The symbiotic mites of some Appalachian Xystodesmidae (Diplopoda : Polydesmida) and the complete mitochondrial genome sequence of the mite *Stylochyrus rarior* (Berlese) (Acari : Mesostigmata : Ologamasidae)

Lynn Swafford[A] *and Jason E. Bond*[A,B]

[A]Department of Biology, Howell Science Complex N211, East Carolina University,
 Greenville, North Carolina 27858, USA.
[B]Corresponding author. Email: bondja@ecu.edu

Abstract.   Millipedes of the family Xystodesmidae (Polydesmida) are often host to several symbiotic mite species, but very little work has been done to identify these acarines or to understand their relationship to the millipedes. In an attempt to better understand these associations, mites found on xystodesmid millipedes, a group for which a species phylogeny has been proposed, were collected in the Appalachian Mountains of Kentucky, Virginia, Tennessee and North Carolina. Mites in the genera *Stylochyrus* Canestrini & Canestrini, 1882 (Mesostigmata: Ologamasidae) and *Schwiebea* Oudemans, 1916 (Sarcoptiformes: Acaridae) were prevalent among millipedes in the genera *Apheloria* Chamberlin, 1921, *Appalachioria* Marek & Bond, 2006, *Boraria* Chamberlin, 1943, *Brachoria* Chamberlin, 1939, *Dixioria* Chamberlin, 1947, *Nannaria* Chamberlin, 1918, *Pleuroloma* Rafinesque, 1820, *Prionogonus* Shelley, 1982, *Rudiloria* Causey, 1955 and *Sigmoria* Chamberlin, 1939. Of the mite taxa collected, the species *Stylochyrus rarior* (Berlese, 1916) was found on the greatest number of sampled millipede taxa. To enhance future coevolutionary studies of xystodesmid millipedes and their mite symbionts, the complete mitochondrial genome of *S. rarior* associated with the millipede genus *Apheloria* (Polydesmida: Xystodesmidae) was sequenced. The genome is 14 899 nucleotides in length, has all the typical genes of an arthropod mitochondrion, differs in gene arrangement from that of the ancestral arthropod, and has a gene order that is unique among mites and ticks. The major difference in *S. rarior* is the placement of the protein-coding gene *nad1*, which is positioned between the rRNA gene 12S and the protein-coding gene *nad2* (tRNA genes and non-coding regions excluded). There are also two non-coding control regions within this mitochondrial genome.

Introduction

The order Mesostigmata, which contains ~12 000 known species, is a hyper-diverse arthropod group that comprises both free-living and symbiotic species of mites (Krantz and Walter 2009). These mite species are commonly predatory, brown in colour and their first pair of legs is often used for sensory purposes in addition to walking (Hunter and Rosario 1988). Mesostigmatid mites, as well as other acarine taxa, are probably poor dispersers as a consequence of their small size and lack of wings. Many groups of mites thus commonly practice phoresy (Krantz and Walter 2009), a short-term symbiotic relationship in which a small animal (the phoretic) increases its ability to disperse by attaching to a larger, more mobile animal (the carrier) (Farish and Axtell 1971). A symbiotic relationship is an association between two or more organisms in which at least one of the organisms benefits; it can be mutual, commensal or parasitic. Phoresy is considered to be a commensal type of symbiotic relationship because the phoretic benefits, but the carrier is usually not affected. Phoretic associations can be facultative or obligatory, and they can range from having only one very specific carrier to having a wide variety of taxa as carriers

(Krantz and Walter 2009). Animals in a phoretic stage often undergo an arrest of basic functions, such as development, reproduction and feeding (Farish and Axtell 1971). Once a suitable new environment is reached, the animals can leave their carrier, and these halted functions can recommence. Phoresy commonly takes place in mites in the deutonymphal stage of development and in adults (Krantz and Walter 2009). Despite the interesting life history characteristics of species attributed to this group and the close evolutionary associations of many of its members with totally unrelated arthropod and other animal species, relatively few studies have focussed on mesostigmatid mites.

