UNIVERSIDADE FEDERAL DO PARANÁ

ALESSANDRO LÍVIO PRANTONI

OLIGOQUETAS MARINHOS: NOVOS REGISTROS GLOBAIS COM ÊNFASE NO ATLÂNTICO SUL

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Orientador: Dr. Paulo da Cunha Lana
Co-orientador: Dr. Marcelo Visentini Kitahara

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em SISTEMAS COSTEIROS E OCEÂNICOS da Universidade Federal do Paraná foram convocados para realizar a arguição da Tese de Doutorado de **ALESSANDRO LIVIO PRANTONI**, intitulada: "**OLIGOQUETAS MARINHOS: NOVOS REGISTROS GLOBAIS COM ÊNFASE NO ATLÂNTICO SUL**", após terem inquirido o aluno e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO.

Pontal do Paraná, 20 de Abril de 2016.

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(Presidente da Banca Examinadora)

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Prof MARCOS SOARES BARBEITOS (UFPR)

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RESUMO

Os oligoquetas “strictu sensu” (i.e. Clitellata, exceto Hirudinea, Branchiobdellida e Acanthobdellida) são encontrados em uma grande variedade de ambientes, desde regiões costeiras como estuários e manguezais até ambientes extremos como as fossas hadais e glaciais. Historicamente, os oligoquetas foram classificados em dois principais grupos, os Microdrilli, formas menores, geralmente associadas à água e os Megadrilli, formas maiores associadas a ambientes terrestres. Aproximadamente 600 das 1700 espécies aquáticas conhecidas são marinhas. As formas marinhas são geralmente pequenas, variando entre menos de 1 milímetro e poucos centímetros de comprimento. As espécies mais abundantes pertencem à controversa família Tubificidae/Naididae, com cerca de 450 marinhas. Outras famílias com representantes marinhos são Enchytraeidae, Capilloventridae e Randiellidae. Os oligoquetas marinhos são numericamente importantes nas associações bênticas marinhas e exercem um importante papel na produtividade secundária, remineralizando nutrientes tornando-os novamente disponíveis para os produtores primários. Entretanto, por serem muito similares ou mesmo indistinguíveis, são raramente identificados em nível especifico, genérico ou mesmo no nível de família, sendo reportados simplesmente como “Oligochaeta” na maioria dos estudos ecológicos. Algumas espécies como *Tubificoides benedii* são conhecidas pela alta tolerância a poluição por enriquecimento orgânico. Os oligoquetas podem se reproduzir tanto sexuada como assexuadamente. O ciclo de vida destes organismos também é muito pouco conhecido e a escassez de estudos deve-se principalmente a: (1) ausência de classes discretas de idade; (2) tanto o tamanho do corpo como o número de segmentos não são características precisas do estagio de maturidade; (3) o tempo consumido na análise do estágio de maturidade de cada espécime em microscópio; (4) os casulos das diferentes espécies de oligoquetas marinhos não são identificáveis por características morfológicas. A diversidade de oligoquetas marinhos é bem conhecida em praticamente todo o hemisfério norte. No entanto, o conhecimento taxonômico dos oligoquetas marinhos no Atlântico Sul é claramente insatisfatório. Apesar de alguns estudos pontuais entre as décadas de 50 e 80 no século passado, o grupo permanece ignorado pela comunidade científica local. Neste contexto, esta tese traz importantes avanços para o conhecimento do grupo na região, sumarizando o conhecimento atual através de uma revisão bibliográfica atualizada e mais cinco capítulos em forma de artigos científicos. O primeiro artigo faz uma visão crítica do conhecimento atual dos oligoquetas marinhos no Brasil. Ainda neste artigo, a distribuição geográfica do naidídeo (sensu Erséus et al., 2008) *Tectidrilus* c.f. *gabriellae*, originalmente descrito para a Ilha Bela, São Paulo e conhecida também para algumas regiões do Caribe é estendida para a Baía de Paranaguá, Paraná. O segundo artigo traz o primeiro registro de uma espécie aqueta (desprovida de cerdas) do gênero *Marionina* para América do Sul. Esta espécie é muito similar a *Marionina nevisensis* descrita para Ilha de Nevis no Caribe, entretanto, análises moleculares preliminares sugerem se tratar de uma nova espécie. No terceiro artigo é proposta uma nova hipótese filogenética além da descrição de nove novas espécies de outro gênero de Enchytraeidae, *Grania.* Sete destas espécies, *Grania brasiliensis* **sp. nov** do Brasil; *Grania bekkouchei* **sp. nov.**, *Grania cryptica* **sp. nov.,** *Grania capensis* **sp. nov.,** *Grania simonae* sp. nov.**,** da África do Sul, *Grania hinojosai* **sp. nov.** e *Grania chilensis* **sp. nov.,** do Chile são o primeiro registro do gênero tanto no continente africano como no sul-americano, e duas, *Grania unitheca* **sp. nov.,** e *Grania carolinensis* **sp. nov.**, novos registros para América do Norte. O quarto artigo fornece a primeira lista das espécies de *Grania*, incluindo dados históricos e recentes de todas as espécies válidas descritas até hoje, suas respectivas sinonímias, além de informações a respeito da sua distribuição geográfica. Ao todo foram registradas 80 espécies, descritas nos cinco continentes, contemplando as bacias oceânicas e o ambiente antártico. No quinto artigo, a monofilia de 80 espécimes do Atlântico sul e Antártica foi estatisticamente testada através da combinação de quatro métodos estatísticos. Foram identificadas 32 espécies potenciais pertencentes a duas familias de oligoquetas marinhos. Neste artigo, as espécies *Doliodrilus fibrisaccus, Limnodriloides pierantonii, L. rubicundus, L. sacculus, Paranais frici, Stephensoniella sterreri e Thalassodrilides gurwitsch,* são registradas pela primeira vez na costa leste da América do Sul (Brasil). Este artigo confirma ainda a utilidade das abordagens moleculares na identificação de oligoquetas marinhos que são dificilmente identificáveis unicamente pela morfologia.

Palavras-chave: Oligoquetas marinhos; marcadores moleculares; filogenia molecular; taxonomia; Atlântico Sul

ABSTRACT

The oligochaetes “strictu sensu” (i.e. Clitellata, exept Hirudinea, Branchiobdellida e Acanthobdellida) are found in a large variety of environments, from coastal regions such as estuaries and mangroves to extreme habitats such as glaciers and hadal trenches. Historically, oligochaetes have been divided into two groups, “Microdrili”, the smaller worms generally associated with the aquatic environment, and “Megadrili”, the larger forms most of which are found in terrestrial habitats. Approximately 600 of 1700 known aquatic species are marine. The marine forms are generally small, ranging from less than 1 millimeter to a few centimeters long. Among the marine taxa, the most abundant belong to the controversial family Tubificidae / Naididae, with about 450 species Other groups with marine representatives are Enchytraeidae, Capilloventridae, and Randiellidae. Marine oligochaetes are numerically important in marine benthic assemblages, and play an important role in the secondary productivity, remineralizing nutrients and making them available again to the primary producers. However, due their morphological similarity, they are rarely identified to species, genus or even to family level, and frequentely reported as "Oligochaeta" in most ecological studies. Some species such as *Tubificoides benedii* are known for the high tolerance to pollution by organic enrichment.The marine oligochaetes are able to reproduce sexually and asexually. The life cycle of these organisms is also poorly known, and the lack of studies is mainly due to: (1) absence of discrete age classes; (2) Neither body size nor number of segments are precise features of maturity stage; (3) the time consumed in the analysis of the stage of maturity of each specimen under a microscope; (4) the cocoons of different species are not identifiable by morphological features. The diversity of marine oligochaetes is well known in the northern hemisphere. However, the taxonomic knowledge of marine oligochaetes in the South Atlantic is clearly unsatisfactory. Although some sporadic studies between the 50’s and 80’s in the last century, the group remains ignored by the local scientific community. In this context, this thesis provides important advances in the knowledge of the group in the South Atlantic, summarizing current knowledge of this fauna through an updated literature review and five chapters written in the form of scientific articles.The first article is a critical overview of the current knowledge of marine oligochaetes in Brazil. In this article, the geographical distribution of the naidid (sensu Erséus et al., 2008) *Tectidrilus c.f. gabriellae*, originally described for Ilha Bela, São Paulo and also known for some regions of the Caribbean is extended to Bay of Paranaguá, Paraná. The second article presents the first record of an achaeta species (species that devoid of chaetae) of the geneus *Marionina* to South America. This species is very similar to Marionina nevisensis described for Nevis Island in the Caribbean however, preliminary molecular analysis suggests it is a new species. In the third article, we provide a new phylogenetic hypothesis and also the description of nine new species of another Enchytraeidae genus, *Grania*. Seven of these species *Grania brasiliensis* **sp. nov.** from Brazil; *Grania bekkouchei* **sp. nov.**; *Grania cryptica* **sp. nov.**; *Grania capensis* **sp. nov.**; *Grania simonae* **sp. nov.**, from South Africa, *Grania hinojosai* **sp. nov.** and *Grania chilensis* **sp. nov.**, from Chile are the first report for for this genus in both, Africa and South America, and two, *Grania unitheca* **sp. nov.**, and *Grania carolinensis* **sp. nov.**, new records for North America. The fourth article provides the first list of *Grania* species, including historical and recent data of all valid species described to date, their respective synonyms, and information about their geographical distribution. A total of 80 species were recorded described in all five continents, covering the ocean basins and Antarctic region. In the fifth article, the monophyly of 80 species of the South Atlantic including Antarctica was statistically tested by combining four statistical methods. We could identify 32 potential species belonging to two families of marine oligochaetes. In this article, the species *Doliodrilus fibrisaccus*, *Limnodriloides pierantonii*, *L. rubicundus*, *L. sacculus*, *Paranais Frici*, *Stephensoniella sterreri*, and *Thalassodrilides gurwitschi* are recorded for the first time from the eastern coast of South America (Brazil). This study also confirms the usefulness of molecular approaches to identify marine oligochaetes which are hardly identifiable through morphology only.

Keywords: Marine oligochaetes; molecular markers; molecular phylogeny; taxonomy; South Atlantic

LISTA DE ARTIGOS

I. A taxonomic overview of marine and estuarine oligochaetes from Brazil.

Prantoni, A.L., Di Domenico, M., Lana, P.C., 2013. **Marine Biodiversity**. 44 (3), 275-278.

II. First record of achaetous Marionina Michaelsen, 1890 (Annelida: Clitellata: Enchytraeidae) in the southern Atlantic

Prantoni, A.L., Di Domenico, M., Lana, P.C., 2014. **Zoosymposia**, 9, 10-13.

III. First reports of *Grania* (Clitellata: Enchytraeidae) from Africa and South America - Molecular phylogeny and descriptions of nine new species

Prantoni, A.L., De Wit, P., Erséus, C., 2016. **Zoological Journal of the Linnean Society,** 176, 485-510.

IV. Global checklist of species of *Grania* (Clitellata: Enchytraeidae) with remarks on their geographic distribution

Prantoni, A. L., Lana, P. C., Erséus, C. Revista alvo: **Zootaxa**

V. Genetic diversity marine oligochaetes from the Southern Atlantic (Annelida: Clitellata) as revealed by DNA-barcoding

Prantoni, A. L., Belmonte-Lopes, R., Lana, P. C, Erséus, C. Revista alvo: **Invertebrate Systematics**

SUMÁRIO

Introdução

Relações filogenéticas de Annelida, Clitellata e Oligochaeta: um campo controverso

O relacionamento filogenético dos Annelida entre si e com outros grupos de invertebrados é motivo de intenso debate na biologia evolutiva dos Metazoa (Rouse & Fauchald 1997; Bartolomaeus et al. 2005; Rousset et al. 2007; Struck et al. 2007; Weigert & Bleidorn, 2016). Com base principalmente em características morfológicas, o filo tem sido tradicionalmente dividido em Polychaeta e Clitellata (Rouse & Fauchald, 1997; Brusca & Brusca, 2003) ou Polychaeta, Oligochaeta e Hirudinea (Marshall & Williams, 1972; Ruppert et al., 2005).

Em uma primeira abordagem filogenética, conduzida a partir de uma matriz de dados morfológicos bastante abrangente, Rouse & Fauchald (1997) propuseram um divisão dos Polychaeta em dois clados distintos, Palpata (subdividido em Canipalpata e Aciculata) e Scolecida (Figura 1). Neste estudo, as famílias Aelosomastidae e Potamodrilidae, até então referidas a Oligochaeta, foram pela primeira vez posicionadas dentro de Polychaeta, enquanto Clitellata, Echiura e Sipuncula foram classificados como grupos monofiléticos, separados dos demais.

` As primeiras tentativas de reconstrução da filogenia dos Annelida com base em dados moleculares datam do final do século XX e começo do XXI. A maioria destes estudos foi baseada em apenas um marcador (18S RNA) (Struck et al., 2002; Blaidorn et al., 2003; Weigert & Bleidorn, 2016). Estes estudos, no entanto, cobriam apenas uma pequena fração da diversidade de anelídeos e não foram suficientemente robustos para reconhecer Palpata, Canipalpata e Scolecida, e mesmo Polychaeta como grupos monofiléticos (Weigert & Bleidorn, 2016). Mesmo assim, a incorporação de dados moleculares já fornecia evidências satisfatórias para inclusão de Siboglinidae (Pogonophora + Vestimentífera) e Echiura dentro de Annelida e um suporte adicional para o posicionamento de Clitellata dentro de Polychaeta (McHugh 2000; Bleidorn et al. 2003; Weigert & Bleidorn, 2016).

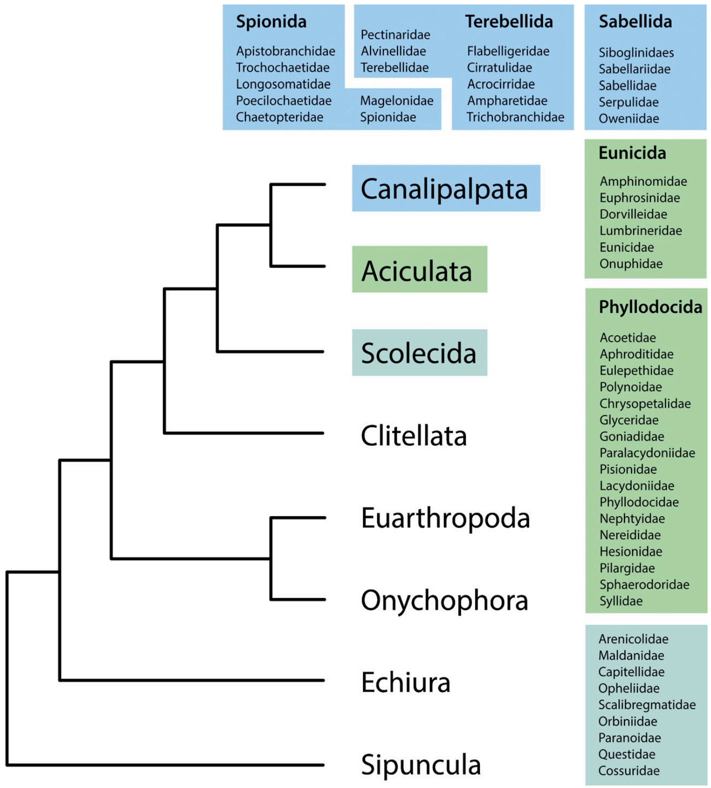

Figure 1: Filogenia proposta por Rouse & Fauchald (1997). (Fonte: Weigert & Bleidorn, 2016).

Na medida em que as técnicas moleculares foram se tornando mais acessíveis e com incorporação de outros genes (e.g. 28S rRNA, 16rRNA, H3, U2 snRNA, e COI), houve um aumento considerável na resolução das análises filogenéticas. Através destas abordagens “multi-locus”, muitas vezes em conjunto com dados morfológicos, algumas relações filogenéticas, como a inclusão de Siboglinidae, Echiura e Clitellata dentro de Annelida/Polychaeta foram resolvidas (Struck et al., 2007; Rousset et al., 2007), com poucas controvérsias (Christoffersen, 2012). Entretanto, a monofilia de Annelida ainda permaneceu controversa (Rousset et al., 2007; Zrzavý et al. 2009; Weigert & Bleidorn, 2016). Embora com um suporte fraco, o primeiro estudo reportando Annelida como grupo monofilético e incluindo Sipuncula foi conduzido por Struck et al. (2007). Entretanto, a posição filogenética de certos táxons como Myzostomida e Diurodrilidae continuou obscura (Struck et al., 2011; Weigert & Bleidorn, 2016).

Finalmente, com o surgimento de novas técnicas moleculares, incluindo os chamados sequenciamentos de nova geração, novas hipóteses filogenéticas robustas e que corroboram a monofilia de Annelida foram propostas (Struck et al., 2011; Weigert et al., 2014; Struck et al., 2015; Weigert et al., 2016; Weigert & Bleidorn, 2016). De acordo com estes estudos, atualmente os anelídeos são compostos por dois grandes grupos monofiléticos: Errantia (incluindo Myzostomida) e Sedentaria (incluindo Clitellata e Echiura), que juntos formam os Pleistoannelida (Struck, 2011), e por mais 5 linhagens consideradas basais, Sipuncula, Amphinomida, Chaetopteridae, Magelonidae e Oweniidae (Weigert et al., 2014; Weigert & Bleidorn, 2016) (Figura 2).

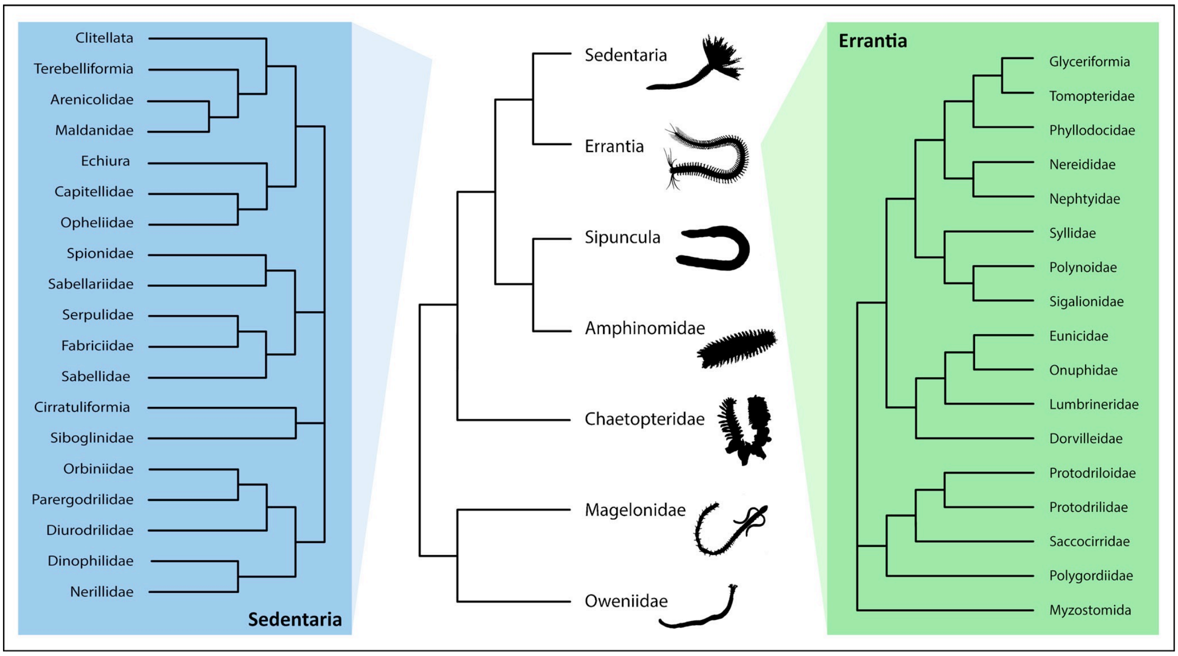

Figure 2: Relações filogenéticas de Annelida de acordo com o conhecimento atual. (Fonte: Weigert & Bleidorn, 2016).

Nesta nova “visão filogenética”, Clitellata, antes considerado um grupo irmão, é visto agora como um clado monofilético dentro de Polychaeta (Struck et al., 2011; Weigert et al., 2014; Struck et al., 2015; Weigert et al., 2016; Weigert & Bleidorn, 2016). Entretanto, se comparados a Polychaeta, os Clitellata em geral, principalmente os marinhos, são pobremente representados em todos os estudos filogenéticos recentes. Como exemplo desta baixa representatividade, Struck et al. (2007) incluiram em sua análise apenas cinco espécies, das quais três lumbricídeos terrestres, um naidídeo dulcícola e um hurudíneo. Zrzavý et al. (2009) incluíram quatro táxons, um capilloventrídeo estuarino/dulcícola, um naidídeo dulcícola, um lumbricídeo terrestre e um hirudíneo. Struck et al. (2011) consideraram oito táxons, três lumbricídeos e um megascolecídeo terrestres, um tubificídeo (naidídeo sensu Erséus et al., 2008) dulcícola e três hirudíneos. Weigert et al. (2014) e Struck et al. (2015) contemplaram dez táxons, três lumbricídeos e dois megascolecídeos terrestres, dois naidídeos dulcícolas e três hirudíneos. O estudo mais abrangente em termos de Clitellata é o de Rousset et al. (2007). Mesmo assim, dos dezoito táxons utilizados, apenas a espécie *Tubificoides amplivasatus* é exclusivamente marinha.

Por outro lado, a monofilia de Clitellata é bem fundamentada tanto por abordagens morfológicas como moleculares (Erséus, 2005; Marotta et al., 2008). Estudos filogenéticos unindo dados moleculares e caracteres morfológicos fornecem fortes evidências de que os oligoquetas pertencentes à ordem Lumbriculida são um grupo irmão dos ectoparasitas compostos por Hirudinida, Acanthobdellida e Branchiobdellida (Martin, 2001; Siddall et al., 2001; Erséus & Källersjö, 2004; Martin et al., 2007; Marotta et al., 2008) (Figura 3). Desta forma, os oligoquetas tornam-se parafiléticos (Rousset et al., 2007), ou todo o grupo, incluindo os tradicionais oligoquetas e hirudíneos, pode ser denominado de Oligochaeta (Siddall et al., 2001; Martin et al., 2007) ou Clitellata (Martin, 2001; Erséus & Kallersjo, 2004; Rousset et al., 2007).

Embora existam argumentos para utilizar as duas nomenclaturas, o nome Clitellata é mais comumente utilizado devido à presença de uma sinapomorfia (clitelo) em hirudíneos e oligoquetas. Nesta tese optei pelo uso do termo Oligochaeta por ser menos inclusivo (i.e. Clitellata, exceto Hirudinea, Branchiobdellida e Acanthobdellida).

A origem aquática de Clitellata foi confirmada na última década (Rousset et al. 2008), entretanto ainda não se sabe se o ancestral comum a todos os clitelados é originário de ambientes marinhos ou de água doce. A família Capilloventridae, posicionada filogeneticamente como grupo irmão dos clitelados atuais (Erséus & Källersjö, 2004), possui representantes tanto marinhos como dulcícolas, o que dificulta bastante as inferências a respeito do habitat do ancestral comum.

Os oligoquetas “stricto sensu” (i.e. Clitellata, exceto Hirudinea, Acanthobdellida e Branchiobdellida) colonizaram uma grande variedade de ambientes, desde regiões costeiras como estuários e manguezais (Erséus, 2005) até ambientes extremos como sedimentos finos das fossas hadais (Rota & Erséus, 2003; Erséus & Rota, 2003) e glaciais (Hartzell et al., 2005). Podem ser encontrados em uma ampla gama de microhabitats como os espaços intersticiais dos sedimentos, vegetação aquática, matéria orgânica em decomposição, etc. Historicamente, os oligoquetas foram classificados em dois principais grupos, relacionados com o tamanho e o habitat, incluindo formas menores, geralmente associadas à água e formas maiores associadas a ambientes terrestres (Erséus, 2005). Benham (1890) chamou estes grupos respectivamente de Microdrili e Megadrili. Utilizando esta nomenclatura, Beddard (1895) redefiniu Microdrili para incluir a família Naidomorpha (Naididae na terminologia recente), um grupo reportado por Benham como uma subclasse, separada de outros oligoquetas (Erséus 2005).

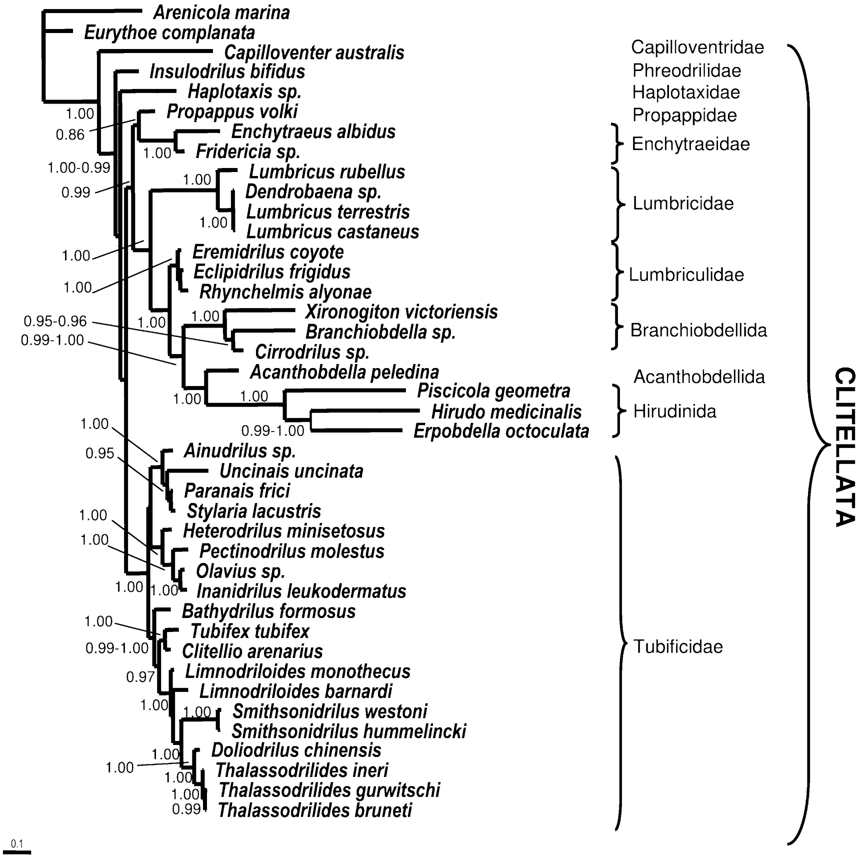

Figura 3: Filogenia de Clitellata. (Fonte: Morotta et. al., 2008)

A maioria dos caracteres diagnósticos que separam os clitelados dos outros anelídeos está relacionada com sua biologia reprodutiva (e.g. presença de clitelo, hermafroditismo, a organização dos órgãos reprodutivos, prostômio reduzido, a ausência de órgãos prostomiais). Entretanto, características como a ausência de larva trocófora, órgãos copulatórios e mesmo o hermafroditismo também são descritas para algumas espécies intersticiais de poliquetas (Westheide, 1971). Portanto, não podem ser considerados atributos exclusivos dos clitelados (Rodriguez & Reinoldson, 2011).

Os oligoquetas também possuem o prostômio (menos conspícuo do que em poliquetas) desprovido de apêndices, uma boca anterior ventral, um ânus posterior no pigídio (figura 4a), intestino recoberto por células peritoniais modificadas, os cloragógenos, que são responsáveis pela síntese de proteínas pela estocagem de glicogênio. Geralmente possuem quatro feixes de cerdas quitinosas segmentalmente dispostas ao longo da maior parte do corpo. Entretanto, em contraste com os poliquetas, estes não são localizados em parapódios (Rodriguez & Reinoldson, 2011).

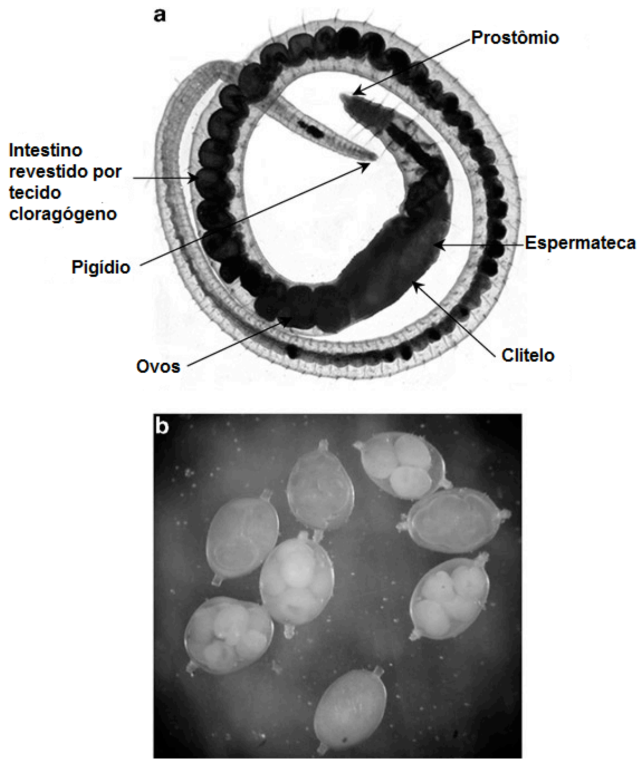

Figura 4: (**a**) Anatomia generalizada de um oligoqueta; (**b**) casulo com embriões. Fonte: Rodriguez & Reinoldson, 2011.

O clitelo é um órgão reprodutivo característico que se desenvolve em forma de anel ou sela, localizado ao redor ou atrás do poro feminino quando o indivíduo atinge a maturidade sexual. O clitelo secreta um casulo onde os ovos são depositados e fertilizados, fornecendo tanto a cobertura externa como o conteúdo interno necessário para o desenvolvimento direto dos embriões (Rodriguez & Reinoldson, 2011) (Figura 4b).

Atualmente, são conhecidas cerca de 1700 espécies de oligoquetas aquáticos, das quais aproximadamente 600 são marinhas (Erséus, 2005; Martin et al. 2007). Com exceção de alguns gêneros, são animais geralmente pequenos, variando entre menos de 1 milímetro e poucos centímetros de comprimento. Por este motivo, parte dos oligoquetas marinhos são também reportados como intersticiais ou meiofaunais (Erséus, 1988).

Arranjos taxonômicos de oligoquetas recentes no nível de famílias

Os oligoquetas marinhos e estuarinos mais frequentes pertencem à controversa família Tubificidae/Naididae, que tem aproximadamente 1000 espécies, das quais cerca de 450 marinhas (Martin et al, 2007). Características morfológicas (Erséus, 1990; Brinkhurst, 1994) e dados moleculares (Christensen & Thiesen, 1998; Erséus et al., 2000, 2002; Siddall et al., 2001; Erséus, 2005; Sjölin et al., 2005; Envall et al., 2006) suportam a premissa de que todos os membros da antiga família Naididae Ehrenberg, 1828 estão filogeneticamente dentro de Tubicifidae Vejdovský, 1876. Por esta razão, Erséus & Gustavsson (2002) propuseram que estes táxons deveriam ser agrupados em uma só família, de maneira a permitir a parafilia de Tubicifidae. Sugeriram então que todos os Naididae fossem tratados como membros de Tubicifidae, reportando Naidinae como uma subfamília dentro deste táxon. Entretanto, não deixaram de reconhecer Naididae como o nome mais antigo das duas famílias, e ressaltaram que o novo arranjo poderia violar o princípio da prioridade como estipulado pelo ICZN (1999). Tentando evitar o problema, Erséus et al., (2005) solicitaram à Comissão Internacional de Nomenclatura Zoológica a precedência de Tubicifidae sobre Naididae, com a justificativa de ser o grupo mais inclusivo entre os dois. O pedido foi rejeitado com base no princípio da prioridade (ICZN, 2007: Opinião, 2167; caso, 3305). Deste modo, permanece válida a família Naididae (sensu Erséus et al., 2008), dividida em oito subfamílias: Naidinae Ehrenberg, 1828, Pristininae Lastockin, 1921 (com um único gênero, *Pristina*), Tubificinae Vejdovský, 1876, Telmatodrilinae Eisen, 1879, Rhyacodrilinae Hrabě, 1963, Phallodrilinae Brinkhurst, 1971, Limnodriloidinae Erséus, 1982, Opistocystinae Černosvitov, 1936 (anteriormente tratada como família) (Erséus et al. 2010), a maioria com representantes marinhos. Limnodriloidinae é quase exclusivamente marinha, com exceção de *Doliodrilus puertoricensis* (Erséus & Milligan, 1988). Phallodrilinae, apesar de compreender algumas espécies exclusivamente dulcícolas, é primariamente marinha (Martin et al, 2007). Nesta revisão, Naididae e Tubificidae são tratadas como famílias distintas na medida em que nenhuma diagnose formal do conceito estendido de Naididae foi publicada até o presente momento (Erséus, com. pess.). As diagnoses de Naididae e Tubifidae a seguir são aquelas de Giere & Pfannkuche (1982).

Diagnose (Tubificidae): Cerdas dorsais e ventrais frequentemente dissimilares, a maioria bífida em forma de pequenos ganchos. Cerdas capilares geralmente presentes. Comumente com cerdas genitais especializadas. Espermateca em X, poros masculinos em XI, bainha penial quitinosa frequente. Funis masculinos em X, átrio pareado e glândulas prostáticas geralmente presentes. A classificação em nível genérico é normalmente baseada na estrutura dos órgãos genitais.

Diagnose (Naididae): Cerdas dorsais e ventrais frequentemente dissimilares a maioria bífida em forma de pequenos ganchos, as ventrais com nódulos. Cerdas capilares dorsais com formato e número variável. A maioria com cerdas peniais quando maduros. Espermateca (a maioria em V) e testículos pareados e funis masculinos no mesmo segmento. Poros masculinos e átrio no segmento subsequente. Pênis ausente, células prostáticas muitas vezes difusas.

Enchytraeidae Vejdovsky 1879 é o segundo maior grupo com representantes marinhos. Com aproximadamente 700 espécies descritas, é a família com distribuição mais ampla entre os oligoquetas, sendo encontrada na maioria dos ambientes, tanto aquáticos como terrestres. As espécies deste grupo são particularmente numerosas em regiões entremarés de praias arenosas e em alguns habitats terrestres (Erséus et al., 2010). Entretanto, também são conhecidos por habitarem sedimentos finos de mar profundo (Rota & Erseus, 2003; Erseus & Rota, 2003). São encontrados ainda em ambientes extremos como glaciais (e.g., Hartzell et al., 2005). As espécies de Enchytraeidae variam de menos de 1 mm a 170 mm (Rota, 2001), mas as mais comuns variam entre 5 e 20 mm (Erséus, 2005).

Diagnose: Formato das cerdas ventrais e dorsais geralmente idêntico, unidentadas, retas ou ligeiramente sigmoides. Espermateca em V. Poros masculinos em XII. Bulbo penial presente, pênis, glândulas prostáticas e atrio ausentes. Geralmente com glândulas septais conspícuas em V – VII.

A família Capilloventridae Harman & Loden 1984 e seu único gênero *Capilloventer*, estabelecidos por Harman & Loden (1984), foram descritos a partir de exemplares de *Capilloventer atlanticus,* coletados na Baia de Guanabara, Rio de Janeiro, Brasil. Posteriormente, mais duas espécies foram descritas por Erséus (1993), *Capilloventer antarcticus* do Mar de Weddell, Antártica, e *Capilloventer australis* no estuário de Hawkesbury River em New South Wales, Austrália. A posição filogenética da família ainda não está satisfatoriamente resolvida. Entretanto, análises moleculares conduzidas por Erséus & Kallersjo (2004) suportam a hipótese, também fundamentada por características morfológicas (Erséus, 1993; Ferraguti et al., 1996), de que Capilloventridae representa um clado basal dos oligoquetas. Este é o único grupo entre os oligoquetas marinhos que possui cerdas capilares ventrais e dorsais. Erséus (1993) sugeriu que isto poderia ser uma simplesiomorfia com poliquetas. Por outro lado, Brinkhurst (1982) argumentou que a presença de cerdas capilares ventrais excluiriam *C. atlanticus* dos oligoquetas. As espécies desta família possuem clitelo na região dos poros genitais, em alguns casos com células clitelares distribuídas de maneira esparsa, mas envolvendo toda a circunferência dos segmentos. Desta forma, podem ser incluídos legitimamente dentro de Oligochaeta (Pinder & Brinkhurst, 1997).

Diagnose: Cerdas dorsais e ventrais presentes, duas por feixe, uma capilar e outra em forma de pequeno gancho. Duto masculino pareado, sem modificações abrindo na câmara copulatória em XII. Poros femininos em XIII.

Randiellidae Erséus & Strehlow 1986 é uma família monogenérica composta por quatro espécies meiofaunais: *Randiella caribaea* e *Randiella minuta*, encontradas nas ilhas de Guadalupe e Martinica, Caribe; *Randiella litoralis*, reportada para costa do Estado de Oregon, USA (Erséus, 1997) e *Randiella multithecata*, encontrada na costa leste dos Estados Unidos da América (Erséus & Strehlow, 1986).

Diagnose: Indivíduos normalmente pequenos com prostômio e pigídio arredondados e simples, sem apêndices. Clitelo extendido em XII e XIII. Cerdas dispostas em quatro feixes por segmento começando em II, geralmente mais de duas por feixe nos segmentos anteriores. . Cerdas genitais modificadas em capilares presentes nas espécies *R. caribaea*, *R. minuta* e *R. multithecata*; em *R litoralis* ausentes. Placa faríngea dorsal. Parede esofageal glandular nos segmentos anteriores. Cérebro alongado com uma profunda fenda na parte posterior. Cordão nervoso com vários gânglios por segmento. Um ou dois espermodutos presentes em X e XI. Dutos masculinos muito curtos e inconspícuos, possivelmente ausentes. Atrio e órgãos copulatórios ausentes. Linhas pareadas de glândulas copulatórias presentes ao longo da parede ventral do corpo na região genital. 3 pares de espermateca em VII e/ou VIII.

Ecologia de oligoquetas marinhos

A importância ecológica dos oligoquetas dulcícolas é bem conhecida por ecólogos e biólogos, mas as espécies marinhas têm sido negligenciadas pela grande maioria dos biólogos marinhos (Erséus, 1994). Em termos de densidade, apesar de serem numericamente importantes nas associações bênticas, as populações de oligoquetas marinhos podem ser muitas vezes subestimadas em estudos ecológicos. As peneiras comumente utilizadas neste tipo de estudo, entre 0,5 a 1 mm, podem não reter a quantidade real de indivíduos, uma vez que muitas espécies são meiofaunais. Por outro lado, em estudos da meiofauna, apesar das malhas serem finas o suficiente para reter os oligoquetas, o tamanho das amostras é normalmente muito pequeno para gerar um número significativo de espécimes (Erséus, 1994). Outro fator que pode subestimar a densidade dos oligoquetas marinhos é a grande similaridade dos tubificídeos marinhos com poliquetas capitelídeos, especialmente *Capitella*. A ocorrência de oligoquetas e estes poliquetas oligoquetóides no mesmo habitat podem causar confusão quando não identificados de forma consistente por especialistas (Giere & Pfannkuche, 1982).

Além disso, os oligoquetas marinhos são morfologicamente muito similares ou mesmo indistinguíveis, como é o caso de algumas espécies crípticas (De Wit & Erséus, 2010; Matamoros et al. 2012; Prantoni et al., 2016). Portanto, uma vez que as espécies marinhas exibem poucas características morfológicas externas conspícuas, a diversidade real pode não ser revelada mesmo em amostragens representativas. Um fator que aumenta as dificuldades de identificações consistentes é o tempo necessário para preparar adequadamente as lâminas e o processo de corar as estruturas internas de maneira que possam ser visualizadas com maior facilidade. Como resultado, o grupo inteiro é geralmente reportado simplesmente como “oligoquetas”, com a recorrente justificativa de ser muito difícil e laboriosa a identificação em um nível taxonômico menos inclusivo (e.g. Ferrando & Mendes, 2011).

A bioturbação é um dos efeitos mais conhecidos da infauna bêntica sobre os sedimentos marinhos e fluviais. Neste sentido, os oligoquetas e outros escavadores como poliquetas, bivalves e crustáceos exercem um importante papel ecológico. Como os oligoquetas podem ser encontrados em grandes densidades em regiões litorais, atingindo até 10^5–10^6 ind. m^{-2} (Giere & Pfannkuche, 1982), os buracos cavados por estes organismos geram uma “porosidade secundária” facilitando a troca de solutos e poluentes na interface água/sedimento (Giere, 2006), além de auxiliar na oxigenação das camadas mais profundas dos sedimentos (Thrush & Dayton, 2002). A infauna bêntica, incluindo os oligoquetas, exerce ainda um importante papel na produtividade secundária. O aproveitamento dos detritos pelos oligoquetas gera subprodutos como o dióxido de carbono, nitrogênio inorgânico, fósforo e sílica, que são remineralizados e “recuperados” para a coluna d’água, tornando-se novamente disponíveis para os produtores primários (Thrush & Dayton, 2002).

Poucos estudos analisaram em detalhe os efeitos de fatores ambientais abióticos sobre os oligoquetas marinhos (Jansson, 1962, 1967, 1968; Lassèrre, 1967, 1969, 1970, 1971; Tyen, 1969; Giere, 1970, 1971, 1973, 1975; Chapman et al., 1982). Através de dados de abundância, biomassa e biovolume, Giere (1975) demonstrou o importante papel ecológico dos oligoquetas em regiões entremarés. Segundo o autor, sua importância numérica e o papel que exercem na produtividade secundária é comparável aos de outros grupos da macro- e meiofauna. Na medida em que os oligoquetas marinhos exibem uma certa “especialização” trófica (e.g. consumidores de bactérias e diatomáceas aderidas a detritos ou grãos de areia), Giere (1975) sugeriu que a disponibilidade de alimento pode controlar sua estrutura populacional e distribuição.

McCann & Levin (1989) hipotetizaram que organismos depositívoros de subsuperfície como o oligoqueta *Monopylephorus evertus* poderiam inibir o assentamento de larvas de organismos suspensívoros e mais sedentários como o espionídeo *Streblospio benedicti*. Através de abordagens manipulativas em campo e em laboratório concluíram que o assentamento larval de *S.benedicti* ou de outros poliquetas, bivalves e gastrópodes não é afetado pela presença de *M. evertus.*

Algumas espécies de oligoquetas são conhecidas pela alta tolerância à poluição (Coates & Ellis 1980; Ferrando & Mendes, 2011). *Tubificoides benedii*, conhecido anteriormente como *Peloscolex benedeni* (Udekem, 1855), é muitas vezes dominante em regiões costeiras fortemente impactadas por enriquecimento orgânico com altos níveis de sulfeto de hidrogênio. Esta espécie, muitas vezes caracterizada como oportunista (Giere, 2006), é capaz de manter a respiração aeróbica mesmo em baixas concentrações de oxigênio (Dubilier et al., 1994; Giere et al., 1999).

Chapman et al. (1982) investigaram a tolerância de 12 espécies de oligoquetas, das quais 4 marinhas (*Monopylephorus cuticulatus, Tubificoides gabriellae* conhecido hoje como *Tectidrilus gabriellae* (Erséus, 1982), *Limnodriloides verrucosus* e *Limnodriloides victoriensis*) a cinco diferentes contaminantes (cádmio, mercúrio, pentaclorofenol, efluentes de uma fábrica de celulose e esgoto doméstico e quatro fatores ambientais (pH, temperatura, salinidade e anoxia). As espécies marinhas foram ranqueadas, generalizadamente quanto à tolerância, da seguinte forma: *M. cuticulatus* > *T. gabriellae* > *L. verrucosus* > *L. victoriensis*. *M. cuticulatus* foi a que apresentou maior tolerância em relação ao cádmio, ao esgoto doméstico, ao “black liquor”, o chorume oriundo da fabricação de celulose e a anoxia, sugerindo que pode ser um indicador destes tipo de contaminação. Resultado semelhante foi encontrado por Coates & Ellis (1980). Entre as duas espécies de *Limnodriloides*, *L. verrucosus* foi consistentemente mais tolerante do que *L. victoriensis* aos poluentes testados. Em geral, os oligoquetas marinhos exibiram forte tolerância ao cádmio entretanto, são pouco tolerantes ao mercúrio.

Um estudo recente realizado em uma laguna costeira no México mostrou que os oligoquetas foram os táxons dominantes nos setores mais impactados tanto por efluentes industriais como por esgoto doméstico (Ferrando & Mendes, 2011). Entretanto, os autores não identificaram estes organismos em nível específico, provavelmente pelas dificuldades descritas acima.

Algumas espécies de oligoquetas podem suportar elevadas variações de salinidade (Chapman & Brinkhurst, 1980). Em um experimento manipulativo em laboratório, *Aktedrilus monospermathecus* suportou salinidades entre 1,25 e 20 e *Marionina preclitellochaeta* entre 2,25 e 10 (Jansson, 1962). Por outro lado, Giere, (1977) demonstrou, em um experimento que envolveu, além da salinidade, o pH e a temperatura, que *A. monospermathecus* pode sobreviver em salinidades entre 2,5 e 40. Entretanto, Birtwell (1972) demonstrou que a temperatura e o pH alteraram a tolerância de três espécies de oligoquetas à salinidade. Conforme demonstrado por Chapman & Brinkhurst (1980), a salinidade intersticial é muito mais conservativa que a da coluna d'água. Neste sentido, os autores recomendaram atenção com trabalhos anteriores à década de 80, em que a salinidade da água sobrejacente era relacionada com a distribuiçãoo da infauna bêntica

Reprodução, ciclo de vida e estrutura populacional

Uma das características mais marcantes dos oligoquetas, especialmente os tubificídeos, é a complexidade dos órgãos reprodutivos. Giere (2006) afirmou textualmente que "*Estamos longe de compreender a biologia reprodutiva destes animais e não podemos sequer imaginar as inter-relações entre a forma e a função das estruturas genitais destes organismos hermafroditas*". A função destas estruturas e o modo como o esperma é transferido permanecem enigmáticas. Os caracteres autapomórficos de muitas estruturas genitais não podem ser extrapolados a partir de observações pretéritas em minhocas terrestres e os "megadrilli" não podem ser considerados como representativos de todos os oligoquetas (Takashima & Mawatari 1998). Giere & Pfannkuche (1982) sumarizaram as dificuldades que explicam a escassez de estudos sobre o ciclo de vida dos oligoquetas: (1) ausência de classes discretas de idade; (2) tanto o tamanho do corpo como o número de segmentos não são características precisas do estagio de maturidade; (3) o tempo consumido na análise do estágio de maturidade de cada espécime em microscópio; (4) os casulos das diferentes espécies de oligoquetas marinhos não são identificáveis por características morfológicas. Somente a incubação em laboratório resolveria este problema. O insucesso ocorre ainda devido a problemas de alimentação e baixa tolerância a alterações químicas da água, como é o caso dos naidídeos, que são bastante sensíveis.

Os oligoquetas podem se reproduzir tanto sexuada como assexuadamente. A paratomia (brotamento) é a forma mais comum de reprodução assexuada nos naidídeos enquanto a arquitomia (fissão transversal) é mais rara (Giere & Pfannkuche, 1982). Quase todas as espécies marinhas desta família atingem seus picos populacionais por reprodução assexuada na primavera (Koene, 1981) ou no outono (Kendall, 1979), estações do ano em que a temperatura é moderada e com farto suprimento alimentar. A dominância da reprodução assexuada nas espécies marinhas e estuarinas é reconhecida por vários autores (Pfannkuche, 1979; Koene, 1981). Existem poucas evidências de que a reprodução sexuada desempenhe uma função importante no crescimento populacional dos naidídeos (Giere & Pfannkuche, 1982). Quando este tipo de reprodução ocorre, somente uma vez ao ano, os adultos morrem logo após a procriação (Learner et al., 1978).

As densidades populacionais dos naidídeos podem sofrer amplas variações sazonais (Giere & Pfannkuche, 1982). Um exemplo disto é a espécie estuarina *Paranais litoralis* que pode desaparecer completamente em determinados períodos do ano (Watling, 1975). Segundo Learner et al. (1978), estes organismos poderiam migrar para as camadas mais profundas do sedimento como forma de evitar condições adversas em determinados períodos do ano. Por outro lado, estudos a respeito da distribuição vertical não fornecem evidências claras que suportem essa premissa (Hughes, 1975; Pfannkuche, 1980). Outra explicação possível é que *P. litoralis* atravessaria estes períodos menos favoráveis em alguma forma resistente, como casulos (Learner et al., 1978).

Em função do estágio de vida, os tubificídeos são geralmente classificados como: (1) imaturos; (2) maduros (com a genitália completamente desenvolvida); (3) reprodutivos (indivíduos com espermatóforos ou ovos); (4) pós reprodutivos, caso de adultos sem espermatóforos ou ovos, com a genitália reabsorvida, mas conservando ainda a bainha penial (Giere & Pfannkuche, 1982).

A reprodução da maioria dos tubificídeos é muito similar. Normalmente atingem a maturidade no final do inverno e começo da primavera e se reproduzem na primavera e começo do verão (Giere & Pfannkuche, 1982). Entretanto, o crescimento, a reprodução, as taxas de fertilidade e o tempo de maturação podem diferir entre regiões geográficas, uma vez que são fortemente influenciados pela temperatura (Kennedy, 1966; Poddubnaya, 1980), concentração de oxigênio e estrutura sedimentar (Palmer, 1964; Hunter & Arthur, 1978), disponibilidade de alimento (Pfannkuche, 1981) e densidade populacional (Poddubnaya, 1980).

Os tubificídeos exibem dinâmica populacional marcadamente diferente dos naidídeos. As populações desta família se mantém relativamente estáveis ao longo do ano, mesmo considerando as variações sazonais descritas por alguns autores (Poddubnaya, 1959; Hunter & Arthur, 1978). Esta relativa constância se deve provavelmente ao comportamento reprodutivo de algumas espécies. No caso de *Tubifex costatus* e *Tubificoides benedii,* somente uma parte da população atinge a maturidade durante as estações de procriação. A outra parte da população permanece imatura, e procriará na estação seguinte. Embora dependente das condições ambientais locais, o intervalo das atividades reprodutivas pode se estender por alguns meses de modo que novas gerações são gradualmente recrutadas durante um longo período (Giere & Pfannkuche, 1982).

Erséus e Diaz (1989), em seu compreensivo estudo a respeito da dinâmica populacional do tubificídeo *Tubificoides amplivasatus* no norte da Europa, mostraram que esta espécie se reproduz irregularmente ao longo do ano, mas apresenta picos de abundância irregulares que podem ser relacionados com temperaturas mais altas no final do verão e no outono. O longo tempo levado por *T. amplivasatus* para atingir a maturidade, em torno de 200 dias, indica que os indivíduos desta espécie podem viver por vários anos. O mesmo padrão foi relatado para o congênere *T.* cf. *brownae* em águas salobras norte americanas. Este é um padrão comum em anelídeos costeiros e estuarinos (Diaz, 1984). Esta relação entre temperatura e recrutamento não foi observada para *Tubificoides benedii* (Bagheri & McLusky, 1982). Neste estudo, as curvas anuais de população irregulares com picos também no inverno não apresentaram sazonalidade clara e nenhum período marcado de recrutamento. Entretanto, ao contrário de *T.benedii*, os picos populacionais dos naidídeos *Amphichaeta sannio* e *Paranais litoralis* pode ser correlacionado com blooms de algas (Bagheri & McLusky, 1982).

