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The relationships of marsupial-dwelling Viannaiidae and description of *Travassostrongylus scheibelorum* sp. n. (Trichostrongylina: Heligmosomoidea) from mouse opossums (Didelphidae) from French Guiana

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Abstract: The trichostrongylid nematode *Travassostrongylus scheibelorum* sp. n. from the Linnaeus' mouse opossum, *Marmosa murina* (Linnaeus) (type host), and the woolly mouse opossum, *Marmosa demerarae* (Thomas), from French Guiana is described. The nematodes have a synlophe with ridges frontally oriented from right to left, six dorsal and six ventral, at midbody; seven dorsal and seven ventral posterior to the vulva, and two cuticular thickenings within the lateral spaces; a long dorsal ray and a pointed cuticular flap covering the vulva. This is the 12th species of *Travassostrongylus* Orloff, 1933, which includes species featuring ridges around the synlophe and a didelphic condition. These traits contrast with those in other genera in the Viannaiidae Neveu-Lemaire, 1934, which feature ventral ridges on the synlophe of adults and a monodelphic condition. Members of the family are chiefly Neotropical and are diagnosed based on the presence of a bursa of the type 2-2-1, 2-1-2 or irregular, and cuticle without ridges on the dorsal side (at least during one stage of their development). Herein, we present a reconstruction of the ancestral states of the didelphic/monodelphic condition and the cuticular ridges that form the synlophe in opossum-dwelling trichostrongyles, namely *Travassostrongylus* and *Viannaia* Travassos, 1914. Our investigations suggest they are not reciprocal sister taxa and that the change from didelphy to monodelphy and the loss of dorsal ridges, occurred in the common ancestor of species of *Viannaia*. These results suggest a synlophe with three ventral ridges is not plesiomorphic in the opossum dwelling trichostrongylids.

Keywords: *Viannaia*, *Hoineffia*, Didelphidae, host-switching, coalescence, coevolution

This article contains supporting information (Table S1, S2) online at http://folia.paru.cas.cz/suppl/2014-3-242.pdf

Viannaiidae Neveu-Lemaire, 1934 includes trichostrongyline nematodes with a combination of the following characteristics: (i) synlophe with either three ventral ridges or with the ridges arranged with a frontal axis of orientation; (ii) a symmetrical or asymmetrical bursa, with rays arranged as type 2-2-1 or 2-1-2; and (iii) with didelphy or monodelphy. The family includes two subfamilies: the monotypic Hydrochoerisnematinae Arantes et Artigas, 1983, which includes *Hydrochoerisnema anomalobursata* Arantes et Artigas, 1980, and Viannaiinae Neveu-Lemaire, 1934, which includes six genera: *Avellaria* Freitas et Lent, 1934, *Hoineffia* Diaw, 1976 *Oswaldonema* Travassos, 1927, *Travassostrongylus* Orloff, 1933, *Viannaia* Travassos, 1914, and *Viannella* Travassos, 1920 (Durette-Desset 1983, Durette-Desset et al. 2006).

Travassostrongylus, *Viannaia* and *Hoineffia* are the three genera known to occur in New World marsupials. However, species of *Travassostrongylus* are morphologically distinct from species in the other genera. *Travassostrongylus* is characterized by a synlophe with five dorsal and five ventral cuticular ridges oriented frontally, from right to left, with the absence of lateral ridges. Males have a bursal ray arrangement of the type 2-1-2 and the females are didelphic with a symmetrical ovejector. Conversely, *Viannaia* is traditionally defined by having a cordiform bursa, often with an elongated dorsal ray, whereas *Hoineffia* is defined by possessing transversally elongated lateral bursal lobes and short dorsal ray. Males of both genera have a synlophe consisting of three ventral ridges and monodelphic females (Durette-Desset 1974, 1983).

Travassostrongylus is currently included in the Heligmosomoidea Travassos, 1914 yet it was historically associated with species currently in Trichostrongyloidea Leiper, 1908 and Molineoidea Skryabin et Schulz, 1937 (see Orloff 1933, Neveu-Lemaire 1934, Travassos 1937, Skryabin et al. 1954, Chabaud 1959, Durette-Desset 1974).

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The genus was placed in Viannaiidae based on a) the taxonomy of their hosts and their biogeography, b) a resemblance between the caudal bursa of species of *Travassostrongylus* and *Hoineffia* (Diaw 1976, Durette-Desset and Chabaud 1977), and c) the presence of three ventral ridges in the synlophe during at least one stage of their development (Diaw 1976). The latter was considered a plesiomorphy for the family since three ridges are present in larvae and adults of all species of *Viannaia* and *Hoineffia*, whereas three ventral ridges are observed in the synlophe of larvae of species of *Travassostrongylus*, which subsequently have 10 ridges in the adults (Diaw 1976). No member of this family has been included in phylogenetic reconstructions of Trichostrongylina based on molecular characters (de Bellocq et al. 2001, Audebert et al. 2005).

The number of ridges in the synlophe has been used as an important character in the taxonomy and the interpretation of the evolution of Herpetostrongylidae Humpherey-Smith (1983), Viannaidae by Diaw (1976), Durette-Desset (1985) and Cassone et al. (1986), and Heligmosomidae by Durette-Desset (1985). A parallel increase in the number of ridges has been inferred for all three lineages, considering the presence of three ventral ridges in the synlophe a plesiomorphic condition for Heligmosomoidea (Cassone et al. 1986). In contrast, the number of uterine branches is considered an unreliable character because it has appeared multiple times in several lineages of trichostrongyloid nematodes (Durette-Desset and Chabaud 1977).

To evaluate the evolution of the opossum-dwelling members traditionally considered part of Viannaiidae, we attempted to reconstruct the ancestral states for the didelphic/monodelphic condition as well as for the cuticular ridges that form the synlophe in Heligmosomoidea. As part of the examination of variability of characters, a new species of nematode was discovered. This new taxon is described herein.

MATERIALS AND METHODS

Collection and isolation of specimens

Specimens were collected between 2004 and 2011 in Argentina, Australia, Costa Rica, French Guiana, Mexico, Panama and the United States of America. Preservation, clearing, and mounting of parasites followed Pritchard and Kruse (1982). Type specimens were deposited in the Collection Helminthologique du Muséum National d'Histoire Naturelle, Paris, France (MNHN), the Harold W. Manter Laboratory of the University of Nebraska, Lincoln, USA (HWML), and the Institute of Parasitology, České Budějovice, Czech Republic (IPCAS). Nematodes were cleared in lactophenol and mounted on temporary slides.

All measurements are given in micrometres; for each character, the range is given first, followed by the average, coefficient of variation, and sample size (when different from number of specimens used in the description). The coefficient of variation is presented to introduce a metric that allows the detection of hypervariable characters and assist in the determination of what measurements and characters are reliable for the discrimination of species (Sokal and Rohlf 1995). Mammalian specimens are part of the holdings of the Muséum d'Histoire Naturelle de la Ville de Genève, Switzerland (MHNG), and the Muséum National d'Histoire Naturelle, Paris, France (MNHN).

DNA extraction and amplification

Photographs of complete specimens were taken prior to DNA extraction. Sections were made just posterior to the oesophageal-intestinal junction and anterior to the spicules. Both anterior and posterior ends were fixed and mounted on temporary slides with alcohol or glycerine for species identification and to serve as museum vouchers. Genomic DNA was extracted from the midsections of male individuals using Chelex (BioRad, Hercules, USA) or QIAGEN DNeasy blood and tissue kit (Qiagen, Valencia, USA), following manufacturer's recommendations. Five nuclear and mitochondrial DNA regions were amplified, including the small subunit 18S rRNA gene, the first and second internal transcribed spacers (ITS-1 and ITS-2) as well as the 5.8S rDNA, the mitochondrial large ribosomal subunit RNA (*rrnL*), cytochrome c oxidase subunit I (*cox1*), and the cytochrome *b* mitochondrial gene (*cob*).