It is now commonplace to use very large sets of molecular data in systematic evolutionary studies, including multiple genes sampled across both nuclear and organellar genomes and sequence data comparisons based upon entire genomes (Boore *et al*. 2005). Complete mitochondrial genome DNA sequences are often used because they are easy to isolate from nuclear DNA and tend to have a constant number of genes across most animal groups (Boore *et al*. 2005). The circular mitochondrial genome of animals typically consists of one or two non-coding control

regions and 37 genes: 13 protein-coding genes, 2 ribosomal RNA (rRNA) genes and 22 transfer RNA (tRNA) genes. Complete mitochondrial genomes have been sequenced for more than 200 arthropod species. Of these, 23 belong to the subclass Acari (mites and ticks). Mitochondrial genomes of acarines range in size from 13 103 nucleotides in *Tetranychus urticae* Koch, 1836 (Tetranychoidea) (Van Leeuwen *et al.* 2008) to 24 961 nucleotides in *Metaseiulus occidentalis* (Nesbitt, 1951) (Phytoseiidae) (Jeyaprakash and Hoy 2007). The ancestral arthropod gene order is considered to be the arrangement found in the horseshoe crab *Limulus polyphemus* Linnaeus, 1758 (Lavrov *et al.* 2000), but some arthropods, including several mites, deviate from this arrangement. A few (such as *M. occidentalis*) differ even in the number of mitochondrial genes as a result of duplications or deletions (Fahrein *et al.* 2007; Jeyaprakash and Hoy 2007). The vast number of differences among mite mitochondrial genomes indicates that there remains considerable work if we are to understand acarine systematics.

Generally speaking, we know very little about the genomes of mesostigmatid mites. The mitochondrial genome has been completely sequenced for only two species, and the synteny and size of their genomes are very different (Evans and Lopez 2002; Navajas *et al.* 2002; Jeyaprakash and Hoy 2007). *M. occidentalis* has a surprisingly large mitochondrial genome that is very divergent from the ancestral arthropod condition (Jeyaprakash and Hoy 2007). It contains both duplicated and triplicated regions, has short tRNAs and may be lacking the two protein-coding genes *nad3* and *nad6* (Jeyaprakash and Hoy 2007). The entire mitochondrial genome of *Varroa destructor* Anderson & Trueman, 2000 (Varroidae) has been sequenced twice with slightly different results (Evans and Lopez 2002; Navajas *et al.* 2002). Evans and Lopez (2002) concluded that the mitochondrial genome is 15 218 nucleotides in length, whereas Navajas *et al.* (2002) estimated the size to be 16 477 nucleotides. The protein-coding genes and rRNA genes of *V. destructor*, however, are located at the same relative positions as in ancestral arthropods (Evans and Lopez 2002; Navajas *et al.* 2002).

Neither *M. occidentalis* nor *V. destructor* belong to the family Ologamasidae, which is a large, widespread family of soil-dwelling, predaceous mites that typically eat small invertebrates and their eggs (Krantz and Walter 2009). Most ologamasid mite taxa are not phoretic; however, deutonymphs of a few genera have been found associated with mammal nests, carabid beetles, dipterans and other arthropods (Krantz and Walter 2009). *Stylochyrus rarior* (Berlese, 1916) is an ologamasid mite commonly found in moist, deciduous forests and is sometimes found associated with millipedes, small mammals and birds (Kethley 1983). Only juveniles in the deutonymphal stage (Fig. 1) have been found to form symbiotic relationships, whereas adults are usually free-living and collected in leaf litter on the forest floor (Kethley 1983). It is therefore believed that *S. rarior* forms temporary or phoretic symbiotic associations only for dispersal purposes (Kethley 1983). Deutonymphs have a distal hyaline extension on one of their cheliceral digits that is often associated with mites that practice phoresy (Kethley 1983). However, very little work has been done to understand the phoretic relationships of

S. rarior or to determine how many taxa these mites use as carriers.

The objectives of this study are: (1) to document the prevalence of symbiotic mites on xystodesmid millipede species of the Appalachian Mountains; and (2) to examine the common mite *S. rarior* and sequence its entire mitochondrial genome. This work will lay the foundation for a future genetic study of the coevolution of these millipedes and their associated mites.

