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The Mongolian Remodeling and the structure of Anoplocephalid cestode diversity

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THE MONGOLIAN REMODELING AND THE STRUCTURE OF ANOPLOCEPHALID CESTODE DIVERSITY

By

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THE MONGOLIAN REMODELING AND THE STRUCTURE OF ANOPLOCEPHALID CESTODE DIVERSITY

This thesis by Mackenzie Grover is recommended for approval by the student's Thesis Committee and Department Head in the Department of Biology and by the Dean of Graduate Studies and Research.

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ABSTRACT

THE MONGOLIAN REMODELING AND THE STRUCTURE OF ANOPLOCEPHALID CESTODE DIVERSITY

By

Mackenzie Grover

Ecological disruption plays an important role in structuring diversity of flora and fauna. At the end of the Eocene, climatic change across Asia resulted in a faunal turnover (the Mongolian Remodeling) as rodents diversified and larger mammals declined. Throughout the Oligocene, the landscape in Asia was characterized by episodic climatic and landscape changes resulting in pulses of rodent diversification. The role of historical ecological disruption in Central Asia in structuring the diversity of parasites of small rodents has not been thoroughly investigated. The hyper-diverse *Paranoplocephala* species complex (family: Anoplocephalidae) infect rodents throughout the Holarctic and present an opportunity to investigate the consequences of ecological disruption on the diversity of parasites. I test the hypotheses that the Mongolian Remodeling initiated diversification and subsequent recurrent episodes of climatic change increased the tempo of diversification within this assemblage. Using mitochondrial genome sequencing, I build the most robust phylogenetic tree of seven known species and eight previously unknown lineages of the *Paranoplocephala* species complex and use a clock calibration to estimate the temporal origin of the group. Results indicated multiple trans-Beringian dispersal events and numerous host-colonization events, consistent with ecological disruption playing an important role in structuring parasite diversity. I then used morphological analysis to investigate the identities of five unknown lineages. Results indicated that these specimens represent previously undescribed species, reflecting the need for additional sample collection and curation in biorepositories to understand the extent of global parasite diversity.

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This thesis is formatted following the guidelines of the Journal of Parasitology.

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ABSTRACT

Ecological disruption plays an important role in structuring diversity of flora, fauna, and their parasites. At the end of the Eocene, climatic change across Asia resulted in a faunal turnover (the Mongolian Remodeling) as rodents diversified and larger mammals declined. Throughout the Oligocene, the landscape in Asia was characterized by episodic climatic and landscape changes resulting in pulses of rodent diversification. The role of historical ecological disruption in Central Asia in structuring the diversity of parasites of small rodents has not been thoroughly investigated. The hyper-diverse *Paranoplocephala* species complex (family: Anoplocephalidae) infect rodents throughout the Holarctic and present an opportunity to investigate the consequences of ecological disruption on the diversity of parasites. Here, I use whole mitogenome sequencing to produce a well-resolved phylogeny showing relationships within the "arvicolinae clade" of the *Paranoplocephala* species complex. I test the hypotheses that the Mongolian Remodeling initiated diversification and subsequent recurrent episodes of climatic change increased the tempo of diversification within this parasite assemblage. Using mitochondrial genome sequencing, I build the most robust phylogenetic tree of seven known species and eight previously unknown lineages. To include additional diversity into the analysis, I constrained analysis of the mitochondrial *cox1* gene to the topology of the mitogenome tree and perform ancestral host association and biogeographic analysis. A clock calibration estimate put the temporal origin of the *Paranoplocephala* species complex approximately 42 MYA and lineage through time tests showed more diversification occurred in deeper time. Results indicated multiple trans-Beringian dispersal events and numerous host-colonization events, consistent with ecological disruption playing an important role in structuring parasite diversity.

INTRODUCTION

The landscape in Central Asia underwent significant transformations between the end of the Eocene and mid-Miocene epochs as a result of changing climates. During the warm and humid Eocene, Central Asian landscapes were densely forested. In the late Eocene, the proto-Paratethys sea, a major source of Central Asian humidity, retreated, which led to aridification (Kaya et al., 2019). With the transition to the Oligocene (36.4 million years ago), the climate became cooler (Barbolini et al., 2020). The most severe cooling occurred just after the Eocene-Oligocene boundary, approximately 33.5 million years ago. This climatic change led to a transition from densely forested landscapes to open grasslands (Meng & McKenna, 1998). The landscape transition resulted in a faunal turnover as the dominant mammals of the Eocene (perissodactyls) declined and smaller mammals such as rodents and lagomorphs diversified (Gomes Rodrigues et al., 2012; Kraatz & Geisler, 2010; Zhang et al., 2012). This biotic shift has been called the Mongolian Remodeling (Meng & McKenna, 1998).

After the onset of the Oligocene, approximately 31 million years ago, the landscape continued to aridify during a period known as the Early Oligocene Aridification Event (EOAE). Throughout this time the mammal communities remained fairly unchanged (Harzhauser et al., 2016). However, after the EOAE certain components of mammal communities started to diversify, including small mammals, ungulates, and carnivores (Harzhauser et al., 2016). Diversity peaked approximately 28 million years ago. Large herbivore and carnivore diversity subsequently declined, resulting in a strong species turnover. The mid-Chattian stage of the Oligocene consisted of warm, stable environmental conditions and another increase in small mammal diversity, followed by the Late Oligocene Extinction Event (LOEE; 25.6 million years ago), which resulted in a drastic loss (approximate 48%) of mammal diversity. This was

probably caused by another aridification event. During the transition into the Miocene, the climate became increasingly variable, with an increase in humidity and precipitation (Harzhauser et al., 2016).

The fluctuating diversity and distribution of rodents in Central Asia was likely accompanied by diversification of their parasites. Because parasites have phylogenetic histories unique from their hosts, they can inform complex patterns of dispersal and diversification (Hoberg et al., 2015; Hoberg & Brooks, 2008; Galbreath et al., 2020; Nieberding et al., 2004). Within the cestode family Anoplocephalidae, the *Paranoplocephala* species complex (*Paranoplocephala* sensu lato) parasitizes voles across the Holarctic and represents a hyper-diverse assemblage consisting of 23 genera and 72 species (Haukisalmi et al., 2014). Investigating the geographic origin and tempo of diversification of this diverse group could provide insight into the role climatic change has on structuring parasite diversity. For example, if an increased rate of diversification is observed in deeper history, around the time of the Mongolian Remodeling, this could indicate that the extreme climatic changes of this period were an initial driver of parasite diversity and faunal assembly.

Based on current understandings of phylogenetic relationships, it has been proposed that the *Paranoplocephala* species complex originated in Central Asia (Haukisalmi et al., 2014). However, prior to that proposal there had been very limited sampling in Central Asia, which is necessary to evaluate the geographic origins of a group that spans the Holarctic. Further, though some past phylogenetic analyses of the *Paranoplocephala* species complex included extensive taxonomic sampling, phylogenies were primarily based only on the mitochondrial protein-coding *cox1* gene (Haukisalmi et al., 2004, 2006, 2008, 2014, 2016; Wickström et al., 2005). Efforts to apply other protein-coding genes, the mitochondrial *nad1* and nuclear 18s and 28s ribosomal

subunits, were only partially successful. Existing primers for the *nad1* gene do not reliably amplify across all *Paranoplocephala* s.l. lineages, whereas the nuclear 18s and 28s ribosomal subunits reveal minimal genetic diversity across species. Phylogenies constructed using only the *cox1* gene show limited resolution beyond clustering of individuals into distinct clades representing separate species. In addition, the use of a single gene for phylogenetic analyses only evaluates one perspective of an organism's evolutionary history, but the different genetic markers are in some instances phylogenetically discordant, producing trees with different topologies (Haukisalmi et al., 2014). Building a more robust phylogeny of this diverse group is necessary to better understand the timing, tempo, and biogeography of diversification, and the factors that structured host-parasite associations across time. Therefore, completing additional gene sequencing is necessary to resolve phylogenetic relationships among anoplocephalid cestodes.

With a more robust phylogeny, we will also be in a better position to estimate divergence dates. A clock calibration has been calculated for concatenated *cytb*, 12s and 16s genes of another lineage of anoplocephalid cestodes, genus *Schizorchis* (Galbreath et al., 2020). However, the *cytb* gene has yet to be sequenced for members of the *Paranoplocephala* species complex. Acquiring additional loci, including *cytb*, will facilitate estimating divergence dates. Considering the significant array of host genera, the *Paranoplocephala* species complex can inhabit, resolving phylogenetic relationships will also provide a framework for mapping out the history of host colonization in the group, which could yield insight into historical episodes of ecological disruption that created opportunities for parasites to colonize new host lineages (e.g., Hoberg and Brooks, 2008; Brooks et al., 2019). For example, arvicoline rodents are the primary definitive hosts of the *Paranoplocephala* species complex. Repeated pulses of diversification have been

observed in murid phylogenetic history (Conroy & Cook, 2000). The genus *Microtus* encompasses the rodent species that are the most common hosts of *Paranoplocephala* s.l., and likely have a geographic origin in Eurasia. Multiple pulses of diversification have been observed in *Microtus*, occurring between 2 to 5 MYA. The second most common host genus of the *Paranoplocephala* species complex, *Myodes*, also dates back to 2 to 3 MYA (Kohli et al., 2014). If the *Paranoplocephala* species complex did originate around the time of the Mongolian Remodeling, their persistence would indicate a long history of host-colonization events. Alternatively, given the relatively recent evolution of the primary host taxa, the *Paranoplocephala* species complex could have evolved around the time their current hosts appeared on the landscape, followed by rapid diversification over the last 5 MY.

In this study, I use whole mitochondrial genome sequencing to acquire sequences for 11 protein coding genes from an array of species within the *Paranoplocephala* species complex in order to resolve phylogenetic relationships. I test the hypothesis that the Mongolian Remodeling played an important role in structuring the diversity of the *Paranoplocephala* species complex. In addition, I apply a clock calibration to the phylogeny and estimate the temporal origin of the group and the tempo of diversification across geologic time, testing the prediction that there will be an increased rate of diversification in deeper time coinciding with the repeating episodes of climatic change throughout the Oligocene and Miocene.

METHODS

Study System:

Mongolia is located in Central Asia and has six ecological zones distributed across it. These can be divided into three major latitudinal zones; alpine, forest, and forest steppe in the north and west, desert in the south and east, and steppe habitats lying between them (Angerer et

al., 2008). It has a continental climate, meaning that it experiences climatic extremes, including severely cold winters and hot, dry summers.

Voles (subfamily Arvicolinae), the primary hosts of the *Paranoplocephala* species complex, can be found throughout Mongolia and occupy an array of habitat types, including open prairies and dense forests. Almost all voles are herbivorous, but they hunt and eat insects as well (Novikovskaya et al., 2021), which increases the potential for cestode infection by trophic transmission of metacestodes in the intermediate hosts, which are oribatid mites for anoplocephalids (Denegri, 1993).

I acquired cestodes from the Museum of Southwestern Biology (MSB) in Albuquerque, New Mexico, and the Northern Museum of Zoology at Northern Michigan University. These specimens had been provisionally identified as anoplocephalids and were collected from 287 hosts representing 28 species (Supplemental Table 7). In addition, I acquired 96 samples of isolated DNA from cestodes putatively identified as anoplocephalids, but not identified to species, collected in Alaska and eastern Siberia. In total, my data represented sampling from 46 localities, 35 in the Nearctic and 11 in the Palearctic (Figs. 1, 2).

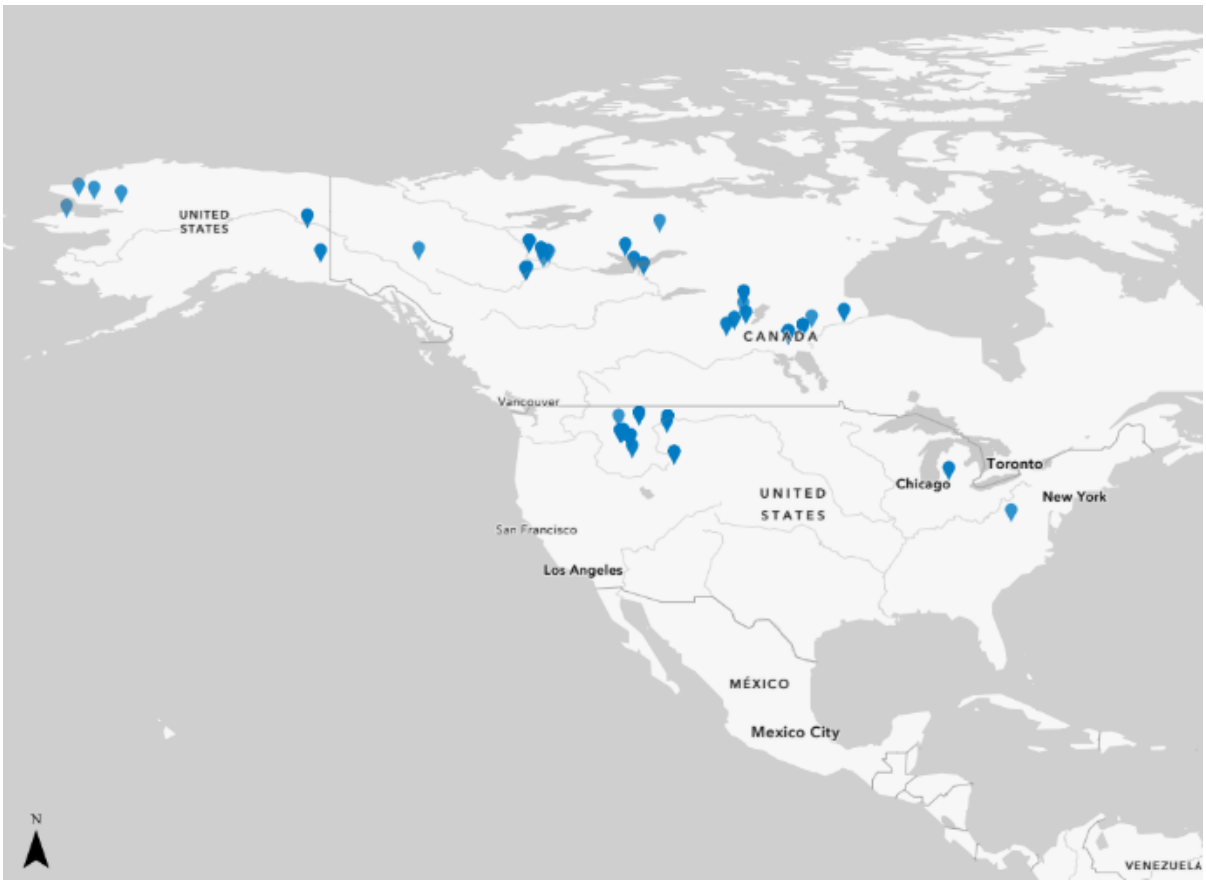

Figure 1. Nearctic sampling localities for anoplocephalids in rodent hosts archived at the Museum of Southwestern Biology and the Northern Museum of Zoology.

Figure 2. Palearctic sampling localities for anoplocephalids in rodent hosts archived at the Museum of Southwestern Biology and the Northern Museum of Zoology.

From the MSB collection, I subsampled one to three distal proglottids and extracted whole genomic DNA using a Masterpure Complete DNA and RNA Purification Kit from Biosearch Technologies. These DNA isolates were then used for PCR amplification of the mitochondrial *cox1* gene using primers COX-F (forward, 5' GATGTTTTCTTTACATTTATCTGGTG 3') and COX-R (reverse, 5' GCCACCACAAATCAAGTATC 3') (Haukisalmi et al., 2004). Thermocycling conditions consisted of initial denaturation at 95 degrees for 8 minutes, 35 cycles of denaturation at 95 degrees for 30 seconds, annealing at 53 degrees for 60 seconds, and primer extension at 72 degrees for 60 seconds, followed by final extension at 72 degrees for 7 minutes (Haukisalmi et

al. 2004). PCR amplification was confirmed by gel electrophoresis in 1.5% Tris-EDTA agarose gel. Ethidium bromide staining was used for PCR product detection. Successfully amplified PCR products were purified using exo-sap before being sent to Elim Biopharmaceuticals, Inc., for bi-directional sequencing. Electropherograms were assembled and evaluated using Geneious v6. Preliminary species identification was made using NCBI's BLAST database. Supplementary *cox1* sequences were acquired from Voitto Haukisalmi and from Genbank, representing samples with morphologically confirmed species identification, which provided a reference for DNA sequence-based species identification.

