

The first phylogenetic reconstruction of Nippostrongylinae (Nematoda: Heligmonellidae) reveals 3 new genera, the polyphyletic nature of *Carolinensis* and *Vexillata*, and identifies 5 clades with varying associations with mammals

F. Agustín Jiménez¹, Guinevere O. Drabik¹, Jorge Falcón-Ordaz², Andrew G. Hope³, Kurt E. Galbreath⁴, Noé U. de la Sancha⁵, John M. Kinsella⁶, Chris T. McCallister⁷, Vasyl Tkach⁸, Whitney Preisser⁹, and Scott L. Gardner¹⁰

¹School of Biological Sciences, Zoology, Southern Illinois University Carbondale, Carbondale IL, USA; ²Laboratorio de Morfología Animal, Universidad Autónoma del Estado de Hidalgo, Mexico; ³Division of Biology, Kansas State University, Manhattan, KS, USA; ⁴Department of Biology, Northern Michigan University, Marquette, MI, USA; ⁵Environmental Science and Studies, DePaul University, Chicago, IL, USA; ⁶HelmWest, Missoula, MT, USA; ⁷Division of Natural Science, Northeast Texas Community College, Mt. Pleasant, TX, USA; ⁸ Department of Biology, University of North Dakota, Grand Forks, ND, USA; ⁹ Department of Ecology, Evolution, and Organismal Biology, Kennesaw State University, GA, USA; ¹⁰The Harold W. Manter Laboratory of Parasitology, University of Nebraska, Lincoln, NE 68588-0514, USA

Corresponding author: F. Agustín Jiménez, Email: agustinjz@siu.edu

This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted re- use, distribution and reproduction, provided the original article is properly cited.

Abstract

The Nippostrongylinae is a group of strongylid nematodes that includes species typically associated with coprophagous mammals. In the New World, the taxon is represented by 82 species within 11 genera. Two main morphological features, the synlophe and the caudal bursa, are used to evaluate the characteristics that allow identification and classification of the organisms in the taxon. However, the analysis of these characters often requires a partial or total destruction of specimens and therefore morphological variation is studied in only a very small subset of organisms per species. To evaluate the phylogenetic signal from these characteristics, we use genetic data to reconstruct the first phylogeny for the Nippostrongylinae using nuclear and mitochondrial genes and include representatives of the most common and diverse genera. The reconstructed phylogeny features five distinct clades and allows us to identify three non-monophyletic taxa including *Carolinensis*, *Vexillata* and *Hassalstrongylus*. From these, *Carolinensis* s. l. is divided into four genera including *Carolinensis*, *Boreostrongylus*, *Neoboreostrongylus* n. gen. and *Tepalcuanema* n. gen. *Stunkardionema* is resurrected to include *Vexillata noviberiae* and *Hassalstrongylus* is divided into two, establishing *Lovostrongylus* n. gen. to include species that are closely related to *Guerrerostrongylus* and *Trichofreitasia*. Organisms in these three genera feature a caudal arrangement of type 2-2-1. Furthermore, species in *Hassalstrongylus sensu stricto* are more closely related to species in *Malvinema* and *Stilestrongylus*. Our results reveal the existence of an additional unnamed genus and underscore the usefulness of framing morphological characters in a comparative framework. A key for genera from the Americas is proposed.

Keywords: *Lovostrongylus*; *Neoboreostrongylus*; *Tepalcuanema*; *Comparative method*; *Heligmonellidae*

Introduction

The Heligmosomoidea Cram, 1927, is a very diverse taxon of nematodes of tetrapods that includes species occurring mainly in rodents. They are monoxenous and upon infection via ingestion or cutaneous penetration, these nematodes feature special-level variation in their patterns of tissue migration. Because of this variation, two rodent-dwelling species, *Heligmosomoides bakeri* Durette-Desset, Kinsella and Forrester, 1972 and *Nippostrongylus brasiliensis* (Travassos, 1914), are widely used as models to study the interactions between the mammalian immune response modulation and immune evasion by the nematodes as they pass through various tissues on their way to their target site in the digestive tract (Maizels & McSorley, 2016).

The Heligmosomoidea has a complex taxonomic history in that groupings for its species diversity have been considered at different taxonomic hierarchies (Cram, 1927; Durette-Desset & Chabaud, 1993; Skrjabin et al., 1952) and used to recognize several infrafamilial taxa (Beveridge et al., 2014; Hodda, 2022). Furthermore, these monoxenous nematodes have been presumed to have a narrow host range, which has been used to justify taxonomic splitting by using the taxon of the host as a “character” (Durette-Desset, 1983; Durette-Desset, 1985). However, the true degree of host range or host-specificity has been seldom tested. Most of the original descriptions offered no information relative to the simultaneous examination of additional mammals in the study site, preventing the characterization of the parasite distribution in one or in several species of sympatric mammals. Furthermore, the degree of hosts specificity has been rarely tested using molecular data.

In their natural state, as adults situated in the intestine of their host, these bursate nematodes are usually coiled, feature a cephalic vesicle and a very small buccal capsule, which in most cases is reduced to the length of a single annulus of the cuticle. These worms feature a synlophe, a system of cuticular structures that run from or near the anterior end

posteriorly the length of the body, these aretes, crests or cuticular ridges are typically continuous and in cross section they appear to be oriented towards the left dorsal quadrant of the body. Presently, Heligmosomoidea is recognized as a superfamily (Beveridge et al., 2014) or a subfamily Heligmosominae (Hodda, 2022) within Trichostrongylidae Leiper, 1908. The complex taxonomic history of this group of nematodes reflects numerous changes dictated by patterns of the bursal rays that are considered of taxonomic significance (Beveridge et al., 2014; Durette-Desset, 1983). The synlophe, has also received attention as it is useful in the determination of major lineages within the bursated nematodes and was used to justify the proposal of infrafamilial subordinate taxa (Durette-Desset & Chabaud, 1977).

Unsurprisingly, the same set of characters is used to determine the relationships among constituent genera and species that make up the diversity of the Heligmosomoidea. Among these subordinate taxa of the Heligmosomoidea, the most diverse is the Heligmonellidae Skrjabin and Schikhobalova, 1952 which includes hundreds of species featuring an array of patterns in the caudal bursa and a spineless tail in females (Durette-Desset et al., 2017). Variation in the caudal bursa includes different patterns of branching in the dorsal ray and differences in the symmetrical arrangement of the lobes that encase the rays. Irrespective of their differences, all heligmonellids feature a buccal capsule reduced to an annulus, a cephalic vesicle and ridges in the synlophe in an oblique axis of orientation. Recent systematic efforts focused on the Heligmonellidae have evaluated cuticular and bursal structures as independent characters and provided various interpretations of their variability and usefulness as character states (Durette-Desset & Digiani, 2005a; Durette-Desset & Digiani, 2012; Durette-Desset et al., 2017), but these have not been rigorously qualified through phylogenetic reconstruction that test the robustness of taxonomic classifications (de Bellocq et al., 2001). Most recently, Durette-Desset et al., (2017) recognized five subfamilies within Heligmonellidae; four of them were included in the monumental monograph of the taxon which excluded the

Nippostrongylinae Durette-Desset, 1971. The Nippostrongylinae is defined by the continuous ridges along the cuticle, ridges which in cross section have a sagittal orientation: from the ventral right quadrant to the dorsal left quadrant or to the left side (Durette-Desset, 1971b; Durette-Desset, 1983). The limited set of characters available to identify more than 400 known species reduces the possible combination of characters useful for accurate diagnosis of genera and species (Durette-Desset & Digiani, 2012).

Herein we employ DNA sequence data to infer a phylogeny for species of the Nippostrongylinae present in the New World. Our objective is to establish a phylogenetic foundation for investigating morphological convergence among lineages and to identify the characters that are most informative for constructing a predictive classification. We aim to clarify classification within the most species-rich groups with emphasis on taxa representative from the Americas.

Materials and methods

Selection of taxa

Taxa used in this study were collected across the New World with some specimens resulting from expeditions led by the authors in both South and North America. Specific collection localities are listed in Table 1. We generated vouchers and sequences for 44 out of 47 operational taxonomic units (OTU) used in our analyses, including outgroups. Sequences of three relevant taxa that are part of the ingroup were downloaded from GenBank; these represent *Nippostrongylus brasiliensis*, *Nippostrongylus magnus* (Mawson, 1961) and *Chisholmia binae* (Beveridge and Durette-Desset, 1992). We used 43 nippostrongyline worms from 10 putative genera with the goal of including at least two representative species per genus (Table 1).

Identification of taxa

For examination, specimens were cleared in diluted glycerin and mounted on temporary

slides in glycerin or glycerin jelly. For observation of the diagnostic genital structures, we dissected four male specimens to clear their posterior ends in lactophenol. Cross sections of these specimens were made to observe the synlophes at the junction of the esophagus (anterior), the midbody (mid) and in the posterior third of the worm (posterior). Preserving the last third of the body allowed us to evaluate reproductive structures of males and monodelphic prodelfic females. Based on characters observed in each individual, worms were assigned to a genus based on characteristics described in the most current diagnosis from available literature (Beveridge et al., 2014; Digiani et al., 2007; Digiani et al., 2003; Durette-Desset, 1970; Durette-Desset, 1983; Durette-Desset & Digiani, 2005a; Durette-Desset & Digiani, 2012; Durette-Desset & Guerrero, 2006).

DNA extraction and sequencing

DNeasy Blood and Tissue spin columns (Qiagen Inc., Madison, WI, USA) were used for tissues excised between the mid-body and posterior end of the worm. The anterior and posterior ends of worms were saved as a voucher and deposited in the Harold W. Manter Laboratory of Parasitology, HWML (Lincoln, NE, USA) or the Parasite Division of the Museum of Southwestern Biology, MSB (Albuquerque, NM, USA). Attempts to extract DNA failed for specimens deposited in collections for periods longer than 15 years, including *Allipistrongylus marki* Drabik, Vivar and Jiménez, 2022. One mitochondrial and two nuclear ribosomal gene regions were targeted to achieve the goals of the study. For amplification of the mitochondrial gene cytochrome c oxidase subunit 1 (*COI*), we used the primers NCO1f 5'-CCT ACT ATG ATT GGT GGT TTT GGT AAT TG-3' and NCO1r2 5'-GTA GCA GCA GTA AAA TAA GCA C-3' (Jiménez et al., 2013) with the following cycling conditions: 94°C/60 s, [94°C/10 s, 60 °C/ 45 s, 72 ° C /60 s] x 34; 72 ° C/600sec. For some reactions, we amplified COI using the universal primers LCO 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3' and HCO 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' (Folmer et al.,

1994) adjusting annealing temperature to 50°C. A continuous region of nuclear ribosomal DNA (nrDNA) including internal transcribed spacer 1 (ITS1), 5.8S and ITS2 (hereafter, *ITS*) was completed using primers NC2 and NC5 following protocols described elsewhere (Chilton et al., 2003; Jiménez et al., 2012). A second continuous region of the nrDNA including the majority of the 28S subunit was amplified using the primers NC2R: 5'-AGC GGA GGA AAA GAA ACT AA-3' and NC28-8R: 5'-GTC TAA ACC CAG CTC ACG TT -3' with the following cycling conditions: 94°C/90 sec; [94°C/30 sec; 53°C/45 sec; 72°C/90 sec] x 34; 72°C/420 (Chilton et al., 2003). SydLabs HY PCR Master Mix (SydLabs, Hopkinton MA, USA) was used for all PCRs. Amplicons were submitted for Sanger sequencing at commercial facilities (MCLab, San Francisco, CA, USA; Eurofins Genomics, Louisville, KY, USA). For most products the primers used for PCR amplification were also used for sequencing. However, because of its length, 28S was sequenced using the internal primers NC28-1, NC28R, NC28-3 NC28-12R, NC28-5, NC28-4R, NC28-6R and NC28-7 described by Chilton et al., (2003). Resulting raw sequences were assembled in Sequencher version 5.4.6 (Sequencher, Ann Arbor, MI, USA) or Geneious Prime v.2020.1.2 (Biomatters, Inc., Newark, NJ, USA).