Methods

Sampling and collecting

Xystodesmid millipedes were collected in October 2007, May to July 2008, and May to June 2009. Most collecting took place at the known localities of the millipede genus *Appalachioria* (Marek and Bond 2006) to gather mite and millipede specimens for future coevolutionary analysis. All xystodesmid millipedes found were examined for mites in the field. The millipedes that harboured mites were placed in individual collecting vials, so that there was no opportunity for the transfer of mites between millipedes. To limit the number of mites lost during transport, mites that were visible on the millipedes were removed with soft forceps and placed in RNAlater (Qiagen Inc., Valencia, CA) in the field. Both millipedes and mites were transported back to the laboratory for identification and study. In the laboratory, millipedes were again examined for mites. If mites were found, they were placed in RNAlater and stored at −80°C. Mites stored in RNAlater can be readily used for DNA extraction and sequencing. Millipede species were identified by morphology of male genitalia or by comparing the region of their mitochondrial DNA sequence spanning the 12S and 16S rRNA genes to those of the previously identified millipedes with GenBank accession numbers DQ4900648 to DQ4900700 (Marek and Bond 2006). Lactic acid or lactophenol was used to clear internal structures and then mites were mounted on microscope slides in Hoyer's mounting medium for identification. Alcohol-preserved xystodesmid specimens from the collection at East Carolina University (ECU) were also examined for the presence of mites; both the millipede specimens and the alcohol content of their vials were inspected. All mites and millipedes collected as part of this study have been assigned unique voucher numbers (mite–millipede associations and geographic data in the Accessory Publication, which is available on the *Invertebrate Systematics* website) and are currently stored in the collections at ECU (to be deposited in the Field Museum of Natural History collection).

Molecular protocols

A modified DNA extraction method using a Qiagen DNeasy Tissue Kit was used to isolate genomic DNA from one individual of *S. rarior*. The purpose of changing this extraction protocol was to ensure that the mite was not destroyed and could later be mounted on a microscope slide for identification purposes. The first modifications to the normal protocol consisted of leaving the mite in the digestion mix at 55°C for 24 h followed by −40°C for another 24 h. After thawing at room temperature, all the liquid was removed and transferred to a new tube so that the mite could be recovered. The digestion mix was never vortexed

Fig. 1. Images of *Stylochyrus rarior* deutonymphs and representative xystodesmid millipedes. (*A*) Whole-mount of *S. rarior* in ventral aspect, (*B*) anterior portion of *S. rarior* venter, (*C*) live individual of *Apheloria* sp. from Kentucky and (*D*) live individual of *Prionogonus* sp. from North Carolina.

while it contained the mite specimen. Instead, it was mixed gently by tapping the side of the tube. To complete the extraction procedure, 100 μL of buffer AE (10 mM Tris Cl and 0.5 mM ethylenediametetraacetic acid; warmed to 55°C) was added to the sample and centrifuged. Then 100 μL of buffer AE at room temperature was added to the sample and centrifugation was repeated to produce ~200 μL of genomic DNA. The extracted DNA from this single mite was used to sequence the entire genome of the mitochondrion.

First, a region of the 16S ribosomal gene was amplified and sequenced using the universal primers LR-J-12887 (5′-CCG GTCTGAACTCAGATCACGT-3′) and LR-N-13398 (5′-CGC CTG TTTATCAAAAACAT-3′). A 50-μL reaction was prepared comprising the following PCR master mix: 25.75 μL ultra-pure water, 5 μL 2.5 mM deoxyribonucleotide triphosphate (dNTP) mixture, 5 μL 10× Taq buffer, 5 μL of 2.5 μM or 10 μM primer, 1 μL dimethyl sulfoxide (DMSO), 1 μL bovine serum albumin (BSA), 0.25 μL Takara Ex Taq DNA polymerase, and 2 μL genomic DNA. The following thermal cycle parameters were used: initial denaturation at 95°C for 2 min; 29 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 1 min, and extension at 72°C for 1 min; and final extension at 72°C for