Sequences were aligned in MEGA-11 (Tamura et al., 2021) using MUSCLE (Edgar, 2004) based on default parameters, and alignments were visually confirmed. Some sequences introduced single or double nucleotide gaps. Single or double nucleotide gaps in a protein coding gene would result in a shift of reading frame and are therefore likely to be the result of sequencing errors. Because electropherograms were not available for sequences provided by Voitto Haukisalmi, I was unable to evaluate sequence quality. Therefore, these insertions were presumed to be spurious results from low-quality sequencing runs and their positions were excluded from the final alignments.

To identify genetic lineages that may represent previously undescribed species, I developed a genetic distance threshold by calculating the mean uncorrected intraspecific pairwise genetic distances among validated species in the dataset. Groups that differed by genetic distances greater than this threshold were considered to potentially represent new species-level diversity and were treated as distinct lineages for species tree analysis. In instances where sequences from multiple individuals of a given species were available, discerned by sequence comparisons that showed a genetic distance less than the uncorrected intraspecific pairwise

distance threshold, I selected a single representative specimen for subsequent phylogenetic analyses.

In order to select samples for whole genome sequencing, I evaluated DNA extract quality for all samples using a NanoDrop instrument. I considered good quality DNA extractions to have concentrations over 10 ng/ul, and a 130/160 value around 2.0. I constructed a neighbor-joining tree in MEGA-11 (Tamura et al., 2021), and then selected samples from each major clade that had acceptable DNA quality. More precise DNA concentration measurements were taken using a Qubit Fluorometer and I selected one to two samples per species (or putative species) with the highest DNA concentration for genomic sequencing. These samples were then sent to New England Biolabs for analysis by Bioanalyzer to quantify DNA fragment sizes in order to determine appropriate reaction conditions for DNA shearing during library preparation. Prior to whole mitochondrial genome sequencing, I prepared libraries using the NEBNext FFPE DNA Library Prep Kit with NEBNext UltraShear Enzymatic Fragmentation kit. First, DNA samples were repaired using NEBNext FFPE DNA Repair v2 mix, followed by fragmentation where the DNA was sheared using NEBNext UltraShear Enzymatic Fragmentation Enzyme mix (15 minute) incubation. Next, the ends of the sheared DNA fragments were prepared using Ultra II End Prep Enzyme mix followed by adaptor ligation. SPRIselect Sample Purification Beads were used to isolate adaptor-ligated DNA fragments from extraneous fragments and free adaptors. Samples then underwent a PCR amplification step, which appended unique identifying barcodes to samples representing separate individuals. SPRIselect beads were again used to isolate barcoded fragments. Desired DNA fragments were eluted using 0.1X TE. Final libraries were sent to the Van Andel Institute in Grand Rapids, MI, for sequencing.

Upon receipt of fastq files for each sequenced sample, reads were trimmed and poor-quality reads removed using fastqp Version 0.3.4. The clean files were then analyzed and the mitochondrial genome assembled using MEANGS (Song et al., 2022). To reduce computational demands of the analysis, only the first three to five million sequences from cleaned data files were used, but this provided ample coverage across the mitochondrial genome. The final assembled sequences were evaluated and edited in Geneious v6. Because non-coding regions contained numerous indels which made it difficult to assess homology between sequences derived from different species, I focused on the protein coding regions, which were isolated using MEGA-11 (Tamura et al., 2021). The coding regions *cox2* and *nad4l* contained sequences with numerous ambiguity codes and were removed from the data set. In total, 11 coding regions were retained for analysis.

The 11 coding sequences were partitioned and aligned to the correct reading frame using MEGA 11 (Tamura et al., 2021). Next, I used BEAST Version 2.7.5 (Bouckaert et al., 2019) to remove third codon positions from the 11 coding sequences, as initial analyses suggested a high degree of homoplasy at these positions. I constructed a phylogeny by linking the trees for all the loci and using a GTR+I+G model of nucleotide evolution. The analysis ran for 500 million generations, sampling every 50000 generations, and I confirmed that the analysis converged on stable parameter values in Tracer Version 1.7.1 (Rambaut et al., 2018). To estimate timing, I applied a clock calibration of 0.00426 mutations per site per million years (Galbreath et al., 2020) to the *cytb* gene in BEAST (Bouckaert et al., 2019) and ran 100 million generations. Analysis convergence was confirmed in Tracer Version 1.7.1 (Rambaut et al., 2018) (Fig.3).

To investigate the relationships between species represented in the mitogenome dataset and other anoplocephalid species that were not included in that dataset, I conducted a

phylogenetic analysis of just the *cox1* dataset, including 49 sequences. I completed this analysis using BEAST Version 2.7.5 (Fig.4) and constrained the topology of the tree to the three major clades that were supported by statistically significant posterior probabilities (>0.95) in the 11CDS analysis. All species within the *Paranoplocephala* species complex were constrained to a monophyletic clade to differentiate between *Anoplocephaloides* Baer, 1923 and *Paranoplocephala* s.l. Further clade constraints were applied by specifying MRCA priors in BEAUti (Bouckaert et al., 2019) and incorporating rogues to allow the analysis to include other taxa within the specified clades. The constrained clades were as follows: *Anoplocephaloides: Anop. dentata* (Galli-Valerio, 1905), *Anop. kontrimavichusi* Rausch, 1976, *Anop. lemmi* (Rausch, 1952), unknown lineage 7; Clade 1: *Microticola spp.* Haukisalmi et al., 2014, unknown lineage 4; Clade 2: *Parano. jarrelli* Haukisalmi, Henttonen & Hardman, 2006, *Parano. macrocephala* (Douthitt, 1915), unknown lineage 2, unknown lineage 3, unknown lineage 11; Clade 3: *Rodentocestus freemani* (Haukisalmi et al., 2014), unknown lineage 5, unknown lineage 10. All other species were set as rogues for the three clades within *Paranoplocephala* sensu lato. No outgroups were used and unknown lineage 9 was removed due to its deep divergence from *Anoplocephaloides* and the *Paranoplocephala* species complex. The analysis was run for 100 million generations, sampling every 5,000 generations using tools in the CIPRES web portal (www.phylo.org; Miller et al., 2010). Analysis convergence was confirmed using Tracer (Rambaut et al., 2018). The consensus tree file was constructed using in TreeAnnotator (Bouckaert et al., 2019).

To test for an elevated tempo of diversification in deeper history, I ran a lineage through time test and gamma test on the constrained *cox1* tree in R (R Core Team, 2021), which tests if a phylogenetic assemblage diversified at a constant rate (Pybus & Harvey, 2000). A gamma value

greater than zero indicates more diversification occurred closer to the tips of the phylogeny, while a gamma less than zero suggests more diversification occurred in deeper time than would be expected under a pure birth process.

Historical host-associations were estimated for nodes within the phylogeny using Bayesian Binary Markov chain Monte Carlo (Ronquist & Huelsenbeck, 2003) implemented in RASP (Yu et al., 2015). Each extant lineage in the tree was assigned to its primary host's tribe, which were Microtini, Dicrostonychini, Lemmini, Myodini, Phenacomyini, Apodemini, Marmotini, Arvicolini, and the maximum number of host tribes that could be assigned to nodes was set to two (Mammal Diversity Database).

To examine the biogeographic history of the group, I used the R program BioGeoBEARS to estimate the ancestral ranges (Matzke, 2018). I used the constrained *cox1* consensus tree and assigned each taxon in the tree to one of four geographic regions: North America, Beringia, Asia, and Europe. The maximum geographic range was set to two, but ranges including North America and Asia, and Beringia and Europe were excluded. I calculated raw and corrected AIC scores to arbitrate among six models; dispersal extinction cladogenesis (DEC), dispersal-vicariance likelihood (DIVALIKE), BayArea likelihood (BAYAREALIKE), and each of these models incorporating the jump parameter, which incorporates jump-dispersal estimation into the biogeographic reconstruction.

RESULTS

The final *cox1* dataset consisted of 365 sequences, 103 of which were unique haplotypes. This dataset represented 19 genera and 36 known species. The mean intraspecific genetic distance for *cox1* sequences from validated species was 2.35%. I used this value as a minimum divergence threshold for inferring interspecific lineage comparisons. I identified 11 unknown

lineages that did not phylogenetically group closely with any described species. The mean genetic distance between all unknown lineages and their closest relatives was greater than the 2.35% threshold, supporting their status as unique species. These lineages were assigned numeric identifiers. From each distinct genetic lineage, one sequence was used for further phylogenetic analysis, resulting in a total of 47 *cox1* sequences.

The phylogeny based on the 11CDS dataset has strong support, with all but one of the nodes having statistically significant posterior probabilities (Fig. 3). Within this tree, Unknown lineage 7 groups with *Anoplocephaloides*, and together these lineages are sister to *Paranoplocephala* sensu lato. The other seven unknown lineages group within the *Paranoplocephala* species complex. Unknown lineages 2, 3, and 11 group with genus *Paranoplocephala* s.s. (Clade 1), lineages 5 and 10 fall out with *Rodentocestus* and *Douthittia nearctica* (Haukisalmi & Henttonen, 2007) (Clade 2), and unknown lineage 4 is sister to *Microticola* (Clade 3). Unknown lineage 6 stems from a deep node and is most closely related to *Paranoplocephala* s.s. in Clade 1. The only node with poor support (70.65% posterior probability) leaves uncertainty regarding the relationships among Clades 1, 2 and 3.

The time calibration estimates put the divergence date between the *Paranoplocephala* species complex and *Anoplocephaloides* approximately 45MYA. The estimated origin of P*aranoplocephala* s.l. is about 39 million years ago.

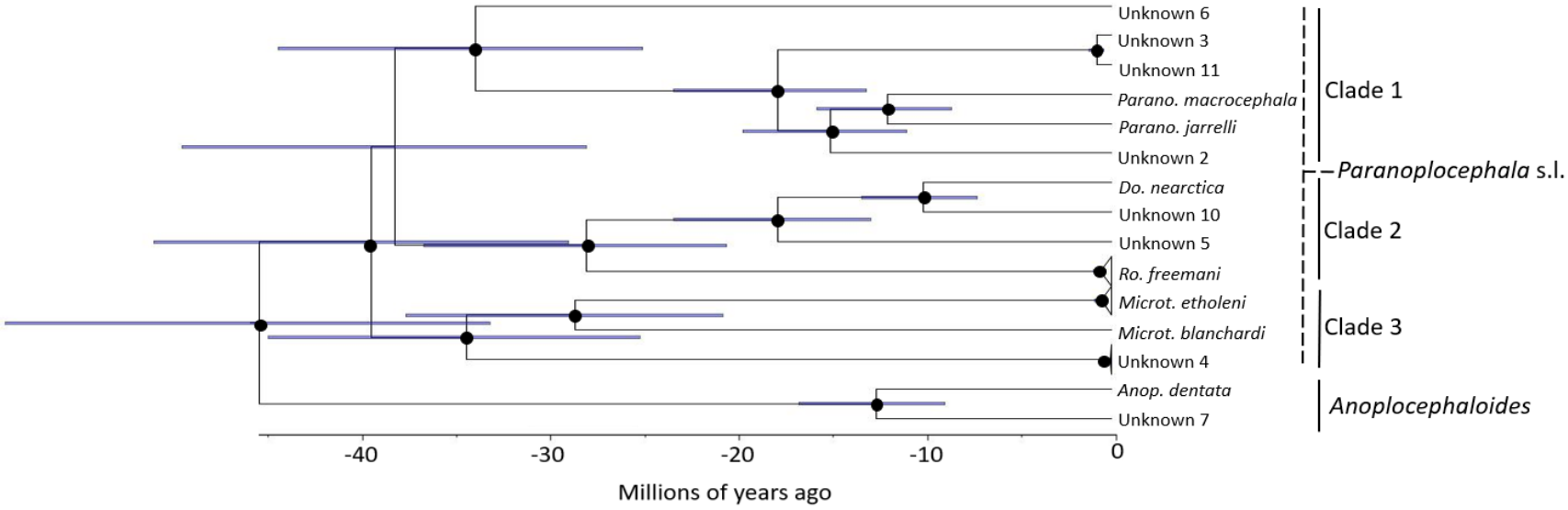

Figure 3. Time calibrated phylogeny based on eleven mitochondrial coding sequences (11CDS) generated in BEAST. Strongly supported relationships with posterior probabilities >95% represented by black circles. Blue bars indicate 95% highest posterior density. Calibrated time scale applied based on an estimate for the age of the basal node of 45 million years. This estimate was calculated using a rate of molecular evolution estimated for *cytb* (Galbreath et al., 2020) applied to an analysis of *cytb* alone.

In the phylogeny based on the *cox1* gene alone I identified four major clades within the *Paranoplocephala* species complex, arbitrarily named Clades 1 through 4, although only monophyly of Clade 1 was strongly supported. This phylogeny shares similarities with the 11CDS tree; the *Anoplocephaloides* diverged from an ancestor common to all of the *Paranoplocephala* species complex, unknown lineage 4 groups with *Microticola,* and lineages 5 and 10 fall out together. However, this tree varies in that lineage 7 is sister to *Eurotaenia gracilis* instead of *Anoplocephaloides,* and lineage 11 does not group with lineages 2 and 3. In addition, some genera are polyphyletic, including *Parano. omphalodes*, *Microticola blanchardi* and *Micro. etholeni.* These issues have been noted in previous studies (Haukisalmi et al., 2014). Interestingly, unknown lineage 9 falls out with an outgroup, *Catenotaenia*, and is deeply divergent from *Anoplocephaloides* and *Paranoplocephala* sensu lato.

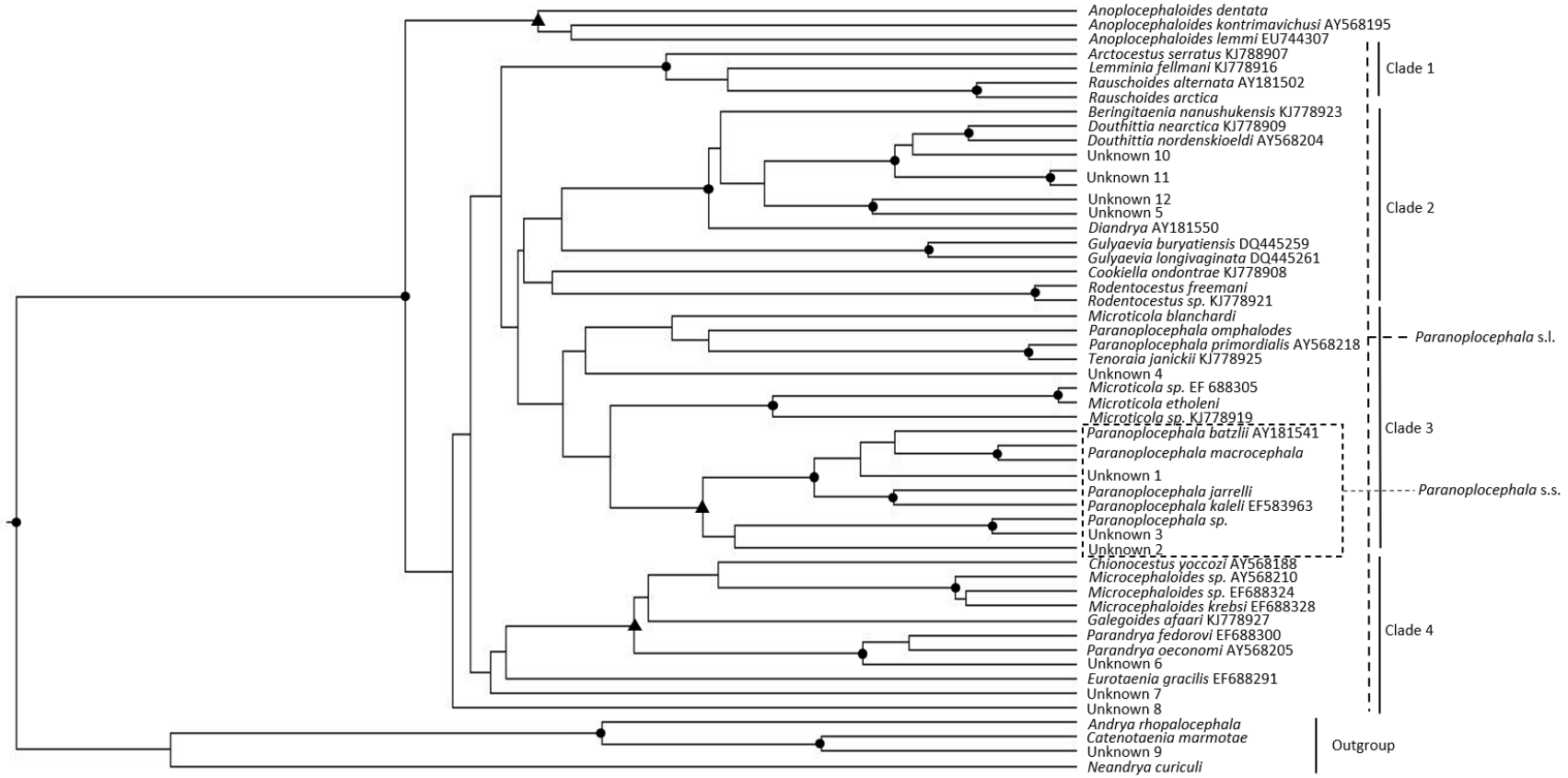

Figure 4. Bayesian phylogeny of anoplocephalid species based on the *cox1* gene. Nodes with statistically significant posterior probabilities (>95%) are indicated with black circles. A node with support >94% is indicated with a black triangle.