Alignment of sequences and phylogenetic analysis

Annotated original sequences were complemented with sequences of the ingroup or relevant taxa published elsewhere and available in GenBank (Alnaqeb et al., 2022a; Alnaqeb et al., 2022b; Audebert et al., 2005; Chilton et al., 2015; Scheibel et al., 2014) The aligned mitochondrial data were analysed for the presence of pseudogenes in Mesquite v.3.5 (Maddison & Maddison., 2018), using the Muscle v.5 alignment program (Edgar, 2004). For ITS and 28S, the alignment was performed using MAFFT software for secondary structure alignment using default QINSI settings (Kato & Standley, 2013). The complete list of sequences generated in this study including their accession numbers are detailed in Table 1.

The models of nucleotide substitution (HKY +I+ G for 28S and GTR +I+G for *ITS* and *COI*) were selected using the best fit criteria according to the corrected Akaike Information Criterion as implemented in jModelTest v.2.1.6 (Posada, 2008). Loci were analysed phylogenetically as a concatenated dataset and the respective models of nucleotide evolution were applied to data partitions representing each locus.

The phylogenetic reconstruction of the Nippostrongylineae was performed under the optimality criteria of Maximum Likelihood using RAXML with 1,000 bootstrap replicates. Branch posterior probability was estimated using MrBayes 3.2 (Minh et al., 2020; Ronquist et al., 2012) running 4 chains for 10 million generations, with sampling every 1,000 generations and a burn-in of 25%. Convergence of the chains was assessed by examining the potential scale reduction factor and visualization of the generated TRACE plot. Analyses were completed in the CIPRES Science Gateway (Miller et al., 2010). Resulting trees were visualized using FigTree v. 1.4.4 (Rambaut, 2018).

To explore the relationships among genera revealed as paraphyletic and to expand on the taxonomic sampling density, we expanded the *ITS* dataset to include 12 additional nippostrongyline species (18 OTUs) that are only represented with this region of nrDNA in GenBank. We analysed this *ITS* datamatrix following the optimality criteria and run parameters described above. We used reciprocal monophyly and the presence of at least one synapomorphy as the criteria to designate new taxa in the genus group.

Results

The aligned *ITS* matrix has a total length of 1,303 positions; of those positions 44% are constant and 14% are variable thus phylogenetically uninformative. The 28S partition is 3,384 positions long, of which 38% are constant and 7% of variable positions were found to be phylogenetically uninformative. The mitochondrial *COI* loci include 677 positions: of those 34% were informative, 30% were phylogenetically uninformative and the rest were

constant.

Results of analyses of the three concatenated data partitions

The phylogenetic reconstruction of the concatenated dataset reveals five strongly supported clades (Figure 1). The first one, Clade 1, reveals species from the Old World, such as *Nippostrongylus brasiliensis*, *Nippostrongylus magnus* and *Chisholmia binae*, as a sister group to a resolved cluster including the North American *cf. Vexillata noviberiae* (Dikmans, 1935), *cf. Carolinensis kinsellai* (Durette-Desset 1969) and *cf. Carolinensis dalrymplei* (Dikmans, 1935). This Clade 1 is the sister group to the rest of the species in the phylogeny; from these, Clade 2 (Figure 1) includes species present in the northern Neotropics including *Vexillata armandae* Gardner, Fong, Al Banna and Raymond, 1994, *Vexillata convoluta* (Caballero and Cerecero, 1943) and *Tepalcuanema perezponcedeleoni* n. comb. (*cf. Carolinensis perezponcedeleoni* Jiménez, 2012). Clade 2 was found to be a sister group for the cluster containing the other 3 clades; among these, Clade 3 (Red in Figure 1) includes species that contain the type species for the genus *Carolinensis*, namely *Carolinensis carolinensis* (Dikmans, 1935) in addition to *Carolinensis neotomae* (Murphy, 1952) and an unnamed *Carolinensis* sp. The rest of the taxa are contained in a clade that is further divided in two: Clade 4 (Yellow in Figure 1), which includes species of *Stilestrongylus* and *Malvinema*, and Clade 5 (Pink in Figure 1), which roughly includes species of *Hassalstrongylus*, *Guerrerostrongylus*, *Trichofreitasia*, *Mazzanema* and a new genus to be described separately.

The phylogeny based on *ITS* is consistent with the topology resulting from the analysis of the concatenated dataset (Figure 2). It reveals the same clades, yet lacks resolution at nodes closer to the root (*Mikenema* + *Vexillata* + *Carolinensis*). Nevertheless, the pattern reveals the same paraphyletic assemblages observed in the multi-locus phylogenetic analysis.

Identification of non-monophyletic groups: new designation of taxa

The phylogeny shows the polyphyletic origin of taxa previously assigned as *Vexillata* Travassos, 1937. The species in Clade 1 (Maroon in Figure 2) *Stunkardionema noviberiae* (Dikmans, 1935) n. comb., was described as a species in *Longistriata* Schulz, 1926, and then transferred to *Vexillata* (cf. *Vexillata noviberiae* (Durette-Desset & Digiani, 2005b)). This same Clade 1 also contains two species formerly assigned to *Carolinensis* including cf. *Carolinensis kinsellai* and cf. *Carolinensis dalrymplei*. Their phylogenetic position warrants them to be transferred to a different taxon and morphologic similarities to species of *Carolinensis sensu stricto* should be considered to be homoplastic.

Stunkardionema noviberiae n. comb., is consistent with the description of the genus proposed by Arnold (1941) and very similar to the description of *Lagostrongylus* Fukumoto, Masao and Masashi, 1986 (Fukumoto et al., 1986); however the position of both species in the phylogeny (Figure 2) suggests that morphological similarities resulted from convergence. The transfer to *Stunkardionema* requires a new taxonomic act, taken herein. The other two species that act as the sister group for *Stunkardionema noviberiae* include cf. *Carolinensis kinsellai* and cf. *Carolinensis dalrymplei*; these two species were transferred to *Boreostrongylus* Durette-Desset (1971b) based on the orientation of ridges in the synlophe. However, in the current reconstruction they do not form a monophyletic group with the type species *Boreostrongylus minutus* (Dujardin, 1845). As a consequence, we propose a new genus to include those two species.

***Neoboreostrongylus* n. gen. Falcón-Ordaz and Jiménez**

Diagnosis: Trichostrongylina: Heligmosomoidea: Heligmonellidae. Synlophe with 13 uninterrupted ridges. Ridges roughly oriented from right to left with dorsal ridges conspicuously smaller than the rest; sinistral (left) and dextral (right) ridges on dorsal side are relatively bigger; ventral ridges with increasing size gradient right to left (Figures 3a, b). Caudal bursa with symmetrical lobes; 2-2-1 arrangement with ray 3 longer than ray 2; ray 3

exceeds cuticular margin of bursa (Figure 3c). Dorsal ray and rays 8 share a common stalk (Figure 3c). Rays 8 split sub-symmetrically from stalk of dorsal ray at midlength; dorsal ray further divided at distal end. Genital cone prominent ($>60\mu\text{m}$), conical in appearance and endowed with fine terminal papillae 7.

Taxonomic summary

Etymology: The genus name uses the Greek prefix *Neo* to the name of the genus, in reference to their location in the New World.

Type species: *Neoboresotrongylus kinsellai* (Durette-Desset, 1969) n. comb.

Type host: *Neofiber alleni*

Type locality: Clewiston, Florida, U.S. A.

Other species: *Neoboresotrongylus dalrymplei* (Dikmans, 1935) n. comb.;
Neoboresotrongylus dikmansi (Durette-Desset, 1974) n. comb.

Other Hosts: *Microtus ochrogaster*

Other localities: Churchill, Manitoba, Canada

Remarks

Neoboreostrongylus features the typical traits of all members included in the Heligmonellidae, namely the presence of a simple buccal cavity, a synlophes made by continuous ridges with oblique axis of orientation, a monodelphic condition as well as having a simple tail without a caudal spine. The disposition and size of the ridges in the synlophes make species of this genus and *Boreostrongylus* relatively easy to differentiate because very few other taxa feature a double gradient in size of the ridges. In these two genera ridges are bigger in the flanks with smaller ridges featuring on the dorsal and ventral surfaces.

In turn, *Boresotrongylus* should include *Boreostrongylus minutus* (Dujardin, 1845), a species distributed in cricetid rodents across Eurasia (Jackson & Friberg, 2022). As a consequence, the diagnosis of *Boreostrongylus* provided by Durette-Desset (1971b) should

be slightly modified to indicate that species in this genus are expected to feature 16 continuous ridges; rays 8 splitting in an asymmetrical manner from the common trunk with dorsal ray. Furthermore, the pairs of rays 7 does not feature prominently on the genital cone.

The presence of a common stalk for the dorsal ray and Rays 8 constitute a conspicuous difference in the diagnosis of *Carolinensis*. Furthermore, the synlophe in species of *Carolinensis* feature smaller ridges on the left side, and slightly larger ridges on the right side.

Relative to the phylogeny of the group, in Clade 2 (Grey Clade), there is a cluster of three species assigned to *Vexillata*, yet these are sister to a species originally assigned to *Carolinensis*, this species requires new genus that is defined below.

***Tepalcuanema* n. gen. Drabik and Jiménez**

Diagnosis: Trichostrongylina: Heligmosomoidea: Heligmonellidae. Synlophe with 13 to 16 uninterrupted ridges; oriented from dextroventral to sinistro-dorsal quadrant. Dextral lateral ridges slightly larger than others (Figures 3d, e). Caudal bursa with symmetrical lobes, pattern of type 2-2-1. Rays 2 and 3, and 5 and 6 share a stalk. Rays 8 and dorsal ray share prominent stalk. Rays 8 split symmetrically from stalk of dorsal ray at midlength; dorsal ray divided at posterior third. Genital cone prominent sub-cylindrical covered by expanded foldable cuticle (Figure 3f); basis of genital cone endowed with ventral membrane. Gubernaculum present. Females monodelphic, tail short and simple, covered by flexible cuticle that covers the tail as a sleeve.

Taxonomic summary

Etymology: The genus is a combination of Nahuatl and Greek. *Tepal* roughly translates into “from someone”; *Cuana* translates into “feeding at the expense of someone” and the Greek word *Nema* means “thread”.