2 min. The reagent ExoSAP-IT (USB Corporation, Cleveland, OH) and its corresponding protocol were used to remove excess dNTPs and primers from the PCR product. This product was then used in a 10-μL sequencing reaction with the following components: 4.35 μL ultra-pure water, 2 μL 5× sequencing buffer, 1 μL BigDye Terminator, 0.65 μL of 2.5 μM or 10 μM primer, and 2 μL PCR product. The following thermal cycle parameters were used: 25 cycles of 96°C for 10 s, 50°C for 15 s, and 60°C for 4 min. This 16S ribosomal gene sequence was used to create primers specific to this gene region for this individual mite that could then be used to amplify the entire mitochondrial genome according to the procedures of Hwang *et al*. (2001). The primers created were HPK16Sbb_mit91 (5′-CATATTGATAAAATAGTTTGCGACCTCGATGTT-3′) and HPK16Saa_mit91 (5′-TCAATACCTTCGCATAGTCAAA ATACCACGGC-3′). The following 50-μL PCR mixture was used: 24.5 μL ultra-pure water, 8 μL 2.5 mM dNTP mixture, 5 μL 10× LA PCR buffer (Takara Bio Inc., Shiga, Japan), 5 μL of 2.5 μM or 10 μM primer, 0.5 μL Takara LA Taq (Takara Bio Inc.), and 2 μL genomic DNA. The thermal cycle parameters described by Hwang *et al*. (2001) were used, and this product was purified using the ExoSAP-IT procedure.

After the entire mitochondrial genome was amplified and cleaned (minus the short region within 16S), an additional amplification was done using a Qiagen Repli-g Ultrafast Mini Kit to increase the amount of mitochondrial DNA. Next, two separate digestions were performed using the restriction enzymes Rsa I (5′-GTAC-3′) and Alu I (5′-AGCT-3′). These digested products were sorted on an agarose gel with a ladder, and fragments between 500 and 1500 nucleotides in size were excised from the gel and purified using a Qiagen MinElute Gel Extraction Kit. Using a Zero Blunt PCR Cloning Kit (Invitrogen, Carlsbad, CA), DNA was inserted into vectors and transformed into *E. coli* cells, which were then grown overnight on kanamycin agar plates. Isolated colonies, each containing a cloned *S. rarior* mitochondrial region, were amplified and sequenced using the primers M13 Reverse and T7 from the cloning kit. A 25-µL PCR reaction was performed using the following mixture: 12.5 µL GoTaq Green Master Mix (Promega, Madison, WI), 9.5 µL ultra-pure water, 1.5 µL of 2.5 µM or 10 µM primer, and 1 swab of an isolated colony. The following thermal cycle parameters were used: initial denaturation at 94°C for 10 min; 29 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min; and final extension at 72°C for 10 min. PCR products were cleaned up with ExoSAP-IT and sequenced using the same protocol described above. Sequences were viewed, edited and assembled in the program Sequencher 4.8 (Gene Codes, Ann Arbor, MI).

To complete the whole mitochondrial genome sequence, primers pairs were designed using Primer3 (Rozen and Skaletsky 2000) to fill in any gaps between the sequence fragments. Genomic DNA from *S. rarior* was amplified and sequenced using these designed primer pairs. Because all gaps were of unknown length, amplifications were done using Takara LA Taq and the corresponding 50-µL PCR mixture described above. The thermal cycle parameters for long amplification described in Hwang *et al.* (2001) were used but with an annealing temperature of 50°C. The same methods as described earlier were used to purify and sequence the PCR products.

Genome annotation

The tRNA genes were identified using tRNAscan-SE 1.21 (Lowe and Eddy 1997); the Cove cutoff score was set to 2 to detect as many potential genes as possible. The tRNA genes that were not found by this method were identified by comparison to known tRNAs of other arthropods and by looking for anticodons. Ribosomal RNA genes were annotated by alignment with available mitochondrial genomes of other Acari. Protein-coding genes were identified by using the National Center for Biotechnology Information (NCBI) ORF Finder (http://www.ncbi.nlm.nih.gov/projects/gorf/) and by running a BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) search against all mite DNA sequences available in GenBank. Regions that were not similar to known genes and that did not have long open reading frames were considered to be non-coding control regions.