O ciclo de vida dos enquitreídeos ainda é pobremente conhecido (Giere & Pfannkuche, 1982). Poucos estudos foram realizados, mesmo em ambientes terrestres, (Dózsa-Farkas, 1973). Apesar de predominantemente sexuada, a reprodução assexual por fragmentação também ocorre em algumas espécies límnicas e terrestres desta família. A taxa de crescimento, fertilidade e a biologia reprodutiva de espécies marinhas e/ou estuarinas de enquitreídeos associadas a bancos de algas marrons e a águas percoladas de esgoto doméstico são caracterizadas pela alta capacidade de adaptação a flutuações na salinidade, temperatura e nutrição (Giere & Pfannkuche, 1982). Apesar da história de vida das espécies meiobênticas ser praticamente desconhecida, Lassèrre (1975), em um estudo realizado na Baia de Arcachon na França sugere a existência de três gerações anuais para as populações de *Marionina* spp. Estes organismos geram entre 2 e 4 casulos por período de maturidade, que permanecem incubados por aproximadamente 13 dias (Giere, 1975).

Até o presente momento, nada se sabe a respeito da reprodução, ciclo de vida e estrutura populacional dos randielídeos e capiloventrídeos. Estima-se que somente 4 ou 5 especialistas tiveram a oportunidade de estudar as espécies destes grupos (Erséus, com. pess.), pelo fato destes animais serem muito raros. Os poucos estudos a respeito destas famílias se resumem a trabalhos unicamente taxonômicos (e.g. Erséus, 1993; Pinder & Brinkhurst, 1997).

Alimentação

A classificação dos oligoquetas aquáticos como depositívoros não seletivos ou herbívoros tem sido generalizada a partir de observações em ambientes dulcícolas. Apesar da escassez de estudos, esta categorização é normalmente utilizada para classificação das espécies marinhas (Giere & Pfannkuche, 1982). A dieta dos oligoquetas inclui microalgas vivas (diatomáceas e flagelados), microorganismos (bactérias, fungos e ciliados), matéria orgânica particulada (detritos), matéria orgânica vegetal fresca (algas) e matéria orgânica dissolvida.

A posição dos naidídeos marinhos como consumidores primários, tendo diatomáceas penadas como principal item alimentar é relativamente bem conhecida (Pfannkuche, 1979). Koene (1981) observou que o conteúdo estomacal do naidídeo *Amphichaeta sannio* era composto somente por diatomáceas. O autor sugere que esta espécie tem certa preferência pela diatomácea *Navicula salinarium*, podendo ingerir uma quantidade desta alga equivalente a mais de duas vezes o seu peso diariamente. Vários autores correlacionaram o ciclo populacional de *Amphichaeta* spp. com o florescimento destas algas (Pfannkuche, 1977; Koene ,1981). Por outro lado, as espécies intersticiais de tubificídeos e enquitreídeos que habitam as camadas sub-superficiais dos sedimentos arenosos não mostram nenhuma especialização trófica, uma vez que a concentração de diatomáceas nestes locais é bastante reduzida (Giere & Pfannkuche, 1982).

Uma característica notável que ocorre nos gêneros *Olavius* e *Inanidrilus*, pertencentes à subfamília Phallolodriline (Tubificidae), é a existência de uma complexa e obrigatória associação simbiótica com bactérias (Erséus, 2003; Giere, 2006). Esta simbiose pode ser considerada análoga à que ocorre em fontes hidrotermais, mas dela difere em termos de diversidade dos organismos simbiontes participantes (Giere & Krieger, 2001). Estas espécies são completamente desprovidas de sistemas digestivos e excretores (Erséus, 2003). Apesar de encontradas em uma grande variedade de habitats, desde regiões tropicais até as de clima temperado, estas espécies parecem ser mais abundantes em regiões costeiras influenciadas pela presença de recifes de coral (Erséus, 2003).

Organização da tese

Existem poucos estudos a respeito da diversidade de oligoquetas marinhos e estuarinos no Atlântico sul e o conhecimento taxonômico do grupo é claramente insatisfatório (ver artigo 5). Apesar de alguns estudos pontuais na costa brasileira, (du Bois-Reymond Marcus, 1950, Marcus, 1965, Erséus, 1980; 1983; Harman & Loden 1984) e na região Antártica (Michaelsen, 1888, Erséus & Lassèrre, 1977; Rota & Erséus, 1996), o grupo continua praticamente ignorado pela comunidade de zoólogos e biólogos marinhos da região.

Neste contexto, esta tese tem como objetivo promover o avanço do conhecimento taxonômico e biogeográfico dos oligoquetas marinhos através do posicionamento filogenético de espécies novas e/ou pouco conhecidas na região. A tese é formada por uma revisão bibliográfica atualizada sobre o conhecimento corrente dos oligoquetas marinhos, seguida de cinco capítulos, sob a forma de artigos científicos, todos com minha autoria primária, em colaboração com cientistas brasileiros e estrangeiros, dos quais três já publicados em revistas internacionais indexadas.

O primeiro artigo apresenta um inventário e uma visão crítica da situação atual do conhecimento taxonômico da fauna de oligoquetas marinhos e estuarinos no Brasil (Prantoni et al., 2013). Neste artigo, a distribuição geográfica do naidídeo (sensu Erséus et al., 2008) *Tectidrilus* c.f. *gabriellae*, originalmente descrita como *Peloscolex gabriellae* (du Bois-Reymond Marcus, 1950) para a Ilha Bela, São Paulo e conhecida também para algumas regiões do Caribe (Erséus, 1990), é estendida para a Baía de Paranaguá, Paraná (S 25º 29’ 04” W 45º 25’ 54”). Este gênero, exclusivamente marinho, foi estabelecido por Erséus (1982) a partir de indivíduos de *Tectidrilus squalidus* para acomodar cinco espécies com as seguintes características diagnósticas: 1) presença de papilas ao longo de toda a parede do corpo; 2) reduzido número de cerdas, com duas por feixe nos segmentos anteriores e uma nos posteriores; 3) clitelo curto ; 4) dutos do átrio pobremente granulados (Erséus, 1982). Além das duas espécies acima mencionadas, outras dez são conhecidas atualmente, *Tectidrilus arabicus* Erséus, 1984, *Tectidrilus bori* (Righi & Kanner, 1979), *Tectidrilus borioides* Erséus & Wang, 2003, *Tectidrilus diversus* Erséus, 1982, *Tectidrilus intermixtus* Finogenova, 1986, *Tectidrilus pictoni* (Erséus, 1984), *Tectidrilus pranzoi* Erséus, 1987, *Tectidrilus probus* Erséus, 1991, *Tectidrilus profusus* Erséus, 1991, *Tectidrilus squalidus* Erséus, 1982, *Tectidrilus verrucosus* (Cook, 1974) (Erséus, com. pess.).

O segundo artigo registra uma espécie de Enchytraeidae do gênero *Marionina*, bastante incomum por não possuir cerdas ventrais ou laterais, coletada no litoral norte de São Paulo e formalmente registrada pela primeira vez no Atlântico sul (Prantoni et al., 2014). Esta espécie é morfologicamente similar a *Marionina nevisensis* Righi & Kanner, 1979, descrita para Ilha de Nevis, no

Caribe. Entretanto, a limitada capacidade de dispersão em conjunto com análises moleculares sugerem fortemente se tratar de uma nova espécie. A filogenia proposta por Erséus et al. (2010) sugere que *Marionina* pode ser dividido em dois grupos, um composto por espécies marinhas e outro por límnicas e terrestres, o que aumenta ainda mais o interesse do registro. O caráter diagnóstico compartilhado pelas espécies de *Marionina* é a bifurcação do vaso sanguíneo dorsal nos segmentos III ou IV. As espécies do gênero desprovidas de cerdas são conhecidas como “achaetous *Marionina*” (Matamoros et al., 2012). De acordo com estes autores, os espécimes identificados como *M. nevisensis* podem ser, na realidade, um complexo de espécies crípticas distribuídas globalmente. Sugerem ainda que as linhagens tropicais (e.g. América Central e Oceania) formam um clado monofilético. Espécies crípticas são aquelas morfologicamente indistinguíveis mas geneticamente divergentes (Bickford et al., 2007). Infelizmente, os espécimes utilizados para as referidas análises de DNA não foram conservados adequadamente para uma análise morfológica acurada e inequívoca. Foram realizadas novas amostragens sistemáticas, sem sucesso, na praia da Ponta do Baleeiro, São Paulo na tentativa de encontrar mais espécimes. Entretanto, quatro indivíduos aqueta de *Marionina* foram recentemente coletados em Guaraqueçaba, Paraná. Análises moleculares preliminares sugerem fortemente se tratar de quatro linhagens distintas (ver artigo 5).

O terceiro artigo apresenta uma nova proposta filogenética de outro gênero de Enchytraeidae, *Grania*, seguida da descrição de nove novas espécies, todas com suporte tanto morfológico como molecular. Sete destas espécies, *Grania brasiliensis* **sp. nov** do Brasil; *Grania bekkouchei* **sp. nov.**, *Grania cryptica* **sp. nov.,** *Grania capensis* **sp. nov.,** *Grania simonae* sp. nov.**,** da África do Sul, *Grania hinojosai* **sp. nov.** e *Grania chilensis* **sp. nov.,** do Chile são o primeiro registro do gênero tanto no continente africano como no sul-americano, e duas, *Grania unitheca* **sp. nov.,** e *Grania carolinensis* **sp. nov.,** novos registros do gênero na América do Norte (Prantoni et al., 2016). Desta forma, o número de espécies do gênero foi expandido de 71 para 80. Ao contrário de *Marionina*, o gênero *Grania* é morfologicamente bem resolvido (Erséus & Lassèrre, 1976), exclusivamente marinho e monofilético (Erséus et al., 2010; et al, 2011; Prantoni et al., 2016). Em todas as espécies, os segmentos I - IV parecem ser “fundidos” com o prostômio.

Cerdas estão ausentes pelo menos nos três primeiros segmentos. Outra característica morfológica interessante do gênero é a disposição das cerdas. Ao contrário da maioria dos oligoquetas que possuem feixes com duas ou mais cerdas, as cerdas ocorrem individualmente em *Grania* (De Wit & Erséus, 2010). Os caracteres sexuais internos variam consideravelmente, com a espermateca e o aparato penial exibindo uma grande variedade de formas (Coates, 1984; Prantoni et al., 2016). As espécies do gênero são intersticiais e bastante pequenas, variando entre 1 - 2 cm de comprimento e aproximadamente 0,1 mm de espessura, com coloração esbranquiçada ou transparente (De Wit et & Erséus, 2010) (Figura 4). Os espécimes de *Grania* são facilmente reconhecidos em amostras intersticiais de macroinvertebrados bênticos pelo seu tipo característico de locomoção. A primeira vista, lembram os vermes nematóides que se locomovem por movimentos laterais ao invés dos movimentos peristálticos, típico dos outros oligoquetas (De Wit et al., 2011). No primeiro estudo filogenético de *Grania*, com apenas 20 espécies sequenciadas, De Wit et al. (2011) mostraram que a distribuição geográfica do grupo é fortemente concordante com a filogenia, o que indica uma capacidade de dispersão bastante restrita. Como a maioria dos oligoquetas, Grania não possui fase larval; os ovos são depositados diretamente no sedimento dentro de um casulo.

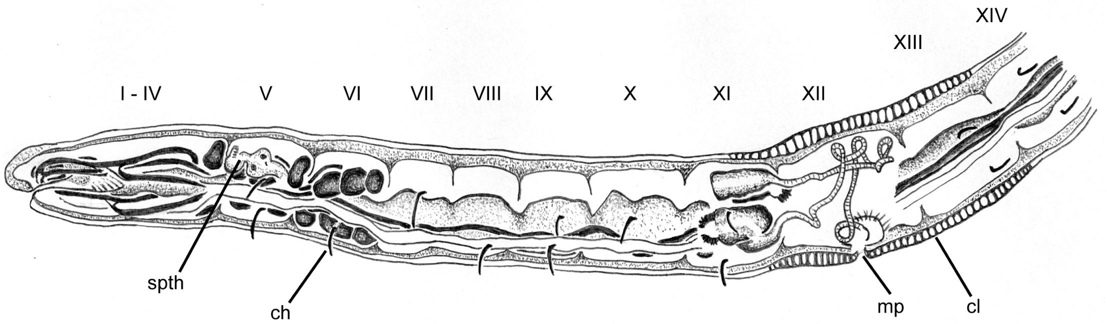

Figura 4: Parte anterior de um espécime de *Grania*. spth, espermateca; ch, cerdas; mp, poro masculino; cl, clitelo. Número de segmentos demarcados em algarismos romanos. (Fonte: Locke & Coates, 1999).

Entretanto a filogenia proposta por De Wit et al. (2011) incluiu apenas dois representantes do Pacífico sul, Grania sp. “Chile 1” e Grania sp. “Chile 2”, posteriormente descritas no artigo 3 por Prantoni et al., (2016) como *Grania chilensis* e *Grania hynojosai* respectivamente, e nenhum do Atlântico sul.

Da mesma forma que o estudo pretérito de De Wit et al., (2011), a filogenia de *Grania* inferida no terceiro artigo da tese é consistentemente relacionada com a distribuição geográfica das espécies, o que corrobora a limitada capacidade de dispersão das espécies deste gênero (De Wit et al., 2011; Prantoni et al., 2016). Entretanto, a adição das novas espécies permitiu observar que as quatro espécies da África formam um clado monofilético e que duas, *Grania bekkouchei* e *Grania cryptica*, são morfologicamente idênticas, com exceção do formato das cerdas, mas geneticamente divergentes. Além disso, também foi observado que *Grania chilensis* é estruturada em pelo menos quatro linhagens maternais distintas, provavelmente devido à capacidade restrita de locomoção associada a ausência de fase larval.

O quarto artigo fornece a primeira lista (checklist) das espécies de *Grania*, incluindo dados históricos e recentes de todas as espécies válidas descritas até hoje, suas respectivas sinonímias, além de informações a respeito da sua distribuição geográfica. Estas listas são importantes no sentido de facilitar análises biogeográficas em grande escala ou pesquisas relacionadas com a biodoversidade (Pagliosa et al., 2012; Shmelz & Collado, 2012). Ao todo foram registradas 80 espécies, descritas nos cinco continentes, contemplando ainda as bacias oceânicas e o ambiente antártico. Considerando o pouco conhecimento da fauna de enquitreídeos (principalmente os marinhos) e o número reduzido de especialistas na área, é de se esperar um número maior de espécies a ser descritas.

O quinto artigo traz um avanço expressivo no conhecimento filogenético dos oligoquetas marinhos no Atlântico sul. Neste artigo, a monofilia de 80 espécimes do Atlântico sul e Antártica foi estatisticamente testada atráves da combinação de quatro métodos, inferência Bayesiana (BI), máxima verossimilhança (ML), Automatic Barcode Gap Discovery (ABGD) e o Generalized Mixed Yule coalescent (GMYC). Foram identificadas 32 espécies potenciais pertencentes a duas familias de oligoquetas marinhos. Todas estas espécies foram associadas a táxons ou gêneros previamente conhecidos através da comparação das sequências obtidas com as presentes nas bases de dados

NCBI/GenBank, Barcoding of Life/BOLD e/ou na base de dados particular, acumulados por Christer Erséus, um dos autores do artigo. Como resultado desta análise, as espécies *Doliodrilus fibrisaccus, Limnodriloides pierantonii, L. rubicundus, L. sacculus, Paranais frici, Stephensoniella sterreri, Thalassodrilides gurwitschi,* foram registradas pela primeira vez na costa brasileira. Este número certamente aumentará com a descrição de quarto potenciais espécies aquetas do gênero *Marionina*, quatro de *Ainudrilus*, duas de *Aktedrilus*, duas de *Limnodriloides*, uma de *Enchytraeus*, uma de *Macquaridrilus*, uma de *Stephensoniella,* além de mais três espécies de *Lumbricillus* do continente antártico. Nossa análise confirma que a abordagem molecular através do gene mitocondrial citocromo oxidase subunidade 1 é um ferramenta excelente para uma primeira delimitação e identificação de espécies de oligoquetas marinhos, que são dificilmente identificados unicamente por análises morfológicas tradicionais.

Referências

Bagheri, E. A. & Mclusky, D. S. (1982). Population dynamics of oligochaetes and small polychaetes in the polluted Forth estuary ecosystem. **Netherlands Journal of Sea Research**, v. 16, p. 55–66.

Bartolomaeus, T; Purschke, G; Hausen, H. (2005). Polychaete phylogeny based on morphological data – a comparison of current attempts. **Hydrobiologia**, v. 535/536, p. 341–356.

Beddard, F. E. (1895). **A monograph of the order of Oligochaeta**, Oxford, Clarendon Press, 806 p.

Benham, W. B. (1890). An Attempt to Classify Earthworms. **Quarterly Journal of Microscopy Science**, v. 31, p. 201–315.

Bickford, D.; lohman, D. J.; Sodhi, N. S.; Ng, P. K. L.; Meier, R.; Winker, K.; Ingram, K. K.; Das, I. (2007). Cryptic species as a window on diversity and conservation. **Trends in Ecology and Evolution**, v. 22(3), p. 148–155.

Birtwell, I. K. (1972). **Ecophysiological aspects of tubificids in the Thames estuary**. Tese (Ph.D). University of London.

Bleidorn, C.; Vogt, L.; Bartolomaeus, T. (2003). New insights into polychaete phylogeny (Annelida) inferred from 18S rDNA sequences. **Molecular Phylogenetics and Evolution**, v. 29(2), p. 279–288.

Brinkhurst, R. 0. (1982). Evolution in the Annllida. **Canadian Journal of Zoology**, v, 60, p. 1043- 1059.

Brinkhurst, R. O. (1994). Evolutionary relationships within the Clitellata: an update. **Megadrilogica,** v. 5, 109–116.

Brusca, R. C. & Brusca, G. J. (2003). **Invertebrados**. Rio de Janeiro: Guanabara Koogan, 968 p.

Chapman, P. M.; Farrell, M. A.; Brinkhurst, R. O. (1982). Relative tolerance of selected aquatic oligochaetes to individual pollents and envirimnental factors. **Aquatic Toxicilogy**, v. 2, p. 47 -67.

Chapman, P. M. & Brinkhurst, R. O. (1980). Salinity Tolerance in Some Selected Aquatic Oligochaetes. **Internationale Revue der gesamten Hydrobiologie und Hydrographie**, v.65, p. 499 – 505.

Christensen, B. & Theisen, B. F. (1998). Phylogenetic status of the family Naididae (Oligochaeta, Annelida) as inferred from DNA analyses. **Journal of Zoological Systematics and Evolutionary Research**, v. 36, p. 169–172.

Coates, K. A. (1984). Specific criteria in Grania (Oligochaeta, Enchytraeidae). **Hydrobiologia** v. 115, p. 45–50.

Coates, K. A. & Ellis, D. V. (1980). Enchytraeid oligochaetes as marine pollution indicators. **Marine Pollution. Bulletin**, v. 11, p.171-174.

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

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** v. 48, p. 285–293.

Diaz, R. J. (1984). Short term dynamics of the dominant annelids in a polyhaline temperate estuary. **Hydrobiologia**, v.115, p. 153–158.

Diaz, R. J.; Erséus, C.; Boesch, D. F. (1987). Distribution and ecology of Middle Atlantic Bight Oligochaeta. **Hydrobiologia**, v. 155, p. 215 – 225.

Dózsa-Farkas, K. (1973). Ananeosis, a new phenomenon in the life history of the Enchytraeids (Oligochaeta). **Opuscula Zoologica**, v. 12, p. 43 – 55.

Dubilier, N.; Giere, O.; Grieshaber, M. K. (1994). Concomitant effects of sulfide and hypoxia on the aerobic metabolism of the marine oligochaete *Tubificoides benedii*. **Journal of Experimental Zoology**, v. 269, p. 287–297.

Du Bois-Reymond Marcus, E. (1950). A marine tubificid from Brazil. **Comunicaciones Zoologicas del Museo de Historia Natural de Montevideo**, v. 59(3), p.1-6.

Envall, I.; Källersjö, M.; Erséus, C. (2006). Molecular evidence for the non-monophyletic status of Naidinae (Annelida, Clitellata, Tubificidae). **Molecular Phylogenetics and Evolution** v. 40, p. 570–584.

Erséus, C. (2005). Phylogeny of oligochaetous Clitellata. **Hydrobiologia** v. 535/536 p. 355–370.

Erséus, C. (1999). Marine Tubificidae (Oligochaeta) from a mangrove habitat in Kenya. **Tropical Zoology**, v. 12, p.137-143.

Erséus, C. (1997). A record of *Randiella* from New Caledonia, the first known occurrence of the marine interstitial family Randiellidae (Annelida; Oligochaeta) in the South Pacific Ocean. **Journal of Natural History**, v. 31(12), p.1745-1750.

Erséus, C. (1994). Marine Tubificidae (Oligochaeta) of Antarctica, with descriptions of three new species of Phallodrilinae. **Zoologica Scripta,** v. 23, p. 217-224.

Erséus, C. (1994). The Oligochaeta. - The Annelida Part 1. Oligochaeta and Polychaeta: Phyllodocida (Phyllodocidae to Paralacydoniidae). In: BLAKE, J. A.; HILBIG, B.; SCOTT, P. H. (Eds.). **Taxonomic atlas of the Santa Maria Basin and western Santa Barbara Channel, vol. 4.** Santa Barbara. Santa Barbara Museum of Natural History, p. 5-38.

Erséus, C. (1993). Taxonomy of *Capilloventer* (Capilloventridae), a little-known group of aquatic Oligochaeta, with descriptions of two new species. **Journal of Natural History**, v. 27, p. 1029-1040.

Erséus, C. (1990). Cladistic analysis of the subfamilies within the Tubificidae (Oligochaeta). **Zoologica Scripta**, v. 19, p. 57–63.

Erséus, C. (1988). Oligichaeta. In: Higgins, R. P.; Thiel, H. (Eds.) **Introductions to the study of meiofauna.** Washington, Smithsonian Intitution Press, p. 349 – 354.

Erséus, C. (1983). Two new species of *Aktedrilus* (Oligochaeta, Tubificidae) from the Western Atlantic. **Bulletin du Muséum national d'histoire naturelle**, v. 4, p.1051-1057.

Erséus, C. (1982). Taxonomic revision of the marine genus *Limnodriloides* (Oligochaeta: Tubificidae). **Verhandlungen des naturwissenschaftlichen Vereins in Hamburg (Neue Folge)**, v. 25, p. 207-277.

Erséus, C. (1980). Taxonomic studies on the marine genera *Aktedrilus* Knöllner and *Bacescuella* Habre (Oligochaeta, Tubificidae), with descriptions of seven new species. **Zoologica Scripta**, v. 9, p.97-111.

Erséus, C.; Envall, I.; Marchese, M.; Gustavsson, L. (2010). The systematic position of Opistocystidae (Annelida, Clitellata) revealed by DNA data. **Molecular Phylogenetics and Evolution**, v. 54, p. 309-313.

Erséus, C.; Rota, E.; Matamoros, L. P. (2010). Molecular phylogeny of Enchytraeidae (Annelida, Clitellata). **Molecular Phylogenetics and Evolution**, v. 57, p. 849-858.

Erséus C.; Wetzel M. J; Gustavsson L. (2008). ICZN rules – a farewell to Tubificidae (Annelida, Clitellata). **Zootaxa** v. 1744 p. 66–68.

Erséus, C., Gustavsson, L. M., and Brinkhurst, R. O. (2005). Case 3305. Tubificidae Vejdovský, 1876 (Annelida, Clitellata): proposed precedence over Naididae Ehrenberg, 1828. *Bulletin of Zoological Nomenclature* **62**, 226–231.

Erséus, C. & Källersjö, M. (2004). 18S rDNA phylogeny of Clitellata (Annelida). **Zoologica Scripta**. v. 33, p. 187–196.

Erséus C. & Rota E. (2003). New findings and overview of the oligochaetous Clitellata (Annelida) of the North Atlantic deep sea. **Proceedings of the Biological Society of Washington**, v. 116, p. 892–900.

Erséus, C. & Gustavsson, L. (2002). A proposal to regard the former family Naididae as a subfamily within Tubificidae (Annelida, Clitellata). **Hydrobiologia**, v. 485, p. 253–254.

Erséus, C.; Källersjö, M.; Ekman, M.; Hovmöller, R. (2002). 18S rDNA Phylogeny of Tubificidae and its constituent taxa (Clitellata): dismissal of the Naididae. **Molecular Phylogenetics and Evolution,** v. 22, p. 414-422.

Erséus, C.; Prestegaard, T.; Källersjö, M. (2000). Phylogenetic analysis of Tubificidae (Annelida, Clitellata) based on 18S rDNA sequences. **Molecular Phylogeny and Evolution**, v. 15, p. 381–389.

Erséus, C. & Diaz, R. J. (1989). Population dynamics of *Tubificoides amplivasatus* (Oligochaeta, Tubificidae) in the Oresund, Denmark. **Hydrobiologia**, v. 180, p. 167- 176.

Erséus, C. & Milligan, M. R. (1988). *Limnodriloides faxatus* and *Doliodrilus puertoricensis*, new Limnodriloidinae (Oligochaeta: Tubificidae) from Puerto Rico**. Proceedings of the Biological Society of Washington**, v.101, p. 11-14.

Erséus, C. & Strehlow, D. R. (1986). Four new interstitial species of marine Oligochaeta representing a new family. **Zoologica Scripta**, v. 15, p. 53-60.

Erséus, C. & Lassarre, P. (1977). Redescription of *Grania* monochaeta (Michaelsen), a Marine Enchytraeid (Oligochaeta) from South Georgia (SW Atlantic). **Zoologica Scripta,** v.6, p. 299–300.

Erséus, C., & Lasserre, P. (1976). Taxonomic Status and Geographic Variationof the Marine Enchytraeid Genus *Grania* Southern (Oligochaeta). **Zoologica Scripta v.** 5, p. 121–132.

Ferraguti, M.; Erséus, C.; Pinder, A. (1996) The spermatozoon of *Capilloventer australis* and the systematic position of the Capilloventridae (Annelida: Oligochaeta). **Australian Journal of Zoology**, v. 44, p. 469-478.

Ferrando, A. & Méndez, N. (2011). Effects of organic pollution in the distribution of annelid communities in the Estero de Urías coastal lagoon, Mexico. **Scientia Marina**, v. 75, p. 351 – 358.

Giere, O. (2006). Ecology and Biology of Marine Oligochaeta – an Inventory Rather than another Review. **Hydrobiologia**, v. 564 (1), p. 103 – 116.

Giere, O. (1977). Biology of benthic organismo. In: Keegan, B. F.; Ceidigh, P. O.; Boaden, P. J. S. (Eds.). **Proceedings of 11th. European Marine Biology Symposium**. Oxford: Pergamon Press, 1977, p. 285 – 296.

Giere, O. (1975). Population structure, food relations and ecological role of marine oligochaetes, with special reference to meiobenthic species. **Marine Biology**, v. 31, p. 139 – 156.

Giere, O. (1973). Oxygen in the marine hygropsammal and the vertical microdistribution of oligochaetes. **Marine Biology**, v. 21, p.180-189.

Giere, O. (1971). Beziehungen zwischen abiotischem Faktoren-system, Zonierung und Abundanz mariner Oligochaeten in einem Kustengebiet der Nord-see. **Thalassia Jugoslavica**, v. 7, p. 67 – 77.

Giere, O. (1970). Untersuchungen zur Mikrozonierung und Okologie mariner Oligochaeten im Sylter Watt. **Veroff Institute Meeresforsch**, 12, 491-529.

Giere, O & Krieger, J. A. (2001). triple bacterial endosymbiosis in a gutless oligochaete (Annelida): ultrastructural and immunocytochemical evidence. **Invertebrate Biology**, v. 120, p. 41 -49.

Giere, O.; Preusse, J. H.; Dubilier, N. (1999). *Tubificoides benedii* (Tubificidae, Oligochaeta) a pioneer in hypoxic and sulfidic environments. An overview of adaptive pathways. **Hydrobiologia**, v. 406, p. 235–241.

Giere, O. & Pfannkuche, O. (1982). Biology and ecology of marine Oligochaeta, a review. **Oceanography and Marine Biology: An Annual Review**, v. 20, p.173–308.

Harman, W. J. & Loden, M. S. (1984). *Capilloventer atlanticus* gen. et sp. n., a member of a new family of marine Oligocheta from Brazil. **Hydrobiologia**, v. 115, p. 51-54.

Hartzell , P. L.; Nghiem, J. V.; Richio, K. J.; Shain , D. H. (2005). Distribution and phylogeny of glacier ice worms (Mesenchytraeus solifugus and Mesenchytraeus solifugus rainierensis). **Canadian Journal of Zoology**, v. 83, p. 1206 – 1213.

Hughes, B. D. A. (1975). Comparison of four samplers for benthic macro-invertebrates inhabiting coarse river deposits. **Water Research**, v. 9, p. 61 – 69.

Hunter, J. B. & Arthur, D. R. (1978). Some aspects of the ecology of *Peloscolex benedeni* Udekem (Oligochaeta: Tubificidae) in the Thames estuary. **Estuarine Coastal Marine Science**, v. 6, p. 197–208.

International Commission of Zoological Nomenclature [ICZN].Opinion 2167 (Case 3305). Naididae Ehrenberg, 1828 (Annelida, Clitellata): precedence over Tubificidae Vejdovský, 1876 maintained (2007). **Bulletin of Zoological Nomenclature**, v. 64, p. 71–72.

International Commission of Zoological Nomenclature [ICZN]: International code of zoological nomenclature (1999). 4th edition. **The International Trust for Zoological Nomenclature**, London: Natural History Museum, 306 p.

Jansson, B. O. (1967). The importance of tolerance and preference experiments for the interpretation of mesopsammon field distributions. **Helgolaender Wissenschaftliche Meeresuntersuchungen**, v. 15, p. 41-58.

Jansson, B. O. (1968). Quantitative and experimental studies of the interstitial fauna in four Swedish sandy beaches. **Ophelia**, v. 5, p. 1-72.

Jansson, B. O. (1962) Salinity resistance and salinity preference of two oligochaetes *Aktedrilus monospermatecus* Knollner and *Marionina preclitellochaeta* n.sp. from the interstitial fauna of marine sandy beaches. **Oikos**, v. 13, p. 293-305, 1962.

Kendall, M. A. (1979). The stability of the deposit feeding community of a mud flat in the River Tees. **Estuarine Coastal Marine Science**, v. 8, p. 15 – 22.

Kennedy, C. R. (1966). The life history of *Limnodrilus hoffmeisteri* Clap. (Oligochaeta, Tubificidae) and its adaptive significance. **Oikos**, v.17, p.158–168.

Koene, H. (1981). **Reproduktie en eetgedrag van *Amphichaeta sannio* (Oligochaeta)**. Tese (Ph.D.). University of Groningen.

Lassèrre, P. (1975). **Reproduction of marine invertebrates vol. 3.** In: Giese, A. C. & Pearse, J. S. (Eds.). Annelids and Echiurans. New York: Academic Press , p. 215 – 275.

Lassèrre, P. (1971). Données écophysiologiques sur la répartition des oligochètes marins meiobenthiques. Incidence des paramètres salinité, température, sur le métabolisme respiratoire de deux espèces euryhalines du genre *Marionina* Michaelsen 1889 (Enchytraeidae, Oligochaeta). **Vie et Milieu**, v. 22, p. 523-540.

Lassèrre, P. (1970). Action des variations de salinité sur le métabolisme respiratoire d'oligochètes euryhalins du genre *Marionina* Michaelsen. **Journal of Experimental Marine Biology and Ecology**, v. 4, p.150-155.

Lassèrre, P. (1969). Relations énergétiques entre le métabolisme respiratoire et la régulation ionique chez une annélide oligochète euryhaline, *Marionina achaeta* Hagen. C. r. hebd. **Seance de lAcadémie de Sciences**, Paris, v. 268, p.1541-1544.

Lassèrre, P. (1967). Oligochètes matins des côtes de France. II. Roscoff, Pempoull, éangs saumâtres de Concarneau: systématique, écologie. **Cahiers de Biologie Marine**, v. 8, p. 273-293.

Learner, M. A.; Lochhead, G.; Hugues, B. D. (1978). A review of the biology of British Naididae (Oligochaeta) with emphasis on the lotic environment. **Freshwater Biology**, v. 8, p. 357–375.

Locke, J. M., & Coates, K. A. (1999). Redescription of Grania americana, G. bermudensis and description of two new species of Grania (Annelida: Clitellata: Enchytraeidae) from Bermuda. **Proceedings of the Biological Society of Washington** v. 112, p. 598–623.

Marcus, E. (1965). Naidomorpha aus brasilianischen Brackwasser. **Beitrage zur Neotropischen Fauna**, v. 4(2), p. 61-83.

Marotta, R.; Ferraguti, M.; Erseus, C.; Gustavsson, L. M. (2008). Combined-data phylogenetics and character evolution of Clitellata (Annelida) using 18S rDNA and morphology. **Zoological Journal of the Linnean Society**. v. 154, p. 1–26.

Marshall, A. J. & Williams, W. D. (1972). **Textbook of zoology invertebrates. 7ª Ed**, v. 1, 874 p.

Martin, P. (2001). On the origin of the Hirudinea and the demise of Oligochaeta. **Proceedings of the Royal Society Serie B**, v. 268, p. 1089–1098.

Martin, P.; Martinez-Ansemil, E.; Pinder, A.; Timm, T.; Wetzel, M. J. (2007). Global diversity of oligochaetous clitellates (“Oligochaeta”; Clitellata) in freshwater. **Hydrobiologia**, v. 595, p. 117–127.

Matamoros, L.; Rota, E.; Erséus, C. (2012). Cryptic diversity among the achaetous *Marionina* (Annelida, Clitellata, Enchytraeidae*),* **Systematics and Biodiversity**, v. 10(4), p. 509-525.

Mccann, L. D, & Levin, L. A. (1989). Oligochaete influence on settlement, growth and reproduction in a surface-deposit-feeding polychaete. **Journal of Experimental Marine Biology and Ecology**, v. 131, p. 233-253.

Michaelsen W. (1888)**.** Die Oligochaeten von Süd-Georgien nach Ausbeute der deutschen Station von 1882–83. **Jahrbuch der Hamburgischen wissenschaftliche Anstalten** v. 5**,** p. 53–73.

Pagliosa, P. R.; Doria, J. G.; Alves, G. F.; Almeida, T. S. M.; Lorenzi, L.; Netto, S. A.; Lana, P. C. (2012). Polychaetes from Santa Catarina State (southern Brazil): checklist and remarks on species distribution. **Zootaxa** v. 3486, p. 1–49.

Palmer, M. (1964). Some aspects of the respiratory physiology of *Tubifex* in relation to its ecology. **Journal of Zoology**, London, v. 154, p. 463–473.

Pinder, A. M. & Brinkhurst, R. O. (1997). The family Capilloventridae (Annelida: Oligochaeta) in Australia, with descriptions of two new species of *Capilloventer*. **Zoologica Scripta** v. 26, p. 255–256.

Poddubnaya, T. L. (1980). Life cycles of mass species of Tubificidae (Oligochaeta). In: **Aquatic Oligochaete Biology**. Brinkhurst, R. O. & COOK, D. G. (Eds). New York: Plenum Publishing Corporation, p. 175–184.

Poddubnaya, T. L. (1959). On the dynamics of the tubificid population (Oligochaeta, Tubificidae) in the Rybinsk reservoir. **Rep. Inst. Reservoir Biol.** Borok, v. 2, p. 102–108.

Prantoni, A. L.; 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**, v.176, p. 485-510.

Prantoni, A. L.; Di Domenico, M.; Lana, P. C. (2014). First Record of Achaetous Marionina Michaelsen, 1890 (Annelida: Clitellata: Enchytraeidae) in the Southern Atlantic. **Zoosymposia**, v. 9(1), p. 10-13

Prantoni, A. L.; Di Domenico, M.; Lana, P. C. (2013). A taxonomic overview of marine and estuarine oligochaetes from Brazil. **Marine Biodiversity**, v. 44(3), p. 275–278.

Pfannkuche, O. (1981). Distribution, abundance and life cycles of aquatic Oligochaeta (Annelida) in a freshwater tidal flat of the Elbe Estuary **Archiv für Hydrobiologie**, v. 43, p. 506-524.

Pfannkuche, O. (1980). Distribution and abundance of Tubificidae and Naididae (Oligochaeta) in a brackish-water fjord, with special reference to the α-mesohaline zone. **Netherlands Journal of Sea Research**, v. 14, p. 78 – 93.

Pfannkuche, O. (1979). Abundance and lifecycle of littoral marine and brackish-water Tubificidae and Naididae (Oligochaeta) In: Naylor, E.; Hartnoll, R.C. (Eds**.). Cyclic phenomena in marine plants and animals**, Oxford: Pergamon Press, p. 103 – 111.

Righi, G. & Kanner, E. (1979) Marine Oligochaeta (Tubificidae and Enchytraeidae) from the Caribbean Sea. **Studies on the Fauna of Curaçao and other Caribbean Islands**, v. 58, p. 44-68.

Rodriguez, P. & Reynoldson, T. B. (2011) **The Pollution Biology of Aquatic Oligochaetes.** Netherland: Springer 261 p.

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

Rota, E. & Erséus, C. (2003). New records of Grania (Clitellata, Enchytraeidae) in the Northeast Atlantic (from Tromsø to the Canary Islands), with descriptions of seven new species. **Sarsia** v. 88, p. 210–243.

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

Rouse, G. W. & Fauchald, K. (1997). Cladistics and polychaetes. **Zoologica Scripta**, v. 26(2), p. 139–204.

Rousset, V.; Plaisance, L.; Erséus, C.; Siddall, M. E.; Rouse, G. W. (2008). Evolution of habitat preference in Clitellata (Annelida). **Biological Journal of the Linnean Society**, v. 95(3), p. 447–464.

Rousset, V.; Pleijel, F.; Rouse, G. W.; Erséus, C.; Siddall, M. E. (2007). A molecular phylogeny of annelids. **Cladistics**, v. 43, p. 41-63.

Ruppert, E. E.; Fox, R. S.; Bernes, R. D. (2005). **Zoologia dos invertebrados: uma abordagem functional-evolutiva.** São Paulo: Roca, 1145 p.

Schmelz, R. M. & R. Collado (2012): An updated checklist of currently accepted species of Enchytraeidae (Oligochaeta). **Agriculture and Forestry Research**, v. 357, p. 67–87.

Siddall, M. E.; Apakupakul, K.; Burreson, E. M.; Coates, K. A., Erséus, C.; Gelder, S. R.; Källersjö , M.; Trapido-Rosenthal, H. (2001). Validating Livanow: molecular data agree that leeches, branchiobdellidans, and *Acanthobdella peledina* form a monophyletic group of Oligochaetes. **Molecular Phylogenetics and Evolution**. v. 21, p. 346–351.

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

Struck, T. H. (2011). Direction of evolution within Annelida and the definition of Pleistoannelida. **Journal of Zoological Systematics and Evolutionary Research**, *49*(4), 340–345.

Struck, T. H.; Golombek, A.; Weigert, A.; Franke, F. A.; Westheide, W.; Purschke, G.; Bleidorn, C.; Halanych, K. M. (2015). The Evolution of Annelids Reveals Two Adaptive Routes to the Interstitial Realm. **Current Biology**.

Struck, T. H.; Paul, C.; Hill, N.; Shartmann, S.; Hösel, C.; Kube, M.; Lieb, B.; Meyer, A.; Tiedemann, R.; Purschke, G.; Bleidorn, C. (2011). Phylogenomic analyses unravel annelid evolution. **Nature,** v. 471, p. 95-98.

Struck, T. H.; Schult, N.; Kusen, T.; Hickman, E.; Bleidorn, C. Mchugh, D.; Halanych, K. M. (2007). Annelid phylogeny and the status of Sipuncula and Echiura. **BMC Evolutionary Biology**, v. 7, p. 57.

Struck, T. H. & Purschke G. (2005). The sister group relationship of Aeolosomatidae and Potamodrilidae (Annelida: 'Polychaeta') – a molecular phylogenetic approach based on 18S rDNA and cytochrome oxidase I. **Zoologischer Anzeiger,** v. 243 p. 281–293.

Takashima, Y & Mawatari, S. F. (1998). *Mitinokuidrilus excavatus* n. g., n. sp., a Marine Tubificid (Oligochaeta) with a Unique Mode of Reproduction. **Zoological Science**, v.15(4), p. 593-597.

Thrush, S. F. & Dayton, P. K. (2002). Disturbance to marine benthic habitats by trawling and dredging: implications to marine biodiversity. **Annual Reviews Ecology Systems**, v. 33, p. 449 – 473.

Watling, L. (1975). Analysis of structural variations in a shallow estuarine deposit-feeding community **Journal of Experimamntal Marine Biology and Ecology**, v. 19, p. 275–313.

Weigert, A. & Bleidorn, C. (2016). Current status of annelid phylogeny. **Organisms Diversity & Evolution**, http://doi.org/10.1007/s13127-016-0265-7.

Weigert, A.; Golombek, A.; Gerth, M.; Schwarz, F.; Struck, T. H.; Bleidorn, C. (2015). Evolution of mitochondrial gene order in Annelida. **Molecular Phylogenetics and Evolution**. v. 94 (A), p. 196–206.

Weigert, A.; Helm, C.; Meyer, M.; Nickel, B.; Arendt, D.; Hausdorf, B.; Santos, S. R.; Halanych, K. M.; Purschke, G.; Bleidorn, C.; Struck, T. H. (2014). Illuminating the Base of the Annelid Tree Using Transcriptomics. **Molecular Biology and Evolution**, v. 31(6), p. 1391–1401.

Zrzavý, J.; Říha, P.; Piálek, L.; Janouškovec, J. (2009). Phylogeny of Annelida (Lophotrochozoa): total-evidence analysis of morphology and six genes. **BMC Evolutionary Biology**, v. 9 (1), p. 189.

CAPÍTULO 1

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A taxonomic overview of marine and estuarine oligochaetes from Brazil

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Abstract Aquatic oligochaetes from South America are still poorly known. As a basis for a general survey of marine oligochaetes from the southwestern Atlantic, we present here a critical overview of the available taxonomic information on marine and estuarine oligochaetes from Brazil. Only 13 of the 600 described species are currently known from Brazil, and 8 exclusively from their type locations. The enchytraeid *Marionina* cf. *nevisensis* is newly reported from São Sebastião (São Paulo State). The distribution range of the naidid *Tectidrilus gabriellae* is expanded to the Paranaguá Bay (Paraná, S Brazil).

Keywords Oligochaeta · Marine oligochaetes · Brazil

Introduction

Among 1,700 species of currently known aquatic oligochaetes, 600 are marine or estuarine (Erséus 2005). Aquatic oligochaetes are small animals, ranging in size from 1 mm to a few centimeters, which explains why most are interstitial or meiofaunal (Erséus 1988). The most diverse and abundant oligochaete group is the Naididae, sensu Erséus et al. (2008), which includes about 1,000 species, 450 of which are marine (Martin et al 2008). Other families with marine taxa are Enchytraeidae, Capilloventridae, Randiellidae, and the predominantly terrestrial Megascolecidae.

Studies on marine and estuarine oligochaetes in Brazil are still scarce. Medeiros and Hadel (1999) and Migotto and Marques (2006) pointed out the major taxonomic impediments and barriers to appropriate evaluation of regional marine oligochaete diversity as lack of experts, lack of appropriate infrastructure, and lack of training and maintenance routines for work in zoological collections. Marine oligochaetes are morphologically very similar and display few conspicuous and diagnostic external characteristics. Consistent identifications rely on internal anatomy, including variations in the position and shape of internal organs, such as the spermatheca, pharyngeal glands, seminal vesicle, and penis, among others.

Brazilian marine oligochaetes have been described by Du Bois-Reymond Marcus (1950), Marcus (1965), Righi (1968), Erséus (1980, 1983), and Harman and Loden (1984). These studies focused primarily on punctual sites in São Paulo and Rio de Janeiro states. No tropical or subtropical geographic sectors have been systematically investigated along the Brazilian coast. More recently, Christoffersen (2007, 2009) provided useful catalogues of aquatic microdrile oligochaetes from South America and assessed their diversity and distribution patterns.

This study presents a critical taxonomic overview of marine and estuarine oligochaetes from Brazil as a basis for an ongoing general survey of marine oligochaetes from the southwestern Atlantic.

Materials and methods

The results of a bibliographic survey of Brazilian records of marine and estuarine oligochaetes are presented as a species list (Table 1). Additional field sampling surveys were carried out along the coasts of São Paulo and Paraná States (SE and S Brazil) to check for previously described and recorded species. Samples were collected using PVC pipes (10 cm in diameter and 10 cm in length) and stored in a plastic bucket

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containing 20 l of seawater. Relaxation was carried out with isotonic $MgCl_2$ with salinity up to 36 in an Erlenmeyer flask that was gently swirled and let to stand for 15 min. The flasks were inverted 3 to 4 times and the supernatant containing numbed animals was washed with fresh seawater in a 0.063-mm sieve and transferred into a Petri dish. Some animals were examined alive and morphotyped; others were preserved in 4 % formaldehyde for morphological analysis. Live animals were subsequently fixed in absolute ethanol for future molecular analyses or fixed in trialdehyde and cacodylate buffer solution for scanning electron microscopy (Higgins and Thiel 1988).

Results and discussion

Only 13 of the approximately 600 species of known marine and estuarine oligochaetes have been recorded from Brazil, and 8 are currently known only from their type locations (Table 1).

Field samplings from southern and southeastern Brazil provided a new species record, an enchytraeid belonging to the genus *Marionina*, characterized by the complete absence of dorsal or ventral chaetae. These animals were collected near the rocky promontory of the Ponta do Baleeiro Beach, in São Sebastião, São Paulo (23º49.689′S, 45º25.392′W; Datum: WGS 84). They are morphologically similar to *Marionina nevisensis* (Righi and Kanner 1979, from samples collected from Nevis Island in the Caribbean). However, Matamoros et al. (2012), in their revision of the achaetous *Marionina*, showed that *M. nevisensis* is in fact a complex of cryptic species. Further ecological information on this finding was provided by Prantoni et al. (2013).

The southern distribution range of the naidid *Tectidrilus gabriellae*, originally described from Cananéia (São Paulo), is expanded. *T. gabriellae* was collected in the Cobras Island, in

Table 1 Species of marine and brackish-water oligochaetes reported from Brazil

Taxon	Environment	Region
NAIDIDAE NAIDINAE		
Paranais evelinae (Marcus 1965)[a]	ES	Cananéia - SP
NAIDIDAE RHYACODRILINAE		
Monopylephorus parvus Ditlevesen, 1904	ES	Cananéia - SP
Jolydrilus jaulus Marcus 1965[a]	ES	Cananéia - SP
NAIDIDAE PHALLODRILINAE		
Aktedrilus dentatus Erséus 1983[a]	OB	Búzios - RJ
Aktedrilus brevis Erséus 1980[a]	OB	Salvador - BA
NAIDIDAE LIMNODRILOIDINAE		
Tectidrilus gabriellae (du Bois-Reymond Marcus, 1950)	ES	Ilha Bela - SP
	ES	Paranaguá – PR
ENCHYTRAEIDAE		
Enchytraeus albidus Henle, 1837	ES	Cananéia - SP
Lumbricillus sadovskyi Marcus 1965[a]	ES	Cananéia - SP
Marionina cana Marcus 1965[a]	ES	Cananéia - SP
Marionina nea Marcus 1965[a]	ES	Cananéia - SP
Marionina cf. *nevisensis* Prantoni; Di Domenico; Lana (in press)	SB	São Sebastião - SP
CAPILLOVENTRIDAE		
Capilloventer atlanticus Harman and Loden 1984[a]	ES	Rio de Janeiro – RJ
MEGASCOLECIDAE		
Pontodrilus litoralis (Grube, 1855)	MG	Cananéia – SP Angra dos Reis –RJ Rio de Janeiro - RJ Florianópolis – SC São Francisco do Sul – SC

For authorship and synonymies see Christoffersen's catalogues (2007, 2009)

ES Estuarine, *OB* oceanic beaches, *SB* sheltered estuarine beaches, *MG* mangrove

[a] Species only known from their original description

the euhaline sector of the Paranaguá Bay (25°28′28.51″S, 48°25′56.28″W; Datum: WGS 84). *T. gabriellae* was originally placed in *Peloscolex* Leidy, 1851, and subsequently transferred to *Tubificoides* Lastočkin, 1937, by Brinkhurst and Baker (1979). However, Erséus (1982) showed that the oesophageal diverticula and the morphology of the male ducts justified its inclusion within *Tectidrilus* Erséus 1982.

The identification of both *M*. cf. *nevisensis* and *T*. cf. *gabriellae* from S and SE Brazil is still preliminary and based only on morphological characters. More accurate morphological and molecular analyses are being performed to confirm their taxonomical status.

The latest descriptions of marine and estuarine oligochaete species from Brazil were published in the 1980s. Harman and Loden (1984) established a new genus and family, first believed to be exclusively marine, based on specimens of *Capilloventer atlanticus* from Guanabara Bay, Rio de Janeiro. This is the only oligochaete group with ventral and dorsal capillary setae. Erséus (1993) suggested that this could be a symplesiomorphy with polychaetes, a hypothesis that was not refuted through molecular analyses (Struck et al 2011). The phylogenetic position of this family has not been satisfactorily resolved. However, molecular analyses conducted by Erséus and Kallersjo (2004) and Rousset et al. (2007) support the hypothesis that Capilloventridae is a basal oligochaete clade. This hypothesis is also substantiated by morphological characteristics (Ferraguti et al. 1996). Erséus (1980, 1983) described two new meiofaunal species, *Aktedrilus brevis* and *A. dentatus*. The first species was collected in the intertidal region of the Farol da Barra beach at the mouth of the Todos os Santos Bay in Salvador, Bahia, and the second one was collected in the João Fernandes beach in Buzios, Rio de Janeiro.

Due to the lack of systematic surveys of Brazilian marine and estuarine oligochaetes, it is premature to make inferences about patterns of endemism. *Monopylephorus parvus* and *Enchytraeus albidus* presumably have cosmopolitan distributions (Erséus 1999). *Pontodrilus litoralis*, originally described as *Lumbricus litoralis*, has a circumtropical and subtropical distribution. Despite belonging to a predominantly continental and worldwide distributed family (Righi 1997), this megascolecid is quite common in estuaries and mangroves, tolerating salinities between 5 and 30 (Blakemore 2007). *Pontodrilus litoralis* is the aquatic oligochaete with the highest number of records in Brazil (Moreira 1903; Michaelsen 1910; Righi 1968). Although having being recorded only once in Brazil (in the original description), *T. gabriellae* is also known from Belize, Panama, Aruba, Bonaire, Barbados, and Curaçao (Erséus 1990). Further surveys of marine oligochaetes from southern and southeastern Brazil will most likely reveal a wider distribution of *T. gabriellae*, taking into account habitat diversity along the coast.

Specimens of the species described from the State of São Paulo are deposited at the Zoology Department from São Paulo University. The type materials described by Erséus (1980, 1983) and Harman and Loden (1984) are deposited at the Muséum National d'Histoire Naturelle (MNHN) in Paris, at the U.S. National Museum of Natural History (USNM 80608-80610), and at the Louisiana State University Museum of Zoology (LSU 3241). (Medeiros and Hadel 1999; Belúcio 1999). Zoological collections of marine and estuarine oligochaetes, formally organized and deposited, are available in two Brazilian institutions, the Institute of Biosciences of Universidade de São Paulo (IBUSP) and Universidade Federal da Paraíba.

The scarcity of studies and experts on Brazilian marine oligochaetes, as pointed out by Medeiros and Hadel (1999), is still evident to date. This is disturbing for a country with about 8,600 km of coastline. Systematic surveys of marine and estuarine oligochaetes in Brazil are sorely needed, as well as the training of taxonomists. Unified efforts between taxonomists and ecologists to include the study of oligochaete diversity as a priority in benthic surveys are fundamental to change this situation.