A region (≈1 629 bp) of the 18S rRNA gene was amplified by PCR as two overlapping fragments. The primers 988F, 5'-CTCAAAGATTAAGCCATGC-3', and 1912R, 5'-TTTACGGTCAGAACTAGGG-3', were used for the first fragment. The set 1813F, 5'-CTGCGTGAGAGGTGAAAT-3', and 2646R, 5'-GCTACCTTGTTACGACTTTT-3', amplified the second fragment (Holterman et al. 2006). Thermal profile used for both sets includes 95 °C/5 min, (95 °C/30 s, 45 °C/30 s, 72 °C/70 s) × 5, (94 °C/30 s, 54 °C/30 s, and 72 °C/70 s) × 35, with a final extension at 72 °C/5 min.

A continuous region of rDNA (≈890 bp) including ITS-1, 5.8S, and ITS-2 was amplified using the primers NC5, 5'-GTAGGTGAACCTGCGGAAGGATCATT-3', and NC2, 5'-TTAGTTTCTTTTCCTCCGCT-3' (Gasser et al. 1993), with a thermal profile of 94 °C/90 s (94 °C/30 s, 53 °C/45 s, 72 °C/90 s) × 34 cycles, followed by an extension at 72 °C/10 min.

A fragment of the mitochondrial large ribosomal subunit RNA (*rrnL*) (≈873 bp) was amplified using the primers C2F3, 5'-CGTCAATGTTCAGAAATTTGTGG-3', and CE16SR, 5'-ATTCTATCTCACAATGAATTAAAC-3' (Jiménez et al. 2012). Alternatively, this region was amplified in two overlapping fragments using primers C2F3 and Tr480R, 5'-ATGTCCTCACGCTAAGACTGCC-3', for the first fragment and Tr480, 5'-GGCAGTCTTAGCGTGAGGACAT-3', and CE16SR for the second fragment. Thermal profile for reactions with the primers C2F3 and CE16SR or C2F3 and Tr480R consisted of 94 °C/3 min (94 °C/60 s, 49 °C/60 s, and 72 °C/90 s) × 34 with a final extension at 72 °C/5 min. Thermal profile for primer set Tr480 and CE16SR consisted of 94 °C/90 s (94 °C/30 s, 48 °C/60 s, and 70 °C/60 s) × 34, followed by an extension at 70 °C/10 min.

A fragment (≈688 bp) of *cox1* was amplified by PCR using the primers NCOIf1, 5'-CCTACTATGATTGGTGGTTTTGGTAATTG-3', and NCOIr2, 5'-GTAGCAGCAGTAAAATAAGCAC-3' (Kanzaki and Futai 2002). Reactions were subject to 94 °C/120 s, [94 °C/30 s, 48 °C/30 s, and 72 °C/60 s] × 34, followed by an extension at 72 °C/10 min.

Finally, a fragment (≈711 bp) of *cob* was amplified using the primers cytb.1Fc, 5'-GRAATTTTGGTAGTATRTTRG-3',

Table 1. Accession numbers of voucher specimens deposited in the Harold W. Manter Laboratory Parasitology of the University of Nebraska, their collection localities and host species, and corresponding GenBank accession numbers

Nematode species	Coll. Nos.	Locality	Host species	18S	ITS-1, 5.8S, ITS-2	*rrnL*	*cox1*	*cob*
Austrostrongylus victoriensis Cassone, 1983	67161	Buangor, Victoria, Australia	*Wallabia bicolor* (Desmarest)	JX877668	JX877697	-	-	-
Austrostrongylus victoriensis	67162	Buangor, Victoria, Australia	*Wallabia bicolor*	JX877684	JX877685	JX877704	JX877727	-
Carolinensis perezponcedeleoni Jiménez, 2012	67163	Catemaco, Veracruz, Mexico	*Nyctomys sumichrasti* Saussure	JX877678	JX877686	JX877708	-	JX877719
Hassalstrongylus sp.	67164	Santa Bárbara, Jujuy, Argentina	*Calomys* sp.	JX877679	JX877694	JX877698	JX877723	JX877713
Odilia bainae Beveridge et Durette-Desset, 1992	67165	Blackwood, Victoria, Australia	*Rattus fuscipes* (Waterhouse)	JX877683	-	JX877703	-	-
Oswaldocruzia sp.	67166	Carbondale, Illinois, USA	*Bufo americanus* Holbrook	JX877669	-	-	-	-
Travassostrongylus callis Travassos, 1914,	67167	Parque Natural Metropolitano, Panamá	*Didelphis marsupialis* Linnaeus	JX877677	-	JX877699	JX877724	JX877714
Travassostrongylus orloffi Travassos, 1935	67168	Temapache, Veracruz, Mexico	*Didelphis marsupialis*	JX877671	-	JX877707	JX877730	JX877718
Travassostrongylus orloffi	67169	Temapache, Veracruz, Mexico	*Didelphis marsupialis*	-	-	-	JX877734	-
Travassostrongylus scheibelorum sp. n.	67170	Cacao, Guyane, French Guiana	*Marmosa demerarae* (Thomas)	JX877670	JX877690	-	-	-
Travassostrongylus scheibelorum sp. n.	67171	Cacao, Guyane, French Guiana	*Marmosa demerarae*	-	JX877691	JX877706	JX877729	-
Vexillata convoluta (Caballero et Cerecero, 1943)	67172	Huitzilac, Morelos, Mexico	*Cratogeomys merriami* (Thomas)	JX877672	JX877692	-	JX877732	-
Viannaia didelphis Travassos, 1914	67173	Colonia Bolanos, Guanacaste, Costa Rica	*Didelphis marsupialis*	JX877676	JX877688	JX877705	JX877728	JX877717
Viannaia didelphis	67174	Parque Natural Metropolitano, Panamá	*Didelphis marsupialis*	JX877674	JX877689	JX877712	JX877731	JX877722
Viannaia hamata Travassos, 1914	67175	Carbondale, Illinois, USA	*Didelphis virginiana*	-	-	JX877709	-	-
Viannaia hamata	67176	Cacao, Guyane, French Guiana	*Marmosa demerarae*	JX877680	JX877695	JX877701	-	JX877720
Viannaia minispicula Guerrero, 1985	67177	Cacao, Guyane, French Guiana	*Marmosa demerarae*	JX877682	JX877696	JX877711	-	-
Viannaia viannai Travassos, 1918	67178	Carbondale, Illinois, USA	*Didelphis virginiana* Kerr	JX877681	-	-	JX877733	-
Viannaia viannai	67179	Colonia Bolanos, Guanacaste, Costa Rica	*Didelphis marsupialis*	JX877675	-	JX877710	-	JX877721
Viannaia viannai	67180	Parque Natural Metropolitano, Panamá	*Didelphis marsupialis*	-	JX877693	JX877702	JX877726	JX877716
Viannaia viannai	67181	Parque Natural Metropolitano, Panamá	*Didelphis marsupialis*	JX877673	JX877687	JX877700	JX877725	JX877715

and cytb.1R, 5'-AGMACGYAAAATWGYAWAAGC-3' (Nieberding et al. 2006). Thermal profile consisted of 94 °C/90 s, (94 °C/30 s, 50 °C/45 s, and 72 °C/90 s) × 40, followed by an extension at 72 °C/10 min.

Reactions were completed in volumes of 20 µl including the primers, DNA template and a mix of reagents included in the Taq DNA Polymerase kit (Qiagen), following manufacturer's recommendations and previously described thermal profiles. PCR products were cleaned using exonuclease I-shrimp alkaline phosphatase (ExoSAP-IT) (GE Healthcare, Cleveland, USA) to remove excess primers and nucleotides. Sequencing was conducted in both directions using BigDye 3.2 (BigDye™ Chemistry Perkin-Elmer Applied Biosystems, Norwalk, USA) and directly sequenced in an ABI 3130xl gene sequencer in the Conservation Genetics Laboratory of Southern Illinois University (Carbondale, IL). Voucher specimens were deposited in the HWML and resulting sequences were uploaded to GenBank (Table 1).

Phylogenetic reconstruction

Resulting sequences were aligned with sequences downloaded from GenBank (Table 2). Sequence alignment was performed with ClustalW (Larkin et al. 2007), using defaults. Ribosomal DNA alignments were further analysed by using GBlocks (Castresana 2000). Differences in rates of evolution and the degree of variability in ITS-1, 5.8S and ITS-2 made GBlocks necessary to confirm an unambiguous alignment of this region. One thousand four hundred and fifty-five (1 455) characters were removed from the alignment, resulting in a dataset of 170 characters.