Type and only species: *Tepalcuanema perezponcedeleoni* (Jiménez, 2012) new combination

Type locality: Adolfo López Mateos, Veracruz, Mexico

Type host: *Nyctomys sumichrasti*

Remarks

Tepalcuanema is the sister taxon of *Vexillata* and yet, both genera are strikingly different because members of the latter feature a prominent *carenee*, whereas members of the former feature ridges that are roughly similar in size. *Tepalcuanema* shares several features with some species in *Carolinensis* Travassos, 1937 listed in Jiménez (2012), including the symmetrical nature of the caudal bursa and the number of ridges in the synloph, which ranges between 13 and 16; nevertheless, while *Tepalcuanema* feature a common stalk between rays 8 and the dorsal ray, in species of *Carolinensis* both dorsal ray and rays 8 bifurcate immediately at the root (see Figures 3h, j). *Tepalcuanema* also resembles species in *Neoboreostrongylus* in that these show a prominent genital cone, ray 8 splitting at mid length of the stalk of dorsal ray, and a dorsal ray that bifurcates into rays 9 and 10 at its distal third. Furthermore, both *Neoboreostrongylus dalrymplei* and *Neoboreostrongylus kinsellai* feature a pair of subterminal papillae -papillae 7- in the genital cone, which are also present, albeit in tandem, in *Tepalcuanema perezponcedeleoni*.

Based on the continuous ridges in the synloph and the presence of a hypertrophied genital cone, *Tepalcuanema* also exhibits similarities with some species in *Malvinema* Digiani, Sutton, and Durette-Desset, 2003, *Stilestrongylus* Freitas, Lent and Almeida, 1937, and *Suttonema* Digiani and Durette-Desset, 2003. However, males in these 3 genera show an asymmetrical caudal bursa and dorsal rays with arrangement different from 2-2-1. *Tepalcuanema* is also different from any of these genera in the relative size of the dorsal ray. The elongated dorsal ray and the symmetrical bursa of *Tepalcuanema* gives this structure the appearance of an inverted heart-shaped cup. In this regard, the bursa of *Tepalcuanema* is very similar to the homologous body parts in *Calypsostrongylus* Schmidt, Myers and Kuntz, 1967

and *Sciurodendrium* Durette-Desset, 1971. However, *Tepalcuanema* is clearly separated from them because it shows continuous ridges in the synlophes and lack of a carenea. In the hierarchical arrangement of heligmonellid nematodes, these structures are used to split the family into subfamilies (Durette-Desset, 1985). The recognition of *Boreostrongylus* and the erection of *Neoboreostrongylus* and *Tepalcuanema*, requires a redefinition of *Carolinensis*, which is represented by Clade 3 in Figure 2.

***Carolinensis* Travassos, 1937**

Diagnosis: Trichostrongylina: Heligmosomoidea: Heligmonellidae. Synlophes with 14 to 16 uninterrupted ridges; oriented from dextroventral to sinistro-dorsal quadrant. Ridges of different sizes; ridge 1 at sinistroventral quadrant larger than ridge 1 at dextrodorsal quadrant; ridges on dorsal side feature a decreasing size gradient from ridge 6 to 2, right to left (Figures 3g, h). Caudal bursa with subsymmetrical lobes, left lobe slightly larger, subventral rays of pattern 2-2-1 or 1-2-2. Rays 8 arising symmetrically from basis of dorsal ray; dorsal ray divided at cranial third. (Figures 3h, j). Genital cone dome shaped or blunt at its distal end. Gubernaculum present or absent. Females monodelphic, with tapering tail.

Taxonomic summary

Type species: Carolinensis carolinensis (Dikmans, 1935) Travassos, 1937.

Type locality: Great Smoky Mountains, North Carolina, U.S.A.

Type host: Peromyscus maniculatus

Other species: Carolinensis norvegica (Dikmans, 1935) Durette-Desset, 1983, *Carolinensis neotomae* (Murphy, 1952); *Carolinensis peromysci* (Durette-Desset, 1974); *Carolinensis petteri* (Denke, 1977) Durette-Desset, 1983, and *Carolinensis huehuetlana* Falcón-Ordaz and Sanabria-Espinoza, 1996.

Distribution: United States of America, Mexico

Remarks

Travassos (1937), noted that in *Longistriata carolinensis* rays 8 and the dorsal ray bifurcated from a common root, thus there was no common trunk shared between them. He used this absence of a common trunk as the sole diagnostic character for the genus. Following, Durette-Desset (1974) transferred *Longistriata carolinensis* Dikmans, 1935, into *Boreostrongylus* Durette-Desset, 1971. As drafting the monumental taxonomic keys for the Trichostrongyloidea, Durette-Desset (1983) transferred all the species of *Boreostrongylus* into *Carolinensis*, with no justification for the combination of species featuring a prominent common trunk -*Boreostrongylus*- and those lacking a trunk -*Carolinensis*-. Subsequently, four more species were described between 1986 to 2012 including *cf. C. eothenomysi* Asakawa, Kamiya and Ohbayashi, 1986; *C. huehuetlana*, *cf. C. tuffi* Durette-Desset and Santos 2000, and *cf. C. perezponcedeleoni*. Based on the phylogeny presented in Figure 1, *Carolinensis* is redefined to include only species with 14 to 16 ridges with a decreasing size gradient from left to right in both ventral and dorsal sides, dome shaped genital cone, subsymmetric bursa, and rays 8 splitting from basis of dorsal ray. The gubernaculum appears to be absent in at least two species currently recognized.

The very general definition provided by Travassos (1937), combined with the lack of a formal redefinition during the last taxonomic rearrangement (Durette-Desset, 1983), resulted in *Carolinensis* becoming a hodgepodge -a combination of species without adequate characterization nor descriptions and with poor diagnoses-. Paradoxically, relative to the copulatory bursa, the bifurcation between rays 8 relative to the stalk of the dorsal ray appears to be a reliable trait that can be used to separate members of this genus from *Boreostrongylus* and *Neoboreostrongylus* in which rays 8 and the dorsal ray share a common trunk in both *Boreostrongylus* and *Neoboreostrongylus*. It is evident that the relevance of this character as diagnostic or a strong synapomorphy was inappropriately abandoned in favor of the characters of the synlophe and caudal bursa championed during the last 40 years (Durette-

Desset, 1983; Durette-Desset & Digiani, 2005a; Durette-Desset & Digiani, 2012). The topology presented in the phylogenies (Figures 1 and 2) indicate that while not exclusive for this group, the proximal bifurcation between dorsal rays and ray 8 (very close to their roots) should be used in combination with the configuration of the synophe and the ray arrangement in the lobes.

For the purposes of this comparison, *Carolinensis eothenomysi* was considered a species *inserta sedis* because neither the arrangement of ridges in the synophe nor the presence of a common stalk supporting rays 8 and dorsal ray fit the diagnosis of the genus (Durette-Desset & Digiani, 2019). Consequently, we consider *cf. C. tuffi* also a species *inserta sedis* as it does not fit the diagnosis; even when rays 8 and dorsal ray bifurcate at their root the numbers of ridges in the synophe is very high (20 in males, 19 in females) and the ridges do not show a clear size gradient.

Clade 4 (Yellow clade) includes *Stilestrongylus*, *Malvinema* and *Hassalstrongylus*; these worms feature asymmetrical bursae with an arrangement 1-4 tending to 1-3-1. In turn, Clade 5 (Pink Clade) includes members of *Trichofreitasia* and *Guerrersostromylus*, as well as organisms that feature characters that make them fit in the definition of *Hassalstrongylus*. In their bursae, rays 3 are longer than ray 2. Because of the polyphyletic distribution of these putative *Hassalstrongylus* we propose to amend its diagnosis and propose a new genus.

***Lovostrongylus* n. gen. Drabik, Falcón-Ordaz and Jiménez**

Diagnosis: Trichostrongylina: Heligmosomoidea: Heligmonellidae. Synophe usually with 19 to 24 uninterrupted ridges at midbody, may reach 31; oriented from sinistroventral to dextrodorsal quadrant. Ridges slightly unequal in size, with one or two prominent ridges on the dextrodorsal quadrant (Figures 4a - d). Axis of orientation of ridges from the dextroventral quadrant to the left or dorsosinistral quadrant. Caudal bursal with asymmetrical lobes; right lobe with pattern of type 2-2-1 tending to 1-3-1; left lobe with pattern of type 2-3

tending to 2-2-1. Rays 2 shorter than rays 3 and curved toward median line; rays 4 and 5 diverging at extremity. Rays 6 diverging from common trunk of rays 2-6. Rays 8 typically arising symmetrically from base of dorsal ray. Dorsal ray thickened at base, dividing within middle third into two branches; dorsal ray typically shorter than rays 8. Genital cone conical or triangular in ventral view. Gubernaculum and telamon present. Females monodelphic, with postvulvar subventral alae (Figures 4d, e); tail short, simple and protrusible as tail is covered by flexible cuticle that acts as a sleeve.

Taxonomic summary

Etymology: The genus name is a combination of the Greek words *lovó* (“*λοβό*” meaning loincloth) and *strongylós* (“*Στρογγυλός*”) meaning round. The name refers to the subventral alae around the vulva.

Type species: *Lovostrongylus argentinus* (Freitas, Lent and de Almeida, 1937) new combination

Type host: *Holochilus brasiliensis*

Type locality: Salta, Argentina

Other species: *Lovostrongylus mazzai* (Freitas, Lent and de Almeida, 1937) n. comb.; *Lovostrongylus dollfusi* (Diaz-Ungria, 1963) n. comb.; *Lovostrongylus hoineffae* (Durette-Desset, 1969) n. comb.; *Lovostrongylus schadi* (Durette-Desset, 1970) n. comb.; and *Lovostrongylus* sp. JX877694.

Distribution: Argentina, Brazil, Colombia and Venezuela

Remarks

Perhaps because of the lack of size gradient in the ridges in the synlophe all species listed in *Lovostrongylus* were included in *Hassalstrongylus* Durette-Desset, 1971. However, species in *Lovostrongylus* can be differentiated based on the presence of postvulvar subventral alae and the flexible caudal cuticle of females, as well as the bursal ray arrangement in males. In

Lovostrongylus, subventral postvulvar alae feature prominently in most species and cuticular expansions in the dorsal and ventral side of the vulva make the tail appear protrusible; the bursa is asymmetrical with a typical ray arrangement 2-2-1; dorsal ray and rays 7 bifurcate at their basis. This genus is closely related to *Guerrerostrongylus* and *Trichofreitasia* (Figures 1 and 2); members of these genera also feature a caudal cuticular expansion on females, which in some cases folds into the cuticle as a sleeve, making it appear protrusible.

Lovostrongylus is clearly differentiated from *Guerrerostrongylus* because in the latter both dorsal ray and rays 8 share a stalk. The dorsal ray is far longer than rays 8 while ray 6 is extremely long. Furthermore, the number of ridges in *Guerrerostrongylus* exceeds 40, nearly twice as many as in most species of *Lovostrongylus*, with the exception of *Lovostrongylus dollfusi* (Serrano et al., 2021). In contrast, *Lovostrongylus* can be differentiated from *Trichofreitasia* in the nature of the bursa, which in the latter is characterized as symmetrical with hypertrophied lobes. However, in members of both genera the ray arrangement is 2-2-1 and there is a similar number of ridges in the synlophe.