References

Belúcio IF (1999) Diagnóstico para avaliação e ações prioritárias da biodiversidade do bentos marinho do Brasil. Relatório técnico, PRONABIO, Ministério do Meio Ambiente

Blakemore RJ (2007) Origin and means of dispersal of cosmopolitan Pontodrilus litoralis (Oligochaeta: Megascolecidae). Eur J Soil Biol 43:S3–S8

Brinkhurst RO, Baker HR (1979) A reviewof the marine Tubificidae (Oligochaeta) of North America. Can J Zool 57:1553–1569

Christoffersen ML (2007) A catalogue of aquatic microdrile oligochaetes (Annelida: Clitellata) from South America. Acta Hydrobiol Sin 31: 59–86

Christoffersen ML (2009) Species diversity and distributions of microdrile earthworms (Annelida, Clitellata, Enchytraeidae) from South America. Zootaxa 2065:52–68

Du Bois-Reymond ME (1950) A marine tubificid from Brazil. Commun Zool Mus Montevideo 59(3):1–6

Erséus C (1980) Taxonomic studies on the marine genera *Aktedrilus* Knöllner and *Bacescuella* Habre (Oligochaeta, Tubificidae), with descriptions of seven new species. Zool Scr 9:97–111

Erséus C (1982) Taxonomic revision of the marine genus *Limnodrioides* (Oligochaeta, Tubificidae). Verh Naturw Ver Hamb 25:207–277

Erséus C (1983) Two new species of *Aktedrilus* (Oligochaeta, Tubificidae) from the Western Atlantic. Bull Mus Nat Hist Nat 4:1051–1057

Erséus C (1988) Oligichaeta. In: Higgins RP, Thiel H (eds) Introductions to the study of meiofauna. Smithsonian Institution Press, Washington, pp 349–354

Erséus C (1990) Marine Tubificidae (Oligochaeta) of the barrier reef ecosystems at Carrie Bow Cay, Belize, and other parts of the Caribbean Sea, with descriptions of twenty–seven. Zool Scr 19:243–303

Erséus C (1993) Taxonomy of Capilloventer (Capilloventridae), a little-known group of aquatic Oligochaeta, with descriptions of two new species. J Nat Hist 27:1029–1040

Erséus C (1999) Marine Tubificidae (Oligochaeta) from a mangrove habitat in Kenya. Trop Zool 12:137–143

Erséus C (2005) Phylogeny of oligochaetous Clitellata. Hydrobiologia 535(53):355–370

Erséus C, Källersjö M (2004) 18S rDNA phylogeny of Clitellata (Annelida). Zool Scr 33:187–196

Erséus C, Wetzel MJ, Gustavsson L (2008) ICZN rules—a farewell to Tubificidae (Annelida, Clitellata). Zootaxa 1744:66–68

Ferraguti M, Erséus C, Pinder A (1996) The spermatozoon of *Capilloventer australis* and the systematic position of the Capilloventridae (Annelida: Oligochaeta). Aust J Zool 44:469–478

Harman WJ, Loden MS (1984) *Capilloventer atlanticus* gen. et sp. n., a member of a new family of marine Oligocheta from Brazil. Hydrobiologia 115:51–54

Higgins RP, Thiel H (1988) Introduction to the study of meiofauna. Smithsonian Institution Press, Washington

Marcus E (1965) Naidomorpha aus brasilianischen Brackwasser. Beitr Neotrop Fauna 4(2):61–83

Martin P, Martinez-Ansemil E, Pinder A, Timm T, Wetzel MJ (2008) Global diversity of oligochaetous clitellates (“Oligochaeta”; Clitellata) in freshwater. Hydrobiologia 595:117–127

Matamoros L, Rota E, Erséus C (2012) Cryptic diversity among the achaetous *Marionina* (Annelida, Clitellata, Enchytraeidae). Syst Biodivers 10(4):509–525

Medeiros LRA, Hadel VF (1999) Oligochaeta. In: Joly CA, Bicudo CEM (orgs). Biodiversidade do estado de São Paulo, Brasil. In: Migotto AE, Tiago CG (eds) síntese do conhecimento ao final do século XX, 3: invertebrados marinhos. FAPESP, São Paulo, pp 177–181

Michaelsen W (1910) Oligochaeten von verschiedenen Gebieten. Mitt Mus Hamburg 27:47–169

Migotto AE, Marques AC (2006) Invertebrados marinhos. In: Lewinsohn T (ed) Avaliação do estado do conhecimento da biodiversidade marinha brasileira. Ministério do Meio Ambiente, Brasilia, pp 149–202

Moreira C (1903) Vermes Oligochaetos do Brasil. Arch Mus Rio de Janeiro 12:125–136

Prantoni AL, Di Dimenico M, Lana PC (in press) First record of achaetous *Marionina* Michaelsen, 1890 (Annelida: Clitellata: Enchytraeidae) in the southern Atlantic. Zoosymposia

Righi G (1968) Sobre alguns Oligochaeta do Brasil. Rev Bras Biol 28(4): 369–382

Righi G (1997) Minhocas da América Latina: diversidade, função e valor. In: Anais do XXVI Congresso Brasileiro de Ciência do Solo. SBCS, Rio de Janeiro. CD-Rom

Righi G, Kanner E (1979) Marine Oligochaeta (Tubificidae and Enchytraeidae) from the Caribbean Sea. Stud Fauna Curaçao Caribbean Isl 58:44–68

Rousset V, Pleijel F, Rouse GW, Erséus C, Siddall ME (2007) A molecular phylogeny of annelids. Cladistics 43:41–63

Struck TH, Paul C, Hill NS, Hartmann S, Hösel C, Kube M, Lieb B, Meyer A, Tiedemann R, Purschke G, Bleidorn C (2011) Phylogenomic analyses unravel annelid evolution. Nature 471: 95–98

CAPÍTULO 2

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First record of achaetous *Marionina* Michaelsen, 1890 (Annelida: Clitellata: Enchytraeidae) in the southern Atlantic

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Short note

Marionina Michaelsen, 1890 is a polyphyletic enchytraeid genus represented by about 100 nominal species (Rota et al., 2008). When the genus was originally described in 1889, it got a preoccupied name, *Marionia*, which was corrected into *Marionina* one year later by Michaelsen himself. According to the ICZN (1999: Art. 60.3), a new replacement name has its own author and date. Thus, the correct nomenclature of the genus should be *Marionina* Michaelsen, 1890 (Rota et al., 2008).

Marionina has marine and non-marine species (Erséus et al. 2010). The former may be found in tidal debris, on mangrove aerial roots, in clean sandy beaches, in sublittoral sediments, and on rocky shores (Healy & Coates 1999). Some of the marine species are unusual in that they lack chaetae, and are grouped under the name "achaetous *Marionina*" (Matamoros et al. 2012). The first published description of an achaetous *Marionina* occurred under the name *Michaelsena achaeta* Hagen, 1954, a taxon later augmented and transferred to *Marionina* by Lasserre (1964). Two other achaetous taxa are currently named: *Marionina arenaria* Healy, 1979, and the former subspecies *Marionina achaeta nevisensis* Righi & Kanner, 1979, later raised to species status as *Marionina nevisensis* by Coates (1983). A comprehensive review of the diversity of "achaetous *Marionina*" was recently performed by combining morphological and molecular data (Matamoros et al. 2012). According to these authors, specimens of achaetous *Marionina* from a number of worldwide localities (Caribbean, Australia and northern Europe) make up a monophyletic grouping comprised of 11 separately evolving lineages, which could be assigned to seven different morphotypes. Only two of these morphotypes could be identified as nominal taxa, *M. nevisensis* Righi & Kanner, 1979 sensu lato and *Marionina nothachaeta* [=*M. achaeta* sensu Lasserre, 1964]. Based on segment numbers, Matamoros et al. (2012) suggested that *Marionina arenaria* and *M. achaeta* sensu Hagen (1954) may be the same species. *M. arenaria* are only available for study at the Natural Museum of Ireland (Natural History Division) in Dublin (NMI). The only *Marionina* species reported from the southern Atlantic are *Marionina cana* Marcus, 1965 and *Marionina nea* Marcus, 1965, both endowed with chaetae (Prantoni et al. in press).

Individuals of *Marionina* without chaetae were collected in July 2012 in an intertidal pond, in bottoms made up by gravel, shell fragments, and coarse sand, close to the rocky promontory of the Ponta do Baleeiro beach, Municipality of São Sebastião, São Paulo State, southeastern Brazil (23°49.689' S; 45° 25.392' W). The site is exposed to waves, and local salinity is around 35 PSU (Figure 1).

Four individuals were analyzed, two of which were mature and devoid of chaetae. Besides being achaetous, they were identified as *Marionina* based on the following diagnostic characteristics: 31–42 segments; total length between 3.6 and 5.5 mm; cuticle thickness between 2 and 3 µm; prostomium conical, wider than long; clitellum diameter of 168 µm, annular in XII–XIII, with glandular cells arranged in transverse lines; seminal vesicle unilateral; dorsal anterior blood vessel bifurcated in III or IV; coelomocytes dispersed, irregular and egg-shaped, with cytoplasm filled with small grains; sperm funnels (Figure 2).

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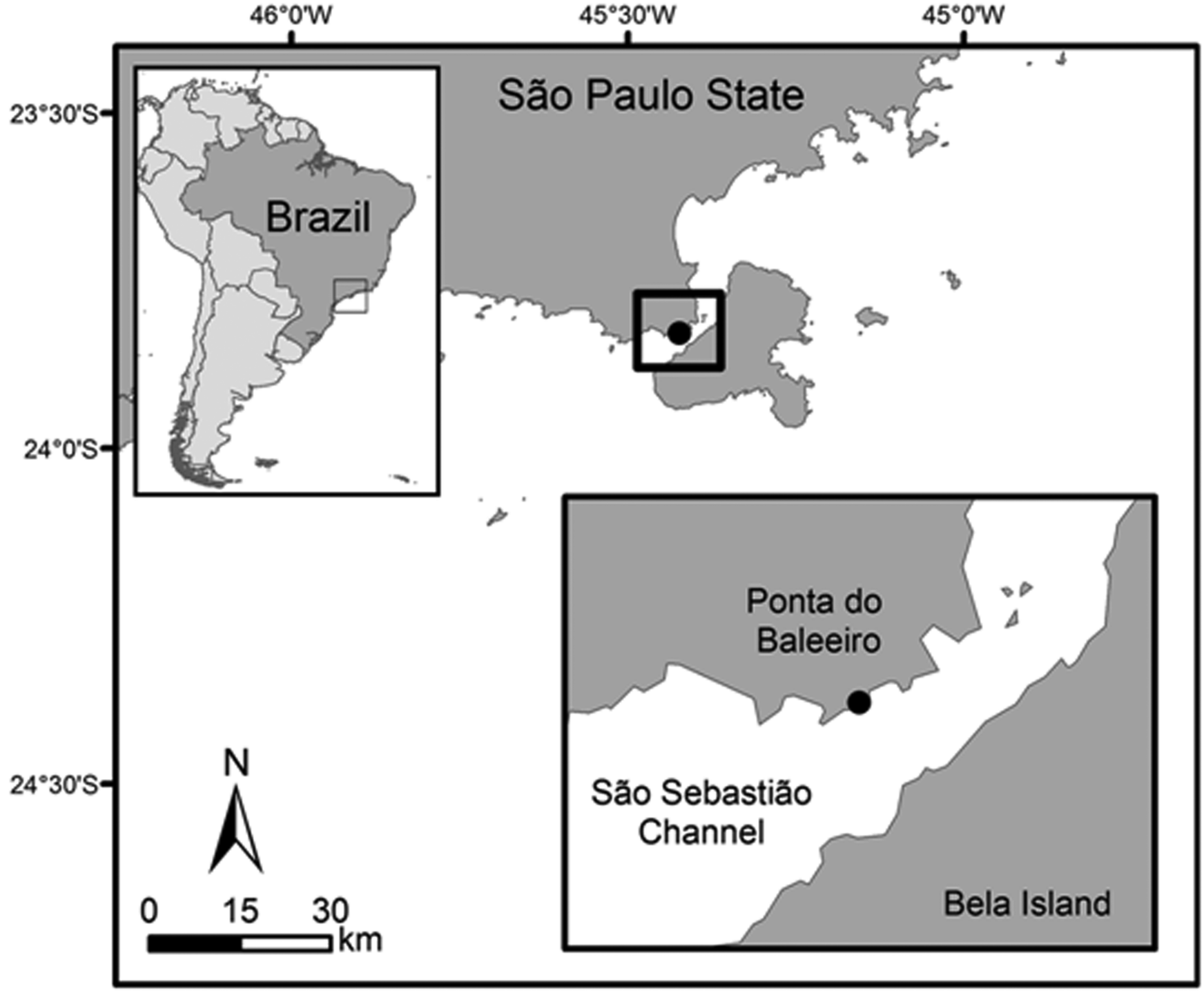

FIGURE 1: Collection site of achaetous *Marionina* in southern Brazil.

This is the first record of an achaetous *Marionina* for the southern Atlantic. The animals collected in São Paulo appear morphologically indistinguishable from *M. nevisensis* Righi & Kanner, 1979 described from Nevis Island in the Caribbean. However, it is possible that *M. nevisensis* as recognized or described subsequently by a number of different authors (Coates, 1983; Erséus, 1990; Erséus et al., 1990; Coates, 1990; Coates & Stacey, 1993; Healy & Coates, 1999) is a complex of globally distributed cryptic species (Matamoros et al. 2012). Further study of the Brazilian specimens is still necessary, including analyses of characters from DNA sequences, and observations with transmission and scanning electron microscopy.

The examination of additional characters will be essential to test whether the shared morphological characteristics are homologous, or whether they represent environmentally convergent adaptations.

Matamoros et al. (2012) suggested that all tropical lineages (e.g., from Central America and Oceania) of achaetous *Marionina* seem to constitute a monophyletic group that originated from ancestors living in temperate climate regions. However, they have also suggested that more extensive sampling, especially in the temperate regions, might reveal a different evolutionary history.

While reporting a new geographical record of achaetous *Marionina*, we emphasize the scarcity of studies on the fauna of brackish-water and marine oligochaetes in the southern Atlantic. Indeed, only 13 of the approximately 600 described species of marine and brackish-water oligochaetes have been reported from Brazil, almost all of them known only from their type localities. A systematic survey of the group in the southern and southeastern Brazil will likely reveal a much higher diversity, considering the variety of regional habitats.

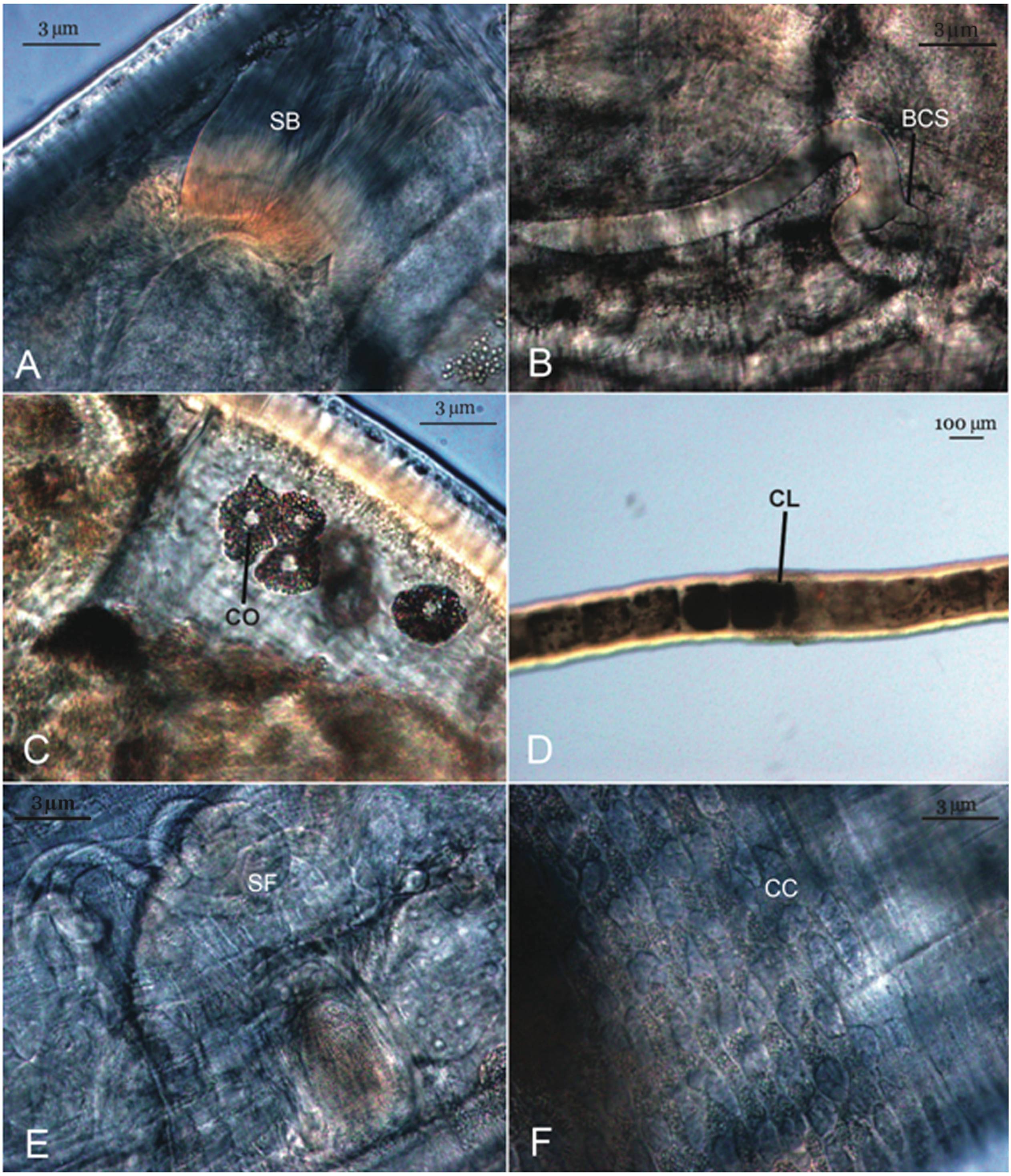

FIGURE 2: Anatomy of the achaetous *Marionina* from Sao Paulo. (A) a bundle of sperm, SB, covering the sperm funnel; (B) bifurcated anterior dorsal blood vessel, BCS; (C) coelomocytes, CO; (D) clitellum, CL; (E) vas deferens; (F) clitellar cells, CC.

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References

Coates, K.A. (1990) Marine Enchytraeidae (Oligochaeta, Annelida) of the Albany area, Western Australia. *In*: Wells, F.E., Walker, D.I., Kirkman, H. & Lethbridge, R., (Eds), *The Marine Flora and Fauna of Albany, Western Australia*. Western Australian Museum, Perth, pp. 13–41.

Coates, K.A. (1983) New records of marine *Marionina* (Oligochaeta, Enchytraeidae) from the Pacific Northeast, with a description of *Marionina klaskisharum* sp. nov. *Canadian Journal of Zoology*, 61, 822–831. http://dx.doi.org/10.1139/z83-108

Coates, K.A. & Stacey, D. (1993) The marine Enchytraeidae (Oligochaeta, Annelida) of Rottnest Island, Western Australia. *In*: Wells, F.E., Walker, D.I., Kirkman, H. & Lethbridge, R., (Eds), *The Marine Flora and Fauna of Rottnest Island, Western Australia*. Western Australian Museum, Perth, pp. 391–414.

Erséus, C. (1990) Marine Oligochaeta of Hong Kong. *In*: Morton, B., (Eds), *Proceedings of the Second International Marine Biological Workshop: The Marine Flora and Fauna of Hong Kong and Southern*. Hong Kong University Press, Hong Kong, pp. 259–335.

Erséus, C., Rota, E., Matamoros, L. & De Wit, P. (2010) Molecular phylogeny of Enchytraeidae (Annelida, Clitellata). *Molecular Phylogenetics and Evolution*, 57, 849–858. http://dx.doi.org/10.1016/j.ympev.2010.07.005

Erséus, C., Daoyuan, S., Yanling, L.& Bin, S. (1990) Marine Oligochaeta of Jiaozhou Bay, Yellow Sea coast of China. *Hydrobiologia,* 202, 107–124. http://dx.doi.org/10.1007/bf00027096

Hagen, G. (1954) *Michaelsena achaeta* nov. sp., ein neuer mariner Oligochaet aus der Kieler Bucht. *Faunistische Mitteilungen aus Norddeutschland*, 1, 12–13.

Healy, B. (1979) Three new species of Enchytraeidae (Oligochaeta) from Ireland. *Zoological Journal of the Linnean Society*, 67, 87–96.

Healy, B. & Coates, K. A. (1999) Finding enchytraeid oligochaetes in hot climates: species occurrence on the shores of Bermuda. *Hydrobiologia*, 406, 111–117.

Lasserre, P. (1964) Notes sur quelques oligochètes Enchytraeidae, presents dans les plages du Bassin d' Archacon. *Procès Verbaux des Séances de La Société Linnéenne de Bordeaux*, 101, 87–91.

Matamoros, L., Rota, E & Erséus, C. (2012) Cryptic diversity among the achaetous *Marionina* (Annelida, Clitellata, Enchytraeidae). *Systematics and Biodiversity*, 10: 4, 509–525. http://dx.doi.org/10.1080/14772000.2012.723640

Michaelsen, W. (1890) *In*: Pfeffer, G. Die niedere, Thierwelt des antarktischen Ufergebietes. Die internationale Polarforschung 1882–1883. *Die Deutschen Expeditionen und ihre Ergebnisse* (5) 2, (17), 455–574.

Prantoni, A.L., Di Domenico, M. & Lana, P.C. (in press). A taxonomic overview of marine and estuarine oligochaetes from Brazil. *Marine Biodiversity.*

Righi, G. & Kanner, E. (1979) Marine Oligochaeta (Tubificidae and Enchytraeidae) from the Caribbean Sea. *Studies of the Fauna of Curaçao and other Caribbean Islands*, 58, 44–68.

Rota, E., Matamoros, L. & Erséus, C. (2008) In search of *Marionina* (Clitellata: Enchytraeidae): a taxonomic history of the genus and re-description of the type species *Pachydrilus georgianus* Michaelsen, 1888. *Italian Journal of Zoology*, 75, 417–436. http://dx.doi.org/10.1080/11250000801930433

CAPÍTULO 3

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Zoological Journal of the Linnean Society, 2016, **176**, 485–510. With 11 figures.

First reports of *Grania* (Clitellata: Enchytraeidae) from Africa and South America: molecular phylogeny and descriptions of nine new species

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In this article, our knowledge of the geographic distribution of *Grania* species is expanded by describing seven new species, ***Grania bekkouchei* sp. nov.**, ***Grania brasiliensis* sp. nov.**, ***Grania capensis* sp. nov.**, ***Grania chilensis* sp. nov.**, ***Grania cryptica* sp. nov.**, ***Grania hinojosai* sp. nov.**, and ***Grania simonae* sp. nov.**, from poorly investigated regions of the Southern Hemisphere, plus two new species, ***Grania carolinensis* sp. nov.** and ***Grania unitheca* sp. nov.**, from off the east coast of the USA. An immature achaetous specimen that we call *Grania* cf. *levis* was also included. The newly generated data were combined with a previously published data set in order to update the hypothesis of phylogenetic relationships among *Grania* species. All new species except ***G. cryptica* sp. nov.** are supported by both morphological and molecular data. In addition, we find that ***G. chilensis* sp. nov.** is structured in at least four distinct populations along the Chilean coast. The species described from South Africa form a monophyletic clade where two are morphologically indistinguishable but diverging in both mitochondrial and nuclear ribosomal genes, and thus we describe them as different species, ***G. bekkouchei* sp. nov.** and ***G. cryptica* sp. nov.** Among the North Carolinian species, ***G. unitheca* sp. nov.** is indicated as a close relative of *Grania monospermatheca* Erséus & Lasserre, 1976, and ***G. carolinensis* sp. nov.** is indicated as a close relative of *G.* cf. *levis*. The updated phylogeny is strongly concordant with geographical species distributions, thus supporting a low level of dispersal within this genus, as has previously been hypothesized.

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ADDITIONAL KEYWORDS: cryptic species – Enchytraeidae – *Grania* – marine Oligochaeta – molecular phylogeny – South Africa – South America.

INTRODUCTION

Grania Southern, 1913 is one of the largest enchytraeid genera (Erséus *et al*., 2010), today comprising 72 nominal species (De Wit, Rota & Erséus, 2011b). Exclusively marine, they are found in a wide range of habitats, from the intertidal zone to the deep sea (Coates, 1984; Erséus & Rota, 2003; Rota & Erséus, 2003; De Wit & Erséus, 2010). Most *Grania* species inhabit the interstitial space of shelly and coarse sediments, and they are generally small and always slender, between 10 and 20 mm long but only about 0.1 mm wide (Erséus & Lasserre, 1976; Rota & Erséus, 2003; De Wit & Erséus, 2010).

The genus is morphologically well separated from other enchytraeid genera. For instance, in *Grania* the cephalization, which is a common feature in the family

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Enchytraeidae, is unusually complex, with a well-developed nervous system that includes, in some species, the so-called 'head organ', which is thought to be a compound georeceptor (Rota *et al*., 1999; Rota & Erséus, 2003). Moreover, in this genus the chaetal bundles are each represented by only one stout chaeta (Erséus & Lasserre, 1976; De Wit *et al*., 2011b), and the circular body musculature is reduced, resulting in a nematode-like, coiling, form of locomotion (Rota, 2001; De Wit, Erséus & Gustavsson, 2011a). Yet species distinction in *Grania* is complex, and a unique combination of multiple morphological features must be used (e.g. chaetal distribution and shape, and details of the penial apparatus and spermatheca) for an accurate and unambiguous identification of morphospecies (Erséus & Lasserre, 1976; De Wit & Erséus, 2010).

In addition, a cryptic species, *Grania occulta* De Wit & Erséus, 2010, which is sympatric with, yet morphologically indistinguishable from, *Grania ovitheca* Erséus, 1977, was recently described from Scandinavian waters using molecular data. This provides a first hint that the species diversity of the genus may be greater than previously thought (De Wit & Erséus, 2010), which highlights the need for including genetic data in species descriptions.

An initial phylogenetic analysis of 20 species of *Grania* was recently performed using a combination of molecular and morphological data (De Wit *et al*., 2011b). According to that study, the genus is well supported as monophyletic and contains three main lineages (referred to as clades A, B, and C by De Wit *et al*., 2011b). Clade A consists of all North Atlantic species, and is further subdivided into one European and one North American subclade. Clade B contains some peculiarly coloured (yellowish green) species from the Indo-Pacific region, as well as Chilean species and *Grania Americana* Kennedy, 1966, whereas clade C comprises species from Australia and Hong Kong (De Wit *et al*., 2011b).

In an ancestral state reconstruction of the total evidence phylogeny (De Wit *et al*., 2011b), most morphological characters, such as chaetal shape, midventral glands, and shape of penial apparati and spermathecae, were found to be highly homoplasious. In addition, a morphology-based phylogeny was found to be incongruent with one based on genetic data from six loci; however, the geographic distribution of the species in the analysis showed great congruence with the phylogeny, suggesting limited dispersal capabilities (De Wit *et al*., 2011b). One caveat, though, was that the geographic sampling was skewed to locations from which recent sampling efforts had been conducted, and it was concluded that including data from other parts of the world, such as Africa and South America, would be crucial to updating the phylogeny of this genus.

This is not a simple task, however, as the biogeographical knowledge of *Grania* species is largely restricted to the North Atlantic (Southern, 1913; Knöllner, 1935; Lasserre, 1966, 1967; Erséus, 1974, 1977; Erséus & Lasserre, 1976; Kossmagk-Stephan, 1983; Locke & Coates, 1999; Rota & Erséus, 2003; De Wit & Erséus, 2010) and some Indo-Pacific regions (Jamieson, 1977; Coates, 1990; Erséus, 1990; Coates & Stacey, 1993, 1997; Rota & Erséus, 2000; Rota, Erséus & Wang, 2003; De Wit & Erséus, 2007; Rota, Wang & Erséus, 2007; De Wit, Rota & Erséus, 2009). Except for some Antarctic (Rota & Erséus, 1996) and sub-Antarctic (Erséus & Lasserre, 1977; Rota & Erséus, 1997) areas, in most parts of the Southern Hemisphere, particularly along the Atlantic and Pacific coasts of South America, and both sides of southern Africa, the genus has remained almost unexplored (De Wit *et al*., 2011b; Prantoni, Di Domenico & Lana, 2013).

Herein we describe nine new species, seven from from coastal areas of Brazil, South Africa, and Chile, and two from eastern USA, combining molecular and morphological information. We also present an updated hypothesis of the phylogenetic relationships among *Grania* species.

MATERIAL AND METHODS

COLLECTION AND IDENTIFICATION

The Chilean specimens of *Grania* were collected by the second author, in February 2009. South African *Grania* were collected by Nicholas Bekkouche, during a workshop on annelid systematics arranged in South Africa by Dr Carol Simon (Stellenbosch University) in December 2011, and the North American species were collected by the third author during a cruise with R/V *Cape Hatteras*, off North Carolina, in May 2011. In Chile and South Africa, sediment samples were collected by hand, stirred with seawater, followed by the decantation of suspension into a 0.25-mm mesh. The North American material was obtained from sieved sediments collected with a box corer from the research vessel.

The Brazilian specimens from Ponta do Baleeiro beach, Sao Paulo State, were sampled in October 2012 during the workshop *Taxonomy and Diversity of Marine Meiofauna – Brazil*. These, as well as the specimens from Paranaguá Bay collected in August 2013, were sampled by taking sediment cores with PVC pipes of 10 cm in length and 10 cm in diameter, stored in a plastic bucket with seawater, relaxed with isotonic $MgCl_2$, and with salinity up to 36 ppt. The supernatant containing numbed animals was washed with fresh seawater in a 0.063-mm sieve and transferred into a Petri dish.

All worms were sorted alive using a stereomicroscope and, with few exceptions, preserved in 80%

ethanol. Some specimens were collected incomplete, but most had a complete body with pygidium. Many of these were intentionally cut and a few (sometimes counted) 'amputated segments' (posterior or middle) were removed and stored in 95% ethanol for subsequent DNA extraction and sequencing. The holotype of *Grania brasiliensis* sp. nov. was killed and stored in 99% ethanol, and then its posterior end was cut for DNA analysis; the paratypes of the same species were fixed in 4% formaldehyde and left uncut. The anterior end of the cut specimens from Chile, South Africa, and Brazil, as well as all complete worms, were stained in alcoholic paracarmine solution and mounted whole in Canada balsam on slides for the purpose of identification. The worms were morphologically examined using light and interference contrast microscopy, and drawings were made with the aid of a camera lucida.

In the descriptions, the chaetal length means the straight line from the distal tip to the furthest proximal point of the chaeta. The chaetal foot length is understood as the maximal breadth of the L-shaped chaetae at their ental end, and the chaetal index was calculated as the average ratio of the chaetal length by the chaetal foot length, ±standard deviation (Rota & Erséus, 2003). Penial bulb lengths were measured as their extension parallel with the long body axis, and widths were taken as perpendicular to the longitudinal body axis.

Type specimens are deposited in the Museu de Zoologia da Universidade Estadual de Campinas 'Adão José Cardoso' (ZUEC), Campinas, Brazil, Iziko South African Museum (SAMC), Cape Town, South Africa, Swedish Museum of Natural History (SMNH), Stockholm, Sweden, and, US National Museum of Natural History (USNM), Washington, DC, USA.

ABBREVIATIONS USED IN THE FIGURES

amp, spermathecal ampulla; cl, coelomocyte; ed, ectal duct of spermatheca; ggc, granular gland cell of clitellum; hgc, hyaline gland cell of clitellum; mp, male pore; pb, penial bulb; sb, sperm bundle; sf, sperm funnel; sr, sperm ring; spp, spermathecal pore.

GENETIC ANALYSES

The DNA of 38 individuals (Table 1) was extracted using the DNeasy Blood & Tissue kit (Qiagen), or the QuickExtract DNA Extraction Solution 1.0 (Epicentre), after which polymerase chain reactions (PCRs) were performed for the mitochondrial cytochrome *c* oxidase subunit I (*COI*) locus, using the universal 'barcoding primers' LCO1490/HCO2198 (Folmer *et al*., 1994). One evolutionary lineage represented by an immature specimen from off North Carolina lacks chaetae completely; we refer to it as *Grania* cf. *levis* Coates & Erséus, 1985, also from the north-west Atlantic Ocean and the only formally described *Grania* with this striking feature. In addition, one specimen (CE20818) of *G. brasiliensis* sp. nov., one specimen (CE21024) of *Grania hinojosai* sp. nov., and nine specimens of *Grania chilensis* sp. nov. (CE21036, CE21037, CE21038, CE21039, CE21040, PDW189, PDW197 PDW200, and PDW202) were *COI* barcoded but not found suitable for closer morphological study. To compare the mitochondrial data with nuclear DNA patterns, 23 individuals were also chosen to be amplified at the *ITS* region (Table 1), using primers ITS5/ITS4 (White *et al*., 1990) and 5.8 mussF and 5.8 mussR (Källersjö *et al*., 2005). Finally, one individual from each putative species was chosen for phylogeny reconstruction (Table 1). For each of these ten individuals, four additional loci were amplified (*18S* rDNA and the D1 region of *28S* rDNA of the nuclear genome; and *12S* and *16S* rDNA of the mitochondrial genome) using primers TimA/TimB for *18S* (Norén & Jondelius, 1999), 28SC1/28SC2 (Dayrat *et al*., 2001) and 12SE1/12SH for *12S* (Jamieson *et al*., 2002), and 16SarL/16SbrH for *16S* (Palumbi *et al*., 1991).

The amplicons were sequenced either by Macrogen Inc. (Seoul, South Korea) or by Eurofins mwg operon (Ebersberg, Germany) using additional sequencing primers for *18S* (600F, 1806R, 4FBK, 4FB, 5f and 7fk; Norén & Jondelius, 1999), assembled using GENEIOUS PRO 4.8.5 (Rozen & Skaletzky, 2000) from Biomatters Ltd (Auckland, New Zealand), and aligned using MAFFT 6 (Katoh & Toh, 2008), applying the L-INS-i setting (slow–accurate). Alignments were tested for models of best fit using the BIC criterion in jModeltest (with the *COI* alignment partitioned by codon position; Guindon & Gascuel, 2003; Darriba *et al*., 2012).

The *COI* and the *ITS* loci were first analyzed individually using Bayesian inference implemented in the parallel version of MrBayes 3.2 (Ronquist & Huelsenbeck, 2003), with two parallel runs of 20 million generations, sampling trees every 1000 generations (*COI* partitions unlinked in all parameters except topology). After examining the output files for convergence using the online software AWTY (Wilgenbusch, Warren & Swofford, 2004), majority-rule consensus trees were created by summarizing all trees after a burn-in period of 5 million generations. Bayesian support values were then plotted on the consensus trees. For *COI*, a distance matrix was plotted (Table S1), which was then used to plot a histogram to examine the prevalence of the so-called 'barcoding gap'. In addition, a haplotype network was created using TCS 1.21 (Clement, Posada & Crandall, 2000) of one of the putative Chilean species, in which there was significant genetic variation.

For the phylogeny reconstruction, the newly generated data were combined with the data set of De Wit *et al*. (2011b), and realigned and tested for models of

Table 1. Specimens of the new species used for genetic analysis, with places of origin, voucher numbers, and GenBank accession numbers

Taxon	Spm	Locality	Voucher	COI	ITS	16S	12S	18S	28S
Grania chilensis	CE21036	Chile, Valdivia	SMNH 147308	KT428079					
Grania chilensis	PDW193	Chile, Valdivia	ZUEC-CLI 13	GU902190	GU902182	GU902141	GU902127	GU902154	GU902168
Grania chilensis	PDW194	Chile, Valdivia	ZUEC-CLI 16	KT428080	KT428058				
Grania chilensis	CE21037	Chile, Valdivia	SMNH 147309	KT428081					
Grania chilensis	CE21038	Chile, Valdivia	SMNH 147310	KT428082					
Grania chilensis	CE21039	Chile, Valdivia	SMNH 147311	KT428083					
Grania chilensis	CE21040	Chile, Valdivia	SMNH 147312	KT428084					
Grania chilensis	PDW190	Chile, Valdivia	ZUEC-CLI 14	KT428085	KT428059				
Grania chilensis	PDW191	Chile, Valdivia	ZUEC-CLI 15	KT428086	KT428060				
Grania chilensis	PDW185	Chile, Coquimbo	ZUEC-CLI 17	KT428087	KT428061				
Grania chilensis	PDW189	Chile, Coquimbo	SMNH 147313	KT428088	KT428062				
Grania chilensis	PDW197	Chile, Concepcion	SMNH 147314	KT428092	KT428066				
Grania chilensis	PDW198	Chile, Concepcion	ZUEC-CLI 18	KT428089	KT428063				
Grania chilensis	PDW199	Chile, Concepcion	ZUEC-CLI 19	KT428090	KT428064				
Grania chilensis	PDW200	Chile, Concepcion	SMNH 147315	KT428091	KT428065				
Grania chilensis	PDW202	Chile, Concepcion	SMNH 147316	KT428093					
Grania hinojosai	PDW177	Chile, Coquimbo	ZUEC-CLI 10	KT428094	KT428067				
Grania hinojosai	PDW182	Chile, Coquimbo	ZUEC-CLI 12	KT428095	KT428068				
Grania hinojosai	PDW186	Chile, Coquimbo	ZUEC-CLI 08	GU902189	GU902181	GU902140	GU902126	GU902153	GU902167
Grania hinojosai	PDW181	Chile, Coquimbo	ZUEC-CLI 11	KT428096	KT428069				
Grania hinojosai	PDW187	Chile, Coquimbo	ZUEC-CLI 09	KT428097	KT428070				
Grania hinojosai	CE21024	Chile, Coquimbo	SMNH 147317	KT428098					
Grania simonae	CE14058	S Africa, Van Dyks Bay	SAMC A82479	KT428101	KT428071 (ITS2 only)	KT375040	KT375048	KT375055	KT375063
Grania simonae	CE14060	S Africa, Van Dyks Bay	SAMC A82480	KT428099					
Grania simonae	CE14110	S Africa, Van Dyks Bay	SAMC A82477	KT428102					
Grania simonae	CE14111	S Africa, Van Dyks Bay	SAMC A82476	KT428100					
Grania simonae	CE14093	S Africa, Van Dyks Bay	SAMC A82481	KT428103					
Grania capensis	CE14015	S Africa, False Bay	SAMC A82474	KT428104	KT428072	KT375041	KT375049	KT375056	KT375064
Grania capensis	CE14016	S Africa, False Bay	SAMC A82475	KT428105					
Grania bekkouchei	CE13975	S Africa, Saldanha Bay	SAMC A82467	KT428106	KT428073	KT375042	KT375050	KT375057	KT375065
Grania bekkouchei	CE13996	S Africa, Saldanha Bay	SAMC A82466	KT428107					
Grania bekkouchei	CE14059	S Africa, Van Dyks Bay	SAMC A82472	KT428108					
Grania cryptica	CE14031	S Africa, False Bay	SAMC A82473	KT428109	KT428074	KT375043	KT375051	KT375058	KT375066
Grania brasiliensis	CE20734	Brazil, Paranagua	ZUEC-CLI 04	KT428110	KT428075	KT375044	KT375052	KT375059	KT375067
Grania brasiliensis	CE20818	Brazil, Baleiro	–	KT428111					
Grania carolinensis	CE11569	USA, N Carolina, 492 m	USNM1283174	KT428112	KT428076	KT375045	KT375053	KT375060	
Grania unitheca	CE11659	USA, N Carolina, 17 m	USNM1283175	KT428113	KT428077	KT375046		KT375061	
Grania cf. *levis*	CE11570	USA, N Carolina, 492 m	USNM1283176	KT428114	KT428078	KT375047	KT375054	KT375062	

Note that for some of the species not all specimens were suitable for closer morphological study.

best fit as described above. The alignments were first concatenated into two loci (mitochondrial and nuclear ribosomal DNA) and run in MrBayes as described above, in order to test whether the two loci share the same phylogeny (Figs S1, S2). After determining that no statistically supported incongruences exist between the gene trees, the two loci were combined into one matrix and analysed again as described above.

RESULTS

TAXONOMY

GRANIA BRASILIENSIS SP. NOV.

FIGURE 1

Holotype

ZUEC CLI 04, individual CE20734, whole-mounted, sexually mature specimen, with some segments amputated, from Ponta do Poço, Paranaguá Bay, Paraná State, southern coast of Brazil, 25°32′54″S, 48°23′18″W, estuarine subtidal, 7 m depth, medium to coarse sand with some mud, and lots of shell and cirriped fragments. Collected by A. L. Prantoni, 19 August 2013. *COI* barcode sequence, GenBank acc. no KT428110; for other sequence data, see Table 1.

Etymology

Named for Brazil.

Paratypes

ZUEC CLI 05–CLI 07, three whole-mounted adult mature specimens, one from the type locality and two from the rocky promontory of the Ponta do Baleeiro beach, São Sebastião, São Paulo State, south-east coast of Brazil, 23°49′41″S, 45°25′23″W, lower intertidal pond, gravel and coarse sand with shell fragments, A. L. Prantoni, 28 October 2012. Paratypes not barcoded.

Description

Holotype > 5.71 mm long, > 38 segments (posterior end used for genetic analyses), 0.18 mm wide at segment III, 0.15 mm wide at segment XII, and 0.12 mm wide at segment XXI. Paratypes (complete adults) 5.28–13.7 mm (*n* = 3), 40–61 segments (*n* = 3), 0.12–0.14 mm wide at segment III, 0.13–0.15 mm wide at segment XII, 0.13–0.17 mm wide at segment XXI (*n* = 2; one paratype not suitable for width measurements). Prostomium small, rounded, 45–65 µm long, 39–81 µm wide (*n* = 4); epidermis not reduced at front tip, 7–16 µm thick. Ventral chaetae from segment IV, lateral chaetae from segment XVII (*n* = 2) or segment XVIII (*n* = 2). Chaetae (Fig. 1A) 35–60 µm long, shaft straight, 3.5–5.0 µm thick at midpoint, L-shaped, proximally bent into a short oblique foot, with low instep and receding heel. Chaetal index (Rota & Erséus, 2003) 3.61 ± 0.56 (*n* = 5). Epidermal gland cells inconspicuous. Clitellum (not well developed in the holotype) maximally 18 µm thick, extending over segment XII and anterior half of segment XIII, comprising more or less regular transverse rows of hyaline cells alternating with granular cells; hyaline cells more abundant than granular cells; both types of cell absent midventrally between male pores (Fig. 1C). Spermathecal pores paired, lateral, somewhat posterior to 4/5. Male pores ventrolateral in middle of segment XII. Female pores ventrolateral, just posterior to 12/13. Anus directed ventrad (paratypes).

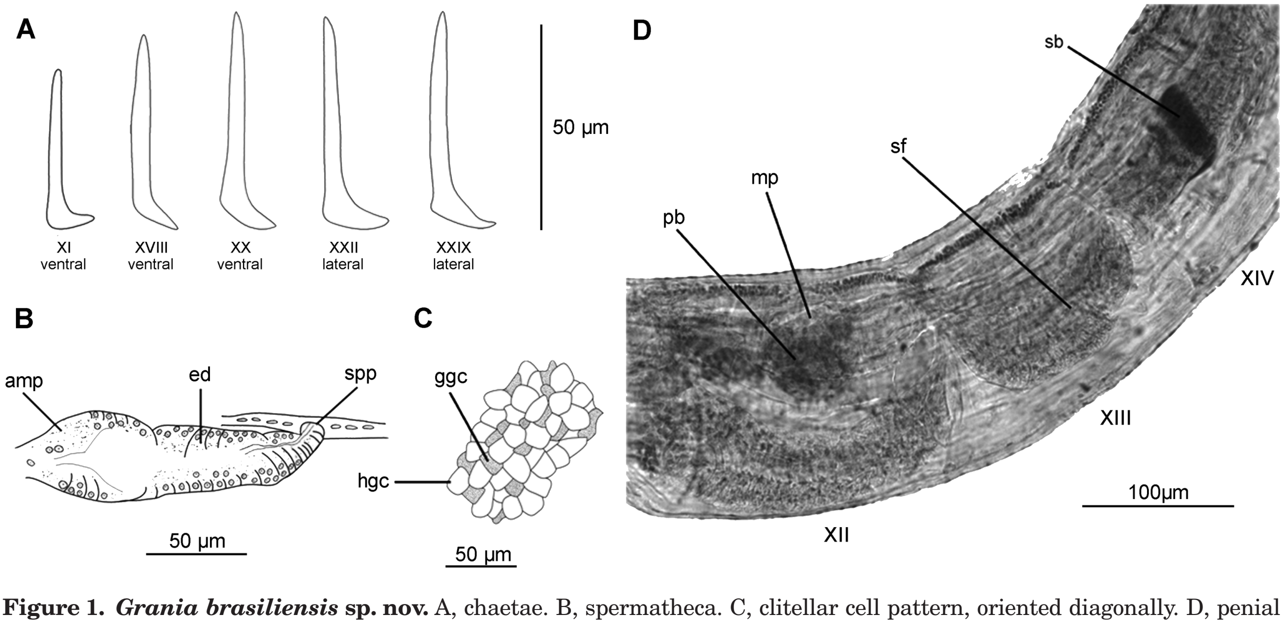

Figure 1. ***Grania brasiliensis* sp. nov.** A, chaetae. B, spermatheca. C, clitellar cell pattern, oriented diagonally. D, penial bulb and sperm funnel. See text for abbreviations.

Brain posteriorly indented. Head organ (*sensu* Rota & Erséus, 1996) absent. Pharyngeal glands in segments IV–VI; dorsal lobes present in segment IV (one pair), segment V (one pair), and in segment VI (one pair), ventral lobes present in segment IV (one pair), segment V (two pairs), and in segment VI (two pairs); glands not connected dorsally. Nephridia not observed. Chloragogen cells inconspicuous. Dorsal blood vessel arising in segment XIX or XX. Coelomocytes sparse, small, up to 11.5 μm long, irregularly oval or circular; cytoplasm with fine granulation around cell nucleus. Sperm sac extending into segments XIV–XVI. Egg sac extending into segments XVII–XXII. Sperm funnels very long, in segments XI–XII (paratype) or segments XI–XIV (holotype), 10–12 times longer than wide, directed posteriorly ($n = 2$) (Fig. 1D). Vasa deferentia 7 μm wide, coiled and reaching at least into segment XV. Penial apparatus type 1 (*sensu* Coates, 1984), with small, round bulb, 59 μm long, 62 μm wide ($n = 1$), or oval bulb, 69 μm long, 38 μm wide ($n = 1$); bulb glandular, surrounding a simple invaginated male pore; stylet absent (Fig. 1D). Midventral copulatory gland (in segment XIV) present. Spermathecae communicating with the oesophagus close to septum 5/6 through narrow ental ducts. Ectal ducts spindle-shaped, thick-walled, muscular, 67–79 μm long, 31–40 μm thick at midcourse, joining ampullar cavity through conspicuous deep conical intrusion. Ampullae 41–66 μm long, 36–59 μm wide; oval, ectally granulated (Fig. 1B). No glands at ectal pores of spermathecae. Sperm rings not observed in ampullae.

Remarks

The combination of the long sperm funnel, presence of a midventral copulatory gland in segment XIV, and the unusual clitellum, with hyaline cells more abundant than granular cells, may differentiate this new species from all other described species of *Grania*.

Grania brasiliensis sp. nov. appears to be similar to the Caribbean species *G. americana*, by the shape of the spermathecae, total length, and number of segments. In addition, the genetic analyses indicate that these two species are closely related (see Phylogenetic analyses). There are at least two obvious morphological differences, however: *G. americana* is distinguished from *G. brasiliensis* sp. nov. by its large glands at the spermathecal pores and the presence of a head organ.

Two species from the South Atlantic, sub-Antarctic island of South Georgia, i.e. *Grania monochaeta* (Michaelsen, 1888) and *Grania lasserrei* Rota & Erséus, 1997, share the presence of a midventral copulatory gland (in segment XIV) with *G. brasiliensis* sp. nov. A third species from the same island, *Grania stephensoniana* Rota & Erséus, 1997, also seems to have this gland, but it was not clearly seen because of the poor quality of the specimens available for the original description (see Rota & Erséus, 1997); *G. stephensoniana* is also larger than all the other species just mentioned. *Grania monochaeta* is similar to *G. brasiliensis* sp. nov. in the location of the spermathecal pores (at some distance from 4/5), but it has glands at its spermathecal pores and stylets in its penial bulbs; *G. brasiliensis* sp. nov. lacks these characters. Both *G. lasserrei* and *G. stephensoniana* possess a head organ (Rota & Erséus, 1996), a structure observed neither in *G. brasiliensis* sp. nov. nor in *G. monochaeta*.

The presence of a copulatory gland in segment XIV is also characteristic of the Tasmanian species *Grania tasmaniae* Rota & Erséus, 2000, but like *G. lasserrei* and *G. stephensoniana*, this species also has a head organ, and furthermore, an unusual type of coelomocytes; according to Rota & Erséus (2000), these cells resemble the spindle-shaped coelomocytes found in *Cernosvitoviella* and some species of *Chamaedrilus* (formerly *Cognettia*; see Martinsson, Rota & Erséus, 2014).

Despite sharing some characters with *G. brasiliensis* sp. nov., all of the species from South Africa described below (see their respective remarks), have sperm funnels that are short, not long as in the *G. brasiliensis* sp. nov., and a clitellar epidermis that seems to contain only granular cells (no rows of hyaline cells as in the Brazilian species).

The length of the sperm funnels and the absence of stylets also distinguish *G. brasiliensis* sp. nov. from the Caribbean *Grania bermudensis* Erséus & Lasserre, 1976, *Grania laxartus* Locke & Coates, 1999, and *Grania hylae* Locke & Coates, 1999. Moreover, these species differ from *G. brasiliensis* sp. nov. by their complex penial apparatus, and the presence of a head organ in both *G. laxartus* and *G. hylae*.

Among the *Grania* species with elongate sperm funnels, a pattern of clitellar cells similar to that in *G. brasiliensis* sp. nov., i.e. alternating rows of granular and hyaline cells, has also been described for the Western Australian *Grania sperantia* Rota, Wang & Erséus, 2007; however, this species lacks lateral chaetae and has an unusually long clitellum (extending over segments XI–XIII and sometimes over a part of segment XIV), with a thicker and more complex 'multiband' pattern, i.e. the hyaline cells are more numerous than the granular cells only at the two ends ('borders of clitellum'; Rota *et al.*, 2007).

The sperm funnels are very long (between nine and 18 times longer than wide), in *G. brasiliensis* sp. nov. and *G. sperantia*, as well as in the other Australian species *Grania conjuncta* Coates & Stacey, 1993, *Grania vacivasa* Coates & Stacey, 1993, *Grania eurystila* Coates & Stacey, 1997, and *Grania integra* Coates & Stacey, 1997; however, all these species are totally devoid of lateral chaetae, which is not the case in *G. brasiliensis* sp. nov.

GRANIA BEKKOUCHEI SP. NOV.

FIGURE 2

Holotype

SAMC A82466, CE13996, whole-mounted, sexually mature specimen, ten posterior segments amputated, from Saldanha Bay, West Coast district, Province of the Western Cape, South Africa, 33°00′25″S, 17°56′45″E, intertidal coarse sand in rock crevice. Collected by N. Bekkouche, 13 December 2011. *COI* barcode sequence, GenBank acc. no. KT428107; for other sequence data, see Table 1.

Etymology

Named for Nicolas Bekkouche, the collector of the type material.