Selection of substitution models was performed using PHYML (Guindon and Gascuel 2003) as implemented in jModelTest v2.1.1 based on the best fitting model indicated by the corrected Akaike information criterion for individual genes (Darriba et al. 2012). The following models of evolution were selected for each matrix: GTR + I for 18S, GTR + G for ITS, and GTR + I + G for *rrnL*, *cob*, and *cox1*.

Table 2. Accession numbers of sequences utilised from GenBank and host species of parasites.

Nematode Species	GenBank accession numbers							Host species
	18S	ITS-1	5.8S	ITS-2	rrnL	cox1	cob	
Haemonchus contortus (Rudolphi, 1803)	EU086374	AB682687	AB682687	AB682687	NC010383	NC010383	NC010383	*Ovis aries* Linnaeus
Mecistocirrus digitatus Dujardin, 1845	-	AB222060	AB222060	AB222060	NC013848	NC013848	NC013848	*Ovis aries*
Nippostrongylus brasiliensis Travassos, 1914	AF036597	AY332646	-	AY333380	-	AF263480	-	*Rattus* sp.
Trichostrongylus axei (Cobbold, 1879)	-	Y15875	Z70742	X78065	GQ888719	GQ888719	GQ888719	*Ovis aries*
Trichostrongylus colubriformis (Giles, 1892)	AJ920350	JF680985	JF680985	JF680985	-	-	-	*Ovis aries*
Trichostrongylus vitrinus (Looss, 1905)	-	JF680986	JF680986	JF680986	GQ888711	GQ888711	GQ888711	*Ovis aries*

Clock-like behaviour was tested for in each of the datasets. Deviation from clock-like rates of substitution in each alignment was determined by using a likelihood-ratio test (LRT) as implemented in the program PAUP*. Each gene sequence alignment showed significant substitution rate variation that was high among lineages. It was apparent with these nucleotide substitution rates that the data sets did not behave as stochastically constant molecular clocks.

Each gene region was analysed for phylogenetic signal independently. Phylogenetic signal was analysed using Maximum Likelihood as optimality criterion, as implemented in the program raxmlGUI (Silvestro and Michalak 2012). The same program was used to calculate a thorough bootstrap support for all branches by running 1 000 samples with 10 replicates. Posterior probabilities of all branches were calculated using Bayesian-based inference as implemented in MrBayes 3.2 (Ronquist et al. 2012). Each phylogeny was rooted with *Trichostrongylus colubriformis* (Giles, 1892), *T. axei* (Cobbold, 1879), *T. vitrinus* (Looss, 1905), *Haemonchus contortus* (Rudolphi, 1803) and *Mecistocirrus digitatus* (von Linstow, 1906).

Separate analyses were performed on each independent data set and the resulting topologies were compared. Data sets with concordant topologies were concatenated, resulting in a nuclear and a mitochondrial data set. These were used to carry out two separate phylogenetic analyses. Each analysis used maximum likelihood as optimality criteria and Bayesian inference as implemented in RAxML and MrBayes, respectively.

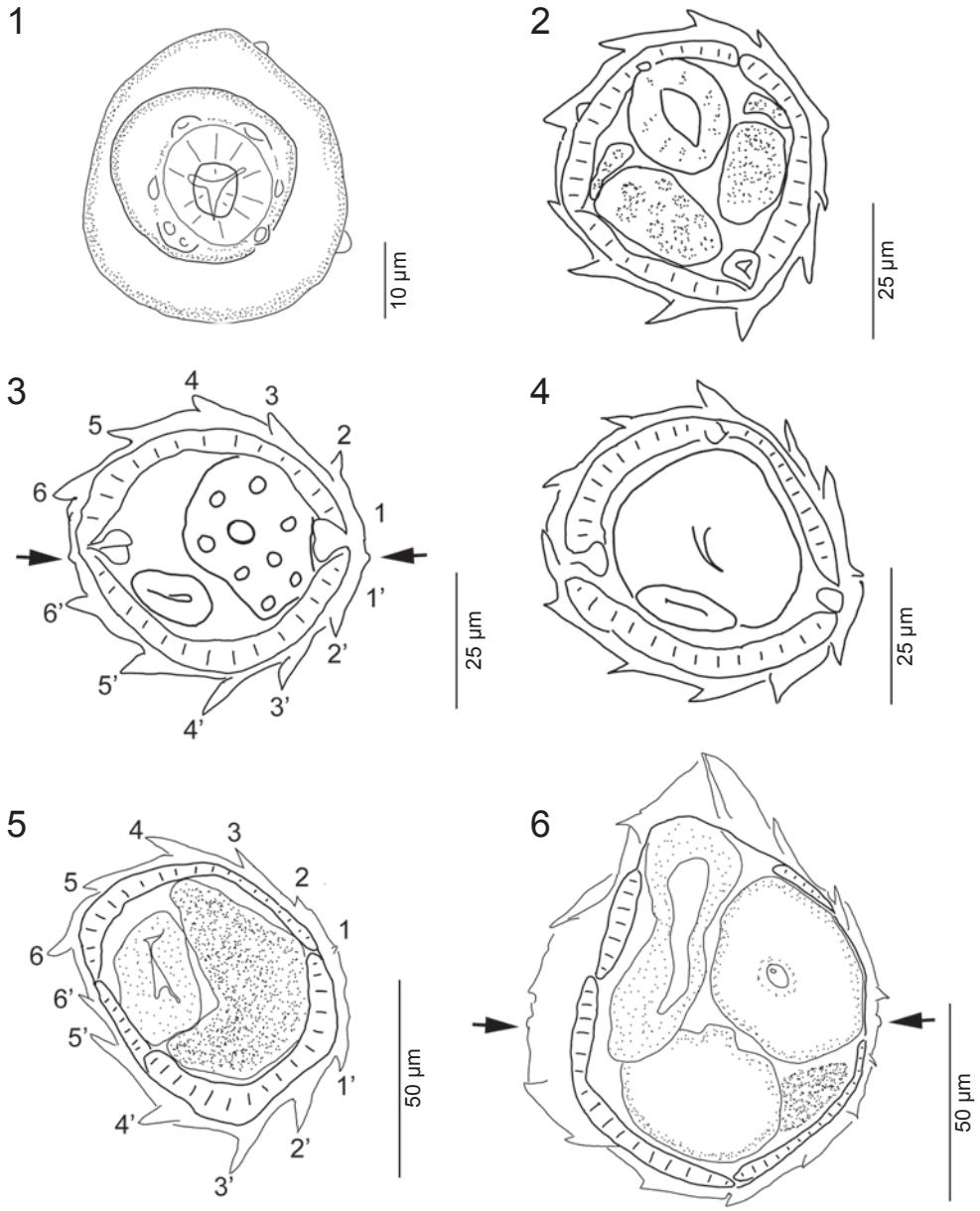
All five data matrices were framed under a coalescent approach. The species tree was calculated by means of algorithms implemented in the program BEAST v1.7.4 (Drummond et al. 2012), by enabling the starBEAST option to estimate the gene trees, which are 'embedded' in a species tree (Heled and Drummond 2010). All five gene sequence alignments were used to reconstruct the species tree under a Yule model (Steel and McKenzie 2001) with the following assumptions: a random local molecular clock, a general time reversible substitution model with an AICc selected site heterogeneity model and four categories for each matrix, three codon site partitions for protein coding regions, and MCMC chain length of 300 million, sampling every 10 000 iterations. Tracer v1.5.0 (Rambaut and Drummond 2009) was utilised to determine Markov chain convergence and adequate sampling. Effective Sample Sizes (ESSs) of traces greater than 300 were accepted. Low ESSs of traces were considered to contain correlated samples and would not adequately represent the posterior distribution.

Character reconstruction

The ancestral states of the major clades in the ingroup were reconstructed by scoring the corresponding character state in each of the terminal taxa used in the resulting phylogenies of Heligmosomoidea. In the first test, corresponding to the reconstruction of the ancestral number of uterine branches, terminal taxa were scored as either monodelphic (0) or didelphic (1). In the second test, corresponding to the reconstruction of the ancestral synlophe configuration, terminal taxa were scored as either having a synlophe with ventral ridges (0) or a synlophe with dorsal and ventral ridges (1). Due to differences in their respective tree topologies, separate reconstructions were carried out using the resulting trees from the nuclear and mitochondrial dataset. An additional reconstruction was carried out using the resulting trees from the calculation of the species tree.