Hassalstrongylus Durette-Desset, 1971

Diagnosis: Trichostrongylina: Heligmosomoidea: Heligmonellidae. Synlophe with 19 to 25 cuticular ridges of different sizes, oriented from sinistroventral to dextrodorsal quadrant with no defined size gradient (Figures 4f - j). Asymmetrical bursa, with pattern 1-4 or 1-3-1, rays 8 split from dorsal ray at the root of their stalk, which is broad. Rays 8 usually as long as dorsal ray. Genital cone dome-shaped. Females monodelphic. Postvulvar cuticle in females is relatively simple (Figure 4i), featuring occasional dorsal expansion or “inflation”.

Taxonomic summary

Type species: Hassalstrongylus aduncus (Chandler, 1932) Durette-Desset, 1971.

Type host: Sigmodon hispidus

Type locality: Houston, Texas, U.S.A.

Other species: *Hassalstrongylus musculi* (Dikmans, 1935) Durette-Desset, 1974; *Hassalstrongylus lichtenfelsi* Durette-Desset, 1974; *Hassalstrongylus forresteri* Durette-Desset, 1974; *Hassalstrongylus chabaudi* Diaw, 1976; *Hassalstrongylus puntanus* Digiani and Durette-Desset, 2003 and *Hassalstrongylus geolayarum* Falcón-Ordaz, Iturbide-Morgado and Martínez-Salazar, 2024.

Distribution: Argentina, Brazil, Ecuador, Mexico, U.S.A.

Remarks

There are four species in *Hassalstrongylus* that are difficult to classify in the genus given the lack of material to characterize their morphological features. These include *Hassalstrongylus dessetae* Pinto, 1978, *Hassalstrongylus musculi* (Dikmans, 1935), *Hassalstrongylus luquei* Costa, Maldonado Jr., Bóia, Lucio and Simões 2014, and *Hassalstrongylus echaileri* Diaw, 1976. From this list, both *H. luquei* and *H. echaileri* feature a caudal bursa of type 2-2-1 as that seen in species of *Lovostrongylus*; however, the female is not known for *H. luquei* and there is no conspicuous postvulvar alae in *H. echaileri*. We suspect these species could be transferred to *Lovostrongylus* once sufficient material collected to properly describe the species and the fact that at least one is morphologically similar to the undescribed species of *Lovostrongylus* included in our phylogenetic analysis (*Lovostrongylus* n. sp. 4 in Figures 1 and 2). Only the examination of the specimens will assist in their accurate determination. From this list, *Hassalstrongylus dessetae*, a species present in eastern Brazil, features 30 ridges in the synloph, which seems consistent with the number of ridges seen in some specimens of *Lovostrongylus dollfusi* collected in Argentina (Serrano et al., 2021). Perhaps the screening of the homologous genes for these two putative species may assist in determining whether they represent a single species with ample morphological variation in the number of spines for these represent coinfection by two species. A key difference between *Hassalstrongylus* and *Lovostrongylus* is the extension and direction of Ray 3 in the

bursa, in *Hassalstrongylus* this ray is typically directed from the midline towards the sides, whereas in *Lovvostrongylus* ray 3 directs anteriorly, almost parallel to ray 2.

Hassalstrongylus appears to be closely related to *Malvinema*, which features a very prominent genital cone and asymmetrical lobes of the copulatory bursa. It is interesting to note that both *Malvinema* and *Hassalstrongylus* are related to *Stilestrongylus*. The common characteristic for these three genera includes the asymmetrical bursa and the ray arrangement that is typically 1-4.

A new genus

The phylogeny reveals what appears to be two lineages related to the clade formed by *Lovostrongylus*, *Trichofreitasia*, and *Guerrerostrongylus*. This lineage includes an undescribed species of *Mazzanema* (*Mazzanema* n. sp 11) and an undescribed genus (New Genus New Species 12). The proper description of these nematodes will be provided separately.

Discussion

Sampling coverage for the reconstruction of the first phylogeny for the Nippostrongylinae

This is the first phylogeny of the Nippostrongylinae based on three gene regions, 1 mtDNA and 2 nrDNA, and it includes 14 of the 18 recognized genera and representatives from genera collected across the Americas spanning the Nearctic and Neotropical regions, nine taxa from Eurasia and one from Australia. Taxonomic coverage for vouchered specimens includes 28 species, of which 10 have to be formally described and named. Because of the taxonomic density in most branches the resulting phylogeny shows good resolution overall, with the exception of some internal nodes representing relationships within certain genera (Clades 4 and 5).

In addition to the specimens used to reconstruct this first phylogeny, 6 species from the

Old World are represented only by sequence data of the ITS 1 and ITS 2 regions, since their 5.8S region was not made available in GenBank. These sequences were included to get a better understanding of the relationships of genera that appear to be distributed across two or more continents, yet a consequence of this inclusion is poor resolution for a few internal nodes. The missing data apparently results in long branches for nodes that are defined by strong support values and high posterior probabilities. Nevertheless, these are considered useful because they allow the clustering of closely related taxa in phylogenetically meaningful groups. Even when they do not allow the proper testing of shared ancestry they act as the foundation to identify diagnostic traits and the framework to test these relationships with additional data and OTUs. This is particularly the case for *Lagostrongylus* and *Boreostrongylus*. The clade of *Boreostrongylus minutus*, *Heligmonoides speciosus* and *Oriostongylus ezoensis* features absolute support, despite all four taxonomic units missing the ribosomal gene 5.8S. Furthermore, the inclusion of these sequences allowed us to include other members of the Heligmonellidae: Nippostrongylineae in the analysis, such as *Ornithostrongylus quadriradiatus* and *Austrostrongylus victoriensis*.

The practice described above underscores the paucity of sequences available for bursated nematodes -and for parasites in general- in the universal genetic data repositories. Furthermore, the data available in GenBank is of limited usefulness for three major reasons. First, the sequences available are seldom linked to vouchered specimens that allow the verification of the parasite identity. We posit that this linkage is necessary because it affords scientists the possibility of correcting identifications, using the specimens for taxonomic decisions and linking the specimens to a geographical location that may assist in the reconstruction of their biogeographical history (De Ley et al., 2005; Jiménez et al., 2012). Second, taxonomic representation is sparse with most species of nematodes being represented by a single sequence typically generated to attempt identification. Since reconstructions of

the phylogeny for the phylum were based on the phylogenetic analysis of 28S, this marker became common (Blaxter et al., 1998; De Ley et al., 2005). As a relatively slowly evolving gene, 28S cannot always help resolve relationships among species or closely related lineages. That task requires of more variable sites resulting from genes/regions characterized by faster rates of substitution or mutations, such as *ITS* or *COI* (Vilas et al., 2005). The problem is currently exacerbated by much of the available genetic data not conforming to a standard marker of choice which can be used to reconstruct phylogenetic relationships at supra-familial levels. This epitomizes a third major issue in that specimen materials -evidence of an infection- are often either not available or are not usable for modern applications. Parasitologists have documented the presence of parasites across centuries, and there are well curated collections that hold resources available to researchers. However, these materials are rarely preserved through methods that allows their use in perpetuity for DNA analysis. Unlike herbarium specimens, which by virtue of being dried preserve their DNA, nematodes must be frozen or fixed and preserved in ethanol. If preserved in ethanol, our experience shows that DNA will degrade over time, even if stable conditions are guaranteed. We urge parasitologists working on nematodes towards standardized workflows (Galbreath et al., 2019) and baseline sequencing, minimally to include gene regions *ITS*, *28S*, *COI* and *16S*, which are commonly used in systematic studies and can be included in expanded reconstructions. Rapid advances in genomic sequencing will likely enable adaptive sampling of entire mitogenomes across robust sample sizes (Badger et al., 2024), where if not already attainable through collaboration should be considered now within funding initiatives.

General patterns of geographical distribution and association with mammalian lineages

From a biogeographic perspective, the phylogeny features two very distinctive clades. The first clade contains 10 Holarctic and 1 Australian species represented by the 11 taxonomic

units in Clade 1. This clade is further divided into three clusters. One cluster is formed by murine-dwelling species in the Far East of Asia and Australia (*Nippostrongylus brasiliensis*, *Nippostrongylus magnus* and *Chisholmia binae*). A second cluster that includes parasites that infect arvicoline rodents (voles) across Eurasia (*Boreostrongylus minutus*) and leporids and murine rodents in eastern Asia (*Lagostrongylus lepori*, *Orientalstrongylus ezoensis*, *Heligmonoides speciosus*). These two clusters appear to be reciprocally monophyletic. The third clade includes three species from the Nearctic region including *Neoboreostrongylus kinsellai* and *Neobeostrongylus dalrymplei* parasites of voles and *Stunkardionema noviberiae* which infect rabbits. The pattern appears to suggest the presence of two independent parasite lineages in the Nearctic and in the Palearctic that follow similar associations with vole and leporid hosts. Also, the branching of these lineages is congruent with the putative origin of this lineage of parasites in murine and arvicoline rodents (Durette-Desset, 1985). To clarify linkages between the Nearctic and Palearctic diversity, further sampling of species from southeast Asia and Beringia (eastern Siberia and western North America) must be included in future biogeographical analyses.

The second clade groups the species from the New World considered as the ingroup in this analysis. It includes eight lineages of which one, *Mikenema lamothei*, is a representative of a different subfamily (Heligmonellinae Skrjabin and Schikobalova, 1952). This and congeneric parasites infect leporids and feature characteristics similar to those seen in members of Nippostrongylinae, including an axis of orientation inclined at 45° to sagittal axis (“from the ventral right quadrant to the dorsal left quadrant”) and about 14 ridges in the synlophe (Durette-Desset et al., 2017). Unfortunately, the poor representation for this taxon does not allow resolving their relationships with the rest of the lineages. The resolution of their relationships may allow scientists to establish the relationships within the entire Family (Heligmonellidae), and to select robust morphological characters that will help to stabilize the

classification.

From the other seven lineages, one includes taxa that occur in both the Nearctic and the Neotropics. In particular, *Vexillata* includes parasites of pocket gophers, pocket mice and neotomine rodents, with records ranging from central USA to northern Venezuela; in large part the distribution of these parasites mirrors the distribution of pocket mice. The other lineage includes the monotypic *Tepalcuanema* (Clade 2), which are known to infect tylomine rodents in the northern Neotropics (Los Tuxtlas). Los Tuxtlas is a relevant Neotropical locality because the extensive helminthological surveys reveal the sympatry of species of both of these lineages (Denke, 1977; Jiménez, 2012).

The vast majority of species included in the other clades were recorded from cricetid rodents. Among them, *Carolinensis* is a clade that includes mainly species associated with Nearctic neotomine and sigmodontine rodents. Few records document their infection in voles and there may be at least two species that occur in the Neotropics (Falcón-Ordaz & Sanabria-Espinoza, 1996). The rest of the species included in the six remaining lineages are chiefly associated with sigmodontine rodents.

Among these, *Malvinema*, *Hassalstrongylus* and *Stilestrongylus* (Clade 4), appear to be essentially Neotropical. In particular most of the species in *Malvinema* are known around the tropical and subtropical regions of Argentina, whereas species of *Hassalstrongylus* range in both northern and southern hemispheres, with three species endemic in the southern Nearctic (Durette-Desset, 1974). Furthermore, two species of *Stilestrongylus* were documented in neotomine rodents in the northern Neotropics (Falcón-Ordaz & Sanabria-Espinoza, 1999).

