**	**GU901973**	**GU902064**	–
M. cf. *nevisensis* Righi & Kanner, 1979	CE260	**GU901744**	**GU901803**	**GU901888**	**GU901974**	**GU902065**	–
M. cf. *riparia* Bretscher, 1899	CE1127	**GU901745**	**GU901836**	**GU901920**	**GU902008**	**GU902096**	SMNH 108427
M. sublitoralis Erséus, 1976	CE183	**GU901750**	**GU901841**	AY365458	**GU902013**	–	–
Mesenchytraeus armatus (Levinsen, 1884)	CE741	**GU901751**	**GU901842**	**GU901925**	**GU902014**	–	SMNH 108428*
M. flavus (Levinsen, 1884)	CE847	**GU901752**	**GU901843**	**GU901926**	**GU902015**	**GU902100**	–
M. pelicensis Issel, 1905	CE742	**GU901753**	**GU901844**	**GU901927**	**GU902016**	**GU902101**	–
M. rhithralis Healy & Fend, 2002	CE554	**GU901754**	**GU901845**	**GU901928**	**GU902017**	–	–
M. solifugus (Emery, 1898)	CE588	**GU901755**	**GU901846**	**GU901929**	**GU902018**	**GU902102**	SMNH 108429*
M. straminicolus Rota, 1995	CE743	**GU901756**	**GU901847**	**GU901930**	**GU902019**	**GU902103**	SMNH 108430*
Oconnorella cambrensis (O'Connor, 1963)	CE788	**GU901757**	**GU901848**	**GU901931**	**GU902021**	**GU902105**	–
O. tubifera (Nielsen & Christensen, 1959)	CE845	**GU901758**	**GU901849**	**GU901932**	**GU902022**	**GU902106**	–
Stephensoniella sterreri (Lasserre & Erséus, 1976)	CE941	**GU901762**	**GU901851**	**GU901934**	**GU902026**	**GU902111**	–
Stercutus niveus Michaelsen, 1888	CE841	**GU901763**	**GU901852**	**GU901935**	**GU902027**	**GU902112**	–
OUTGROUPS							
Capilloventer australis Erséus, 1993	CE437	**GU901683**	AY340448	AY365455	AY340384	–	–
Haplotaxis cf. *gordioides* (Hartmann, 1821)	CE438	**GU901728**	AY340461	AY365456	AY340398	**GU902079**	SMNH 108431
Pontodrilus litoralis (Grube, 1855)	CE130	**GU901759**	AY340473	AY365462	AY340410	**GU902107**	–
Dendrodrilus rubidus (Savigny, 1826)	CE522	**GU901691**	**GU901784**	**GU901868**	**GU901955**	**GU902046**	–
Criodrilus lacuum Hoffmeister, 1845	CE288	**GU901690**	**GU901783**	AY365461	**GU901954**	–	–
Insulodrilus bifidus Pinder & Brinkhurst, 1997	CE271	DQ459882	AY885636	AF411906	**GU901997**	–	–
Antarctodrilus proboscidea (Brinkhurst & Fulton, 1979)	CE436	**GU901679**	AY340447	AY365465	AY340383	–	–
Propappus volki Michaelsen, 1916	CE299	**GU901761**	AY340475	AY365457	AY340412	**GU902109**	–
Eclipidrilus frigidus Eisen, 1881	CE557	**GU901692**	GU592329[a]	**GU901869**	–	GU592300[a]	SMNH 105624
Lumbriculus variegatus (Müller, 1774)	CE27	DQ459885	AY885578	AF209457	–	FJ639298	–
Rhynchelmis tetratheca (Michaelsen, 1920)	CE322	–	AY340477	AY365464	AY340414	GU592316[a]	–
Pristina longiseta Ehrenberg, 1828	CE1588	**GU901760**	**GU901850**	**GU901933**	**GU902024**	**GU902108**	SMNH 108432
Nais alpina Sperber, 1948	CE529	DQ459906	DQ459943	DQ45997	**GU902020**	**GU902104**	–
Tubifex ignotus (Stolc, 1886)	CE211	DQ459921	AY885610	AF411879	**GU902029**	**GU902114**	–
Thalassodrilides bruneti Erséus, 1990	CE79	**GU901764**	AY885625	AF411904	**GU902028**	**GU902113**	–
Rhyacodrilus coccineus (Vejdovsky, 1875)	CE623	DQ459888	DQ459931	DQ459969	**GU902025**	**GU902110**	–
Pirodrilus minutus (Hrabe, 1973)	CE36	DQ459880	DQ459958	DQ45998	**GU902023**	AF064043	–

