Morphological, molecular and phylogenetic analyses of the spirurid nematode *Stegophorus macronectes* (Johnston & Mawson, 1942)

Virginia Vidal [1,2,3], Juana Ortiz[1,3], Julia Ines Diaz[4], Basilio Zafrilla[5], Maria Jose Bonete[5], Maria Rocio Ruiz De Ybañez[1,3], Maria Jose Palacios[6], Jesus Benzal[6], Francisco Valera[6], Carlos De La Cruz[7], Miguel Motas[3,8], Vanesa Bautista[5], Annie Machordom[9], Andres Barbosa[2,3,6*]

1.- Departamento de Sanidad Animal (Parasitología), Facultad de Veterinaria, Universidad de Murcia. Campus de Excelencia Internacional Regional "Campus Mare Nostrum". 30100, Espinardo (Murcia), Spain

2.- Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales, MNCN-CSIC. José Gutiérrez Abascal, 2, 28006, Madrid, Spain

3.- Unidad Asociada de Ecología e Inmunología parasitaria. CSIC-Universidad de Murcia, Spain

4.- Centro de Estudios Parasitológicos y de Vectores (CCT CONICET La Plata- CONICET-UNLP), Calle 120 s/n, entre 61 y 62, 1900 La Plata, Argentina

5.- Departamento de Agroquímica y Bioquímica, Facultad de Ciencias, Universidad de Alicante. Ctra. San Vicente del Raspeig s/n, 03690, San Vicente del Raspeig (Alicante), Spain

6.- Departamento de Ecología Funcional y Evolutiva, Estación Experimental de Zonas Áridas, EEZA-CSIC. Ctra. de Sacramento s/n, La Cañada de San Urbano, 04120, Almería, Spain

7.- Departamento de Biología Animal, Facultad de Ciencias, Universidad de Extremadura. 06006, Badajoz, Spain

8.- Departamento de Ciencias Sociosanitarias (Toxicología), Facultad de Veterinaria, Universidad de Murcia. Campus de Excelencia Internacional Regional "Campus Mare Nostrum". 30100, Espinardo (Murcia), Spain.

9.- Departamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales, MNCN-CSIC. José Gutiérrez Abascal, 2, 28006, Madrid, Spain.

*Corresponding author: A. Barbosa. Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales, MNCN-CSIC. José Gutiérrez Abascal, 2, 28006, Madrid, Spain. Tel: +34 914111328 – 1226. Fax: +34 915645078.

E- mail: barbosa@mncn.csic.es

Running Title: Morphology and phylogeny of *Stegophorus macronectes*

Abstract

Stegophorus macronectes (Johnston & Mawson, 1942) is a gastrointestinal parasite found in Antarctic seabirds. The original description of the species, which was based only on females, is poor and fragmented with some unclear diagnostic characters. This study provides new morphometric and molecular data on this previously poorly described parasite.Nuclear rDNA sequence (18S, 5.8S, 28S and ITS regions) was isolated from *S. macronectes* specimens collected from the chinstrap penguin *Pygoscelis antarctica* Forster on Deception Island, Antarctica. Using 18S rDNA sequences, phylogenetic analyses (Maximum Likelihood, Maximum Parsimony and Bayesian Inference) of the order Spirurida were performed to determine the phylogenetic location of this species. Primer pairs of the ITS regions were designed for genus-level identification of specimens, regardless of its cycle, as an alternative to coprological methods. The utility of this molecular method for identification of morphologically altered specimens is also discussed.

Introduction

Stegophorus macronectes (Johnston & Mawson, 1942) (Nematoda, Acuariidae) is a gastrointestinal parasite found in Australian, Subantarctic and Antarctic bird species (Barbosa & Palacios, 2009; Vidal et al., 2012; Diaz et al., 2013). The taxonomic classification of the species has changed since being first described. Originally described as *Paryseria macronectes* in the southern giant petrel *Macronectes giganteus* (Gmelin) and the grey-headed albatross *Thalassarche chrysostoma* (Forster) in South Australia (Johnston & Mawson, 1942), the species was later redescribed by Zdzitowiecki & Dróżdż (1980) based on specimens found in the type host *M. giganteus*, the subantarctic skua *Catharacta lonnbergi* (Mathews) and the sheathbill *Chionis alba* (Gmelin) all collected on King George Island, South Shetland Islands, Antarctica. These authors considered *Stegophorus paradeliae* Johnston & Mawson, 1945 and *Stegophorus adeliae* Johnston, 1938 *sensu* Petter 1959, both collected from penguins (*Pygoscelis adeliae* (Hombron & Jacquinot) and *P. papua*, respectively), to be identical to *S. macronectes* (see Johnston & Mawson, 1945; Mawson, 1953; Petter, 1959; Zdzitowiecki & Dróżdż, 1980). Although most reports given under this name or its synonyms contribute to the morphological description of the species (Johnston & Mawson, 1945; Mawson, 1953; Petter, 1959; Zdzitowiecki & Dróżdż, 1980), many were made on the basis of badly preserved, fragmented, and/or exclusively female specimens. Therefore, some of the more commonly used diagnostic characters may not be appropriate for species identification (e.g., the number of collarette teeth or the position of deirids or nerve ring). An updated morphological description of this species is, therefore necessary.

Accurate identification of parasites at any point of the life cycle is crucial for diagnosing infection. However, parasite identification using morphological characters can be problematic when only larvae or small portions of an individual are available (Zhu et al., 1998). Also, in some instances, preservation methods, such as freezing, can break the weak eggshell or cause morphological deformities making identification difficult (Pritchard and Kruse, 1982). Molecular

information from DNA sequences provides a high level of specificity for the diagnosis and identification of parasite species (Prichard & Tait, 2001). Thus, specific molecular probes for identification may provide a more reliable diagnosis compared with traditional techniques. With this in mind, we report the first molecular characterization of *S. macronectes.*

To date, only six of the over 150 currently described species of the family have been molecularly characterized, and only four species share a common molecular marker (Nadler et al., 2007; Honisch & Krone, 2008; Perera et al., 2013). Furthermore, previous phylogenetic analyses have only been done at the order level, with uneven representation of the main families (e.g., Blaxter et al., 1998; Nadler et al., 2007; Černotíková et al., 2011). Therefore, phylogenetic studies using molecular data from *S. macronectes*, among other species of the order, may reveal new evolutionary relationships for this understudied group of parasites.

The aims of this paper are as follows: a) describe the morphological features of *Stegophorus macronectes*, providing an updated description of the species; b) evaluate the reliability of the morphological traits used for identification; c) molecularly characterize the species; d) develop primer pairs for molecular diagnoses; and e) determine the phylogenetic position of the species within Spirurida.

Materials and methods

Collection and examination of nematodes

Acuarioid nematodes (n = 1157) were collected from 64 gastrointestinal tracts of recently deceased due to natural causes chinstrap penguins *Pygoscelis antarctica* (61 chicks and 3 adults). from the Vapour Col breeding colony on Deception Island (63°00' S, 60°40' W), South Shetland Islands, Antarctica, during the austral summers (December-February) from 2005 to 2009. Gastrointestinal packages were extracted, placed in labeled plastic bags and frozen at -20°C until analysis. In the laboratory, nematodes were recovered from the stomach and preserved in 70% ethanol. Parasite identification was based on morphometric features following a specific

bibliography (Johnston & Mawson, 1942, 1945; Petter, 1959; Yamaguti, 1961; Chabaud, 1974; Zdzitowiecki & Dróżdż, 1980).