Finally, *Lovostrongylus*, *Guerrerostrongylus* and *Trichofreitasia* (Clade 5) plus *Mazzanema* and a new genus yet to be named are essentially Neotropical and restricted to South American sigmodontines. The resolution of this clade may be possible with the inclusion of representatives of different lineages from Brazil.

Convergence in both bursal arrangement and structure of synlophes

In general terms, the phylogenetic pattern underscores the homoplastic nature of the structures in the *carenee*, size of genital cone and the number of ridges, which in several cases had been used as diagnostic for genera (Durette-Desset, 1983). The phylogeny appears to offer enough resolution to support general conclusions about the diversity of the Nippostrongylineae in the New World; and represents the diversity of the parasites clustered in five clades.

The phylogenetic pattern suggests that *Vexillata* is not related to *Ornithostrongylus*. This conclusion is supported by the analysis of the *ITS* dataset alone (Figure 2), which shows that none of the species of *Vexillata*, namely *Vexillata armandae*, *Vexillata convoluta* and *Vexillata dessetae* share an immediate common ancestor with *Ornithostrongylus quadriradiatus*. By including a representative of the Ornithostrongylidae in this analysis, we are now able to provide an answer to the hypotheses suggested elsewhere (Falcón-Ordaz & García-Prieto, 2004; Guerrero, 1984), which posited that the genus does not belong to the Ornithostrongylineae. Furthermore, our results show that *Stunkardionema noviberiae*, a species formerly included in *Vexillata* does not share a common ancestor with species in *Vexillata*. Of the species included in the analysis, *Stunkardionema noviberiae* shares some similarities with *Lagostrongylus leporis* Fukumoto, Kamiya and Ohbayashi, 1986, these similarities include the structure of the *carenee* and pattern of the bursal rays (Fukumoto et al., 1986; Yamaguti, 1935). However, the topology based on *ITS*, makes it appear as if these similarities resulted from convergence. It is important to expand on the character and taxon sampling for these taxa since they may show greater taxonomic diversity across the Holarctic.

Neoboreostrongylus dalrymplei, *Neoboreostrongylus kinsellai*, *Boreostrongylus minutus* and *Boreostrongylus seurati* were included in *Boreostrongylus* by Durette-Desset (1971b). Subsequently these and the remaining three species making up the genus were transferred to

Carolinensis Travassos, 1937 based on the fact that *Longistriata carolinensis* was proposed as the type species for *Carolinensis* (Durette-Desset, 1983) and that *Carolinensis carolinensis* was inadvertently included in *Boreostrongylus* in the proposal of the latter genus (Durette-Desset, 1974). The present phylogenetic reconstruction shows that *Neoboreostrongylus dalrymplei*, *Neoboreostrongylus kinsellai* and *Boreostrongylus minutus* do not share a common ancestor with *Carolinensis carolinensis*; further, support for *Neoboreostrongylus dalrymplei* and *Neoboreostrongylus kinsellai* is absolute (100%/1), yet the clustering of these two species with *Boreostrongylus minutus* is not supported based on the analysis of the *ITS* phylogeny alone. These three species are arvicoline-dwelling nematodes and they feature characters that are very similar to those present in the genus *Carolinensis*. The phylogeny underscores that those similarities are the result of convergence and highlight the relevance of the shared origin for rays 8 and dorsal ray.

The phylogeny also reveals *Carolinensis sensu lato* Durette-Desset (1983) as polyphyletic because *Longistriata carolinensis* Dikmans, 1935 (type for *Carolinensis*), *Strongylus minutus* Dujardin, 1845, (Type for *Boreostrongylus*), and *cf. Carolinensis perezponcedeleoni* do not share a common ancestor. *Carolinensis sensu stricto* must be restricted to *Carolinensis carolinensis*, *Carolinensis neotomae*, and two undescribed species of *Carolinensis* collected in Mexico and Illinois. This clade appears to act as the sister group to the clade that includes all the diversity of species present in the Neotropics. The species included in this analysis show a similar number of ridges making up the synlophes (between 15 and 16) and feature rays 8 that do not reach the margin of the bursa and a prominent, yet not hypertrophied genital cone.

The relative position of *Vexillata dessettae* makes the genus paraphyletic. Although the support for the clade is strong, the analysis of *ITS* sequences shows a polytomy, which suggests that additional taxa and genetic markers may be required to resolve relationships

within this clade. Alternatively, the inclusion of the 28S gene for *Vexillata dessettae* may help resolving these relationships since this conservative gene may feature greater similarity with the other two species of the genus included in the analysis. We opted to establish a new genus in this clade because the morphology of species included in the clade is so strikingly different from typical characters used to define *Vexillata*. As a consequence we propose *Tepalcuanema* as a new genus to include *Tepalcuanema perezponcedeleoni* (Jiménez, 2012) Drabik and Jiménez, 2025. We predict that increasing the taxon and character sampling from members of this clade will help resolve the genus as monophyletic.

Since its inception *Hassalstrongylus* included species occurring in sigmodontine rodents across North and South America (*i.e.*, *Hassalstrongylus aduncus*; *cf.* *Hassalstrongylus argentinus*) featuring a relatively simple synlophe with no clear size gradient in their ridges. However, species in the genus have disparate morphological traits in the female tail and the arrangement of the bursal rays (Durette-Desset, 1971b; Durette-Desset, 1983). The phylogeny reveals that species formerly assigned to *Hassalstrongylus* represent two distant clades.

The clade that includes *Hassalstrongylus aduncus*, the type species for the genus, is closely related to *Stilestrongylus* and *Malvinema*. This clade includes several species across North America, chiefly as part of *Hassalstrongylus*, and feature a bursal ray arrangement of type 1-4 and rays 8 splitting from the dorsal ray at their root. Their asymmetrical bursa appears to be a shared character with *Stilestrongylus* and *Malvinema*, in which the asymmetry of the bursa is markedly different. This clade features very strong support. In particular, we note that the morphological similar *Malvinema* and *Stilestrongylus* are not reciprocally monophyletic, even when both of them include species that can be assigned to this genus by the asymmetrical nature of the bursa, rays 8 and elongated genital cone. Rather, *Malvinema* includes taxonomic units that act as the sister group for species on *Hassalstrongylus*.

The species included in the rest of the clades feature subventral postvulvar alae or rays 8 and dorsal ray that also split from their root, and a constant bursal ray arrangement of type 2-2-1. From these structures, the 2-2-1 arrangement is a trait shared with members of *Guerrerostrongylus* and *Trichofreitasia*. Nevertheless, the dorsal ray and ray 8 in species of the latter two genera feature a relatively prominent common stalk. In this clade, an interesting problem arises in the evaluation of the phylogeny based on *ITS*, namely the lack of resolution to separate *Trichofreitasia* sp., *Guerrerostrongylus zeta* and *Guerrerostrongylus marginalis*. Rather than suggesting the splitting of *Guerrerostrongylus*, we apply the conservative approach to retain the name until further evidence in the form of additional characters and samples are included to test their relationships.

Identification of structures suggestive of “parental care”

The females of *Lovostrongylus* and *Guerrerostrongylus* feature interesting modifications in the tail, which confer them the ability to fold the cuticle to cover the vulva. These structures were illustrated in detail for *Lovostrongylus dollfusi* by Serrano et al., (2021) and made evident in *Guerrerostrongylus zeta* and in *Guerrerostrongylus marginalis* by others (Digiani & Serrano, 2024; Weirich et al., 2016). In particular, the presence of subventral alae in females of *Lovostrongylus* suggests that these structures may be used in the retention of eggs upon oviposition. In this genus, the character is linked to a small number of eggs maturing in the uterus, which contrasts with the relatively high fecundity seen in most of the species of trichostrongylids. Although these cuticular structures are not unique to *Lovostrongylus* -they are also present in females of *Mikenema*- their presence in combination with an apparent low fecundity raises the question if these worms feature a form of parental care. Furthermore, these subventral alae and the cuticular fold are not the only structures that may be involved in the manipulation of eggs among the Neotropical Nippostrongylinae, since females of the three known species of *Alippistrongylus* feature an expansion that may retain eggs or assist in

attachment to the small intestine (Digiani & Kinsella, 2014; Drabik et al., 2022; Lemes et al., 2024).

The inclusion of representatives of *Alippistrongylus* in the analysis may help test this hypothesis, but most importantly, they may assist in a more robust reconstruction for the genus and a better understanding of the apparent diversity of body forms that is present across South American Nippostrongylineae. Considering the hypothesized origin of the Nippostrongylineae, which posits that the lineage spread from the Palearctic into the Nearctic in the lower and middle Pliocene and then into the Neotropics in the upper Pliocene (Durette-Desset, 1971a; Durette-Desset, 1985), it seems counterintuitive that the greater diversity of body forms and genera is present across the Neotropics, rather than in the Nearctic.

Key to genera of Nippostrongylineae occurring in coprophagous mammals, chiefly cricetids in the New World.

Common characteristics of these nematodes include the presence of a cephalic vesicle with buccal capsule reduced to an annulus; cuticular ridges along the body form a synlophe, typically with a sagittal axis of orientation directed from right to left. Subsymmetrical or asymmetrical bursa endowed with a genital cone, paired spicules and gubernaculum. Monodelphic females with postanal end conical in shape.

- 1 Tail endowed with caudal appendage..... *Alippistrongylus*
- 1' Tail with no appendage 2
- 2 Synlophe inconspicuous at midbody, if present ridges barely emergent *Hypocristata*
- 2' Synlophe conspicuous..... 3
- 3 Carene present 4
- 3' Carene absent 6
- 4 Rays arranged 1-3-1. Ray 8 and dorsal with no common stalk *Mazzanema*
- 4' Rays arranged 2-2-1. Ray 8 and dorsal ray share common stalk 5

5 Ray 3 directed anteriorly, emerges from margin of bursa. Females with prominent lateral ridges posterior to anus. Parasites of leporids

Stunkardionema

5' Ray 3 directed anteriorly, does not emerge from margin of bursa. Cuticle of tail in females with ridges of uniform size. Parasites of Heteromyids and geomyids *Vexillata*

6 Bursa asymmetrical in size: one lobe more prominent7

6' Bursa Subsymmetrical: both lobes similar in size and shape 11

7 Different ray arrangement in right and left lobes of bursa 8

7' Ray arrangement in both lobes of bursa are the same, typically 4-1 10

8 Ray arrangement right lobe 1-3-1 tending to 4-1; 3-1-1 for left lobe. Fourteen ridges in synlophe at midbody. Parasites of invasive muroids.....*Nippostrongylus*

8' Ray arrangement right lobe 2-2-1 tending to 1-3-1; 2-3 for left lobe tending to 2-2-1 9

9 Genital cone hypertrophied: length is at least half the length of caudal bursa; rays 8 with asymmetrical branching from stem of dorsal ray

Stilestrongylus

9' Genital cone triangular in ventral view, rays 8 branch symmetrically from dorsal ray; females feature sublateral ad anal alae

Lovostrongylus

10 Hypertrophied right lobe, synlophe with 9 ridges at midbody *Suttonema*

10' Synlophe with 17 to 24 ridges at midbody *Malvinema*

11 Ray arrangement 1-4..... 12

11' Ray arrangement 1-3-1, rarely 2-2-1 13

12 Synlophe with 14 ridges, adanal ridges form alae. Parasites of leporids *Mikenema*

12' Synlophe with 19 to 25 ridges, uniform ridges reach tail of females ... *Hassalstrongylus*

- 13 Synlophe at midbody between 13 to 16 ridges 14
- 13' Synlophe at midbody with more than 20 ridges 16
- 14 Ray 8 bifurcates immediately at root of dorsal ray *Carolinensis*
- 14' Ray 8 and dorsal ray share stalk, ray 8 bifurcates at least in distal third 15
- 15 Genital cone hypertrophied: more than half of length of bursa.....*Tepalcuanema*
- 15' Genital cone less than half length of bursa, ray 3 emerges from bursa
.....