Cox1 tree constrained to 11CDS:

The *cox1* tree constrained to conform to the topology of the 11CDS tree (Fig.5) has more support at the basal and more recent nodes but loses resolution at the interior relationships. Compared to the unconstrained *cox1* tree, there are overall fewer well supported nodes. Within the *Paranoplocephala* species complex, five deeply divergent clades are apparent, although none with strong support. None of these clades are characterized by apparent unifying host-associations, distributions, or morphological characteristics (as described in Haukisalmi et al., 2014).

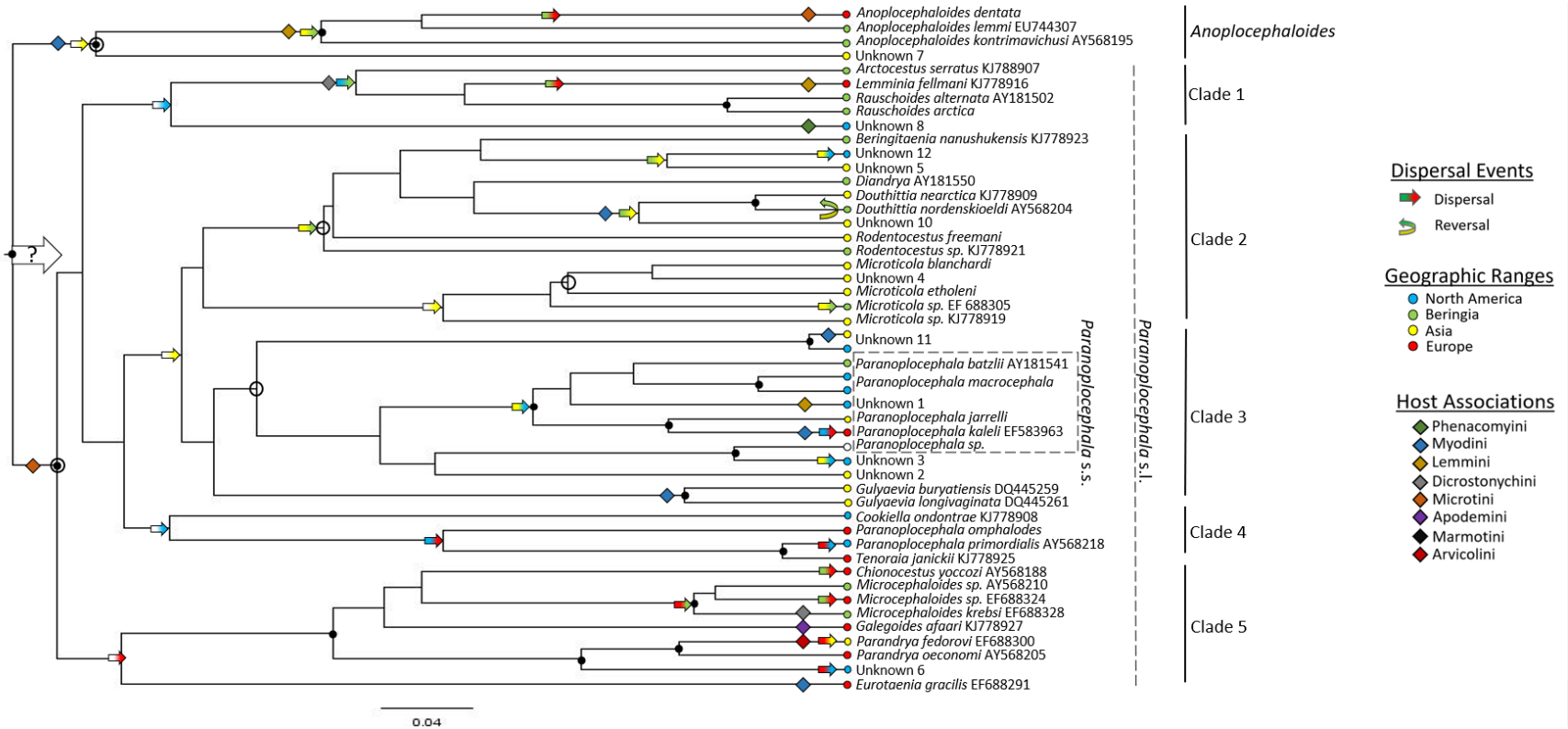

Figure 5. Phylogeny for anoplocephalid species based on *cox1* gene constrained to the topology of well-supported relationships in the 11CDS tree. Constrained relationships are indicated by a black circle on the node of the most recent common ancestor of constrained taxa. Strongly supported relationships with posterior probabilities >95% are represented by black circles. Dispersal events inferred by RASP are indicated by colored arrows, where the leftmost color indicates the area the organism dispersed from and the rightmost color indicates the area dispersed to. Arrows with white portions represent dispersal from unknown regions. Colored circles at the tips of the tree indicate the current geographic distribution of taxa. Colored diamonds indicate host-colonization events inferred by RASP where color corresponds to the colonized host tribe.

Tempo of diversification:

Lineage through time tests based on the constrained *cox1* phylogeny showed greater diversification occurred early in the phylogeny than would be expected under exponential growth (Fig.6). The Pybus and Harvey gamma value of -3.966 and a significant p-value of 0.0001 supports an increased rate of diversification occurring towards the root of the tree as opposed to the tips.

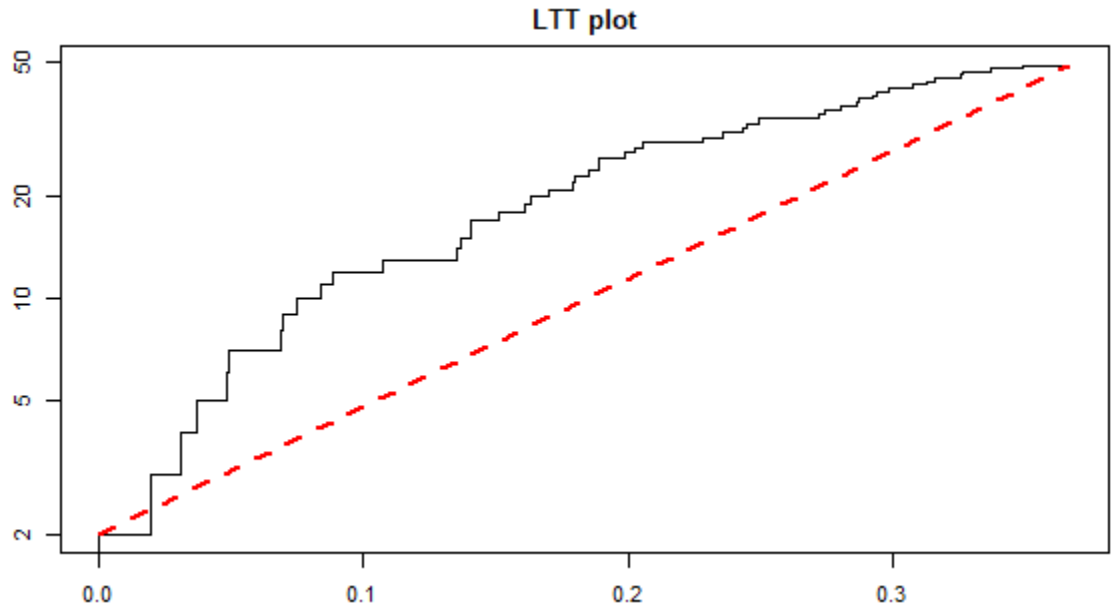

Figure 6. Lineage through time test for anoplocephalid cestodes based on the constrained *cox1* phylogeny. The observed accrual of species is indicated by the black line, while the expectation under the exponential speciation model is indicated by the red dotted line.

Biogeography:

The DIVALIKE+J model had the strongest support by raw and corrected AIC scores. Under this model, the ancestral range is ambiguous. Estimated ranges in the Palearctic followed by a geographic range inferred to be in Beringia or the Nearctic were inferred as eastward dispersal, and vice-versa for westward dispersal. The total number of trans-Beringian dispersal events included six eastward and two westward dispersal events with one and no reversals

respectively (Fig.5). European dispersal events included six dispersing from the Nearctic to Europe, and three from Europe to the Nearctic. Five of these dispersal events coincide with host-colonization events.

Host Associations:

The ancestral host-associations of the *Paranoplocephala* species complex are with tribes Microtini and Myodini. Five colonization events of Myodini from Microtini are inferred, as well as three colonization events of tribe Lemmini, and two of Dicrostonychini. All other host tribes have one inferred colonization event.

DISCUSSION

Systematics

The phylogeny of the *Paranoplocephala* species complex generated from the 11 mitochondrial coding sequences is the most robust phylogenetic reconstruction of the arvicolinae-associated clade of *Paranoplocephala* s.l. to date and demonstrates the existence of previously undescribed lineages. Most of the lineages are primarily associated with *Microtus* hosts and are found in Asia. Within each clade there are several morphological types evident (as described in Haukisalmi et al., 2014, see Supplemental Table 6 for body type categories), and species with similar anatomies do not cluster into monophyletic groups.

The well-resolved 11CDS tree unambiguously clarified some of the questionable taxonomic arrangements among the *Paranoplocephala* species complex and related taxa. For example, phylogenetic reconstructions using the *nad1* gene previously showed *Paranoplocephala* s.l. to be paraphyletic with respect to the *Anoplocephaloides* clade while *cox1* data suggests that these two groups are sister (Haukisalmi et al., 2014). The 11CDS tree

generated in this study supports reciprocal monophyly of *Paranoplocephala* s.l. and *Anoplocephaloides* (including lineage 7) with respect to each other. Morphological characteristics also indicate that unknown lineage 7 belongs to genus *Anoplocephaloides*, as it has a wedge-shaped strobila and lack of a neck, which are characteristic of the genus. However, this lineage is sister to *E. gracilis* in the *cox1* tree. The strong nodal support of the 11CDS tree confirms the generic assignment of this lineage. *Microticola blanchardi* and *Microt. etholeni* are thought to belong to the same genus based on 28s, *nad1*, and morphological data, but they are polyphyletic in the *cox1* dataset. These two taxa were sister in the 11CDS tree with strong support, confirming their assignment to *Microticola*. However, given the limited number of taxa represented in the 11CDS dataset, it does not fully address questionable relationships in the *cox1* phylogeny. For example, *P. primordialis* and *T. janickii* are only represented in the *cox1* phylogeny, where they appear to have diverged from the same clade as *Microticola*, and more recently than the divergence between *Microt. etholeni* and *Microt. blanchardi*. It is unlikely this reflects the true taxonomic relationships of *Tenoraia* and *Microticola* as they are considerably different morphologically. These questionable taxonomic relationships in the *cox1* phylogeny reflect the limitations of *cox1* as a phylogenetic marker to resolve relationships across diverse genera.

Biogeography

The history of the Holarctic has been characterized by the outcomes of climatic cycling with alternating episodes of ecological perturbation and relative stability (Barnosky, 2005; Hoberg et al., 2017). Recurrent climatic oscillations established thermal gradients and subsequently resulted in the episodic opening and closing of the Bering Land Bridge, an intermittent connection between present-day north-eastern Siberia and Alaska, which is also

known as Beringia (Hultén, 1937). Beringia provided a dispersal corridor, with thermal gradients, for organisms to cross between the contents. Because Beringia opened and closed multiple times, organisms crossed it in pulses. Evidence for multiple events of faunal expansion and isolation across the Bering Land Bridge is consistent with the biogeography and mosaic assembly of other cestode taxa parasitizing small mammals, including some of the same host groups (Cook et al., 2004; Hoberg et al., 2012; Galbreath et al., 2023; Repenning, 2001). Most evidence suggests that small mammals and their parasites primarily dispersed eastward across Beringia into the Nearctic (Galbreath et al., 2020; Rausch, 1994; Waltari et al., 2007). For example, five independent and three temporally distinct trans-Beringian dispersal events are inferred for another lineage of cestodes, genus *Arostrilepis,* parasitizing the host genera *Myodes, Microtus,* and *Lemmus* (Haas et al., 2020). This group of cestodes colonized North America relatively recently; once in association with *Microtus* hosts during the early Pleistocene, and twice with *Myodes* hosts. The first North American colonization in association with *Myodes* occurred during the mid-Pleistocene and a more recent North American colonization with *M. rutilus* prior to the last glacial maximum (Hass et al., 2020; Galbreath et al., 2023).

Range estimates using the constrained *cox1* phylogeny suggested multiple dispersal events in both eastward and westward directions, though the analysis left ambiguity regarding the geographic range of ancestral nodes. Therefore, my results support multiple trans-Beringian dispersal events, which is similar to the history of *Arostrilepis*, although the inferred reversal into Asia is unique to the *Paranoplocephala* species complex.

Multiple waves of dispersal across Beringia have been recorded in the biogeographic history of the hosts of the *Paranoplocephala* species complex. The first recognized trans-Beringian dispersal by arvicoline rodents from Asia to North America was likely by a progenitor

of *Lemmiscus* around 4 MYA (Abramson et al., 2021), while the earliest fossils of *Myodes rutilus* found in North America date back only to the Holocene (Cook et al., 2004). The broad range of time in which these dispersal events occurred support multiple waves of trans-Beringian dispersal by the *Paranoplocephala* species complex. However, without a time calibration to the *cox1* tree, estimating specific timing of dispersals is problematic. Additional whole mitochondrial genome sequencing efforts, incorporating the breadth of diversity within *Paranoplocephala* s.l. and the application of a time calibration, may further clarify these patterns.

Dispersal events from Beringia and North America to Europe are also implied by the biogeographic results. Due to the uncertainty around which direction dispersal from the Nearctic to Europe occurred (westward across Beringia via Asia or eastward across the Thulean land bridge), the directionality of European/Nearctic dispersal events could not be identified. These results may indicate that the Thulean land bridge, which once connected North America and Europe via Greenland (Brikiatis, 2014), played a role in the dispersal and diversification of this group. For this to be the case, the ancestors of the *Paranoplocephala* species complex would have evolved with now long extinct rodent faunas, a phenomenon that has been observed in other studies (for example, Galbreath et al., 2020). However, timing estimates put the two Thulean bridge formations around 57 and 55.8 MYA. Because the divergence of the *Paranoplocephala* species complex and *Anoplocephaloides* is estimated to be 45MYA, a European colonization directly from North America is unlikely.

An Asian origin of the *Paranoplocephala* species complex has been proposed in other studies. The Qinghai-Tibet Plateau (QTP) in China is characterized by rich rodent diversity shaped by the uplift of the plateau leading to allopatric speciation. The plateau lifted between 20

and 13 MYA, although an earlier uplift around 40 MYA has also been proposed (Chung et al., 1998). It has been suggested that diversification in the order Cyclophyllidea was driven by the uplift of the plateau (Wu et al., 2022). The point estimate of the *Paranoplocephala* species complex origin, 42 MYA, is close to the initial inferred uplift of the QTP.

Diversification

The results of this study support a temporal origin of the *Paranoplocephala* species complex of approximately 42 MYA, which is inconsistent with my hypothesis of an origin coinciding with the Mongolian Remodeling, approximately 30 MYA. However, my results do show an increase in the tempo of diversification between approximately 27 and 15 MYA. Repeating climatic shifts continued through the end of the Oligocene and into the Miocene, and therefore my results are consistent with the hypothesis that ecological disruption in Central Asia contributed to structuring the diversity of the group through repeating episodes of refugial isolation and range expansion.

In contrast to my results, a 2022 study also attempted to identify the geographic and temporal origin of some Cyclophyllidean cestodes using a clock calibration calculated from the divergence dates of two *Taenia* species and two *Schistosoma* species applied to available mitochondrial protein coding genes. They placed the temporal origin of *Paranoplocephala* s.s. to the Pleistocene (Wu et al., 2022). In contrast my results show a divergence date between *Parano. jarrelli* and *Parano. macrocephala* to be around 12 MYA. The clock calibration used in my study was generated for the *cytb* gene from another lineage of anoplocephalid cestode (Galbreath et al., 2020), and may provide a more accurate estimate of timing, though an internally derived clock calibration for *Paranoplocephala* would undoubtedly improve the estimate.