The ancestral states in the nodes of the phylogeny of Heligmosomoidea were reconstructed using a Bayesian reversible-jump Markov chain Monte Carlo simulation as implemented in BayesTraits 1.0 (Pagel et al. 2004) and for each of the 30 000 trees that were produced from both chains of Bayesian analyses. This program derives the posterior probabilities of character states or traits at internal nodes of phylogenies in order to indicate the trait or character state that best fits. The ancestral reconstruction of the female reproductive system and the synlophe were performed with a hyperprior exponential seeded between 0 and 30. The rate deviation was set at 30 after multiple preliminary analyses enabled us to anticipate that resulting acceptance rates would be between 20% and 40% (Pagel et al. 2004).

Analyses were conducted through 100 million iterations with the first 100 000 samples discarded as burn-in with sampling every 1 000th generation. The harmonic means over three repetitions of the analysis of one constraint were averaged and compared to the harmonic means of the alternative constraint. Harmonic means were used since they can approximate marginal likelihood. More specifically, the integral of the model likelihoods over the values of the model parameters as well as over potential trees is equal to the marginal likelihood. The harmonic means were used to calculate Bayes factors. Strong support for the reconstruction of a particular character state at a node was indicated by a Bayes factor greater than two units (Kass and Raftery 1995).



Figs. 1–6. *Travassostrongylus scheibelorum* sp. n. from *Marmosa demerarae*. **Fig. 1.** Frontal view of anterior end. **Figs. 2–4**. Synlophe of male; levels of nerve ring, midbody and anterior to bursa, respectively. **Figs. 5**, **6.** Synlophe of female; levels of midbody and anterior to anus, respectively. Arrows in Figs. 3 and 5 show lateral thickenings, integers with apostrophe denote ventral ridges.

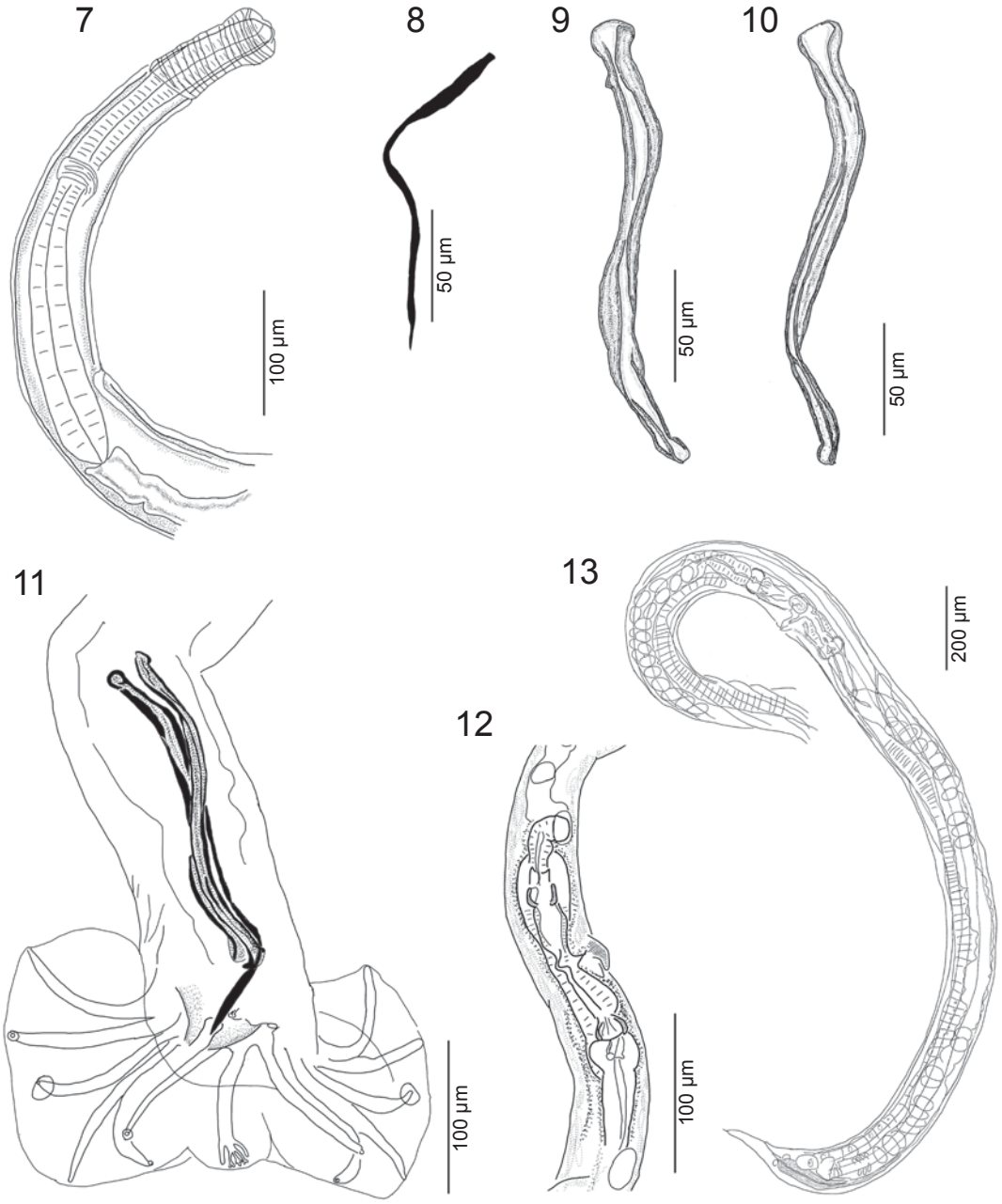
RESULTS

Travassostrongylus scheibelorum sp. n. Figs. 1–13

General: (based on holotype, allotype and 46 paratypes) Medium sized, coiled nematodes. Sexually dimorphic, females larger than males. Excretory pore posterior to nerve ring.

Anterior end: Striated cephalic vesicle present, divided in 2 portions; anterior portion slightly swollen, posterior portion cylindrical, ventral side shorter than dorsal (Fig. 7). Two lateral amphids and 4 submedial cephalic papillae (Fig. 1). Delicate buccal ring present.

Synlophe (based on 5 males and 9 females, Figs. 2–6): Cuticle with continuous, longitudinal ridges beginning posterior to cephalic vesicle, ending anterior to bursa in males and anus in females. Axis of synlophe frontal; ridges oriented from right to left gradually increasing in size at anterior, midbody and posterior levels. Ridges absent from lateral fields; lateral fields contain 2 thickenings (Figs. 3, 6), differing from ridges. Six dorsal and six ventral ridges at anterior and midbody in both sexes (Figs. 3, 6). Seven dorsal and seven ventral ridges posterior to vulva in females (Fig. 6). Ridges of variable size (Figs. 2–6), shortest on right side, flanking right cuticular process; size increases slightly towards left.



Figs. 7–13. *Travassostrongylus scheibelorum* sp. n. from *Marmosa demerarae*. **Fig. 7.** Anterior end, lateral view, showing cephalic vesicle, nerve ring and excretory pore. **Fig. 8.** Gubernaculum. **Fig. 9.** Left spicule. **Fig. 10.** Right spicule. **Fig. 11.** Ventral view of bursa showing the 2-1-2 arrangement, dorsal ray, spicules and gubernaculum. **Fig. 12.** Body at level of ovejector showing branching structures and cuticular flap covering vulva. **Fig. 13.** Posterior end showing amphidelphic uterus and relative placement of the vulva.