Neoboreostrongylus

- 16 Synlophe at midbody with more than 30 ridges, bursa type 1-3-1 *Guerrerostrongylus*
- 16' Synlophe at midbody with 20 ridges, bursa arrangement 2-2-1 *Trichofreitasia*

Data availability. DNA alignments are available at DOI: 10.5061/dryad.p2ngf1w3f

Acknowledgements. Kurt Neubig and Frank Anderson assisted in early stages of the phylogenetic analyses. Linden Reid, Haitham Alnaqeb, Mitzi Fabiola Aquino-Camacho and Matthew Walker assisted in laboratory work. Ian Beveridge provided specimens representing outgroups and encouragement. Dr. Carlos Carrión-Bonilla (Museo de Zoología QCAZ, Pontificia Universidad Católica del Ecuador, Quito), Dr. Pablo Moreno (Instituto Nacional de Biodiversidad, Quito, Ecuador) and Dr. Joseph Cook (University of New Mexico, Albuquerque, New Mexico, USA) for their invaluable help with collecting rodents in Ecuador. Marcelo Knoff (Oswaldo Cruz Institute, Rio de Janeiro, Brazil), Gabor Racz (University of Nebraska, USA) and Sara Brant (University of New Mexico, USA) provided access to collection resources including archival numbers.

Author's contribution. FAJ and GOD conceived and designed the study, conducted data gathering and performed phylogenetic analyses. SLG, VT, MK, WP, KEG, FAJ, GOD, JFO, AEH, NdS and CTM collected and preserved material and identified the host taxon and field identity of the worms. Everyone wrote the article.

Financial support. This work was funded by The Hagan Funds of the University of Nebraska, Vice Chancellor for Research of Southern Illinois University, the National Science Foundation (NSF) through the Division of Undergraduate Education award number 1564969; DEB-9496263., BSR-9024816.; DEB-0097019; DBI-0646356; DBI-1756397 to S. L. G.; BSR8408923 to T. L. Yates; BSR8316740 to S. Anderson; DEB 0196095, 0415668 and 1258010 and USDA Forest Service and US Fish and Wildlife Service contracts to J. A. Cook and DEB 1256943 to K. E. Galbreath. V. V. Tkach was funded by the grant number R15AI092622 from the National Institutes of Health (NIH).

Competing interests. The authors declare there are no conflicts of interest.

Ethical standards. Wild specimens were collected under state-associated scientific collection permits issued to authors. The Arkansas Game and Fish Commission, Illinois Department of Natural Resources. Research methods were approved under Southern Illinois University, Carbondale Institutional Animal Care and Use Committee Protocol 21-017 (Assurance Number D16-00044).

References

- Alnaqeb, H., Galbreath, K. E., Koehler, A. V., Campbell, M. L. and Jiménez, F. A.** (2022a). *Citellinema* (Nematoda: Heligmosomidae) from western North America with descriptions of 2 new species from the red squirrel *Tamiasciurus hudsonicus* and 1 from the Canadian woodchuck, *Marmota monax*. *Parasitology*, **149**, 1199 – 1218. doi: <https://doi.org/10.1017/S0031182022000737>.
- Alnaqeb, H., Greiman, S., Vandegrift, K. J., Campbell, M. L., Meagher, S. and Jiménez, F. A.** (2022b). A molecular reconstruction of Holarctic Heligmosomidae reveals a new species of *Heligmosomoides* (Nematoda: Heligmosomidae) in *Peromyscus maniculatus* (Neotominae) from Canada. *Systematics and biodiversity*, **20**, 2046199. doi: <https://doi.org/10.1080/14772000.2022.2046199>.
- Arnold Jr. , J. G.** (1941). A new rabbit nematode, *Stunkardionema halla*. *Transactions of the American Microscopical Society*, **60**, 65 – 68.
- Audebert, F., Chilton, N., Justine, J. L., Gallut, C., Tillier, A. and Durette-Desset, M. C.** (2005). Additional molecular evidence to support a sister taxon relationship between Heligmosomoidea and Molineoidea nematodes (Trichostrongylini). *Parasitology Research*, **96**, 343-346. doi: Doi 10.1007/S00436-005-1402-Y.
- Badger, J. H., Giordano, R., Zimin, A., Wappel, R., Eskipehlivan, S. M., Muller, S., Donthu, R., Soto-Adames, F., Vieira, P., Zasada, I. and Goodwin, S.** (2024). Direct sequencing of insect symbionts via nanopore adaptive sampling. *Current Opinion in Insect Science*, **61**. doi: 10.1016/j.cois.2023.101135.
- Beveridge, A., Spratt, D. M. and Durette-Desset, M. C.** (2014). Order Strongylida (Railliet & Henry, 1913). In *Handbook of zoology. Gastrotricha, Cycloneuralia and Gnathifera. Volume 2: Nematoda.*, Vol. 2 (ed. Schmidt-Rhaesa, A.), pp. 557 - 612. De Gruyter, Boston, Massachusetts.

- Blaxter, M. L., De Ley, P., Garey, J. R., Liu, L. X., Scheldeman, P., Vierstraete, A., Vanfleteren, J. R., Mackey, L. Y., Dorris, M., Frisse, L. M., Vida, J. T. and Thomas, W. K.** (1998). A molecular evolutionary framework for the phylum Nematoda. *Nature*, **392**, 71 - 75. doi: 10.1038/32160.
- Chilton, N. B., Huby-Chilton, F. and Gasser, R. B.** (2003). First complete large subunit ribosomal RNA sequence and secondary structure for a parasitic nematode: phylogenetic and diagnostic implications. *Molecular and Cellular Probes*, **17**, 33 - 39. doi: 10.1016/S0890-8508(02)00107-X.
- Chilton, N. B., Huby-Chilton, F., Koehler, A. V., Gasser, R. B. and Beveridge, I.** (2015). The phylogenetic relationships of endemic Australasian trichostrongylin families (Nematoda: Strongylida) parasitic in marsupials and monotremes. *Parasitology Research*, **114**, 3665 - 3673. doi: 10.1007/s00436-015-4594-9.
- Cram, E. B.** (1927). Bird parasites of the nematode suborders Strongylata, Ascaridata, and Spirurata. *Smithsonian Institution United States National Museum Bulletin*, 1 - 465.
- de Bellocq, J. G., Ferté, H., Depaquit, J., Justine, J. L., Tillier, A. and Durette-Desset, M. C.** (2001). Phylogeny of the Trichostrongylina (Nematoda) inferred from 28S rDNA sequences. *Molecular Phylogenetics and Evolution*, **19**, 430-442.
- De Ley, P., De Ley, I. T., Morris, K., Abebe, E., Mundo-Ocampo, M., Yoder, M., Heras, J., Waumann, D., Rocha-Olivares, A., Burr, A. H. J., Baldwin, J. G. and Thomas, W. K.** (2005). An integrated approach to fast and informative morphological vouchering of nematodes for applications in molecular barcoding. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **360**, 1945-1958. doi: Doi 10.1098/Rstb.2005.1726.
- Denke, M. A.** (1977). Quatre nouveaux nématodes héligmosomes parasites de rongeurs du Mexique. *Bulletin du Muséum National d'Histoire Naturelle*, **327**, 777 - 787.
- Digiani, M. C. and Kinsella, J. M.** (2014). A new genus and species of Heligmonellidae

- (Nematoda: Trichostrongylina) parasitic in *Delomys dorsalis* (Rodentia: Sigmodontinae) from Misiones, Argentina. *Folia Parasitologica*, **61**, 473 - 478.
- Digiani, M. C., Navone, G. T. and Durette-Desset, M. C.** (2007). The systematic position of some nippostrongyline nematodes (Trichostrongylina : Heligmosomoidea) parasitic in Argentinean sigmodontine rodents. *Systematic Parasitology*, **67**, 87 - 92. doi: 10.1007/s11230-006-9071-5.
- Digiani, M. C. and Serrano, M.** (2024). Synonymy, redescription, molecular characterisation, and new distribution data of species of *Stilestrongylus* and *Guerrerostrongylus* (Nematoda, Heligmonellidae) parasitic in sigmodontine rodents from Argentina and Uruguay: a collection-based survey. *Zoosystematics and Evolution*, **100**, 1315 – 1331.
- Digiani, M. C., Sutton, C. A. and Durette-Desset, M. C.** (2003). A new genus of Nippostrongylinae (Nematoda : Heligmonellidae) from the water rat *Scapteromys aquaticus* (Sigmodontinae) in Argentina. *Journal of Parasitology*, **89**, 124-132.
- Drabik, G. O., Vivar, E. and Jiménez, F. A.** (2022). New species of *Alippistrongylus* (Trichostrongylina: Nippostrongylinae) from the elegant rice rat, *Euroryzomys nitidus*, of La Convención, Peru. *Journal of Parasitology*, **108**, 435 – 440. doi: DOI: 10.1645/22-10.
- Durette-Desset, M. C.** (1970). Nématodes héligmosomes d'Amérique du sud VIII. Description de six nouvelles espèces, parasites de Cricétiés. *Bulletin du Muséum National d'Histoire Naturelle 2e Serie*, **42**, 730 - 744.
- Durette-Desset, M. C.** (1971a). Essai d'interprétation de l'évolution de la famille des Héligmosomes (Nématodes Strongylida) en fonction de la paléobiogéographie de leurs hôtes. *Bulletin de la Société de Ecologie*, **2**, 178 - 188.
- Durette-Desset, M. C.** (1971b). Essai de classification des nématodes Héligmosomes. Corrélation avec la paléobiogéographie des hôtes. *Mémoires du Muséum National*

d'Histoire Naturelle. Série A. Zoologie, **69**, 1 - 126.

Durette-Desset, M. C. (1974). Nippostrongylinae (Nematoda: Heligmosomidae) néarctiques.

Annales de parasitologie humaine et comparee, **49**, 435 - 450.

Durette-Desset, M. C. (1983). *CIH Keys to the nematode parasites of vertebrates. No.10.*

Keys to genera of the Superfamily Trichostrongyloidea, 1st edn. Commonwealth Agricultural Bureaux International, Farnham Royal, UK.

Durette-Desset, M. C. (1985). Trichostrongyloid nematodes and their vertebrate hosts: reconstruction of the phylogeny of a parasitic group. *Advances in Parasitology*, **24**, 239-306.