[a] Sequence published as new by Zhou et al. (2010).

analyzed by Bayesian inference (MCMCMC) using the parallel version of MrBayes 3.1.2 (Altekar et al., 2004; Huelsenbeck et al., 2001; Ronquist and Huelsenbeck, 2003) on an Apple MacPro with 8 processors of 3.0 GHz each. The two files were each run twice with four chains in each run for 50,000,000 generations, sampling once every 1000 generations, using the default MCMC setting for MrBayes except for a change in the branch length prior [Unconstrained:Exponential(100)], to avoid inflation of branch lengths, which has been shown to be an issue, particularly in partitioned Bayesian inference analyses (Brown et al., 2010). The resulting output tree files were examined for convergence using the online software AWTY (Wilgenbusch et al., 2004; Nylander et al., 2008), and were determined to have reached stationarity after 10,000,000 generations. The trees were then summarized into majority-rule consensus trees with the "sumt" command, using burn-ins of 10,000,000 generations. After this, the trees within the 95% confidence limit were tested for congruence using the SH-test function in PAUP* 4.0b (Shimodaira and Hasegawa, 1999; Swofford, 2002) in three different maximum-likelihood environments (all genes together, all mitochondrial genes, and all nuclear genes), using the GTR + G model of base substitution (with an empirically determined α for the gamma distribution of site rate variation) and empirical base frequencies. The test showed that the trees were not incongruent in any of the three environments ($P = 0.000$), and thus it was determined that all five loci could be used for a simultaneous analysis. The combined matrix was analyzed using the same procedure as above.

For comparison, a parsimony Jackknife analysis was conducted within PAUP* on the combined dataset, using 1000 replicates with 35% deletion probability. Each replicate consisted of 10 heuristic searches, using random addition sequence and TBR branch swapping. Jackknife frequencies were calculated on a majority-rule consensus tree and compared to the nodal supports (posterior probabilities) generated by the Bayesian inference analysis.

All trees analysed were rooted at *Capilloventer australis*, which previously has been found as the likely sister group of all other clitellates (Erséus and Källersjö, 2004; Erséus, 2005; Marotta et al., 2008).

All new sequences were submitted to GenBank (http://www.ncbi.nlm.nih.gov); accession nos. of these, as well as of previously published ones, are given in Table 1. The alignments were submitted to TreeBase (http://www.treebase.org).

3. Results

The separate Bayesian inference analyses of the mitochondrial and nuclear data sets generated trees largely congruent with each other and therefore not shown here. Both trees support monophyly of Enchytraeidae (mtDNA tree with posterior probability, pp 0.95; nDNA tree with pp 1.00). However, the resolution differs between these trees. In the nDNA tree, only 30 nodes receive maximum support (pp 1.00), whereas the one based on mtDNA has 41 nodes with pp 1.00. Moreover, the nuclear genes (which are slowly evolving) give more resolution among the outgroups than the more rapidly evolving mitochondrial genes.

The majority-rule consensus tree of the Bayesian inference analysis of the combined data set is shown in Fig. 1, with the most strongly supported nodes marked with black dots (posterior prob-