The evolution of the hosts of the *Paranoplocephala* species complex is generally characterized by pulses of diversification over the last 5-10 MYA (Kohli et al., 2014). Arvicolinae diversity first radiated around 7 MYA (Abramson et al., 2021). Estimates of host diversification have suggested that modern species appeared on the landscape in the last 3 to 5 MYA (Conroy & Cook, 1999). The extant species of the Microtini tribe are estimated to have originated around the late Pleistocene and experienced rapid diversification. The Myodini tribe diverged from *Microtus* and *Dicrostonyx*-like species between 5 and 7 MYA, followed by a steady rate of diversification of genera within the tribe (Kohli et al., 2014). Several host-colonization events are inferred close to the tips of the constrained *cox1* tree, which may correlate to pulses of host diversification. However, due to the lack of a time calibration on the constrained *cox1* gene, this is speculative.

The recent evolution of the hosts relative to the apparent antiquity of the *Paranoplocephala* species complex suggests these cestodes initially evolved with host taxa that are now extinct. This pattern has been noted in other parasite assemblages as well (Hoberg and Brooks 2008). For example, the biogeographic history of another genus of anoplocephalid cestode, *Schizorchis*, revealed an ancient association between the parasite and a now-extinct host species of the genus *Ochotona*. This "ghost assemblage" persisted until it encountered another host species that acquired the ancient cestode lineage before the original host went extinct (Galbreath et al., 2020).

Therefore, my results indicating the *Paranoplocephala* species complex experienced many host-colonization events are consistent with previously observed patterns of host-parasite association assembly. These host-colonization events could have facilitated diversification through factors proposed in the Stockholm Paradigm (Hoberg and Brooks, 2015).

Stockholm Paradigm and the diversification of *Paranoplocephala* s.l.

Repeating patterns of ecological disruption and stability drive diversification and structure complex host-parasite associations (Hoberg and Brooks, 2008, 2015; Brooks et al., 2019; Galbreath et al., 2023; Kafle et al., 2020). The Stockholm Paradigm (Brooks et al., 2019) is a synthesis providing an explanation of host-parasite evolution through the integration of four evolutionary and ecological concepts: The taxon pulse (Erwin, 1985), ecological fitting (Janzen, 1985), oscillation (Janz & Nylin, 2008), and the geographic mosaic of coevolution (Thompson, 2005). Temporally repeating cycles of ecological disruption result in echoing shifts of the geographic ranges of species is referred to as taxon pulses (Erwin, 1985). When conditions change, parasites may have opportunities to encounter new hosts resulting in expansion in host range. This reflects the capacity for colonization based on their existing suite of characteristics if the host offers the necessary resources for them to complete their life cycle. The potential for organisms to colonize new environments without significant adaptive change describes ecological fitting (Janzen, 1985). Oscillations refer to an oscillating pattern of apparent trends in host range by parasites. As parasites coevolve with their hosts during periods of ecological stability (exploitation mode; Agosta and Brooks, 2020), they appear to be host-specific and when they encounter and colonize new hosts during periods of ecological disruption they appear to be host-generalists (exploration mode; Agosta and Brooks, 2020; Janz & Nylin, 2008). The microevolutionary processes that result in apparent coevolution between organisms describes the geographic mosaic of coevolution (Thompson, 2005). Together, these evolutionary and ecological processes describe the mechanisms that structure diverse and complicated host-parasite associations and can be observed by studying the phylogenetic and biogeographic

histories of organisms and their parasites (Brooks and McLennan, 2002; Araujo et al., 2015; Erwin, 1985; Hoberg & Brooks, 2010; Hoberg & Brooks, 2008; Janz & Nylin, 2008).

The evolutionary significance of my results can be better understood through the synthesis outlined and proposed in the Stockholm Paradigm. The multiple events of host-colonization I identified are consistent with ecological fitting and reflect taxon pulse dynamics as parasites entered novel geographic space and encountered new potential hosts. The increased rate of diversification coinciding with periods of climatic instability suggest oscillations between host-parasite coevolution in isolated populations, leading to allopatric speciation, and host generalization via colonization during geographic expansion, the alternating phases of the taxon pulse. Therefore, the climatic and environmental oscillation(s) in Central Asia since the middle Cenozoic played an important role in structuring parasite diversity.

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Chapter 2: Morphological analysis of undescribed anoplocephalid cestode diversity

ABSTRACT

It has been estimated that only one quarter of global parasite diversity has been described. The use of molecular systematics has facilitated the discovery of parasite diversity that is morphologically cryptic. For example, one group of cestodes, *Paranoplocephala* sensu lato (family Anoplocephalidae), was partitioned across a species complex that includes 12 genera and 26 species. It is likely that there is additional undescribed diversity within this assemblage across large, unsampled regions of the Holarctic. Here I investigate the diversity of specimens consistent with *Paranoplocephala* s.l. that were collected from North America and present morphological data for five putative and previously unrecognized species. My results highlight the need for continued widespread sampling and museum archiving of cestode specimens in order to better understand the scope of parasite diversity in terrestrial systems across spatial scales.

INTRODUCTION

Parasitism is among the most successful life-history strategies, as indicated by the number of independent origins of parasitism and the number of parasitic species (Poulin & Morand, 2000). Cyclophyllidean cestodes are found in every vertebrate group except teleostean and chondrichthyan fishes (Hoberg, 1999). However, the full extent of parasite diversity is not yet known. Current estimates put the number of cestode species at approximately 20,000, but only about 4,000 species are currently described (Caira & Jensen, 2017), leaving a vast gap in our understanding of cestode diversity.

The Anoplocephalidae family of cyclophyllidean cestodes is a diverse, Holarctic group that can infect diverse host lineages including rodents, lagomorphs, and ungulates. Within this family is the hyper-diverse *Paranoplocephala* species complex. Species in this complex are usually found in rodents, most commonly voles of tribes Microtini and Myodini (sub-family Arvicolinae), and use oribatid mites as intermediate hosts (Denegri, 1993). Cestodes within the *Paranoplocephala* species complex have short necks and scolexes lacking armed rostella.

The type species of *Paranoplocephala* s.s. was first described as *Anoplocephaloides omphalodes* in 1783 (Hermann, 1783), as a parasite in *Microtus*, before being attributed to the newly erected genus *Paranoplocephala* Luhe, 1910. Subsequently, two other species of *Anoplocephaloides, Anoplocephaloides variabilis* (Douthitt, 1915), which is now under genus *Microcephaloides* Haukisalmi et al., 2008, and *Anoplocephaloides infrequens* (Douthitt, 1915), were also assigned to *Paranoplocephala*, and the subspecies *Paranoplocephala variabilis borealis* was raised to species level (*P. borealis)* (Rausch & Schiller, 1949).

In the following years, investigations into the morphological differences between species, their distributions, and host specificity led to a restructuring of the genera *Paranoplocephala* and *Anoplocephaloides* (Rausch, 1976). Species with a tubular early uterus were transferred to *Anoplocephaloides*, which then consisted of 17 species. These were considered to be from diverse lines that had achieved similar morphology through convergent evolution. However, lack of clarity regarding phylogenetic relationships and uncertainty regarding the taxonomic significance of morphological characteristics precluded further taxonomic reorganization, such as establishment of additional genera. Species within *Anoplocephaloides* were organized into two categories; those that parasitize squirrels and have long, ribbon-like strobilae (*A. transversaria* (Krabbe, 1879), *A. ryjikovi* (Spasskii, 1950), and *A. wigginsi* (Rausch, 1954); and those that parasitize arvicolids, leporids, or murid rodents, and have short, wedge-shaped strobilae, (*A. dentata* (Galli-Valerio, 1905), *A. troeschi* (Rausch, 1946), *A. lemmi* (Rausch, 1952), *A. kontrimavichusi* Rausch, 1976, *A. wimerosa* (Moniez, 1880), and *A. baeri* (Rausch, 1976). The genus *Aprostatandrya* Kirshenblat, 1938 was reduced as a synonym of *Paranoplocephala*, which then consisted of *Parano. omphalodes, Parano. macrocephala* (Douthitt, 1915), along with species from *Aprostatandrya* and *Andrya* Railliet, 1893. An accurate reflection of species diversity was confounded by inadequate taxonomic resolution of these genera based on incomplete and misinterpreted morphological data. Subsequent morphological analyses supported the synonymy of *Aprostatandrya* and *Paranoplocephala* (Haukisalmi et al., 2004; Haukisalmi & Henttonen, 2003), and *Aprostatnadrya* was reduced to junior synonym.

Paranoplocephala macrocephala was originally described in pocket gophers of the genus *Geomys* and assigned to *Andrya* (Douthitt, 1915), but probably represents a species complex given wide variation in the distribution and number of testes, a diverse array of hosts, and a

broad geographic distribution (Rausch, 1976). It was later reported and characterized from three different host families across North America and Europe (Haukisalmi & Henttonen, 2003).

Molecular systematic analysis of the mitochondrial *cox1* gene clarified some phylogenetic relationships within *Paranoplocephala* and also revealed extensive hidden diversity (Haukisalmi et al., 2004, 2014). For example, it led to the description of three new species within *Paranoplocephala omphalodes*, including *Parano. jarrelli* Haukisalmi, Henttonen & Hardman, 2006, *Parano. batzlii* Haukisalmi, Henttonen & Hardman, 2006, *Parano. freemani* (Haukisalmi, Henttonen & Hardman, 2006) (Haukisalmi et al., 2004). Ultimately, the genus *Paranoplocephala* was revised and divided into 12 genera and 26 species (Haukisalmi et al., 2014).

Molecular systematics also provided a gateway for unambiguous species distinctions that clarified which morphological characteristics are phylogenetically informative (Haukisalmi et al., 2004, 2014; Haukisalmi, Hardman, et al., 2006). Many morphological similarities were found to demonstrate homoplasy (convergence) and therefore are not informative for reconstructing relationships among the genera (Haukisalmi et al., 2014). However, certain characters are informative. For example the structure and position of the early uterus and position of the testes distinguish among genera that had been subsumed within *Paranoplocephala* and other anoplocephalids, including species in *Andrya, Andryoides* Haukisalmi, 2023, and *Neandrya* Haukisalmi & Wickstrom, 2005 (Haukisalmi, 2023).

Within the *Paranoplocephala* species complex, the morphology of the scolex and neck are most informative for discriminating between genera. A narrow neck and large scolex with large, protruding, and anteriorly directed suckers are characteristic of genus *Paranoplocephala* s.s., and characterize the first of three groups (Haukisalmi et al., 2014). The second group contains cestodes with a wide neck relative to the scolex and non-protruding suckers, and

includes the genera *Gulyaevia, Chionocestus, Microticola, Beringitaenia, Parandrya,* and *Hunkeleriella.* The third group includes cestodes with a narrow neck and non-protruding suckers, and can be further subdivided into two categories based on the length of the vagina; 3.1, medium/long vagina; 3.2, short vagina relative to cirrus sac. Group 3.1 includes genera *Arctocestus, Rauschoides, Eurotaenia, Douthittia, Afrojoyeuxia,* and *Lemminia,* and group 3.2 includes the genera *Tenoraia, Cookiella,* and *Rodentocestus*. Additional anatomical characteristics useful in discerning between genera and species includes the pattern of testes distribution (extensive, reaching the poral osmoregulatory canals; overlapping ovary; restricted, reaching antiporal margin of ovary) , and the distribution of the female glands (poral or median).

Recent studies evaluating the evolutionary relationships and diversity of the *Paranoplocephala* species complex have used samples primarily collected from Beringia, Europe, and Russia (for example, Haukisalmi et al., 2004, 2007, 2009; Haukisalmi, Henttonen, et al., 2006; Haukisalmi & Henttonen, 2005; Vlasenko et al., 2019; Wickström et al., 2005). By comparison, fewer studies have investigated the diversity of the *Paranoplocephala* species complex in North America; there also remains a general paucity of comprehensive knowledge of helminth faunas among rodents across continental North America (e.g., Hoberg et al., 2012, 2016). This represents a weakness in our current understanding of diversity and evolutionary history within the assemblage. Given the diversity within this assemblage and the limited sampling of parasite diversity across the planet (Cook et al., 2005, 2017; Haukisalmi et al., 2014), it is likely that additional species are yet to be described. In this study I present morphological data contributing to characterization of a series of previously unrecognized species of anoplocephalids collected from localities across northwestern North America.

METHODS

Previous research identified nine unknown genetic lineages within the *Paranoplocephala* species complex based on comparison of *cox1* gene sequences from described anoplocephalid species (see Chapter 1). Twelve specimens, including representatives of five of the unknown lineages and 4 described species, were available for morphological analysis. However, representatives of the other four unknown lineages were not available for analysis. The unknown specimens were sampled from the northwestern region of the contiguous United States and were collected in four different host species (Supplemental Table 7). All specimens were stored in -20-degree freezer in 70% ethanol, at either the Museum of Southwestern Biology in Albuquerque, University of New Mexico, or the Northern Museum of Zoology, Northern Michigan University. Slide-mounted specimens are archived in permanent collections at the Museum of Southwestern Biology in Albuquerque, New Mexico.

Morphological Methods:

Prior to staining, specimens were soaked in distilled water for up to 30 minutes to remove traces of ethanol from the tissues. Specimens were then stained in Mayer's hematoxylin and de-stained in a 1% solution of HCl and distilled water. Contrast of internal anatomical features was improved by transferring stained worms to 0.5% ammonium hydroxide for up to 5 minutes before straightening in 70% ETOH and slide mounting. Worms were dehydrated through a series of ethanol washes up to absolute ethanol, then soaked in xylene to clear. A solution of Canada balsam and xylene was used as mounting medium and prepared slides were left to dry in a slide oven for several weeks. Drawings of scoleces and mature proglottids were sketched on paper after slide mounting using a drawing-tube, then edited in Adobe Illustrator using a drawing tablet. Morphological measurement data was collected using an Olympus UC90 camera mounted

on an Olympus BX53 microscope. Proglottid measurements were averaged across the number of mature proglottids examined (tables 1-5).

RESULTS

Of the twelve specimens morphologically examined, seven represented four known species: *Paranoplocephala jarrelli, Parano. omphalodes, Microticola blanchardi,* and *Rodentocestus freemani.* The morphology of these specimens was consistent with published species descriptions and redescriptions (Haukisalmi et al., 2014).

The five unknown lineages available for morphological examination included lineages 1, 3, 6, 8, and 12 (see Chapter 1). Of these, unknown lineages 3 and 8 were morphologically distinct relative to known genera and species, while lineages 1, 6, and 12 appeared to belong to genera *Paranoplocephala, Parandrya,* and *Douthittia* respectively. Here I describe the morphology of the five lineages for which specimens were available.

Paranoplocephala sp. *Unknown Lineage 1* (Fig.7, Table 1)

Description: Small anoplocephalid cestodes. Maximum body length 28 mm, maximum width of proglottids 1.1 mm. Scolex large with prominent, anteriorly directed, crateriform suckers. Neck narrow relative to scolex. Proglottids consistently wider than long; average length to width of mature proglottids 0.108X0.625 mm, length: width ratio averages 1:5.77. Genital ducts passing dorsal to osmoregulatory canals. Genital pore, dextral in dorsal view, in middle of segment margin. Osmoregulatory system with wide posterior anastomosing canal. Testes antiporal, overlapping ovary, extending across ventral osmoregulatory canal. Cirrus sac attains dorsal osmoregulatory canal. Vagina approximately half the length of cirrus sac, covered by a glandular

cell layer. Seminal receptacle elongate. Ovary lobate, slightly poral of midline. Vitellarium globular, median, ventral, with respect to ovary.

Host: Lemmiscus curtatus (Tomas, 1912) (catalog number NMU:Mamm:2683)

Locality: from Double Springs Pass in central Idaho, U.S.A.; georef coordinates: 44.26392004, -113.806273; date: 10 Aug, 2018; collector: Kurt Galbreath.

Specimen: NMU:Para:903, strobila barcode NT11AE. One sample collected; one sample examined.

Remarks: Testes in the specimen studied reach the antiporal margin of the vitellarium and substantially overlap the antiporal ventral osmoregulatory canal. In contrast, testes in *Parano. maseri*, as originally described, extend to the poral margin of the vitellarium and do not overlap with the antiporal ventral osmoregulatory canal. Further, the specimen characterized here is morphologically inconsistent with other species of *Paranoplocephala* for which molecular data is not available; those being *Parano. microti* and *Parano. kirbyi* which have a poral position for the ovary and vitellarium and *Parano. rauschi* in which the testes do not overlap the antiporal ventral osmoregulatory canal. Morphology and phylogeny are consistent with this specimen belonging to *Paranoplocephala*, but it likely represents a previously undescribed species. Genetic sampling of *Parano. maseri* would allow a test of this hypothesis.

Phylogenetics: Based on *cox1* sequences, lineage 1 groups within the *Paranoplocephala* s.s. clade in the constrained and unconstrained *cox1* tree (see Chapter 1).