Paratypes

Six whole-mounted, sexually mature specimens, all collected by N. Bekkouche. SAMC A82467, CE13975, with 13 posterior segments amputated; SAMC A82468, CE13995, with some segments amputated, SAMC A82469, CE13997, with 11 middle body segments amputated; SAMC A82470, CE13998, with nine middle body segments amputated; all from the type locality and the type date. SAMC A82471, CE14035, with some segments amputated, from Glencairn Heights, False Bay, City of Cape Town, Province of the Western Cape, South Africa, 34°09′29″S, 18°26′01″E, lower intertidal rocky pool, 15 December 2011. SAMC A82472, CE14059, with seven middle body segments amputated from Van Dyks Bay, Overberg District, Overstrand Local Municipality, Province of the Western Cape, South Africa, 34°37′00″S, 19°21′21″E, rocky beach, shallow subtidal, 16 December 2011. For *COI* barcodes of paratypes, see Table 1.

Description

Body > 6.6–12.5 mm long (*n* = 5), comprising 55–72 segments (*n* = 5) (including the segments used for DNA analysis), 0.16–0.17 mm wide at segment III, and 0.15–0.23 mm wide at segment XII (*n* = 7). Prostomium rounded, 50–77 µm long, 55–80 µm wide, occasionally epidermis slightly reduced at front tip, 7–12 µm thick (*n* = 7). Ventral chaetae from segment IV, lateral chaetae from segment XIV (*n* = 4) or segment XV (*n* = 3), sometimes present in preclitellar segments VII–VIII (*n* = 1), VII–IX (*n* = 1), or VII–X (*n* = 1). Chaetae (Fig. 2A) 54–74 µm long, shaft straight, 3.7–6.2 µm thick at midpoint, L-shaped, proximally bent into a foot with broad instep and indistinct heel. Chaetal index (Rota & Erséus, 2003) 4.92 ± 0.68 (*n* = 5). Free chaetae (partly resorbed?) scattered in coelomic cavity, mostly in preclitellar segments. Epidermal gland cells inconspicuous. Clitellum 12–19 µm thick, extending from segment XII to middle of segment XIII, formed by more or less regular transverse rows of granular cells, absent between male pores, hyaline cells not observed. Spermathecal pores in lateral lines, at short distance from 4/5. Male pores ventrolateral in mid segment XII. Female pores lateral (?) in 12/13.

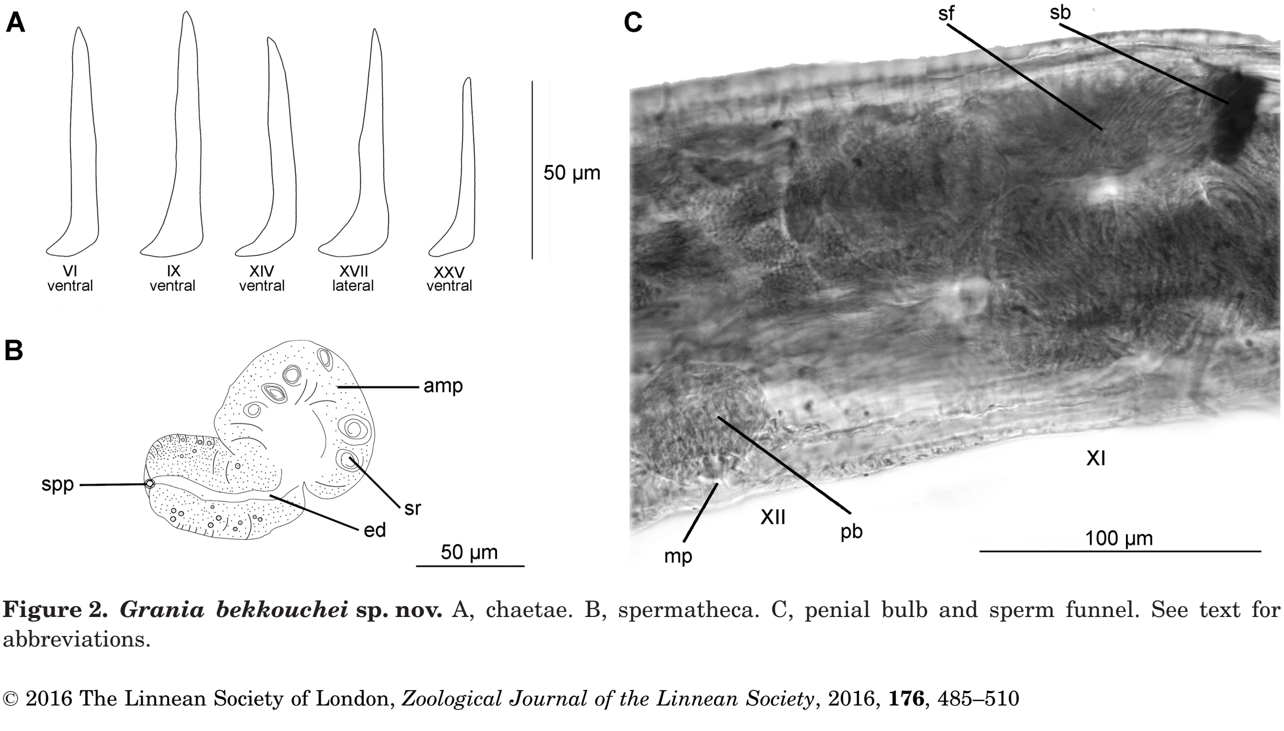

Figure 2. ***Grania bekkouchei* sp. nov.** A, chaetae. B, spermatheca. C, penial bulb and sperm funnel. See text for abbreviations.

Brain posteriorly indented. Head organ (*sensu* Rota & Erséus, 1996) absent. Pharyngeal glands in segments IV–VI; dorsal lobes present in segments IV (one pair), V (one pair), and VI (one pair), ventral lobes present in segments IV (one pair), V (two pairs), and VI (two pairs); glands not connected dorsally. Nephridia not observed. Chloragogen cells inconspicuous. Dorsal blood vessel commencing in segments XVIII–XX. Coelomocytes not observed. Sperm sac reaching XVII. Egg sac extending into segments XVII–XXII. Sperm funnels about 2.5 times longer than wide ($n = 5$; Fig. 2C). Vasa deferentia 7.5 μm wide, internally ciliated, coiled, extending into segments XII–XV. Penial apparatus type 1 (*sensu* Coates, 1984), with oval or round bulb, 55–77 μm long, 45–87 μm wide ($n = 7$); bulb glandular, surrounding a simple invaginated male pore; stylet absent (Fig. 2C). Midventral copulatory gland (in segment XIV) present. Spermathecae communicating with oesophagus at posterior end of segment V. Ectal duct of spermatheca 75–110 μm long, 27–43 μm wide, muscular, maintaining uniform width over its entire length, proximally curved to enter ampulla, devoid of glands at pore. Spermathecal ampulla 60–75 μm long, 60–82 μm wide, dome-shaped with granular walls, containing sperm rings, each maximally 15 μm wide (Fig. 2B).

Remarks

Although noted for some unidentified specimens from the Marion and Crozet islands in the Southern Indian Ocean (see Rota & Erséus, 1997), the presence of lateral chaetae in preclitellar segments is rather unusual in this genus, and is only formally reported for *G. lasserrei* and one specimen of *G. monochaeta*, both from South Georgia Island (Rota & Erséus, 1997). According to Erséus & Lasserre (1976) and Rota & Erséus (1997), the beginning of the chaetal distribution, particularly that of the lateral chaetae, is subject to considerable intraspecific variation. Even so, *G. lasserrei* differs from *G. bekkouchei* sp. nov. by its spermatheca, which has a dorsal, thin-walled, and sacciform diverticulum on the spermathecal ampulla. In *G. monochaeta* the ectal duct of the spermatheca narrows at both ends. The ampulla of *G. bekkouchei* sp. nov. is dome-shaped and the ectal duct maintains the same width along its entire length. In addition, *G. lasserrei* possesses a head organ, which is absent in *G. bekkouchei sp. nov.*

Although seldom noted in descriptions of Enchytraeidae, we observed a lateral ring of histologically distinct cells indicating a small pore-like structure in the intersegmental furrows 12/13 of the holotype: we interpret these structures as the female pores.

Among the new South African species described here, *G. bekkouchei* sp. nov., and its cryptic sister species *Grania cryptica* sp. nov., differ from *Grania capensis* sp. nov. in the morphology of the spermathecae. Unlike the former two species, *G. capensis* sp. nov. has a pear-shaped ampulla and an ectal duct that narrows near the pores. The species are also separated by the chaetal distribution, although some anterior lateral chaetae are irregularly distributed in all three (see the preceding paragraph). The postclitellar lateral chaetae start in segments XIV–XV in *G. bekkouchei* sp. nov., in segment XVIII in *G. cryptica* sp. nov., and in segments XXI–XXII in *G. capensis* sp. nov. In addition, the chaetae of *G. bekkouchei* sp. nov. have a broad instep and no prominent heel, which make them morphologically different from those of *G. capensis* sp. nov. (see Figs 3, 5).

Grania simonae sp. nov., also from South Africa, is easily distinguished from *G. bekkouchei* sp. nov. by the absence of lateral chaetae, the absence of a midventral copulatory gland (in segment XIV), the morphology of the spermathecae, and the start of the dorsal blood vessel (see remarks for *G. simonae* sp. nov. below).

A cryptic form, morphologically similar to *G. bekkouchei* sp. nov., is described as a separate species below (*G. cryptica* sp. nov.).

GRANIA CRYPTICA SP. NOV.

FIGURE 3

Holotype

SAMC A82473, CE14031, whole-mounted, sexually mature specimen, with 11 midbody segments amputated, from Glencairn Heights, False Bay, City of Cape Town, Province of the Western Cape, South Africa, 34°09′29″S, 18°26′01″E, lower intertidal rocky pool, 15 December 2011. Collected by N. Bekkouche, 15 December 2011. *COI* barcode sequence, GenBank acc. No. KT428109; for other sequence data, see Table 1.

Etymology

Named *cryptica* because it is morphologically ‘hidden’ (cryptic) vis-à-vis *G. bekkouchei* sp. nov.

Description

Body > 11.6 mm long, 64 segments (including 11 segments used for DNA analysis), 0.17 mm wide at segment III, and 0.18 mm wide at segment XII. Prostomium rounded, 72 μm long, 62 μm wide, epidermis 15 μm thick, reduced to 10 μm at front tip. Ventral chaetae from segment IV, lateral chaetae in preclitellar segments in segments VI–IX; in postclitellar segments from segment XVIII. Chaetae (Fig. 3A) 66–75 μm long, shaft straight, 5–6 μm thick at midpoint, entally hook-shaped. Free chaetae (partly resorbed?) scattered in the coelomic cavity, present in both preclitellar and postclitellar segments. Epidermal gland cells inconspicuous. Clitellum 10 μm thick, extending from segment XII to middle of segment XIII, formed by more or less regular transverse rows of granular

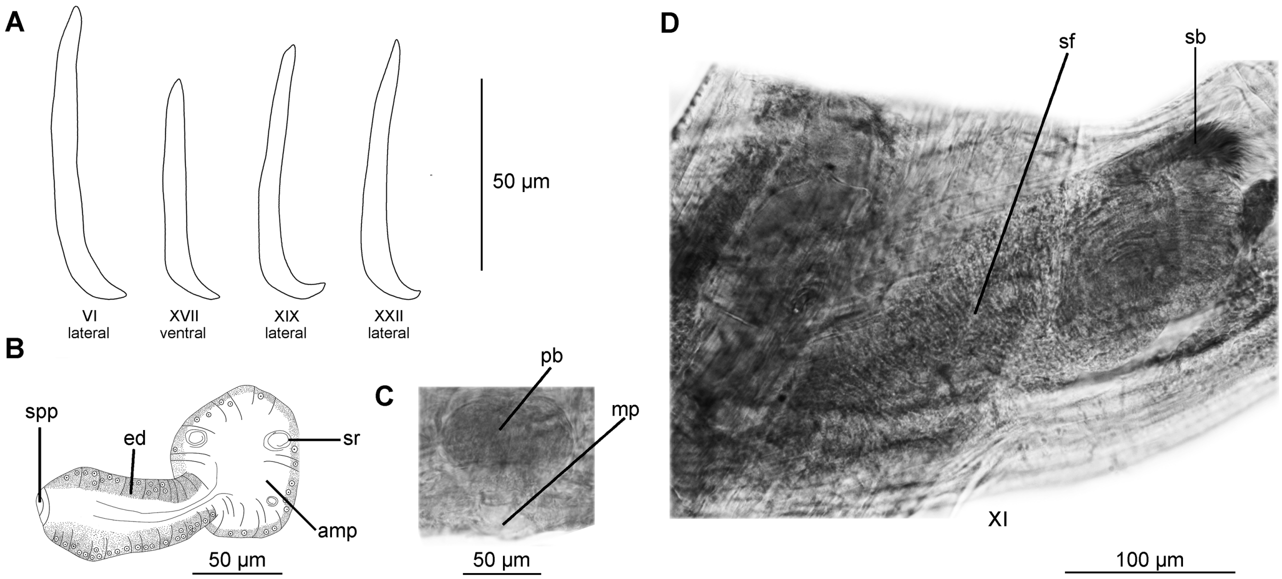

Figure 3. ***Grania cryptica* sp. nov.** A, chaetae. B, spermatheca. C, penial bulb. D, sperm funnel. See text for abbreviations.

cells, absent between male pores, hyaline cells not observed. Spermathecal pores in lateral lines, located at short distance from 4/5. Male pores ventrolateral in mid segment XII. Female pores not observed.

Brain posteriorly indented. Head organ (*sensu* Rota & Erséus, 1996) absent. Pharyngeal glands in segments IV–VI; dorsal lobes present in segment IV (one pair), in segment V (one pair), and in segment VI (one pair), ventral lobes present in segment IV (one pair), in segment V (two pairs), and in segment VI (two pairs); glands not connected dorsally. Nephridia not observed. Chloragogen cells inconspicuous. Dorsal blood vessel commencing in segment XVIII. Coelomocytes not observed. Sperm sac reaching segment XVII. Egg sac not developed. Sperm funnels about 2.5 times longer than wide (Fig. 3D). Vasa deferentia 10 µm wide, internally ciliated, coiled, extending into segments XII–XVI. Penial apparatus type 1 (*sensu* Coates, 1984), with round bulb, 88 µm long, 88 µm wide; bulb glandular, surrounding a simple invaginated male pore; stylet absent (Fig. 3C). Midventral copulatory gland (in segment XIV) present. Spermathecae communicating with oesophagus at posterior end of segment V. Ectal duct of spermatheca 94 µm long, 40 µm wide, muscular, curved, and slightly narrowing at distal end, devoid of glands at pore. Spermathecal ampulla 77 µm long, 80 µm wide, dome-shaped with granular walls, enclosing sperm rings, each maximally 16 µm wide (Fig. 3B).

Remarks

The only distinguishing morphological feature observed between *G. cryptica* sp. nov. and *G. bekkouchei* sp. nov. is the shape of the chaetae. The latter has L-shaped chaetae with a broad instep (Fig. 4A, B, C), whereas in *G. cryptica* sp. nov. the chaetae are hook-shaped (Fig. 4D, E, F). The shape of the spermatheca is virtually identical in *G. bekkouchei* sp. nov. and *G. cryptica* sp. nov., with a possible difference, suggested by the single specimen of *G. cryptica* sp. nov., that the ectal duct gently narrows to its junction with the ampulla in the latter species. This, however, may be an artifact from slide mounting.

Although *G. bekkouchei* sp. nov. and *G. cryptica* sp. nov. are difficult to distinguish morphologically, they genetically differ from each other in all loci investigated, clearly indicating that they are separately evolving lineages. This, in combination with their sympatric distribution, strongly supports them to be two different species.

GRANIA CAPENSIS SP. NOV.

FIGURE 5

Holotype

SAMC A82474, CE14015, whole-mounted, sexually mature specimen, with 11 midbody segments amputated, from Glencairn Heights, False Bay, City of Cape Town, Province of the Western Cape, South Africa, 34°09′29″S, 18°26′01″E, lower intertidal rocky pool. Collected by N. Bekkouche, 15 December 2011. *COI* barcode sequence, GenBank acc. no. KT428104; for other sequence data, see Table 1.

Etymology

Named *capensis* for the Cape Town area.

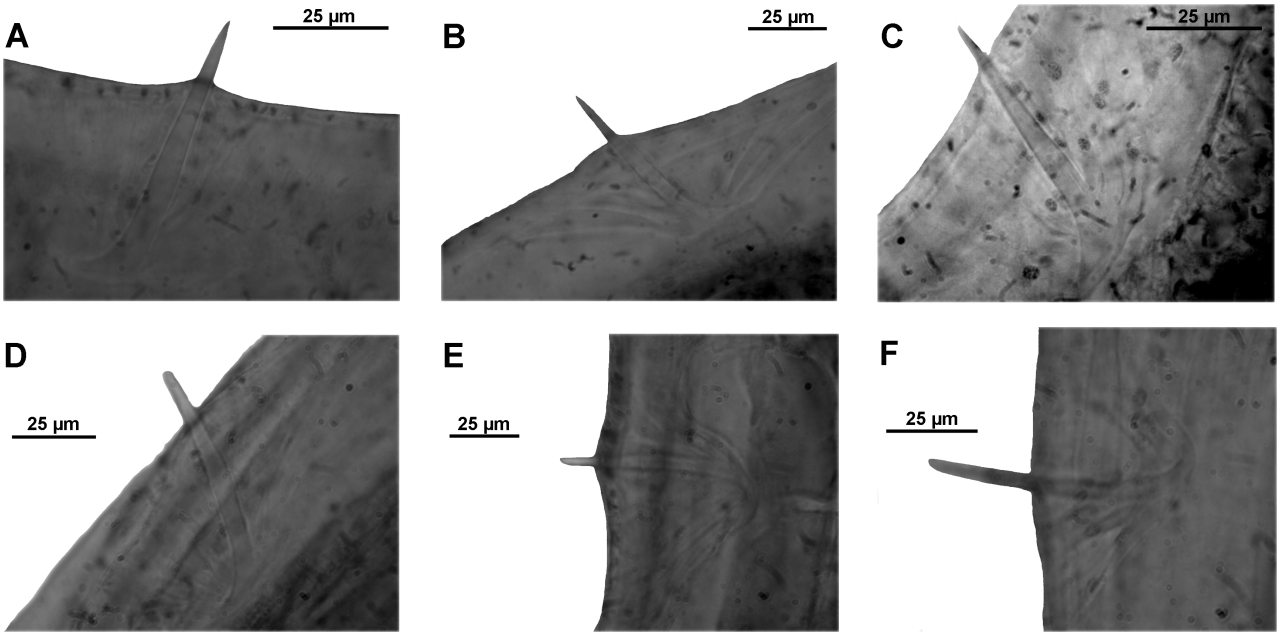

Figure 4. Chaetae of ***Grania bekkouchei* sp. nov.** A, ventral, from segment VII. B, ventral, from segment X. C, ventral, from segment XXXI. Chaetae of ***Grania cryptica* sp. nov.** D, lateral, from segment XXI. E, ventral, from segment XXVII. F, ventral, from segment XVIII.

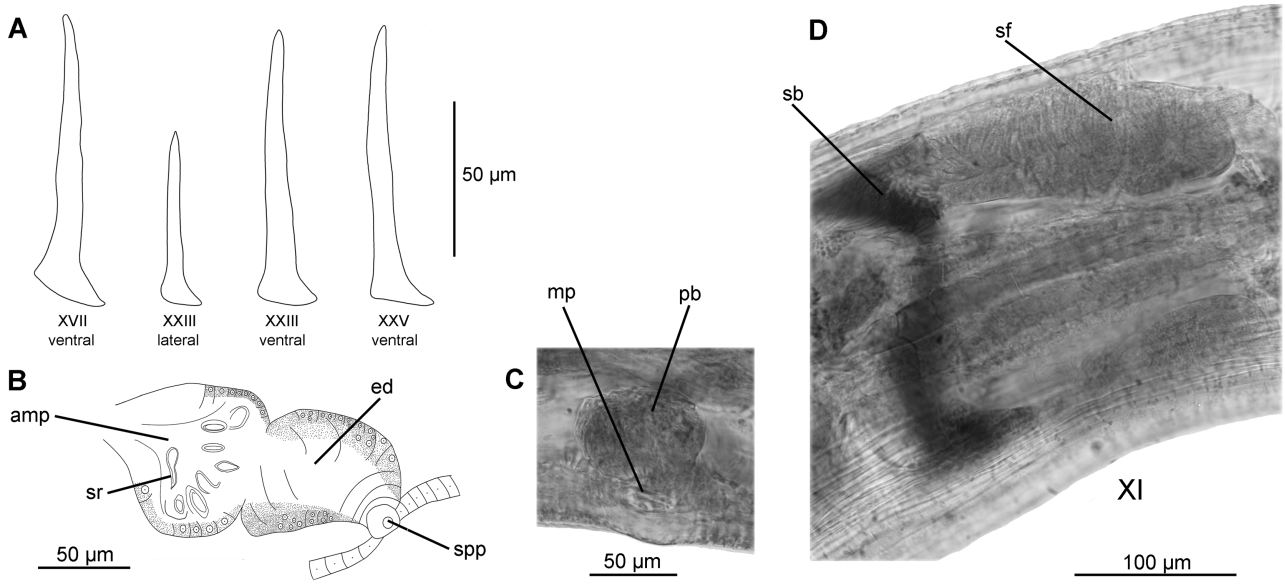

Figure 5. ***Grania capensis* sp. nov.** A, chaetae. B, spermatheca. C, penial bulb. D, sperm funnel. See text for abbreviations.

Paratype

SAMC A82475, CE14016, one whole-mounted, sexually mature specimen, ten middle body segments amputated, from type locality, N. Bekkouche, 15 December 2011. For *COI* barcode of paratype, see Table 1.

Description

Body > 9.2 and 9.9 mm long, 49 and 63 segments ($n = 2$) (including segments used for DNA analysis), 0.22 and 0.24 mm wide at segment III, 0.20 and 0.26 mm wide at segment XII ($n = 2$). Prostomium rounded, 83 and 120 μm long, 100 and 110 μm wide; epidermis 17 and

12 μm thick ($n = 2$), not notably reduced at front tip. Ventral chaetae from segment IV, lateral chaetae occasionally present in segment VII ($n = 1$), but otherwise from segments XXI or XXII. Chaetae (Fig. 5A) 45–90 μm long, shaft straight, 5.0–7.5 μm thick at midpoint, L-shaped, proximally curving into an 11–25 μm long foot with indistinct heel. Chaetal index (Rota & Erséus, 2003) 4.42 ± 0.88 ($n = 4$). Free chaetae sometimes present, scattered in coelomic cavity. Epidermal gland cells inconspicuous. Clitellum not well developed, 12.5 μm thick, extending from segment XII to two-thirds of segment XIII, formed by more or less regular transverse rows of granular cells, absent between male pores, hyaline cells not observed. Spermathecal pores in lateral lines, slightly posterior to 4/5. Male pores ventrolateral in mid-XII. Female pores not observed.

Brain posteriorly indented. Head organ (*sensu* Rota & Erséus, 1996) absent. Pharyngeal glands in segments IV–VI; dorsal lobes present in segment IV (one pair), in segment V (one pair), and in segment VI (one pair), ventral lobes present in segment IV (one pair), in segment V (two pairs), and in segment VI (two pairs); glands not connected dorsally. Nephridia not observed. Chloragogen cells not observed. Dorsal blood vessel originating in segments XIX (paratype) or XX (holotype). Coelomocytes not observed in available specimens. Sperm sac extending into segments XVI–XVII. Egg sac not developed. Sperm funnels cylindrical, about three times longer than wide (Fig. 5D). Vasa deferentia 15 μm wide, internally ciliated, coiled, reaching at least segment XV. Penial apparatus type 1 (*sensu* Coates, 1984), consisting of a glandular bulb, 75–100 μm long, 75–87 μm wide ($n = 2$) next to epidermal invaginations; stylet absent (Fig. 5C). Midventral copulatory gland (in segment XIV) present. Ectal duct of spermatheca short, thick, 53–75 μm long, 39–55 μm wide, muscular, slightly curved, and narrowing towards pore. No gland at pore. Spermathecal ampulla 57–75 μm long, 75–87 μm wide, pear-shaped, attached to oesophagus in posterior half of segment V. Sperm rings few, maximally 15 μm wide (Fig. 5B).

Remarks

Grania capensis sp. nov. differs from its South African congeners by the shape of the spermathecae, the distribution of the lateral chaetae (see remarks for *G. bekkouchei* sp. nov. and *G. cryptica* sp. nov. above, and *G. simonae* sp. nov. below), and wide vasa deferentia; the latter are 15.0 μm wide in *G. capensis* sp. nov., but only 7.5–10.0 μm wide in the others. The spermathecae of *G. capensis* sp. nov. are pear- or heart-shaped, resembling those of the South Atlantic species *G. stephensoniana* (see Rota & Erséus, 1997), but these two species are distinguished by the length of the sperm funnel (about three times longer than wide in *G. capensis* sp. nov., and six or seven times longer than wide in *G. stephensoniana*).

Although geographically distant, two species from the north-east Atlantic, *Grania roscoffensis* Lasserre, 1967 and *Grania pusilla* Erséus, 1974, also share some characteristics with *G. capensis* sp. nov., such as the spermathecal morphology, the length of the sperm funnel, and the distribution of the lateral chaetae. This South African species is distinct from all of these, however, by the simple penial bulb (the other species have a stylet), and the presence of a midventral copulatory gland in segment XIV (absent in the others).

***GRANIA SIMONAE* SP. NOV.**

FIGURE 6

Holotype

SAMC A82476, CE14111, whole-mounted, sexually mature specimen, 11 posterior segments amputated, from Van Dyks Bay, Overberg District, Overstrand Local Municipality, Province of the Western Cape, South Africa, 34°37′00″S, 19°21′21″E, intertidal crevice between rocks. Collected by N. Bekkouche, 16 December 2011. *COI* barcode sequence, GenBank acc. no. KT428100; for other sequence data, see Table 1.

Etymology

Named for the South African annelid specialist, Dr Carol Simon, who arranged and generously assisted with the workshop/fieldwork that led to the collection of this species.

Paratypes

Six whole-mounted, sexually mature specimens, all from the type locality and date, and collected by N. Bekkouche. SAMC A82482, complete, not DNA barcoded specimen. SAMC A82477, CE14110, with 14 midbody segments amputated. SAMC A82478, CE14112, with 14 middle body segments amputated. SAMC A82479, CE14058, with some segments amputated. SAMC A82480, CE14060, with eight posterior segments amputated. SAMC A82481, CE14093, with some segments amputated, from the type locality too, but in shallow subtidal. For *COI* barcodes of paratypes, see Table 1.

Description

Body of only complete specimen 16 mm long, 79 segments (paratype SAMC A82482), 0.16–0.20 mm wide at segment III, 0.15–0.22 mm wide at segment XII ($n = 7$). Prostomium rounded, 55–87 μm long, 62–80 μm wide, epidermis 7–10 μm thick ($n = 7$), not reduced at front tip. Ventral chaetae present from segment IV, lateral chaetae absent. Chaetae (Fig. 6A) L-shaped, 45–90 μm long, 5–6 μm thick at midpoint, shaft straight and broad at the base, foot 16–20 μm

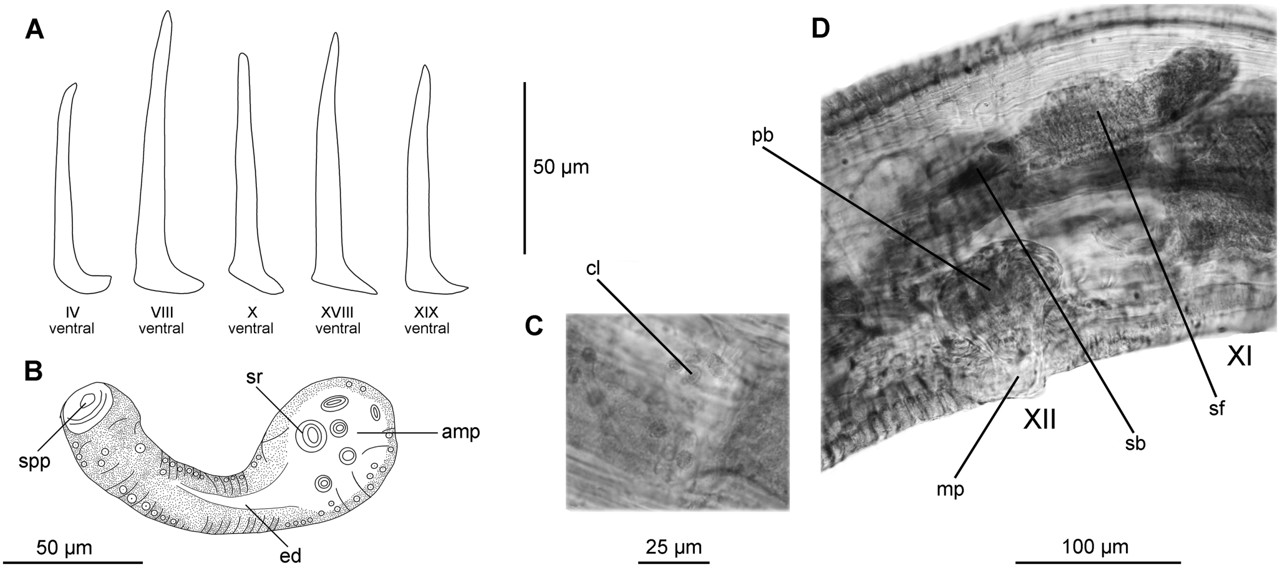

Figure 6. ***Grania simonae* sp. nov.** A, chaetae. B, spermatheca. C, coelomocytes. D, penial bulb and sperm funnel. See text for abbreviations.

long, with slight heel, and sometimes with slight toe. Chaetal index (Rota & Erséus, 2003) 4.70 ± 0.39 (*n* = 5). Free chaetae scattered in the coelomic cavity, sometimes numerous. Epidermal gland cells inconspicuous. Clitellum maximally 22 µm thick, extending from segments XII to XIII, comprising rows of large granular cells, absent between male pores. Spermathecal pores in lateral lines, immediately posterior to 4/5. Male pores located ventrolaterally in mid-segment XII. Female pores not observed.

Brain posteriorly indented. Head organ (*sensu* Rota & Erséus, 1996) absent. Pharyngeal glands in segments IV–VI, dorsal lobes in segment IV (one pair), in segment V (one pair), and in segment VI (one pair), ventral lobes present in segment IV (one pair), in segment V (two pairs), and in segment VI (two pairs); glands not connected dorsally. Nephridia not observed. Chloragogen cells from segment VII distinctive, containing dark granules, dense in posterior segments. Dorsal blood vessel from segments XXI or XXIV (*n* = 3). Coelomocytes irregular, at least 10 µm wide, most abundant posteriorly (in complete specimen) (Fig. 6C). Sperm sac extending into segment XVI, egg sac reaching segment XX. Sperm funnels in segment XI about two times longer than wide, folded, occasionally directed posteriorly (holotype) (Fig. 6D). Vasa deferentia reaching at least segment XIV, coiled, internally ciliated, 15 µm wide near sperm funnel, narrowing to about 10 µm. Glandular penial bulb type 1 (*sensu* Coates, 1984), 75–80 µm long, 50–68 µm wide (*n* = 6); stylet absent (Fig. 6D). Copulatory gland (in segment XIV) absent. Spermathecal ampulla small, more or less spherical, 37–55 µm long, 37–52 µm wide (Fig. 6B). Sperm rings up to 15 µm wide. Ectal duct slender, 90–115 µm long, 17–25 µm wide, not narrowing at ends. No gland at spermathecal pore.

Remarks

Grania simonae sp. nov. is unique among the South African species described here and all other members of the genus by the combination of the following characters: lack of lateral chaetae, spermathecae with small and spherical ampulla, dense chloragogen cells, dorsal blood vessel commencing in segment XXIV, and absence of head organ and a midventral copulatory gland in segment XIV. This is the only South African species with numerous and conspicuous coelomocytes scattered throughout the body, but as stated by Rota *et al.* (2007) and De Wit & Erséus (2007), the apparent lack of coelomocytes in the other species described herein may be an artifact, caused by the fixation and staining procedures.

The absence of lateral chaetae has been recorded for at least 13 different species of *Grania* to date. Among them, *G. simonae* sp. nov. is reminiscent of the Antarctic species *Grania carchinii* Rota & Erséus, 1996, by the absence of penial stylets and head organ, and the possession of dense chloragogen cells; however, the adult specimens of *G. simonae* sp. nov. are at least twice as large as those of *G. carchinii* known so far. These two species are also distinguished from each other by the morphology of the spermatheca. *Grania carchinii* has a larger (pear-shaped) spermathecal ampulla and a shorter spermathecal duct than *G. simonae* sp. nov.; the latter has a spherical spermathecal ampulla.

Moreover, *G. carchinii* has a midventral copulatory gland in segment XIV, which is lacking in *G. simonae* sp. nov.

The Western Australian *G. conjuncta* and *G. vacivasa* also resemble *G. simonae* sp. nov. by the absence of lateral chaetae, stylets, and head organ, but they both differ from the latter by possessing a midventral copulatory gland in segment XIV and by extremely long sperm funnels; the funnels are only about twice longer than wide in *G. simonae* sp. nov., but 19 times longer than wide in *G. conjuncta*, and 11–15 times longer than wide in *G. vacivasa*.

GRANIA HINOJOSAI SP. NOV.

FIGURE 7

GRANIA SP. CHILE 1; DE WIT *ET AL*., 2011B

Holotype

ZUEC CLI 08, PDW186, whole-mounted, sexually mature specimen, with some segments amputated, from Puerto Aldea, Coquimbo, Elqui, Chile, 30°18′19″S, 71°39′33″W. Intertidal, sand among rocks, P. De Wit and I. Hinojosa, 6 February 2009. *COI* barcode sequence, GenBank acc. no. GU902189; for other sequence data, see Table 1.

Etymology

Named for Ivan Hinojosa, who was instrumental to all of the Chilean fieldwork.

Paratypes

Four whole-mounted, sexually mature specimens, and all collected by P. De Wit. ZUEC CLI 09, PDW187, with some segments amputated, from type locality. ZUEC CLI 10–CLI 12, PDW177, PDW181, PDW182, with some segments amputated, from Pampilla Point, Coquimbo, Elqui, Chile, 29°57′23″S, 71°21′39″W, heterogeneous sand with organic material, 6 February 2009. For *COI* barcodes of paratypes, see Table 1.

Description

Body > 4.15–5.85 mm long, > 18–28 segments ($n = 5$) (posterior ends used for DNA extractions), 0.17–0.20 mm wide at segment V, 0.16–0.20 mm at segment XII ($n = 5$). Prostomium conical or rounded, 67–87 μm long, 100–110 μm wide, epidermis not reduced at front tip, 10–12 μm thick ($n = 5$). Ventral chaetae from segment IV, lateral chaetae from segments XVII–XIX. Chaetae (Fig. 7A) increasing in size towards the posterior, 30–65 μm long, shaft straight, 3.7–6.2 μm thick at midpoint, L-shaped, proximally bent into a foot, with low instep and indistinct heel. Chaetal index (Rota & Erséus, 2003) 3.99 ± 0.42 ($n = 5$). Epidermal gland cells inconspicuous. Clitellum maximally 7–10 μm thick, extending from posterior half of segment XI to anterior half of segment XIII, formed by more or less regular transverse rows of granular cells, absent between male pores. Spermathecal pores in lateral lines, somewhat posterior to 4/5. Male pores ventrolateral in mid-segment XII. Female pores not observed.

Brain posteriorly indented. Head organ (*sensu* Rota & Erséus, 1996) absent. Pharyngeal glands in segments IV–VI, dorsal lobes in segment IV (one pair), in segment V (one pair), and in segment VI (one pair), ventral lobes present in segment IV (one pair), in

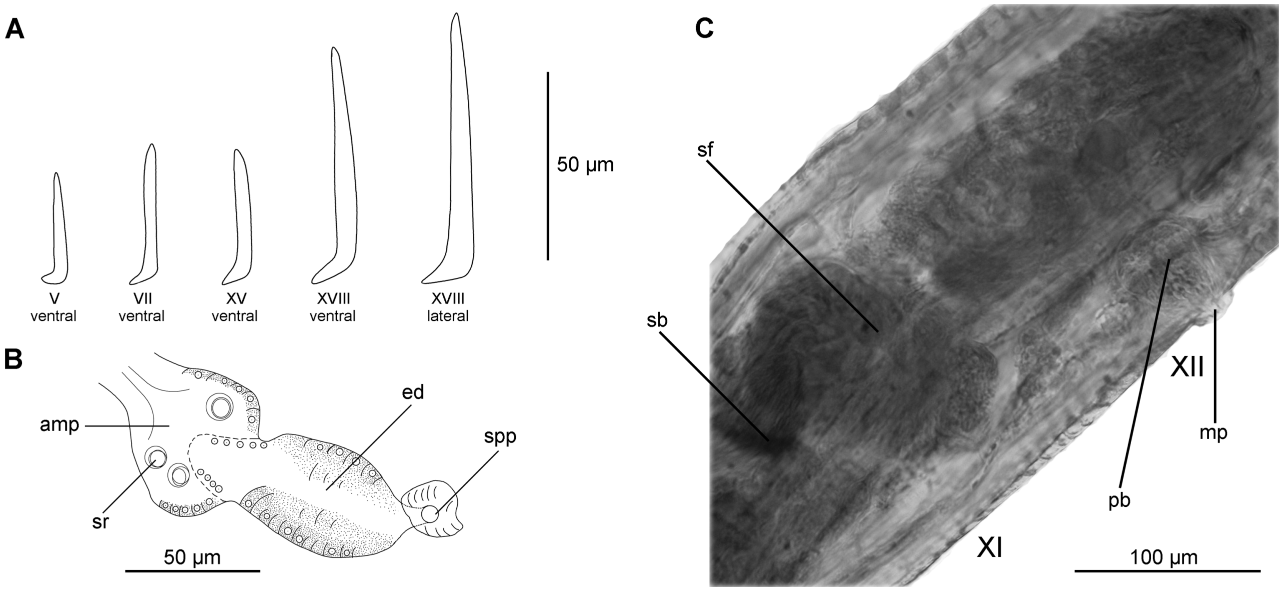

Figure 7. ***Grania hinojosai* sp. nov.** A, chaetae. B, spermatheca. C, penial bulb and sperm funnel. See text for abbreviations.

segment V (two pairs), and in segment VI (two pairs); glands not connected dorsally. Nephridia not observed. Chloragogen cells not observed. Dorsal blood vessel commencing in segments XV or XVI. Coelomocytes not observed. Sperm sac extending into segment XIV, egg sac extending into segment XVI (holotype). Sperm funnels about 1.5 times longer than wide (Fig. 7C). Vasa deferentia observed in segments XI–XII, internally ciliated, tightly coiled near sperm funnel, 10 µm wide. Penial apparatus type 1 (*sensu* Coates, 1984), small, compact glandular bulb, 47–50 µm long, 55–67 µm wide ($n = 5$); stylet absent. Midventral copulatory gland (in segment XIV) present. Each spermatheca attached to oesophagus in posterior half of segment V through narrow ental duct. Ampulla 'heart-shaped', 37–55 µm long, 37–52 µm wide (Fig. 7B). Sperm rings maximally 17 µm wide, but few ($n = 5$). Ectal duct narrowing at both ends, 80–92 µm long, 37–40 µm wide, joining ampulla through a conical intrusion, with a prominent gland attached near pore (Fig. 7B).

Remarks

The diagnostic characters for *G. hinojosai* sp. nov. are the unique combination of the glands at the spermathecal pores, the location of these pores, at some distance from 4/5, the short sperm funnels, and the small and compact penial bulbs.

Among the group of *Grania* species possessing glands at the spermathecal pores, *Grania novacaledonia* De Wit & Erséus, 2007 resembles this Chilean species by sharing characters such as the distribution of lateral chaetae, the chaetal size distribution (increasing in size posteriorly), presence of a midventral copulatory gland (in segment XIV), and the absence of copulatory stylets; however, *G. hinojosai* sp. nov. is distinguished by its shorter sperm funnel and its spermathecal ducts narrowing ectally. Spermathecal glands have also been reported for some species in the West Indian Ocean [*Grania ersei* Coates, 1990 and *Grania darwinensis* (Coates & Stacey, 1997)], North Pacific waters [*Grania paucispina* (Eisen, 1904)], and the North (*G. americana*) as well as the South Atlantic Ocean (*G. monochaeta*). The heart-shaped spermathecal ampulla, the somewhat spindle-shaped spermathecal duct, and the more posterior distribution of the lateral chaetae appear to distinguish *G. hinojosai* sp. nov. from *G. ersei* and *G. paucispina*; the lateral chaetae begin in segments XVII–XIX in *G. hinojosai* sp. nov., but in segments XIV or XV in the other two taxa. *Grania ersei* has a top-shaped spermathecal ampulla, and a long, coiled spermathecal duct, whereas *G. paucispina* has a spermatheca with an ovoid ampulla. Moreover, *G. ersei* possesses very long copulatory stylets, structures not even present in *G. hinojosai* sp. nov.

The Western Australian *G. darwinensis* has a more complex penial apparatus than *G. hinojosai* sp. nov., i.e. it has accessory glands, covered by muscle layers, at both sides of the bulb, whereas in *G. hinojosai* sp. nov., the whole apparatus is smaller and compact.

Grania americana is easily differentiated from *G. hinojosai* sp. nov. by the length of the sperm funnel (eight times longer than wide in the former, about 1.5 times longer than wide in the latter) and the presence of a head organ, absent in *G. hinojosai* sp. nov.

Although *G. monochaeta* and *G. hinojosai* sp. nov. share the somewhat unusual, more posterior, position of the spermathecal pores, and the presence of a midventral copulatory gland in segment XIV (Rota & Erséus, 1997), the lateral chaetae start in segment XIII in *G. monochaeta*, but start in segments XVII–XIX in *G. hinojosai* sp. nov. Finally, *G. hinojosai* sp. nov. differs from all species mentioned above by its short sperm funnel.

GRANIA CHILENSIS SP. NOV.

FIGURE 8

GRANIA SP. CHILE 2; DE WIT *ET AL*., 2011B

Holotype

ZUEC CLI 13, PDW193, whole-mounted, sexually mature specimen, with some segments amputated, from Punta Loncoyen, Valdivia, Chile, 39°49′27″S, 73°24′25″W. Lower intertidal, sand among rocks. Collected by P. De Wit, 9 February 2009. *COI* barcode sequence, GenBank acc. no. GU902190; for other sequences, see Table 1.

Etymology

Named for Chile.

Paratypes

Six whole-mounted, sexually mature specimens, collected by P. De Wit. ZUEC CLI 14–CLI 16, PDW190, PDW191, PDW194, with some segments amputated, from type locality. ZUEC CLI 17, PDW185, with some segments amputated, from Puerto Aldea, Coquimbo, Elqui, Chile, 30°18′19″S, 71°39′33″W, intertidal, sand among rocks, 6 February 2009. ZUEC CLI 18–CLI 19, PDW198, PDW199, with some segments amputated, from Caleta Tumbes, Talcahuano, Concepción, Chile, 36°38′00″S, 73°05′27″W, lower intertidal, heterogeneous sand with organic material between boulders, 16 February 2009. For *COI* barcodes of paratypes, see Table 1.

Description

Body > 3.85–7.45 mm long, > 26–40 segments ($n = 6$) (posterior ends used for DNA extractions), 0.13–0.17 mm wide at segment V, 0.11–0.19 mm at segment XII ($n = 6$). Prostomium conical, 55–80 µm long, 60–80 µm wide, epidermis not reduced at front tip, 7 µm thick ($n = 6$). Ventral chaetae from segment IV, lateral chaetae beginning in segment XVI ($n = 1$), in

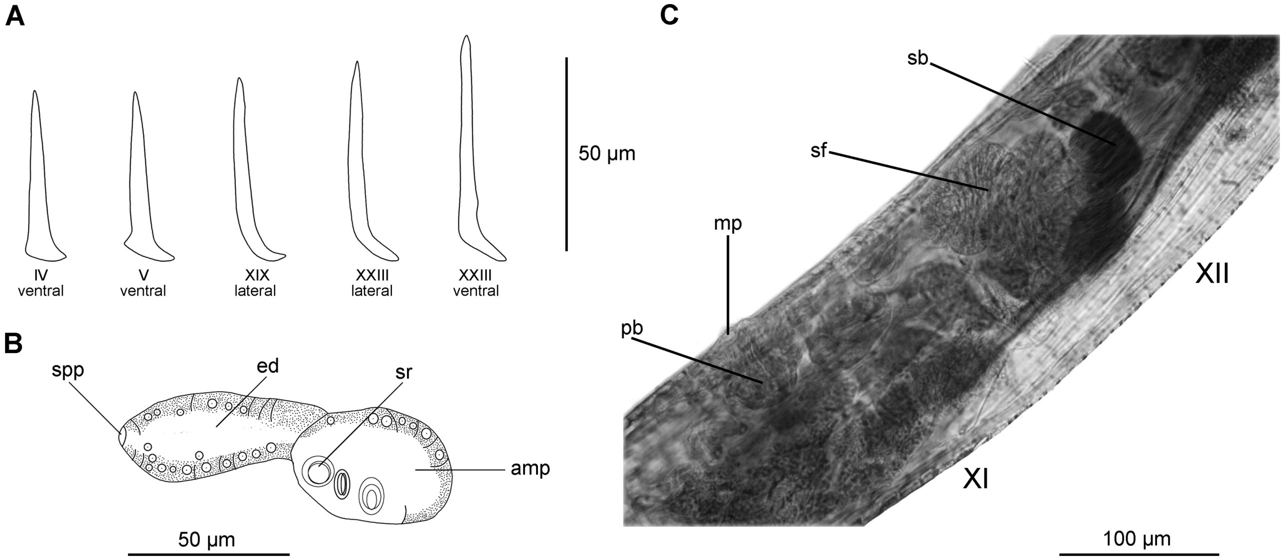

Figure 8. ***Grania chilensis*** **sp. nov.** A, chaetae. B, spermatheca. C, penial bulb and sperm funnel. See text for abbreviations.

segment XVII ($n = 3$), in segment XVIII ($n = 1$), or segment XIX ($n = 1$). Chaetae (Fig. 8A) of uniform size, 44–59 µm long, shaft straight, 3.7–5.0 µm thick at midpoint, L-shaped, proximally bent into a foot with low instep, and with distinct heel only in preclitellar segments. Chaetal index (Rota & Erséus, 2003) 4.03 ± 0.52 ($n = 5$). Epidermal gland cells inconspicuous. Clitellum maximally 10–12 µm thick ($n = 4$), extending from segment XII to anterior half of segment XIII, consisting of transverse rows of granular gland cells interspersed with hyaline cells; with the latter, however, absent ventrally. Spermathecal pores in lateral lines, just posterior to 4/5. Male pores ventrolateral in middle of segment XII. Female pores not observed.

Brain posteriorly indented. Head organ (*sensu* Rota & Erséus, 1996) absent. Pharyngeal glands in segments IV–VI, dorsal lobes in segment IV (one pair), in segment V (one pair), and in segment VI (one pair), ventral lobes present in segment IV (one pair), in segment V (two pairs), and in segment VI (two pairs); glands not connected dorsally. Nephridia not observed. Chloragogen cells not observed. Dorsal blood vessel commencing in segments XVI–XVIII. Coelomocytes not observed. Sperm sac extending to segments XIII–XV, egg sac extending to segments XV–XVII. Sperm funnels about 1.5–2.5 times longer than wide (Fig. 8C). Vasa deferentia observed in segments XI–XIII, internally ciliated, coiled, 10 µm wide. Penial apparatus type 1 (*sensu* Coates, 1984), small, compact glandular bulb, 35–55 µm long, 37–50 µm wide ($n = 6$); stylet absent. Midventral copulatory gland (in segment XIV) present. Spermatheca attached to oesophagus in posterior half of segment V by narrow ental duct. Ampulla oval, 42–65 µm long, 30–42 µm wide (Fig. 8B). Sperm rings maximally 17 µm wide, but few. Ectal duct, 54–95 µm long, 13–30 µm wide at midcourse. No gland at spermathecal pore.

Remarks

The compact and small penial bulb, the sperm funnels being 1.5–2.5 times longer than wide, the absence of penial stylets, the presence of a midventral copulatory gland in segment XIV, and the distribution of the lateral chaetae, starting from segments XVI–XIX, make *G. chilensis* sp. nov. reminiscent of *G. hinojosai* sp. nov.; however, *G. hinojosai* sp. nov. has a spermathecal ampulla that is heart-shaped, a distinct chaetal size distribution (chaetae becoming larger towards the posterior end), and possesses a gland at the spermathecal pore, whereas in *G. chilensis* sp. nov. the ampulla is ovoid, the chaetal size is uniform, and there are no glands at the spermathecal pore.

Three species from South Pacific, *Grania galbina* De Wit & Erséus, 2007, *Grania breviductus* De Wit, Rota & Erséus, 2009, and *G. novacaledonia* share a similar distribution of the lateral chaetae to that in *G. chilensis* sp. nov. Moreover, as in *G. chilensis* sp. nov., a midventral copulatory gland is present in *G. breviductus* and *G. novacaledonia* too. *Grania chilensis* sp. nov., however, differs from all the species mentioned above by the short length of the sperm funnel; in the other three species these funnels are about four or five times longer than wide. Furthermore, *G. galbina* has its male pore surrounded by a large granular mass, *G. breviductus* has the communication between the spermatheca and the oesophagus in the middle of segment V, and

G. novacaledonia possesses glands at the spermathecal pores, none of which is the case for *G. chilensis* sp. nov.

The ovoid spermathecal ampulla, the length of the sperm funnel, the distribution of the lateral chaetae, and the absence of a penial stylet make *G. chilensis* sp. nov. (see discussion above) similar to the Irish *Grania mira* Locke & Coates, 1998, but in addition to being geographically distant from this species, *G. chilensis* sp. nov. differs from *G. mira* by the presence of a midventral copulatory gland in segment XIV, and the lack of thickened muscles along the male ducts.

GRANIA UNITHECA SP. NOV.

FIGURE 9

Holotype

USNM1283175, CE11659, whole-mounted, sexually mature specimen, eight posterior segments amputated, from North Carolina, USA, 34°47′22″N, 76°13′43″W. Sublitoral, 17 m in depth, sand. Collected by C. Erséus, 24 May 2011. *COI* barcode sequence, GenBank acc. no. KT428113; for other sequences, see Table 1.

Etymology

Named *unitheca* for its single spermatheca.

Description

Body > 8.56 mm long, 61 segments (including segments used for DNA analysis), 0.09 mm wide at segment V, 0.09 mm at segment XII. Prostomium rounded, 40 μm long, 60 μm wide, epidermis not reduced at front tip, 6 μm thick. Ventral chaetae from segment XV, lateral chaetae absent. Chaetae (Fig. 9A) 50–62 μm long, shaft straight, 3.7–5.0 μm thick at midpoint, hook-shaped. Epidermal gland cells inconspicuous. Clitellum not well developed. Spermathecal pore unpaired, mid dorsal, just posterior to 4/5. Male pores ventrolateral in middle of segment XII. Female pores not observed.

Brain posteriorly indented. Head organ (*sensu* Rota & Erséus, 1996) absent. Pharyngeal glands in segments IV–VI, dorsal lobes in segment IV (one pair), in segment V (one pair), and in segment VI (one pair), ventral lobes present in segment IV (one pair), in segment V (two pairs), and in segment VI (two pairs); glands not connected dorsally. Nephridia not observed. Chloragogen cells not observed. Dorsal blood vessel commencing in segment XV. Coelomocytes not observed. Sperm sac extending to segment XIV, egg sac in segment XVII. Sperm funnels as long as wide (Fig. 9D). Vasa deferentia observed in segments XII–XIII, internally ciliated, coiled, 8 μm wide. Penial apparatus type 1 (*sensu* Coates, 1984), small, compact glandular bulb, 44 μm long, 39 μm wide; stylet absent (Fig. 9C). Midventral copulatory gland (in segment XIV) present. Spermatheca attached to oesophagus near 5/6. Ampulla nearly spherical, 52 μm long, 54 μm wide. Sperm rings maximally 9 μm wide (Fig. 9B). Ectal duct, 61 μm long, 35 μm wide at midcourse. No gland at spermathecal pore.