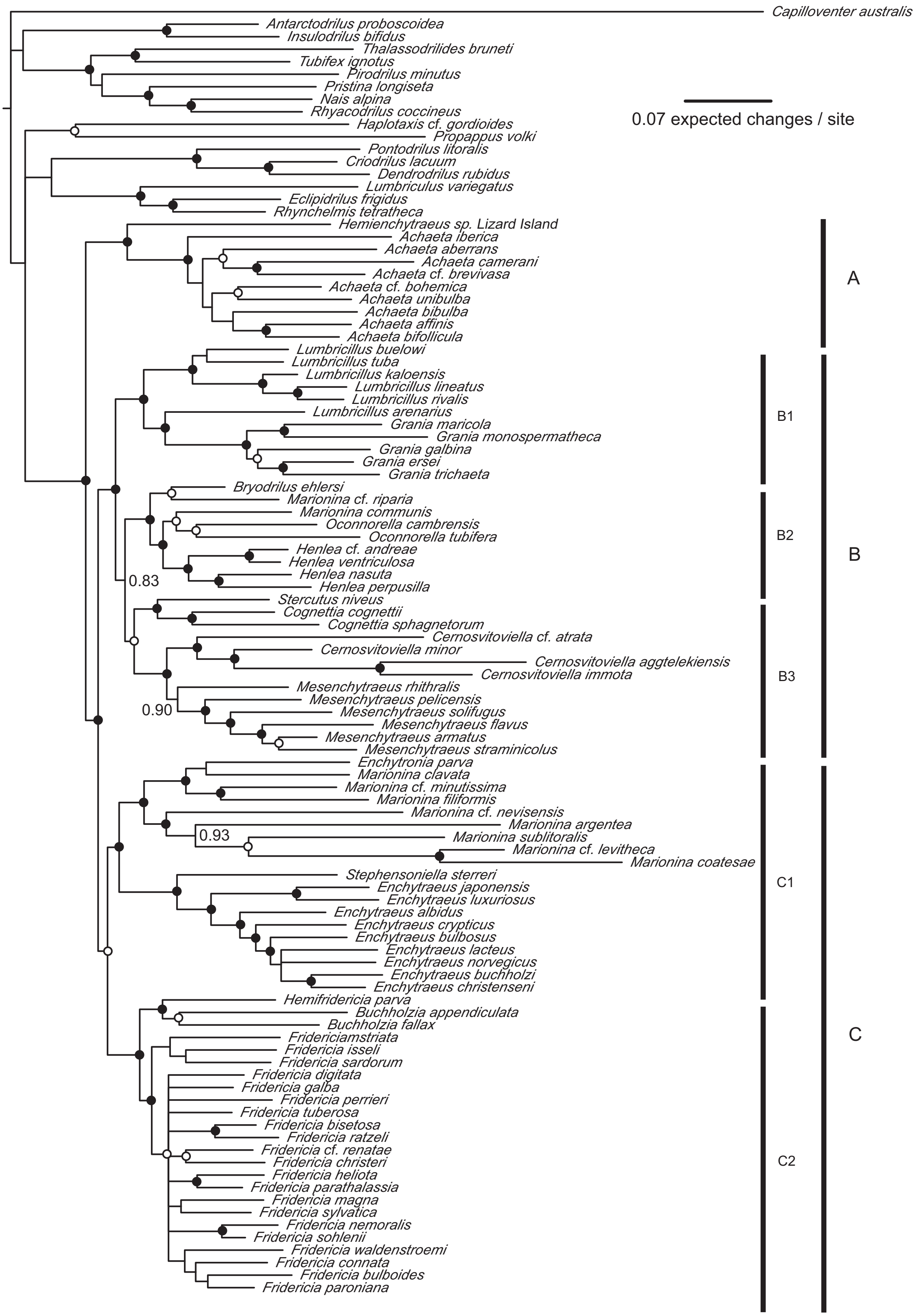

Fig. 1. Majority-rule consensus tree of Bayesian inference analysis of combined mitochondrial and nuclear gene sequences. Nodes with posterior probabilities (pp) of 1.00 marked with black dots, those with pp 0.95–0.99 with open circles, and a few others (discussed in text) marked with actual pp value. This means that a node shown as resolved, but without a particular value, has a pp between 0.50 and 0.94. The vertical bars denote ingroup clades discussed in the text.

ability, pp 1.00) or open circles (pp 0.95–0.99). In this tree, 57 nodes receive maximum support (1.00), and one of them is Enchytraeidae. A basal dichotomy of the family shows *Hemienchytraeus* + *Achaeta* as the sister group (Clade A) of all remaining enchytraeid genera (Clade [B + C]), both branches with pp 1.00. *Achaeta* is monophyletic (pp 1.00), while *Hemienchytraeus* is represented in this study by a single (undescribed) species only.

The remaining enchytraeid taxa are divided into two main clades, Clade B (supported by pp 1.00) and Clade C (pp 0.98). If pp ⩾0.95 is regarded as a cut-off level, Clade B is a trichotomy of three smaller clades (B1–B3), each with good support. Clade B1 (pp 1.00) contains all *Lumbricillus* and *Grania* species. *Grania* is supported (pp 1.00), and (also with pp 1.00) sister to *L. arenarius*, and these together form the sister group to the remaining *Lumbricillus* spp. (which also have pp 1.00).

Clade B2, which is fully resolved (using pp ⩾0.95 as cut-off), comprises *Bryodrilus* (one species investigated), *Oconnorella* (pp 0.95), *Henlea* (pp 1.00), and two species of *Marionina*, *M.* cf. *riparia* and *M. communis*, but the latter two are not forming a group. Instead, *Bryodrilus* + *M.* cf. *riparia* (sisters supported by pp 0.98) are the sister group to the other taxa, and *Oconnorella* + *M. communis* (pp 0.95) are most closely related to *Henlea* (the three together with pp 1.00).

