The mitochondrial genome of the oribatid mite *Paraleius leontonychus*: new insights into tRNA evolution and phylogenetic relationships in acariform mites

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Bilaterian mitochondrial (mt) genomes are circular molecules that typically contain 37 genes. To date, only a single complete mitogenome sequence is available for the species-rich sarcoptiform mite order Oribatida. We sequenced the mitogenome of *Paraleius leontonychus*, another species of this suborder. It is 14,186 bp long and contains 35 genes, including only 20 tRNAs, lacking tRNAGly and tRNATyr. Re-annotation of the mitogenome of *Steganacarus magnus* increased the number of mt tRNAs for this species to 12. As typical for acariform mites, many tRNAs are highly truncated in both oribatid species. The total number of tRNAs and the number of tRNAs with a complete cloverleaf-like structure in *P. leontonychus*, however, clearly exceeds the numbers previously reported for Sarcoptiformes. This indicates, contrary to what has been previously assumed, that reduction of tRNAs is not a general characteristic for sarcoptiform mites. Compared to other Sarcoptiformes, the two oribatid species have the least rearranged mt genome with respect to the pattern observed in *Limulus polyphemus*, a basal arachnid species. Phylogenetic analysis of the newly sequenced mt genome and previously published data on other acariform mites confirms paraphyly of the Oribatida and an origin of the Astigmata within the Oribatida.

Studies on the description of complete mitochondrial (mt) genomes have accumulated in recent years, as, with the advent of high-throughput sequencing methods, it has become much easier to quickly obtain accurate mitogenome assemblies from any target species of choice¹ ⁴. Moreover, free user-friendly software or online tools are available, providing easy and fast automated gene annotation, which in turn enables generation of a draft mitogenome in a matter of weeks⁴ ⁶.

Bilaterian animals have a circular mitogenome, usually 13–17 kb in size, with a relatively conserved gene content, usually comprising 37 genes. These are 13 protein coding genes (PCGs), 2 ribosomal RNA (rRNA) genes, and 22 different transfer (tRNA) genes. In addition, animals also have an A + T-rich control region⁷ ⁸. Interspecific length variation of mitogenomes is usually due to length variation in the control region and/or presence/absence of particular tRNA genes, but also due to length differences in the PCGs⁹. Complete mitogenomes represent important molecular resources not only for phylogenetic, phylogeographic and population genetic studies¹⁰ ¹², but are also interesting and relevant with respect to gene order evolution¹³ ¹⁴ or adaption to novel environments¹⁵ ¹⁶. Although there are several bioinformatics tools available, identifying mt-tRNAs is not always straightforward, in particular in case of unknown codon/anticodon rules, post-transcriptional modifications, deviations from the standard genetic code or unconventional secondary structures¹⁷ ¹⁸. Additionally, it is well known that the presence of the complete 22 tRNAs is not universal as there are several mitogenomes (also in metazoans) lacking one to all tRNA genes¹⁹ ²⁰.
In general, more than 90% of the metazoan mt-tRNAs are inferred to possess the conventional four-armed cloverleaf secondary structure. There is at least one well-known exception, the D–arm lacking tRNA59 (AGNY)31, a feature shared among nearly all Metazoa32,33. So far, there are several known mitogenomes whose encoded tRNAs are non-canonical which means that they have either reduced D- or T-arms (truncated tRNAs), or even lack these two elements at all (“armless” tRNA), resulting in very short encoding genes. An extreme case of armless tRNAs has been found in the mitochondria of the nematode class Enoplea, where encoded transcripts have a length of only 42 nucleotides (nts)34,35, representing the world’s smallest tRNA (typical tRNA length is 70–100 nts). Biological activity of these extremely short tRNAs was demonstrated by Wende et al.36 by verification of in vitro transcription and 3'- and 5'-processing of several mt-tRNAs of the nemathid Romanomeris culicivorax. Beside nematodes, evidence for truncated tRNAs has been found in several other groups, but most frequently in arthropods25,26 and in particular in mites. While the majority of species from the superorder Parasitiformes do not have more than two truncated tRNAs24–26, reduction of RNA-D- and/or T-arms has been shown in all published mitogenomes of the second superorder Acariformes22,27,28 known so far. In general, only the tRNA36 shows the typical cloverleaf structure in all known acariform species, except for the orbibatid mite Steganacarus magnus29. Three further tRNAs lack the T-arm in all Acariformes, representing a potential ancestral feature, while all remaining 18 tRNAs vary in their secondary structure among the same mite species25. In contrast to the aberrant secondary structures, losing a tRNA is not typical for arthropods36. However, there are a few documented examples, as e.g. in the Chinese scorpion Mesobuthus martensi37, in some isopods34,35 and also in mites. Whereas all published parasitiform mitogenomes have the full set of tRNA genes, four species in the Acariformes are known to have a reduced number of mt-tRNA, namely S. magnus29, Sarcoptes scabiei31 and two Tyrophagus species30. While the mt genomes of S. scabiei and Tyrophagus spp. lack two, respectively, three tRNAs, S. magnus lost 16 tRNAs29.