Remarks

An unpaired spermatheca is unusual in enchytraeids and, among *Grania* species, it has been only reported for *Grania monospermatheca* Erséus & Lasserre, 1976, also known from the continental shelf off the

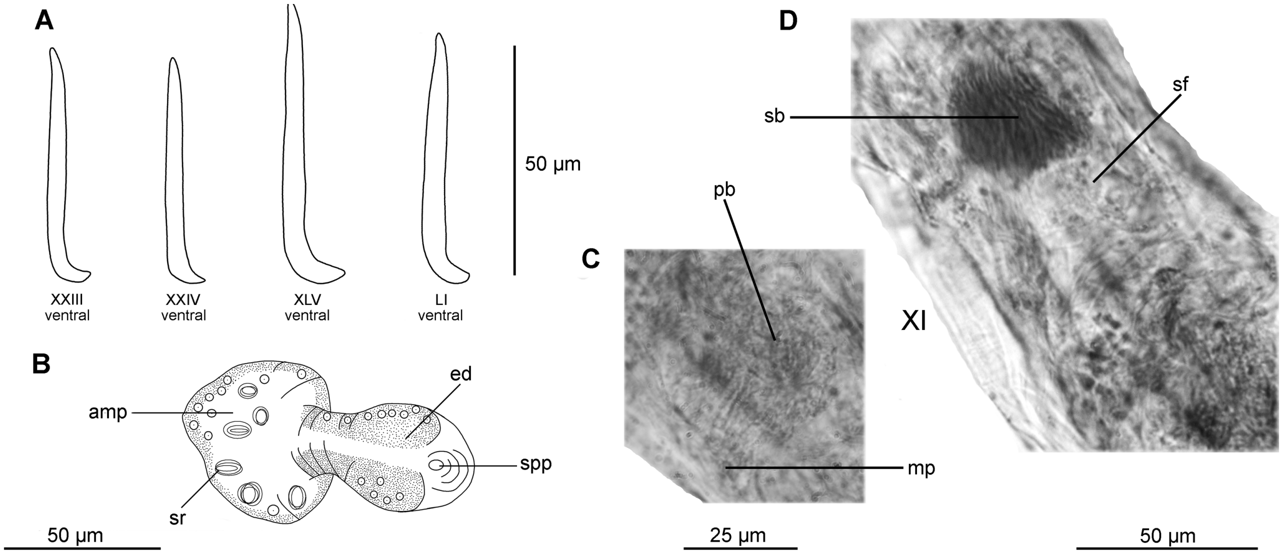

Figure 9. *Grania unitheca* sp. nov. A, chaetae. B, spermatheca. C, penial bulb. D, sperm funnel. See text for abbreviations.

eastern USA. In fact, *G. unitheca* sp. nov. is morphologically similar to *G. monospermatheca* in almost all characters observed; however, it may be distinguished from *G. monospermatheca* by its small and compact penial bulb, with no stylet. *Grania monospermatheca* has a more complex penial apparatus, with a large glandular structure attached to the penial bulb, and the presence of a stylet. Furthermore, *G. unitheca* sp. nov. possesses a midventral copulatory gland in segment XIV. This gland was not observed in *G. monospermatheca* (see Erséus & Lasserre, 1976 and Coates & Erséus, 1985).

Genetically, these two species are well distinguished from each other; however, the phylogenetic analysis, as well as the fact that the two species are from the same geographical area, suggest that *G. unitheca* sp. nov. and *G. monospermatheca* are closely related (see 'Phylogenetic analysis' below).

GRANIA CAROLINENSIS **SP. NOV.**

FIGURE 10

Holotype

USNM 1283174, CE11569, whole-mounted, sexually mature specimen, with some segments amputated, from off North Carolina, USA, 33°10′23″N, 76°45′23″W. Continental shelf slope, 492 m in depth, sand. Collected by C. Erséus, 20 May 2011. *COI* barcode sequence, GenBank acc. no. KT428112; for other sequences, see Table 1.

Etymology

Named for North Carolina.

Description

Body > 5.10 mm long, > 30 segments (posterior end used for DNA extractions), 0.08 mm wide at segment V, 0.09 mm wide at segment XII. Prostomium rounded, 51 µm long, 66 µm wide, epidermis 10 µm thick, reduced to 4 µm at front tip. Ventral chaetae from segment XIII, lateral chaetae absent. Chaetae (Fig. 10A) 42–50 µm long, shaft straight, 3.7 µm thick at midpoint, L-shaped, proximally bent into a foot with low instep and slight heel. Chaetal index (Rota & Erséus, 2003) 3.60 ± 0.33 ($n = 4$). Epidermal gland cells inconspicuous. Clitellum not well developed. Spermathecal pores in lateral lines, immediately posterior to 4/5. Male pores ventrolateral in mid-segment XII. Female pores not observed.

Brain posteriorly indented. Head organ (*sensu* Rota & Erséus, 1996) absent. Pharyngeal glands in segments IV–VI, dorsal lobes in segment IV (one pair), in segment V (one pair), and in segment VI (one pair), ventral lobes present in segment IV (one pair), in segment V (two pairs), and in segment VI (two pairs); glands not connected dorsally. Nephridia not observed. Chloragogen cells not observed. Dorsal blood vessel commencing in segment XVII. Coelomocytes not observed. Sperm sac extending into half of segment XV, egg sac extending into segment XVII. Sperm funnels about six times longer than wide (Fig. 10C). Vasa deferentia observed in segments XII–XIII, internally ciliated, coiled, 7 µm wide. Penial apparatus type 1 (*sensu* Coates, 1984), small, glandular bulb, 40 µm long, 31 µm wide; stylet absent. Midventral copulatory gland (in segment XIV) not observed. Each spermatheca attached to oesophagus in posterior half of segment V. Ampulla large, oval, 100 µm long, 44 µm wide. Sperm

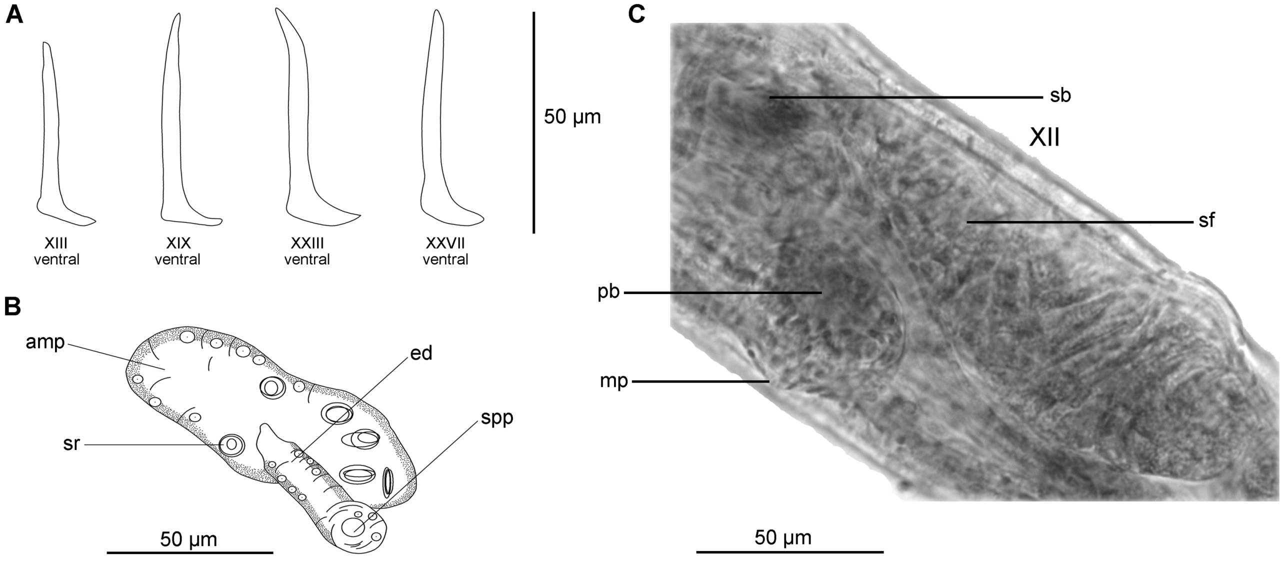

Figure 10. ***Grania carolinensis*** **sp. nov.** A, chaetae. B, spermatheca. C, penial bulb and sperm funnel. See text for abbreviations.

rings maximally 11 μm wide, but few. Ectal duct slender, 53 μm long, 14 μm wide at midcourse, narrowing at distal end Figure 10B). No gland at spermathecal pore.

Remarks

Grania carolinensis sp. nov. is similar to the north-east Atlantic, *Grania postclitellochaeta* (Knöllner, 1935), *Grania ovitheca* Erséus, 1977, and the cryptic congener to *G. ovitheca*, *Grania occulta* De Wit & Erséus, 2010, a species constellation also supported by the genetic data (see 'Phylogenetic analyses' below). All of these four species have the same distribution of ventral chaetae (beginning in segment XIII), and they lack lateral chaetae and the midventral copulatory gland in segment XIV. *Grania carolinensis* sp. nov., *G. ovitheca*, and *G. occulta* also share the shape of the spermatheca, which has a short and slender ectal duct and a large oval ampulla. In *G. ovitheca* and *G. occulta*, however, the sperm funnels are longer (between eight and 15 times longer than wide, as opposed to six times longer than wide in *G. carolinensis* sp. nov.) and they lack the ventral lobes of the pharyngeal glands in segment IV that are present in *G. carolinensis* sp. nov. and also in *G. postclitellochaeta*. *Grania postclitellochaeta* differs from *G. carolinensis* sp. nov. by possessing a racket-shaped diverticulum in the spermathecal ampulla, which is absent in *G. carolinensis* sp. nov. In addition, *G. carolinensis* sp. nov. differs from all the species mentioned above by its penial apparatus, with a simple invagination surrounded by a small and glandular bulb. In *G. postclitellochaeta* the penial bulbs are covered by a large supplementary glandular body, whereas in *G. ovitheca* the bulbs instead have lateral aglandular sacs directed posteriorly (Rota & Erséus, 2003). The aglandular sacs were not mentioned in the original description of *G. occulta* (see De Wit & Erséus, 2010), but the authors noted that the single available specimen possessed oval structures next to the epidermal invaginations. No accessory glands or sacs could be observed near the male pores of *G. carolinensis* sp. nov.

The deep-sea Atlantic *Grania atlantica* Coates & Erséus, 1985 also resembles *G. carolinensis* sp. nov. by the proportions of the sperm funnel and the morphology of the penial apparatus, but the former differs from the latter by its spermathecal ampulla, which has a large sacciform diverticulum. Moreover, *G. atlantica* possesses lateral chaetae, a head organ, as well as a midventral copulatory gland in segment XIV, all of which are absent in the new species.

GRANIA CF. *LEVIS* COATES & ERSÉUS, 1985
PROBABLY *GRANIA LEVIS* COATES & ERSÉUS, 1985: 111–112, FIG. 6

Material examined

USNM 1283176, CE11570, whole-mounted, sexually immature specimen, with some segments amputated, from off North Carolina, USA, 33°10′23″N, 76°45′23″W. Continental shelf slope, 492 m in depth, sand. Collected by C. Erséus, 20 May 2011. *COI* barcode KT428114; for other genes, see Table 1.

Remarks

This barcoded, but immature specimen, and thus unsuitable for complete morphological description, was included in the phylogenetic analysis, to enlarge the taxonomic sampling from the north-western Atlantic region. Phylogenetically, this specimen came out as closely related to *G. carolinensis* sp. nov. (Fig. 15), but it is morphologically distinct by its complete lack of chaetae. The latter trait suggests that this specimen could belong to *Grania levis* Coates & Erséus, 1985, originally described from somewhat further north, from Georges Bank, south-east of Massachusetts, USA.

GENETIC ANALYSES

COI clustering

The Bayesian inference of the *COI* sequences divide the 38 individuals into ten well-supported clades (Fig. 11), four of which are found in South Africa, two in Chile, one in Brazil, and three in the North Atlantic. Within-clade variation is generally low, but in one clade, i.e. all specimens referred to the new taxon *G. chilensis* sp. nov., there is a notable subclustering pattern, dividing this clade into four subclades. A haplotype network (Fig. 12) indicates that *G. chilensis* sp. nov. is structured geographically, with two subclades found in the southernmost site (Valdivia), one subclade in the northernmost site (Coquimbo), and an intermediate subclade in the intermediately located site (Concepcion). Pairwise genetic distances indicate that in general there is a strong barcoding gap present between lineages within this group. In the *G. chilensis* sp. nov. clade, however, there is higher than average within-species divergence, although not nearly as great as the lowest between-species differences (Fig. 13).

ITS clustering

The Bayesian inference analysis of 23 *ITS* sequences supports all ten clusters found in the mitochondrial data (Fig. 14); however, although there is also variation within the *G. chilensis* sp. nov. cluster in the *ITS* region, the geographic substructuring is not seen here. Instead, the variation seems to be randomly distributed with respect to geography.

Phylogenetic placement of new species

The updated phylogeny is completely congruent with that described in De Wit *et al*. (2011b), containing three main clades (A, B, C in Fig. 15). All of the South African species form one strongly supported clade within clade A,

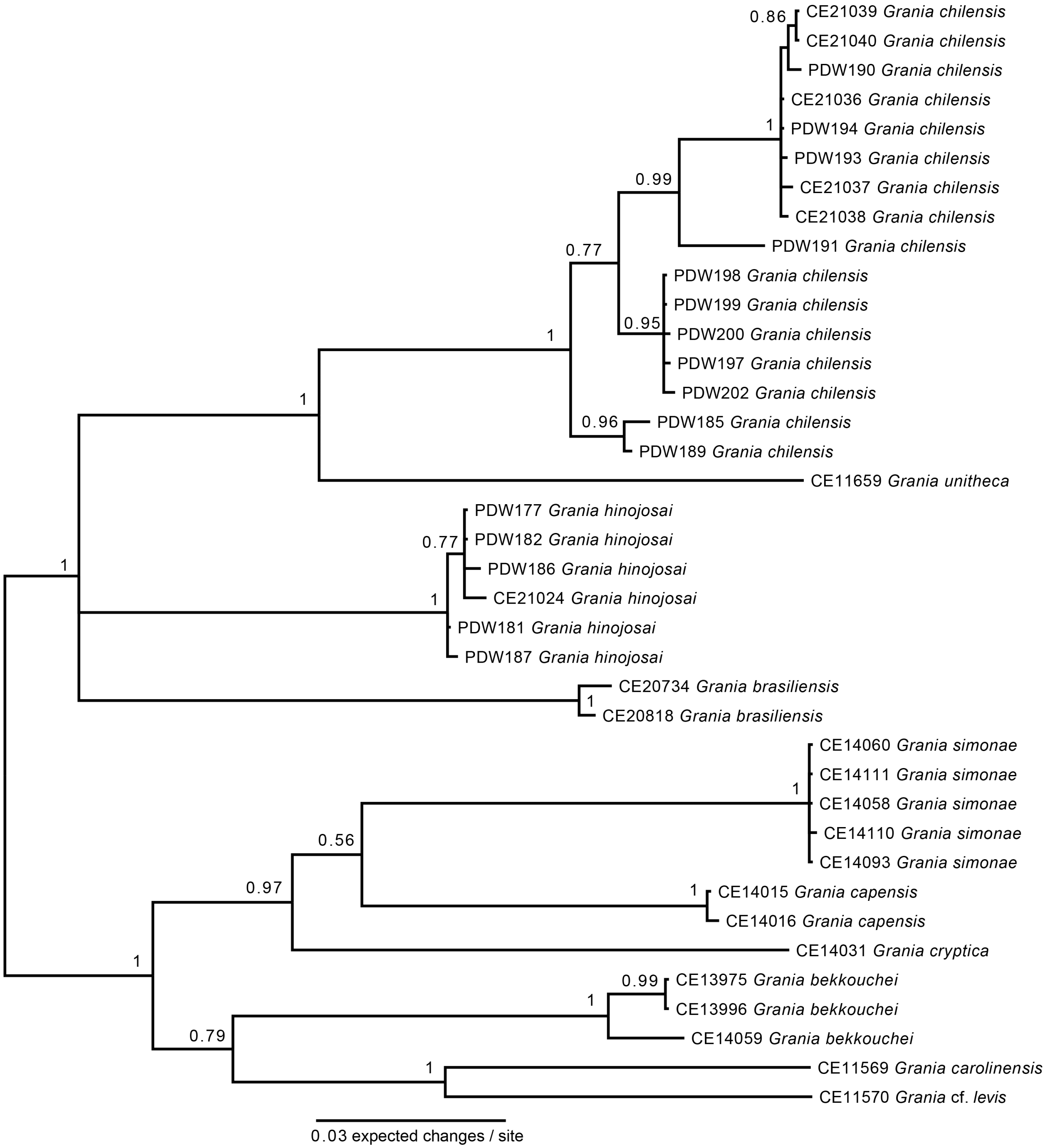

Figure 11. Majority-rule consensus gene tree of cytochrome *c* oxidase subunit I (*COI*) locus from all species described in this study. Posterior probabilities calculated from Bayesian Markov chain Monte Carlo (MCMC) inference are noted above the branches. Note the four clades within ***Grania chilensis* sp. nov.**

together with all the North Atlantic species. By contrast, the Chilean and the Brazilian species are placed in clade B, together with *Grania curta* De Wit & Erséus, 2007 and *G. americana*. *Grania unitheca* sp. nov. from shallow water in North Carolina (North Atlantic) is placed together with the other North American species *G. monospermatheca* (its sister taxon) and *G. laxartus*; however, *G. carolinensis* sp. nov. and the closely related immature specimen of *Grania* cf. *levis*, both found in deep water off the North Carolinian coast, are placed

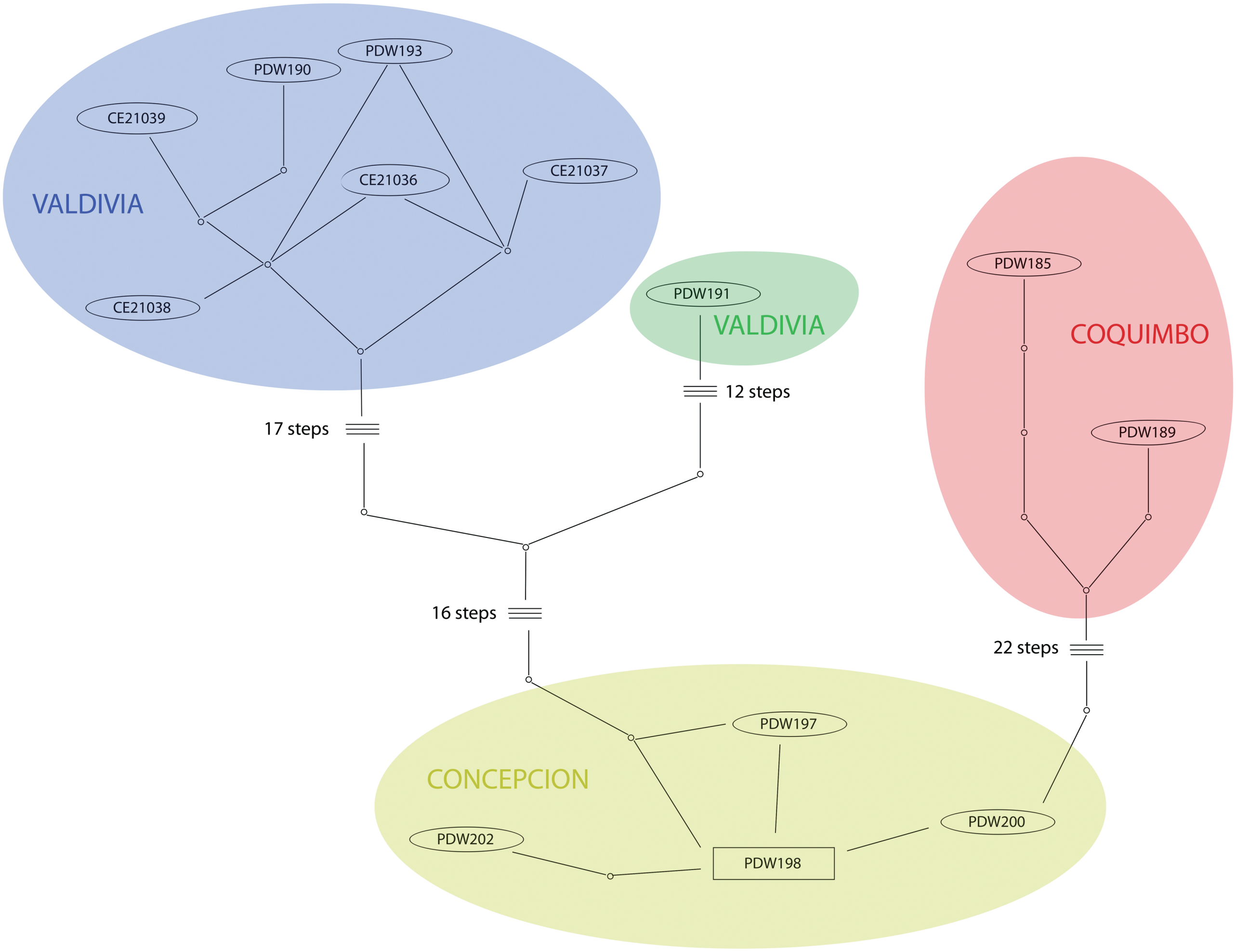

Figure 12. Haplotype network of individuals of ***Grania chilensis* sp. nov.** Note the long distance (35 steps) between the two groups found in Valdivia.

as the sister clade of *G. postclitellochaeta* and the cryptic *G. occulta*, whereas *G. ovitheca*, which is morphologically identical to *G. occulta*, is strongly supported as the sister to this four-taxon group (Fig. 15).

DISCUSSION

Based on molecular and morphological data, species of *Grania* from Brazil, South Africa, and Chile are described for the first time and, together with the two new species described for north-western Atlantic, the number of nominal species belonging to this genus is increased from 72 to 81. The phylogeny of the group was updated by the addition of these representatives from poorly known areas in the southern hemisphere, plus the species from the North Atlantic Ocean. This study clearly shows that within *Grania* commonly used morphological characters are unsuitable for phylogenetic reconstruction, supporting the previous conclusion (De Wit *et al.*, 2011b) that homoplasy is abundant within the genus.

The finding of a new cryptic species in South African waters again illustrates the importance of an integrative taxonomic approach to the delimitation of closely related clitellates; for other similar cases see Gustafsson, Price & Erséus (2009), De Wit & Erséus (2010), and Martinsson *et al.* (2013). As *G. cryptica* sp. nov. and *G. bekkouchei* sp. nov. are sympatric and hard to distinguish without genetic analyses, they probably would otherwise be identified as the same species, leading to an underestimation of the diversity of the group. Interestingly, *G. cryptica* sp. nov. and *G. bekkouchei* sp. nov. do differ in their chaetal shape (L- versus hook-shaped), which constitutes the only observed distinguishing difference between these two species (Fig. 4A, B, C, D, E, F). Chaetal shape has previously been indicated as one of the few morphological characters showing some degree of congruence with

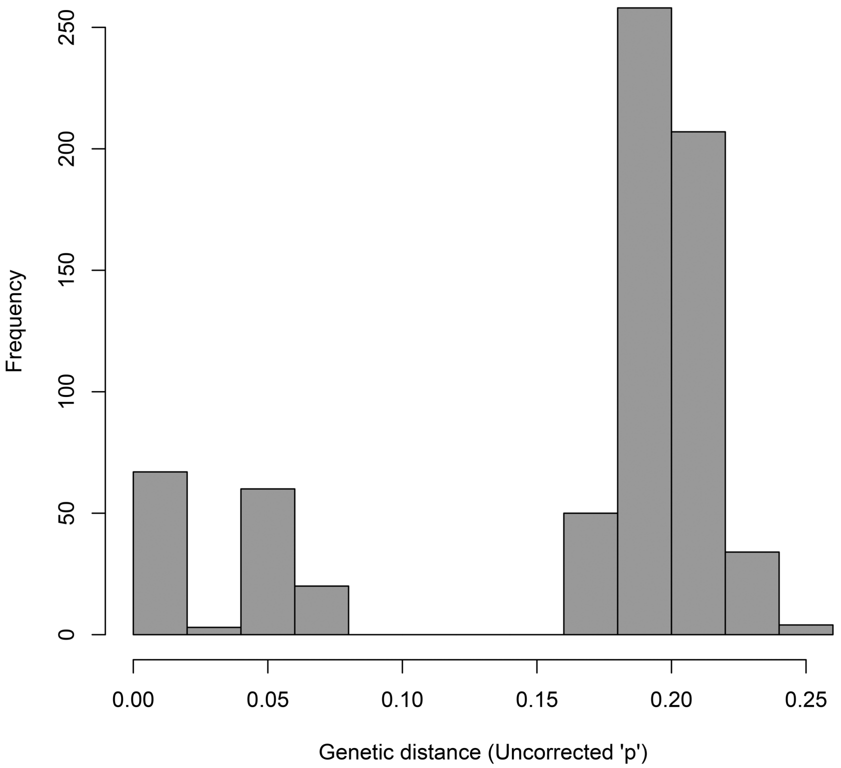

Figure 13. Distance histogram of pairwise uncorrected p distances between all individuals analysed genetically in this study. Within-species distances range from 0 to 8% (0–3% if not counting ***Grania chilensis* sp. nov.**); between-species distances range from 16 to 25%.

molecular data (De Wit *et al*., 2011b), and could thus be an interesting avenue for future research.

The high differences of 19.5% in *COI* between *G. cryptica* sp. nov. and *G. bekkouchei* sp. nov. strongly support that these taxa represent two separately evolving lineages. A similar situation was found in Scandinavian species of *Grania*, where a molecular analysis suggested that no gene flow occurs between the cryptic *G. ovitheca* and *G. occulta* populations (De Wit & Erséus, 2010). In these two cases, the genetic differences suggest that the process of speciation is complete, despite their morphological similarities. Most of the species concepts (i.e. biological, ecological, evolutionary, and phylogenetic) share the common point that species are separately evolving metapopulation lineages (De Queiroz, 2007). According to De Queiroz, the species will acquire different properties over the speciation process, for instance, reciprocal monophyly, phenetic differences, and reproductive incompatibility, although not necessarily in the same order or on the same time scale. Thus, for a complete speciation event, the criteria mentioned above should be fulfilled (De Queiroz, 2007; Nygren, 2014). Although not tested, in this study we assume that *G. cryptica* sp. nov. and *G. bekkouchei* sp. nov. are not reproductively compatible, because of the great difference in both mitochondrial and nuclear markers between these two species. In a study that combined genetic data with reproductive crosses, *Ophryotrocha japonica* Paxton & Åkesson, 2010 and *Ophryotrocha notoglandulata* Pfannenstiel, 1972, two sympatric species of Polychaeta, only a 5% difference in *COI* was shown to be associated with full reproductive isolation (Wiklund *et al*., 2009; Nygren, 2014).

Unfortunately, only one specimen each of *G. brasiliensis* sp. nov., *G. cryptica* sp. nov., *G. unitheca* sp. nov., *G. carolinensis* sp. nov., and *G.* cf. *levis* was available for genetic analysis, and thus it is not possible to draw any conclusions regarding intraspecific variation in these taxa. Even so, it is clear that the morphological features (and differences) observed suggest that these individuals represent different taxa, of which at least four are new to science. Moreover, the combined DNA analysis (Fig. 15) shows that all these four specimens are genetically distinct from at least all other species of *Grania* genetically analysed to date.

On the other hand, *G. chilensis* sp. nov. is structured in four different maternal lineages (as shown by *COI*; Fig. 12): two at Valdivia and one each at Coquimbo and Concepción. At a first glance, the *G. chilensis* sp. nov. populations match an isolation-by-distance (IBD) pattern (Wright, 1943; Malécot, 1948), indicating a decrease

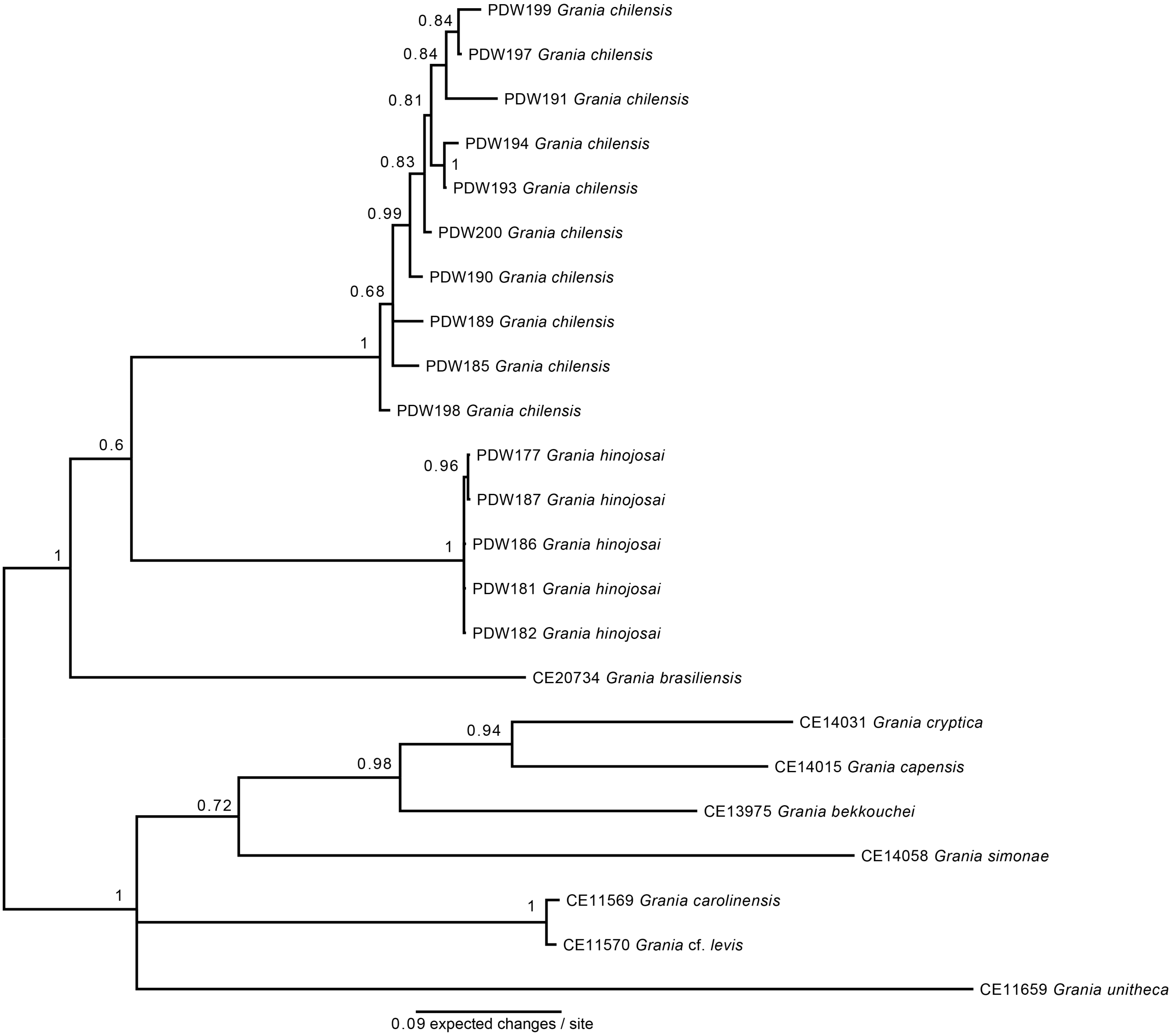

Figure 14. Majority-rule consensus gene tree of the *ITS* region (*ITS1-5.8S* rDNA-*ITS2*) from all species described in this study. Posterior probabilities calculated from Bayesian Markov chain Monte Carlo (MCMC) inference are noted above the branches. Note that the four ***Grania chilensis*** **sp. nov.** clades seen in the *COI* locus are absent here.

in gene flow with increasing distance, and migration probabilities depend only on distances between birthplaces of offspring and their parents (Ishida, 2009). A likely cause for this population structure is the limited mobility of these organisms, which is associated with their infaunal/interstitial lifestyle and the lack of dispersal phases in their life cycle (Costa *et al.*, 2013). Interestingly, however, two genetically distinct populations of *G. chilensis* sp. nov. are observed in sympatry at the southernmost site, Valdivia. There are several potential explanations for this pattern: it could result from an ancient separation with secondary contact, ecological niche separation with respect to local environmental variables that were not assessed herein, or two separate colonization events from a more northern site. In any case, the four population clusters are not supported by the more slow-evolving, recombining nuclear *ITS* locus, suggesting that the separation is fairly recent or alternatively that there is a limited rate of gene flow at present.

The updated phylogenetic tree (Fig. 15) maintained the three main clades (A, B, and C) found already by De Wit *et al.* (2011b), although some differences are seen in clades A and B. All the South African species come out as a strongly supported monophyletic group, which (with low support: posterior probability, PP 0.81) clusters together with the north-west Atlantic *G. monospermatheca*, *G. laxartus*, and

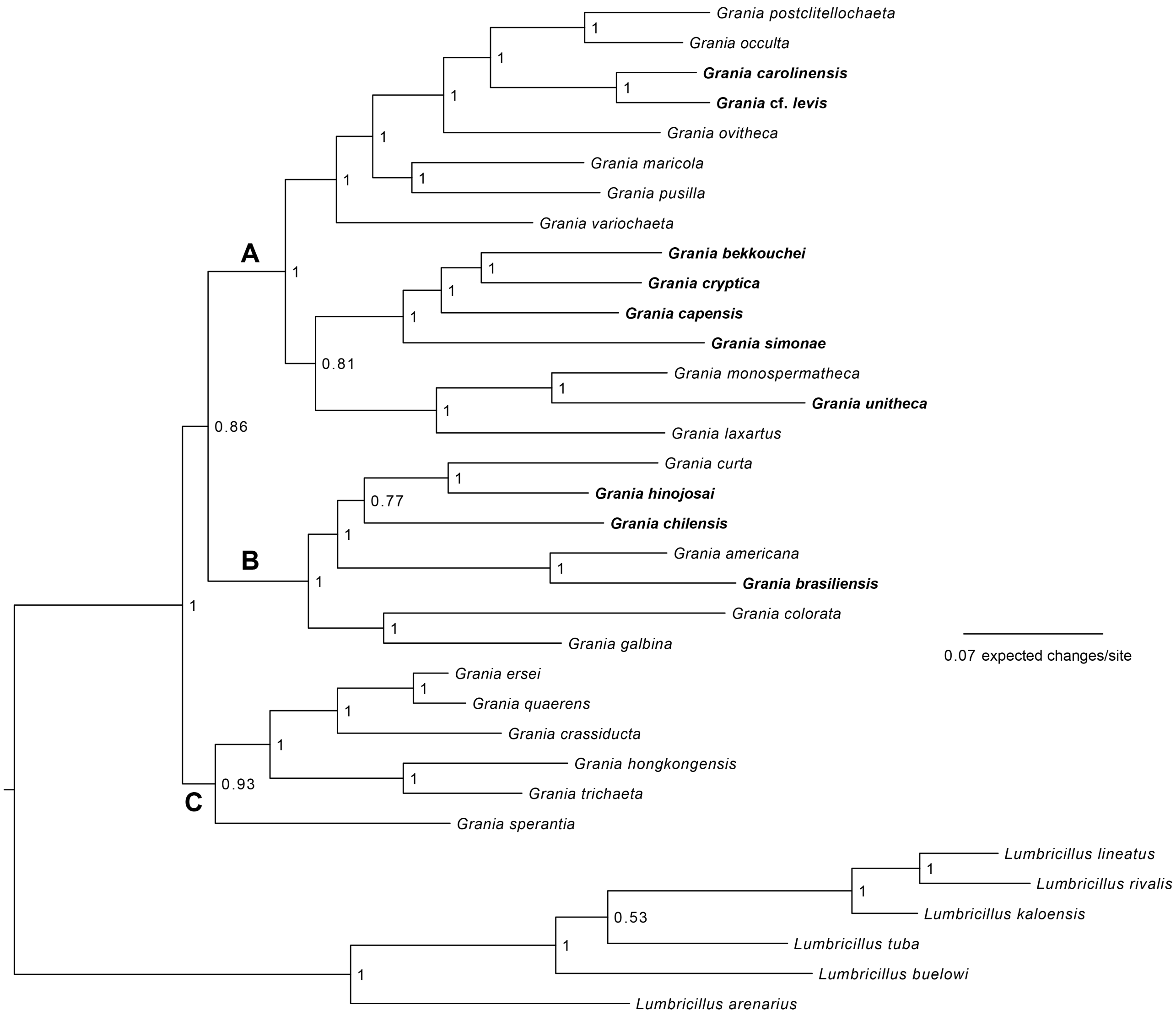

Figure 15. Multi-locus phylogeny of all available genetic data in *Grania* to date, using three mitochondrial and three nuclear loci. New species from this study are marked in bold. Majority-rule consensus tree from Bayesian Markov chain Monte Carlo (MCMC) inference; clade posterior probabilities are given next to the nodes.

G. carolinensis sp. nov. Consequently, the former western and eastern Atlantic subclades within clade A (see De Wit *et al.*, 2011b) now appear as one unique pan-Atlantic clade. In this clade, *G. monospermatheca* and *G. unitheca* sp. nov. are sister taxa. The morphology and the geographic distribution (north-west Atlantic) of these two species are strongly concordant with phylogeny. The main diagnostic character (the unpaired spermatheca) is found only in these species, supporting that they share a common ancestor.

The inclusion of the seven additional species in the phylogeny (*G. hinojosai* sp. nov. and *G. chilensis* sp. nov. have already been included as *G.* sp. Chile 1 and *G.* sp. Chile 2, respectively, in the analysis described in De Wit *et al.*, 2011b) raises another interesting aspect. The two Chilean species as well as the Western Atlantic *G. brasiliensis* sp. nov. and *G. americana* are placed within clade B, which largely is an Indo-West-Pacific clade. As suggested by De Wit *et al.* (2011b), the ancestor of *G. americana* probably migrated from the eastern Pacific to North Atlantic through Central America before the closing of the Isthmus of Panama (3 Mya). The addition of the new Brazilian species to the phylogenetic analysis suggests that this ancestor is shared by *G. americana* and *G. brasiliensis* sp. nov. The origin of the ancestor of the two Chilean species remains unclear (De Wit *et al.*, 2011b).

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REFERENCES

Clement M, Posada D, Crandall K. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9:** 1657–1659.

Coates KA. 1984. Specific criteria in *Grania* (Oligochaeta, Enchytraeidae). *Hydrobiologia* **115:** 45–50.

Coates KA. 1990. Marine Enchytraeidae (Oligochaeta, Annelida) of the Albany area, Western Australia. In: Wells FE, Walker DI, Kirkman H, Lethbridge R, eds. *Proceedings of the third international marine biological workshop: the marine flora and fauna of albany*. Western Australia: Western Australian Museum, 13–41.

Coates KA, Erséus C. 1985. Marine Enchytraeids (Oligochaeta) of the Coastal Northwest Atlantic (Northern and Mid U.S.A.) *Zoologica Scripta*, **14:** 103–116.

Coates KA, Stacey DF. 1993. The marine Enchytraeidae (Oligochaeta, Annelida) of Rottnest Island, Western Australia. In: Wells FE, Walker DI, Kirkman H, Lethbridge R, eds. *Proceedings of the fifth international marine biological workshop: the marine flora and fauna of rottnest island*. Western Australia: Western Australian Museum, 391–414.

Coates KA, Stacey DF. 1997. Enchytraeids (Oligochaeta: Annelida) of the lower shore and shallow subtidal of Darwin Harbour, Northern Territory, Australia. In: Hanley J, Caswell G, Megirian D, Larson H, eds. *Proceedings of the sixth international marine biological workshop: the marine flora and fauna of Darwin Harbour, Northern Territory, Australia*. Darwin: Museums and Art Galleries of the Northern Territory and the Australian Marine Sciences Association, 67–79.

Costa D, Timmermans MJTN, Sousa JP, Ribeiro R, Roelofs D, Van Straalen NM. 2013. Genetic structure of soil invertebrate populations: collembolans, earthworms and isopods. *Applied Soil Ecology* **68:** 61–66.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9:** 772.

Dayrat B, Tillier A, Lecointre G, Tillier S. 2001. New clades of euthyneuran gastropods (Mollusca) from 28S rRNA sequences. *Molecular Phylogenetics and Evolution* **19:** 225–235.

De Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology* **56:** 879–886.

De Wit P, Erséus C. 2007. Seven new species of *Grania* (Annelida: Clitellata: Enchytraeidae) from New Caledonia, South Pacific Ocean. *Zootaxa* **50:** 27–50.

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, Erséus C, Gustavsson LM. 2011a. Ultrastructure of the body wall of three species of *Grania* (Annelida: Clitellata: Enchytraeidae). *Acta Zoologica* **92:** 1–11.

De Wit P, Rota E, Erséus C. 2009. *Grania* (Annelida: Clitellata: Enchytraeidae) of the Great Barrier Reef, Australia, including four new species and a re-description of *Grania trichaeta* Jamieson, 1977. *Zootaxa* **38:** 16–38.

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

Eisen G. 1904. Enchytraeidae of the west coast of North America. *Harriman Alaska Expedition Series* **12:** 1–166.

Erséus C. 1974. *Grania pusilla* sp.n. (Oligochaeta, Enchytraeidae) from the west coasts of Norway and Sweden with some taxonomic notes on the genus *Grania*. *Sarsia* **56:** 87–94.

Erséus C. 1977. Marine Oligochaeta from the Koster area, west coast of Sweden, with descriptions of two new enchytraeid species. *Zoologica Scripta* **6:** 293–298.

Erséus C. 1990. Marine Oligochaeta of Hong Kong. In: Morton B, ed. *The marine flora and fauna of Hong Kong and Southern China 2, Vol. 1, Introduction and taxonomy*. Hong Kong: Hong Kong University Press, 259–335.

Erséus C, Lasserre P. 1976. Taxonomic status and geographic variation of the marine enchytraeid genus *Grania* Southern (Oligochaeta). *Zoologica Scripta* **5:** 121–132.

Erséus C, Lasserre P. 1977. Redescription of Grania monochaeta (Michaelsen), a Marine Enchytraeid (Oligochaeta) from South Georgia (SW Atlantic). *Zoologica Scripta* **6:** 299–300.

Erséus C, Rota E. 2003. New findings and an overview of the oligochaetous Clitellata (Annelida) of the North Atlantic deep sea. *Proceedings of the Biological Society of Washington* **116:** 892–900.

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

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

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52:** 696–704.

Gustafsson DR, Price DA, Erséus C. 2009. Genetic variation in the popular lab worm *Lumbriculus variegatus* (Annelida: Clitellata: Lumbriculidae) reveals cryptic speciation. *Molecular Phylogenetics and Evolution* **51:** 182–189.

Ishida Y. 2009. Sewall wright and gustave malécot on isolation by distance. *Philosophy of Science* **76:** 784–796.

Jamieson BGM. 1977. Marine meiobenthic Oligochaeta from Heron and Wistari Reefs (Great Barrier Reef) of the genera *Clitellio*, *Limnodriloides* and *Phallodrilus* (Tubificidae) and *Grania* (Enchytraeidae). *Zoological Journal of the Linnean Society* **61:** 329–349.

Jamieson BGM, Tillier S, Tillier A, Justine J-L, Ling E, James S, McDonald K, Hugall AF. 2002. Phylogeny of the Megascolecidae and Crassiclitellata (Annelida, Oligochaeta): combined versus partitioned analysis using nuclear (28S) and mitochondrial (12S, 16S) rDNA. *Zoosystema* **24:** 707–734.

Katoh K, Toh H. 2008. Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* **9:** 286–298.

Källersjö M, von Proschwitz T, Lundberg S, Eldenäs P, Erséus C. 2005. Evaluation of ITS rDNA as a complement to mitochondrial gene sequences for phylogenetic studies in freshwater mussels: an example using Unionidae from northwestern Europe. *Zoologica Scripta* **34:** 415–424.

Knöllner FH. 1935. Ökologische und systematische Untersuchungen über litorale und marine Oligochäten der Kieler Bucht. *Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere* **66:** 425–512.

Kossmagk-Stephan K-J. 1983. Marine Oligochaeta from a sandy beach of the island of Sylt (North Sea) with description of four new enchytraeid species. *Akademie der Wissenschaften und der Literatur Mainz Mathematisch – Naturwissenschaftlichen Klasse Mikrofauna des Meeresbodens* **89 (**Suppl.): 1–28

Lasserre P. 1966. Oligochétes marins des côtes de France. I. Bassin d'Arcachon: systématique. *Cahiers de Biologie Marine* **7:** 295–317.

Lasserre P. 1967. Oligochétes marins des côtes de France. II. Roscoff, penpoull, étangs saumâtres de Concarneau: systématique, écologie. *Cahiers de Biologie Marine* **8:** 273–293.

Locke JM, Coates KA. 1998. A new species of *Grania* (Enchytraeidae, Clitellata, Annelida) and redescription of *Grania pusilla* from a rocky shore in SE Ireland. *Journal of Natural History* **32:** 1101–1114.

Locke JM, Coates KA. 1999. Redescriptions of *Grania americana*, *G. bermudensis* and descriptions of two new species of *Grania* (Annelida: Clitellata: Enchytraeidae) from Bermuda. *Proceedings of the Biological Society of Washington* **112:** 598–623.

Malécot G. 1948. *Les mathematiques de l'heredid*. Paris: Masson.

Martinsson S, Achurra A, Svensson M, Erséus C. 2013. Integrative taxonomy of the freshwater worm *Rhyacodrilus falciformis* s.l. (Clitellata: Naididae), with the description of a new species. *Zoologica Scripta* **42:** 612–622.

Martinsson S, Rota E, Erséus C. 2014. Revision of *Cognettia* (Clitellata, Enchytraeidae): re-establishment of *Chamaedrilus* and description of cryptic species in the *sphagnetorum* complex. *Systematics and Biodiversity* **13:** 257–277.

Michaelsen W. 1888. Die Oligochaeten von Süd-Georgien nach Ausbeute der deutschen Station von 1882–83. *Jahrbuch der Hamburgischen wissenschaftliche Anstalten* **5:** 53–73.

Norén M, Jondelius U. 1999. Phylogeny of the Prolecithophora (Platyhelminthes) Inferred from 18S rDNA Sequences. *Cladistics: The International Journal of the Willi Hennig Society* **15:** 103–112.

Nygren A. 2014. Cryptic polychaete diversity: a review. *Zoologica Scripta* **43:** 172–183.

Palumbi SR, Martin SA, Romano S, McMillan WO, Stice L, Grabowski G. 1991. *The simple fool's guide to PCR*. Honolulu, HI: University of Hawaii press.

Prantoni AL, Di Domenico M, Lana PC. 2013. A taxonomic overview of marine and estuarine oligochaetes from Brazil. *Marine Biodiversity* **44:** 275–278.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: bayesian phylogenetic inference under mixed models. *Bioinformatics (Oxford, England)* **19:** 1572–1574.

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, 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* **2:** 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, Erséus C. 2000. Two new and peculiar species of *Grania* (Annelida : Clitellata : Enchytraeidae) inhabiting Tasmanian estuaries. *New Zealand Journal of Zoology* **27:** 245–254.

Rota E, Erséus C. 2003. New records of *Grania* (Clitellata, Enchytraeidae) in the Northeast Atlantic (from Tromsø to the Canary Islands), with descriptions of seven new species. *Sarsia* **88:** 210–243.

Rota E, Erséus C, Wang H. 2003. *Grania ocarina* sp. n., *G. darwinensis* (Coates & Stacey) comb. n., and other marine Enchytraeidae (Oligochaeta) from the Dampier area, Western Australia. In: Wells FE, Walker DI, Jones DS, eds. *The marine flora and fauna of dampier, Western Australia*. Perth: Western Australian Museum, 497–511.

Rota E, Wang H, Erséus C. 2007. The diverse *Grania* fauna (Clitellata : Enchytraeidae) of the Esperance area, Western Australia, with descriptions of two new species. *Journal of Natural History* **41:** 999–1023.

Rozen S, Skaletzky HJ. 2000. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S, eds. *Bioinformatics methods and protocols: methods in molecular biology*. Tottowa, NJ: Humana Press, 365–386.

Southern R. 1913. Part 48: oligochaeta. clare island survey. *Proceedings of the Royal Irish Academy. Section B. Biological, Geological and Chemical Science* **31:** 1–14.

White TJ, Bruns TD, Lee SB, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR protocols: a guide to methods and applications*. New York: Academic Press, Inc., 315–322.

Wiklund H, Glover AG, Johannessen PJ, Dahlgren TG. 2009. Cryptic speciation at organic-rich marine habitats: a new bacteriovore annelid from whale-fall and fish farms in the North-East Atlantic. *Zoological Journal of the Linnean Society* **155:** 774–785.

Wilgenbusch JC, Warren DL, Swofford DL. 2004. AWTY: a system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. http://ceb.csit.fsu.edu/awty

Wright S. 1943. Isolation by distance. *Genetics* **28:** 114–138.

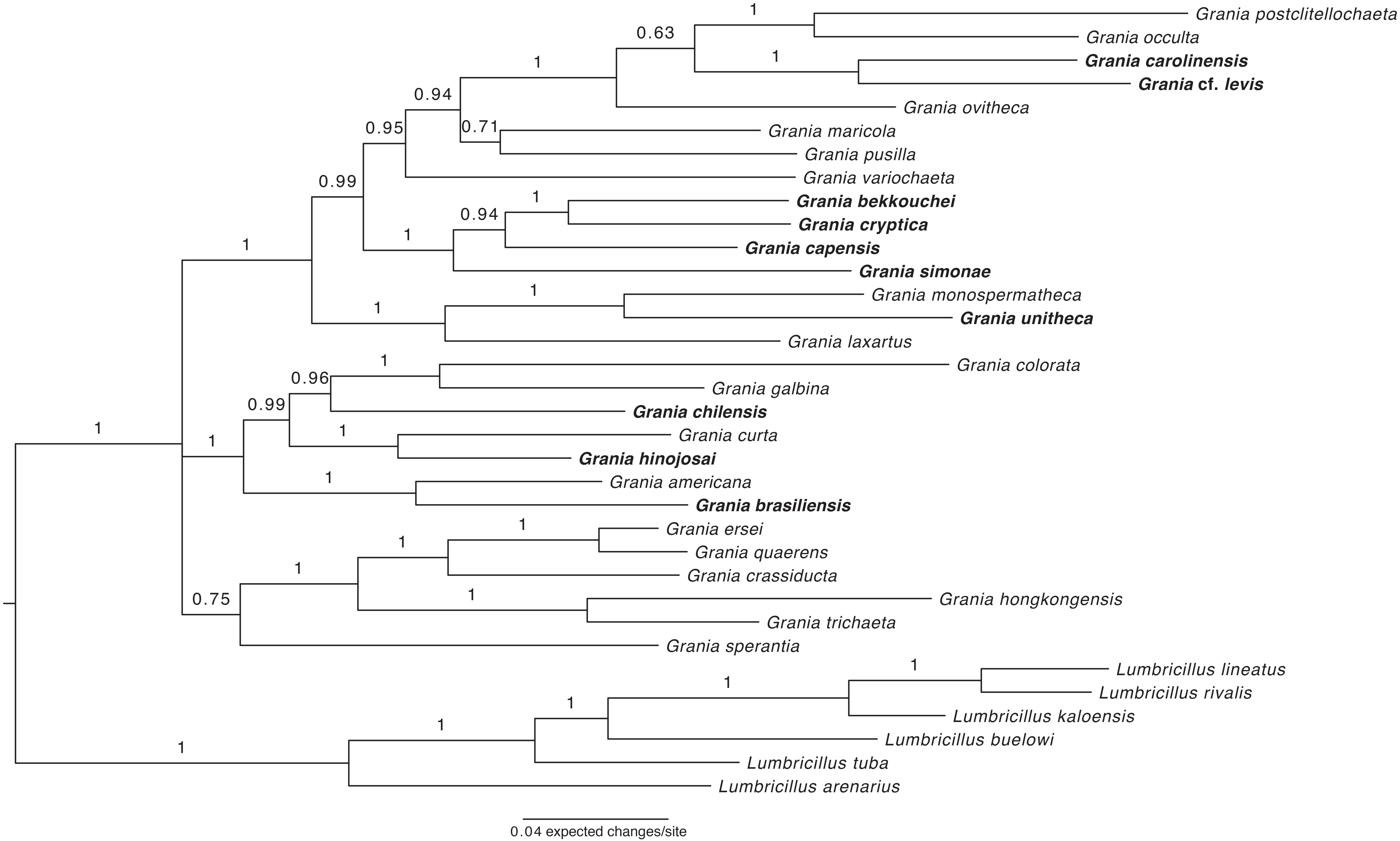
SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Figure S1. Mitochondrial DNA phylogeny of all available genetic data in *Grania* to date, using three loci (*12S* rDNA, *16S* rDNA, and COI).