Ten male and ten female relaxed and well-preserved specimens were measured. Rigid and/or badly preserved specimens that had morphological alterations were also analyzed for comparison. Nematodes were cleared with Amman lactophenol or 25% glycerin ethanol prior to observation under an optical microscope. Drawings were made with the aid of a camera lucida. Several specimens were dried using the critical point method (Bray, 2000), examined by scanning electron microscopy (JEOL-6100®) and photographed. Measurements (in micrometers unless otherwise stated) are reported as means with standard deviations in parentheses. In addition, collarette teeth in 815 individuals (556 females, 239 males and 20 immature specimens) were counted and differences analyzed using the Kruskal-Wallis test.

Molecular analysis

Several relaxed, well preserved individuals with precise morphological identification were chosen for DNA isolation following the protocol by Floyd et al. (2002). Nuclear rDNA sequences (18S, 5.8S, 28S and ITS regions) for *S. macronectes* were amplified using seven primer pairs (Table 1), three for 18S, one spanning the ITS regions and 5.8S and three for 28S. PCR amplifications were made using 50 µl of 1x Ecogen Taq buffer, 3 mM $MgCl_2$, 0.2 mM of each dNTP, 10 µM of each primer and 1 U of Taq polymerase (Ecogen). Two µl of isolated DNA were used as the template for each reaction. The PCR conditions began with an initial denaturation step at 94°C for five minutes followed by 36 cycles at 94ºC for one minute, 54ºC for one minute, and 72ºC for two minutes, and a final extension step at 72ºC for eight minutes. Five µl of each PCR product were checked in agarose gels stained with GelRed™ (Biotium) and then purified with the GFX™ PCR DNA and Gel Band purification kit (GE Healthcare). Fragments were then cloned into the pGEM® T-Easy vector (Promega) and sequenced with M13 primers in an "ABI PRISM™ 310 Genetic Analyzer" (Applied Biosystems) automatic sequencer.

The "ABI PRISM™ BigDye Terminator" (Applied Biosystems) method was used with a 60 second injection time and 120 minute run time. The "ABI Prism™ 310 Collection v.1.1.2" program was used for data acquisition and "v.3.0 Sequencing Analysis" for sequence analysis.

To diagnostically test for the presence of *S. macronectes*, species-specific primer pairs against the two ITS regions (ITS1 and ITS2), were designed. Primer pairs were validated in ten different worms by positive PCR amplification. Seven of the worms showed morphological alterations while the other three showed the morphology typically described for this species. PCR amplifications conditions were as stated above. In addition, primer pairs were also tested using an egg solution prepared from gravid females.

Primer pairs specificity was assessed by testing these primers in another species of the *Stegophorus* genus, *Stegophorus diomedeae* (Johnston & Mawson, 1942), isolated from *Thalassarche melanophris* Temminck (Chubut 2009), and in two other marine bird parasite genera of the family Acuariidae, *Syncuaria* sp., isolated from *Phalacrocorax brasilianus* Gmelin in Buenos Aires, Argentina in 2011, and *Paracuaria adunca* (Creplin, 1846) obtained from *Larus dominicanus* Lichtenstein in Chubut, Argentina in 2012. These specimens were collected, identified and provided by JID. DNA was isolated using the QIAamp® DNA Mini Kit (Qiagen), and PCR amplifications were performed using the aforementioned conditions.

Phylogenetic analyses

Sequences of 18S rDNA from Spirurida (n = 106) and from other orders (Strongylida, Oxyurida, Ascaridida and Rhabditida) were retrieved from GenBank (see supplementary material Table S1). Sequences, including *S. macronectes* were aligned in ClustalX (Thompson et al., 1997) using default settings. The resulting alignment was checked and adjusted with Se-Al v2.0a11 (Rambaut, 2002). A matrix with the final alignment was generated (available upon request from the corresponding author). The ITS regions, 5.8S and 28S sequences were not used

due to the small number of Spirurida sequences available in the GenBank. Gblocks (Castresana, 2000) was used to analyze the matrix. The complete and Gblocks matrices were compared.

The best fit model for nucleotide substitution in the resulting matrix was GTR+I+G, determined by the Akaike Information Criterion (AIC) in a jModelTest (Posada, 2008). Phylogenetic analyses were performed using PhyML v3.0 (Guindon & Gascuel, 2003) for Maximum Likelihood (ML), PAUP*v4.0b10 (Swofford, 2002) for Maximum Parsimony (MP), and MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003) for Bayesian Inference (BI).

Supports for ML and MP analyses were determined by performing 1000 bootstrap replicates. For BI analyses, five million generations were performed in two parallel runs, sampling trees at 1000-generation intervals. The first 10% of sampled trees were discarded as burn-in, and the remaining trees were used to calculate the posterior probabilities. The maximum clade credibility tree was generated by TreeAnnotator (Drummond & Rambaut, 2007).

Results

Morphology

Stegophorus macronectes (Johnston & Mawson, 1942). Spirurida, Acuariidae, Seuratinae. Synonyms: *Stegophorus paradeliae* Johnston & Mawson, 1945; *Stegophorus adeliae* Johnston, 1938 *sensu* Petter, 1959. General morphology (Fig. 1 A-F). Cuticle with fine transverse striations. Well developed pseudolabia. Cephalic papillae at the same level as amphids and a short distance posterior to the oral opening. Pronounced apical process on each pseudolabium. Cephalic ornamentation appears as a collarette composed of two lateral lobes (hemi-collarettes) (Fig. 1 A-B). Each lobe emerges from the commissures of the buccal lips and has a continuous series of a varying number of teeth on its posterior border (Fig. 1 A-B). A short buccal capsule is lined with fine transverse striations. Large deirids, tridentate, with a sharp or blunt-ended middle denticle, sometimes bifid at the tip and shorter than the lateral denticle (Fig. 1 C). Deirids, almost always symmetrical, are usually located behind the junction of the buccal capsule and the

oesophagus, but may sometimes occur at the same level (Fig. 1 A-B). Nerve ring usually located immediately posterior to buccal capsule-oesophagus junction, although occasionally located at the same level or less frequently anterior to it. Excretory pore posterior to nerve ring. Oesophagus straight, divided into muscular and glandular parts.

Males: Body length 7.15±1.26 mm, maximum width 135±25. Cephalic collarette length 56±2.6. Deirids at 184±30 from anterior end, 19±2 long by 18±1 wide. Nerve ring and excretory pore at 224±57.4 and 250 from anterior end, respectively. Buccal capsule length 196±55, muscular oesophagus length 602±41 and glandular oesophagus length 1570±30. Total oesophagus length 2170±23. Long caudal alae present. Four pairs of precloacal papillae, first and third pairs smaller than second and fourth pairs (Fig. 1 D). Six pairs of postcloacal papillae, first and second pairs close to each other. Last three pedunculated pairs equally distant from each other. Inconspicuous sessile pair (sixth) of papillae at the base of fifth pedunculated pair (Fig. 1 E). Phasmids just behind the last pair of papillae. Left spicule very thin, 1034±56 long, slightly dilated at distal end, ending in a sharply pointed tip. Right spicule 100±17 long, slightly bent, ending in a crescent-like process pointing toward the anterior region. Tail 199±20 in length (Fig. 1 D).