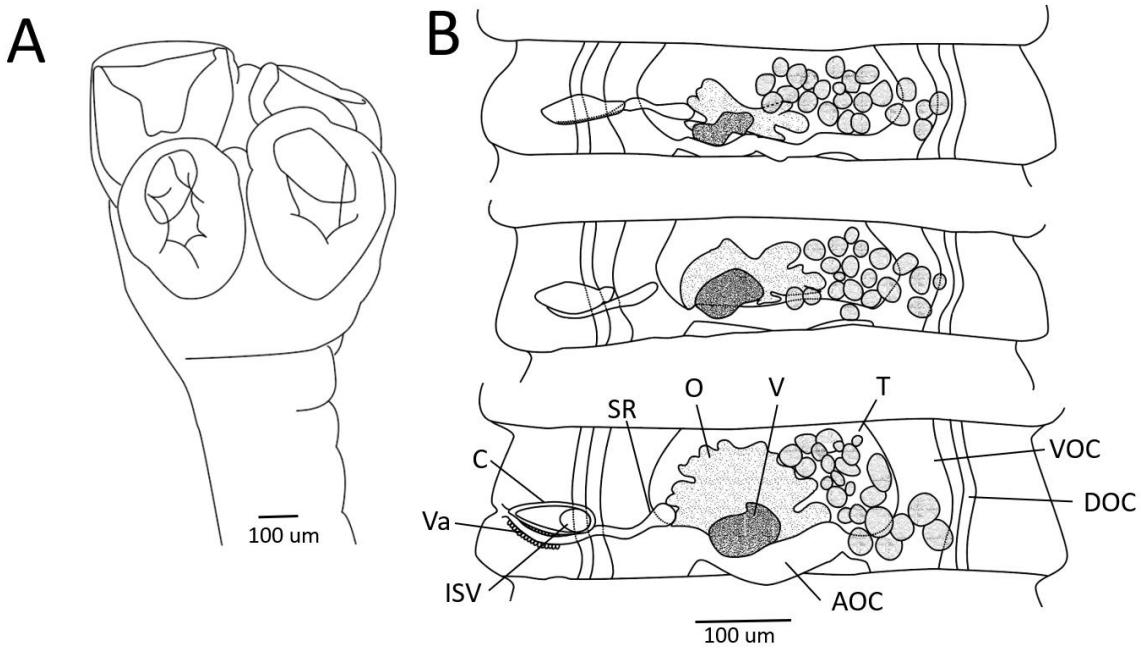

Figure 7. *d* A. Scolex and neck. B. Mature proglottids, in ventral view. C: cirrus sac, Va: vagina, ISV: internal seminal vesicle, SR: seminal receptacle, O: ovary, V: vitellaria, T: testes, VOC: ventral osmoregulatory canal, DOC: dorsal osmoregulatory canal, AOC: anastomosing osmoregulatory canal.

Table 1. Meristic data for *Paranoplocephala* sp. *Unknown lineage 1*, specimen NT11AE. All measurements are in micrometers except BL which is in millimeters. Abbreviations are as follows. BL: strobila length; ScD: scolex diameter; SuD: sucker diameter; Length by width (LxW)- T: testes; CS: cirrus sac; ISV: internal seminal vesicle; O: ovary; Vi: vitellarium; Va: vagina; SR; seminal receptacle.

	NT11AE		
	Mean	Range	No. meas
BL (mm)	28	-	1
ScD	586	599-572	2
SuD	269	285-253	4
T (LxW)	24x20	25x25-22x17	6
CS (LxW)	122x31	185x39-66x24	12
ISV (LxW)	29x22	57x27-17x18	4
O (LxW)	162x84	173x95-147x67	8
Vi (LxW)	85x20	111x28-64x11	8
Va (LxW)	89x6	106x7-71x5	7
SR (LxW)	112x34	163x54-81x22	7

Anoplocephalidae gen. sp., Unknown Lineage 3 (Fig. 8, Table 2)

Description: Small to medium anoplocephalid cestodes. Maximum body length 70 mm, maximum proglottid width 1.2 mm. Scolex large with prominent, protruding, crateriform anteriorly directed suckers. Neck narrow relative to scolex. Proglottids consistently wider than long; average length to width measurements of mature proglottids 0.127x0.896 mm, length to width ratio of mature proglottids averages 1:7.05. Genital pores irregularly alternating. Genital pore near middle of segment margin. Genital ducts passing dorsal to osmoregulatory canals. Testes antiporal, overlapping ovary but not antiporal ventral osmoregulatory canal. Cirrus sac not

attaining ventral osmoregulatory canal. Seminal vesicle short, not attaining ventral osmoregulatory canal. Vagina greater than half the length of cirrus sac, cell layer present. Seminal receptacle elongated with long, narrow neck. Ovary sparsely lobed, on midline. Vitellarium poral with respect to ovary.

Hosts: Specimen in *Microtus longicaudus* (catalog number NMU:Para:3267).

Localities: In *Microtus longicaudus* (Merriam, 1888) from Pilot Knob in western Wyoming; georef coordinates: 43.71213801, -110.036841; date: 12 Aug. 2018; collector: Kurt Galbreath; and in *Myodes sp.*, from Northwest Territories, Canada; georef coordinates: 60.31699, -123.31075; date: 26 July, 2013; collector: Kurt Galbreath.

Specimen: NMU:Para:3267, strobila barcode NT11YT. One specimen available for morphological analysis.

Remarks: Specimens representing lineage 3 are consistent with *Paranoplocephala* in possessing a large scolex, prominent, anteriorly directed suckers, and narrow neck. These characteristics are unique to *Paranoplocephala* s.s. and can differentiate this genus from all other genera in *Paranoplocephala* sensu lato (Haukisalmi et al., 2014).

There are three species of *Paranoplocephala* s.s. for which molecular data do not exist. Of these, the specimen from our survey is most morphologically similar to *Parano. microti*, although the testes of this specimen do not overlap the ventral osmoregulatory canal whereas the testes of *Parano. microti* do. The reported host of *Parano. microti* is *Microtus ochrogaster*, but the host species of the current specimen is unknown. Additional specimens phylogenetically similar to this one are necessary for additional morphological analysis to confirm if this specimen is conspecific with *Parano. microti*.

Of the other two species lacking molecular data, the current specimen is dissimilar to *Parano. kirbyi* in that the testes do not overlap with the antiporal ventral osmoregulatory canal. The scolex and suckers of *Parano. rauschi* are much larger, the strobila much smaller, and the osmoregulatory canals are much narrower than these attributes in the current specimen (Table 2, Supplemental Tables 3-10).

Phylogenetics: Based on *cox1* sequences, lineage 3 is sister to an unknown lineage of *Paranoplocephala* identified by Voitto Haukisalmi, and groups with *Paranoplocephala* s.s., although not with strong support.

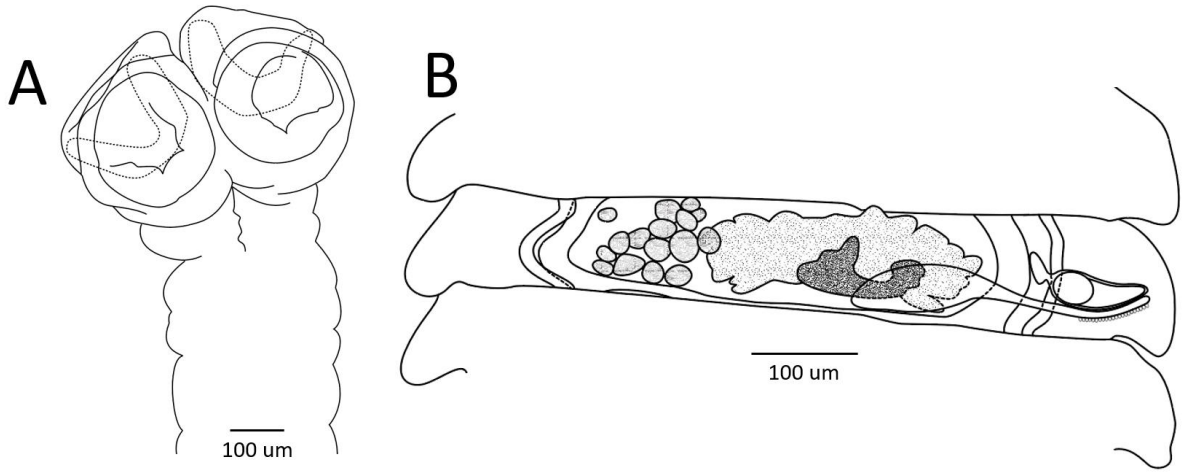

Figure 8. Anoplocephalidae gen. sp., Unknown Lineage 3, in dorsal view. A. Scolex and neck. B. Mature proglottid, dorsal view. Details are the same as in Figure 7.

Table 2. Meristic data for Anoplocephalidae gen. sp., Unknown Lineage 3; Specimen NT11YT. Details are the same as in Table 1.

	NT11YT		
	Mean	Range	No. meas
BL (mm)	70	-	1
ScD	530	538-521	2
SuD	233	235-230	4
T (LxW)	78x79	86x89-64x66	4
CS (LxW)	114x41	129x49-97x33	5
ISV (LxW)	24x24	-	1
O (LxW)	276x93	290x95-255x88	4
Vi (LxW)	123x19	131x22-108x16	4
Va (LxW)	101x9	-	1
SR (LxW)	216x81	241x91-185x72	6

Parandrya sp. *Unknown lineage 6* (Fig. 9, Table 3)

Description: Large anoplocephalid cestodes. Maximum body length 7000 mm, maximum proglottid width 0.86 mm. Scolex small with laterally directed suckers partially embedded within scolex. Neck wide relative to scolex. Proglottids consistently wider than long; average length to width measurements of mature proglottids 0.172x1.099 mm, length to width ratio of mature proglottids averages 1:6.37. Genital pores irregularly alternating, opening in middle of segment margin. Genital ducts dorsal to osmoregulatory canals. Testes entirely antiporal, marginal to ovary, not overlapping antiporal osmoregulatory canals. Cirrus sac long, wide, reaching margin of ventral osmoregulatory canal. Seminal vesicle elongate, extending anteriad along margin of ovary. Vagina approximately ¾ length of cirrus sac, covered by cell layer. Seminal receptacle

elongate. Ovary lobate, median, not extending to osmoregulatory canals. Vitellarium poral of midline relative to ovary.

Host: Specimen in *Microtus richardsoni* (catalog number NMU:Mamm:2509).

Locality: Sabe Mountain, northern Idaho, U.S.A.; georef coordinates: 45.64064596, -114.905908; date: 2 Aug, 2028; collector: Kurt Galbreath.

Specimen: NMU:Para:3273. One specimen available for morphological analysis.

Remarks: A single specimen examined appears attributable to *Parandrya* based on presence of a wide neck, transversely elongate proglottids, primarily antiporal distribution of the testes, and an elongate seminal receptacle. as a putative undescribed species. Independent status as a putative undescribed taxon appears demonstrated based on a sister-species relationship and deep genetic divergence relative to *Parand. fedorovi* and *Parand. oeconomi* (see Chapter 1) In contrast to genera morphologically similar to *Parandrya*, the lack of a "neck swelling", prominent cirrus spines, and the primarily antiporal distribution of testes distinguishes it from *Microticola, Beringitaenia,* and *Chionocestus* respectively. This is apparently the first record of this genus from North America (Haukisalmi et al., 2014).

Phylogenetics: Based on *cox1* sequences, lineage 6 is sister to *Parandrya* with strong support, and groups within a clade including *Microcephaloides* and *Galegoides.*

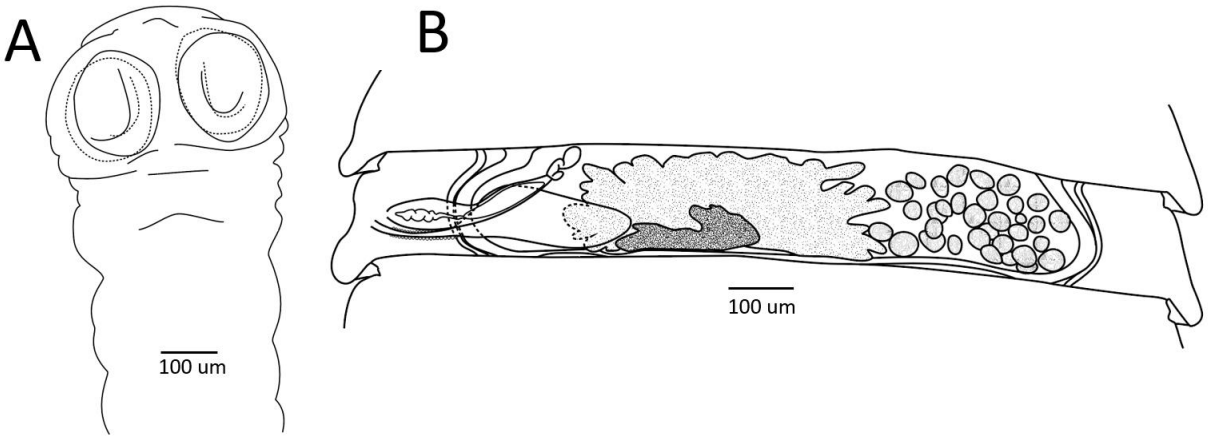

Figure 9. *Parandrya* sp. Unknown lineage 6, in dorsal view. A. Scolex and neck. B. Mature proglottid, ventral view. Details are the same as in Figure 7.

Table 3. Meristic data for *Parandrya* sp. *Unknown lineage 6*; (NMU:Para:3273). Details are the same as in Table 1.

	NMU:Para:3273		
	Mean	Range	No. meas
BL (mm)	7000	-	1
ScD	455	-	1
SuD	170	-	1
T (LxW)	24x21	28x26-22x18	6
CS (LxW)	125x48	147x52-110x43	8
ISV (LxW)	-	-	-
O (LxW)	386x149	654x229-191x98	17
Vi (LxW)	113x17	119x23-106x13	5
Va (LxW)	135x10	149x12-119x9	5
SR (LxW)	194x77	389x145-116x47	8

Anoplocephalidae gen sp., Unknown Lineage 8 (Fig.10, Table 4)

Description: Small anoplocephalid cestodes. Maximum body length 54 mm, maximum body width 0.6mm. Scolex of medium dimensions with anteriorly directed, partially embedded suckers. Neck narrow relative to scolex. Proglottids consistently wider than long; average length to width measurements of mature proglottids are 0.091x0.345 mm and of gravid proglottids are 0.411x0.595 mm, length to width ratio of mature proglottids averages 1:5.99. Genital pores irregularly alternating; opening in middle of segment margin. Genital ducts passing dorsal to osmoregulatory canals. Testes largely antiporal of ovary, overlapping antiporal ventral osmoregulatory canal; extending in poorly defined row anterior to ovary. Cirrus sac large, extending past ventral osmoregulatory canal. Vagina lacking prominent cellular layer. Seminal receptacle with elongated neck. Ovary slightly lobed, poral of midline, vitellarium poral of midline.

Host: Specimen in *Phenacomys intermedius* (catalog number NMU:Mamm:2543).

Locality: Salmon Mountain in north-central Idaho, U.S.A.; georef coordinates: 45.70361903, -114.620431; date: 4 Aug. 2018; collector: Kurt Galbreath.

Specimen: NMU:Para:918, strobila barcode NT11XN. Four specimens of this lineage identified; one specimen morphologically examined, the remaining 3 specimens preserved in 80% ETOH at the Northern Museum of Zoology.

Remarks: An apparently unknown anoplocephalid represented by lineage 8 is phylogenetically most closely related to *Lemminia*, *Arctocestus*, and *Rauschoides* based on DNA sequences (see Chapter 1). Further, specimens of lineage 8 are morphologically inconsistent with species of these genera (Haukisalmi et al., 2014) as the testes of this specimen do not extend beyond the antiporal ventral osmoregulatory canal, as they do in these other genera. In contrast, the elongate

vagina, large cirrus sac, and small, rounded seminal receptacle are consistent with species in the genus *Douthittia* (Haukisalmi et al., 2014). The current specimen, however, lacks a distinct vaginal cell-layer and the scolex is more strongly pronounced with larger, protruding suckers. Consequently, the taxonomic placement of this specimen remains unclear. The unique morphological characteristics and deep phylogenetic divergence may support a proposal for a novel generic level taxon for lineage 8.

Phylogenetics: Based on *cox1* sequences, lineage 8 is sister to a clade including *Rauschoides,* *Lemminia*, and *Arctocestus,* although not with strong support and it is deeply divergent.