Clade B3 (pp 0.97) contains four genera, separated in two strongly supported subclades (both with pp 1.00). The first of these is *Stercutus* (monotypic) plus *Cognettia* (pp 1.00), the second *Cernosvitoviella* (pp 1.00) plus *Mesenchytraeus* (but latter with pp 0.90 only).

Clade C is basally divided into two smaller ones, C1 and C2 (both with pp 1.00). Clade C1 is further divided into one group (pp 1.00) containing eight species of *Marionina* plus *Enchytronia parva*, and another (pp 1.00) with *Stephensoniella* (one species investigated) sister to *Enchytraeus* (pp 1.00). In the first group, *Enchytronia parva* is nested with *M. clavata, M. filiformis* and *M.* cf. *minutissima* (pp 1.00), and these four taxa together are the sister group to another (pp 1.00) containing *M.* cf. *nevisensis*, *M. argentea, M. sublitoralis, M.* cf. *levitheca* and *M. coatesae*.

Finally, Clade C2 (pp 1.00) encompasses all *Fridericia* species (supported by pp 1.00), plus *Hemifridericia* (one species investigated) and *Buchholzia* (pp 0.98), and the latter are sister groups (supported by pp 1.00).

Thus, regarding traditionally recognized genera for which at least two species are included in the study, *Achaeta, Grania, Oconnorella, Henlea, Cognettia, Cernosvitoviella, Enchytraeus, Buchholzia,* and *Fridericia* are well supported (pp 0.95–1.00). *Marionina* (polyphyletic) and *Lumbricillus* (paraphyletic), however, are rejected by the analysis.

Propappus volki (family Propappidae) was not found closely related to Enchytraeidae. The Bayesian analysis placed it (with pp 0.95) as sister to *Haplotaxis* cf. *gordioides* (Haplotaxidae).

The results of the parsimony jackknife analysis of the combined data set (Supplementary Fig. 1) were compared to those of the Bayesian analysis. In the jackknife tree, no nodes with any substantial support (cut-off arbitrarily set to 70%) are incongruent with clades supported by ⩾0.95 in the Bayesian analysis, with a single exception. The placement of *Marionina argentea*, which in the jackknife tree is the sister taxon to *Cernosvitoviella aggtelekiensis* + *C. immota* (jackknife support 81%); it is not placed even near to the other *Marionina* species that are members of Clade C1 in the Bayesian tree (Fig. 1). This unexpected position in the parsimony-based tree may be the effect of long branch attraction; *M. argentea* has the longest of all terminal ingroup branches.

Further, in the parsimony analysis, Enchytraeidae is supported by jackknife 96%, but the *Hemienchytraeus* + *Achaeta* group (Clade A in the Bayesian tree; Fig. 1) is supported by 64% only. Clade [B + C] (all other genera) also comes out as a group, but with a mere 51% jackknife support. Clades B1, B2 and C2 have values between 70% and 100%, B3 and C1 are unresolved. Only three (multi-species) genera are supported (with cut-off 70%): *Achaeta* (97%), *Cognettia* (99%), and *Grania* (100%); others are polyphyletic (*Marionina*; see above) or unresolved.

4. Discussion

This study strongly supports the monophyly of Enchytraeidae and the notion that Propappidae (one species investigated) is not nested within it (Coates, 1986), and our molecular data fail to recover the sister group relationship between the two families, found by, e.g., Marotta et al. (2008).

Within Enchytraeidae, two main lineages are recognized (Clades A and B + C in Fig. 1), and they both have maximum support by the molecular data. The first group (A) is here represented by *Hemienchytraeus* Černosvitov, 1934, and *Achaeta* Vejdovský, 1878, two genera with much of their distribution in tropical regions, and both placed in Achaetinae by Černosvitov (1937). In this subfamily, Černosvitov also included *Guaranidrilus* Černosvitov, 1937 (with northern and southern species in both the Old and New World), the monotypic, West African, *Aspidodrilus* Baylis, 1914, and the monotypic, European *Stercutus* Michaelsen, 1888. In our study, only the last-mentioned of these three genera was studied, and it came out nested inside Clade B3 and not with *Hemienchytraeus* and *Achaeta*. On morphological grounds, Coates (1990) concluded that the similarities of *Aspidodrilus* to achaetines (ventral anterior chaetae, and structure of the nephridia) must be regarded as plesiomorphic in the family, whereas this genus seems to have derived similarities, especially in gut diverticula and penial apparati, to *Henlea*. On the other hand, it seems likely that *Guaranidrilus*, along with *Tupidrilus* Righi, 1974, and certain nominal species of *Marionina* Michaelsen, 1890 (see Rota et al., 2008) are also members of Clade A, since they all share with *Achaeta* and *Hemienchytraeus* a distinct ganglionation of the nerve cord combined with the prostomial location of the head pore, free spermathecae, and nephridia with large anteseptals.