Females: (all measurements are for specimens with uteri containing mature eggs). Body length 17.7±3 mm, maximum width 300±71. Cephalic collarette length 87±10. Deirids at 201 ±30 from anterior end, 25.6±1 long by 24±3 wide. Nerve ring and excretory pore 239±32.6 and 331±71 from anterior end, respectively. Buccal capsule 201±41 long. Muscular and glandular oesophagus 1500±30 and 1350±68 long, respectively. Vulva located at the end of the second third of body length, 9.96 mm from anterior end (45% to 64% of body length) on a small cuticular protuberance. Vagina divided into vagina vera and vagina uterine (Fig. 1 F). Mature larvated eggs (measured in the uterus near the ovejector) 42±1 x 22±1. Tail 159±20 long.

Statistical analyses showed significant differences between the number of collarette teeth in males, females and immature specimens ($H_{2,813} = 35.25$ $p < 0.0001$). Immature specimens had

fewer teeth on each hemi-collarette (mean = 15±2.4) compared with adult males (mean = 18±2.6) and females (mean = 17±2.4).

Taxonomic summary

Type Host: *Macronectes giganteus* (Gmelin)

Site of infection: stomach

Type Locality: South Australia

Other Hosts: *Thalassarche chrysostoma* (Forster); *Pygoscelis papua* (Foster); *Eudyptes chrysolophus* (Brandt); *Eudyptes chrysocome* (Forster); *Catharacta lonnbergi* (Mathews); *Chionis alba* (Gmelin); *Pygoscelis adeliae* (Hombron & Jacquinot); *Pygoscelis antarctica* (Foster).

Other Localities: Heard Island; King George Island.

Host and locality of present material: *Pygoscelis antarctica* Forster; Deception Island (South Shetland Islands).

Mean intensity x ± SD and prevalence: 24.3±28.9 and 72% in chicks (n=61); 39.5±43. 9 and 67% in adults (n=3) (Vidal et al., 2012).

Voucher specimens were deposited in the Helminthological Collection, Museo de La Plata, La Plata, Argentina (MLP 6513) and in the Museo Nacional de Ciencias Naturales, Madrid, Spain (MNCN 11.01/403 and MNCN 11.01/404).

Remarks

The general morphology and measurements of the specimens described here fully agree with those of *S. macronectes* provided by other authors (see Zdzitowieki & Drodz, 1980; Table 2).

According to the measurements given by other authors, the species is characterized as having a variable number of teeth, between 15 and 21, on the posterior border of each hemi-

collarette (Table 2). Most specimens isolated in this study were within this range, however a few individuals had 11 teeth, while others had 27. Deirids are described as tridentate structures with the three cusps approximately equal in length, although the middle tooth is sometimes bifid (Johnston & Mawson, 1942, 1945; Zdzitowiecki & Dróżdż, 1980). We observed some deirids with a bifid middle tooth, and some with one or two external bifid teeth. We also observed some deirids with two smaller protrusions between the main teeth (Fig. 1 C). In one specimen, we observed a deirid with a bifid middle tooth, while the deirid located on the opposite side had non-bifid teeth (Fig. 1 C).

Previous authors have described the deirid position at the buccal capsule-oesophagus junction, or posterior to it (Johnston & Mawson, 1942, 1945; Mawson, 1953; Zdzitowiecki & Dróżdż, 1980), and in fact, have used this feature to distinguish the species (see Johnston & Mawson, 1945; Zdzitowiecki & Dróżdż, 1980). However, some specimens may have been deformed, especially at the anterior end. In this study, depending on the degree of contraction and specimen condition, we observed deirids appearing either before or after the buccal capsule-oesophagus junction..

Morphological features of *S. macronectes* match those of specimens studied by Petter (1959), who identified them as *S. adeliae* (Johnston, 1938). At present, the validity of *S. adeliae* appears doubtful. The original description was based on two distorted females, one which was incomplete, consisting of only the posterior part (Johnson, 1938). Later, one of the two type specimens was assigned to a new species, *S. paradeliae* (Mawson, 1945). Subsequently, other specimens, including males, were reported under this name (Mawson, 1953). Petter (1959), based on specimens from *P. papua*, considered *S. paredeliae* to be a synonym of *S. adeliae*. However, Zdzitowieki & Drodz (1980) considered *S. paradeliae* to be identical to *S. macronectes*. One author of this study (JID) examined ten female specimens found in *P. papua* from the Petter nematode collection at the Museum d'Histoire Naturelle de Paris and found them to be identical to *S. macronectes,* supporting the finding of Zdzitowieki & Drodz (1980).

Molecular analysis

Nuclear rDNA sequences (18S, 5.8S, 28S and ITS regions) for *S. macronectes* were cloned and sequenced. Sequences from seven PCR products were assembled resulting in a 6670 bp fragment (GenBank accession number HE793715) delimiting the 18S, 5.8S and 28S rDNA and ITS regions. Four species-specific primer pairs were then designed within the ITS regions for the molecular diagnosis of *S. macronectes* (Table 1).

Adult *S. macronectes* having either the typical or altered morphologies resulted in positive PCR amplifications with the four primer pairs in all analyses. Positive amplifications were also obtained with a *S. macronectes* egg solution and in *S. diomedeae,* supporting the use of these primer pairs for diagnosis of *Stegophorus* spp., regardless of life cycle stage or preservation condition. These four primer pairs were also tested in other Acuariidae genera.In *Syncuaria* sp., all reactions were negative. However, in *Paracuaria adunca* faint bands (slight amplification) were observed in reactions using the Steg2-ITS1 and Steg4-ITS2 primer pairs.

Phylogenetic analyses

The final alignment for the 18S rDNA matrix consisted of 119 sequences of 1933 bp (718 bp were variable and informative characters). Using Gblocks on the complete matrix yielded a matrix consisting of 1523 characters (525 bp were variable and informative characters). Phylogenetic analyses of the complete and Gblocks matrices resulted in similar findings for the major groups. However, the complete matrix consisted of more informative characters, resulting in trees that showed greater resolution. Results of the phylogenetic analyses with the complete matrix using BI, ML and MP approaches are summarized in Fig. 2.

Stegophorus macronectes was situated within the cluster (A), corresponding to the Spirurina suborder. Within this clade, *S. macronectes* appeared in a highly supported cluster (A2) that included other representatives of from the family Acuariidae, and representatives from the

Rhabdochonidae, Cystidicolidae and Physalopteridae families. However, most species from the Physalopteridae (collapsed in Fig. 2) family grouped together (A3) outside of this monophyletic assemblage.

There was no clear structure among the different families in Cluster A2 (Fig. 2). The Acuariidae family cluster, which *S. macronectes* belongs to, was not highly supported, and was related to *Ascarophis adioryx* (Cystidicolidae) with high bootstrap and posterior probability. In fact, species considered as belonging to the Cystidicolidae family were distributed among different clusters.

The Cluster A2 sister group was not clearly established due to a polytomy at this level. The relationships among clusters A2 and A3 (comprising the Physaloptera and Turgida (Physalopteridae) genera) and A1 (comprising representatives of the Onchocercidae, Tetrameridae, Thelaziidae, Setariidae, Diplotriaenidae, Spirocercidae, Habronematidae families, and Gongylonematidae) were not resolved.