Results and discussion

Prevalence of mites on millipede hosts

In total, 136 xystodesmid millipedes (Fig. 1) were collected in the Appalachian Mountains of North Carolina, Virginia, Kentucky and Tennessee during the sampling period (see Accessory Publication on the *Invertebrate Systematics* website). Of these millipedes, 89 had symbiotic mites. More than 400 mites (MIT00138 to MIT00226 in Accessory Publication), representing the two orders Sarcoptiformes (including the genus *Schwiebea*) and Mesostigmata (including the genus *Stylochyrus*), were extracted from these millipedes. All *Stylochyrus* and *Schwiebea* individuals collected were in the deutonymphal stage of development. More than 100 *S. rarior* deutonymphs were collected from 43 millipedes including the following genera: *Apheloria*, *Appalachioria*, *Brachoria*, *Dixioria*, *Nannaria*, *Pleuroloma*, *Prionogonus* and *Sigmoria*. For those millipedes that harboured this species of mite, the number of *S. rarior* individuals per millipede ranged from 1 to 13 with an average of 2.49 (s.d. = 2.58). About 51% (22 out of 43) of these millipedes had only a single individual of *S. rarior* associated with them. Deutonymphal mites of the genus *Schwiebea* were collected from 57 xystodesmid millipedes from the genera *Apheloria*, *Appalachioria*, *Brachoria*, *Boraria*, *Dixioria*, *Nannaria*, *Rudiloria* and *Sigmoria*. The number of *Schwiebea* individuals per millipede ranged from 1 to 26 with an average of 5 (s.d. = 5.72). Although these mites were found in greater numbers on individual millipedes, they were not found on as many species of millipede as *S. rarior*. Fifteen millipedes had both *Schwiebea* and *S. rarior*. A few unidentified mesostigmatid mites were also collected in very small numbers from xystodesmids.

Symbiotic mites were discovered on many different body parts of their millipede carriers. Some mites were found attached near the anterior end of the millipede, whereas others were found on the legs or near the bases of the legs. Others seemed to be actively moving along the dorsal and ventral surfaces of the millipedes. It is not clear whether this activity is natural or whether it occurred because of human interference. *Stylochyrus* was typically active upon the millipede, whereas *Schwiebea* was usually inactive and attached under the head or near the legs of the millipede. This was expected because *Schwiebea* deutonymphs have sucker-like attachment plates on their ventral surfaces and have been found tightly attached to other arthropods (Purrington and Drake 2008). *Stylochyrus* deutonymphs lack attachment plates, so they must hang on to a carrier with their legs, claws or chelicerae.

Upon examination of the millipede collection at ECU, more mite taxa were found to be associated with xystodesmids. In addition to *Schwiebea* and *Stylochyrus*, the taxa *Viedebanttia* Oudemans, 1929 (Sarcoptiformes: Acaridae) and Heterozerconidae (Mesostigmata) were also found. All the mites found (MIT00011 to MIT00137 in Accessory Publication) were detached from their associate millipede specimen, which indicates that they fell off in storage. Mites from the ECU millipede collection could have been lost or even transferred between millipedes, which means that the number and kinds of mites found may not be representative of natural populations. Mites were found associated with 119 preserved xystodesmid specimens. Of these millipedes, 72 were associated with *S. rarior* (1 to 9 individuals per millipede), 34 with *Schwiebea* (1 to more than 20 per millipede), 19 with *Viedebanttia* (1 to 7 per millipede) and 4 with heterozerconids (only 1 per millipede). Some of these mite taxa were found

associated with the same millipede specimen. All individuals belonging to the genera *Stylochyrus*, *Schwiebea* and *Viedebanttia* were juveniles in the deutonymphal stage. The four heterozerconid mites were adults. Heterozerconidae is an acarine family found primarily on juliform millipedes (Gerdeman *et al.* 2000). In the USA, heterozerconid mites are not known to be associated with any species of Polydesmida; therefore, this xystodesmid-associated heterozerconid may be an undescribed species.