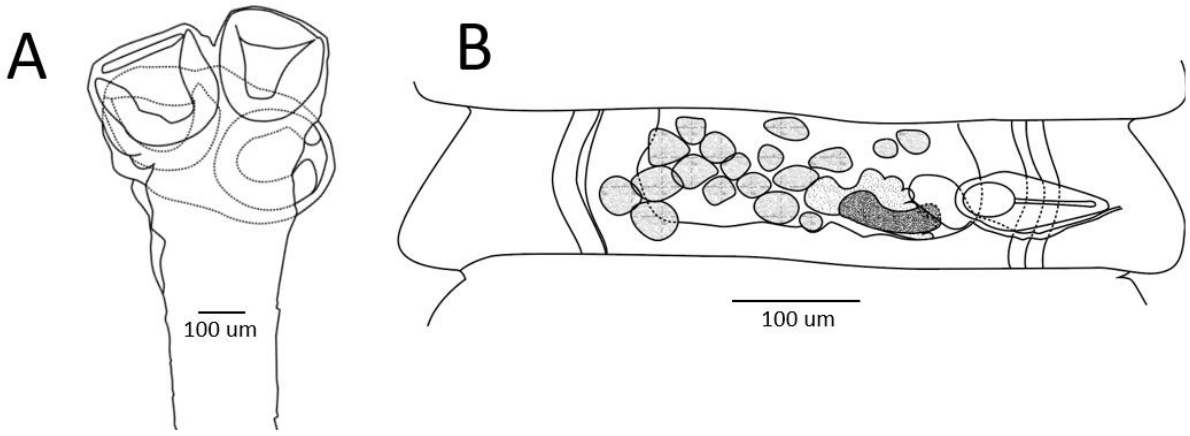

Figure 10. Anoplocephalidae gen. sp., Unknown Lineage 8, in dorsal view. A. Scolex and neck. B. Mature proglottid, dorsal view. Details are the same as in Figure 7.

Table 4. Meristic data for Anoplocephalidae gen. sp., Unknown Lineage 8. NT11XN. Details are the same as in Table 1.

	NT11XN		
	Mean	Range	No. meas
BL (mm)	54	-	1
ScD	0.483	0.516-0.449	2
SuD	0.234	0.243-0.225	4
T (LxW)	0.037x0.026	0.043x0.048-0.032x0.020	6
CS (LxW)	0.097x0.038	0.100x0.041-0.093x0.036	3
ISV (LxW)	0.023x0.022	-	1
O (LxW)	0.229x0.081	0.239x0.090-0.223x0.074	2
Vi (LxW)	0.119x0.017	0.134x0.021-0.105x0.011	5
Va (LxW)	0.085x0.004	0.108x0.08-0.062x0.002	3
SR (LxW)	0.088x0.044	0.096x0.048-0.077x0.037	6

Douthittia sp., Unknown Lineage 12 (Fig.11, Table 5)

Description: Small anoplocephalid cestodes. Maximum body length 45 mm, maximum body width 3 mm. Scolex small with antero-laterally directed, partially embedded suckers. Neck wide relative to scolex. Proglottids consistently wider than long; average length to width measurements of mature proglottids are 0.185x0.882 mm, length to width ratio of mature proglottids averages 1:4.74. Genital pores irregularly alternating, opening marginally in middle of segment. Genital ducts passing dorsal to osmoregulatory canals. Ventral osmoregulatory canals, strongly distended in mature proglottids. Testes entirely anterior to ovary, attaining midline in distribution; overlapping antiporal ventral osmoregulatory canal. Cirrus sac elongate, overlapping poral ventral osmoregulatory canal. Vagina approximately half length of cirrus sac,

cell layer present. Seminal receptacle short with elongated neck. Center of female organs on median line; ovary lobate, vitellarium positioned in posterior of proglottid.

Host: Specimen in *Microtus longicaudus* (catalog number NMU:Mamm:2578).

Locality: Nez Perce Peak, Wyoming, U.S.A.; georef coordinates: 45.70603897, -114.616046; date: 4 Aug, 2018; collector: Kurt Galbreath.

Specimen: NMU:Para:933, strobila barcode NT11EM. One sample available for morphological analysis.

Remarks: Consistent with genus *Douthittia* based on narrow strobila of moderate length; the scolex gradually merging with the neck; anterior testes distribution, overlapping with antiporal ventral osmoregulatory canal; a wide cirrus sac, extending across the osmoregulatory canals; a vagina as long as the cirrus sac; a spherical seminal receptacle; median ovary filling up most of the space between the osmoregulatory canals. Therefore, given the morphological similarities and the close phylogenetic relationship to *Douthittia* (see Chapter 1) this specimen appears to be a previously unrecognized species attributable to *Douthittia.*

This specimen differs further from species among genera morphologically similar to *Douthittia* relative to distribution of the testes. In this apparently unknown species of *Douthittia,* the testes do not extend laterally beyond the antiporal osmoregulatory canals (versus testes that extend beyond the antiporal osmoregulatory canal in *Arctocestus, Lemminia,* and *Rauschoides*).

Phylogenetics: Based on *cox1* sequences, lineage 12 is sister to *Beringitaenia,* although not with strong support, and falls out in a clade including *Douthittia*, *Diandrya,* and *Rodentocestus*.

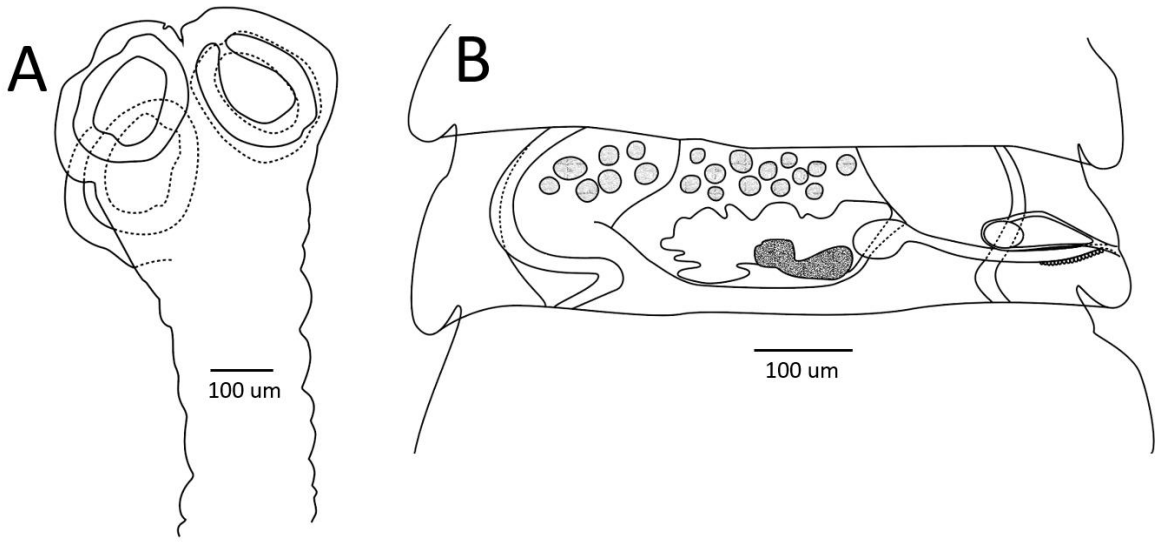

Figure 11. Anoplocephalidae gen. sp., Unknown Lineage 12, in dorsal view. A. Scolex and neck. B. Mature proglottid. Details are the same as in Figure 7

Table 5. Meristic data for Anoplocephalidae gen. sp., Unknown Lineage 12. NT11EM. Details are the same as in Table 1.

	NT11EM		
	Mean	Range	No. meas
BL (mm)	45	-	1
ScD	310	-	1
SuD	114	147-142	4
T (LxW)	27x23	29x25-25x21	2
CS (LxW)	141x51	150x57-119x45	5
ISV (LxW)	45x29	61x65-26x25	4
O (LxW)	259x71	281x82-234x62	6
Vi (LxW)	130x32	138x39-124x28	5
Va (LxW)	100x7	107x7-88x6	3
SR (LxW)	240x62	326x99-189x42	8

DISCUSSION

Nine unknown lineages of cestode belonging to the *Paranoplocephala* species complex have been identified (see Chapter 1). Comparative morphological analyses for five of these unknown lineages provisionally supported delineation of five previously unrecognized species and potentially two new genera. Current analyses and a robust integrated pathway to fully characterize these putative taxa, along with patterns of morphological variation, are hindered by a paucity of specimens held in museum collections (e.g., Haukisalmi et al., 2014; Hoberg et al., 2016). Additional samples suitable for morphological analysis are necessary for completion and evaluation of species descriptions for unknown lineages 3, 8, and 12; only one specimen of each was used for this project. Furthermore, specimens are needed for morphological analyses of the 4 genetic lineages for which no strobilae were available to assess their status as potentially distinct species. All the unknown lineages were from regions that were not represented in earlier investigations (for example, Haukisalmi et al., 2007, 2014; Haukisalmi & Henttonen, 2005; Wickström et al., 2003). The northwest region of the contiguous U.S.A., where these specimens were collected, represents a poorly studied area where considerable cestode diversity apparently remains to be discovered (for example, Makarikov & Hoberg, 2016; Makarikov et al., 2020).

The first record for a species of *Parandrya* in North America suggests considerable additional diversity within this genus is likely to be recognized. Species of *Microtus* and *Arvicola* from Eurasia (Haukisalmi et al., 2014) are the hosts of *Parand. oeconomi* and *Parand. fedorovi*. The host of unknown lineage 6, *Microtus richardsoni¸* has a relatively narrow distribution in North America (Ludwig, 1984), and the oldest fossil record of this species in North America dates back to the Pleistocene (Burns, 1982). Given the deep phylogenetic history of lineage 6

(see Chapter 1) the persistence and presence of this assemblage in North America may imply numerous episodes of geographic expansion and host-colonization linking faunas in Eurasia. In addition, presuming a Eurasian origin of *Parandrya* (see Chapter 1), their presence in North America reflects trans-Beringian dispersal. The ecological instability that punctuated the time periods around the emergence of the Bering Land Bridge, and numerous geographic and host-colonization events likely played an important role in promoting diversification of this assemblage (e.g., Hoberg et al., 2012). Additional geographically extensive and site intensive sampling of mammalian hosts and parasites is necessary to understand the full extent of diversity and an extended history for the mosaic assembly of the North American and Holarctic fauna.

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APPENDIX

Supplemental Table 6. Species diversity within the *Paranoplocephala* species complex and *Anoplocephaloides*. Body type score described in Haukisalmi et al., (2014).

Paranoplocephala s.l.	Body type	Host Species	Range
Afrojoyeuxia gundii Haukisalmi, 2013	3.1	*Ctenodacylus gundi*	Afrotropical
Arctocestus serratus (Haukisalmi & Henttonen, 2000)	*3.1*	*Dicrostonyx groenlandicus*	Amphiberingian
Beringitaenia nanushukensis Haukisalmi *et al*., 2014	*2*	*Microtus miurus*	Amphiberingian
Chionocestus yoccozi (Haukisalmi & Henttonen, 2005)	*2*	*Chionomys nivalis*	Palearctic
Cookiella ondatrae (Rausch, 1948)	*3.2*	*Microtus Montanus*	Nearctic
Douthittia bairdi (Schad, 1954)	*3.1*	*Phenacomys sp., Arborimus sp.*	Nearctic
Do. nordenskioeldi (Haukisalmi *et al*., 2001)	*3.1*	*Dicrostonyx groenlandicus, Microtus oeconomus, Microtus miurus*	Amphiberingian
Do. nearctica (Haukisalmi & Henttonen, 2007)	*3.1*	*Myodes rutilus*	Amphiberingian
Do. primordialis (Douthitt, 1915)	*3.1*	*Sciurus hudsonica*	Nearctic
Eurotaenia gracilis (Tenora & Murai, 1980)	*3.1*	*Myodes glareolus*	Palearctic
Gulyaevia buryatiensis (Haukisalmi *et al.*, 2007)	*2*	*Myodes rufocanus*	Palearctic
Gu. Longivaginata (Chechulin & Gulyaev, 1998)	*2*	*Myodes rutilus*	Palearctic
Hunkeleriella dasymidis (Hunkeler, 1972)	*2*	*Dasymys incomtus*	Afrotropical
Lemminia fellmani (Haukisalmi & Henttonen, 2001)	*3.1*	*Lemmus lemmus*	Palearctic
L. gubanovi (Gulyaev & Krivopalov, 2003)	*3.1*	*Mypous schizticolor*	Palearctic
Microticola etholeni (Haukisalmi *et al*., 2002)	*2*	*Microtus pennsylvanicus*	Amphiberingian
Microt. Blanchardi (Moniez, 1891)	*2*	*Microtus sp.*	Palearctic
Parandrya fedorovi Gulyaev & Chechulin, 1996	*2*	*Microtus sp.*	Palearctic
Parand. oeconomi (Gubanui & Murai, 2002)	*2*	*Microtus oeconomus*	Palearctic
Paranoplocephala batzlii Haukisalmi, Henttonen & Hardman, 2006	*1*	*Microtus miurus*	Amphiberingian
Paran. Omphalodes (Hermann, 1783)	*1*	*Microtus agrestis*	Palearctic

Paran. jarrelli Haukisalmi, Henttonen & Hardman, 2006	*1*	*Microtus oeconomus*	Holarctic
Paran. kaleli Tenora, Haukisalmi & Henttonen, 1985	*1*	*Myodes rufocanus*	Palearctic
Paran. kirbyi Voge, 1948	*1*	*Microtus californicus californicus*	Nearctic
Paran. macrocephala (Douthitt, 1915)	*1*	*Microtus longicaudus*	Nearctic
Paran. maseri Tenora, Gubanyi & Murai, 1999	*1*	*Lemmiscus curtatus*	Nearctic
Paran. microti (Hansen, 19947)	*1*	*Microtus ochrogaster*	Nearctic
Paran. rauschi (Fair, Schmidt & Wertheim, 1990)	*1*	*Microtus guentheri*	Palearctic
Rodentocestus aquaticus (Genov, Vasileva & Georgiev, 1996)	*3.2*	*Arvicola terrestris, Ondontra zibethica*	Palearctic
Ro. Freemani (Haukisalmi, Henttonen & Hardman, 2006)	*3.2*	*Microtus xanthognathus*	Amphiberingian
Ro. sciuri (Rausch, 1947)	*3.2*	*Glaucomys sbaarinus macrotis*	Nearctic
Rauschoides arctica (Rausch, 1952)	*3.1*	*Dicrostonyx groenlandicus*	Amphiberingian
Ra. alternata (Haukisalmi et al., 2001)	*3.1*	*Dicrostonyx groenlandicus*	Amphiberingian
Tenoraia janickii (Tenora, Murai &Vaucher, 1985)	*3.2*	*Microtus arvalis*	Palearctic
Diandrya composita Darrah, 1930		*Marmota caligata*	Amphiberingian
Gallegoides arfaai (Mobedi & Ghadirian, 1977)		*Apodemus sylvaticus*	Palearctic
Microcephaloides variabilis (Douthitt, 1915)		*Geomys, Thomomys*	Nearctic
Microcephaloides neofibrinus (Rausch, 1952)		*Neofiber alleni*	Nearctic
Microcephaloides mascomai (Murai, Tenora & Rocamora, 1980)		*Microtus cabrerae*	Palearctic
Microcephaloides krebsi (Haukisalmi *et al*., 2001)		*Dicrostonyx groenlandicus*	Amphiberingian
Other species			
Anoplocephaloides dentata (Galli-Valerio, 1905)		*Microtus sp., Chionomys nivalis*	Palearctic
Anop. lemmi (Rausch, 1952)		*Lemmus sibiricus*	Amphiberingian
Anop. kontrimavichusi Rausch, 1976		*Synaptomys borealis*	Amphiberingian
Anop. troeschi (Rasuch, 1946)		*Microtus sp.*	Nearctic
Anop. dentatoides Sato *et al.*, 1993		*Myodes rufocanus*	Palearctic

Supplemental Table 7. Sampling data from the Museum of Southwestern Biology (MSB) and the Northern Museum of Zoology. NK, AF, and NMU:Mamm numbers are host specimen identifiers. Consecutive rows with shaded/unshaded cells indicate specimens derived from a single host. NK#: MSB Genomic Resources collection number; Lot Barc: barcode for the parent vial from which individual cestode specimens were acquired; Stro Barc: individual specimen barcode; DNA Ex: DNA extraction barcode.