In the molecular study by Christensen and Glenner (2010), however, *Achaeta* is placed, with high support, in a group also comprising *Fridericia* Michaelsen, 1889, *Buchholzia* Michaelsen, 1886, *Cognettia* Nielsen and Christensen, 1959 and *Henlea* Michaelsen, 1889. As discussed further below, this contradictory result may be due to the different selection of outgroup taxa.

In this study, the sister group to Clade A (i.e., all remaining enchytraeid taxa) is well resolved with good support for most of its basal nodes. Clades B (pp 1.00) and C (pp 0.98) have good support, and although the suggested monophyly of B2 + B3 is poorly supported (pp 0.83), the subordinate Clades B1–B3 and C1–C2 are each strongly supported (pp 0.97 for B3, 1.00 for all others).

In Clade B1 we find a well-supported paraphyly of the genus *Lumbricillus* Örsted, 1844. The majority of our sampled species form a distinct group, whereas *L. arenarius* is the sister to *Grania* Southern, 1913. Nielsen and Christensen (1959, p.110) moved *Enchytraeus arenarius* Michaelsen, 1889 to *Lumbricillus* from one of several groups that had earlier been lumped into *Marionina*, and they did not seem to regard it as deviant in their [then] "well defined" genus *Lumbricillus* (op. cit., p. 97). They did, however, describe its testis sacs as only "somewhat lobed" and the nephridial postseptale as being covered by a layer of large hyaline peritoneal cells. Neither of these traits, however, is typical of *Grania*, and only some species of the latter share with *L. arenarius* the unusual proportions of the sperm funnels (ratio length/width up to 15). Within *Lumbricillus*, straight or slightly sigmoid chaetae are a condition shared by *L. arenarius, L. tuba* and *L. buelowi*, and this appears to be plesiomorphic to the markedly sigmoid chaetae of *L. lineatus,* *L. kaloensis* and *L. rivalis*. *Lumbricillus* is a vast genus with more than 80 species worldwide (Rodriguez and Rico, 2008), and much work remains, preferably incorporating molecular data, to resolve it completely. However, as our study includes the type species, *L. lineatus*, the bifurcation in Clade B1 provides evidence that *Lumbricillus* is a good genus, providing that *L. arenarius* (at least) is excluded from it. *Grania*, on the other hand, is one of the most homogeneous of the larger enchytraeid genera. Its 71 species (De Wit, unpublished compilation) are slender, nematode-like worms, with large straight chaetae (absent in one species) arranged singly and not in bundles; moreover, the chaetae are totally absent from at least a few anteriormost segments.

Both *Lumbricillus* and *Grania* are marine genera, the former being restricted to littoral and brackish-water habitats, the latter being truly marine with a range that includes the deep sea (Rota and Erséus, 2003). Other marine enchytraeids are found in *Stephensoniella* Černosvitov, 1934, *Enchytraeus* and *Marionina*, but according to our tree these genera are not closely related to *Lumbricillus* and *Grania*. Of these "marine" genera, Christensen and Glenner's (2010) only included *Enchytraeus* and *Lumbricillus* in their recent molecular study, and found them to form a paraphyletic group at the base of Enchytraeidae. From this they suggested that these two genera represent early successful attempts to utilize decaying seaweed on seashores, possibly predating the emergence of land plants. In effect, their tree indicates that the family originated on seashores and that all the seven terrestrial (or freshwater) genera included are more closely related to *Enchytraeus* than to *Lumbricillus*. In our tree (Fig. 1), the data of the 17 outgroups determined a root of Enchytraeidae in a radically different position than the corresponding root in Christensen and Glenner's tree. These latter authors used only one lumbriculid, *Lumbriculus variegatus* (also used in our study), and a lumbricid, *Lumbricus terrestris* (replaced by *Dendrodrilus rubidus* in our outgroup selection), whereas all our outgroup taxa represent also the families Naididae, Phreodrilidae, Propappidae, Almidae, Megascolecidae, Haplotaxidae and Capilloventridae. This extended outgroup sampling has given a stronger basis for a correct estimation of the position of the enchytraeid root; our results suggest that *Enchytraeus* and *Lumbricillus* are not part of an ancestral enchytraeid assemblage, but rather are derived groups, each with possible relationships to other marine genera (see further below).