In general, acariform mites typically show high levels of mt-gene rearrangement, loss of tRNAs and unconventional secondary structures of tRNAs, which makes them an ideal model system for studying gene order and tRNA evolution. Mite systematics, in general, is complex and controversial and also for acariform mites there are several classification schemes present in the literature36–39. In the current study we refer to the classification scheme of Lindquist et al.37 who divide the Acariformes into the two orders Trombidiiformes and Sarcoptiformes. These two orders are subdivided in several families and are subdivided into various suborders and/or supercohorts.

The species investigated here, Paraleius leontonychus, is a very unusual member of the sarcoptiform suborder Oribatida. Its special feature is undoubtedly not only the typical arboreal life-style; but especially its unusual form and/or supercohorts.

In the present study we sequenced and analyzed the complete mitogenome of P. leontonychus (as part of an ongoing whole genome assembly and annotation project) to investigate its impact on the evolution of tRNAs within the Acariformes as well as on the phylogeny of the Sarcoptiformes. We compared the new mitogenome with those of other closely related species and performed gene rearrangement analyses relative to Limulus polyphemus, as the hypothetical ancestor of arachnids. The mt genome of S. magnus was originally described by Domes et al.30 to exhibit an unexpectedly great loss of tRNAs (only 6 of 22 present). In a later study, Klimov & O'Connor provided an improved tRNA prediction in the house dust mite Dermatophagoides farinae including tRNAs of S. magnus for a comparison. Like Domes et al.30, Klimov and O'Connor used tRNAscan-SE and ARWEN to predict tRNAs and infer their secondary structure. In contrast to Domes et al.30, the minimum free energy (MFE) of the constrained and unconstrained secondary structure was additionally calculated to select the most probable of alternative predicted structures. Klimov and O'Connor identified another two tRNAs and re-annotated three of the previously described ones based on manual sequence annotation and MFE calculations. With this background, we decided to re-annotate the tRNAs in the S. magnus mitogenome once more using the same programs and prediction methods as for P. leontonychus and compared our results with those from the former studies37. Beyond that, S. magnus is of particular interest as it belongs to the orbibatid supercohort Mixonomatidae, which represents a phylogenetically more basal group compared to species of the supercohort Desmonomatomidae38, which includes P. leontonychus. Considering the presumed close relationship of S. magnus and P. leontonychus, we expect a similarly extensive loss of tRNA genes in P. leontonychus.

**Results**

The mitogenome of P. leontonychus is a closed circular DNA molecule that is 14,186 nts long and encodes for 35 genes, 13 PCGs, two ribosomal RNAs and 20 tRNAs (Fig. 1, Table 1). While PCGs and tRNAs are located on both strands, the two rRNAs are encoded on the (−)-strand. Ten of the 13 PCGs start with the mt start codons ATA, ATC or ATT, while nad4 and nad1 use TTG and nad6 the start codon GTG. The stop codons are either TAA or TAG, and incomplete stop codons, T or TA, are present in those PCGs that overlap with other coding genes or tRNAs. The nucleotide composition of the leading (+)-strand is A = 38.8%, C = 22.0%, G = 13.3% and T = 25.8%, resulting in a positive AT-skew (0.201) and a negative GC-skew (−0.245).