The Philometridae, Dracunculidae, Skyrjabillanidae, Daniconematidae and Camallanidae families comprised a second large cluster in the Spirurida order (B), corresponding to the Camallanina suborder (this appears collapsed in Fig. 2). Of the four different orders taken as outgroups (each one represented by three different species), two (Rhabditida and Strongylida) were at the base of the tree; the other two (Ascaridida and Oxyurida) were more closely related to the two main Spirurida clusters (A and B) than to Cluster E, which consisted of two families also considered Spirurida (Anguillicolidae and Gnathostomatidae), thereby breaking the Spirurina and Camallanina suborder monophyly.

Discussion

The presence of a variable number of teeth on the posterior border of the collarette has been used as a diagnostic feature for delimiting species of the *Stegophorus* genera. However, our

results in *S. macronectes* show that the number of teeth can exceed the range described in the literature (see Table 2), suggesting that this feature increases asymmetrically during nematode development. Therefore, variations in the number of teeth on the collarette make this criterion a poor diagnostic feature, unless the difference in the number of teeth on each hemi-collarette is clearly delineated in the different species (e.g., ~10 in *S. diomedeae vs.* ~20 in *S. macronectes*). Moreover, deirid morphology and position are highly variable in this species. Deirids commonly are found at the level of the buccal capsule-oesophagus junction but can also be found at other levels, depending on specimen contraction and preservation.

The morphological deformities observed in some specimens are likely due to poor preservation, either because of the time elapsed from host death to collection or preservation by freezing. Parasites undergo internal and external changes, including internal decomposition and detachment of the cuticle when frozen (Pritchard & Kruse, 1982). During this process, the cuticle tends to move frontally while the body of the parasite retracts backwards, hindering specific identification of some traits, such as deirid position. In addition, the oesophagus and buccal capsule can move back and sometimes, the lateral lobes appear folded. The same problem was reported by Zdzitowiecki & Dróżdż (1980), who provided morphometric data for two *S. macronectes* specimens, one straight and one contracted. Using altered traits (i.e. deirid position relative to the buccal capsule and oesophagus) could lead to erroneously describing different species. However, our molecular results show that, although relaxed and contracted specimens may appear different, they belong to the same species.

Primer pairs designed from the sequences of ITS regions were used to test for the molecular detection of *S. macronectes*. Four primer pairs were validated against different species of *Stegophorus spp.* with positive results, thus proving useful at the genus level. However, two of the primer pairs (Steg2-ITS1 and Steg4-ITS2) were also positive for another genus of the Acuariidae family. Therefore, we recommend that the other two primer pairs (Steg1-ITS1 and Steg3-ITS2) be used for greater specificity.

The phylogenetic relationships of spirurid nematodes have been studied for many years (Blaxter et al., 1998; Nadler et al., 2007; Černotíková et al., 2011). Our results show that analyses of 18S rDNA sequences correctly classified *S. macronectes* within the Acuariidae family, though this was not strongly supported. The robustness of analyses were likely hampered by the scarcity of available sequences, for instance, for the 21 genera in this family (Skrjabin, 1949), there are only four 18S rDNA sequences available in the NCBI database. More sequences are therefore necessary to improve our knowledge of the relationships within the Acuariidae family.

The composition of the main Spirurida clades in our phylogenetic reconstructions is in agreement with previous studies (Černotíková et al., 2011): Clade A corresponds to Spirurina (except for the Gnathostomatidae family), Clade B to Camallanina (except for *Anguillicoloides crassus*), and Clade E is comprised of *A. crassus* and Gnathostomatidae, supporting the non-monophyly of Dracunculoidea and Spirurina. However, the relationships within these clades differ slightly from those in others studies (Nadler et al., 2007; Van Megen, 2009; Černotíková et al., 2011). More Spirurida sequences were used in this study and may account for these differences. Results for species belonging to other orders, such as Strongylida, Oxyurida, Ascaridida and Rhabditida, were not always in agreement with previous phylogenetic studies. This is because only three species per order were chosen at random as outgroups for these analyses.

In any case, our analyses only consisted of a single gene (18S), which may account for the inconsistencies observed between our phylogenetic reconstructions and the classical taxonomy of these groups. Additional genes should be included to clarify whether these results are a consequence of homoplasy of some morphological characters or simply represent the phylogenetic relationships of a particular gene.

Acknowledgements

We thank the Spanish Antarctic research base “Gabriel de Castilla”, the Spanish Polar ship “Las Palmas”, the Argentinean Antarctic Institute, and the Maritime Technology Unit (UTM-CSIC) for logistical support and transport. Permission to work and collect samples in the study area was given by the Spanish Polar Committee. We thank Maria Cristina Estivariz (CEPAVE), for help with the drawings. This is a contribution to International Polar Year Project 172 BirdHealth, the PINGUCLIM project and to the Unidad Asociada de Ecología e Inmunología parasitaria of the University of Murcia and the Spanish Council of Scientific Research (CSIC). Melinda Modrell corrected the English language usage. We greatly appreciate the reviewers’ comments, which have improved an early version of the manuscript.

Financial support

This study was funded by the Spanish Ministry of Economy and Competiveness and the European Regional Development Fund Projects CGL2004-01348, POL2006-05175, CGL2007-60369, and CTM2011-24427. VV was supported by a PhD grant from the Spanish Council of Scientific Research (CSIC) and the European Social Fund (JAEPre08-01053). MJP was supported by a PhD grant from the Spanish Ministry of Science and Innovation (BES2005-8465). JID is a member of CONICET and is partially supported by PIP 698 (CONICET), N628 and N726 (UNLP).

Statements of interest

No

References

Barbosa, A. & Palacios, M.J. (2009) Health of antarctic birds: a revision of their parasites, pathogens and diseases. *Polar Biology* **32**, 1095-1115.

Blaxter, M.L., De Ley, R., Carey, J.R., Liu, L.X., Scheldeman, R., Vierstraete, A., Vanfleteren, J.R., Mackey, L.Y., Dorris, M., Frisse, L.M., Vida, J.T. & Thomas, W.K. (1998) A molecular evolutionary framework for the phylum Nematoda. *Nature* **392**, 71-75.

Bray, D. (2000) Critical point drying of biological specimens for scanning electron microscopy. pp. 235–243 in Williams, J.R. & Clifford, A.A. (*Eds*) *Supercritical Fluid Methods and Protocols*. Inc, Totowa, NJ. Humana Press.

Castresana, J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17**, 540-552.

Černotíková, E., Horák, A., Moravec, F. (2011) Phylogenetic relationships of some spirurine nematodes (Nematoda: Chromadorea: Rhabditida: Spirurina) parasitic in fishes inferred from SSU rRNA gene sequences. *Folia Parasitologica* **58**, 135–148.

Chabaud, A.G. (1974) Keys to Genera of the Order Spirurida, Nº3, Part 2. pp. 29-58. in Anderson, R.C. , Chabaud, A.G. & Willmott, S. (*Eds.*) *Keys to the Nematode Parasites of Vertebrates*. Commonwealth Agricultural Bureaux, Farnhman Royal, Buckinghamshire, United Kingdom.

Diaz, J.I., Fusaro, B., Longarzo, L., Coria, N.R., Vidal, V., Jerez, S., Ortiz, J. & Barbosa, A. (2013) Gastrointestinal helminths of Gentoo penguins (*Pygoscelis papua*) from Stranger Point, 25 de Mayo/King George Island, Antarctica. *Parasitology Research* **112**, 1877–1881.