Stylochyrus rarior taxonomy and mitochondrial genome organisation

Superorder **PARASITIFORMES**

Order **MESOSTIGMATA**

Suborder **MONOGYNASPIDA**

Superfamily **RHODACAROIDEA**

Family **OLOGAMASIDAE** Ryke, 1962

Genus *Stylochyrus* Canestrini & Canestrini, 1882

Type species: *Stylochyrus rovennensis* Canestrini & Canestrini, 1882: 31–82.

Table 1. Primer pairs and their locations

Primer pair	Name	Primer sequence	Location
1	Mit162R	5′-CTGTCAAATTAAACCCTCCAGC-3′	*nad1*
	Mit289L	5′-GAAGATAATTCTTGCTCTAGATTGAAA-3′	*nad2*
2	Mit289R2	5′-CCAATTTACTTTTGACTTCCTCAAATA-3′	*nad2*
	Mit194L2	5′-TTAGGGGATAACTAATACGGAGATAAA-3′	*nad2*
3	Mit194R2	5′-TTTATAGTAATACCAGCCATAATTGGG-3′	*cox1*
	Mit160L2	5′-ATCTAATTCCTGCTAAGTGAAGTCTAA-3′	*cox1*
4	Mit160R	5′-CGACACCCCTATTCTTTGATCT-3′	*cox1*
	Mit247L	5′-TATCGCCGAGGTATTCCTCTTA-3′	*cox1*
5	Mit247R	5′-AAACAAAACAAACTTTATATCAACCA-3′	*atp8*
	Mit191L	5′-AGAAATTAATAGGTGGCCAGCA-3′	*atp6*
6	Mit191R	5′-TTCGACTTGGCTCTTGAATACA-3′	*nad3*
	Mit291L	5′-GGAGTGTAAATTCAATCACGGA-3′	*nad5*
7	Mit291R	5′-CTCTTAAAAATGGAAACCCCG-3′	*nad5*
	Mit199L	5′-GGTAGAATGTGGGGTTGGATAG-3′	*nad4*
8	Mit291R2	5′-CATCAAACACCCAAATAAAATAACC-3′	*nad5*
	Mit199L2	5′-GTTAGCTGCAAATTTTGAGAGTGAT-3′	*nad4*
9	Mit199R	5′-ATTACCCAATCCTCCCCATAAT-3′	*nad4*
	Mit209L	5′-GGTTATTATGGGTTAGTGGGGTG-3′	*cob*
10	Mit209R	5′-TTACCTTCATATCGGTCGAGGT-3′	*cob*
	Mit175L	5′-AACAGCGAGTACAAAAGGAAGG-3′	*cob*
11	Mit175R	5′-CTAACACCCCTCCTCAAGAACA-3′	*cob*
	Mit16SL	5′-AATAGTTTGCGACCTCGATGTT-3′	*16S*
12	Mit16SR	5′-TCCATTCTCTTAGCACCCAATT-3′	*16S*
	Mit303L	5′-ATCAGGGGGCTTCAATAAAATT-3′	*16S*
13	Mit303R	5′-CAATTATTCATGAGAGCGACGG-3′	*16S*
	Mit12SL	5′-TTTGGCGGTATTTCAATCTTTT-3′	*12S*
14	Mit12SR2	5′-AACCTTAAAAACAAAACTAAAACTGCC-3′	*12S*
	MitND1L	5′-GGTAGTAATTTTAGCTGAAACAAATCG-3′	*nad1*

Stylochyrus rarior (Berlese, 1916)

Gamasiphis (*Epiphis*) *rarior* Berlese, 1916: 289–338.
Epiphis rarior Berlese, 1916. – Vitzthum, 1942: 756; Kethley 1983: 2598.
Stylochyrus rarior (Berlese, 1916). – Lee, 1970: 94.

Diagnosis

All of the *S. rarior* individuals collected are morphologically identical deutonymphs (Fig. 1) and correspond to Kethley's (1983) descriptions. However, there were a few discrepancies between Kethley's (1983) illustrations and diagnosis of *S. rarior* deutonymphs, and they are clarified here. All dorsal setae are very short except for s4, Z3 and Z5, which are substantially (more than five times) longer. The fixed digit of the chelicera has an elongate, distal hyaline process and is longer than the movable digit.