Durette-Desset, M. C. and Chabaud, A. G. (1977). Essai de classification des Nématodes Trichostrongyloidea. *Annales de parasitologie humaine et comparee*, **52**, 539 - 558.

Durette-Desset, M. C. and Chabaud, A. G. (1993). Nomenclature des Strongylida au-dessus du groupe-famille. *Annales de parasitologie humaine et comparee*, **68**, 111-112.

Durette-Desset, M. C. and Digiani, M. C. (2005a). The axis of orientation of the synophe in the Heligmosomoidea (Nematoda, Trichostrongylina): a new approach. *Parasite*, **12**, 195 - 202.

Durette-Desset, M. C. and Digiani, M. C. (2005b). Systematic position of some nearctic Heligmosomoidea (Nematoda: Trichostrongylina) from the U.S. National Parasite collection and their description. *Journal of Parasitology*, **91**, 893-899. doi: 10.1645/GE-3446.1.

Durette-Desset, M. C. and Digiani, M. C. (2012). The caudal bursa in the Heligmonellidae (Nematoda: Trichostrongylina). Characterization and hypothesis on its evolution. *Parasite*, **19**, 3 - 18.

Durette-Desset, M. C. and Digiani, M. C. (2019). Taxonomic revision of the Heligmonellidae (Skrjabin & Schikhobalova, 1952 tribe) Durette-Desset & Chabaud,

1977 (Nematoda) parasitic in arvicoline rodents "*Eothenomys*" (*sensu lato*) (Cricetidae) from Japan and Thailand. *Systematic Parasitology*, **96**, 1 – 13. doi: 10.1007/s11230-018-9836-7.

Durette-Desset, M. C., Digiani, M. C., Kilani, M. and Geffard-Kuriyama., D. (2017). *Critical Revision of the Heligmonellidae (Nematoda: Trichostrongylina: Heligmosomoidea)*, Publications Scientifiques,, Paris, France.

Durette-Desset, M. C. and Guerrero, R. A. (2006). A new species of *Hypocristata* (Nematoda, Trichostrongylina, Heligmosomoidea) a parasite of *Sigmodon hispidus* (Cricetidae, Sigmodontinae) from Venezuela. *Parasite*, **13**, 201 - 204.

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, **32**, 1792 – 1797. doi: 10.1093/nar/gkh340.

Falcón-Ordaz, J. and Garcia-Prieto, L. (2004). Morphology of some trichostrongilinae (Strongylida) from the National Helminth Collection, Institute of Biology, UNAM, Mexico. *Revista de Biología Tropical*, **52**, 377-386.

Falcón-Ordaz, J., Iturbe-Morgado, J. C. and Martínez-Salazar, E. A. (2024). Redescription of *Hassalstrongylus aduncus* Chandler, 1932 (Nematoda: Heligmonellidae), with the description of a new species of the genus and their phylogenetic position with other Heligmosomoidea Travassos, 1914. *Journal of Parasitology*, **110**, 455 – 470.

Falcón-Ordaz, J. and Sanabria-Espinoza, M. A. (1996). Especie nueva del género *Carolinensis* (Nemata: Heligmosomidae) de *Peromyscus difficilis* de Hidalgo, México. *Anales del Instituto de Biología*, **67**, 67 - 75.

Falcón-Ordaz, J. and Sanabria-Espinoza, M. A. (1999). Dos nuevas especies de *Stilestrongylus* (Nematoda: Heligmonellidae) parásitos de *Peromyscus* (Rodentia: Cricetidae) de México. *Revista de Biología Tropical*, **47**, 929-937.

- Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R.** (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294-299.
- Fukumoto, S.-i., Kamiya, M. and Ohbayashi, M.** (1986). A new genus of the intestinal nematode, *Lagostrongylus* (Heligmonellidae) from lagomorphs : a review of related genera of subfamilies Heligmonellinae and Brevistriatinae. *Japanese Journal of Veterinary Research*, **34**, 241 – 250. doi: 10.14943/jjvr.34.3-4.241.
- Galán-Puchades, M. T., Gosálvez, C., Trelis, M., Gómez-Samblás, M., Solano-Parada, J., Osuna, A., Sáez-Durán, S., Bueno-Marí, R. and Fuentes, M. V.** (2024). Parasite fauna and coinfections in urban rats naturally infected by the zoonotic parasite *Angiostrongylus cantonensis*. *Pathogens*, **13**. doi: 10.3390/pathogens13010028.
- Galbreath, K. E., Eric P. Hoberg, Joseph A. Cook, Blas Armién, Kayce C. Bell, Mariel L. Campbell, Jonathan L. Dunnun, Altangerel T. Dursahinhan, Ralph P. Eckerlin, Scott L. Gardner, Stephen E. Greiman, Heikki Henttonen, F. Agustín Jiménez, Anson V. A. Koehler, Batsaikhan Nyamsuren, Vasyl V. Tkach, Fernando Torres-Pérez, Albina Tsvetkova and Hope, A. G.** (2019). Building an integrated infrastructure for exploring biodiversity: field collections and archives of mammals and parasites. *Journal of Mammalogy*, **100**, 382 – 393. doi: 10.1093/jmammal/gyz048.
- Guerrero, R.** (1984). Nematoda - Trichostrongyloidea parasites of Venezuelan wild mammals . 3. The genus *Vexillata* Travassos, 1937. *Annales de parasitologie humaine et comparee*, **59**, 253 - 261.
- Hodda, M.** (2022). Phylum Nematoda: a classification, catalogue and index of valid genera, with a census of valid species. *Zootaxa*, **5114**, 1 – 289. doi: 10.11646/zootaxa.5114.1.1.
- Jackson, J. A. and Friberg, I. M.** (2022). *Carolinensis minutus* (Dujardin, 1845) Travassos 1937 (Nematoda, Heligmonellidae) in *Microtus agrestis* in the United Kingdom.

Checklist, **18**, 845 – 848. doi: <https://doi.org/10.15560/18.4.845>.

Jiménez, F. A. (2012). A new species of *Carolinensis* (Nematoda: Heligmonellidae) from Sumichrast's vesper rat *Nyctomys sumichrasti*. *Comparative Parasitology*, **79**, 208 - 213.

Jiménez, F. A., Carreno, R. A. and Gardner, S. L. (2013). *Aspidodera kinsellai* n. sp. (Nematoda: Heterakoidea) from nine-banded armadillos in Middle America with notes on phylogeny and host-parasite biogeography. *Journal of Parasitology*, **99**, 1056 - 1061.

Jiménez, F. A., Gardner, S. L., Navone, G. T. and Ortí, G. (2012). Four events of host-switching in Aspidoderidae (Nematoda) involve convergent lineages of mammals. *Journal of Parasitology*, **98**, 1166 - 1175.

Katoh, K. and Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution*, **30**, 772-780. doi: 10.1093/molbev/mst010.

Ledwoń, A., Szotowska, I., Dolka, I. and Szeleszczuk, P. (2023). Molecular characterization of *Ornithostrongylus quadriradiatus* isolated from a racing pigeon (*Columba livia domestica*). *Journal of Parasitology*, **109**, 543 - 549. doi: 10.1645/22-103.

Lemes, D. S., de Andrade Silva, B. E., Maldonado, A., Vilela, R. V., Luque, J. L. and de Oliveira Simões, R. (2024). New species of the genus *Alippistrongylus* (Nematoda: Trichostrongylina) parasitic in *Delomys dorsalis* (Rodentia: Sigmodontinae) in the Atlantic Forest of Rio de Janeiro, Brazil. *Journal of Helminthology*, **98**, e58. doi: 10.1017/S0022149X24000464.

Maddison, W. P. and Maddison., D. R. (2018). Mesquite: a modular system for evolutionary analysis.

Maizels, R. M. and McSorley, H. J. (2016). Regulation of the host immune system by helminth parasites. *Journal of Allergy and Clinical Immunology*, **138**, 666-675. doi:

<https://doi.org/10.1016/j.jaci.2016.07.007>.

- Miller, M. A., Pfeiffer, W. and Schwartz, T.** (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *Proceedings of the Gateway Computing Environments Workshop (GCE)* pp. 1 – 8. New Orleans, LA.
- Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A. and Lanfear, R.** (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, **37**, 1530 – 1534. doi: 10.1093/molbev/msaa015.
- Posada, D.** (2008). jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253-1256. doi: Doi 10.1093/Molbev/Msn083.
- Rambaut, A.** (2018). FigTree v1.4.4. University of Edinburgh.
- Ramírez-Cañas, S. A., López-Caballero, J. D. and Mata-López, R.** (2021). Morphological and molecular data reveal two new species of *Viannaia* (Nematoda: Viannaiidae), parasitizing opossums (Mammalia: Didelphidae) in Mexico. *Journal of Parasitology*, **107**, 388 – 403. doi: 10.1645/18-199.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. and Huelsenbeck, J. P.** (2012). MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**, 539 - 542. doi: Doi 10.1093/Sysbio/Sys029.
- Scheibel, R. P., Catzeflis, F. and Jiménez, F. A.** (2014). The relationships of marsupial-dwelling Viannaiidae and description of *Travassostrongylus scheibelorum* n. sp. (Trichostrongylina: Heligmosomoidea), from mouse opossums (Didelphidae) from French Guiana. *Folia Parasitologica*, **61**, 242 - 254.
- Serrano, P. C., Digiani, M. C., Gómez-Muñoz, M. d. I. A., Notarnicola, J., Robles, M. d. R. and Navone, G. T.** (2021). *Hassalstrongylus dollfusi* (Nematoda, Heligmonellidae):

rediscovery in native South American rodents, six decades after its description. *Parasite*, **28**, 80.

Skrjabin, K. I., Shikhobalova, N. P., R. S. Schulz, T. I. Popova, S. N. Boev and Delyamure, S. L. (1952). *Opredelitel Parazit. Nematode vol. III. Strongyliati*, Izdatel'stvo Akademii Nauk, SSSR, Moscow.

Travassos, L. (1937). Revisão da família Trichostrongylidae Leiper, 1912. *Monographias do Instituto Oswaldo Cruz*, **1**, 1 - 512.