Male (measurements based on 20 worms, unless otherwise noted)*:* Male loosely coiled with as many as 6 coils. Body length 2.7–6.3 mm (4.9 mm, 19%, n = 16), width at midbody 45–104 (80, 18%, n = 16). Cephalic vesicle 82–128 (106, 12%) long dorsally, 61–108 (88, 13%) long ventrally; 29–57 (42, 17%) wide at anterior inflation. Nerve ring and excretory pore located 159–325 (207, 19%) and 215–471 (366, 17%, n = 19) from anterior end, respectively. Oesophagus 274–543 (385, 16%) long, 23–52 (34, 20%) wide, near base. Spicules subequal, delimited by walls of irregular thickness, not convergent at distal end (Figs. 9–11); proximal and distal longitudinal thickenings in lamina present; distal thickening merges with marginal wall; distal end of spicule ends with exposed lamina (Figs. 9, 10). Left spicule 164–246 (198, 12%, n = 16) long by 8–17 (13, 21%, n = 16) wide at manubrium (Fig. 10). Right spicule 169–241 (197, 11%, n = 16) long by 8–19 (13, 22%, n = 16) wide at manubrium (Fig. 9). Gubernaculum 117–193 (157, 13%, n = 16) long, curved dorsally (Fig. 8). Caudal bursa with symmetrical

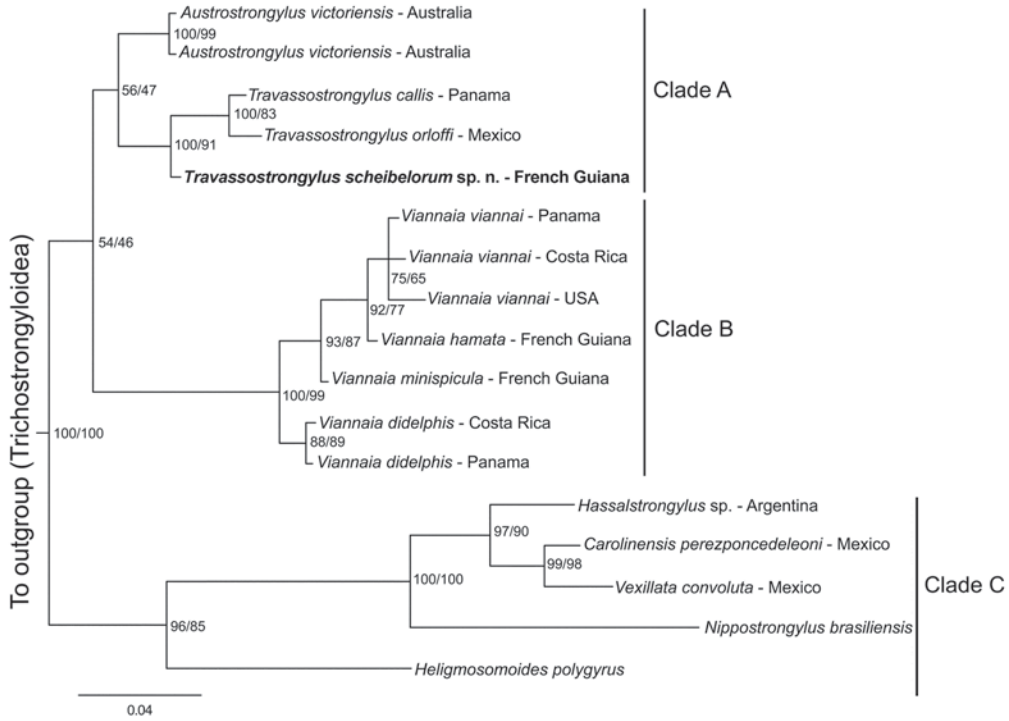


Fig. 14. Phylogenetic reconstruction for didelphid-dwelling Viannaiidae based on nuclear sequences of 18S, ITS-1, 5.8S and ITS-2. The tree is rooted in *Trichostrongylus colubriformis*, *T. axei*, *T. vitrinus* (Trichostrongylinae), *Haemonchus contortus* and *Mecistocirrus digitatus* (Haemonchinae). Values on the tree nodes are posterior probabilities and bootstrap proportions (%), respectively. The scale bar represents the number of substitutions per site.

lobes (Fig. 11). Lateral ray arrangement 2-1-2. Rays 2 and 3 and rays 5 and 6 paired, respectively, ray 4 isolated, not reaching bursal margin. Long, narrow externodorsal rays at base of dorsal trunk (Fig. 11). Well-developed dorsal lobe with dorsal ray 58–98 (83, 15%, n = 16) long, measured from basis to terminal bifurcation, usually branching symmetrically into rays 9 and 10 (Fig. 11). Prominent genital cone 47–80 (61, 13% n = 16) long (Fig. 11).

Holotype: Body length 4.1 mm, width at midbody 45. Cephalic vesicle 96 long dorsally, 85 long ventrally; 29 wide anterior half. Nerve ring and excretory pore located 162 and 330 from anterior end, respectively. Oesophagus 274 long, 26 wide. Spicules subequal, left 174 long by 8 wide at manubrium; right spicule 169 long by 8 wide at manubrium. Gubernaculum 124 long. Well-developed dorsal lobe with dorsal ray 58 long. Genital cone 47.

Female (measurements based on 28 worms, unless otherwise noted): Female coiled in 3 to 8 coils. Body length 4.8–12.7 mm (7.9 mm, 30%), width at midbody 54–135 (94, 26%). Cephalic vesicle 50–136 (110, 17%, n = 23) long dorsally, 44–121 (90, 18%) long ventrally, 31–96 (49, 26%) wide at anterior inflation. Nerve ring and excretory pore located 138–315 (210, 18%) and 273–538 (391, 17%) from anterior end, respectively. Oesophagus 317–517 (399, 17%, n = 23) long, 26–55 (40, 23%) wide, near base. Posterior end tapers to caudal terminus (Fig. 13). Distance from posterior end to vulva 817–1 934 (1 318, 29%); to anus 62–119 (96, 14%, n = 23). Pointed, cuticular flap directed posteriad, covers vulva (Fig. 12). Didelphic, amphidelphic. Vagina vera 30–77 long (50, 24%); vestibule 94–206 (146, 26%); anterior sphincter 13–29 (21, 18%) long, 23–53 (38, 21%) wide; posterior sphincter 13–45 (22, 29%) long, 25–57 (35, 20%) wide (Figs. 12, 13). Eggs ovoid, 38–61 (n = 105, 10%) long by 23–38 (n = 105, 11%) wide.

Allotype: Body length 6 mm, width at midbody 65. Cephalic vesicle 95 long dorsally, 78, long ventrally, 31 wide at anterior inflation. Nerve ring and excretory pore located 235 and 312 from anterior end, respectively. Oesophagus 312 long 28 wide, near base. Distance from posterior end to vulva 958, to anus 92. Pointed, cuticular flap directed posteriad, covers vulva. Didelphic, amphidelphic. Vagina vera 44 long; vestibule 101; anterior sphincter 20 long, 29 wide; posterior sphincter 20 long, 32 wide. Eggs ovoid, 47–54 long by 26–29 wide.

Type host: Linnaeus's mouse opossum, *Marmosa murina* (Linnaeus) (Didelphidae: Didelphinae). Symbiotype MNHN 2001–2241, a juvenile male collected on 23 May 1999 in xerophitic vegetation of a rocky outcrop near Les Nouragues field station.

Other hosts: Woolly mouse opossum, *Marmosa demerarae* Thomas (Didelphidae: Didelphinae).

Type locality: French Guiana: Municipality of Regina: Nouragues (4°05'N; 52°42'W).

Other localities: Montagne du Tigre (4°54'N; 52°18'W); Saül (3°37'N; 53°13'W), Route de Kaw (4°37'N; 52°17'W).