DNA processing

Extracted genomic DNA from the *S. rarior* specimen MIT00091-4 was used to amplify and sequence the entire mitochondrial genome (GenBank accession number GQ927176). This mite was associated with a millipede of the genus *Apheloria* (SPC001176) from the Appalachian Mountains of Tennessee. After both restriction enzyme digestions were completed and selected DNA fragments were cloned and sequenced, DNA sequences were assembled into 13 contigs in Sequencher 4.8. These contigs contained partial sequences of all the 13 protein-coding genes and the 2 rRNA genes normally present within a mitochondrial genome. Fourteen pairs of primers (Table 1) were designed to obtain DNA sequences from the missing regions between these 13 DNA fragments.

Genome organisation

The mitochondrial genome of *S. rarior* is circular, consists of 14 899 nucleotides and contains 13 protein-coding genes,
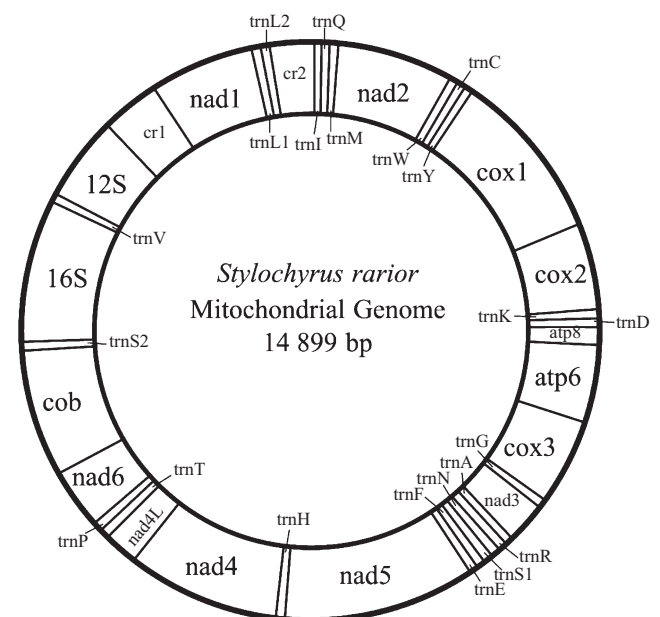

Fig. 2. Mitochondrial genome map for *Stylochyrus rarior*.

Table 2.   Position and length (nt) of genes in the mitochondrion of *Stylochyrus rarior*

Gene	Gene product	Position	Length	Strand
trnM	Transfer RNA methionine	1–63	63	+
nad2	NADH dehydrogenase subunit 2	64–1026	963	+
trnW	Transfer RNA tryptophan	1025–1085	61	+
trnC	Transfer RNA cysteine	1078–1140	63	–
trnY	Transfer RNA tyrosine	1129–1190	62	–
cox1	Cytochrome *c* oxidase subunit 1	1202–2725	1524	+
cox2	Cytochrome *c* oxidase subunit 2	2725–3400	676	+
trnK	Transfer RNA lysine	3401–3463	63	+
trnD	Transfer RNA aspartic acid	3464–3524	61	+
atp8	ATP synthase F0 subunit 8	3525–3683	159	+
atp6	ATP synthase F0 subunit 6	3680–4345	666	+
cox3	Cytochrome *c* oxidase subunit 3	4359–5123	765	+
trnG	Transfer RNA glycine	5124–5186	63	+
nad3	NADH dehydrogenase subunit 3	5187–5525	339	+
trnA	Transfer RNA alanine	5525–5586	62	+
trnR	Transfer RNA arginine	5587–5645	59	+
trnN	Transfer RNA asparagine	5646–5706	61	+
trnS1	Transfer RNA serine 1	5707–5760	54	+
trnF	Transfer RNA phenylalanine	5761–5821	61	+
trnE	Transfer RNA glutamic acid	5820–5879	60	–
nad5	NADH dehydrogenase subunit 5	5880–7548	1669	–
trnH	Transfer RNA histidine	7549–7610	62	–
nad4	NADH dehydrogenase subunit 4	7606–8934	1329	–
nad4L	NADH dehydrogenase subunit 4 L	8936–9208	273	–
trnT	Transfer RNA threonine	9225–9284	60	+
trnP	Transfer RNA proline	9285–9347	63	–
nad6	NADH dehydrogenase subunit 6	9335–9781	447	+
cob	Apocytochrome *b*	9788–10898	1111	+
trnS2	Transfer RNA serine 2	10899–10961	63	+
16S	Large subunit rRNA	10962–12161	1200	–
trnV	Transfer RNA valine	12162–12233	72	–
12S	Small subunit rRNA	12234–12886	653	–
cr1	Control region 1	12887–13357	471	N/A
nad1	NADH dehydrogenase subunit 1	13358–14260	903	–
trnL1	Transfer RNA leucine 1	14261–14319	59	–
trnL2	Transfer RNA leucine 2	14313–14376	64	–
cr2	Control region 2	14377–14775	399	N/A
trnI	Transfer RNA isoleucine	14776–14836	61	+
trnQ	Transfer RNA glutamine	14837–14899	63	–