A control region (CR) with a length of 435 nts was predicted, which is comparable to annotated CR sequences of other published sarcoptiform mitogenomes (except for S. magnus [1019 nts] and Tyrophagus longior [50 nts]). The AT content of the CR is 59.3%, which is considerably lower than for the other Sarcoptiformes (69.0–91.6%). Together with the lack of longer A and T stretches this leads to smaller stemloop structures compared to the related species. The position and number of the stemloops do not seem to be conserved.

For P. leontonychus, eighteen tRNAs were detected by at least one of the programs used (Supplementary Table S1, Supplementary Fig. S1), while tRNAala and tRNAtyr were identified manually only based on the antico-don sequences and the conserved secondary structure. tRNAarg and tRNVal could not be identified. In general,
most of the tRNAs are short and do not show the typical clover-leaf secondary structure. They are highly truncated and miss either the D-, T-arm or both arms (="armless" tRNA). About half of them have mismatched base pairs and/or truncated acceptor stems (with less than seven paired bases, e.g. tRNAala, tRNALeu2, tRNAMet, tRNASer1, tRNAVal; Fig. 2). Furthermore, we observed tRNA genes overlapping with other tRNAs (tRNALeu2/tRNAPhe, tRNAMet/tRNACys, and tRNAIle/tRNAGln), but no overlap between tRNAs and PCGs. The annotated sequence has been deposited in the European Nucleotide Archive under the accession number LT984407.

The re-annotation of the tRNAs of the S. magnus mitogenome predicted 16 novel tRNAs (tRNA Asp, tRNAMet, tRNASer1, tRNAPhe, tRNAThr, tRNASer2, tRNACys, tRNAGln, tRNATyr, tRNATrp, tRNAGlu, tRNAGly, tRNAIle, tRNAArg, and tRNALeu1). Filtering the novel tRNAs based on the constrained MFE values left nine reliable predictions (tRNA Asp, tRNASer1, tRNAThr, tRNASer2, tRNACys, tRNATrp, tRNALys, tRNAArg, and tRNALeu1), including three changed tRNA assignments (tRNA Pro > tRNAThr, tRNATrp > tRNASer2, tRNASer2 > tRNATrp; Supplementary Table S2, Supplementary Fig. S2). Together with the three correctly annotated tRNAs in Domes et al., this lead to a new mitogenome arrangement of S. magnus with a total of 12 tRNAs (Fig. 3).

The phylogenetic reconstructions based on Maximum likelihood (ML) and Bayesian Inference (BI) analyses revealed identical topologies for both datasets, nucleotide (ND) and amino acid (AAD) sequences of the PCGs. Almost all nodes were statistically well supported by high bootstrap values and high BI posterior probabilities (Fig. 4 and Supplementary Fig. S3). All analyses unambiguously supported the monophyly of the two superorders (Acariformes and Parasitiformes) and the orders Sarcoptiformes, Ixodida and Mesostigmata. The new sequence of P. leontonychus was placed as sister group of the Astigmata, rendering the Oribatida paraphyletic. In addition, the order Trombidiformes was recovered as paraphyletic due to the separate placement of the two eriophyoid species, Epitrimerus sabinae and Phyllocoptes taishanensis at the base of the Acariformes.

The Neighbor joining (NJ) tree obtained from gene rearrangement analyses also suggested that the Sarcoptiformes evolved within the Trombidiformes (Fig. 5). The exact branching order, however, differed from the tree topologies obtained from ND and AAD dataset. Interestingly, the two oribatid species P. leontonychus and S. magnus clustered together despite their obvious differences in the number of tRNA genes present. Histiosoma blomquisti was placed as sister taxon of the two oribatid species, rendering the Astigmata paraphyletic based on gene rearrangement patterns.