Drummond, A.J. & Rambaut A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 214.

Floyd, R., Abebe, E., Papert, A. & Blaxter, M. (2002) Molecular barcodes for soil nematode identification. *Molecular Ecology* **11**, 839-850.

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

Honisch, M. & Krone, O. (2008) Phylogenetic relationships of Spiruromorpha from birds of prey based on 18S rDNA. *Journal of Helminthology* **82**, 129-133.

Johnston, T.H. (1938) Parasitic Nematoda. Australasian Antarctic Expedition 1911-14 under the leadership of Sir Douglas Mawson. *Scientific Reports Series C, Zoology and Botany*, Vol X, Part I, 20 p.

Johnston, T.H. & Mawson, P.M. (1942) Nematodes from Australian albatrosses and petrels. *Transactions of The Royal Society of South Australia* **66**, 66–70.

Johnston, T.H. & Mawson, P.M. (1945) Parasitic Nematodes. *British Australian and New Zealand Antarctic Research Expedition Reports*, Series B. **5**, 73-160.

Mawson, P. (1953) Parasitic nematoda collected by the Australian National Antarctic Research Expedition: Heard Island and Mcquarie Island, 1948-1951. *Parasitology* **43**, 291-297.

Nadler, S.A., Carreno, R.A., Mejía-Madrid, H., Ullberg, J., Pagan, C., Houston, R., & Hugot, J-P. (2007) Molecular phylogeny of clade III nematodes reveals multiple origins of tissue parasitism. *Parasitology* **134**, 1421–1442.

Petter, A.J. (1959) [Redescription of *Paryseia adeliae* Johnston 1938; remarks on the genus *Paryseria* and the related genera *Rusguniella*, *Aviculariella*, *Proyseria* (gen. nov.), *Seuratia*]. *Annales de Parasitologie Humaine et Comparee* **34**, 322-330. (In French.).

Perera, J.P., Maia, M.C., Jorge, F. & Harris, D.J. (2013) Molecular screening of nematodes in lacertid lizards from the Iberian Peninsula and Balearic Islands using 18S rRNA sequences. *Journal of Helminthology* **87**, 189–194.

Posada, D. (2008) jMODELTEST: phylogenetic model averaging. *Molecular Biology and Evolution* **25**, 1253–1256.

Pritchard, M.H. & Kruse, G.O.W. (1982) *The Collection and Preservation of Animal Parasites*. Lincoln, Nebraska. University of Nebraska Press.

Prichard, R. & Tait, A. (2001) The role of molecular biology in veterinary parasitology. *Veterinary Parasitology* **98**, 169-194.

Rambaut, A. (2002) Se-Al. alignment editor. Version 2.0all, University of Oxford, Oxford, UK. Available: http://tree.bio.ed.ac.uk/software/seal/

Ronquist, F. & Huelsenbeck, J.P. (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19**, 1572–1574.

Skrjabin, K.I. (1949) *Key to parasitic nematodes*. IPST Press, Jerusalem.

Swofford, D.L. (2002) PAUP*: phylogeny analysis using parsimony (*and other methods). Version 4.0b10. Sunderland, M.A.: Sinauer Associated Inc.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analyses tools. *Nucleic Acids Research* **25**, 4876–4882.

Van Megen, H., Van den Elsen, S., Holterman, M., Karssen, G., Mooyman, P., Bongers, T., Holovachov, O., Bakker, J. & Helder, J. (2009) A phylogenetic tree of nematodes based on about 1200 full-length small subunit ribosomal DNA sequences. *Nematology* **11**, 927–950.

Vidal, V., Ortiz, J., Díaz, J.I., Ruiz de Ybañez, M.R., Amat, M.T., Palacios, M.J., Benzal, J., Valera, F., De la Cruz, C., Motas, M. & Barbosa, A. (2012) Gastrointestinal parasites in Chinstrap Penguins from Deception Island, South Shetlands, Antarctica. *Parasitology Research* **111**, 723-727.

Yamaguti, S. (1961) Nematodes of Birds. pp. 183-349 in Yamaguti, S. (*Ed.*) *Systema Helminthum*. New York, Interscience publishers INC.

Zdzitowiecki, K. & Dróżdż, J. (1980) Redescription of *Stegophorus macronectes* (Johnston et Mawson, 1942) and description of *Stegophorus arctowskii* sp. n. (Nematoda, Spirurida) from birds of South Shetlands (the Antarctic). *Acta Parasitologica* **26**, 205-212.

Zhu, X.Q., Gasser, R.B., Podolska, M. & Chilton, N.B. (1998) Characterisation of anisakid nematodes with zoonotic potential by nuclear ribosomal DNA sequences. Int. *Journal of Parasitology* **28**, 1911-1921.

Figure legends

Fig.1. *Stegophorus macronectes* from *Pygoscelis antarctica* to show. **A.** Female, anterior view. **B.** Male, anterior view. **C**. Deirids. **D**. Male, posterior end showing spiculae, papillae distribution, and sessile papilla (black arrow). (ls) left spicule, (rs) right spicule. **E.** Male, detail of postcloacal papillae showing sessile papillae (black arrow) and phasmids (white arrow). **F**. Female, detail of vulva (black arrow), vagina vera, vagina uterine, and eggs. (d) deirid, (bc) buccal capsule, (nr) nerve ring, (ep) excretory pore, (me) muscular oesophagus, (vv) vagina vera, (vu) vagina uterine.

Fig. 2. Phylogenetic relationship between species of the Spirurida order. Tree topology was inferred by Bayesian analysis, based on 18S rDNA. A, B, C, D, E and F show the main clades. The numbers on the main branches show the Bayesian posterior probability and bootstrap support found under Maximum Parsimony and Maximum Likelihood criteria, respectively. 1 shows strongly very well supported (pp=1; bootstrap=100). Stars mark other well-supported clades (pp≥0.95; bootstrap ≥ 70). Circles mark pp≥0.8; bootstrap ≥ 50. Triangles indicate pp≥0.8 and bootstrap ≥ 50 for at least one method).

Table legends

Table 1. Primers pairs used for sequencing (molecular characterization) 18S, 5.8S, 28S and molecular diagnosis of ITS regions of *Stegophorus macronectes*

Table 2. Measurements (means followed by range) of *Stegophorus macronectes*, given in the present study and by Zdzitowieki & Drozdz (1980); measurements in µm unless otherwise stated, n - number of specimens examined, n/a - number not given

Table 1. Primers pairs used for sequencing

Primer name	Sequence 5'→3'	Fragment length (bp)
18SF.1	CYG CGA AYG GCT CAT T	496
18SR.1	TTA CCG CGG CTG CTG G	
18SF.2	GGG CAA GTC TGG TGC C	643
18SR.2	TTG AGT CAA ATT AAG CCG	
18SF.3	CGG AAG GGC ACC ACC AGG	495
18SR.3	CGA CGG GCG GTG TGT AC	
5.8SF	GAT TAC GTC CCT GCC CTT TG	1795
5.8SR	CTT TCC CTY RCG GTA CTT G	
28SF.1	ACA AGT ACC GYR AGG GAA AG	1243
28SR.1	CGG CAG GTG AGT TGT TAC ACA C	
28SF.2	CCG CYA AGG AGT GTG TAA C	1449
28SR.2	AGG GTC TTC TTT CCC CGC	
28SF.3	GTA GCC AAA TGC CTC GTC	881
28SR.3	ACT TAG AGG CGT TCA G	
Steg1-ITS1F	GAT CAA ATG ATT GCA GCA TA	245
Steg1-ITS1R	GCA GCA GCA CAA TAA TAA TC	
Steg2-ITS1F	CGG TAG TGA TGA AGG ATA AGG A	196
Steg2-ITS1R	GAG AGC AAA TCA ATG CTA CAC A	
Steg3-ITS2F	CGC ATT TAA TGG CGT ATT TTC	166
Steg3-ITS2R	ATT AAT TGC GGC TAC AAA CG	
Steg4-ITS2F	GTT TGT AGC CGC AAT TAA TGA T	230
Steg4-ITS2R	AGA GAG AAA AAT TAT GCG CAA G	