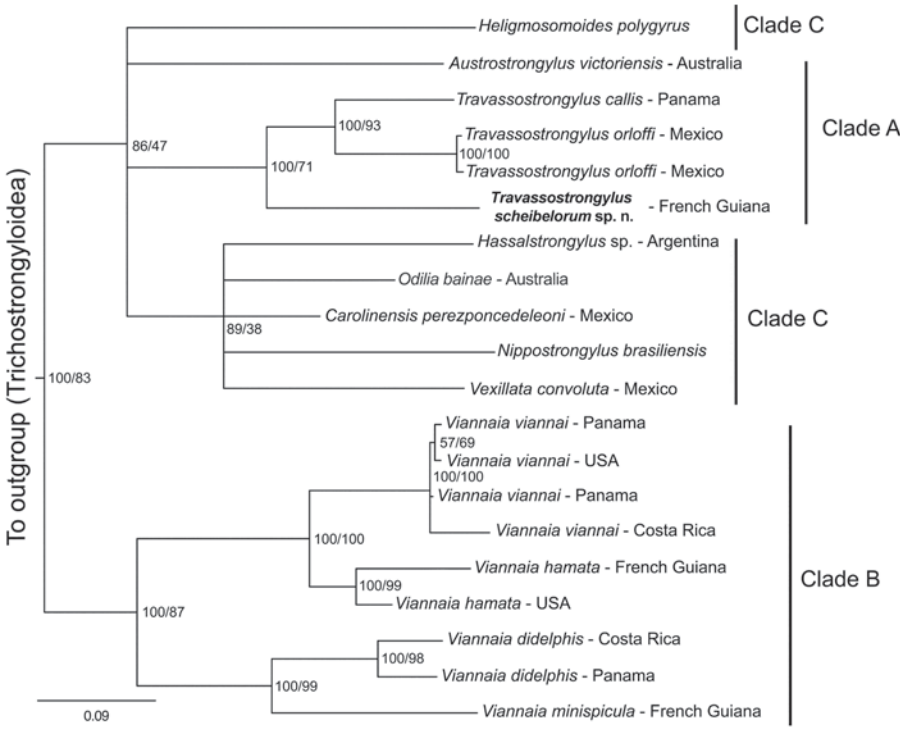


Fig. 15. Phylogenetic reconstruction for didelphid-dwelling Viannaiidae based on mitochondrial sequences of *rrnL*, *cox1* and *cob*. The tree is rooted in Trichostrongyloidea. Values on the tree nodes are posterior probabilities and bootstrap proportions (%), respectively. The scale bar represents the number of substitutions per site.

Prevalence: *Marmosa murina*: 28%, 5 of 18 individuals; *Marmosa demerarae*: 28%, 7 of 25 individuals.

Specimens deposited: Holotype, male MNHN22YT. Allotype, female MNHN65YT; paratypes from *Marmosa murina* MNHN25YT, MNHN27YT, MNHN29YT, MNHN66YT; HWML67183, HWML67188, HWML67190–HWML67192; from *Marmosa demerarae* MNHN23YT, MNHN24YT, MNHN26YT, MNHN28YT; HWML67170, HWML67171, HWML67184–HWML67187, and IPCAS N–1031.

Site of infection: Small intestine.

Etymology: This species is named after Melania and Dr. L.W. (Bill) Scheibel, parents of the first author, in recognition of Dr. Scheibel's contribution to parasitology and to credit both of them for fostering RPS's interest in biology.

Remarks. *Travassostrongylus scheibelorum* differs from the 11 known species in the genus by displaying 12 ridges and 2 lateral thickenings on the synlophe. The 12 dorsal-ventral synlophe ridges vary in size, and are small in comparison to the extended ridges observed on *T. callis* Travassos, 1914, *T. orloffi* Travassos, 1935, *T. paraquintus* Durette-Desset, 1974 and *T. tourei* Diaw, 1976, which are species that also occur in French Guiana. The two cuticular thickenings run parallel to the hypodermal cords and appear to be positioned within the lateral spaces of the synlophe. These structures have not been characterised for any other species in the genus. In females of *T. scheibelorum*, ridges and lateral thickenings in the synlophe reduce in height posterior to the vulva, yet 2 additional ridges are apparent, giving a total of 14 ridges (Fig. 7).

The spicules observed in *T. scheibelorum* are similar in size and overall shape with those in *T. orloffi*, *T. callis*, *T. sextus* Freitas, 1937 and *T. travassosi* Durette-Desset, 1968. However, they can be clearly differentiated from *T. orloffi* and *T. travassosi* because the distal portion of the lamina in *T. scheibelorum* is not indented. The continuous terminal lamina in *T. scheibelorum* resembles the homologous structure in both *T. callis* and *T. sextus*, yet the spicules of *T. scheibelorum* are longer. In addition, the length of the gubernaculum and dorsal ray allows discrimination among these five species.

Cuticular flaps covering the vulva, which were observed in *T. callis*, *T. travassosi* and *T. didelphis*, are blunt and rounded. The cuticular flap of *T. scheibelorum* is pointed and covered with striations. In addition, the size of the female of *T. scheibelorum* is substantially larger than that of females of *T. callis*, *T. travassosi* and *T. didelphis*, and can be recognized by multiple loose coils throughout the length of the body. Females for *T. quatuor* Freitas, 1937, *T. quintus* Freitas, 1937, *T. sextus* and *T. tertius* Freitas, 1937 are unknown.

Phylogenetic reconstruction

A total of 17 individuals were successfully sequenced for 18S, 13 for ITS, 15 for *rrnL*, 12 for *cox1*, and 10 for

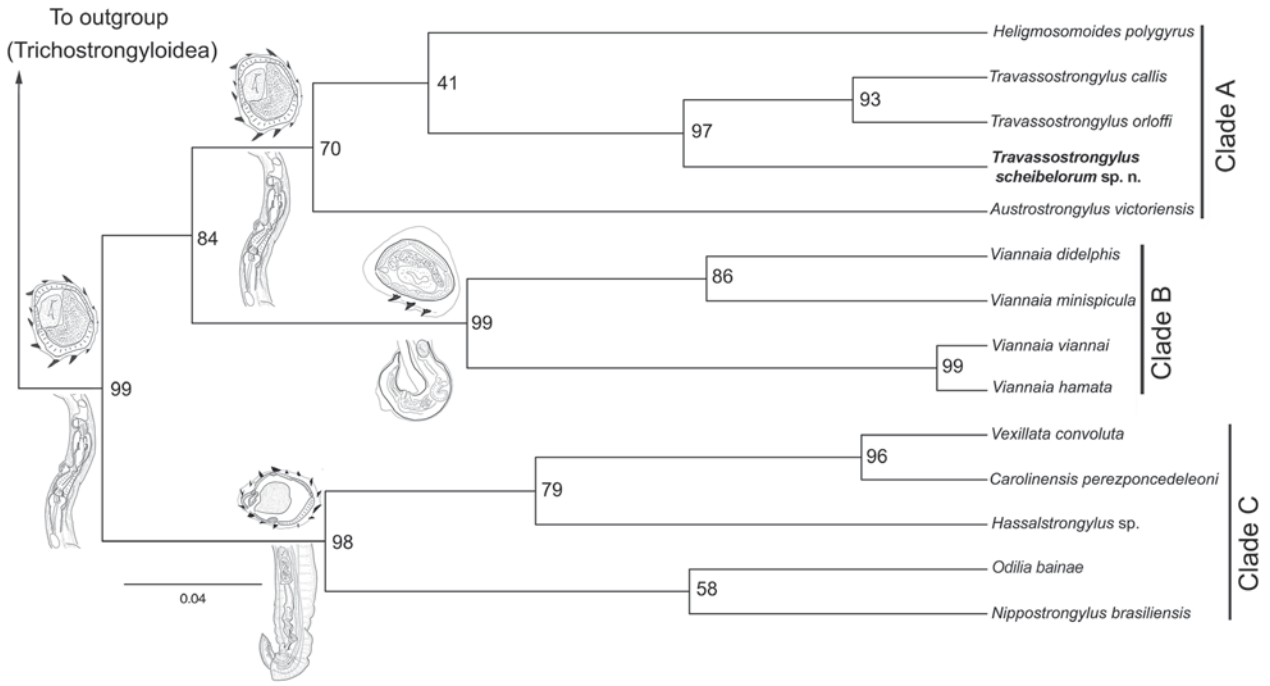


Fig. 16. The species tree of didelphid-dwelling Viannaiidae based on nuclear and mitochondrial genes (18S, ITS-1, 5.8S, ITS-2, rrnL, cox1 and cob). The tree is rooted in Trichostrongyloidea. Values on the tree nodes are posterior probabilities. The scale bar represents the number of substitutions per site.

cob (Table 1). Sixty-seven out of 1 741 sites for 18S were variable and 51 were parsimony-informative. The ITS GBlocks cured matrix contained 65 variable and 45 parsimony informative sites out of 170 total sites. The *rrnL* dataset contained 455 out of 915 sites that were variable and 356 were parsimony informative. From the 710 sites in the *cox1* dataset, 209 were variable and 176 were parsimony informative. Two hundred and eighty out of 751 sites for *cob* were variable and 244 were parsimony informative.