Figure S2. Nuclear ribosomal DNA phylogeny of all available genetic data in *Grania* to date, using three loci [*18S* rDNA, *28S* rDNA (D1 region), and the *ITS* region (*ITS1-5.8S* rDNA-*ITS2*)].

Table S1. Distance matrix of uncorrected p distances between all individuals analysed genetically in this study.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1
0.94
1
0.95
0.71
0.99
1
0.94
1
1
1
1
0.96
1
0.99
1
1
1
1
1
1
1
0.75
1
1
1
1
1
1
Grania postclitellochaeta
Grania occulta
Grania carolinensis
Grania cf. levis
Grania ovitheca
Grania maricola
Grania pusilla
Grania variochaeta
Grania bekkouchei
Grania cryptica
Grania capensis
Grania simonae
Grania monospermatheca
Grania unitheca
Grania laxartus
Grania colorata
Grania galbina
Grania chilensis
Grania curta
Grania hinojosai
Grania americana
Grania brasiliensis
Grania ersei
Grania quaerens
Grania crassiducta
Grania hongkongensis
Grania trichaeta
Grania sperantia
Lumbricillus lineatus
Lumbricillus rivalis
Lumbricillus kaloensis
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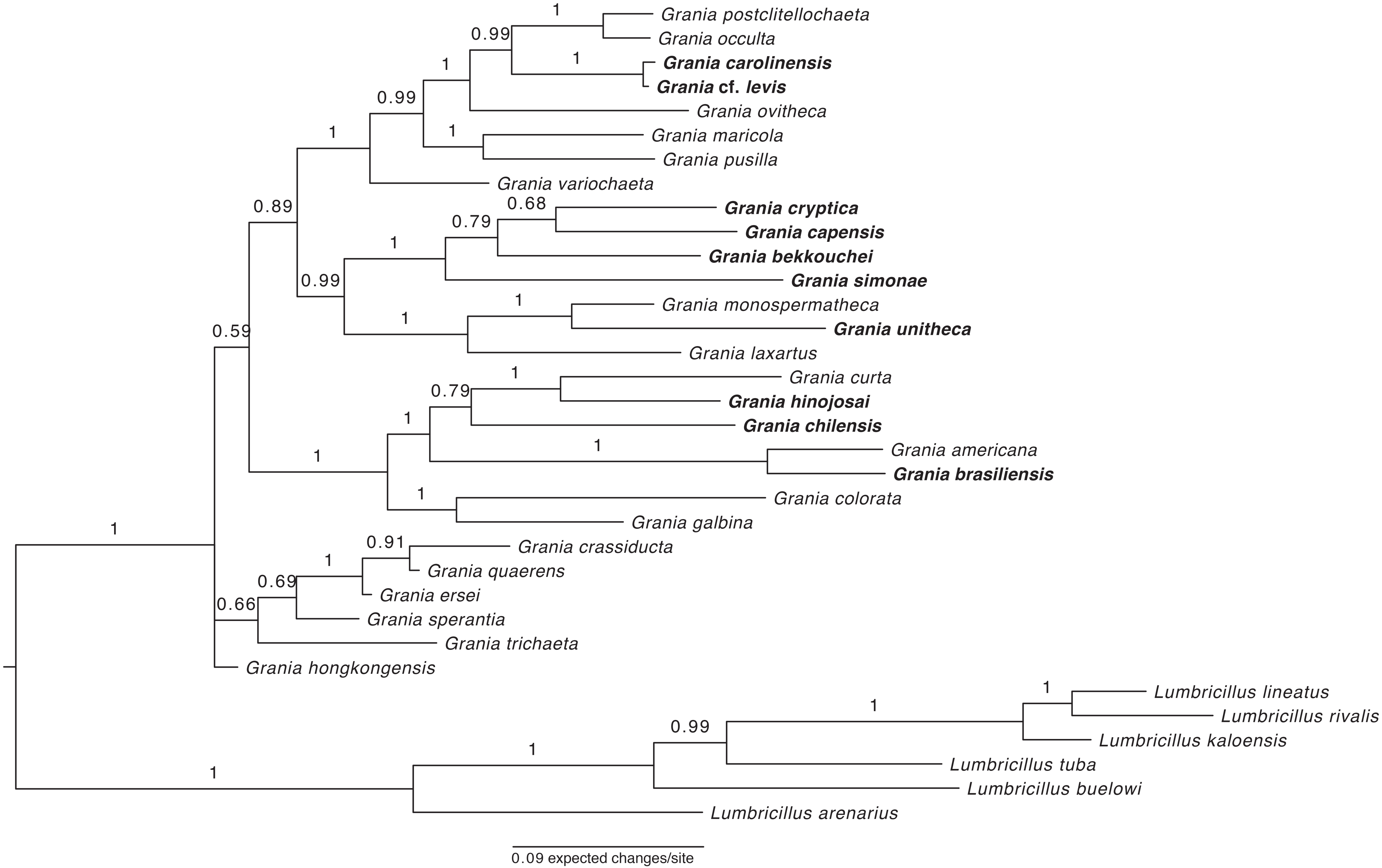
Lumbricillus tuba
Lumbricillus arenarius
0.04 expected changes/site


Grania postclitellochaeta
Grania occulta
Grania carolinensis
Grania cf. levis
Grania ovitheca
Grania maricola
Grania pusilla
Grania variochaeta
Grania cryptica
Grania capensis
Grania bekkouchei
Grania simonae
Grania monospermatheca
Grania unitheca
Grania laxartus
Grania curta
Grania hinojosai
Grania chilensis
Grania americana
Grania brasiliensis
Grania colorata
Grania galbina
Grania crassiducta
Grania quaerens
Grania ersei
Grania sperantia
Grania trichaeta
Grania hongkongensis
Lumbricillus lineatus
Lumbricillus rivalis
Lumbricillus kaloensis
Lumbricillus tuba
Lumbricillus buelowi
Lumbricillus arenarius
1
0.99
1
1
0.99
1
1
0.89
0.68
0.79
1
0.99
1
1
0.59
1
0.79
1
1
1
1
1
0.91
1
0.69
0.66
1
1
0.99
1
1
0.09 expected changes/site

Supplementary Table 1. Distance matrix of uncorrected ‘p’ distances between all individuals analyzed genetically in this study.

	CE21036	PDW194	PDW193	CE21037	CE21038	CE21039	CE21040	PDW190	PDW191	PDW185	PDW189	PDW198	PDW199	PDW200	PDW197	PDW202	PDW177	PDW182	PDW186	PDW181	PDW187	CE21024	CE14060	CE14111	CE14058	CE14110	CE14093	CE14015	CE14016	CE13975	CE13996	CE14059	CE14031	CE20734	CE20818	CE11569	CE11570	CE11659
CE21036	–																																				
PDW194	0	–																																			
PDW193	0.00304	0.00304	–																																		
CE21037	0.00304	0.00304	0.00304	–																																	
CE21038	0.00304	0.00304	0.00304	0.00608	–																																
CE21039	0.00456	0.00456	0.00456	0.0076	0.00456	–																															
CE21040	0.00456	0.00456	0.00456	0.0076	0.00456	0	–																														
PDW190	0.00608	0.00608	0.00608	0.00912	0.00608	0.00456	0.00456	–																													
PDW191	0.05319	0.05319	0.05319	0.05319	0.05319	0.05471	0.05471	0.05623	–																												
PDW185	0.06687	0.06687	0.06687	0.06687	0.06687	0.06687	0.06687																														

```
0.06687 0.05927 -


PDW189  0.06687 0.06687 0.06687 0.06687 0.06687 0.06687 0.06687
0.06687 0.05927 0.00912 -


PDW198  0.05623 0.05623 0.05927 0.05927 0.05927 0.05623 0.05623
0.05927 0.05015 0.04255 0.04103 -


PDW199  0.05623 0.05623 0.05927 0.05927 0.05927 0.05623 0.05623
0.05927 0.05015 0.04255 0.04103 0       -


PDW200  0.05775 0.05775 0.06079 0.06079 0.06079 0.05775 0.05775
0.06079 0.05167 0.04103 0.03951 0.00152 0.00152 -


PDW197  0.05623 0.05623 0.05927 0.05927 0.05927 0.05623 0.05623
0.05927 0.05015 0.04255 0.04103 0.00152 0.00152 0.00304 -


PDW202  0.05623 0.05623 0.05927 0.05927 0.05927 0.05623 0.05623
0.05927 0.05015 0.04255 0.04103 0.00304 0.00304 0.00456
0.00456 -

PDW177  0.18997 0.18997 0.18997 0.18997 0.18997 0.19149 0.19149
0.19301 0.19605 0.18693 0.18389 0.18237 0.18237 0.18237 0.18237
0.18237 -

PDW182  0.18997 0.18997 0.18997 0.18997 0.18997 0.19149 0.19149
0.19301 0.19605 0.18693 0.18389 0.18237 0.18237 0.18237 0.18237
0.18237 0       -

PDW186  0.19149 0.19149 0.19149 0.19149 0.19149 0.19301 0.19301
0.19453 0.19757 0.18845 0.18541 0.18389 0.18389 0.18389 0.18389
0.18389 0.00456 0.00456 -

PDW181  0.18997 0.18997 0.18997 0.18997 0.18997 0.19149 0.19149
0.19301 0.19757 0.18237 0.18085 0.18389 0.18389 0.18389 0.18389
0.18389 0.00456 0.00456 0.00912 -
```

```
PDW187  0.18997 0.18997 0.18997 0.18997 0.18997 0.19149 0.19149
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0.18237 0.0076  0.0076  0.01216 0.00304 -


CE21024 0.19048 0.19048 0.19048 0.19048 0.19048 0.19201 0.19201
0.19352 0.19658 0.1874  0.18435 0.18287 0.18287 0.18286 0.18287
0.18287 0.0061  0.0061  0.01067 0.01067 0.01372 -


CE14060 0.20974 0.20974 0.20974 0.20968 0.20974 0.20976 0.20976
0.21113 0.20518 0.20034 0.19716 0.20391 0.20391 0.20551 0.20387
0.20387 0.17804 0.17804 0.17648 0.17969 0.17967 0.18355 -


CE14111 0.20669 0.20669 0.20669 0.20669 0.20669 0.20669 0.20669
0.20669 0.20213 0.19453 0.19149 0.20365 0.20365 0.20517 0.20365
0.20365 0.18237 0.18237 0.18085 0.18389 0.18389 0.18749
0       -

CE14058 0.20669 0.20669 0.20669 0.20669 0.20669 0.20669 0.20669
0.20669 0.20213 0.19453 0.19149 0.20365 0.20365 0.20517 0.20365
0.20365 0.18237 0.18237 0.18085 0.18389 0.18389 0.18749 0
0       -

CE14110 0.20821 0.20821 0.20821 0.20821 0.20821 0.20821 0.20821
0.20821 0.20365 0.19605 0.19301 0.20517 0.20517 0.20669 0.20517
0.20517 0.18389 0.18389 0.18237 0.18541 0.18541 0.18902 0.00161
0.00152 0.00152 -

CE14093 0.20309 0.20309 0.20309 0.20304 0.20309 0.2031  0.2031
0.20309 0.20134 0.19179 0.18858 0.20159 0.20159 0.20319 0.20155
0.20154 0.18242 0.18242 0.17901 0.18403 0.18399 0.18531 0
0       0       0.00165 -

CE14015 0.20213 0.20213 0.20213 0.20365 0.20061 0.20517 0.20517
0.20365 0.18997 0.20213 0.20061 0.19149 0.19149 0.19149 0.19149
0.18845 0.18085 0.18085 0.17933 0.18541 0.18389 0.18142 0.17344
0.17629 0.17629 0.17477 0.17384 -

CE14016 0.20365 0.20365 0.20365 0.20517 0.20213 0.20669 0.20669
0.20517 0.19149 0.20365 0.20213 0.19301 0.19301 0.19301 0.19301
0.18997 0.18237 0.18237 0.18085 0.18693 0.18541 0.18295 0.17503
0.17781 0.17781 0.17629 0.17383 0.00304 -

CE13975 0.18997 0.18997 0.18997 0.19149 0.18845 0.18997 0.18997
0.18845 0.19149 0.19149 0.19453 0.19757 0.19757 0.19909 0.19605
0.19757 0.17173 0.17173 0.17325 0.17021 0.16717 0.17534 0.19888
0.19909 0.19909 0.20061 0.20005 0.17629 0.17933 -

CE13996 0.18997 0.18997 0.18997 0.19149 0.18845 0.18997 0.18997
0.18845 0.19149 0.19149 0.19453 0.19757 0.19757 0.19909 0.19605
0.19757 0.17173 0.17173 0.17325 0.17021 0.16717 0.17534 0.19888
0.19909 0.19909 0.20061 0.20005 0.17629 0.17933 0       -
```

```
CE14059  0.19453  0.19453  0.19453  0.19605  0.19301  0.19453  0.19453
0.18997  0.19453  0.19757  0.20061  0.18541  0.18541  0.18693  0.18389
0.18845  0.17173  0.17173  0.17325  0.17021  0.16717  0.17381  0.20376
0.20213  0.20213  0.20365  0.20262  0.17933  0.17933  0.03647
0.03647  -
CE14031  0.21581  0.21581  0.21733  0.21429  0.21581  0.21733  0.21733
0.21581  0.21581  0.21277  0.21429  0.20669  0.20669  0.20669  0.20517
0.20669  0.20365  0.20365  0.20669  0.20517  0.20365  0.20736  0.19243
0.19301  0.19301  0.19453  0.20196  0.18237  0.18237  0.18237  0.18237
0.18541  -
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0.21581  0.21733  0.20365  0.20061  0.20061  0.20061  0.20061  0.19909
0.20213  0.20061  0.20061  0.20213  0.20061  0.20061  0.2012   0.19439
0.19757  0.19757  0.19909  0.19196  0.19453  0.19605  0.19605  0.19605
0.19149  0.21884  -
CE20818  0.22492  0.22492  0.2234   0.22492  0.2234   0.2234   0.2234
0.22036  0.21733  0.19757  0.19757  0.20061  0.20061  0.20061  0.19909
0.20213  0.19149  0.19149  0.19301  0.19149  0.19149  0.19206  0.19443
0.19605  0.19605  0.19757  0.19033  0.19453  0.19605  0.19149  0.19149
0.18389  0.21884  0.01368  -
CE11569  0.21733  0.21733  0.21429  0.21733  0.21733  0.21581  0.21581
0.21733  0.21429  0.21581  0.21581  0.21733  0.21733  0.21733  0.21733
0.21733  0.20365  0.20365  0.20669  0.20213  0.20061  0.2073   0.2071
0.20821  0.20821  0.20821  0.20974  0.19757  0.19757  0.18845  0.18845
0.18693  0.21733  0.20669  0.20365  -
CE11570  0.23556  0.23556  0.2386   0.23556  0.2386   0.24012  0.24012
0.24164  0.2386   0.231    0.231    0.23252  0.23252  0.23252  0.23252
0.23404  0.21429  0.21429  0.20973  0.21125  0.20973  0.21641  0.22488
0.22036  0.22036  0.21884  0.21317  0.19909  0.20061  0.20821  0.20821
0.20973  0.22492  0.23404  0.23252  0.18389  -
CE11659  0.18389  0.18389  0.18389  0.18085  0.18389  0.18541  0.18541
0.18389  0.17781  0.17781  0.17325  0.17477  0.17477  0.17325  0.17325
0.17629  0.20973  0.20973  0.21125  0.21277  0.21125  0.21037  0.22104
0.21581  0.21581  0.21733  0.21157  0.21125  0.21125  0.22036  0.22036
0.21733  0.24012  0.20973  0.20821  0.22796  0.21581  -
```

CAPÍTULO 4

Global checklist of species of *Grania* (Clitellata: Enchytraeidae) with remarks on their geographic distribution

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Abstract

A checklist of all currently accepted species of *Grania* (Clitellata, Enchytraeidae) is presented. Remarks on their geographical distribution, habitat, synonymies and museum catalogue numbers are also provided. The genus is widespread over the world and comprises eighty species described to date.

Introduction

Grania Southern, 1913 is a morphologically homogeneous genus of marine Enchytraeidae with a world-wide distribution. The worms are typically small, only a few mm long, with a filiform, nematode-like body, and a characteristic pattern of few, stout chaetae. Most species live interstitially in intertidal or subtidal sands, a few taxa in the deep sea.

The genus was originally established for specimens of *Grania maricola* Southern, 1913, sampled from the west coast of Ireland. Later on, Pierantoni (1915) regarded *Grania maricola* and *Michaelsena macrochaeta* (Pierantoni, 1901) as synonyms. In the opposite direction, Stephenson, (1930) considered them as separate species within *Michaelsena* Ude, 1896. Nielsen & Christensen (1959) pointed out, however, that Stephenson's concept of *Michaelsena* was artificial, and they transferred both species to the genus *Enchytraeus* Henle, 1837.

Interestingly, two authors independently rehabilitated the genus *Grania* in 1966. Kennedy (1966) redescribed *G. maricola* and *Grania macrochaeta* and described *Grania americana* Kennedy, 1966 from off the coast of Florida, whilst Lasserre (1966) transferred *Michaelsena postclitellochaeta* (Knöllner, 1935) to *Grania*. One year later, considering the similarities among *G. maricola*, *G. macrochaeta* and G*. americana*, Lassserre (1967) proposed to divide *G. macrochaeta* into four subspecies, *G. m. macrochaeta*, *G. m. americana*, *G. m. maricola* and *G. m. roscoffensis* Lasserre, 1967. This situation remained so for about 10 years, after which a high number of additional species of *Grania* have been described from different parts of the world (Erséus and Lasserre 1976; Erséus, 1977; Jamieson 1977 Erséus, 1980; Coates & Erséus, 1985; Coates, 1990; Erséus, 1990; Rota & Erséus, 1996; Rota & Erséus, 1997; Locke & Coates, 1998, 1999; De Wit & Erséus, 2007; Rota et al., 2007; De Wit et al., 2009; Prantoni et al., 2016), bringing a better understanding of species-specific characters and raising the subspecies back to species status (Coates, 1984; De Wit, 2010).

The genus is morphologically and genetically well separated from other enchytraeid genera (Erséus et al., 2010; De Wit et al., 2011; Prantoni et al., in press). Its monophyly was corroborated by a DNA-based phylogeny by De Wit et al (2011). These authors also found that the phylogeny is strongly concordant with geographical distribution and suggested a limited dispersal capability within *Grania*. More recently, Prantoni et al. (in press) updated the genus phylogeny together with the descriptions and genetic data of nine new species, including for the first time species from Africa and east coast of South America (Brazil).

To provide a taxonomical overview of *Grania* over the world, the present checklist summarizes historical and recently published data, including all valid species described to date.

Material and Methods

The checklist is arranged in chronological order and based on a bibliographic survey. All *Grania* records from published papers, and monographs were reviewed. When available, additional information on habitat, geographical distribution, and museum catalogue numbers were included.

Results and discussion

We recorded 80 currently accepted species of *Grania*, of which 49 from the southern and 31 from the northern hemisphere. Almost half of the southern species (24) occur in Australia. On the other hand, 15 of the 32 species described from the northern hemisphere to date are European. The only species found in both hemispheres (Atlantic Ocean) is the deep-sea *Grania atlantica.*

Despite the many species described from the Australian continent and other southern regions of the globe (e.g. New Caledonia and Antarctica), the African and South American continents were completely ignored until recently. The situation has changed with seven new species described from Brazil (1 species), Chile (2 species) and South Africa (4 species) (Prantoni et al., in press). Even so, the number of *Grania* species described to date certainly does not represent the actual diversity of the group. The lack of specialists around the world is one of the probable causes for this. As a partial solution, the combined efforts of taxonomists and ecologists may come as a first and necessary step towards a better understanding of the group as well as marine clitellate species diversity as a whole.

List of species

***Grania monochaeta* (Michaelsen, 1888)**

Enchytraeus monochaetus Michaelsen, 1888: 66-68, figs. 6A-C, part; Beddard, 1895: 339; Michaelsen, 1900: 91.
Non *Michaelsena monochaeta*; Michaelsen, 1921: 3; Stephenson, 1932: 263, fig. 14.
Marionina monochaeta; Nielsen & Christensen, 1959: 109.
Hemigrania monochaeta; Lasserre, 1971: 454.

Non *Grania monochaeta*; Erséus and Lasserre, 1977: 299-300, figs. 1A-D.
Grania monochaeta; Erséus & Lasserre, 1976: 200-300, figs. 1 A-D; Rota & Erséus, 1997: 29-34, fig. 2, table 1.
Type material: Lectotype, MZUT 123.1, Museo ed Instituto de Zoologia del' Universitá de Torino, Turin. Paralectotypes, MZUT Olig. Coll. 123.2-123.4.
Other material: SMNH 362, Swedish Museum of Natural History, Stockholm.
Type locality: South Georgia, Southwest Atlantic.
Habitat: Intertidal, and subtidal to 20 m, shelly detritus, among the roots of seaweeds and in the canal system of sponges.
Distribution: Only known from type locality.

***Grania macrochaeta* (Pierantoni, 1901)**

Enchytraeus macrochaetus Pierantoni, 1901: 201-202; Nielsen & Christensen, 1959: 89-91, table 9.
Michaelsena macrochaeta; Pierantoni, 1903: 409-444, figs. 1-28; Pierantoni, 1915: 48-50; Stephenson, 1930: 776-777.
Grania macrochaeta macrochaeta; Lasserre, 1967: 280.
Grania macrochaeta; Kennedy, 1966: 403-404; Lasserre, 1966: 312-314; Erséus, 1974: 90-93, table 1; Rota, 1995: table 2.
Type material: Not designated.
Other material: MNHM AH 61-63. Muséum National d'Histoire Naturelle, Paris. This refers to three specimens of *G. macrochaeta* collected in June, 1967 by J. Renaud-Mornant and examined by Erséus & Lasserre (1976).
Type locality: Bay of Naples, Italy.
Habitat: Subtidal, 4-13 m, coarse sand.
Distribution: Only known from type locality.

***Grania paucispina* (Eisen, 1904)**

Michaelsena paucispina Eisen, 1904: 74, fig. 43; Michaelsen, 1907: 130.
Marionina paucispina; Nielsen & Christensen, 1959: 109; Lasserre, 1971: 454.

Grania paucispina; Erséus & Lasserre, 1976, p. 127; Coates & Erséus, 1980: 1037-1038, fig. 1; Coates & Ellis, 1981: 2134.

Type material: Typus amissus. According to Coates and Erséus (1980), the type material deposited in the California Academy of Science in San Francisco was destroyed during the earthquake and fire in 1906.

Other material:USNM 58906-58907, National Museum of Natural History, Washington, D.C.

Type locality: Santa Barbara, California, USA.

Habitat: 2-6 m in muddy sand with much organic material, brackish water.

Distribution: California, USA and British Columbia.

Grania principissae **(Michaelsen, 1907)**

Michaelsena principissae Michaelsen, 1907: 129-131, plate 1, figs. 1-2.

Hemigrania principissae; Lasserre, 1971: 454.

Grania principissae; Erséus & Lasserre, 1976: 128; Coates, 1990: 28-30, figs 2, 8.

Type material: Not designated.

Other material: WAM 69-89, Western Australian Museum, Perth. ROMIZ 11277, Invertebrate Zoology, Royal Ontario Museum, Toronto.

Type locality: Princess Royal Harbour, Albany area, Western Australia.

Habitat: Intertidal, sand with organic debris accumulated in mussel bed.

Distribution: Only known from type locality.

Grania maricola **Southern, 1913**

Michaelsena macrochaeta; Pierantoni, 1915: 48-50.

Michaelsena maricola; Stephenson, 1930: 776-777.

Enchytraeus maricolus; Nielsen & Christensen, 1959: 89-91, table 9.

Grania macrochaeta maricola; Lasserre, 1967: 280.

Grania maricola Southern, 1913: 14 figs 1-7; Kennedy, 1966: 400-402, fig. 2; Lasserre, 1966: 312-314; Erséus, 1974: 90-93; Erséus, 1976: 35, table 3; Coates, 1984: 40, fig. 7A table 1; Rodriguez, 1986: 82-83, fig. 2; Rota, 1995:

table 2; Locke & Coates, 1998: 1107-1112, figs. 6-12, table 1; De Wit, 2006: 25.

Type material: NMINH 1909.151, NMINH 1913.415 and NMINH 1914.313, National Museum of Ireland (Department of Natural History), Dublin.

Type locality: Dingle Bay, Co Kerry, Ireland.

Habitat: Subtidal, 8-80 m, coarse shell sand and shell gravel.

Distribution: Ireland, Iberian Peninsula, Italy, Norway and Sweden.

Grania postclitellochaeta **(Knöllner, 1935)**

Michaelsena postclitellochaeta Knöllner, 1935: 449-455, figs. 19-25, table 1; Hagen, 1954: 12-13.

Marionina postclitellochaeta; Nielsen & Christensen, 1959: 109-110, table 11.

Grania postclitellochaeta postclitellochaeta; Lasserre, 1966, pp. 299-300, 312-314, tables 1-2; Erséus, 1976: 35, table 3; Erséus & Lasserre, 1976: 124, Table 1.

Hemigrania postclitellochaeta; Lasserre, 1971, 454-456, fig. 3C.

Grania postclitellochaeta; Kossmagk-Stephan 1983: 598; Rota & Erséus, 2003: 232-234, figs. 10C-D.

Type material: Not designated.

Other material: MNHM AH 66-68, from France, Muséum National d'Histoire Naturelle, Paris, (see Erséus & Lasserre, 1976). SMNH 45646–45651 from France, SMNH 45652–45654, from North sea, off Belgium, SMNH 45655–45660, from Iceland, SMNH 45661–45665 (see Rota & Erséus, 2003), SMNH 107730,107736, 107738-107745, 108220, from Sweden, SMNH 107746-107749, from Norway (see De Wit & Erséus, 2010), Swedish Museum of Natural History, Stockholm. MCZR Oligochaeta 0116-0117 from France, MCZR Oligochaeta 0118 from North sea, off Belgium, MCZR Oligochaeta 0119-0120 from Iceland (see Rota & Erséus, 2003), Museo Civico di Zoologia di Roma, Rome.

Type locality: Kiel Bay, Germany.

Habitat: Intertidal, and Subtidal, 20-100 m in coarse shell sand, shell gravel, sometimes in brackish water (Baltic Sea), tolerating a wide range of salinity (11-35 ppt).

Distribution: Baltic Sea, Iceland (see Rota & Erséus, 2003), North Sea (Germany and Belgium), France, Norway and Sweden.

Grania americana Kennedy, 1966

Grania americana Kennedy, 1966: 404-405, fig. 3. Erséus, 1974: 90-93, table 1; Healy & Coates, 1999: 111,114, table 1; Locke & Coates, 1999: 598-623, figs. 16-20; Locke & Coates, 2000: 619-620, 625-626, figs. 4A, 5.

Grania macrochaeta americana; Lasserre, 1967: 78-280.

“*Grania americana*, nomen dubium”; Erséus & Lasserre, 1976: p. 123.

Type material: USNM 33005, 33039, United States National Museum of Natural History, Washington, D.C.

Other material: USNM 185957- 85960, United States National Museum of Natural History, Washington, D.C.

Type locality: 0.5 km from North Entrance Point, West side of North Bimini, Bahamas.

Habitat: Intertidal, and subtidal to 10 m, fine to coarse sand.

Distribution: Belize, Bermuda, Bahamas, Florida.

Grania roscoffensis Lasserre, 1967

Grania macrochaeta roscoffensis Lasserre, 1967: 277-280.

Grania roscoffensis; Erséus, 1974: 90-93, table 1; Erséus, 1976: 125, fig. 6; Coates, 1984: 49; Rota & Erséus, 2003: 218-221, fig. 4.

Non *Grania roscoffensis*; Erséus 1977: 294, table 1 (see *Grania vikinga* Rota & Erséus, 2003).

Type material: MNHM AH 64 and AH 65, Muséum National d'Histoire Naturelle, Paris.

Type locality: Harbour of Roscoff, France.

Habitat: Intertidal, coarse sand.

Distribution: France and Sweden.

Grania pusilla Erséus, 1974

Grania pusilla Erséus, 1974: 87-94, figs. 1-6, table 1; Erséus, 1976: 34, table 3; Locke & Coates, 1998: 1107-1112, figs. 6-12; De Wit et al., 2011: 513, figs. 1-5, table 1.
Grania macrochaeta pusilla; Erséus & Lasserre, 1976: 122, fig 2; Erséus, 1977: 294, table 1; Coates, 1984: 49; Kossmagk-Stephan, 1985: 77–78.
Type material: ZMUB 55050-55051, Zoological Museum of University of Bergen, Bergen.
Other material: SMNH 107775-107796, Swedish Museum of Natural History, Stockholm.
Type locality: Vågegrunnen, Hjeltefjorden, near Bergen, Norway.
Habitat: Subtidal, 35-500 m, fine to coarse shelly sand.
Distribution: West coasts of Norway and Sweden, Morocco.

Grania bermudensis Erséus & Lasserre, 1976

Grania macrochaeta bermudensis Erséus & Lasserre, 1976: 122-124, fig. 3 table 1; Lasserre & Erséus, 1976: 453; Coates, 1984, p. 49, fig. 8A.
Grania bermudensis; Locke & Coates, 1999: 609-614, figs. 6, 12-15, table 1; Locke & Coates, 1999: 609-614, figs. 6,12-15, table 1; Locke & Coates, 2000: 619-621, 626, fig. 6C.
Type material: USNM 53202-53203, United States National Museum of Natural History, Washington, D.C.
Type locality: Castle Island, Bermuda.
Habitat: Subtidal, 8-15 m, medium to coarse coral sand and gravel.
Distribution: Only known from Bermuda.

Grania longiducta Erséus & Lasserre, 1976

Grania postclitellochaeta longiducta Erséus & Lasserre, 1976: 127, fig 7; Erséus, 1977: 296-297; Coates, 1984: 49.

Grania longiducta; Coates & Erséus, 1985: 113-114, fig. 8; Diaz et al., 1987: table 1, 3; Locke & Coates, 2000: 619, 625.

Type material: USNM 43482, 53201, United States National Museum of Natural History, Washington, D.C.

Type locality: Cape Cod Bay, Massachusetts, USA.

Habitat: Subtidal, 42-78 m, medium to coarse sand.

Distribution: Cape Cod Bay, Massachusetts, Georges Bank (SE of Massachusetts), off the coast of New Jersey, Delaware and Maryland.

***Grania monospermatheca* Erséus & Lasserre, 1976**

Grania monospermatheca Erséus & Lasserre, 1976: 127, fig. 9, table 1; Coates, 1984: 49, fig. 8B; Coates & Erséus, 1985: 114-115, fig. 9; Diaz et al., 1987: tables 1-3; Locke & Coates, 2000: 619, 626-628.

Type material: USNM 53204-53205, United States National Museum of Natural History, Washington, D.C.

Type locality: Cape Cod Bay, Massachusetts, USA.

Habitat: Subtidal, 3-48 m, fine to coarse well sorted sands and sand mixed with shell or shell gravel.

Distribution: Along North American Atlantic coast, from Cape Cod Bay, Massachusetts to Biscayne Bay, Miami, Florida.

***Grania variochaeta* Erséus & Lasserre, 1976**

Grania variochaeta Erséus, 1976: 35, table 3; Erséus & Lasserre, 1976: 125-126, figs. 10-11, table 1; Erséus, 1977: 297-298, table 1; Coates, 1984: 46, fig. 6; Rota & Erséus, 2003: 211, 234-235, fig. 11.

Type material: SMNH 3132-3136, Swedish Museum of Natural History, Stockholm.

Type locality: East of Yttre, Vattenholmen, Kosterfjorden, Bohuslãn, Bohuslän, west coast of Sweden.

Habitat: Subtidal, 20-140 m, heterogeneous sand.

Distribution: West coast of Norway and Sweden.

Grania ovitheca Erséus, 1977

Grania ovitheca Erséus, 1977: 125, figs. 5-7; Bonomi & Erséus, 1984: 209, table1; Rota, 1995: table 2; Rota & Erséus, 2003: 230-233, figs. 10A, B.

Type material: SMNH 3071-3073, Swedish Museum of Natural History, Stockholm.

Type locality: Kosterfjorden, north of Kostergrund**,** Bohuslän, west coast of Sweden.

Habitat: Subtidal, 15-30 m, shell sand with gravel and pebbles or coarse sand with stones, pebbles and shells.

Distribution: West coasts of Norway and Sweden.

Grania trichaeta Jamieson, 1977

Grania macrochaeta trichaeta Jamieson, 1977: 345–347, fig. 5, plate 1G; Coates 1984: 46, fig. 5A.

Grania trichaeta; De Wit et al., 2009: 28-30, figs. 8-10E.

Type material: QM 8863-8866, Queensland Museum, Brisbane. BNMH 1976.1.21-23, Natural History Museum, London. BJ 1975.7.74-75, BJ 1975.7.84 BJ 1975.7.76-78, Jamieson collection.

Other material: SMNH 105540-105559, SMNH 105560-105584, Swedish Museum of Natural History, Stockholm (De Wit et al., 2009).

Type locality: Wistari Reef, southern part of Great Barrier Reef, Australia.

Habitat: Intertidal, and subtidal to 7 m, fine to medium heterogeneous sand.

Distribution: Lizard Island, Heron Island and Wistari Reef, Great Barrier Reef, Australia.

Grania pacifica Shurova, 1979

Grania pacifica Shurova, 1979: 84-86, fig. 6.

Type material: Cat. nº 16018, ?Russian Museum.

Type locality: Sea of Okhotsk, Kuril Islands, Far East of Russia.

Habitat: Subtidal, 15-20 m, gravelly sediment.

Distribution: Only known from type locality.

Grania incerta Coates & Erséus, 1980

Grania incerta Coates & Erséus, 1980: 1038-1040, fig. 2; Coates & Ellis, 1981: 2134-2135; Coates, 1984: 46, fig. 4.

Type material: USNM 58908-58910, United States National Museum of Natural History, Washington, D.C.

Type locality: Santa Barbara, California, USA.

Habitat: Subtidal, 3-17 m, well sorted fine sand.

Distribution: California and British Columbia (Canada).

Grania parvitheca Erséus, 1980

Grania parvitheca Erséus, 1980: 27-28, fig. 1.

Type material: USNM 58738-58739, United States National Museum of Natural History, Washington, D.C.

Type locality: Northeast Bay, Ascension Island (South Atlantic Ocean).

Habitat: Intertidal, among rocks and clumps of Sabellaridae tubes.

Distribution: Only known from type locality.

Grania atlantica Coates & Erséus, 1985

Grania atlantica Coates & Erséus, 1985; 112-113, fig. 7; Diaz, et al., 1987: 222-224, tables 1, 3, 4; Locke & Coates, 2000: 619, 626; Rota & Erséus, 1996: 182; Erséus & Rota, 2003: 898, table 1; Rota & Erséus, 2003: 210-211, 235-237, fig. 10E.

Type material: USNM 96503-96508, United States National Museum of Natural History, Washington, D.C.

Type locality: Continental slope off Massachusetts.

Habitat: Continental slope, 744-1796 m, fine ooze to silty deep-sea sediments.

Distribution: Widely distributed in the Atlantic Ocean from 48º35.4’N to 09º05'S in the east and from 39º51.2’N to 08º58.0'N in the west.

Grania levis Coates & Erséus, 1985

Grania levis Coates & Erséus, 1985: 111-112, fig. 6; Diaz et al., 1987: tables 1, 4; Locke & Coates, 2000: 626.

?Grania cf. *levis*; Prantoni et al., *in press*.

Type material: USNM 96509-96511, United States National Museum of Natural History, Washington, D.C.

Type locality: Georges Bank (SE of Massachusetts), Northwest Atlantci Ocean.

Habitat: Intertidal, and subtidal to 79 m (probably to 492 m, see Prantoni et al., in press) medium to coarse sand.

Distribution: Georges Bank SE of Massachusetts, on the continental shelf (and slope?) off New Jersey and North Carolina.

Grania reducta Coates & Erséus, 1985

Grania reducta Coates & Erséus, 1985: 110-111, fig. 5; Diaz et al., 1987: tables 3-4; Locke & Coates, 2000: 626, 628.

Type material: USNM 96512-96513, United States National Museum of Natural History, Washington, D.C.

Type locality: Off Maryland, USA.

Habitat: Intertidal, and subtidal to 65 m, medium to coarse sand.

Distribution: Continental shelf off New Jersey, Maryland and Delaware.

Grania ascophora Coates, 1990

Grania ascophora Coates, 1990: 23-25, fig. 5.

Type material: WAM 69.89, Western Australian Museum, Perth. ROMIZ I2880, Invertebrate Zoology, Royal Ontario Museum, Toronto.

Type locality: Barker Bay, King George Sound, Western Australia.

Habitat: Subtidal, 4 m, fine sand with shells and seagrass.

Distribution: Barker Bay, and Princess Royal Harbour, Albany area, Western Australia.

Grania bykane **Coates, 1990**

Grania bykane Coates, 1990: 21–23, figs. 2, 4A–D; Rota et al., 2007: 1001-1004, figs. 1A-G, 2A.

Type material: WAM 55.89-56.89, Western Australian Museum, Perth.

Type locality: Princess Royal Harbour, Albany area, Western Australia.

Habitat: Intertidal, and subtidal to at least 6 m, fine to coarse sand and in sediments under boulders and in seagrass beds.

Distribution: Southern coast of Western Australia (Albany and Esperance areas).

Grania crassiducta **Coates 1990**

Grania crassiducta Coates, 1990: 20–21, figs. 2, 3A–D; Coates and Stacey, 1993: 404–406, figs. 9A–F; Rota et al., 2007: 1004-1006, figs. 2B, 3A–F.

Type material: WAM 51.89-53.89, Western Australian Museum, Perth. ROMIZ I1279, Invertebrate Zoology, Royal Ontario Museum, Toronto.

Type locality: South Point, Princess Royal Harbour, Albany area, Western Australia.

Habitat: Intertidal, muddy coarse sand, gravel, and mixed sand with pebbles and coral.

Distribution: Southern (Albany and Esperance) and western (Rottnest Island) coasts of Western Australia.

Grania ersei **Coates, 1990**

Grania ersei Coates, 1990: 17–20, figs. 1A–D, 2; Coates & Stacey 1993: 406–408, figs. 10A–F; Rota et al., 2007: 1008-1011, figs. 4D–G, 5A.

Type material: WAM 61.89-68.89, Western Australian Museum, Perth. ROMIZ I1273-I1276, Invertebrate Zoology, Royal Ontario Museum, Toronto.

Type locality: Brable Point, Princess Royal Harbour, Albany area, Western Australia.

Habitat: Intertidal, and subtidal to 26 m, sand among boulders and pebbles, and with algal debris.

Distribution: South (Albany, Esperance) and west (Rottnest Island) coasts of Western Australia.

Grania hastula **Coates, 1990**

Grania hastula Coates, 1990: 26-28, fig. 7.

Type material: USNM 120714, United States National Museum of Natural History, Washington, D.C.

Type locality: Middleton Beach, Albany, Western Australia.

Habitat: Intertidal, sand among rocks in algal wash.

Distribution: Only known from type locality.

Grania hyperoadenia **Coates, 1990**

Grania hyperoadenia Coates, 1990: 25–26, fig. 6; De Wit et al., 2009: 30-31, fig. 9.

Type material: WAM 54.85, Western Australian Museum, Perth.

Type locality: Barker Bay, King George Sound, Western Australia.

Habitat: Subtidal, 1.5-4 m in sand.

Distribution: Albany area, Western Australia and Lizard Island, Great Barrier Reef, Queensland.

Grania hongkongensis **Erséus, 1990**

Grania hongkongensis Erséus, 1990: 311-12, fig. 22.

Type material: BMNH 1987.3.39-40, Natural History Museum, London. SMNH 3717, Swedish Museum of Natural History, Stockholm.

Type locality: Crooked Island, Mirs Bay, Hong Kong (New Territories), China.

Habitat: Intertidal, and subtidal to 15 m, shelly sand.

Distribution: Only known from type locality.

Grania inermis **Erséus, 1990**

Grania inermis Erséus, 1990: 314-315, fig. 24.

Type material: BMNH 1987.3.42-43, Natural History Museum, London. SMNH 3719, Swedish Museum of Natural History, Stockholm.

Type locality: Kung Chao (off Tap Mun), Mirs Bay, Hong Kong (New Territories), China.

Habitat: 7-14 m in shelly sand.

Distribution: Only known from type locality.

Grania stilifera **Erséus, 1990**

Grania stilifera Erséus, 1990: 312-314, fig. 23.

Type material: BMNH 1987.3.41, Natural History Museum, London. SMNH 3718, Swedish Museum of Natural History, Stockholm.

Type locality: Round Island, Mirs Bay, Hong Kong (New Territories), China.

Habitat: Subtidal, 5-8 m, shelly sand.

Distribution: Only known from type locality.

Grania alliata **Coates & Stacey, 1993**

Grania alliata Coates & Stacey, 1993: 397-399, figs. 3-4.

Type material: WAM 192-92, Western Australian Museum, Perth.

Type locality: North side of west end of Rottnest Island, Western Australia.

Habitat: Intertidal, gravelly sand.

Distribution: Only known from type locality.

Grania conjuncta Coates & Stacey, 1993

Grania conjuncta Coates & Stacey, 1993: 402-404, figs. 7-8.

Type material: WAM 193-92, Western Australian Museum, Perth.

Type locality: Fishhook Bay, Rottnest Island, Western Australia.

Habitat: Subtidal, 2 m, medium to coarse sand.

Distribution: Only known from type locality.

Grania longistyla Coates & Stacey, 1993

Grania longistyla Coates & Stacey, 1993: 394-397, figs. 1-2.

Type material: WAM 194-92-195-92, Western Australian Museum, Perth.

Type locality: North side of west end of Rottnest Island, Western Australia.

Habitat: Intertidal, gravelly sand.

Distribution: Only known from type locality.

Grania vacivasa Coates & Stacey, 1993

Grania vacivasa Coates & Stacey, 1993: 400-402, figs. 5-6; Rota et al., 2007: 1018-1020, figs. 8C, 9A-E.

Type material: WAM 196-92-197-92, Western Australian Museum, Perth.

Type locality: Salmon Bay, Rottnest Island, Western Australia.

Habitat: Subtidal, 1 m in coarse sand.

Distribution: South (Esperance) and west (Rottnest Island) coasts of Western Australia.

Grania acanthochaeta Rota & Erséus, 1996

Grania acanthochaeta Rota & Erséus, 1996: 174-175, fig. 4, table 1.

Type material: USNM 172142, USNM 172193.

Other material:USNM 172194-172397, United States National Museum of Natural History, Washington, D.C.

Type locality: McMurdo Sound, south of Hut Point, Ross Island, Antarctica.

Habitat: Subtidal, 38 m, greyish brown gravelly mud with sponge spicules and *Limatula* valves.

Distribution: Only known from type locality.

Grania algida Rota & Erséus, 1996

Grania algida Rota & Erséus, 1996: 179-181, fig. 8, table 1.

Type material: USNM 172398, United States National Museum of Natural History, Washington, D.C. Oligochaeta 0065, Museo Civico di Zoologia di Roma, Rome.

Type locality: McMurdo Sound, off beach in front of Scott's Hut, northern shore of Cape Evans, Ross Island, Antarctica.

Habitat: Subtidal, 14-40 m, volcanic gravel and cobble.

Distribution: Only known from type locality.

Grania angustinasus Rota & Erséus, 1996

Grania angustinasus Rota & Erséus, 1996: 177-178, figs. 3B, 6, table 1.

Type material: MCZR Oligochaeta 0059-0063, Museo Civico di Zoologia di Roma, Rome. SMNH 4759-4761, Swedish Museum of Natural History, Stockholm.

Type locality: Terra Nova Bay, between Faraglione and Cape Russell, Ross Island, Antarctica.

Habitat: Subtidal, 35-126 m, fine sand.

Distribution: Only known from type locality.

Grania antarctica Rota & Erséus, 1996

Grania antarctica Rota & Erséus, 1996: 178-179, figs. 3C, 7, table 1.

Type material: USNM 172400-172402, United States National Museum of Natural History, Washington, D.C. MCZR Oligochaeta 0059, Museo Civico di Zoologia di Roma, Rome.

Type locality: McMurdo Sound, off beach in front of Scott's Hut, northern shore of Cape Evans, Ross Island, Antarctica.

Habitat: Subtidal, 14-31 m, volcanic gravel and cobble.

Distribution: Only known from type locality.

Grania carchinii Rota & Erséus, 1996

Grania carchinii Rota & Erséus, 1996: 175-177, fig. 5, table 1.

Type material: MCZR Oligochaeta 0057-0058, Museo Civico di Zoologia di Roma, Rome.

Type locality: Terra Nova Bay, between Faraglione and Cape Russell, Ross Island, Antarctica.

Habitat: Subtidal, 35 m, fine sand with mica shales, shell debris and some pebbles.

Distribution: Only known from type locality.

Grania hirsuticauda Rota & Erséus, 1996

Grania hirsuticauda Rota & Erséus, 1996: 175-177, fig. 5, table 1.

Type material: USNM 172136-172138, United States National Museum of Natural History, Washington, D.C.

Type locality: McMurdo Sound, near tip of Cape Armitage, Ross Island, Antarctica.

Habitat: Subtidal, 5-585 m, volcanic gravel, sandy mud, small rocks, ectoproct and sponge debris.

Distribution: Only known from type locality.

Grania darwinensis (Coates & Stacey, 1997)

Randidrilus darwinensis Coates and Stacey, 1997: 70-72, fig. 1.

Grania darwinensis; Rota et al., 2003: 504-509, fig. 3.

Type material: NTM Wo 0084-0087, Museum and Art Gallery of the Northern Territory, Darwin. ROMIZ I2457-I2458, Invertebrate Zoology, Royal Ontario Museum, Toronto.

Type locality: Darwin Harbour, Northern Territory, Australia.

Habitat: Intertidal, and subtidal to 16 m, medium to coarse sand, clay or silty sediments.

Distribution: Northern Territory and Western Australia.

Grania eurystil Coates & Stacey, 1997

Grania eurystil Coates & Stacey, 1997: 73-74, fig. 2.

Type material: NTM Wo 0081-0083, Museum and Art Gallery of the Northern Territory, Darwin. ROMIZ I2479, Invertebrate Zoology, Royal Ontario Museum, Toronto.

Type locality: Darwin Harbour, Northern Territory, Australia.

Habitat: Intertidal rock pool.

Distribution: Only known at two locations in the inner part of Darwin Harbour.

Grania integra Coates & Stacey, 1997

Grania integra Coates & Stacey, 1997: 74–76, fig. 3; Rota et al., 2003: 499-501, fig. 1.

Type material: NTM Wo 0079-0080, Museum and Art Gallery of the Northern Territory, Darwin.

Type locality: Darwin Harbour, Northern Territory, Australia.

Habitat: Intertidal crevice with sand gravel, pebbles and heterogeneous sediments.

Distribution: Darwin Harbour, Northern Territory and Nickol Bay, Dampier area, Western Australia.

Grania lasserrei Rota & Erséus, 1997

Enchytraeus monochaetus; Michaelsen, 1888: 66, figs. 6a-c; part., plate 2.

Grania lasserrei Rota & Erséus, 1997: 34-37, fig. 3, table1.

Michaelsena monochaeta; Michaelsen, 1921: 3.

Grania monochaeta; Erséus and Lasserre 1977: 299-300, figs. 1A-D.

Type material: SMNH 4803-4806, Swedish Museum of Natural History, Stockholm. MZUT 1078, Museo ed Intituto de Zoologia del' Universitá de Torino, Turin. BMNH 1996:916, Natural History Museum, London.

Type locality: East Cumberland Bay, South Georgia (S Atlantic Ocean).

Habitat: Intertidal, and subtidal to 20 m.

Distribution: Cumberland and Royal Bays, South Georgia.

Grania stephensoniana Rota & Erséus, 1997

Grania stephensoniana Rota & Erséus, 1997: 37-39, figs. 4-5, table1.

Michaelsena monochaeta; Stephenson, 1932: 263, fig. 14.

Type material: BMNH 1931.6.23.78, BMNH 1933.2.23.946-1933.2.23.948, Natural History Museum, London.

Type locality: South Georgia, South Atlantic Ocean

Habitat: 160 m in rocky bottom

Distribution: Only known from type locality.

Grania mira Locke & Coates, 1998

Grania mira Locke & Coates, 1998: 1103-1107, figs. 1-5.

Grania sp.; Healy, 1996a: 53, 56-57, fig.1, tables 1, 2; Healy, 1996b: 1287.

Type material: NMI 4.1998, NMI 5.1998 and NMI 6.1998, National Museum of Ireland (Department of Natural History), Dublin.

Type locality: Carnsore Point, County Wexford, Ireland.

Habitat: Intertidal, sediments trapped in dense turf of *Corallina officinalis* on horizontal or gently sloping rock.

Distribution: Only known from type locality.

Grania hylae Locke & Coates, 1999

Grania hylae Locke & Coates, 1999: 605-609, figs. 6-7, 11, table 1; Locke & Coates, 2000: 619-621, 626, fig. 4B.

Type material: USNM 185954-185956, National Museum of Natural History, Washington, D.C. BAMZ 199 180 007, Bermuda Aquarium, Museum and Zoo.

Type locality: Paget Island, Bermuda.

Habitat: Intertidal, and subtidal to 17 m, medium to coarse sand with rocks.

Distribution: Rocky Hill Park, Castle Island and Paget Island, Bermuda, and Fowey Rocks, Miami, Florida.

Grania laxartus **Locke & Locke & Coates, 1999**

Grania laxartus Locke & Locke & Coates, 1999: 602-605, figs. 2-6, 11, table 1; Locke & Coates, 2000: 619-621, 626-627, figs. 4C, 6A.

Type material: USNM 185951-185953, National Museum of Natural History, Washington, D.C. BAMZ 199 180 006, Bermuda Aquarium, Museum and Zoo.

Type locality: Ferry Point Bridge, Bermuda.

Habitat: Intertidal pools with accumulation of sand and fine to mediumcoarse calcareous sand.