Table 2. Measurements of *Stegophorus macronectes*

Characteristics	Present study		Zdzitowieki & Drozdz (1980)	
	Males (n = 10)	Females (n = 10)	Males (n = 34)	Females (n/a)
Total length (mm)	7.15 (5.5-8.6)	17.7 (12.75-20.92)	3.7-7.2	6.4-15.4
Maximum width	135 (100-160)	300 (220-460)	82-173	131-298
Collarette	56 (53-60)	87 (70-97)	51-74	71-109
Teeth	20 (18-22)	20 (18-22)	15-21 (both sexes)	15-21 (both sexes)
Buccal capsule	196 (140-300)	201 (150-270)	125-189	152-204
Nerve ring	224 (140-300)	239 (200-280)	161-224	190-263
Deirids (from anterior end)	184 (130-220)	201 (150-240)	134-230	151-265
Excretory pore (from anterior end)	250 (n=1)	331 (270-410)	229-339	268-390
Muscular oesophagus	602 (570-670)	1500 (1150-1710)	420-730	540-760
Glandular oesophagus (mm)	1.57 (1.49-1.62)	1.35 (1.30-1.43)	1.1-1.8	1.4-2.3
Right spicule	100 (67-120)		74-98	
Left spicule	1034 (960-1140)		710-1230	
Spicule ratio	9.7 (6.7-11.4)			
Precloacal papillae	4		4	
Postcloacal papillae	6		6	
Tail	199 (171-230)	159 (130-195)	125-185	140
Vulva (from anterior end) (mm)		9.96 (6.98-13.35)		4.1-8.6 (53-67%)
Egg length		22 (19-26)		42-46
Egg width		20 -24		20 -24

Figure 1
Click here to download high resolution image

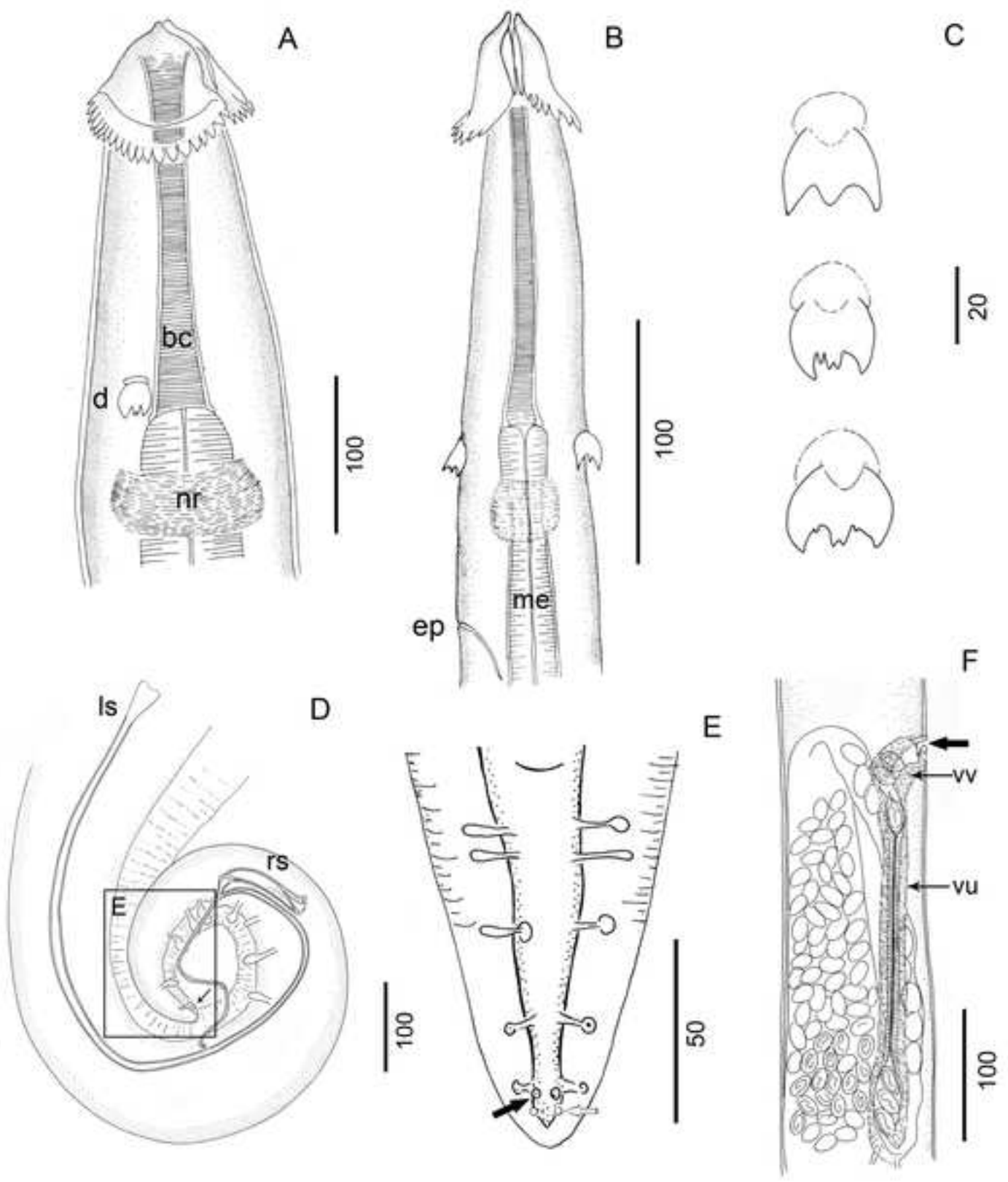
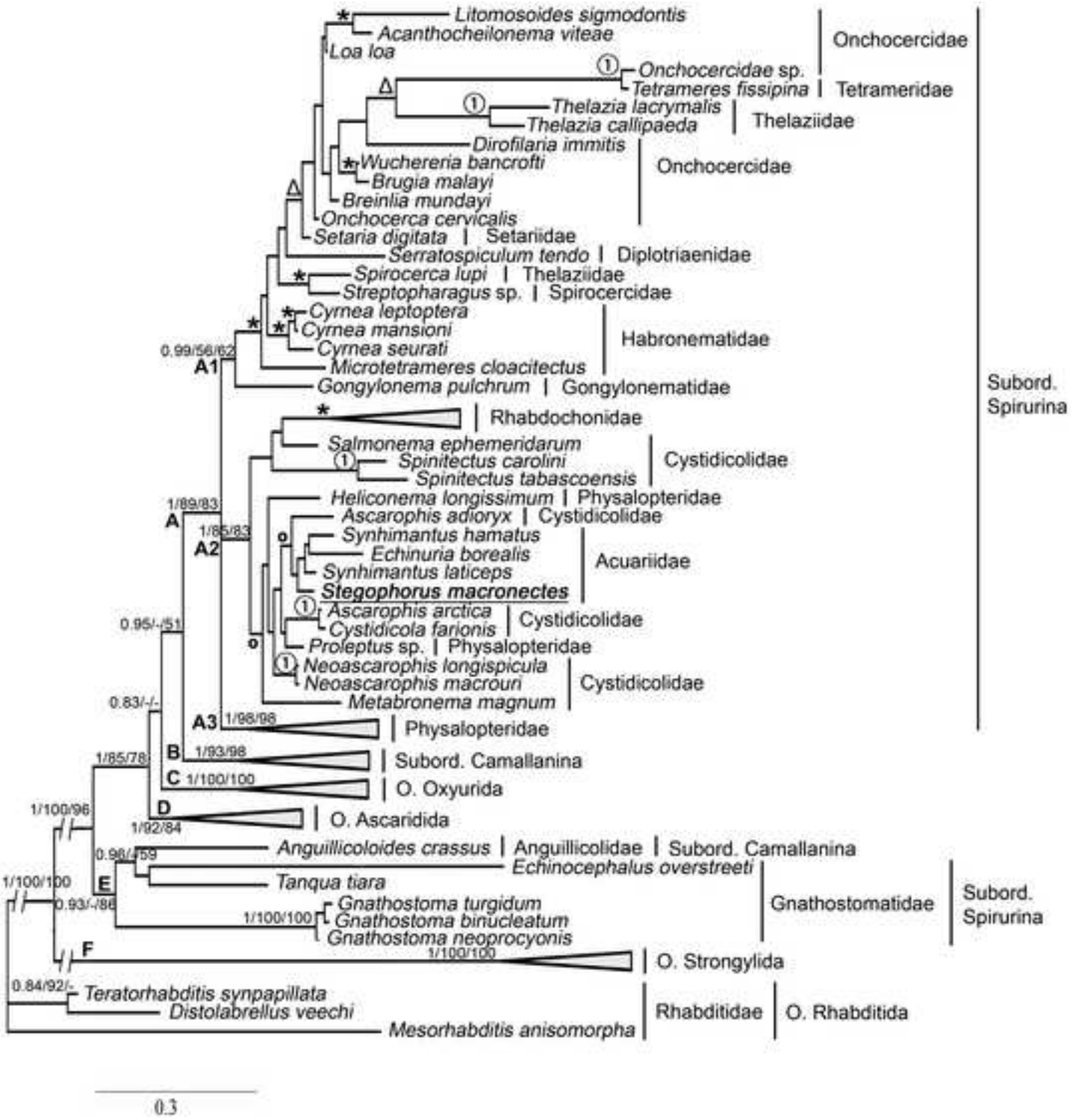