The next clade to consider, B2, has maximum support by our data. It contains *Bryodrilus* Ude, 1892, *Oconnorella* Rota, 1995, *Henlea* Michaelsen, 1889, and two species still placed in *Marionina* (*M.* cf. *riparia* and *M. communis*). *Oconnorella* was established by Rota (1995) to accommodate species originally regarded as members of *Marionina*. Rota considered *Oconnorella* most closely related to *Henlea*, to which it is similar in several characters (the fan-wise, straight chaetae, the transversal orientation of the head pore, the occurrence of oesophageal appendages, the structure of the nephridia and, partly, the shape of spermathecae), but she also pointed out its great resemblance to *Bryodrilus*. Thus, Clade B2 has much support in morphology, but our study also adds two more of the former species of the heterogeneous genus *Marionina* to this assemblage. It is remarkable that *M. communis* has chaetae of unequal length in bundles of three (a pattern easily derived from a fan-wise arrangement by loss of the medial element), and nephridia with efferent ducts arising anteroventrally (as typical of *Henlea, Oconnorella* and *Bryodrilus*), although with a marionine anteseptale. This gives further strength to Rota's suggestion (1995) that also *Marionina libra* Nielsen and Christensen, 1959 may be close to *Oconnorella*. Both *M.* cf. *riparia* and *M. communis* have unusually numerous preclitellar nephridia for *Marionina*, from 6/7 through 9/10 (Rota, pers. obs.), but while *M. communis* goes with *Oconnorella* and *Henlea* (straight chaetae), *M.* cf. *riparia* goes with *Bryodrilus* (sigmoid chaetae). Černosvitov (1937) already placed *Henlea* and *Bryodrilus* in the same subfamily, Henleainae, but on the other hand, he regarded *M. riparia* [together with several other species within "*Pachydrilus* (subgenus *Marionina*)"] as a member of another subfamily, Enchytraeinae. Christensen and Glenner (2010) concluded that *Henlea* is close to *Cognettia*, but this relationship was only supported by pp 0.82 in their tree, and not supported at all in ours (Fig. 1).

Clade B3 corroborates that *Stercutus* is the sister group of *Cognettia* Nielsen and Christensen, 1959, a position already suggested by Dózsa-Farkas (1973). In addition, we observe that the chromosome number is similar in these two genera and unusually high in the family ($n = 50$ in *Stercutus*; $n = 54$ in *Cognettia*) (Nielsen and Christensen, 1959; Dózsa-Farkas, 1973). Further, Clade B3 supports that *Stercutus* and *Cognettia* are the sister group of *Mesenchytraeus* Eisen, 1878, plus *Cernosvitoviella* Nielsen and Christensen, 1959. The latter two genera may be sister taxa, but the monophyly of *Mesenchytraeus* depends on the inclusion of *M. rhithralis*, which here is only moderately supported (pp 0.90). Healy and Fend (2002) described *M. rhithralis* as being peculiar in possessing an intersegmental septum and annexed pharyngeal glands at 3/4 (septa anterior to 4/5 are normally missing in enchytraeids) and unmodified vasa deferentia (lacking the ectal expansion or 'atrium') and simple penial bulbs (devoid of accessory glands). The same features had been reported before only in another member of *Mesenchytraeus*, *M. kuril* Healy and Timm, 2000. This issue should be further investigated as the two species may deserve to be allocated in a separate genus. Although the species of *Mesenchytraeus* are considerably larger than those of *Cernosvitoviella*, both genera have characteristic sigmoid chaetae, with distinct nodes, and much reduced interstitial tissue between the loops of the nephridial canal (Nielsen and Christensen, 1959). A close relationship between *Mesenchytraeus* and *Cernosvitoviella* was also found by Christensen and Glenner (2010), who noted that they are the only enchytraeid genera with nephridia similar to those found in more typical aquatic oligochaete families.