Distribution: Ferry Point Bridge, Whalebone Bay, Pearl Island, Ferry Reach and Smith’s Sound, Bermuda, and Carrie Bow Cay, Belize.

Grania dolichura **Rota & Erséus, 2000**

Grania dolichura Rota & Erséus, 2000: 249-252, fig. 3; Rota et al., 2007: 1006-1008, figs. 4A-C.

Type material: QVM 14: 3889-14: 3889, Queen Victoria Museum, Launceston, Tasmania. SMNH 5203-5206, Swedish Museum of Natural History, Stockholm. MCZR Oligochaeta 0085-0088, Museo Civico di Zoologia di Roma, Rome.

Type locality: Little Musselroe Bay, Tasmania, Australia.

Habitat: Intertidal, silt-clay sediments.

Distribution: Widespread around Tasmania.

Grania tasmaniae **Rota & Erséus, 2000**

Grania tasmaniae Rota & Erséus, 2000: 247-249, fig. 2.

Type material: QVM 14: 3887-14: 3888, Queen Victoria Museum, Launceston, Tasmania.

Type locality: Tamar Estuary, Tasmania, Australia.

Habitat: Intertidal, silt-clay sediments.

Distribution: Only known from type locality.

Grania aquitana **Rota & Erséus, 2003**

Grania aquitana Rota & Erséus, 2003: 226-229, fig. 7, table 1.

Type material: SMNH 5729-5733, Swedish Museum of Natural History, Stockholm.

Type locality: Bassin d'Arcachon, Atlantic coast of France.

Habitat: Subtidal, 2-5 m, fine sand

Distribution: Only known from the type locality.

Grania canaria **Rota & Erséus, 2003**

Grania canaria Rota & Erséus, 2003: 213-215, fig. 1, table 1.

Type material: ZMA V.OL 9344, Zoological Museum of Amsterdam, The Netherlands, Amsterdam. SMNH 5710–5711, Swedish Museum of Natural History, Stockholm.

Type locality: Punta del Hidalgo, Tenerife, Canary Islands.

Habitat: Intertidal, sand and gravel.

Distribution: Only known from type locality.

Grania fortunata **Rota & Erséus, 2003**

Grania fortunata Rota & Erséus, 2003: 215-218, fig. 2, table 1.

Type material: SMNH 5712-5713, Swedish Museum of Natural History, Stockholm. MCZR Oligochaeta 0103-0104, Museo Civico di Zoologia di Roma.

Type locality: Ensenada de los Abades, Tenerife, Canary Islands**.**

Habitat: Subtidal, 12-17 m, fine and muddy sands associated with beds of the seagrass *Cymodocea nodosa*.

Distribution: Only known from type locality.

Grania mauretanica **Rota & Erséus, 2003**

Grania mauretanica Rota & Erséus, 2003: 224-226, fig. 6, table 1.

Type material: SMNH 5718-5720, Swedish Museum of Natural History, Stockholm. MCZR Oligochaeta 0107, Museo Civico di Zoologia di Roma, Rome.

Type locality: Off Casablanca, on the Moroccan coast.

Habitat: Subtidal, 173 m, mud with shell debris.

Distribution: Only known from type locality.

Grania papillinasus **Rota & Erséus, 2003**

Grania papillinasus Rota & Erséus, 2003: 239-240, fig. 13.

Type material: SMNH 5726-5728, Swedish Museum of Natural History, Stockholm. MCZR Oligochaeta 0124-0126, Museo Civico di Zoologia di Roma, Rome.

Type locality: Northeast Atlantic, Gulf of Gascogne, continental slope off France.

Habitat: Deep sea, 2630-2885 m, most likely very fine sediment.

Distribution: Gulf of Gascogne, off France and off the eastern USA (i.e., both sides of the North Atlantic) (Erséus & Rota, 2003).

Grania torosa **Rota & Erséus, 2003**

Grania torosa Rota & Erséus, 2003: 237-239, fig. 12.

Type material: SMNH 5721-5725, Swedish Museum of Natural History, Stockholm. MCZR Oligochaeta 0123, Museo Civico di Zoologia di Roma, Rome.

Type locality: Rockall Trough, off the coast of Scotland**.**

Habitat: Continental slope, 1170-1800 m, fine sandy and hemi-pelagic ooze.

Distribution: Northern Rockall Trough, off the coast of Scotland, to near the entrance to the English Channel (NE Atlantic Ocean).

Grania vikinga Rota & Erséus, 2003

Grania vikinga Rota & Erséus, 2003: 222-224, fig. 5.

Non *Grania roscoffensis* Lasserre, 1967.

Grania roscoffensis, part; Erséus1977: 294, table1.

Type material: SMNH 5714-5717, Swedish Museum of Natural History, Stockholm. MCZR Oligochaeta 0105-0106, Museo Civico di Zoologia di Roma, Rome.

Type locality: Skagerrak, Bohuslän, southeast of Bredholmen, in archipelago at entrance of Gullmar Fjord, west coast of Sweden.

Habitat: Subtidal, 10-18 m, sand.

Distribution: West coast of Sweden.

Grania ocarina Rota, Erséus & Wang, 2003

Grania ocarina Rota, Erséus & Wang, 2003: 502-504, fig. 2.

Type material: WAM V 4351-4352, Western Australian Museum, Perth. SMNH 5868, Swedish Museum of Natural History, Stockholm. MCZR Oligochaeta 0128, Museo Civico di Zoologia di Roma, Rome.

Type locality: Withnell Bay, Dampier Area, Burrup Peninsula, Western Australia.

Habitat: Barely subtidal, 0.5 m, medium to coarse sand.

Distribution: Only known from type locality.

Grania cinctura De Wit & Erséus, 2007

Grania cinctura De Wit & Erséus, 2007: 33-36, fig. 3, table 1.

Type material: SMNH 6559-6568, 6572, Swedish Museum of Natural History, Stockholm.

Type locality: Baie de Chateaubriand, Lifou, Loyality Islands, New Caledonia.

Habitat: Intertidal, and subtidal to 6 m, fine to coarse sand.

Distribution: Touho and Nouméa areas, and Loyalty Islands, New Caledonia.

Grania curta **De Wit & Erséus, 2007**

Grania curta De Wit & Erséus, 2007: 38-40, fig. 5, table 1.

Type material: SMNH 6583-6588,Swedish Museum of Natural History, Stockholm.

Type locality: Ejengen (off village of St. James), Lifou, Loyalty Islands, New Caledonia.

Habitat: Barely subtidal, 0.5 m, heterogeneous sand.

Distribution: Only known from type locality.

Grania fiscellata De Wit & Erséus, 2007

Grania fiscellata De Wit & Erséus, 2007: 45-47, fig. 9, table 1.

Type material: SMNH 6610-6613, 6617, Swedish Museum of Natural History, Stockholm.

Type locality: Touho area (between Tiéti and Poindimié), New Caledonia.

Habitat: Intertidal, and subtidal to 3 m, heterogeneous sand.

Distribution: Touho area, and Lifou (Loyalty Islands), New Caledonia.

Grania fustata **De Wit & Erséus, 2007**

Grania fustata De Wit & Erséus, 2007: 40-42, fig. 6, table 1.

Type material: SMNH 6589-6598, Swedish Museum of Natural History, Stockholm.

Type locality: Touho area (south end of Grand Récif Mengalia, east of Ilot Ain), New Caledonia.

Habitat: Intertidal, and barely subtidal, coarse sand and gravel.

Distribution: Only known from type locality.

Grania galbina **De Wit & Erséus, 2007**

Grania galbina De Wit & Erséus, 2007: 36-38, fig. 4, table 1.

Type material: SMNH 6573-6582, Swedish Museum of Natural History, Stockholm.

Type locality: Baie de Chateaubriand, Lifou, New Caledonia.

Habitat: Intertidal, and subtidal to 13 m, heterogeneous sand.

Distribution: Nouméa area and Lifou (Loyalty Islands), New Caledonia.

Grania novacaledonia **De Wit & Erséus, 2007**

Grania novacaledonia De Wit & Erséus, 2007: 31-33, fig. 2, table 1.

Type material: SMNH 6549-6558, Swedish Museum of Natural History, Stockholm.

Type locality: East of Baie de Touho (north side of middle of large intertidal bank north of Kombounou)**,** Touho area, New Caledonia.

Habitat: Intertidal, and subtidal to 21 m, heterogeneous sand.

Distribution: Touho and Nouméa areas, New Caledonia.

Grania papillata **De Wit & Erséus, 2007**

Grania papillata De Wit & Erséus, 2007: 42-45, figs. 7-8, table 1.

Type material: SMNH 6599-6606, 6608-6609, Swedish Museum of Natural History, Stockholm.

Type locality: South of Baie du Santal (between Jua Wekutr and Jua Wajez) Lifou, New Caledonia.

Habitat: Intertidal, and subtidal to 22 m, heterogeneous sand.

Distribution: Touho area and Lifou (Loyalty Islands), New Caledonia.

Grania quaerens **Rota, Wang & Erséus, 2007**

Grania quaerens Rota, Wang & Erséus, 2007: 1011-1013, figs. 5B-D, 6A-I.

Type material: WAM V 7315-7319, Western Australian Museum, Perth. SMNH 6803-6808, Swedish Museum of Natural History, Stockholm. MCZR Oligochaeta 0146-0149, Museo Civico di Zoologia di Roma, Rome.

Type locality: North of New Island, Cape Le Grand, Western Australia.

Habitat: Intertidal, medium to coarse sand.

Distribution: South coast of Western Australia.

Grania sperantia **Rota, Wang & Erséus, 2007**

Grania sperantia Rota, Wang & Erséus, 2007: 1014-1017, figs. 7A-H, 8A, B.

Type material: WAM V 7320-7326, Western Australian Museum, Perth. SMNH 6809-6817, Swedish Museum of Natural History, Stockholm. MCZR Oligochaeta 0150-0155, Museo Civico di Zoologia di Roma, Rome.

Type locality: Southeast of Cowerie Bay at Cheyne Point, Western Australia.

Habitat: Barely subtidal, 0.5-2 m, medium to coarse sand.

Distribution: Esperance area, South coast of Western Australia.

Grania breviductus **De Wit, Rota & Erséus, 2009**

Grania breviductus De Wit, Rota & Erséus, 2009: 19-21, figs. 2, 10A.

Type material: AMS W.35536- 35542, Australian Museum, Sydney. SMNH 7761-7766, Swedish Museum of Natural History, Stockholm.

Type locality: Heron Island, Great Barrier Reef, Queensland, Australia.

Habitat: Intertidal in coarse sand.

Distribution: Only known from type locality.

Grania regina **De Wit, Rota & Erséus, 2009**

Grania regina De Wit, Rota & Erséus, 2009: 21-23, figs. 3-4, 10B.

Type material: AMS W.35543, Australian Museum, Sydney.

Type locality: Heron Island, Great Barrier Reef, Queensland, Australia.

Habitat: Subtidal, 15 m, fine sand.

Distribution: Only known from type locality.

Grania homochaeta De Wit, Rota & Erséus, 2009

Grania homochaeta De Wit, Rota & Erséus, 2009: 23-25, figs. 5, 10C.

Type material: AMS W.35544, Australian Museum, Sydney. SMNH 7767, Swedish Museum of Natural History, Stockholm.

Type locality: Heron Island, Great Barrier Reef, Queensland, Australia.

Habitat: Subtidal, 18 m, gravelly fine sand.

Distribution: Only known from type locality.

Grania colorata De Wit, Rota & Erséus, 2009

Grania colorata De Wit, Rota & Erséus, 2009: 25-27, figs. 6-7, 10D.

Type material: AMS W.35545-35553, Australian Museum, Sydney. SMNH 7768-7772, Swedish Museum of Natural History, Stockholm.

Type locality: Heron Island, Great Barrier Reef, Queensland, Australia.

Habitat: Subtidal, 7 m in heterogeneous sand.

Distribution: Only known from type locality.

Grania brasiliensis Prantoni, De Wit & Erséus, 2016

Grania brasiliensis Prantoni, De Wit & Erséus, 2015: fig. 1.

Type material: ZUEC CLI 04-07, Museu de Zoologia da Universidade Estadual de Campinas, Campinas, Brazil.

Type locality: Paranaguá Bay, Southern coast of Brazil.

Habitat: Intertidal, and subtidal to 7 m, medium to coarse sand with some mud, and lots of shell and cirriped fragments.

Distribution: Coast of Paraná and São Paulo States, Brazil.

Grania bekkouchei Prantoni, De Wit & Erséus, 2016

Grania bekkouchei Prantoni, De Wit & Erséus, 2015: fig. 2.

Type material: SAMC A82466, Iziko South African Museum, Cape Town.

Type locality: Saldanha Bay, West Coast district, Province of the Western Cape, South Africa.

Habitat: Intertidal, coarse sand in rock crevice**.**

Distribution: Only known from type locality.

Grania cryptica Prantoni, De Wit & Erséus 2016

Grania cryptica Prantoni, De Wit & Erséus, 2015: fig. 3.

Type material: SAMC A82473, Iziko South African Museum, Cape Town.

Type locality: Glencairn Heights, False Bay, City of Cape Town, Province of the Western Cape, South Africa.

Habitat: Lower intertidal, rock pool.

Distribution: Only known from type locality.

Grania capensis Prantoni, De Wit & Erséus, 2016

Grania capensis Prantoni, De Wit & Erséus, 2015: fig. 5.

Type material: SAMC A82474- A82475, Iziko South African Museum, Cape Town.

Type locality: Glencairn Heights, False Bay, City of Cape Town, Province of the Western Cape, South Africa.

Habitat: Lower intertidal, rock pool.

Distribution: Only known from type locality.

Grania simonae Prantoni, De Wit & Erséus, 2016

Grania simonae Prantoni, De Wit & Erséus, 2015: fig. 6.

Type material: SAMC A82476-A82482, Iziko South African Museum, Cape Town.

Type locality: Van Dyks Bay, Overberg District, Overstrand Local Municipality, Province of the Western Cape, South Africa.

Habitat: Intertidal, crevice between rocks.

Distribution: Only known from type locality.

Grania hinojosai Prantoni, De Wit & Erséus, 2016

Grania hinojosai Prantoni, De Wit & Erséus, 2015: fig. 7.

Grania sp. Chile 1; De Wit et al., 2011: 513.

Type material: ZUEC CLI 08-CLI 12, Museu de Zoologia da Universidade Estadual de Campinas, Campinas, Brazil..

Type locality: Puerto Aldea, Coquimbo, Elqui, Chile.

Habitat: Intertidal, sand among rocks.

Distribution: Puerto Aldea to Pampilla Point, Coquimbo, Elqui, Chile.

Grania chilensis Prantoni, De Wit & Erséus, 2016

Grania chilensis Prantoni, De Wit & Erséus, 2015: fig. 8.

Grania sp. Chile 2; De Wit et al., 2011: 513, 517.

Type material: ZUEC CLI 13-CLI 19, Museu de Zoologia da Universidade Estadual de Campinas, Campinas, Brazil.

Type locality: Punta Loncoyen, Valdivia, Chile.

Habitat: Intertidal, sand among rocks and heterogeneous sand with organic material.

Distribution: Punta Loncoyen, Valdivia, Puerto Aldea, Coquimbo, Caleta Tumbes, Concepción, Chile.

Grania unitheca Prantoni, De Wit & Erséus, 2016

Grania unitheca Prantoni, De Wit & Erséus, 2015: fig. 9.

Type material: USNM 1283175, National Museum of Natural History, Washington, D.C.

Type locality: NE of Morehead City, off North Carolina**,** USA**.**

Habitat: Subtidal, 17 m, sand.
Distribution: Only known from type locality.

***Grania carolinensis* Prantoni, De Wit & Erséus, 2016**

Grania carolinensis Prantoni, De Wit & Erséus, 2015: fig. 10.
Type material: USNM 1283174, National Museum of Natural History, Washington, D.C.
Type locality: Off North Carolina**,** USA**.**
Habitat: Continental shelf slope, 492 m, sand.
Distribution: Only known from type locality.

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References

Beddard, F.E. (1895) *A Monograph of the Order Oligochaeta*. Clarendon Press, Oxford, 769 pp.

Bonomi, G. & Erséus, C. (1984) A taxonomic and faunistic survey of the marine Tubificidae and Enchytraeidae (Oligochaeta) of Italy. Introduction and preliminary results. *Hydrobiologia*, 115, 207–210.

Coates, K.A. (1984) Specific criteria in *Grania* (Oligochaeta, Enchytraeidae). *Hydrobiologia*, 115, 45–50.

Coates, K.A. (1990) Marine Enchytraeidae (Oligochaeta, Annelida) of the Albany area, Western Australia. Pages 13–41 in F. Wells, D. Walker, H. Kirkman, and R. Lethbridge, eds. *Proceedings of the Third International Marine Biological Workshop: The Marine Flora and Fauna of Albany, Western Australia*. Western Australian Museum, Perth.

Coates, K.A. & Erséus, C. (1980) Two species of *Grania* (Oligochaeta, Enchytraeidae) from the Pacific Coast of North America. *Canadian Journal of Zoology*, 58, 1037–1041.

Coates, K.A. & Ellis, D. V. (1981) Taxonomy and distribution of marine Enchytraeidae (Oligochaeta) in British Columbia. *Canadian Journal of Zoology*, 59, 2129–2150.

Coates, K.A. & Erséus, C. (1985) Marine enchytraeids (Oligochaeta) of the Coastal Northwest Atlantic (Northern and Mid U.S.A.). *Zoologica Scripta*, 14, 103–116.

Coates, K.A. & Stacey, D. (1997) Enchytraeids (Oligochaeta: Annelida) of the lower shore and shallow subtidal of Darwin Harbour, Northern Territory, Australia. Pages 67–79 in J. Hanley, G. Caswell, D. Megirian, and H. Larson, eds. *Proceedings of the Sixth International Marine Biological Workshop: The Marine Flora and Fauna of Darwin Harbour, Northern Territory, Australia*. Museums and Art Galleries of the Northern Territory and the Australian Marine Sciences Association: Darwin, Australia.

Coates, K.A. & Stacey, D. (1993) The marine Enchytraeidae (Oligochaeta, Annelida) of Rottnest Island, Western Australia. Pages 391–414 in F. Wells, D. Walker, H. Kirkman, and R. Lethbridge, eds. *Proceedings of the Fifth International Marine Biological Workshop: The Marine Flora and Fauna of Rottnest Island, Western Australia*. Western Australian Museum, Perth.

De Wit, P. (2006) *Grania maricola. Fauna & Flora*, 101(2), 25.

De Wit, P. (2010) *Systematics of Grania (Clitellata: Enchytraeidae), an interstitial annelid taxon*. PhD thesis, University of Gothenburg, Gothenburg.

De Wit, P. & Erséus, C. (2007) Seven new species of *Grania* (Annelida:

Clitellata: Enchytraeidae) from New Caledonia, South Pacific Ocean. *Zootaxa*, 1426, 27–50.

De Wit, P., Rota, E & Erséus C. (2009) *Grania* (Annelida: Clitellata: Enchytraeidae) of the Great Barrier Reef, Australia, including four new species and a re-description of *Grania trichaeta* Jamieson, 1977. *Zootaxa*, 38, 16–38.

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.

Diaz, R. J., Erséus, C. & Boesch, D. F. (1987) Distribution and ecology of Middle Atlantic Bight Oligochaeta. *Hydrobiologia*, 155, 215–225.

Eisen, G. (1904) *Enchytraeidae of the west coast of North America. Harriman Alaska Expedition, Alaska XII, 1904.* New York, pp. 1-166.

Erséus, C. (1974) *Grania pusilla* sp.n. (Oligochaeta, Enchytraeidae) from the west coasts of Norway and Sweden with some taxonomic notes on the genus *Grania.Sarsia*, 56, 87–94.

Erséus, C. (1976) Marine subtidal Tubificidae and Enchytraeidae (Oligochaeta) of the Bergen area, western Norway. *Sarsia*, 62, 25–48.

Erséus, C. (1977) Marine Oligochaeta from the Koster area, west coast of Sweden, with descriptions of two new enchytraeid species. *Zoologica Scripta*, 6, 293–298.

Erséus, C. (1980) A new species of Grania (Oligochaeta, Enchytraeidae) from Ascension Island, South Atlantic. *Sarsia*, 65, 27–28.

Erséus, C. (1990) Marine Oligochaeta of Hong Kong. Pages 259–335 in B. Morton, ed. *Proceedings of the Second International Marine Biological Workshop: The Marine Flora and Fauna of Hong Kong and Southern China*. Hong Kong University Press, Hong Kong.

Erséus, C. & Lasserre, P. (1976) Taxonomic status and geographic variation of the marine enchytraeid genus *Grania* Southern (Oligochaeta). *Zoologica Scripta*, 5, 121–132.

Erséus, C. & Lasserre, P. (1977) Redescription of *Grania monochaeta* (Michaelsen), a Marine Enchytraeid (Oligochaeta) from South Georgia (SW Atlantic), *Zoologica Scripta*, 6, 299–300.

Erséus, C. & Rota, E. (2003). New findings and an overview of the oligochaetous Clitellata (Annelida) of the North Atlantic deep sea. *Proceedings of the Biological Society of Washington*, 116(4), 892–900.

Erséus, C., Rota, E., Matamoros, L., & De Wit, P. (2010) Molecular phylogeny of Enchytraeidae (Annelida, Clitellata). *Molecular Phylogenetics and*

Evolution, 57(2), 849–858.

Healy, B. (1996a) The distribution of Oligochaeta on an exposed rocky shore in southeast Ireland, *Hydrobiologia*, 334, 51–62.

Healy, B. (1996b) New species of *Marionina* (Oligochaeta: Enchytraeidae) from a wave-exposed rocky shore in SE Ireland. *Journal of Natural History*, 30, 1287–1295.

Healy, B. & Coates, K.A. (1999) Finding enchytraeid oligochaetes (Clitellata) in hot climates: species occurrence on the shores of Bermuda. *Hydrobiologia*, 406, 111–117.

Henle, F. (1837) Über *Enchytraeus*, eine neue Anneliden-Gattung. *Archiv für Anatomie, Physiologie und Wissenschaftliche Medicin*, 1837, 74–90.

Jamieson, B.G.M. (1977) Marine meiobenthic Oligochaeta from Heron and Wistari Reefs (Great Barrier Reef) of the genera *Clitellio, Limnodriloides* and *Phallodrilus* (Tubificidae) and *Grania* (Enchytraeidae). *Zoological Journal of the Linnean Society*, 61, 329–349.

Kennedy, C. (1966) A taxonomic revision of the genus *Grania* (Oligochaeta: Enchytraeidae). *Journal of Zoology*, 148, 399–407.

Knöllner, F.H. (1935) Ökologische und systematische Untersuchungen über litorale und marine Oligochäten der Kieler Bucht. *Zoologische Jahrbücher (Systematik)*, 66, 425–512.

Kossmagk-Stephan K.J. (1983) Marine Oligochaeta from a sandy beach of the Island of Sylt (North Sea) with description of four new enchytraeid species. *Mikrofauna des Meeresbodens*, 89, 593–618.

Kossmagk-Stephan K.J. (1985) *Systematik, Faunistik und Lebenszyklus mariner Oligochaeta der Nord- und Ostseeküste.* Dissertation, Georg-August-Universität, Göttingen.

Lasserre, P. (1966) Oligochétes marins des côtes de France. I. Bassin d'Arcachon: Systématique. *Cahiers de Biologie Marine*, 7, 295–317.

Lasserre, P. (1967) Oligochétes marins des côtes de France. II. Roscoff, Penpoull, étangs saumâtres de Concarneau: Systématique, Écologie. *Cahiers de Biologie Marine*, 8, 273–293.

Lasserre, P. (1971) The Marine Enchytraeidae (Annelida, Oligochaeta) of the eastern coast of North America with notes on their geographical distribution and habitat. *Biological Bulletin*, 140, 440–460.

Locke, J.M. & Coates, K.A. (1998) A new species of *Grania* (Enchytraeidae, Clitellata, Annelida) and redescription of *Grania pusilla* from a rocky shore in SE Ireland. *Journal of Natural History*, 32, 1101–1114.

Locke, J.M. & Coates, K.A. (1999) Redescriptions of *Grania americana, G.*

bermudensis and descriptions of two new species of *Grania* (Annelida: Clitellata: Enchytraeidae) from Bermuda. *Proceedings of the Biological Society of Washington*, 112, 598–623.

Locke, J.M. & Coates, K. A. (2000) An illustrated key to the species of *Grania* and *Randidrilus* (Annelida: Clitellata: Enchytraeidae) of eastern North America, Bermuda, and the Caribbean area. *Proceedings of the Biological Society of Washington*, 113, 617–632.

Michaelsen W. (1888) Die Oligochaeten von Süd-Georgien nach Ausbeute der deutschen Station von 1882–83. *Jahrbuch der Hamburgischen wissenschaftliche Anstalten,* 5, 53–73.

Michaelsen W. (1900) Oligochaeta. *Das Tierreich*, 10, 1–575.

Michaelsen W. (1907) Oligochaeta. Die Fauna Südwest-Australiens. Pages117–232 In Michaelsen W, Hartmeyer R, editors. *Ergebnisse der Hamburger südwest-australischen Forschungsreise*. Jena: Fischer.

Michaelsen W. (1921) Neue und wenig bekannte Oligochäten aus skandinavischen Sammlungen. *Arkiv for Zoologi,* 13, I-25.

Nielsen, C. O. & Christensen, B. (1959) The Enchytraeidae - critical revision and taxonomy of European species*. Naturhistorisk Museum*, 8–9, 1–160.

Pierantoni, U. (1901) Sopra una nuova specie di oligochete marino (*Enchytraeus macrochaetus* n. sp.). *Monitore Zoologico Italiano*, 12, 201–202.

Pierantoni, U. (1903) Studii anatomici su *Michaelsena macrochaeta* Pierant. *Mitteilungen aus dem Zoologischen Station zu Neapel*, 16, 409–444.

Pierantoni, U. (1915) Per l'ídentità di *Grania maricola* Southern con *Michaelsena macrochaeta* Pierant. *Bollettino Della Società Dei Naturalisti in Napoli*, 27, 1–10.

Prantoni, A.L., 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.

Rodriguez, P. (1986) Nuevos resultados acerca de la fauna de Oligoquetos acuáticoas del País Vasco y cuenca altal del Ebro II. Enchytraeidae. *Munibe Ciencias Naturales Natur Zientziak*, 38, 81–87.

Rota, E. (1995) Italian Enchytraeidae (Oligochaeta). I. *Bolletino Di Zoologia*, 62(2), 183–231.

Rota, E. & Erséus, C. (1996) Six new species of *Grania* (Oligochaeta, Enchytraeidae) from the Ross Sea, Antarctica. *Antarctic Science*, 2, 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. & Erséus, C. (2000) Two new and peculiar species of *Grania* (Annelida: Clitellata: Enchytraeidae) inhabiting Tasmanian estuaries. *New Zealand Journal of Zoology*, 27, 245–254.

Rota, E. & Erséus, C. (2003) New records of *Grania* (Clitellata, Enchytraeidae) in the Northeast Atlantic (from Tromso to the Canary Islands), with descriptions of seven new species. *Sarsia*, 88, 210–243.

Rota, E., Erséus, C. & Wang, H. (2003) *Grania ocarina* sp.n., *G. darwinensis* (Coates and Stacey) comb.n., and other marine Enchytraeidae (Oligochaeta) from the Dampier area, Western Australia. Pages 497–511 in D. Walker and D. Jones, eds. *The Marine Flora and Fauna of Dampier, Western Australia*. Western Australian Museum, Perth.

Rota, E., Wang, H. & Erséus, C. (2007) The diverse *Grania* fauna (Clitellata: Enchytraeidae) of the Esperance area, Western Australia, with descriptions of two new species. *Journal of Natural History*, 41, 999–1023.

Shurova, N.M. (1979) Enchitreidy (Oligochaeta) dal'nevostocnych morej SSSR (Enchytraeids of the far eastern seas of the USSR*) Inissledovannja pelagiceskich i donnych organirmov dal'nevostocnych morej. Vladivostok*, 75–90.

Southern, R. (1913) Part 48: Oligochaeta. Pages 1–14. *Clare Island Survey*. Proceedings of the Royal Irish Academy (B).

Stephenson, J. (1930) *The Oligochaeta.* Clarendon Press, Oxford, 978 pp.

Stephenson J. (1932) Oligochaeta. Part I. Microdrili. *Discovery Reports,* 4, 233–264.

Ude, H. (1896) Enchytraeiden. *Ergebnisse der Hamburger Magelhaensischen Sammelreise*, 1, 1-43.

CAPÍTULO 5

Genetic diversity marine oligochaetes from the Southern Atlantic (Annelida: Clitellata) as revealed by DNA-barcoding

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Abstract

The marine oligochaetes are poorly investigated in the South Atlantic, especially in the east coast of South America. These animals are generally very similar to each other, or even indistinguishable. The lack of specialists and modern identification guides has been pointed out as the main reason for the scarcity of studies in this geographical region. To increase the knowledge of this group in South Atlantic, the monophyly of 80 specimens from Brazil and Antarctica was statistically tested by combining the Bayesian Inference (BI), Maximum Likelihood (ML), the Automatic Barcode Gap Discovery (ABGD) and the generalized mixed Yule coalescent (GMYC) approaches. A total of 80 COI sequences, with about 658 bp representing 32 distinct potential species were obtained. The ABGD established a barcoding gap between 3% and 14% uncorrected *p* distances. GYMC was concordant with ABGD, and only two cases of discrepancy were detected. Then, with both methods the clusters obtained are separates by more than 10% of genetic divergence threshold in COI sequences. All these species could be associated with previously known species or genera. This study confirms the usefulness of the COI barcoding approach combined with an intraspecific genetic divergence threshold at about 10% and was able to recognize all potential species analyzed.

Keywords: Marine oligochaetes; species delimitation; Southern Atlantic; DNA-barcode

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Introduction

The Clitellata Michaelsen, 1919 is a large monophyletic taxon, primarily distinguished from other annelids by the presence of a glandular, ring- or saddle-shaped structure developed around a specific part of the body called the clitellum. This structure is a part of the reproductive system, and it secretes a cocoon, into which the eggs are laid (Erséus, 2005; Martin *et al*., 2007).

Traditionally, Clitellata has been classified into Oligochaeta (earthworms, sludge worms) and Hirudinea (leeches). However, molecular data supports Clitellata as a synonym of Oligochaeta, which includes not only Hirudinea, but also Branchiobdellida (epizoans on crayfish) and Acanthobdellida (parasitizing fish), i.e., two taxa traditionally classified near Oligochaeta (Rousset *et al*., 2007, 2008; Marotta *et al*., 2008; Struck *et al*., 2011; Weigert *et al*., 2014; Struck *et al*., 2015). In this paper we consider oligochaetes in the old sense (i.e., clitellates except Hirudinea, Branchiobdellida and Acanthobdellida).

Historically, oligochaetes have been divided into two groups related to body size and habitat preference. Benham (1890) named these groups “Microdrili”, i.e., the smaller worms generally associated with the aquatic environment, and “Megadrili”, i.e., the larger forms most of which are found in terrestrial habitats (Erséus, 2005). The oligochaete worms have colonized a large variety of environments, from coastal regions such as estuaries and mangroves (Erséus, 2002a, 2005) to extreme habitats such as hadal trenches (Rota and Erséus, 2003; Erséus and Rota 2003) and glaciers (Hartzell *et al*., 2005; Erséus *et al*., 2010).

Aquatic oligochaetes are usually small, from 1 mm to a few centimeters long, and currently comprise about 1700 valid species, of which about one third are marine and/or estuarine (Erséus, 2005; Martin *et al*., 2007; Prantoni *et al*., 2013). Among the marine taxa, Naididae sensu Erséus *et al*. (2008) is the most speciose and diverse family, with about 450 species (Martin *et al*., 2007; Prantoni *et al*., 2013). Other groups with marine representatives are Enchytraeidae and the monogeneric families Capilloventridae Harman and Loden, 1984 and Randiellidae Erséus and Strehlow, 1986.

Most of the knowledge on marine oligochaetes is concentrated in the North Atlantic (Beddard, 1889; Pierantoni, 1901, 1903ab; Moore, 1902, 1905; Southern, 1913; Knöllner, 1935; Backlund, 1948; Brinkhurst, 1963; Lasserre, 1967; Cook, 1969; Erséus, 1974, 1975, 1976ab, 1977ab, 1978, 1979abcd, 1981a, 1982abc; 1983a, 1984, 1988, 1989ab; Healy, 1975, 1979; Erséus and Lasserre, 1976; Giere, 1979; Righi and Kanner, 1979; Kossmagk-Stephan, 1983; Milligan, 1991; Locke and Coates, 1998; Rota and Erséus, 2003; De Wit and Erséus, 2010) and some Indo-Pacific regions (Jamieson, 1968,1977; Coates, 1990; Erséus, 1981b, 1983b, 1989cd, 1990, 1999; Coates and Stacey, 1993, 1997; Rota and Erséus, 2000; Wang and Erséus, 2003; De Wit and Erséus, 2007; Rota, *et al*., 2007; De Wit *et al*., 2009).

With the exception of some Antarctic and sub-Antarctic areas (Michaelsen, 1888, Erséus and Lasserre, 1977; Rota and Erséus, 1996, 1997), scattered sites along the east coast of South America (Du Bois-Reymond Marcus 1950; Marcus 1965, Righi 1968; Erséus 1980, 1983c; Harman and Loden, 1984; Prantoni *et al*., 2013, Prantoni *et al*., 2016) and the west coast of South Africa (Prantoni *et al*., 2016), there is scant knowledge about the taxonomy and biogeography of oligochaetes from the South Atlantic Ocean.

Marine oligochaetes have often been neglected in ecological studies. They are rarely identified to species, genus or even to family, mainly due to taxonomic difficulties, the lack of specialists and modern identification keys (Martin *et al*., 2007; Prantoni *et al*., 2013). Furthermore, many marine oligochaetes are similar to each other, sometimes even indistinguishable (cryptic diversity) (De Wit and Erséus, 2010; Matamoros *et al*., 2012; Prantoni *et al*., 2016). As a result, the whole group has just been reported as “Oligochaeta" in various studies (Negrello-Filho *et al*., 2006; Martin *et al*., 2007; Souza, *et. al*., 2013; Sandrini-Neto and Lana, 2014; Morais *et al*., 2016). In this context, the DNA barcoding approach is a useful and cost-effective framework to resolve the taxonomic problems and also for delineating new species (Hebert *et al*., 2003; Achurra and Erséus, 2013; Martinsson *et al*., 2015; Prantoni *et al*., 2016). The delimitation of putative species based on DNA barcode data also increases objectivity, accelerate and improve taxonomy workflow and, in many cases, can decrease the risk of synonymy by exposing cryptic lineages (De Wit and Erséus, 2010; Kekkonen *et al*., 2014; Prantoni *et al*., 2016).

The cytochrome c oxidase subunit I (COI) is the most common marker used for molecular identification of species in various studies. This gene is considered useful for a preliminary and exploratory hypothesis of the species boundaries and has been proposed as a standard "DNA barcode" for the identification of most animal taxa (Herbert *et al*., 2003; Puillandre *et al*., 2011 Taylor and Harris, 2012). The identification is made by the expected gap between intraspecific and interspecific diversity, the so-called "barcoding gap", and it is performed by comparing the sequence of an unknown animal with previously established sequence libraries in databases (Herbert *et al*., 2003; Puillandre *et al*., 2011; Taylor and Harris, 2012).

Herein we use Bayesian inference and Maximum Likelihood methods in order to investigate the genetic diversity and statistically test the monophyly of 80 specimens of marine and estuarine oligochaetes from the South Atlantic Ocean, using DNA-barcoding. We also provide the first records of the genera *Ainudrilus*, *Limnodriloides*, *Thalassodrilides*, *Doliodrilus*, *Macquaridrilus*, and *Stephensoniella* from along the eastern coast of South America.

Material and Methods

Taxon sampling and collection of specimens

The specimens from the southeastern coast of South America (Brazil) were collected from October 2012 to April 2015 by the first author and Nálita Scamparle. South African species were collected by Nicholas Bekkouche in December 2011, and Antarctic species were collected by Karla Paresque in January 2015 during the XXXIII Brazilian Antarctic Expedition (see Appendix 1 for detailed site description).

South African sediment samples were collected by hand, stirred with seawater, followed by the decantation of suspension into a 0.25-mm mesh. The specimens from South America were sampled by taking sediment cores with PVC pipes of 10 cm in length and 10 cm in diameter, stored in a plastic bucket with seawater, and relaxed with isotonic $MgCl_2$. The supernatant containing numbed animals was washed with clean seawater in a 0.063-mm sieve and transferred into a Petri dish. The samples of Antarctic specimens were taken from algae by scraping off the rocky intertidal substratum and transferred to a plastic bag with seawater for lab work.

All worms were sorted alive using a stereomicroscope and, with the exception of *Grania brasiliensis* Prantoni, De Wit and Erséus, 2016, preserved in 80% ethanol. Posterior or middle sections of each worm were cut, removed and stored in 95% ethanol for subsequent DNA extraction and sequencing. *Grania brasiliensis* was killed and stored in 99% ethanol, and then its posterior end was cut for DNA analysis.

In this study, the material was used only for molecular purposes, but the anterior ends (vouchers) of all specimens were stained in alcoholic paracarmine solution and mounted whole in Canada balsam on slides, following Erséus (1994), for future morphological analysis and description of the possible new species. The Linnean taxonomic allocations of most specimens are, therefore, preliminary and based either on BLAST searches in public databases (NCBI/GenBank and Barcoding of Life Database/BOLD), or on matches with unpublished barcodes in the database accumulated by the last author (CE).

Extraction, gene amplification and sequencing

DNA of all 80 specimens was extracted using the DNeasy Blood & Tissue® kit (Qiagen), or the NucleoSpin® Tissue kit (Macherey-Nagel), following the protocol supplied by the manufacturers. Polymerase chain reactions (PCRs) were performed for the mitochondrial cytochrome *c* oxidase subunit I (COI) locus, using the universal ‘barcoding primers’ LCO1490/HCO2198 and reaction conditions described in Folmer *et al*. (1994). The amplicons were sequenced at Macrogen Inc. (Seoul, South Korea), bi-directionally to verify accuracy, with the consensus sequences being obtained using the software Staden (Staden *et al*., 2003).

Phylogenetic analyses

The obtained sequences were aligned in the software Mafft v. 7.2 (Katoh and Standley, 2013), with the best fit DNA substitution model and partitioning scheme being selected with the software PartitionFinder v. 1.1 (Lanfear *et al*. 2012), using the Bayesian Information Criterion (Schwarz, 1978), as a simulation study (see Luo *et al*., 2010) suggests that it can outperform the Akaike Information Criterion (Akaike, 1974). The partitioning scheme selected by PartitionFinder was by codon, with the model selected for the Bayesian Inference (BI) being the HKY+G for the first position, the GTR+G+I for the second position, and GTR+G for the third position, while the maximum likelihood (ML) analysis was also partitioned by codon, but with the GTR+G model for all partitions. For both methods we rooted the tree with a GenBank COI sequence of *Capilloventer australis* Erséus, 1993. The ML phylogeny was estimated using RAxML v. 8.2.4 (Stamatakis, 2014), a GTR+G DNA substitution model, and 1,000 bootstrap replicates.

Tree inference by BI methods was performed in MrBayes 3.2.6. (Ronquist *et al*., 2012) using a birth-death clocklike prior to generate an ultrametric tree that could be used in species delimitation analyses. The Bayesian analyses were performed with four replicates, each with four chains, for 5×10^6 generations with sampling each 1,000, and a temperature of 0.05 for chain swap. The results were checked for convergence using Potential Scale Reduction Factor (Gelman and Rubin, 1992), estimates of effective sample size (ESS), and AWTY (Wilgenbusch *et al*. 2004, Nylander *et al*. 2008). After discarding the burn-in of 33%, the trees from all replicates were combined and used to obtain a majority-rule consensus tree. The same set of trees were also used to generate a fully bifurcating ultrametric tree using TreeAnnotator v. 1.8.1 (Drummond and Rambault, 2013). All phylogenetic analyses were performed at the Cipres server (Miller *et al*. 2010).

Species delimitation analyses

DNA barcoding works for species identification if the intraspecific diversity is lower than the interspecific one (i.e., the “barcoding gap”), and if there is a reference database of known barcodes for most of the taxa found. However, it can also be used as an exploratory tool for unstudied groups, with groups predicted from the barcodes representing a set of species hypothesis (Puillandre *et al*. 2012). To evaluate the performance of DNA barcoding gaps in the studied Oligochaeta, we calculated an uncorrected pair-wise DNA distance matrix in the software MEGA v.7 (Tamura *et al*., 2013). This matrix was used as input to perform an automated analysis of DNA barcoding gaps in the software Automatic Barcode Gap Discovery (ABGD) (Puillandre *et al*. 2012). For this analysis, we used a *P* (maximum prior intraspecific distance) range between 0.001-0.1, a relative gap width of 1.0, and 30 recursive steps.

The fully bifurcating ultrametric tree obtained by the BI was used to analyze the data under the generalized mixed Yule coalescent (GMYC) approach to species delimitation (Fujisawa and Barraclough, 2013). This method was presented for the first time in Pons *et al*. (2006) and Fontaneto *et al*. (2007), being formally described by Fujisawa and Barraclough (2013). This single loci method tries to locate independent evolution (mutations that not spread rapidly in other species) along the tree branches (Templeton 1989; De Queiroz 2007, Barraclough *et al*. 2009). The method uses a maximum likelihood model of branching between and within species on a gene tree, combining the Yule diversification model and an intraspecific genealogy based on a neutral coalescent model (Fujisawa and Barraclough 2013). The analyses by this method were performed in R v. 3.2.3 (R Development Core Team, 2015) with the package 'splits' (Fujisawa and Barraclough 2013), using the single threshold method.

Results

Phylogenetic analyses

We obtained a total of 80 COI sequences, representing 32 potentially distinct species from two families of marine oligochaetes (see species delimitation section below). Sequences were about 658 bp, with the partitioning scheme selected by the PartitionFinder analyses being by codon position (three partitions).

The Bayesian inference (BI) analysis of the COI sequences divided the southern Atlantic oligochaetes into two main clades (A and B) (Fig. 1). Clade

A is a monophyletic group containing all species of family Naididae with maximum support. This clade is further subdivided into Limnodriloidinae with *Ainudrilus* sp.1, sp. 2, sp. 3, sp. 4, *Aktedrilus* sp. 1, sp. 2, *Macquaridrilus* sp.1, Naididae sp.1 and *Paranais frici* placed as a sister to this subfamily.

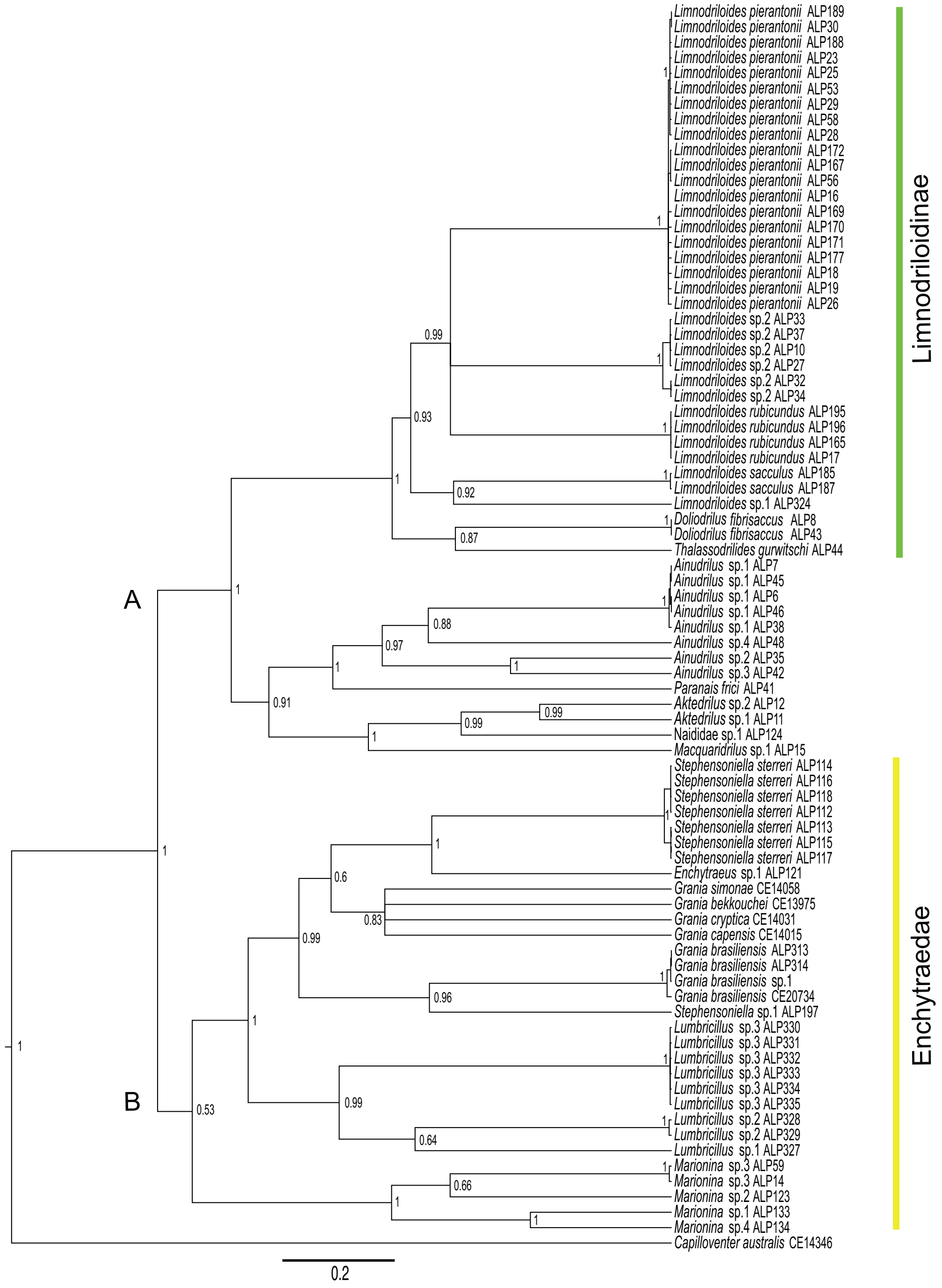

Fig1. Bayesian Inference majority-rule consensus tree generated in MrBayes with the obtained oligochaetes COI sequences. Values of nodal support are posterior probabilities.

Clade B is formed by all species of Enchytraeidae, but with weak support. The analysis does not support the monophyly of *Grania* and *Stephensoniella*. *Grania bekkouchei* Prantoni, De Wit & Erséus, 2016, *G. capensis* Prantoni, De Wit & Erséus, 2016, *G. cryptica* Prantoni, De Wit & Erséus, 2016, and *G. simonae* Prantoni, De Wit & Erséus, 2016, all from South Africa, cluster together.

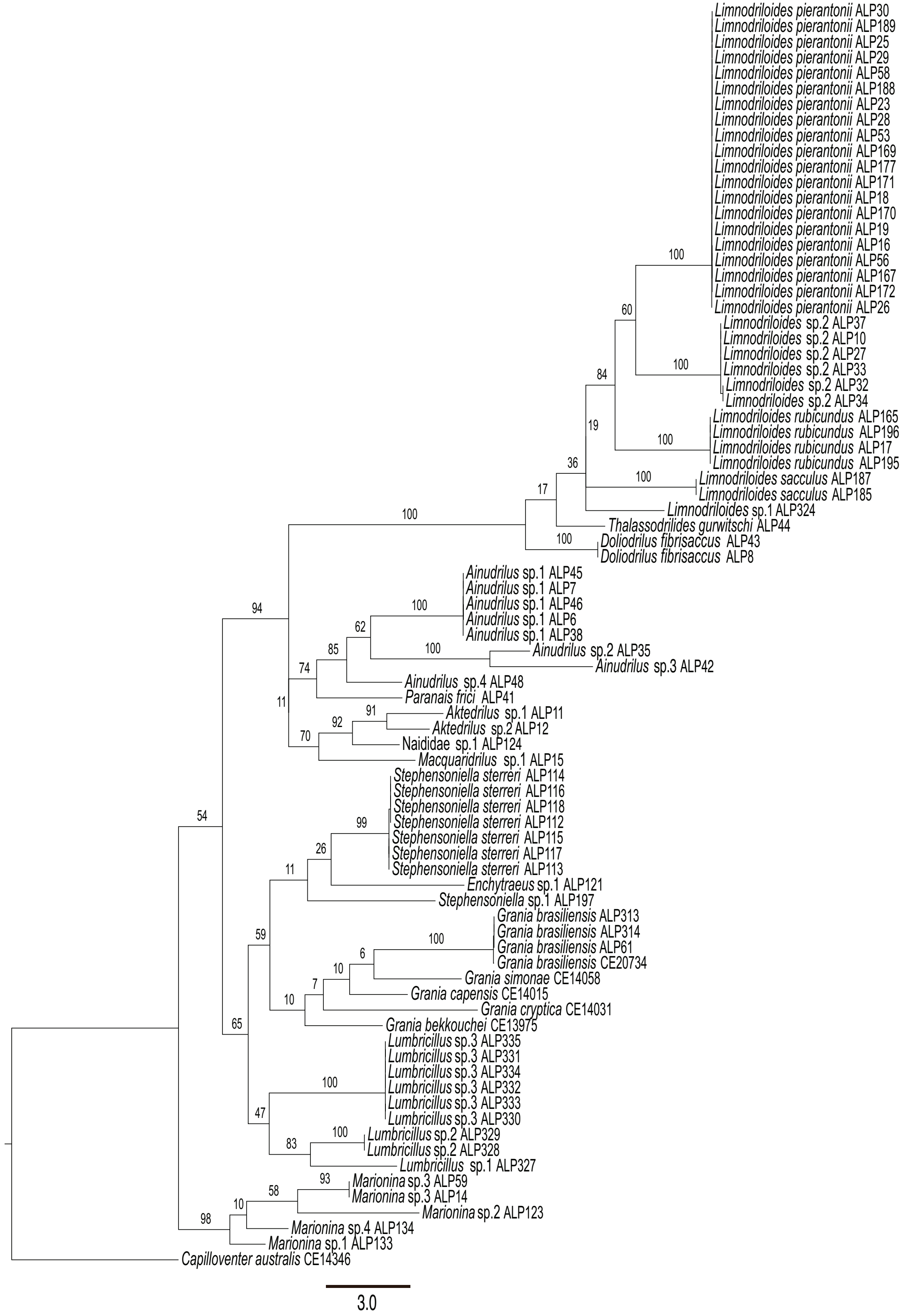

Fig 2. Maximum likelihood majority-rule consensus tree inferred by RAxML for the sampled oligochaetes COI sequences. Values of nodal support were obtained by 1,000 bootstrap replicates.

By contrast, *G. brasiliensis* comes out as a sister taxon of this four-taxon group, and *Stephensoniella* sp. 1 as a sister species of *G. brasiliensis.*

Monophyly of the Antarctic *Lumbricillus* spp., the “achaetous” species of *Marionina* (i.e., those completely lacking chaetae), Limnodriloidinae, and *Ainudrilus*, respectively, is strongly supported (Fig 1).

The ML-estimate tree also corroborates the monophyly of Limnodriloidinae*,* and *Ainudrilus* (Fig. 2). However, it shows a lower support for certain nodes, and does not support the basal topology (A and B) in Fig. 1. The analysis also does not support monophyly of *Grania*, *Stephensoniella*, and *Lumbricillus*. The achaetous *Marionina* spp. come out as a monophyletic group forming a basal branch with high support (Fig. 2).