Figure 2

Click here to download high resolution image



Journal of Helminthology

Morphological, molecular and phylogenetic analyses of the spirurid nematode *Stegophorus macronectes* (Johnston & Mawson, 1942)

Virginia Vidal, Juana Ortiz, Julia Ines Diaz, Basilio Zafrilla, Maria Jose Bonete, Maria Rocio Ruiz De Ybañez, Maria Jose Palacios, Jesus Benzal, Francisco Valera, Carlos De La Cruz, Miguel Motas, Vanesa Bautista, Annie Machordom, Andres Barbosa

Table S1. GenBank information, species, families, and orders of nematodes used in phylogenetic analysis.

GenBank Accesion number	Species	Family	Suborder	Order
DQ094171	*Acanthocheilonema viteae*	Onchocercidae	Spirurina	Spirurida
JF803946	*Afrophilometra hydrocyoni*	Philometridae	Camallanina	Spirurida
DQ442672	*Alinema amazonicum*	Philometridae	Camallanina	Spirurida
DQ118535	*Anguillicoloides crassus*	Anguillicolidae	Camallanina	Spirurida
U94366	*Ascaris lumbricoides*	Ascarididae	-	Ascaridida
JF803930	*Ascarophis adioryx*	Cystidicolidae	Spirurina	Spirurida
DQ094172	*Ascarophis arctica*	Cystidicolidae	Spirurina	Spirurida
JF934735	*Breinlia mundayi*	Onchocercidae	Spirurina	Spirurida
AF036588	*Brugia malayi*	Onchocercidae	Spirurina	Spirurida
EF180071	*Camallanus cotti*	Camallanidae	Camallanina	Spirurida
JF803915	*Camallanus hypophthalmichthys*	Camallanidae	Camallanina	Spirurida
DQ442663	*Camallanus lacustris*	Camallanidae	Camallanina	Spirurida
DQ503463	*Camallanus oxycephalus*	Camallanidae	Camallanina	Spirurida
DQ442664	*Camallanus sp.*	Camallanidae	Camallanina	Spirurida
JF803939	*Caranginema americanum*	Philometridae	Camallanina	Spirurida
EU004815	*Cyrnea leptoptera*	Habronematidae	Spirurina	Spirurida
AY702701	*Cyrnea mansioni*	Habronematidae	Spirurina	Spirurida
EU004816	*Cyrnea seurati*	Habronematidae	Spirurina	Spirurida
JF803919	*Cystidicola farionis*	Cystidicolidae	Spirurina	Spirurida
DQ442673	*Dentiphilometra sp.*	Philometridae	Camallanina	Spirurida
AF036638	*Dirofilaria immitis*	Onchocercidae	Spirurina	Spirurida
AF082999	*Distolabrellus veechi*	Rhabditidae	-	Rhabditida

AY947719	*Dracunculus insignis*	Dracunculidae	Camallanina	Spirurida
JF934737	*Dracunculus lutrae*	Dracunculidae	Camallanina	Spirurida
AY947720	*Dracunculus medinensis*	Dracunculidae	Camallanina	Spirurida
AY852268	*Dracunculus medinensis*	Dracunculidae	Camallanina	Spirurida
AY852269	*Dracunculus oesophageus*	Dracunculidae	Camallanina	Spirurida
JF934729	*Echinocephalus overstreeti*	Gnathostomatidae	Spirurina	Spirurida
EF180064	*Echinuria borealis*	Acuariidae	Spirurina	Spirurida
Z96946	*Gnathostoma binucleatum*	Gnathostomatidae	Spirurina	Spirurida
Z96947	*Gnathostoma neoprocyonis*	Gnathostomatidae	Spirurina	Spirurida
Z96948	*Gnathostoma turgidum*	Gnathostomatidae	Spirurina	Spirurida
AB495401	*Gongylonema pulchrum*	Gongylonematidae	Spirurina	Spirurida
JF803949	*Heliconema longissimum*	Physalopteridae	Spirurina	Spirurida
GU245692	*Krefftascaris sharpiloi*	Ascarididae	-	Ascaridida
EF180073	*Leidynema portentosae*	Thelastomatidae	-	Oxyurida
AF227233	*Litomosoides sigmodontis*	Onchocercidae	Spirurina	Spirurida
DQ094173	*Loa loa*	Onchocercidae	Spirurina	Spirurida
AB185161	*Margolisianum bulbosum*	Philometridae	Camallanina	Spirurida
AF083013	*Mesorhabditis anisomorpha*	Rhabditidae	-	Rhabditida
JF803918	*Metabronema magnum*	Cystidicolidae	Spirurina	Spirurida
HM566089	*Mexiconema cichlasomae*	Daniconematidae	Camallanina	Spirurida
DQ442678	*Micropleura australiensis*	Dracunculidae	Camallanina	Spirurida
EU004814	*Microtetrameres cloacitectus*	Habronematidae	Spirurina	Spirurida
DQ442668	*Molnaria intestinalis*	Skyrjabillanidae	Camallanina	Spirurida
U01230	*Nematodirus battus*	Molineidae	-	Strongylida
JF803921	*Neoascarophis longispicula*	Cystidicolidae	Spirurina	Spirurida
DQ442660	*Neoascarophis macrouri*	Cystidicolidae	Spirurina	Spirurida
DQ442671	*Nilonema senticosum*	Philometridae	Camallanina	Spirurida
DQ094174	*Onchocerca cervicalis*	Onchocercidae	Spirurina	Spirurida
DQ103704	*Onchocercidae sp.*	Onchocercidae	Spirurina	Spirurida
EF180062	*Oxyuris equi*	Oxyuridae	-	Oxyurida
JF803948	*Philometra bagri*	Philometridae	Camallanina	Spirurida
JF803943	*Philometra brevispicula*	Philometridae	Camallanina	Spirurida
DQ442675	*Philometra cyprinirutili*	Philometridae	Camallanina	Spirurida
JF803942	*Philometra diplectri*	Philometridae	Camallanina	Spirurida
JF803928	*Philometra floridensis*	Philometridae	Camallanina	Spirurida