NK #	Host ID	Geography	Lot Barc.	Stro Barc.	DNA Ex	Identification
270946	*Mic. oeconomus*	Uvs, Mongolia	A3NLY	NT11U5	A014287	*Parano. jarrelli*
270946	*Mic. oeconomus*	Uvs, Mongolia	A3NLY	NT11W2	A014285	*Parano. jarrelli*
272072	*Myo. rufocanus*	Uvs, Mongolia	A3OHI	NT11T5	A014284	*Parano. jarrelli*
272072	*Myo. rufocanus*	Uvs, Mongolia	A3OHI	NT11C6	A014286	*Parano. jarrelli*
270505	*Mic. oeconomus*	Bayan-Olgii, Mongolia	A3MXO	NT11Z4	A014283	unknown 4
270505	*Mic. oeconomus*	Bayan-Olgii, Mongolia	A3MXO	NT11W4	A014282	unknown 4
270505	*Mic. oeconomus*	Bayan-Olgii, Mongolia	A3MXO	NT11DC	A014281	unknown 4
270505	*Mic. oeconomus*	Bayan-Olgii, Mongolia	A3MXO	NT11YR	A014280	unknown 4
270508	*Mic. oeconomus*	Bayan-Olgii, Mongolia	A3MY5	NT11B3	A014279	unknown 4
270525	*Mic. oeconomus*	Bayan-Olgii, Mongolia	A3MY8	NT11Y3	A014112	unknown 5
270892	*Microtus sp.*	Uvs, Mongolia	A3NMB	NT11UB	A014113	*Parano. jarrelli*
270884	*Microtus sp.*	Uvs, Mongolia	A3NIF	NT11EG	A014117	unknown 5
270884	*Microtus sp.*	Uvs, Mongolia	A3NIF	NT11C5	A014122	unknown 5
270884	*Microtus sp.*	Uvs, Mongolia	A3NIF	NT11T1	A014124	unknown 5
270446	*Myo. rufocanus*	Bayan-Olgii, Mongolia	A3MXY	NT1105	A014119	unknown 5
270514	*Myo. rutilus*	Bayan-Olgii, Mongolia	-	NT11T3	A014508	*Do. nearctica*
270514	*Myo. rutilus*	Bayan-Olgii, Mongolia	-	NT11D6	A014274	*Do. nearctica*
270193	*Alticola strelzowi*	Bayan-Olgii, Mongolia	A3MTC	NT11T8	A014066	unknown 2
270269	*Myo. rufocanus*	Bayan-Olgii, Mongolia	A3MU5	NT11SL	A014074	unknown 7
270269	*Myo. rufocanus*	Bayan-Olgii, Mongolia	A3MU5	NT11SL	A014074	unknown 7
270269	*Myo. rufocanus*	Bayan-Olgii, Mongolia	A3MU5	NT11SL	A014074	unknown 7
270701	*Mic. gregalis*	Uvs, Mongolia	A3N76	NT11AX	A014064	*An. dentata*
270336	*Myo. rufocanus*	Bayan-Olgii, Mongolia	A3MWL	NT11X7	A014082	unknown 2
270419	*Myo. rufocanus*	Bayan-Olgii, Mongolia	A3MY2	NT11V7	A014080	unknown 7
270419	*Myo. rufocanus*	Bayan-Olgii, Mongolia	A3MY2	NT11AJ	A014088	unknown 7

270376	*Myo. rufocanus*	Bayan-Olgii, Mongolia	A3MWO	NT11YI	A014095	unknown 7
272007	*Mic. oeconomus*	Uvs, Mongolia	A3NLH	NT11AZ	A014096	*Parano. jarrelli*
272007	*Mic. oeconomus*	Uvs, Mongolia	A3NLH	NT119Y	A014097	*Parano. jarrelli*
270301	*Myo. rufocanus*	Bayan-Olgii, Mongolia	A3MW8	NT11YB	A014100	unknown 10
270862	*Mic. oeconomus*	Uvs, Mongolia	A3NIE	NT11ZA	A014183	unknown 5
272228	*Mic. oeconomus*	Khovsgol, Mongolia	A3OHV	NT11UV	A014184	*Parano. jarrelli*
272168	*Mic. oeconomus*	Khovsgol, Mongolia	A3OIL	NT11YV	A014185	*Parano. jarrelli*
272167	*Mic. oeconomus*	Khovsgol, Mongolia	A3OLD	NT11YK	A014186	*Parano. jarrelli*
270526	*Mic. gregalis*	Bayan-Olgii, Mongolia	A3N0I	NT11CF	A014208	unknown 2
289444	*Mic. mongolicus*	Khentii, Mongolia	A4I1R	NT11UL	A014511	*Parano. jarrelli*
289293	*Mic. oeconomus*	Khentii, Mongolia	A4I0I	NT11TR	A014512	*Parano. jarrelli*
289327	*Mic. maximowiczii*	Khentii, Mongolia	A4I0N	NT11AL	A014515	*Microt. blanchardi*
289359	*Mic. maximowiczii*	Khentii, Mongolia	A4I13	NT11WV	A014516	*Parano. jarrelli*
289234	*Mic. mongolicus*	Khentii, Mongolia	A4HOM	NT11XV	A014519	*Parano. omphalodes*
289382	*Mic. oeconomus*	Khentii, Mongolia	A4I15	NT11SU	A014520	*Parano. omphalodes*
289247	*Mic. mongolicus*	Khentii, Mongolia	A4HOU	NT11VB	A014521	*Parano. jarrelli*
289042	*Mic. oeconomus*	Bulgan, Mongolia	A4HFR	NT11AI	A014522	*Parano. jarrelli*
289667	*Microtus*	Dornod, Mongolia	A4HZW	NT11D1	A014524	*Parano. jarrelli*
289354	*Mic. mongolicus*	Khentii, Mongolia	A4HTL	NT11D9	A014525	*Parano. jarrelli*
289354	*Mic. mongolicus*	Khentii, Mongolia	A4HTL	NT11YY	A014526	*Parano. jarrelli*
289357	*Mic. maximowiczii*	Khentii, Mongolia	A4HTM	NT11AT	A014527	*Parano. jarrelli*
289357	*Mic. maximowiczii*	Khentii, Mongolia	A4HTM	NT11VY	A014528	*Parano. jarrelli*
289311	*Mic. maximowiczii*	Khentii, Mongolia	A4I0L	NT11DR	A014529	*Parano. jarrelli*
289342	*Mic. maximowiczii*	Khentii, Mongolia	A4I2Q	NT11VP	A014530	*Parano. jarrelli*
289388	*Mic. mongolicus*	Khentii, Mongolia	A4HTQ	NT11SK	A014535	*Parano. jarrelli*
289036	*Myo. rufocanus*	Bulgan, Mongolia	A4HG9	NT11BC	A014537	unknown 7
289036	*Myo. rufocanus*	Bulgan, Mongolia	A4HG9	NT11YZ	A014538	unknown 7
289036	*Myo. rufocanus*	Bulgan, Mongolia	A4HG9	NT11SH	A014539	unknown 7
289366	*Mic. mongolicus*	Khentii, Mongolia	A4I2U	NT11AW	A014540	*Microt. blanchardi*
289225	*Mic. maximowiczii*	Khentii, Mongolia	A4HOR	NT11UE	A014541	*Parano. jarrelli*
289119	*Myo. rufocanus*	Bulgan, Mongolia	A4HJ1	NT11BL	A014543	*G. buryatiensis*

289229	*Mic. maximowiczii*	Khentii, Mongolia	A4HLI	NT11A6	A014544	*Parano. jarrelli*
289702	*Microtus*	Dornod, Mongolia	A4I23	NT11UM	A014615	*Parano. jarrelli*
289835	*Mic. fortis*	Dornod, Mongolia	A4IL1	NT11CB	A014547	*Parano. jarrelli*
289248	*Mic. maximowiczii*	Khentii, Mongolia	A4HOV	NT11U6	A014550	*Parano. jarrelli*
289759	*Mic. fortis*	Dornod, Mongolia	A4IAL	NT11V2	A014551	*Parano. jarrelli*
289562	*Mic. fortis*	Dornod, Mongolia	A4HZB	NT11XT	A014552	*Parano. jarrelli*
289562	*Mic. fortis*	Dornod, Mongolia	A4HZB	NT11XZ	A014555	*Parano. jarrelli*
289562	*Mic. fortis*	Dornod, Mongolia	A4HZB	NT11BX	A014554	*Parano. jarrelli*
289562	*Mic. fortis*	Dornod, Mongolia	A4HZB	NT11WW	A014553	*Parano. jarrelli*
289827	*Mic. fortis*	Dornod, Mongolia	A4IIA	NT11CX	A014556	*Parano. jarrelli*
289100	*Mic. oeconomus*	Bulgan, Mongolia	A4HH7	NT11VO	A014557	*Parano. jarrelli*
289766	*Mic. fortis*	Dornod, Mongolia	A4IA8	NT119R	A014558	*Parano. jarrelli*
289478	*Mic. mongolicus*	Khentii, Mongolia	A4I3D	NT11DO	A014563	*Microt. blanchardi*
289264	*Mic. mongolicus*	Khentii, Mongolia	A4HOW	NT11EH	A014564	*Microt. blanchardi*
289264	*Mic. mongolicus*	Khentii, Mongolia	A4HOW	NT11WB	A014565	*Microt. blanchardi*
289655	*Microtus*	Dornod, Mongolia	A4HZU	NT11EK	A014566	*Parano. jarrelli*
289477	*Mic. maximowiczii*	Khentii, Mongolia	A4HU8	NT11TO	A014570	*Microt. blanchardi*
289477	*Mic. maximowiczii*	Khentii, Mongolia	A4HU8	NT11B5	A014571	*Microt. blanchardi*
289558	*Microtus*	Dornod, Mongolia	A4HYW	NT11XU	A014572	*Parano. jarrelli*
289558	*Microtus*	Dornod, Mongolia	A4HYW	NT11A2	A014573	*Parano. jarrelli*
289362	*Mic. mongolicus*	Khentii, Mongolia	A4I2T	NT11WJ	A014575	*Parano. jarrelli*
289358	*Mic. maximowiczii*	Khentii, Mongolia	A4HTO	NT11A9	A014576	*Parano. jarrelli*
289358	*Mic. maximowiczii*	Khentii, Mongolia	A4HTO	NT11TS	A014577	*Parano. jarrelli*
289303	*Mic. maximowiczii*	Khentii, Mongolia	A4HP2	NT11C7	A014578	unknown 7
289281	*Mic. mongolicus*	Khentii, Mongolia	A4HOZ	NT11VN	A014579	*Parano. jarrelli*
289281	*Mic. mongolicus*	Khentii, Mongolia	A4HOZ	NT11TV	A014581	*Parano. jarrelli*
289333	*Microtus*	Khentii, Mongolia	A4HPD	NT11XS	A014582	*Parano. jarrelli*
289360	*Mic. mongolicus*	Khentii, Mongolia	A4I12	NT11AB	A014584	*Parano. jarrelli*
289914	*Microtus*	Dornod, Mongolia	A4IJJ	NT11DA	A014589	*Ro. freemani*
289893	*Mic. maximowiczii*	Dornod, Mongolia	A4IM9	NT11BP	A014592	*Parano. jarrelli*
289244	*Mic. mongolicus*	Khentii, Mongolia	A4HT8	NT11YH	A014596	*Parano. jarrelli*

289244	*Mic. mongolicus*	Khentii, Mongolia	A4HT8	NT11VK	A014597	*Parano. jarrelli*
289771	*Mic. fortis*	Dornod, Mongolia	A4ILB	NT11Z2	A014598	*Parano. jarrelli*
289771	*Mic. fortis*	Dornod, Mongolia	A4ILB	NT11AP	A014599	*Parano. jarrelli*
289771	*Mic. fortis*	Dornod, Mongolia	A4ILB	NT11WF	A014600	*Parano. jarrelli*
289600	*Mic. fortis*	Dornod, Mongolia	A4HZ4	NT11SN	A014602	*Ro. freemani*
289600	*Mic. fortis*	Dornod, Mongolia	A4HZ4	NT11VZ	A014601	*Ro. freemani*
289600	*Mic. fortis*	Dornod, Mongolia	A4HZ4	NT11DX	A014603	*Parano. jarrelli*
289600	*Mic. fortis*	Dornod, Mongolia	A4HZ4	NT11SS	A014604	*Parano. jarrelli*
289600	*Mic. fortis*	Dornod, Mongolia	A4HZ4	NT11EE	A014605	*Ro. freemani*
289796	*Mic. fortis*	Dornod, Mongolia	A4IL6	NT11TH	A014606	*Parano. jarrelli*
289796	*Mic. fortis*	Dornod, Mongolia	A4IL6	NT11X4	A014607	*Parano. jarrelli*
289796	*Mic. fortis*	Dornod, Mongolia	A4IL6	NT11AQ	A014608	*Parano. jarrelli*
289810	*Mic. fortis*	Dornod, Mongolia	A4IL5	NT11WS	A014609	*Parano. jarrelli*
289778	*Mic. fortis*	Dornod, Mongolia	A4IC8	NT11B2	A014610	*Parano. jarrelli*
289778	*Mic. fortis*	Dornod, Mongolia	A4IC8	NT11VW	A014611	*Parano. jarrelli*
289778	*Mic. fortis*	Dornod, Mongolia	A4IC8	NT11DG	A014612	*Parano. jarrelli*
289044	*Mic. oeconomus*	Bulgan, Mongolia	A4HGC	NT11CZ	A014614	*Parano. jarrelli*
272233	*Mic. oeconomus*	Khovsgol, Mongolia	A3OLT	NT11V1	A010600	*Parano. jarrelli*
289044	*Mic. oeconomus*	Bulgan, Mongolia	A4HGC	NT11UH	A010578	*Parano. jarrelli*
289814	*Mic. fortis*	Dornod, Mongolia	A4IAS	NT119W	A010502	*Parano. jarrelli*
289802	*Mic. fortis*	Dornod, Mongolia	A4ICD	NT11X6	A010595	*Parano. jarrelli*
289829	*Mic. fortis*	Dornod, Mongolia	A4ICK	NT11DU	A010556	*Parano. jarrelli*
289316	*Mic. maximowiczii*	Hentiy	A4HPC	NT11VL	A010596	*Parano. jarrelli*
289287	*Mic. mongolicus*	Hentiy	A4HP0	NT11Y6	A010575	*Parano. jarrelli*
289563	*Mic. maximowiczii*	Dornod, Mongolia	A4HZQ	NT1287	A014717	*Ro. freemani*
289587	*Mic. fortis*	Dornod, Mongolia	A4HZ5	NT128N	A014725	*Parano. jarrelli*
289672	*Mic. fortis*	Dornod, Mongolia	A4I07	NT1289	A010571	*Parano. jarrelli*
289672	*Mic. fortis*	Dornod, Mongolia	A4I07	NT128K	A010562	*Parano. jarrelli*
289205	*Mic. mongolicus*	Hentiy	A4HLD	NT128M	A010574	*Parano. jarrelli*
289313	*Mic. maximowiczii*	Hentiy	A4HP5	NT1293	A010754	*Parano. jarrelli*
289696	*Mic. fortis*	Dornod, Mongolia	A4IA0	NT124L	A010623	*Parano. jarrelli*

289462	*Mic. mongolicus*	Hentiy	A4I1T	NT126F	A010753	*Parano. jarrelli*
289270	*Mic. maximowiczii*	Hentiy	A4HT9	NT123X	A010770	*Parano. jarrelli*
289218	*Mic. oeconomus*	Hentiy	A4HOG	NT12G5	A010784	*Parano. jarrelli*
231448	*Mic. pennsylvanicus*	Saskatchewan, Canada	-	C1	A013609	*Parano. macrocephala*
231397	*Myo. gapperi*	Saskatchewan, Canada	-	C2	A013606	unknown 11
231468	*Mic. pennsylvanicus*	Saskatchewan, Canada	-	C1	A013607	*Parano. macrocephala*
231605	*Mic. pennsylvanicus*	Manitoba, Canada	-	C1A	A013608	*Parano. macrocephala*
231461	*Myo. gapperi*	Saskatchewan, Canada	-	C2	A013622	*Parano. macrocephala*
231463	*Myo. gapperi*	Saskatchewan, Canada	-	C1B	A013652	unknown 11
231463	*Myo. gapperi*	Saskatchewan, Canada	-	C1A	A013636	unknown 11
231434	*Tamiasciurus hudsonicus*	Saskatchewan, Canada	-	C1A	A013653	unknown 11
231515	*Mic. pennsylvanicus*	Manitoba, Canada	-	C1A	A013656	*Parano. macrocephala*
231517	*Mic. pennsylvanicus*	Manitoba, Canada	-	C1A	A013657	*Parano. macrocephala*
231447	*Mic. pennsylvanicus*	Saskatchewan, Canada	-	C1A	A013638	*An. dentata*
231537	*Myo. gapperi*	Manitoba, Canada	-	C1	A013643	unknown 11
231381	*Myo. gapperi*	Saskatchewan, Canada	-	C1	A013646	*Do. nearctica*
231478	*Mic. pennsylvanicus*	Manitoba, Canada	-	C1	A013655	*Parano. macrocephala*
231019	*Myodes sp.*	NWT, Canada	-		A013675	*Parano. omphalodes*
231280	*Mic. pennsylvanicus*	NWT, Canada	-	C1A	A013668	*Microt. etholeni*
231216	*Mic. pennsylvanicus*	NWT, Canada	-	C1	A013669	*Parano. macrocephala*
231291	*Mic. pennsylvanicus*	NWT, Canada	-	SIA	A013684	*Microt. etholeni*
231292	*Mic. pennsylvanicus*	NWT, Canada	-	SIA	A013672	*Parano. macrocephala*
231276	*Mic. pennsylvanicus*	NWT, Canada	-	C1B	A013678	*Microt. etholeni*
231276	*Mic. pennsylvanicus*	NWT, Canada	-	C2A	A013673	*Microt. etholeni*
231287	*Mic. pennsylvanicus*	NWT, Canada	-	A	A013679	*Microt. etholeni*
270190	*Alticola sp.*	Bayan-Olgii, Mongolia	-	A3JTN	A013909	*Parano. macrocephala*
270190	*Alticola sp.*	Bayan-Olgii, Mongolia	-	A3JTL	A013932	unknown 2
270407	*Arvicola amphibius*	Bayan-Olgii, Mongolia	-	A3JTS	A013910	*Parano. omphalodes*
270192	*Alticola strelzowi*	Bayan-Olgii, Mongolia	-	A3JSF	A013920	unknown 2
270192	*Alticola strelzowi*	Bayan-Olgii, Mongolia	-	A3JSC	A013907	unknown 2
270251	*Alticola strelzowi*	Bayan-Olgii, Mongolia	-	A3JSJ	A013922	unknown 2