Species delimitation

The Automatic Barcode Gap Discovery (ABGD) analyses established a barcoding gap between 3% and 14% uncorrected *p* distances (Fig. 3). The recursive iterations recovered 43 groups with P = 0.001, 34 groups with P = 0.01, and 32 groups with P = 0.1 (Tab.1).

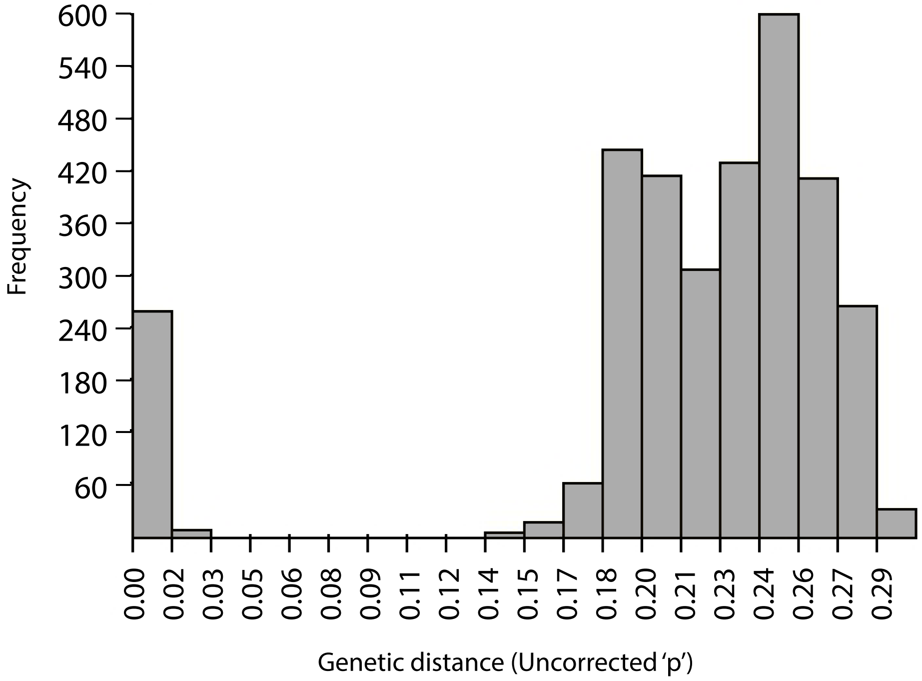

Fig 3. Distribution of the pairwise uncorrected *p* genetic distances generated by the ABGD analysis of the COI sequences of the sampled oligochaetes.

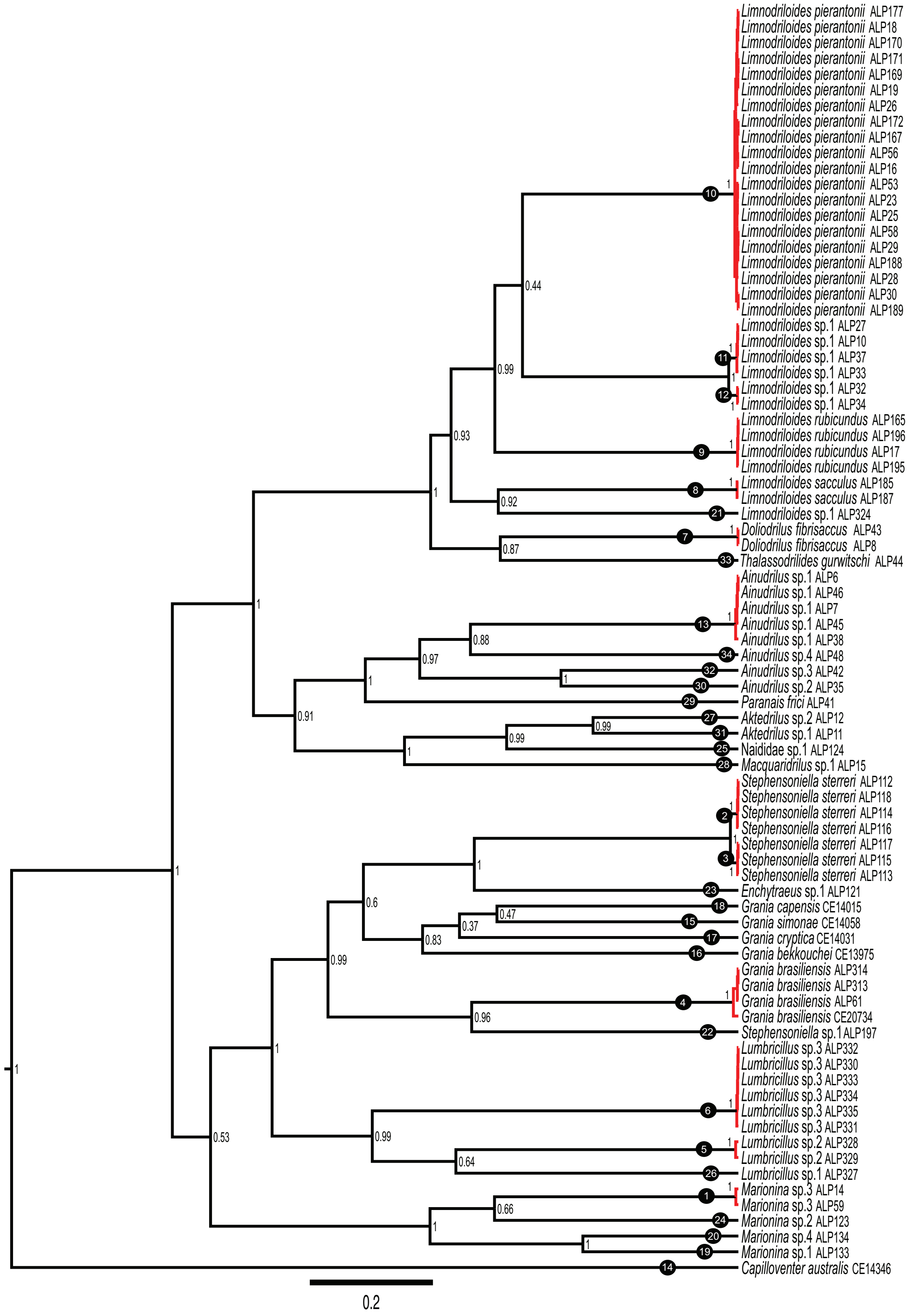

Fig 4. Results of the Generalized Mixed Yule Coalescent (GMYC) analysis over a strictly bifurcating ultrametric tree obtained in TreeAnotator using the same set of Bayesian Inference trees used for the consensus tree of Fig.1. The maximum likelihood entities (MLE) (or operational taxonomic units) defined in the analysis are indicated by the numbers in the black circles (same as in Table XX). MLEs with multiple individuals are indicated in red. Support values are nodal posterior probabilities.

The GMYC analysis returned a total of 13 ML independent genetic clusters (confidence interval 11-13), and a total of 34 ML entities or potential species (c.i. 32-43) (Fig. 4). The results of the likelihood test suggest significant differences between the obtained clustering and a null model (threshold time = -0.008; Likelihood ratio = 249.69; LR test $p < 0.001$). Both methods recovered a similar number of taxa, with the grouping resulting from a $P = 0.01$ in ABGD being the same as the one obtained by the GMYC analysis.

Discussion

This study, based on a COI barcode approach, was consistent in defining species boundaries with maximum support in both Bayesian inference (BI) and Maximum likelihood (ML) methods.

BI, ML, and ABGD (considering maximum prior interspecific divergence $P = 0.1$) and GYMC analyzes all together allowed for the identification of 32 lineages or operational taxonomy units (OTUs) from the 80 specimens analyzed (see discussion below). All these lineages could be associated with previously known species or genera. Consequently, the number of marine oligochaetes from the southeastern coast of South America (Brazil) increased from 14 to 21 by reporting the species *Doliodrilus fibrisaccus* Wang & Erséus, 2004*, Limnodriloides pierantonii* (Hrabě, 1971) *, L. rubicundus* Erséus, 1982*, L. sacculus* Erséus, 1990*, Paranais frici* Hrabě, 1941*, Stephensoniella sterreri,* lasserre & Erséus, 1976 and *Thalassodrilides gurwitschi* (Hrabě, 1971) for the first time. The number of species recorded from the South Atlantic areas as a whole will probably increase with the descriptions of the potentially new species of *Ainudrilus*, *Aktedrilus*, *Enchytraeus*, *Limnodriloides*, *Macquaridrilus*, *Stephensoniella* and the four candidate species belonging to the "achaetous" *Marionina* complex (Matamoros, *et al*., 2012) from Brazil, as well as with the three species of *Lumbricillus* from Antarctica.

The two clustering methods, ABGD and GYMC were strongly concordant and only two cases of minor discrepancies were observed considering the most conservative prior interspecific divergence ($P = 0.1$) for the ABGD. In GYMC, the sympatric specimens of *Limnodriloides* sp.2 were split into two distinct linages or OTUs, and the same occurred with

Stephensoniella sterreri, also sympatric. Conversely, ABGD grouped all specimens of *Limnodriloides* sp.2 and *S. sterreri* into only two lineages (*P* = 0.1) (Fig. 3, Tab. 1). These two methods showed the same result (34 OUT, *P* = 0.01), but ABGD clearly overestimated the number of species (43), when using *P* = 0.001 (Tab. 1). Simulations performed by Puillandre *et al*., 2012 have shown that the results of ABGD and GYMC can be influenced by the evenness of the sampled dataset. The authors pointed out that these two methods may overestimate or underestimate the number of species in even or uneven sampling and more efforts must be made to investigate the effects of uneven sampling in species delimitation. Increasing the sampling effort as an attempt to ”standardize” the number of species could be a methodological solution, but it is often not feasible due to the prevalence of rare species in most ecological datasets (Puillandre *et al*., 2012).

However, the mean genetic divergences within *Limnodriloides* sp.2 and *S. sterreri* are low 2.3 ±0,6 and 1.4 ±0,5 respectively. Thus, the low genetic divergence, combined with the sympatric distribution of the two species, strongly supports that they are only two instead of four species, as suggested by GYMC and ABGD (with *P* 0.01). The COI barcode approach and the genetic distance threshold for species delimitation boundaries are still subject to debate (Erséus and Gustavsson, 2009; Dasmahapatra *et al*., 2010; Collins and Cruickshank, 2013; Martinsson *et al*., 2013). Achurra and Erséus (2013) argued that the use of only COI genetic distance as a threshold to define species could overestimate the number of lineages and they recommended to use at least one additional nuclear marker. In their study, the single oligochaete species *Stylodrilus heringianus* Claparède, 1862 was divided into at least six distinct lineages when only COI barcodes were used, but most of these lineages showed allopatric distribution. On the other hand, even when using a multi-locus approach for establishing the species boundaries, COI barcodes and using a 10% threshold value for intraspecific genetic divergence were by themselves enough to identify nine new species of the enchytraeid genus *Grania*, and this included two cryptic lineages (Prantoni *et al*., 2016). Similar results were obtained by Vivien *et al.* (2015) in a study of freshwater oligochaetes from Switzerland. They were able to identify 41 distinct lineages using COI barcode approach with 10% threshold of intraspecific genetic divergence, while only 28 taxa could be morphologicaly identified.

This study confirms that a COI barcoding approach combined with a intraspecific genetic divergence threshold at about 10% was able to recognize all potential species in our samples. DNA barcoding may therefore become a very useful framework for species delimitation. It is especially recommended for the large datasets frequently used in ecological studies of poorly known taxa and also for the groups, which are hard to identify by traditional morphological analysis only.

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References

Achurra, A., and Erséus, C. (2013). DNA barcoding and species delimitation: the *Stylodrilus heringianus* case (Annelida: Clitellata: Lumbriculidae). *Invertebrate Systematics* **27(1)**, 118.

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactionson Automatic Control* **19**, 716–723.

Backlund, H. O. (1948). *Lumbricillus reynoldsoni* n. sp., an enchytraeid from the beaches of north Wales. *Journal of Marine Biological Association of the United Kingdom* **27**, 710–717.

Barraclough, T. G., Hughes, M., Ashford-Hodges, N., and Fujisawa, T. (2009). Inferring evolutionarily significant units of bacterial diversity from broad environmental surveys of single-locus data. *Biology Letters* **5**, 425–428.

Beddard, F. E. (1889). Notes on the Marine Oligochaeta of Plymouth. *Journal of the Marine Biological Association of the United Kingdom* **1**, 69–71.

Brinkhurst, R. O. (1963). Notes on the Brackish-Water and Marine Species of Tubificidae. *Journal of the Marine Biological Association of the United Kingdom* **43**, 709–715.

Coates, K. A. (1990). Marine Enchytraeidae (Oligochaeta, Annelida) of the Albany area, Western Australia. In 'Proceedings of the third international marine biological workshop: the marine flora and fauna of Albany.' (Eds F. E. Wells, D. I. Walker, H. Kirkman, and R. Lethbridge.) pp. 13–41. (Western Australian Museum: Albany.)

Coates, K. A., and Stacey, D. F. (1997). Enchytraeids (Oligochaeta: Annelida) of the lower shore and shallow subtidal of Darwin Harbour, Northern Territory, Australia. In 'Proceedings of the sixth international marine biological workshop: the marine flora and fauna of Darwin Harbour, Northen Territory, Australia'. (Eds J. Hanley, G. Caswell, D. Megirian, and H. Larson.) pp. 391–414. (Darwin Museums and Art Galleries of the Northern Territory and the Australian Marine Sciences Association: Darwin.)

Coates, K. A., and Stacey, D. F. (1993). The Marine Enchytraeidae (Oligochaeta, Annelida) of Rottnest Island, Western Australia. In '*Proceedings* *of the Fifth International Marine Biological Workshop: The Marine Flora and Fauna of Rottnest Island, Western Australia*'. (Eds F. E. Wells, D. I. Walker, H. Kirkman, and R. Lethbridge.) pp. 391–414. (Western Australian Museum: Perth.)

Collins, R. A., and Cruickshank, R. H. (2012). The seven deadly sins of DNA barcoding. *Molecular Ecology Resources*, **13**, 969-975.

Cook, D. G. (1969). The Tubificidae (Annelida, Oligochaeta) of Cape Cod Bay with a Taxonomic Revision of the Genera *Phallodrilus* Pierantoni, 1902, *Limnodriloides* Pierantoni, 1903 and *Spiridion* Knollner, 1935. *Biological Bulletin* **136**, 9–27.

Dasmahapatra, K. K., Elias, M., Hill, R. I., Hoffman, J. I., and Mallet, J. (2010). Mitochondrial DNA barcoding detects some species that are real, and some that are not. *Molecular Ecology Resources* **10**, 264–273.

De Queiroz, K. (2007). Species Concepts and Species Delimitation. *Systematic Biology* **56**, 879–886.

De Wit, P., and 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(4)**, 285–293.

De Wit, P., Rota, E., and Erséus, C. (2009). *Grania* (Annelida: Clitellata: Enchytraeidae) of the Great Barrier Reef, Australia, including four new species and a re-description of Grania trichaeta Jamieson, 1977. *Zootaxa* **2165**, 16–38.

De Wit, P., and Erséus, C. (2007). Seven New Species of *Grania* (Annelida: Clitellata: Enchytraeidae) From New Caledonia, South Pacific Ocean. *Zootaxa* **1426**, 27–50.

Drummond, A. J., and Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 214.

Du Bois-Reymond Marcus, E. (1950). A Marine Tibificid from Brazil. *Comunicaciones Zoologicas del Museo de Historia Natural de Montevideo* **3**, 1–6.

Erséus, C. (2005). Phylogeny of oligochaetous Clitellata. *Hydrobiologia* **535/536,** 357–372.

Erséus, C. (2002). Mangroves and marine oligochaete diversity. *Wetlands Ecology and Management* **10**, 197–202.

Erséus, C., Prestegaard, T., and Källersjö, M. (2000). Phylogenetic Analysis of Tubificidae (Annelida, Clitellata) Based on 18S rDNA Sequences. *Molecular Phylogenetics and Evolution* **15**, 381–389.

Erséus, C. (1999). Marine Tubificidae (Oligochaeta) from a mangrove habitat in Kenya. *Tropical Zoology* **12**, 137–143.

Erséus, C. (1994). Oligochaeta and Polychaeta: Phyllodocida (Phyllodocidae to Paralacydoniidae). In 'Taxonomic Atlas of the Benthic Fauna of the Santa Maria Basin and Western Santa Barbara Channel'. (Eds J. A. Blake and B. Hilbing.) pp. 1–55. (Santa Barbara Museum of Natural History: Santa Barbara.)

Erséus, C. (1990). Marine Oligochaeta of Hong Kong. In 'Procceedings of the Second International Marine Biological Workshop: The Marine Flora and Fauna of Hong Kong and Southern China, Hong Kong, 1986'. (Ed B. Morton.) pp. 259–335. (Hong Kong University Press: Hong Kong.)

Erséus, C. (1989a). Four new West Atlantic species of *Tubificoides* (Oligochaeta, Tubificidae). *Proceedings of the Biological Society of Washington* **102**, 878–886.

Erséus, C. (1989b). *Phallodrilus vescus*, new species (Oligochaeta, Tubificidae) from the Gulf of Mexico. *Proceedings of the Biological Society of Washington* **102**, 134–136.

Erséus, C. (1989c). *Phallodrilus hessleri*, new species (Oligochaeta, Tubificidae), from abyssal depths in the Western Pacific Ocean. *Proceedings of the Biological Society of Washington* **102**, 131–133.

Erséus, C. (1989d). Two new species of the marine genus *Limnodriloides* and a record of *Tubificoides fraseri* Brinkhurst (Oligochaeta: Tubificidae) from New Zealand. *New Zealand Journal of Marine and Freshwater Research* **23**, 557–561.

Erséus, C. (1988). Deep sea Tubificidae (Oligochaeta) from the Gulf of Mexico. *Proceedings of the Biological Society of Washington* **101**, 67–71.

Erséus, C. (1984). Taxonomy of some species of *Phallodrilus* (Oligochaeta, Tubificidae) from the Northwest Atlantic, with description of 4 new species. *Proceedings of the Biological Society of Washington* **97**, 812–826.

Erséus, C. (1983a). *Duridrilus tardus* gen. et sp.n., a marine tubificid (Oligochaeta) from Bermuda and Barbados. *Sarsia* **68**, 29–32.

Erséus, C. (1983b). Taxonomic Studies of the Marine Genus *Marcusaedrilus* Righi & Kanner (Oligochaeta, Tubificidae), with Descriptions of Seven New Species from the Caribbean area and Australia. *Zoologica Scripta* **12**, 25–36.

Erséus, C. (1983c). Two new species of *Aktedrilus* (Oligochaeta, Tubificidae) from the Western Atlantic. *Bulletin du Muséum d'histoire naturelle* **4,**1051–1057.

Erséus, C. (1982a). *Atlantidrilus*, a new genus of deep-sea Tubificidae (Oligochaeta). *Sarsia* **67**, 43–46.

Erséus, C. (1982b). Three new species of the marine genus *Coralliodrilus* (Oligochaeta, Tubificidae) from Italy. *Bolletino di Zoologia* **49**, 241–247.

Erséus, C. (1982c). *Parakaketio longiprostatus* gen. et sp.n., a marine tubificid (Oligochaeta) from Florida, U.S.A. *Zoologica Scripta* **11**, 195–197.

Erséus, C. (1981a). Taxonomic studies of Phallodrilinae (Oligochaeta, Tubificidae) from the Great Barrier Reef and the Comoro Islands with descriptions of ten new species and one new genus. *Zoologica Scripta* **10**, 15–31.

Erséus, C. (1981b). Taxonomic studies of Phallodrilinae (Oligochaeta, Tubificidae) from the Great Barrier Reef and the Comoro Islands with descriptions of ten new species and one new genus. *Zoologica Scripta* **10**, 15–31.

Erséus, C. (1980). Taxonomic studies on the marine genera *Aktedrilus* Knöllner and *Bacescuella* Hrabe' (Oligochaeta, Tubificidae), with descriptions of seven new species. *Zoologica Scripta* **9**, 97–111.

Erséus, C. (1979a). Taxonomic Revision of the Marine Genus *Phallodrilus* Pierantoni (Oligochaeta, Tubificidae), with descriptions of thirteen new Species. *Zoologica Scripta* **8**, 187–208.

Erséus, C. (1979b). *Inanidrilus bulbosus* gen. et sp.n., a Marine Tubificid (Oligochaeta) from Florida, USA. *Zoologica Scripta* **8**, 209–210.

Erséus, C. (1979c). Taxonomic Revision of the Marine Genera *Bathydrilus* Cook and *Macroseta* Erséus (Oligochaeta, Tubificidae), with Descriptions of Six New Species and Subspecies. *Zoologica Scripta* **8**, 139–151.

Erséus, C. (1979d). *Coralliodrilus leviatriatus* gen. et sp.n., a marine tubificid (Oligochaeta) from Bermuda. *Sarsia* **63**, 179–182.

Erséus, C. (1978). Two New Species of the Little-known Genus *Bacescuella* Hrabě (Oligochaeta, Tubificidae) from the North Atlantic. *Zoologica Scripta* **7**, 263–267.

Erséus, C. (1977a). New species of Adelodrilus and a revision of the genera *Adelodrilus* and *Adelodriloides* (Oligochaeta, Tubificidae). *Sarsia* **63**, 135–144.

Erséus, C. (1977b). Marine Oligochaeta from the Koster area, west coast of Sweden, with descriptions of two new enchytraeid species. *Zoologica Scripta* **6**, 293–298.

Erséus, C. (1976a). Littoral Oligochaeta (Annelida) from Eyjafjördur, North coast of Iceland. *Zoologica Scripta* **5**, 5–11.

Erséus, C. (1976b). Marine subtidal Tubificidae and Enchytraeidae (Oligochaeta) of the Bergen area, western Norway. *Sarsia* **62**, 25–48.

Erséus, C. (1975). On the Systematic Position of *Rhyacodrilus prostatus* Kölner (Oligochaeta, Tubificidae). *Zoologica Scripta* **4**, 33–35.

Erséus, C. (1974). *Grania Pusilla* sp.n. (Oligochaeta, Enchytraeidae) from the west coast of Norway and Sweden with some taxonomic notes on genus *Grania*. *Sarsia* **56**, 87–94.

Erséus, C., and Gustafsson, D. (2009). Cryptic speciation in clitellate model organisms. In ‘Annelids in Modern Biology’. (Ed D. H. Shain.) pp. 1–16. (John Wiley & Sons, Inc.: Hoboken.)

Erséus, C., and Lassarre, P. (1977). Redescription of *Grania monochaeta* (Michaelsen), a Marine Enchytraeid (Oligochaeta) from South Georgia (SW Atlantic). *Zoologica Scripta* **6**, 299–300.

Erséus, C., and Lasserre, P. (1976). Taxonomic Status and Geographic Variationof the Marine Enchytraeid Genus *Grania Southern* (Oligochaeta). *Zoologica Scripta* **5**, 121–132.

Erséus, C., Rota, E., Matamoros, L., and De Wit, P. (2010). Molecular phylogeny of Enchytraeidae (Annelida, Clitellata). *Molecular Phylogenetics and Evolution* **57(2)**, 849–858.

Erséus, C., Wetzel, M. J., and Gustavsson, L. M. (2008). ICZN rules-a farewell to Tubificidae (Annelida, Clitellata). *Zootaxa* **1744**, 66–68.

Erséus, C., and Rota, E. (2003). New findings and an overview of the oligochaetous Clitellata (Annelida) of the North Atlantic deep sea. *Proceedings of the Biological Society of Washington* **116(4)**, 892–900.

Fontaneto, D., Herniou, E. A., Boschetti, C., Caprioli, M., Melone, G., Ricci, C., and Barraclough, T. G. (2007). Independently evolving species in asexual bdelloid rotifers. *PLoS biology* **5**, e87.

Fujisawa, T., and Barraclough, T. (2013). Delimiting species using single-locus data and the Generalized Mixed Yule Coalescent (GMYC) approach: a revised method and evaluation on simulated datasets. *Systematic Biology* **62**, 707–724.

Gelman, A. and Rubin, D. R. (1992). Inference from Iterative Simulation Using Multiple Sequences. *Statistical Science* **7(4)**, 457-472.

Giere, O. (1979). Studies on marine Oligochaeta from Bermuda, with emphasis on new *Phallodrilus* species (Tubificidae). *Cahiers de Biologie Marine* **3**, 301–314.

Harman, W. J., and Loden, M. S. (1984). Capilloventer atlanticus gen. et sp.n., a member of a new family of marine Oligochaeta from Brazil. *Hydrobiologia* **115**, 51–54.

Hartzell, P. L., Nghiem, J. V., Richio, K. J., and Shain, D. H. (2005). Distribution and phylogeny of glacier ice worms (Mesenchytraeus solifugusand Mesenchytraeus solifugusrainierensis). *Canadian Journal of Zoology* **83(9)**, 1206–1213.

Healy, B. (1979). Three new species of Enchytraeidae (Oligochaeta) from Ireland. *Zoological Journal of the Linnean Society* **67**, 87–95.

Healy, B. (1975). A description of five new species of Enchytraeidae (Oligochaeta) from Ireland. *Zoological Journal of the Linnean Society* **56**, 315–326.

Hebert, P. D. N., Ratnasingham, S., and Waard, J. R. (2003). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society B: Biological Sciences* **270**, 96–99.

Jamieson, B. G. M. (1977). Marine meiobenthic Oligochaeta from Heron and Wistari Reefs (Great Barrier Reef) of the genera *Clitellio*, *Limnodriloides* and *Phallodrilus* (Tubificidae) and Grania (Enchytraeidae). *Zoological Journal of the Linnean Society* **61**, 329–349.

Jamieson, B. G. M. (1968). *Macquaridrilus*: a new genus of Tubificidae (Oligochaeta) from Macquarie Island. *University of Queensland Papers, Department of Zoology* **3**, 55–69.

Katoh, K., and Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution* **30**, 772–780.

Kekkonen, M., and Hebert, P. D. N. (2014). DNA barcode-based delineation of putative species: efficient start for taxonomic workflows. *Molecular Ecology Resources* **14**, 706–715.

Knöllner, F. H. (1935). Ökologische und systematische Untersuchungen über litorale und marine Oligochäten der Kieler Bucht. *Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere* **66,** 425–512.

Kossmagk-Stephan K-J. (1983). Marine Oligochaeta from a sandy beach of the island of Sylt (North Sea) with description of four new enchytraeid species. *Akademie der Wissenschaften und der Literatur Mainz Mathematisch–Naturwissenschaftlichen Klasse Mikrofauna des Meeresbodens* **89,**1–28

Lasserre, P. (1966). Oligochétes marins des côtes de France. I. Bassin d'Arcachon: systématique. *Cahiers de Biologie Marine* **7:** 295–317.

Lasserre, P. (1967). Oligochétes marins des côtes de France. II. Roscoff, penpoull, étangs saumâtres de Concarneau: systématique, écologie. *Cahiers de Biologie Marine* **8:** 273– 293.

Lanfear, R., Calcott, B., Ho, S. Y. W., and Guindon, S. (2012). PartitionFinder: Combined Selection of Partitioning Schemes and Substitution Models for Phylogenetic Analyses. *Molecular Biology and Evolution* **29**, 1695–1701.

Locke, J. M., and Coates, K. A. (1998). A new species of *Grania* (Enchytraeidae, Clitellata, Annelida) and redescription of *Grania pusilla* from a rocky shore in SE Ireland. *Journal of Natural History* **32**, 1101–1114.

Luo, A., Qiao, H., Zhang, Y., Shi, W., Ho, S. Y. W., Xu, W., Zhang, A., and Zhu, C. (2010). Performance of criteria for selecting evolutionary models in phylogenetics: a comprehensive study based on simulated datasets. *BMC Evolutionary Biology* **10**, 1–13.

Marotta, R., Ferraguti, M., Erséus, C., and Gustavsson, L. M. (2008). Combined-data phylogenetics and character evolution of Clitellata (Annelida) using 18S rDNA and morphology. *Zoological Journal of the Linnean Society* **154**, 1–26.

Matamoros, L., Rota, E., and Erséus, C. (2012). Cryptic diversity among the achaetous Marionina (Annelida, Clitellata, Enchytraeidae). *Systematics and Biodiversity* **10(4)**, 509–525.

Marcus, E. (1965). Naidomorpha aus brasilianischem Brackwasser. *Beitrage zur Neotropischen Fauna* **4**, 61–83.

Martin, P., Martínez-Ansemil, E., Pinder, A., Timm, T., and Wetzel, M. J. (2007). Global diversity of oligochaetous clitellates (“Oligochaeta;” Clitellata) in freshwater. *Hydrobiologia* **595(1)**, 117–127.

Martinsson, S., Cui, Y., Martin, P. J., Pinder, A., Quinlan, K., Wetzel, M. J., and Erséus, C. (2015). DNA-barcoding of invasive European earthworms (Clitellata: Lumbricidae) in south-western Australia. *Biological Invasions* **17(9)**, 2527–2532.

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

Michaelsen, W. (1919). Über die Beziehungen der Hirudineen zu den Oligochäten*. Mitteilungen aus dem Naturhistorischen Museum in Hamburg* **36**, 131–153.

Michaelsen W. (1888)**.** Die Oligochaeten von Süd-Georgien nach Ausbeute der deutschen Station von 1882–83. *Jahrbuch der Hamburgischen wissenschaftliche Anstalten* **5,** 53–73.

Miller, M. A., Pfeiffer, W., and Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In Gateway Computing Environments Workshop (GCE), 2010. pp. 1–8. (IEEE.)

Milligan, M. R. (1991). Two new species of *Tubificoides* (Oligochaeta, Tubificidae) and new records of *T. brownae* and *T. imajimai* from the Gulf of

Mexico and Caribbean, with a redescription of *T. bakeri*. *Zoologica Scripta* **20**, 339–345.

Moore, J. P. (1905). Some Marine Oligochaeta of New England. *Proceedings of the Academy of Natural Sciences of Philadelphia* **57**, 373–399.

Moore, J. P. (1902). Some Bermuda Oligochaeta, with a Description of a New Species. *Proceedings of the Academy of Natural Sciences of Philadelphia* **54**, 80–84.

Morais, G. C., Camargo, M. G., and Lana, P. (2016). Intertidal assemblage variation across a subtropical estuarine gradient: How good conceptual and empirical models are? *Estuarine, Coastal and Shelf Science* **170**, 91–101.

Negrello-Filho, O. A., Underwood, A. J., and Chapman, M. G. (2006). Recolonization of infauna on a tidal flat: An experimental analysis of modes of dispersal. *Journal of Experimental Marine Biology and Ecology* **328(2)**, 240–250.

Nylander, J. A. A., Wilgenbusch, J. C., Warren, D. L., and Swofford, D. L. (2008). AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* **24**, 581–583.

Pierantoni, U. (1903a). Due nuovi generi di Oligochaeti marini rinvenuti nel Gulfo di Napoli. *Bollettino della Società dei naturalisti in Napoli* **16**, 113–117.

Pierantoni, U. (1903b). Altri nuovi oligocheti del Golfo di Napoli *(Limnodriloides* n. gen). *Bollettino della Società dei naturalisti in Napoli* **17**, 185–192.

Pierantoni, U. (1901). Sopra una nuova specie di oligochete marino (*Enchytraeus macrochaetus* n. sp.). *Monitore zoologico italiano* **12**, 201–202.

Pons, J., Barraclough, T., Gomez-Zurita, J., Cardoso, A., Duran, D., Hazell, S., Kamoun, S., Sumlin, W., and Vogler, A. (2006). Sequence-Based Species Delimitation for the DNA Taxonomy of Undescribed Insects. *Systematic Biology* **55**, 595–609.

Prantoni, A. L., De Wit, P., and 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 (3)**, 485-510.

Prantoni, A. L., Di Domenico, M., and Lana, P. C. (2013). A taxonomic overview of marine and estuarine oligochaetes from Brazil. *Marine Biodiversity* **44(3)**, 275–278.

Puillandre, N., Lambert, A., Brouillet, S., and Achaz, G. (2011). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology* **21**, 1864–1877.

R Development Core Team (2015) 'R: A language and environment for statistical computing. R Foundation for Statistical Computing'. Available at http://www.R-project.

Righi, G. (1968). Sobre alguns Oligochaeta do Brasil. *Revista Brasileira de Biologia* **28**, 369–382.

Righi, G., and Kanner, E. (1979). Marine Oligochaeta (Tubifidae and Enchytraeidae) from the Caribbean Sea. *Studies on the Fauna of Curaçao and other Caribbean Islands* **58**, 44–68.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D., Darling, A., Hohna, S., Larget, B., Liu, L., Suchard, M. A., and Huelsenbeck, J. P. (2012). 'MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space'. *Software for Systematics and Evolution* **61**, 539–542.

Rota, E., Wang, H., and Erséus, C. (2007). The diverse *Grania* fauna (Clitellata: Enchytraeidae) of the Esperance area, Western Australia, with descriptions of two new species. *Journal of Natural History* **41**, 999–1023.

Rota, E., and Erséus, C. (2003). New records of Grania (Clitellata, Enchytraeidae) in the Northeast Atlantic (from Tromsø to the Canary Islands), with descriptions of seven new species. *Sarsia* **88(3)**, 210–243.

Rota, E., and Erséus, C. (2000). Two new and peculiar species of *Grania* (Annelida: Clitellata: Enchytraeidae) inhabiting Tasmanian estuaries. *New Zealand Journal of Zoology* **27**, 245–254.

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

Rouse, G. W., and Fauchald, K. (1997). Cladistics and polychaetes. *Zoologica Scripta* **26(2)**, 139–204.

Rousset, V., Plaisance, L., Erséus, C., Siddall, M. E., and Rouse, G. W. (2008). Evolution of habitat preference in Clitellata (Annelida). *Biological Journal of the Linnean Society* **95(3)**, 447–464.

Rousset, V., Pleijel, F., Rouse, G. W., Erséus, C., and Siddall, M. E. (2007). A molecular phylogeny of annelids. *Cladistics* **23(1)**, 41–63.

Sandrini-Neto, L., and Lana, P. C. (2014). Journal of Experimental Marine Biology and Ecology. *Journal of Experimental Marine Biology and Ecology* **452(C)**, 9–21.

Southern, R. (1913). Oligochaeta. *Proceedings of the Royal Irish Academy* **31**, 1–14.

Souza, F. M., Brauko, K. M., Lana, P. C., Muniz, P., and Camargo, M. G. (2013). The effect of urban sewage on benthic macrofauna: a multiple spatial scale approach. *Marine Pollution Bulletin* **67(1-2)**, 234–240.

Staden, R., Judge, D. P., and Bonfield, J. K. (2003). Analysing Sequences Using the Staden Package and EMBOSS. In ´ Introduction to Bioinformatics. A Theoretical and Practical Approach´. (Eds S. A. Krawetz and D. D. Womble.) (Human Press Inc., Totawa, NJ.)

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 1–2.

Struck, T. H. (2011). Direction of evolution within Annelida and the definition of Pleistoannelida. *Journal of Zoological Systematics and Evolutionary Research* **49(4)**, 340–345.

Struck, T. H., Golombek, A., Weigert, A., Franke, F. A., Westheide, W., Purschke, G., Bleidorn, C., Halanych, K. M. (2015). The Evolution of Annelids Reveals Two Adaptive Routes to the Interstitial Realm. *Current Biology* **25(15)**, 1993–1999.

Struck, T. H., Paul, C., Hill, N., Hartmann, S., Hösel, C., Kube, M., Lieb, B., Meyer, A., Tiedemann, R., Purschke, G., and Bleidorn, C. (2011). Phylogenomic analyses unravel annelid evolution *Nature* **470**, 95–98.

Struck, T. H., Schult, N., Kusen, T., Hickman, E., Bleidorn, C., McHugh, D., and Halanych, K. M. (2007). Annelid phylogeny and the status of Sipuncula and Echiura. *BMC Evolutionary Biology* **7(1)**, 57.

Schwarz, G. (1978). Estimating the domension model. *The annals of Statistics* **6**, 461–464.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* **30**, 2725–2729.

Tamura, K., and Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**, 512–526.

Taylor, H. R., and Harris, W. E. (2012). An emergent science on the brink of irrelevance: a review of the past 8 years of DNA barcoding. *Molecular Ecology Resources* **12**, 377–388.

Templeton, A. R. (1989). The meaning of species and speciation: A genetic perspective. In ´Speciation and Its Consequences´. (Eds Otte and J. A. Endler.) pp. 3-27. (Sinauer Associates, Sunderland, Massachusetts.)

Voss, G. L. (1988). Evolution and phylogenetic relationships of deep-sea octopods (Cirrata and Incirrata). In ´The Mollusca. Vol. 12. Palaeontology and Neontology of Cephalopods´. (Eds M. R. Clarke and E. R. Trueman.) pp. 253-276. (Academic Press: London, UK.)

Weigert, A., Golombek, A., Gerth, M., Schwarz, F., Struck, T. H., and Bleidorn, C. (2015). Evolution of mitochondrial gene order in Annelida. *Molecular Phylogenetics and Evolution* **94 A**, 196–206.

Wang, H., and Erséus, C. (2003). Marine species of *Ainudrilus* and *Heterodrilus* (Oligochaeta: Tubificidae: Rhyacodrilinae) from Hainan Island in southern China. *New Zealand Journal of Marine and Freshwater Research* **37**, 205–217.

Weigert, A., and Bleidorn, C. (2016). Current status of annelid phylogeny. *Organisms Diversity & Evolution*. http://doi.org/10.1007/s13127-016-0265-7.

Weigert, A., Helm, C., Meyer, M., Nickel, B., Arendt, D., Hausdorf, B., Santos, S. R., Halanych, K. M., Purschke, G., Bleidorn, C., and Struck, T. H. (2014). Illuminating the Base of the Annelid Tree Using Transcriptomics. *Molecular Biology and Evolution* **31(6)**, 1391–1401.

Wilgenbusch, J. C., Warren, D. L., and Swofford, D. L. (2004). AWTY: a system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. Avaibale at: http://ceb.csit.fsu.edu/awty.

Zrzavý, J., Říha, P., Piálek, L., and Janouškovec, J. (2009). Phylogeny of Annelida (Lophotrochozoa): total-evidence analysis of morphology and six genes. *BMC Evolutionary Biology* **9(1)**, 189.

Appendix 1: List of species, collection sites, date, coordinates of 80 barcoded specimens, GenBank Accession nos. of COI barcodes and ML-entities (operational taxonomic units) inferred by GMYC (see Fig. 4), and the putative species nos. found by the three ABDG analyses.

ID	Taxa	Collection site	Date month/ year	Coordinates	GenBank Accession nº	GMYC	ABGD *P* = 0.1	ABGD *P* = 0.01	ABGD *P* = 0.001
ALP6/CE20837	*Ainudrilus* sp.1	Paranaguá Bay - Brazil	Oct-13	25°27'54"S; 48°25'12"W		13	24	24	24
ALP7/CE20742	*Ainudrilus* sp.2	Paranaguá Bay - Brazil	Oct-13	25°27'54"S; 48°25'12"W		13	24	24	24
ALP8/CE20838	*Doliodrilus fibrisaccus*	Paranaguá Bay - Brazil	Sep-13	25°27'54"S; 48°25'12"W		7	29	29	29
ALP10/CE20840	*Limnodriloides* sp.2	Paranaguá Bay - Brazil	Oct-13	25°27'54"S; 48°25'12"W		11	26	34	43
ALP11/CE20826	*Aktedrilus* sp.1	Florianópolis - Brazil	Jul-13	27°45′58″S; 48°34′27″W		31	28	28	28
ALP12/CE20737	*Aktedrilus* sp.2	Florianópolis - Brazil	Jul-13	27°45′58″S; 48°34′27″W		27	22	22	22
ALP14/CE20827	*Marionina* sp.3	Florianópolis - Brazil	Jul-13	27°45′58″S; 48°34′27″W		1	2	2	2
ALP15/CE20739	*Macquaridrilus* sp.1	Florianópolis - Brazil	Jul-13	27°45′58″S; 48°34′27″W		28	23	23	23
ALP16/CE20918	*Limnodriloides pierantonii*	Paranaguá Bay - Brazil	Aug-13	25º30'01"S: 48º29'17" W		10	7	7	39
ALP17/CE20829	*Limnodriloides rubicundus*	Paranaguá Bay - Brazil	Aug-13	25º30'01"S: 48º29'17" W		9	14	14	14
ALP18/CE20830	*Limnodriloides pierantonii*	Paranaguá Bay - Brazil	Aug-13	25º30'01"S: 48º29'17" W		10	7	7	7
ALP19/CE20831	*Limnodriloides pierantonii*	Paranaguá Bay - Brazil	Aug-13	25º30'01"S: 48º29'17" W		10	7	7	7
ALP23/CE20740	*Limnodriloides pierantonii*	Paranaguá Bay - Brazil	Aug-13	25º30'01"S: 48º29'17" W		10	7	7	35

ID	Taxa	Collection site	Date month/ year	Coordinates	GenBank Accession nº	GMYC	ABGD *P* = 0.1	ABGD *P* = 0.01	ABGD *P* = 0.001
ALP25/CE20832	*Limnodriloides pierantonii*	Paranaguá Bay - Brazil	Aug-13	25º30'01"S: 48º29'17" W		10	7	7	36
ALP26/CE20833	*Limnodriloides pierantonii*	Paranaguá Bay - Brazil	Aug-13	25º30'01"S: 48º29'17" W		10	7	7	37
ALP27/CE20847	*Limnodriloides* sp.2	Antonina Bay - Brazil	Sep-13	25º25'12"S; 48º42'10" W		27	26	34	43
ALP28/CE20920	*Limnodriloides pierantonii*	Antonina Bay - Brazil	Sep-13	25º25'12"S; 48º42'10" W		10	7	7	40
ALP29/CE20848	*Limnodriloides pierantonii*	Antonina Bay - Brazil	Sep-13	25º25'12"S; 48º42'10" W		10	7	7	34
ALP30/CE20849	*Limnodriloides pierantonii*	Antonina Bay - Brazil	Sep-13	25º25'12"S; 48º42'10" W		10	7	7	34
ALP32/CE20820	*Limnodriloides* sp.2	Paranaguá Bay - Brazil	Sep-13	25°27'54"S; 48°25'12"W		12	26	26	26
ALP33/CE20821	*Limnodriloides* sp.2	Paranaguá Bay - Brazil	Sep-13	25°27'54"S; 48°25'12"W		11	26	34	43
ALP34/CE20822	*Limnodriloidinae* sp.1	Paranaguá Bay - Brazil	Sep-13	25°27'54"S; 48°25'12"W		12	26	26	26
ALP35/CE20823	*Ainudrilus* sp.2	Paranaguá Bay - Brazil	Sep-13	25°27'54"S; 48°25'12"W		30	27	27	27
ALP37/CE20824	*Limnodriloides* sp.2	Paranaguá Bay - Brazil	Sep-13	25°27'54"S; 48°25'12"W		11	26	34	43
ALP38/CE20825	*Ainudrilus* sp.1	Paranaguá Bay - Brazil	Sep-13	25°27'54"S; 48°25'12"W		13	24	24	42
ALP41/CE20743	*Paranais frici*	Paranaguá Bay - Brazil	Oct-13	25°27'54"S; 48°25'12"W		29	25	25	25
ALP42/CE20841	*Ainudrilus* sp.3	Paranaguá Bay - Brazil	Oct-13	25°27'54"S; 48°25'12"W		32	30	30	30
ALP43/CE20842	*Doliodrilus fibrisaccus*	Paranaguá Bay - Brazil	Oct-13	25°27'54"S; 48°25'12"W		7	29	29	29
ALP44/CE20843	*Thalassodrilides gurwitschi*	Paranaguá Bay - Brazil	Oct-13	25°27'54"S; 48°25'12"W		33	31	31	31

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ALP45/CE20844	*Ainudrilus* sp.1	Paranaguá Bay - Brazil	Oct-13	25°27'54"S; 48°25'12"W		13	24	24	24
ALP46/CE20845	*Ainudrilus* sp.1	Paranaguá Bay - Brazil	Oct-13	25°27'54"S; 48°25'12"W		13	24	24	24
ALP48/CE20846	*Ainudrilus* sp.4	Paranaguá Bay - Brazil	Oct-13	25°27'54"S; 48°25'12"W		34	32	32	32
ALP53/CE20834	*Limnodriloides pierantonii*	Paranaguá Bay - Brazil	Aug-13	25º30'01"S: 48º29'17" W		10	7	7	38
ALP56/CE20836	*Limnodriloides pierantonii*	Paranaguá Bay - Brazil	Aug-13	25º30'01"S: 48º29'17" W		10	7	7	33
ALP58/CE20850	*Limnodriloides pierantonii*	Antonina Bay - Brazil	Sep-13	25º25'12"S; 48º42'10" W		10	7	7	34
ALP59/CE20921	*Marionina* sp.3	Antonina Bay - Brazil	Sep-13	25º25'12"S; 48º42'10" W		1	2	2	2
ALP61/CE20818	*Grania brasiliensis*	S. Sebastião - Brazil	Oct-12	23°49′41″S; 45°25′23″W		4	11	11	11
ALP 112	*Stephensoniella sterreri*	Paranaguá Bay - Brazil	Sep-14	25°25'18"S; 48°24'28"W		2	8	33	41
ALP 113	*Stephensoniella sterreri*	Paranaguá Bay - Brazil	Sep-14	25°25'18"S; 48°24'28"W		3	8	8	8
ALP 114	*Stephensoniella sterreri*	Paranaguá Bay - Brazil	Sep-14	25°25'18"S; 48°24'28"W		2	8	33	41
ALP 115	*Stephensoniella sterreri*	Paranaguá Bay - Brazil	Sep-14	25°25'18"S; 48°24'28"W		3	8	8	8
ALP 116	*Stephensoniella sterreri*	Paranaguá Bay - Brazil	Sep-14	25°25'18"S; 48°24'28"W		2	8	33	41
ALP 117	*Stephensoniella sterreri*	Paranaguá Bay - Brazil	Sep-14	25°25'18"S; 48°24'28"W		3	8	8	8
ALP 118	*Stephensoniella sterreri*	Paranaguá Bay - Brazil	Sep-14	25°25'18"S; 48°24'28"W		2	8	33	41
ALP 121	*Enchytraeus* sp.1	Paranaguá Bay - Brazil	Sep-14	25°27'47'S; 48°34'13'W'		23	16	16	16

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ALP 123	*Marionina* sp.2	Paranaguá Bay - Brazil	Sep-14	25°27'47'S; 48°34'13'W'		24	17	17	17
ALP 124	*Naididae* sp.1	Paranaguá Bay - Brazil	Sep-14	25°27'47'S; 48°34'13'W'		25	18	18	18
ALP 133	*Marionina* sp.1	Paranaguá Bay - Brazil	Sep-14	25º25'12"S; 48º42'10" W		19	9	9	9
ALP 134	*Marionina* sp.4	Paranaguá Bay - Brazil	Sep-14	25º25'12"S; 48º42'10" W		20	10	10	10
ALP 165	*Limnodriloides rubicundus*	Paranaguá Bay - Brazil	Mar-15	25°27'54"S; 48°25'12"W		9	14	14	14
ALP 167	*Limnodriloides pierantonii*	Paranaguá Bay - Brazil	Mar-15	25º30'01"S: 48º29'17" W		10	7	7	33
ALP 169	*Limnodriloides pierantonii*	Paranaguá Bay - Brazil	Mar-15	25º30'01"S: 48º29'17" W		10	7	7	7
ALP 170	*Limnodriloides pierantonii*	Paranaguá Bay - Brazil	Mar-15	25º30'01"S: 48º29'17" W		10	7	7	7
ALP 171	*Limnodriloides pierantonii*	Paranaguá Bay - Brazil	Mar-15	25º30'01"S: 48º29'17" W		10	7	7	7
ALP 172	*Limnodriloides pierantonii*	Paranaguá Bay - Brazil	Mar-15	25º30'01"S: 48º29'17" W		10	7	7	33
ALP 177	*Limnodriloides pierantonii*	Paranaguá Bay - Brazil	Mar-15	25º30'01"S: 48º29'17" W		10	7	7	7
ALP 185	*Limnodriloides sacculus*	Paranaguá Bay - Brazil	Mar-15	25°27'54"S; 48°25'12"W		8	13	13	13
ALP 187	*Limnodriloides sacculus*	Paranaguá Bay - Brazil	Mar-15	25°27'54"S; 48°25'12"W		8	13	13	13
ALP 188	*Limnodriloides pierantonii*	Paranaguá Bay - Brazil	Mar-15	25°27'54"S; 48°25'12"W		10	7	7	34
ALP 189	*Limnodriloides pierantonii*	Paranaguá Bay - Brazil	Mar-15	25°27'54"S; 48°25'12"W		10	7	7	34
ALP 195	*Limnodriloides rubicundus*	Paranaguá Bay - Brazil	Mar-15	25°27'54"S; 48°25'12"W		9	14	14	14

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ALP 196	*Limnodriloides rubicundus*	Paranaguá Bay - Brazil	Mar-15	25°27'54"S; 48°25'12"W		9	14	14	14
ALP 197	*Stephensoniella* sp.1	Paranaguá Bay - Brazil	Mar-15	25°25'18"S; 48°24'28"W		22	15	15	15
ALP 313	*Grania brasiliensis*	S. Sebastião - Brazil	Apr-15	23°49′41″S; 45°25′23″W		4	11	11	11
ALP 314	*Grania brasiliensis*	S. Sebastião - Brazil	Apr-15	23°49′41″S; 45°25′23″W		4	11	11	11
ALP 324	*Limnodriloides* sp.1	S. Sebastião - Brazil	Apr-15	23°49′41″S; 45°25′23″W		21	12	12	12
ALP 327	*Lumbricillus* sp.1	Pico Varréal - Antarctica	Jan-15	62°10′21″S; 58º08'10″W		26	19	19	19
ALP 328	*Lumbricillus* sp.2	Snow Island - Antarctica	Jan-15	62°46'31″S; 61°17'09″W		5	20	20	20
ALP 329	*Lumbricillus* sp.2	Snow Island - Antarctica	Jan-15	62°46'31″S; 61°17'09″W		5	20	20	20
ALP 330	*Lumbricillus* sp.3	Snow Island - Antarctica	Jan-15	62°46'31″S; 61°17'09″W		6	21	21	21
ALP 331	*Lumbricillus* sp.3	Snow Island - Antarctica	Jan-15	62°46'31″S; 61°17'09″W		8 6	21	21	21
ALP 332	*Lumbricillus* sp.3	Snow Island - Antarctica	Jan-15	62°46'31″S; 61°17'09″W		6	21	21	21
ALP 333	*Lumbricillus* sp.3	Snow Island - Antarctica	Jan-15	62°46'31″S; 61°17'09″W		6	21	21	21
ALP 334	*Lumbricillus* sp.3	Snow Island - Antarctica	Jan-15	62°46'31″S; 61°17'09″W		6	21	21	21
ALP 335	*Lumbricillus* sp.3	Snow Island - Antarctica	Jan-15	62°46'31″S; 61°17'09″W		6	21	21	21
CE13975	*Grania bekkouchei*	Saldanha Bay - South Africa	Dec-11	33°00′25″S; 17°56′45″E		16	4	4	4
CE14015	*Grania capensis*	False Bay - South Africa	Dec-11	34°09′29″S; 18°26′01″E		18	6	6	6

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CE14031	*Grania cryptica*	False Bay - South Africa	Dec-11	34°09′29″S; 18°26′01″E		17	5	5	5
CE14058	*Grania simonae*	Van Dyks Bay - South Africa	Dec-11	34°37′00″S; 19°21′21″E		15	3	3	3
CE20734	*Grania brasiliensis*	S. Sebastião - Brazil	Oct-12	23°49′41″S; 45°25′23″W		4	11	11	11
CE14346	*Capilloventer australis*					14	1	1	1