Clade C contains two maximally supported clades, one (C1) including several marine littoral species (within genera *Marionina*, *Stephensoniella* and *Enchytraeus*), the other (C2) with only typical terrestrial taxa (*Hemifridericia, Buchholzia* and *Fridericia*). One of the two sister groups of Clade C1 is a strongly supported group of nine small enchytraeid taxa: *Enchytronia* (one species investigated), and eight species of *Marionina*. All these taxa have long branches indicating large interspecific genetic variation, but it is impossible to know whether this reflects that the taxa sampled are only a few terminal members of an old, much diversified group, or if there has been rapid evolution (high substitution rates) in the individual lineages. Possibly, both factors are in operation. The first alternative is supported by the fact that the taxa included in our study are indeed only a few examples of the large assemblage of small species currently assigned to *Marionina*, and it is reasonable to anticipate that many of the other members of this taxon would fall within this clade if they were to be added in a molecular study. The two sister taxa, *M.* cf. *levitheca* and *M. coatesae*, are morphologically distinguished only by minor differences in their spermathecae (Erséus, 1990), and at the same time they are genetically well separated (see Fig. 1).

Interestingly, the *Marionina*/*Enchytronia* group is divided in two well-supported subclades, one of which contains largely marine littoral species, i.e., *M.* cf. *nevisensis* through *M. coatesae* in Fig. 1, although the ubiquitous *M. argentea* is also found in terrestrial and limnic habitats. The other subclade (*M. clavata* through *M.* cf. *minutissima*, including *E. parva*) is exclusively non-marine. The marine subclade receives morphological support from the pharyngeal pattern of bifurcation of the dorsal blood vessel (no data available for *M. sublitoralis* however).

Marionina Michaelsen, 1890 is an artificial taxon containing more than one evolutionary lineage, and is in great need of revision. For this specific reason, it was deliberately excluded from the molecular analysis by Christensen and Glenner (2010). The type species of *Marionina*, the subantarctic *Pachydrilus georgianus* Michaelsen, 1888, was recently redescribed (Rota et al., 2008; Schmelz and Collado, 2008), and a lectotype was designated (by Rota et al.). The two redescriptions are surprisingly similar(!) in terms of taxonomic criteria and characters examined but the final portraits of the species are not totally identical. Nevertheless they both lead to a combination of morphological features that does not completely overlap with that of any other genus defined today. Furthermore, few of the many nominal species currently included in *Marionina* will probably prove to share this combination (see Rota et al., 2008, p. 434; Schmelz and Collado, 2008). *Marionina georgiana* is similar to *Lumbricillus* with regard to chaetal morphology, patterns of nervous and circular systems, and simplicity of the alimentary system, but differs from this genus in coelomocyte appearance, testes and penial bulb morphology (the difference from a lumbricilline penial bulb holds true, both for the reconstruction provided by Rota et al. and for that given by Schmelz and Collado). At the same time, the species differs from most of its own nominal congeners in nephridial anatomy and gland patterns of the clitellum. *Marionina georgiana* thus seems to represent an evolutionary lineage, the phylogenetic position of which is more likely to be in the vicinity of Clade B1, rather than in close relationship with Clades B2 or C1 where its nominal congeners are situated (see Fig. 1).

Despite its great genetic variation, the *Marionina*/*Enchytronia* part of Clade C1 is a strongly supported group, and it can be predicted that many additional nominal species of *Marionina*, if genetically investigated, will be found to belong to this lineage. Moreover, if *M. georgiana* is not among these other members, another generic name needs to be established for it; more than one name, if the lineage is to be further divided. Among the nine taxa assessed here, *Enchytronia parva* is the only species that is the type of a genus (*Enchytronia*). One option is thus to expand the definition of *Enchytronia* to include also all species of the *Marionina* assemblage in Clade C1, or to restrict this genus to those species that belong to the "non-marine" subclade within it, so far represented also by *M. clavata*, *M. filiformis* and *M.* cf. *minutissima*. However, considering the limited taxon sampling in this study, it would be premature to formally propose these nomenclatural actions at this point.

The second part of the bifurcation of Clade C1 contains *Stephensoniella* and *Enchytraeus*. *Stephensoniella* was established for a marine littoral species originally placed in *Enchytraeus*, *E. marinus* Moore, 1902 (Černosvitov, 1934). Coates (1983) added two species, including *S. sterreri* that represents the genus here, and supported Černosvitov's view that *Stephensoniella* has similarities with both *Lumbricillus* and (the heterogeneous) *Marionina*. She also repeated Černosvitov's notion that *Stephensoniella* differs from *Enchytraeus* by its compact penial bulbs and lack of peptonephridia. This study indeed corroborates that *Stephensoniella* is closely related to, but not a part of *Enchytraeus*. Christensen and Glenner (2010) noted that *Enchytraeus* and *Lumbricillus* have particular testis sacs enclosing the maturing sperm, a feature present also in *Stephensoniella* (Černosvitov, 1934; Rota et al., 2008).