AF#	Host ID	Geography	Lot Barc.ode #	Stro A Barc.	DNA Ex	ID
38157	*Myo. rufocanus*	Magadan Oblast, Russia	-	c1	-	*An. dentata*
46204	*Dicrostonyx groenlandicus*	Alaska, U.S.A.	-	c2	-	*Ra. alternata*
46389	*Mic. oeconomus*	Alaska, U.S.A.	-	c2	-	*Parano. jarrelli*
48486	*Mic. oeconomus*	Alaska, U.S.A.	-	c1	-	*Parano. jarrelli*
48684	*Mic. oeconomus*	Alaska, U.S.A.	-	c1	-	*Parano. jarrelli*
49642	*Mic. oeconomus*	Alaska, U.S.A.	-	-	-	*Parano. jarrelli*
49675	*Mic. oeconomus*	Alaska, U.S.A.	-	-	-	*Parano. jarrelli*
54313	*Mic. oeconomus*	Alaska, U.S.A.	-	c1	-	*Parano. jarrelli*
NMU: Para#	**Host ID**	**Geography**	**Lot Barc.**	**Stro A Barc.**	**DNA Ex**	**ID**
903	*Lemmiscus curtatus*	Idaho, U.S.A.	A08668	NT11AE	A013440	unknown 1
908	*Mic. longicaudus*	Idaho, U.S.A.	A08667	NT11DZ	A013452	*An. dentata*
915	*Myo. gapperi*	Idaho, U.S.A.	A08066	NT11TA	A013457	unknown 11
918	*Phenacomys intermedius*	Idaho, U.S.A.	A08069	NT11XN	A013458	unknown 8
923	*Phenacomys intermedius*	Idaho, U.S.A.	A08253	NT11WO	A013462	unknown 8
2504	*Phenacomys intermedius*	Idaho, U.S.A.	A08253	NT11SW	A013447	unknown 8
2504	*Phenacomys intermedius*	Idaho, U.S.A.	A08253	NT11TT	A013461	unknown 8
926	*Mic. pennsylvanicus*	Michigan, U.S.A.	A09633	NT11WQ	A013474	*An. dentata*
929	*Mic. longicaudus*	Montana, U.S.A.	A08006	NT11A1	A013480	*An. dentata*
933	*Mic. longicaudus*	Wyoming, U.S.A.	A08508	NT11EM	A013490	unknown 12
2715	*Mic. longicaudus*	Wyoming, U.S.A.	A013168	NT11YT	A013488	unknown 3
2715	*Mic. longicaudus*	Wyoming, U.S.A.	A013159	NT11UC	A013489	unknown 11
3273	*Mic. richardsoni*	Idaho, U.S.A.	A08272	-	A013467	unknown 6

Supplemental Table 8. GenBank accession numbers for nine of the eleven coding sequences.

DNA Ex	atp6	cob	cox2	nad1	nad2	nad3	nad4l	nad5	nad6
A014282	OR787578	OR804556	OR804572	OR804588	OR804606	OR804624	OR804641	OR804659	OR804677
A014279	OR787577	OR804555	OR804571	OR804587	OR804605	OR804623	OR804640	OR804658	OR804676
A014124	OR787576	OR804554	-	OR804586	OR804604	OR804622	OR804639	OR804657	OR804675
A014521	OR787579	OR804558	OR804573	OR804590	OR804608	OR804626	OR804643	OR804661	OR804679
A014538	OR787580	OR804559	OR804574	OR804591	OR804609	OR804627	OR804644	OR804662	OR804680
A014540	OR787581	OR804560	-	OR804592	OR804610	OR804628	OR804645	OR804663	OR804681
A014589	OR787582	OR804561	OR804575	OR804593	OR804611	OR804629	OR804646	OR804664	OR804682
A014605	OR787583	OR804562	OR804576	OR804594	OR804612	OR804630	OR804647	OR804665	OR804683
A013638	OR787570	OR804548	OR804566	OR804580	OR804598	OR804616	OR804634	OR804651	OR804669
A013646	OR787571	OR804549	OR804567	OR804581	OR804599	OR804617	OR804635	OR804652	OR804670
A013668	OR787572	OR804550	OR804568	OR804582	OR804600	OR804618	OR804636	OR804653	OR804671
A013684	OR787574	OR804552	OR804570	OR804584	OR804602	OR804620	OR804637	OR804655	OR804673
A013672	OR787573	OR804551	OR804569	OR804583	OR804601	OR804619	-	OR804654	OR804672
A013922	OR787575	OR804553	-	OR804585	OR804603	OR804621	OR804638	OR804656	OR804674
A013488	OR787567	OR804546	OR804564	OR804578	OR804596	OR804614	OR804632	OR804649	OR804667
A013489	OR787568	OR804547	OR804565	OR804579	OR804597	OR804615	OR804633	OR804650	OR804668
A013467	OR787566	OR804545	OR804563	OR804577	OR804595	OR804613	OR804631	OR804648	OR804666

Supplemental Table 9. Measurement data for *Rauschoides arctica* from three sampling localities in Siberia (Yamal Penninsula and Western Kolyma) and Canada (Nunavet). BL: body length; ScD: scolex diameter; SuD: sucker diameter; T: testes; CS: cirrus sac; ISV: internal seminal vesicle; O: ovary; Vi: vitellarium; Va: vagina; SR; seminal receptacle; (L): length; (LxW): length by width. Measurement data in micrometers, except BL which is in millimeters. Source: Wickstrom et al. (2001).

	Rauchoides arctica (Rausch, 1952)								
	Yamal Penninsula			Western Kolyma			Nunavut		
	Mean	Range	N	Mean	Range	N	Mean	Range	N
BL (mm)	106	74-123	3	112.9	90-136	7	94	74-123	9
ScD	275	260-290	6	339	300-390	8	280	240-320	10
SuD	154	150-165	5	166	155-180	8	159	140-180	10
T (LxW)	-	-	-	-	-	-	-	-	-
CS (L)	483	430-503	7	340	400-500	8	449	400-500	12
ISV (LxW)	-	-	-	-	-	-	-	-	-
O (LxW)	-	-	-	-	-	-	-	-	-
Vi (LxW)	-	-	-	-	-	-	-	-	-
Va (LxW)	-	-	-	-	-	-	-	-	-
SR (L)	519	400-610	7	657	400-800	8	610	450-900	12

Supplemental Table 10. Measurement data for *Arctocestus serratus* from three sampling localities. BL: body length; ScD: scolex diameter; SuD: sucker diameter; T: testes; CS: cirrus sac; ISV: internal seminal vesicle; O: ovary; Vi: vitellarium; Va: vagina; SR; seminal receptacle; (L): length; (W): width; (LxW): length by width. Measurement data in micrometers, except BL which is in millimeters. Source: Haukisalmi & Henttonen (2000)

	Arctocestus serratus (Haukisalmi & Henttonen, 2000)								
	Mainland Siberia			Wrangel Island, Siberia			North America		
	Mean	Range	No. meas	Mean	Range	No. meas	Mean	Range	No. meas
BL (mm)	127	106-186	5	100		1	124.4	95-146	5
ScD	390	280-470	8	380	350-370	2	380	370-390	2
SuD	180	160-20	8	170	160-170	2	170	170-180	2
T (LxW)	-	-	-	-	-	-	-	-	-
CS (L)	350	270-380	25	270	250-300	5	240	200-330	18
CS (W)	110	90-130	27	107	100-120	6	10	80-130	18
ISV (L)	90	50-130	26	80	70-100	6	70	40-120	17
ISV (W)	70	40-110	26	60	50-90	6	60	40-80	17
O (L)	240	180-280	10	240	200-270	3	220	190-250	12
O (W)	480	370-590	13	310	300-320	3	360	290-440	12
Vi (L)	120	90-170	27	110	90-130	6	90	60-160	16
Vi (W)	210	120-300	27	150	130-180	6	160	120-210	16
Va (L)	260	220-320	19	200	200-210	2	170	130-240	10
Va (W)	50	40-60	16	50	40-60	4	40	30-50	9
SR (L)	230	180-310	26	220	180-250	6	220	150-320	16
SR (W)	130	100-170	26	120	100-160	6	120	80-200	6

Supplemental Table 11. Measurement data for *Parandrya oeconomus.* BL: body length; ScD: scolex diameter; SuD: sucker diameter; T: testes; CS: cirrus sac; ISV: internal seminal vesicle; O: ovary; Vi: vitellarium; Va: vagina; SR; seminal receptacle; (L): length; (W): width; (LxW): length by width. Measurement data in micrometers, except BL which is in millimeters. Source: Haukisalmi et al. (2003).

	Parandrya oeconomi (Gubanyi & Murai, 2002)					
	Finland			Alaska		
	Mean	Range	No. meas	Mean	Range	No. meas
BL (mm)	183.1	142-235	-	167	141-195	-
ScD	775	505-910	-	853	690-1030	-
SuD	338	280-378	-	351	295-390	-
T (LxW)	-	-	-	-	-	-
CS (L)	217	170-265	-	232	200-257	-
ISV (LxW)	-	-	-	-	-	-
O (W)	608	390-818	-	578	500-716	-
Vi (W)	280	200-348	-	287	230-390	-
Va (LxW)	-	-	-	-	-	-
SR (LxW)	-	-	-	-	-	-

Supplemental Table 12. Measurement data for *Gulyaevia longivaginata* and *G. buryatiensis* in *Myodes rutilus* from South-Central Russia and in *Myodes rufocanus* from Buryatia respectively. BL: body length; ScD: scolex diameter; SuD: sucker diameter; T: testes; CS: cirrus sac; ISV: internal seminal vesicle; O: ovary; Vi: vitellarium; Va: vagina; SR; seminal receptacle; (L): length; (W): width; (LxW): length by width. Source: Haukisalmi et al. (2006).

	Gulyaevia longivaginata (Chechulin & Gulyaev, 1998)			*Gulyaevia buryatiensis* (Haukisalmi et al., 2007)		
	Mean	Range	N	Mean	Range	N
BL (mm)	-	198-225	3	-	148-277	-
ScD	-	650-770	4	-	660-770	-
SuD	-	250-330	16	-	240-350	-
T (LxW)	-	-	-	-	-	-
CS (L)	-	300-430	4	-	260-420	-
ISV (LxW)	-	-	-	-	-	-
O (W)	-	40-70	7	-	60-1100	-
Vi (W)	-	210-360	7	-	230-410	-
Va (L)	-	410-510	7	-	470-650	-
SR (L)	-	150-350	12	-	200-450	-

Supplemental Table 13. Measurement data for *Paranoplocephala sciuri* from two sampling localities in North America. BL: body length; ScD: scolex diameter; SuD: sucker diameter; T: testes; CS: cirrus sac; ISV: internal seminal vesicle; O: ovary; Vi: vitellarium; Va: vagina; SR; seminal receptacle; (L): length; (W): width; (LxW): length by width. Source: Rausch & Haukisalmi (2007).

	Rodentocestus sciuri (Rausch, 1947)					
	Wisconsin (Rausch, 1947)			Oregon		
	Mean	Range	No. meas	Mean	Range	No. meas
BL (mm)	-	170	-	-	160	1
ScD	-	380	-	-	500-590	4
SuD	-	150	-	-	200-240	136
T (LxW)	-	-	-	-	-	-
CS (L)	200	-	-	-	560-790	14
ISV (LxW)	-	-	-	-	-	-
O (W)	-	-	-	-	560-790	14
Vi (W)	-	-	-	-	210-330	13
Va (W)	-	-	-	-	340-590	6
SR (L)	-	420	-	-	500-650	6

Supplemental Table 14. Measurement data for *Paranoplocephala batzlii* and *Parano. jarrelli* in *Microtus miurus* from Alaska and *Microtus oeconomus* from Finland respectively. BL: body length; ScD: scolex diameter; SuD: sucker diameter; T: testes; CS: cirrus sac; ISV: internal seminal vesicle; O: ovary; Vi: vitellarium; Va: vagina; SR; seminal receptacle; (L): length; (W): width; (LxW): length by width. Source: Measurement data in micrometers, except BL which is in millimeters. Haukisalmi et al. (2006).

	Paranoplocephala s.s.					
	Paranoplocephala batzlii Haukisalmi et al., 2006			*Paranoplocephala jarrelli* Haukisalmi et al., 2006		
	Mean	Range	No. meas	Mean	Range	No. meas
BL (mm)	-	170-246	-	-	96-235	-
ScD	-	910-1220	-	-	550-1180	-
SuD	-	370-510	-	-	270-470	-
T (LxW)	-	-	-	-	-	-
CS (L)	-	160-320	-	-	170-320	-
ISV (LxW)	-	-	-	-	-	-
O (W)	-	-	-	-	-	-
Vi (W)	-	-	-	-	-	-
Va (LxW)	-	170-220	-	-	130-210	-
SR (LxW)	-	270-400	-	-	200-540	-

Supplemental Table 15. Measurement data for *Paranoplocephala omphalodes* and *Parano. microti* in *Microtus agrestis* from the Palearctic and *Microtus ochrogaster* in the Nearctic respectively. BL: body length; ScD: scolex diameter; SuD: sucker diameter; T: testes; CS: cirrus sac; ISV: internal seminal vesicle; O: ovary; Vi: vitellarium; Va: vagina; SR; seminal receptacle; (L): length; (W): width; (LxW): length by width. Measurement data in micrometers, except BL which is in millimeters. Source: Haukisalmi et al. (2006).

	Paranoplocephala s.s.					
	Paranoplocephala omphalodes (Herman, 1747)			*Paranoplocephala microti* (Hansen, 1947)		
	Mean	Range	N	Mean	Range	N
BL (mm)	-	112-204	-	-	157	-
ScD	-	840-1130	-	-	230	-
SuD	-	340-500	-	-	1160	-
T (LxW)	-	-	-	-	410x690	-
CS (L)	-	160-320	-	-	178-233	-
CS (W)	-	-	-	-	550-690	-
ISV (LxW)	-	-	-	-	-	-
O (L)	-	-	-	-	247-575	-
O (W)	-	-	-	-	164-274	-
Vi (W)	-	-	-	-	-	-
Va (L)	-	110-180	-	-	140-168	-
SR (LxW)	-	80-350	-	-	20-370	-

Supplemental Table 16. Measurement data for *Paranoplocephala kirbyi* from *Microtus californicus californicus* in California. BL: body length; ScD: scolex diameter; SuD: sucker diameter; T: testes; CS: cirrus sac; ISV: internal seminal vesicle; O: ovary; Vi: vitellarium; Va: vagina; SR; seminal receptacle; (L): length; (W): width; (LxW): length by width. Measurement data in micrometers, except BL which is in millimeters. Source: Haukisalmi et al. (2006).

	Paranoplocephala s.s.		
	Paranoplocephala kirbyi Voge, 1948		
	Mean	Range	N
BL (mm)	-	16000-27000	-
ScD	940	880-1029	-
SuD	365	332-398	-
T (diameter)	34	25-49	-
CS (LxW)	166x64	144x46-199x83	-
ISV (LxW)	-	-	-
O (W)	-	-	-
Vi (W)	-	-	-
Va (LxW)	-	-	-
SR (LxW)	-	-	-