The phylogenetic reconstruction based on nuclear genes (18S, ITS-1, 5.8S, and ITS-2) is shown in Fig. 14. The support for the monophyly of Heligmosomoidea is 100% for both Bayesian inference and maximum likelihood. *Austrostrongylus victoriensis* Cassone, 1983 is clustered with species of *Travassostrongylus* with a posterior probability of 56% and a bootstrap support of 47%. This group, Clade A, is sister to the *Viannaia* species, Clade B, with posterior probability and bootstrap support of 54% and 46%, respectively. Clade C, consisting of *Heligmosomoides* Hall, 1916, Heligmonellidae, and *Vexillata* Travassos, 1937, is recovered with a 95% posterior probability and 85% bootstrap support.

The phylogenetic reconstruction based on mitochondrial genes (*rrnL*, *cox1* and *cob*) is shown in Fig. 15. Heligmosomoidea is supported with a posterior probability of 100% and 83% bootstrap support. Clade B is shown to be resolved with a posterior probability of 100% and a bootstrap support value of 87%. However, Clade A and Clade C were unresolved and resulted in a polytomy. *Vexillata convoluta* (Caballero et Cerecero, 1943), *Nippostrongylus brasiliensis* Travassos, 1914, *Carolinensis perezponcedeleoni* Jiménez, 2012, *Odilia bainae* Beveridge et Durette-Desset, 1992, and *Hassalstrongylus* sp. formed a group. The species of *Travassostrongylus* were supported as a monophyletic group with 100% posterior probability and maximum likelihood bootstrap support above 70%.

The species tree for the marsupial-dwelling Viannaiidae is shown in Fig. 16. Heligmosomoidea is shown to be monophyletic with support of 99% with clades A, B and C. Clade A, including *Austrostrongylus* and *Travassostrongylus*, shows a support of 70%. However, this group also contains *Heligmosomoides polygyrus* Dujardin, 1845 with a posterior probability of 41%. Clade B, the *Viannaia* species, is monophyletic with a posterior probability of 99% and appears as the sister group to Clade A with a support of 84%. Clade C, excluding *H. polygyrus*, is shown to be monophyletic with a support of 98%.

Character reconstruction

Tables S1 and S2 (part of supplementary information) summarize the reconstruction of ancestral states described below. For the reconstruction of characters using the trees generated from the analyses of nuclear genes, the monodelphic/didelphic condition as well as the cuticular ornamentation on the dorsal surface (Table S1) were reconstructed for the common ancestor of all species included in the superfamily (didelphic condition 5.36; dorsal and

ventral ridged synlophe 4.14). The results do not show a significant difference in the character reconstruction for the common ancestor for *Heligmosomoides*, Heligmonellidae and *Vexillata* as either monodelphic or didelphic (0.74), however, the dorsal and ventral ridged synlophe is reconstructed as the ancestral state for the same node (6.22). The internal node including *Viannaia*, *Austrostrongylus* and *Travassostrongylus* supports a didelphic condition (5.82) with a dorsal and ventral ridged synlophe (4.10). The ancestor of the species in *Viannaia* – Clade B – is suggested to be monodelphic (8.80) with only ventral ridges (10.44). Finally, the results show a significant difference in the reconstruction of the common ancestor for *Austrostrongylus* and *Travassostrongylus* – Clade A – as didelphic (8.04) with a synlophe consisting of dorsal and ventral ridges (6.40).

The solution for the reconstruction of the association among trichostrongyles in Heligmosomoidea using the trees generated from the analysis of the mitochondrial data set indicates significant differences in the reconstruction of synlophe morphology for two nodes (Table S1). These include the reconstruction of the ancestor of *Viannaia* as possessing a ventral ridged synlophe (5.02) and an ancestor with a dorsal and ventral ridged synlophe for the common ancestor of *Austrostrongylus*, *Travassostrongylus*, *Heligmosomoides*, Heligmonellidae and *Vexillata* (4.02). A monodelphic ancestor of *Viannaia* was reconstructed with strong support (2.72).

The reconstruction of characters using the resulting trees from the calculation of the species tree indicates significant differences at four nodes (Table S2). A monodelphic ancestor of Heligmonellidae and *Vexillata* was reconstructed with strong support (3.27); this ancestor also showed a synlophe with ventral and dorsal ridges (1.68). The results show a significant difference in the reconstruction of the common ancestor for *Austrostrongylus* and *Travassostrongylus* as possessing a synlophe with dorsal and ventral ridges (3.01). The common ancestor for the species in *Viannaia* is suggested to be monodelphic (3.61) with a ridge-less dorsal synlophe (2.40). Finally, the ancestor of the species of *Travassostrongylus* is reconstructed as didelphic (4.64) with a dorsal and ventral synlophe (4.97).

DISCUSSION

Phylogenetic reconstruction

All resulting topologies suggest that Viannaiidae is not monophyletic. The phylogeny resulting from the analysis of nuclear sequences resolves each of the three clades (Fig. 14). In addition, the topology conflicts with the phylogeny of the mitochondrial genes in that this tree shows a polytomy involving Clade A and Clade C. The relative position of Clade A and C is contrary to the topology derived from nuclear genes, as well as previous hypotheses based on morphology. This association may have been an artifact of the reduced number of species of Herpetostrongylidae from Sahul (Australia and New Guinea) included in the present analysis.

The species tree (Fig. 16) is most similar to the phylogeny based on nuclear markers except for the placement of *Heligmosomoides polygyrus*, which appeared closer to species of *Travassostrongylus*. The position of *H. polygyrus* is not consistent with the nuclear phylogeny or morphology and shows a posterior probability of 41%. Despite the appearance of this rogue taxon, Clade A contains *Austrostrongylus victoriensis* and species of *Travassostrongylus* with support greater than 70% posterior probability. The placement of *H. polygyrus* relative to species of *Travassostrongylus* invokes a relationship with at least some species currently considered part of Heligmosomidae.

This relationship was found in the analysis of all nuclear markers and the coalescent approach to the reconstruction of the species tree. Although using multiple genes was an effective method for testing the hypothesis of this study, it did reveal differences in the resulting phylogenies of nuclear and mitochondrial data. A conclusive explanation of the appearance of *H. polygyrus* as a rogue taxon within Clade A will require additional individuals of this species and eventually the inclusion of more genes. Additional sequences of gene regions and species from Heligmosomidae are needed for future analysis of their relationships, and to test the influence in the topology of the trees of mitochondrial genes.

Other studies in nematodes have shown that the phylogenies resulting from the analysis of nuclear and mitochondrial datasets may conflict (Nadler et al. 2006, Park et al. 2011) and there have been cases in which nematode mitochondrial DNA was estimated to be more homoplastic than nuclear characters (Blouin et al. 1998, Nadler and Hudspeth 2000). To better understand incongruence in topologies based on different loci, trichostrongyle systematists should investigate single copy nuclear genes, such as Hsp90, which has been shown to be phylogenetically useful in certain nematodes (Skantar and Carta 2004). Comparisons of genetic loci, especially with whole mitochondrial genomes of members of Heligmosomidae, will be valuable in confidently estimating nematode evolutionary history.

Although taxon sampling does not include all species currently recognized in Viannaiidae by Durette-Desset et al. (2006), it is possible to suggest that Viannaiidae is not monophyletic based on the fact that *Viannaia* does not share a common ancestor with *Travassostrongylus*. It is apparent that the group closest to *Travassostrongylus* includes species of Herpetostrongylidae and, together, they form a lineage separate from the species in *Viannaia*.

Character reconstruction

The reconstruction we present shows that characters observed in *A. victoriensis* and *Travassostrongylus* were

inherited from a putative common ancestor. This putative ancestor was likely didelphic and featured a synlophe with dorsal and ventral ridges. The reconstruction also shows that the putative ancestor of species included in *Viannaia* was monodelphic and had a synlophe with only ventral ridges. This suggests that the common ancestor of *A. victoriensis* and *Travassostrongylus* was morphologically very different to the ancestor of the species in *Viannaia*. As a consequence two lineages of opossum-dwelling trichostrongyles of very different origins may be recognized. These include: the lineage of *Travassostrongylus*, defined by the didelphic condition of females and the presence of ridges on the dorso-ventral surface of the body, and the lineage of *Viannaia*, defined by females with a monodelphic reproductive system and presence of only ventral ridges on the synlophe.