The final Clade to be discussed, C2, is the strongly supported group comprising the largely terrestrial genera *Hemifridericia* Nielsen and Christensen, 1959, *Buchholzia* Michaelsen, 1886, and *Fridericia* Michaelsen, 1889. Černosvitov (1937) placed *Fridericia* in a monotypic subfamily, Fridericinae, while he regarded *Buchholzia* as a member of Henleinae; *Hemifridericia* being unknown at the time. Christensen and Glenner (2010), however, also obtained maximum support for a clade containing *Fridericia* and *Buchholzia*. In terms of morphology, the synapomorphy uniting *Fridericia*, *Hemifridericia* and *Buchholzia* is obvious and exclusive: the three genera possess (without exceptions) small anucleated hyaline corpuscles floating in the coelomic fluid along with the ordinary type of coelomocytes. Only the latter, larger nucleated cells, are homologous to the coelomocytes of the other genera. The controversial *Christensenidrilus blocki* Dózsa-Farkas & Convey, 1998 (see Rota et al., 2008), characterized by possessing only anucleate, small, stick-like, hyaline coelomocytes could also fall in this clade.

Interestingly, *Hemifridericia bivesiculata* Christensen and Dózsa-Farkas, 2006, from the Arctic Archipelago of Canada, has been described to differ from the type species *H. parva* in possessing ventral oesophageal appendages, in the form of two almost spherical hollow sacs with short unpaired stalk in III (or IV?). *Fridericia* and *Buchholzia* also have oesophageal appendages (peptonephridia) in IV, but always paired: in the former they are hollow, elongate and with ventrolateral roots, in the latter they are hollow or solid and with dorsolateral stalks. To be noted, however, is that other enchytraeid species, notably in *Marionina* (see Xie and Rota, 2001), are known to possess oesophageal pouches in IV (including *M. clavata*), which suggests either convergent evolution, or that these structures are plesiomorphic, at least to Clade C.

In our analysis, *Fridericia* itself has maximum support, but within *Fridericia*, resolution is low and terminal branches are short, particularly if compared to the corresponding features in the "*Enchytronia*/*Marionina*" part of Clade C1 (Fig. 1; discussed above). This indicates that *Fridericia* has had a recent process of extensive radiation, as suggested also by a high number of nominal species and genetic variants described to date (Rota, 1994b, 1995; Rota et al., 1998; Rota and Healy, 1999; Schmelz, 2003; Cech and Dózsa-Farkas, 2005; Dózsa-Farkas, 2009).

To summarize, the molecular data set analyzed in this study has enabled us to recognize a number of well-supported evolutionary lineages among the Enchytraeidae, and to show that a majority of the nominal genera analysed are monophyletic. The phylogeny of some of the groups, however, need to be further scrutinized, and more extensive taxon and gene sampling will be needed to resolve the evolutionary relationships among basal as well as more terminal lineages.

Acknowledgments

We are indebted to Philippe Bouchet, and the Total Foundation, for the invitation (to C.E.) to participate in the LIFOU 2000 expedition to New Caledonia; to the staffs of the Caribbean Marine Research Center on Lee Stocking Island (Bahamas), the Smithsonian Marine Station at Fort Pierce (Florida), and the Lizard Island Research Station (Great Barrier Reef), for use of their excellent facilities; to Anna Ansebo, Leyla Arsan, Stephen Atkinson, Anders Boström, Achille Casale, Bent Christensen, Steve Fend, Lena Gustavsson, Andreas Haller, Paula Hartzell, (the late) Brenda Healy, Anne Hoggett, Stefan Lundberg, Yoshio Nakamura, Henning Petersen, Valentin Petushkov, Adrian Pinder, Sherry Reed, Jörg Römbke, Tarmo Timm, Björn Tunberg, Hongzhu Wang, and Lyle Vail, for assisting in field work or otherwise providing specimens; to Anna Ansebo, Erik Boström, Bodil Cronholm, Jeffrey Hunt, Sebastian Kvist, Maria Lindström, and Erica Sjölin, for technical assistance in the molecular lab; and to the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS), the Swedish Taxonomy Initiative, and The Royal Society of Arts and Sciences in Göteborg, for financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2010.07.005.

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