22 tRNA genes, 2 rRNA genes and 2 non-coding control regions (Fig. 2, Table 2). Genes are encoded on both DNA strands (Table 2) as in other arthropods. The nucleotide composition of this mitochondrial genome consists of 38.3% adenine, 34.4% thymine, 17.8% cytosine and 9.5% guanine. Approximately 72% of this entire mitochondrial genome sequence codes for proteins. Only 18 tRNA genes were identified and located by the program tRNAscan-SE. The other four (*trnL2*, *trnC*, *trnR*, *trnS1*) were located by comparison to known tRNA sequences of other arthropods and by determining their appropriate anticodons. There are two *trnL* genes and two *trnS* genes present. Several of the genes of the mitochondrial genome have short overlapping regions between them (Table 2).

The order of genes in the mitochondrion of *S. rarior* differs from the assumed ancestral arthropod synteny of *L. polyphemus*. This gene arrangement is also novel among acarines owing to the placement of the protein-coding gene *nad1*, which is located between 12S and *nad2* (tRNAs and control regions excluded).

When compared with *L. polyphemus*, the positions of the tRNA genes *trnF* and *trnE* are swapped in *S. rarior*. The following two regions have also been transposed: 16S–*trnV*–12S and *nad1*–*trnL1*–*trnL2*. There are two non-coding control regions, one on each side of the *nad1*–*trnL1*–*trnL2* region.

Conclusions

Stylochyrus rarior appears to be considerably more common among the xystodesmid millipede species than other mite taxa. This is one of the reasons why *S. rarior* was chosen for further study and use in future coevolutionary analysis. It is also large in size and soft-bodied, which allows for easier DNA extraction. *S. rarior* is the first ologamasid and the first rhodacaroid mite to have its entire mitochondrial genome sequenced. This genome contains all 37 genes that are typical of the animal mitochondrion, and it also contains two non-coding regions. The mitochondrial gene order of *S. rarior* is different from the ancestral arthropod

arrangement and is unique among the acarines. Acarine mitochondrial genomes often have gene rearrangements, but it is unclear whether these gene order differences have any phylogenetic significance because very few mitochondrial genomes of mites and ticks have been completely sequenced. *S. rarior* is only the third mesostigmatid mite for which the whole mitochondrial DNA sequence is known and thus may help to improve the understanding of acarine evolution.

There still remains much to learn about the ecology of phoretic mites and their millipede carriers. Phoresy could be beneficial to millipede mites in ways other than just enhanced dispersal. For example, xystodesmid millipedes are not typically eaten because they produce a hydrogen cyanide defence secretion (Marek and Bond 2006). Perhaps the symbiotic mites of xystodesmid millipedes could also be protected from predation; it is unknown if phoretic mites are resistant to these cyanide secretions. Another uncertainty is how the millipedes respond to the association with their mite commensals; that is, do they benefit by the relationship, is there a cost, or is the association neutral?

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