JF803916	*Philometra gymnosardae*	Philometridae	Camallanina	Spirurida
FJ161972	*Philometra lateolabracis*	Philometridae	Camallanina	Spirurida
JX456388	*Philometra lateolabracis*	Philometridae	Camallanina	Spirurida
JF803945	*Philometra lati*	Philometridae	Camallanina	Spirurida
FJ161974	*Philometra madai*	Philometridae	Camallanina	Spirurida
JF803933	*Philometra morii*	Philometridae	Camallanina	Spirurida
FJ161975	*Philometra nemipteri*	Philometridae	Camallanina	Spirurida
AY852267	*Philometra obturans*	Philometridae	Camallanina	Spirurida
JF803929	*Philometra ocularis*	Philometridae	Camallanina	Spirurida
DQ442677	*Philometra ovata*	Philometridae	Camallanina	Spirurida
JF803920	*Philometra saltatrix*	Philometridae	Camallanina	Spirurida
FJ161971	*Philometra sciaenae*	Philometridae	Camallanina	Spirurida
JF803944	*Philometra spiriformis*	Philometridae	Camallanina	Spirurida
JF803941	*Philometroides grandipapillatus*	Philometridae	Camallanina	Spirurida
DQ442676	*Philometroides sanguineus*	Philometridae	Camallanina	Spirurida
FJ155811	*Philometroides seriolae*	Philometridae	Camallanina	Spirurida
DQ442670	*Philonema oncorhynchi*	Philometridae	Camallanina	Spirurida
AY702703	*Physaloptera alata*	Physalopteridae	Spirurina	Spirurida
EU004817	*Physaloptera apivori*	Physalopteridae	Spirurina	Spirurida
JF934734	*Physaloptera thalacomys*	Physalopteridae	Spirurina	Spirurida
DQ503459	*Physaloptera turgida*	Physalopteridae	Spirurina	Spirurida
JF803932	*Procamallanus annulatus*	Camallanidae	Camallanina	Spirurida
JF803914	*Procamallanus fulvidraconis*	Camallanidae	Camallanina	Spirurida
JF803934	*Procamallanus laeviconchus*	Camallanidae	Camallanina	Spirurida
JF803931	*Procamallanus monotaxis*	Camallanidae	Camallanina	Spirurida
DQ442665	*Procamallanus pacificus*	Camallanidae	Camallanina	Spirurida
DQ442666	*Procamallanus pintoi*	Camallanidae	Camallanina	Spirurida
DQ442667	*Procamallanus rebecae*	Camallanidae	Camallanina	Spirurida
JF934733	*Proleptus sp.*	Physalopteridae	Spirurina	Spirurida
U94380	*Pseudoterranova decipiens*	Anisakidae	-	Ascaridida
DQ442659	*Rhabdochona denudata*	Rhabdochonidae	Spirurina	Spirurida
JF934732	*Rhabdochona guerreroensis*	Rhabdochonidae	Spirurina	Spirurida
JF803913	*Rhabdochona hellichi hellichi*	Rhabdochonidae	Spirurina	Spirurida
JF803937	*Rhabdochona hellichi turkestanica*	Rhabdochonidae	Spirurina	Spirurida
JF803938	*Rhabdochona hospeti*	Rhabdochonidae	Spirurina	Spirurida

JF803936	*Rhabdochona mazeedi*	Rhabdochonidae	Spirurina	Spirurida
JF803923	*Rumai rumai*	Philometridae	Camallanina	Spirurida
JF803927	*Salmonema ephemeridarum*	Cystidicolidae	Spirurina	Spirurida
AY702704	*Serratospiculum tendo*	Diplotriaenidae	Spirurina	Spirurida
DQ094175	*Setaria digitata*	Setariidae	Spirurina	Spirurida
DQ442669	*Skrjabillanus scardinii*	Skyrjabillanidae	Camallanina	Spirurida
DQ503464	*Spinitectus carolini*	Cystidicolidae	Spirurina	Spirurida
JF803922	*Spinitectus tabascoensis*	Cystidicolidae	Spirurina	Spirurida
EF180076	*Spirocamallanus istiblenni*	Camallanidae	Camallanina	Spirurida
JF934736	*Spirocamallanus philippinensis*	Camallanidae	Camallanina	Spirurida
DQ494195	*Spirocamallanus rarus*	Camallanidae	Camallanina	Spirurida
JF803912	*Spirocamallanus rarus*	Camallanidae	Camallanina	Spirurida
AY751497	*Spirocerca lupi*	Thelaziidae	Spirurina	Spirurida
HE793715	*Stegophorus macronectes*	Acuariidae	Spirurina	Spirurida
AJ920345	*Stephanurus dentatus*	Stephanuridae	-	Strongylida
HM067977	*Streptopharagus sp.*	Spirocercidae	Spirurina	Spirurida
EU004819	*Synhimantus hamatus*	Acuariidae	Spirurina	Spirurida
EU004818	*Synhimantus laticeps*	Acuariidae	Spirurina	Spirurida
JF934728	*Tanqua tiara*	Gnathostomatidae	Spirurina	Spirurida
AF083015	*Teratorhabditis synpapillata*	Rhabditidae	-	Rhabditida
EF180077	*Tetrameres fissipina*	Tetrameridae	Spirurina	Spirurida
EF180068	*Thelastoma krausi*	Thelastomatidae	-	Oxyurida
AB538282	*Thelazia callipaeda*	Thelaziidae	Spirurina	Spirurida
DQ503458	*Thelazia lacrymalis*	Thelaziidae	Spirurina	Spirurida
EF180069	*Turgida torresi*	Physalopteridae	Spirurina	Spirurida
AY843436	*Wuchereria bancrofti*	Onchocercidae	Spirurina	Spirurida
AJ920338	*Zoniolaimus mawsonae*	Cloacinidae	-	Strongylida