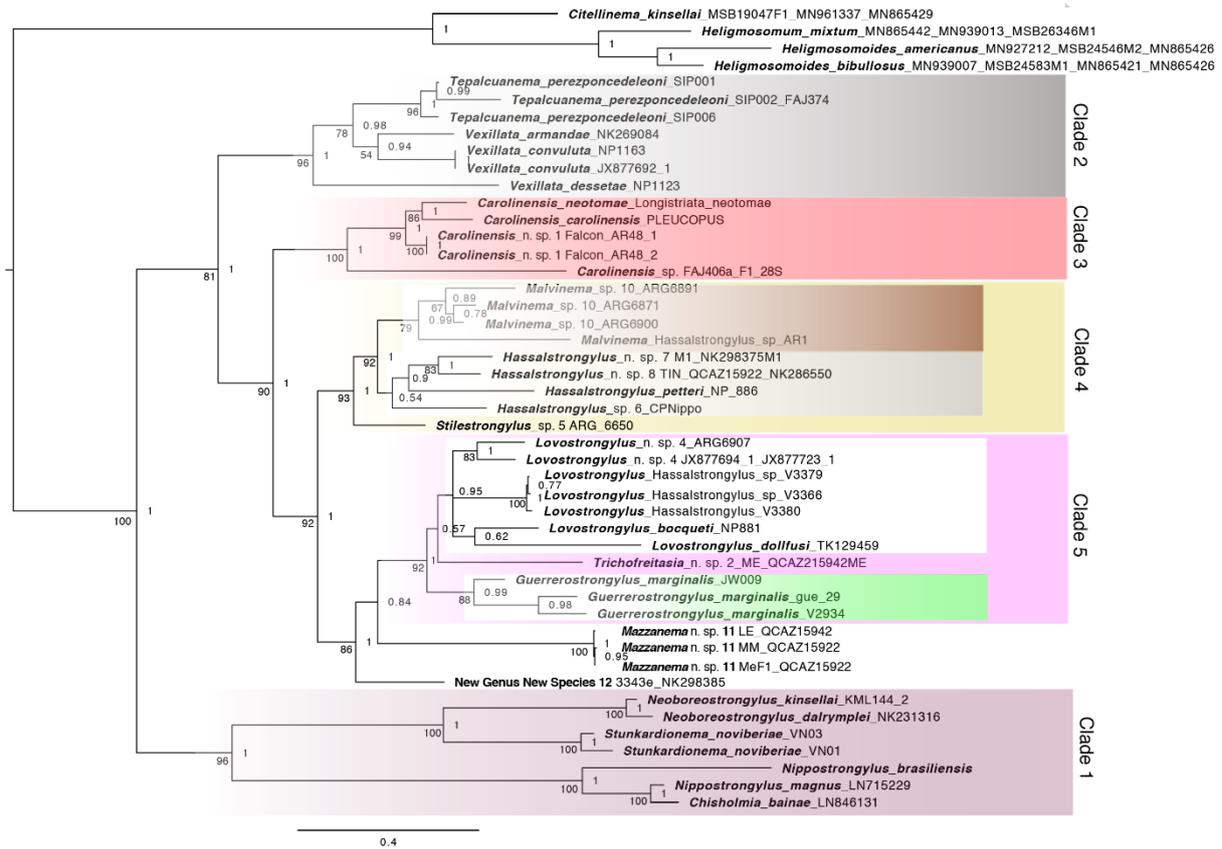
Vilas, R., Criscione, C. D. and Blouin, M. S. (2005). A comparison between mitochondrial DNA and the ribosomal internal transcribed regions in prospecting for cryptic species of platyhelminth parasites. *Parasitology*, **131**, 839 - 846. doi: Doi 10.1017/S0031182005008437.

Weirich, J. M., Catzefflis, F. M. and Jiménez, F. A. (2016). *Guerrerostrongylus marginalis* n. sp. (Trichostrongyloidea: Heligmosomoidea) from the Guianan *Oecomys* (*Oecomys auyantepui*) from French Guiana. *Parasite*, **23**, 10. doi: <http://dx.doi.org/10.1051/parasite/2016009>.

Yamada, S., Yoshida, A., Yoshida, K., Kuraishi, T., Hattori, S., Kai, C., Nagai, Y., Sakoda, T., Tatara, M., Abe, S. and Fukumoto, S. (2012). Phylogenetic relationships of three species within the family Heligmonellidae (Nematoda; Heligmosomoidea) from Japanese rodents and a lagomorph based on the sequences of ribosomal DNA internal transcribed spacers, ITS-1 and ITS-2. *Japanese Journal of Veterinary Research*, **60**, 15 - 21.

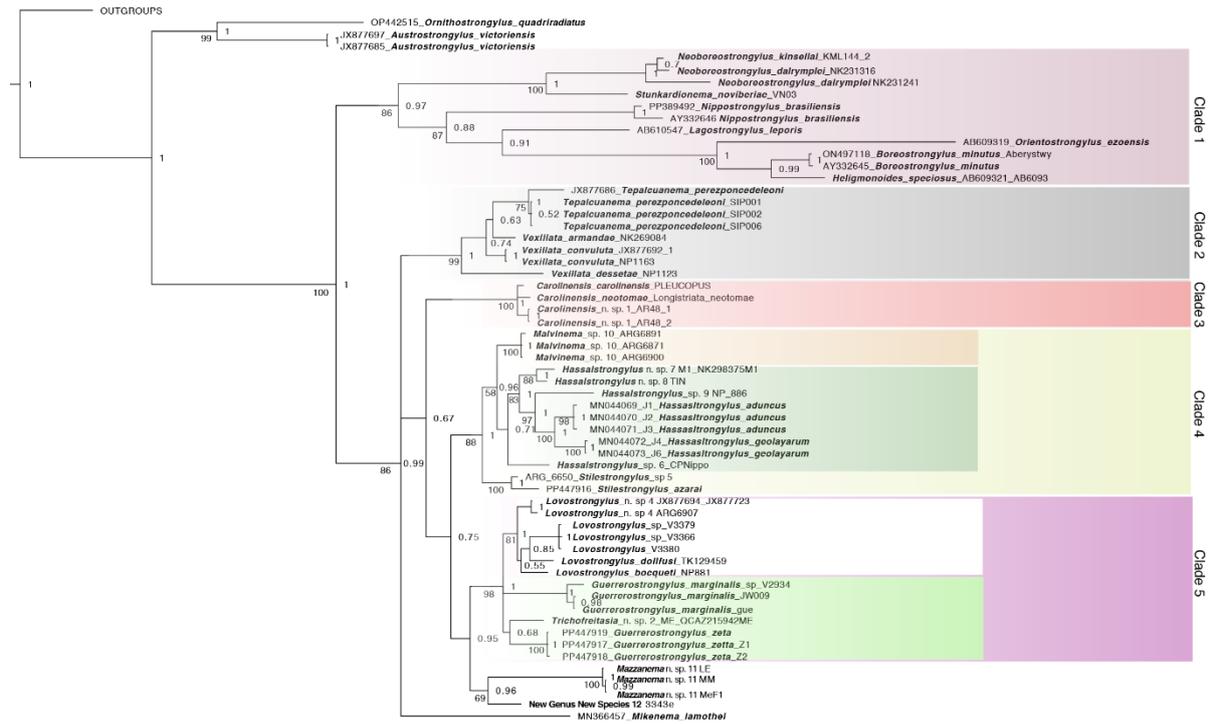
Yamaguti, S. (1935). Studies on the helminth fauna of Japan Part 13. Mammalian nematodes. *Japanese Journal of Zoology*, **6**, 433 – 456.

Figure 1. Maximum likelihood phylogeny based on concatenated nDNA (*ITS1*, 5.8, and *ITS2*) and mtDNA (*COI*) sequences. Numbers on branches indicate bootstrap support values (ML) followed by posterior probabilities (Bayesian) for major nodes. Tips are labeled with species names, followed by museum catalogue numbers and GenBank accession numbers as appropriate (Table 1).



ACCEPT

Figure 2. Maximum likelihood phylogeny based on the ribosomal nuclear DNA (*ITS*) sequences. Numbers on branches indicate bootstrap support values (ML) followed by posterior probabilities (Bayesian) for major nodes. Tips are labeled with species names, followed by museum catalog numbers and GenBank accession numbers as appropriate (Table 1).



ACCEPTED

Figure 3. Comparison of synlophes and bursae of three genera in Nippostrongylinae. **a**, cross section of male and **b**, female of *Neoboreostromylus kinsellai*, collected from *Neofiber alleni* in Florida. **c**, Bursa of *Neoboreostromylus dalrymplei* collected from *Microtus pennsylvanicus* in Canada. **d**, Cross section of male; **e**, female and **f**, bursa of *Tepalcuanema perezponceleoni* collected from *Nyctomys sumichrasti* in Los Tuxtlas locality. **g**, Cross section of male and **h**, bursa of *Carolinensis carolinensis* collected from *Peromyscus maniculatus* in Arkansas, U.S.A. **i**, Cross section of female and **j**, bursa of *Carolinensis neotomae*, collected from *Neotoma floridana* in Arkansas, U.S.A. Scale bars **a**, **b**, **e**, **g**, and **h** = 30µm. Scale bars for **c** = 200 µm. Scale bars for **d**, **h** and **j** = 50µm. Scale bar for **f** = 100µm

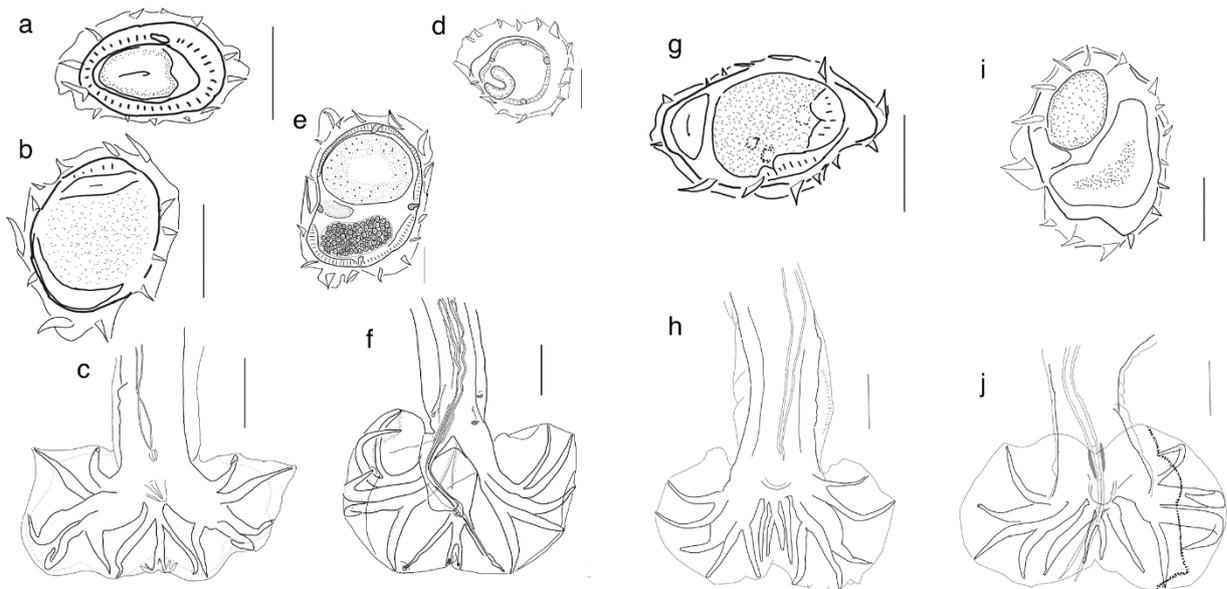


Figure 4. Comparison of synlophe and caudal ornamentation between *Lovostrongylus* and *Hassalstrongylus*. **a – e**, Female of *Lovostrongylus* n. sp. 4 collected from *Calomys* sp. in Argentina; **a**, cross section at esophageal level; **b**, midbody, **c**, uterus, **d**, anal region and **e**, posterior end in lateral view featuring postanal ala. **f – j**, female of *Hassalstrongylus geolayarum* collected from *Sigmodon* sp in Mexico; **f**, cross section at esophageal level, **g**, midbody; **h**, uterus, **i**, anal region and, **j**, posterior end in lateral view. Scale bars **a – d**, and **f – i** = 30µm; **e, j**= 50µm

Accepted Manuscript