In regards to the reconstruction of the distribution of ridges in the synlophe, the results suggest that in the clade including *Viannaia* and *Travassostrongylus*, the three ventral ridges are not plesiomorphic. This appears to contradict the notion that a synlophe composed of three ventral or left ventral ridges is the most 'primitive' type in a transformation series. This type of synlophe is characteristic of species in *Viannaia*, *Woolleya* Mawson, 1973 and *Suncinema* Durette-Desset, 1973, genera proposed as the origin of each major lineage in Heligmosomoidea, including Viannaiiadae, Herpetostrongylidae and Heligmosomidae (Durette-Desset 1985). It was proposed that groups with three ventral ridges, such as *Woolleya* and *Viannaia*, led to derived species with synlophes armed in both dorsal and ventral surfaces such as *Patricialina* Inglis, 1968 and *Travassostrongylus*, respectively (Cassone et al. 1986, Durette-Desset 1985).

The reconstruction summarized in Tables S1 and S2 and Fig. 16, contradicts the transformation series proposed for the synlophe in Viannaiidae, which includes the increase in the number of cuticular ridges in the synlophe (Durette-Desset 1979, Humphery-Smith 1983). This suggests that the presence of ventral ridges may not be an ancestral condition, since the common ancestor of all taxa included in this analysis is reconstructed as didelphic with a synlophe consisting of dorsal and ventral ridges. It is still possible to sustain the multiple origin of a three ventral ridge synlophe, or the loss of dorsal ridges for that matter, during the evolution of Heligmosomoidea. The amplification of several genes from individuals of *Woolleya* and *Suncinema* and their inclusion into proposed data matrices will assist in testing this hypothesis and provide a better understanding of the evolution of the synlophe in heligmosomoid nematodes.

The results suggest that the ancestor to *Viannaia*, *Austrostrongylus* and *Travassostrongylus* was didelphic and had a synlophe with dorsal and ventral ridges. During the evolution of Heligmosomoidea there was a likely event of morphological change in which the lineage containing *Viannaia* became monodelphic and underwent a loss of the dorsal ridges on the synlophe. The loss of the posterior uterine branch is considered common in trichostrongylids and other rhabditid nematodes (Durette-Desset 1985). A more taxon-inclusive phylogeny will assist on the reconstruction of the evolutionary changes pertaining to the uterine branches and the evolution of the ridges on the synlophe.

Taxonomic considerations

Viannaia, which is the type genus for Viannaiidae, is shown to be monophyletic. There are, however, conspicuous morphological differences between nematodes similar in form to *Viannaia viannai* Travassos, 1918 and those like *Viannaia didelphis* Travassos, 1914, especially in the spicules and bursa. *Viannaia didelphis* is important, as it has the widest distribution of any viannaiid, found from Illinois, USA to the southern coast of Brazil in multiple species of opossums.

Considering the diversity of the suborder, it is possible for trichostrongyles of opossums to be part of distinct evolutionary lineages. The phylogenies suggest cohesion between *Travassostrongylus* and Herpetostrongylidae and imply that Viannaiidae is not monophyletic. The results of this study impel the investigation into the associations between the species included in *Travassostrongylus* and the species of parasites infecting reptiles and marsupials in Australia. Their relationship may be an ancient one dating before continental break-up of South America, Antarctica and Australia. There are species in the latter continent that infect dasyurid marsupials and show a synlophe, caudal bursa and cephalic cap almost identical to those seen in *Travassostrongylus scheibelorum* – see Durette-Desset and Beveridge (1981), Beveridge and Durette-Desset (2009). However, based largely on biogeography, those species have been assigned to a different family (Durette-Desset and Beveridge 1981). The inclusion of species in *Copemania* Durette-Desset et Beveridge, 1981 in a phylogenetic analysis of marsupial dwelling trichostrongyles will enable researchers to test whether shared-ancestry or convergence is responsible for this similarity. This would facilitate the taxonomic arrangement for the groups. In addition, investigations into the trichostrongyle fauna of extant marsupials present in South America during the Cretaceous besides Didelphidae (Caenolestidae and Microbiotheriidae) could provide insight into the evolution of these animals.

At this stage, the inclusion of *Avellaria* Freitas et Lent, 1934, *Hydrochoerisnema* Arantes et Artigas, 1980, *Oswaldonema* and *Viannella* is necessary to draw a firm conclusion about the monophyly of the family relative to the nematodes that primarily infect caviomorph rodents. A test of the affinities of trichostrongyles from caviomorph rodents is important because these mammals invaded South America after the putative diversification of

the major lineages of didelphid marsupials (Opazo 2005, Voss and Jansa 2009, Ezcurra and Agnolín 2012).

Clade C contains Heligmosomidae, Heligmonellidae, and Ornithostrongylidae. Most trichostrongyles in these families infect rodents and, morphologically, these taxa are similar with monodelphic reproductive systems and multiple, often numerous ridges, around the synlophe. Based on these characteristics, it is possible that the viannaiid rodent parasites (*Avellaria*, *Hydrochoerisnema*, *Oswaldonema* and *Viannella*) may actually be members of this clade. If these parasites of caviomorph rodents are more closely related to either of those families in Clade C, then Viannaiidae would consist of a polyphyletic broad-spectrum conglomerate of species that can be found across the major lineages of the superfamily.

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Field-N°	Espece	Museum-catalog	Localite	Political Unit (commune)		sexe	Date	Collecteur
V-1076	M. demerarae	ISEM-V-1076	Cayenne, Montagne du Tigre	Cayenne	04°54' N; 52°18' W	f	10 june 1999	A. Talarmin
V-1309	M. demerarae	MHNG-1885.053	Cayenne, Montagne du Tigre	Cayenne	04°54' N; 52°18' W	m	07-mars-01	F. Catzeflis & JF Mauffrey
V-1338	M. demerarae	MHNG-1885.057	Cayenne, Montagne du Tigre	Cayenne	04°54' N; 52°18' W	f	10-mars-01	F. Catzeflis & JF Mauffrey
V-1352	M. demerarae	MHNG-1885.059	Cayenne, Montagne du Tigre	Cayenne	04°54' N; 52°18' W	f	11-mars-01	F. Catzeflis & JF Mauffrey
V-1353	M. demerarae	MHNG-1885.060	Cayenne, Montagne du Tigre	Cayenne	04°54' N; 52°18' W	m	11-mars-01	F. Catzeflis & JF Mauffrey
V-2943	M. demerarae	MNHN: 2011-879	Cacao	Roura	04°34' N ; 52°27' W	m	13-juin-11	F. Catzeflis
V-2950	M. demerarae	MHNG-1979.079	Cacao	Roura	04°34' N ; 52°27' W	f	21-juin-11	F. Catzeflis
V-954	M. murina	MNHN: 2001-2249	Nouragues	Regina	04°05' N; 52°42' W	m	21-mai-99	F. Catzeflis
V-1056	M. murina	MHNG-1885.038	Route de Kaw	Roura	04°37' N; 52°17' W.	m	august 2000	M. Blanc
V-1107	M. murina	MHNG-1885.039	Saül	Saül	03°37' N; 53°13' W	m	31-oct-00	J.-F. Mauffrey & C. Steiner
V-1085	M. murina	ISEM-V-1085	Cayenne, Montagne du Tigre	Cayenne	04°54' N; 52°18' W	f	late-june 1999	A. Talarmin
V-1386	M. murina	MHNG-1885.063	Cayenne, Montagne du Tigre	Cayenne	04°54' N; 52°18' W	f	14-mars-01	F. Catzeflis & JF Mauffrey
Abbreviations:								
ISEM = Institut des Sciences de l Evolution de Montpellier, Université de Montpellier								
MNHN = Museum National Histoire Naturelle, Paris								
MHNG = Museum Histoire Naturelle de Geneve, Switzerland								
M. = Marmosa								

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The relationships of marsupial-dwelling Viannaiidae and description of *Travassostrongylus scheibelorum* sp. n. (Trichostrongylina: Heligmosomoidea) from mouse opossums (Didelphidae) from French Guiana

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