Figure 1. Mitochondrial genome of P. leontonychus. Genes transcribed on the leading (+)-strand are on the outside of the circles, those on the lagging (−)-strand on the inside of the circles. Color codes for the genes are given in the box; tRNAs are abbreviated by the one-letter code for the corresponding amino acid. All abbreviations are the same as in Table 1.
Table 1. Mitochondrial genome organization of *Paraleius leontonychus*.

| Gene Product/Description | Start | End | Strand | Length | Gap | Startcodon | Stopcodon |
|--------------------------|-------|-----|--------|--------|-----|------------|-----------|
| cox1 cytochrome c oxidase subunit I | 1 | 1536 | + | 1536 | 9 | ATA | TAA |
| cox2 cytochrome c oxidase subunit II | 1546 | 2202 | + | 657 | 5 | ATA | TAA |
| trnD tRNA-Asp(gtc) | 2208 | 2267 | + | 60 | 1 |
| atp8 ATP synthase F0 subunit 8 | 2269 | 2413 | + | 145 | 1 | ATC | T(AA) |
| atp6 ATP synthase F0 subunit 6 | 2415 | 3079 | + | 665 | 0 | ATA | TAA |
| cox3 cytochrome c oxidase subunit III | 3080 | 3865 | + | 786 | 41 | ATA | TAA |
| nad3 NADH dehydrogenase subunit 3 | 3907 | 4248 | + | 342 | 13 | ATT | TAA |
| trnA tRNA-Ala(ttg) | 4262 | 4306 | + | 45 | 1 |
| trnL2 tRNA-Leu(taa) | 4308 | 4370 | + | 63 | 1 |
| trnF tRNA-Phe(gaa) | 4370 | 4424 | − | 55 | 0 |
| nad5 NADH dehydrogenase subunit 5 | 4425 | 6033 | − | 1609 | 0 | ATT | T(AA) |
| trnH tRNA-His(gig) | 6034 | 6088 | − | 55 | 1 |
| nad4 NADH dehydrogenase subunit 4 | 6090 | 7130 | − | 1041 | 255 | TTG | TAA |
| nad4L NADH dehydrogenase subunit 4L | 7386 | 7655 | − | 270 | 0 | ATA | TAA |
| trnT tRNA-Thr(tgt) | 7656 | 7710 | + | 55 | 0 |
| nad6 NADH dehydrogenase subunit 6 | 7711 | 8136 | + | 426 | 1 | GTG | TAA |
| cytB cytochrome b | 8138 | 9232 | + | 1095 | 1 | ATA | TAA |
| trnS2 tRNA-Ser(tga) | 9234 | 9287 | + | 54 | 2 |
| nad1 NADH dehydrogenase subunit 1 | 9286 | 10182 | − | 897 | 1 | TTG | TAG |
| trnK tRNA-Lys(itt) | 10184 | 10247 | + | 64 | 2 |
| trnW tRNA-Trp(tca) | 10250 | 10311 | + | 62 | 28 |
| trnM tRNA-Met(cat) | 10340 | 10397 | + | 58 | 5 |
| trnC tRNA-Cys(gca) | 10393 | 10463 | − | 71 | 1 |
| nad2 NADH dehydrogenase subunit 2 | 10465 | 11406 | + | 942 | 7 | ATA | TAA |
| trnI tRNA-Ile(gat) | 11414 | 11470 | + | 57 | 2 |
| trnQ tRNA-Gln(stg) | 11469 | 11531 | − | 63 | 34 |
| trnP tRNA-Pro(tgg) | 11566 | 11629 | − | 64 | 13 |
| trnL2 large ribosomal RNA | 11633 | 12751 | − | 1109 | 0 |
| trnV tRNA-Val(tac) | 12752 | 12798 | − | 47 | 4 |
| trnS3 small ribosomal RNA | 12795 | 13477 | − | 683 | 2 |
| trnN tRNA-Asn(gtt) | 13476 | 13531 | − | 56 | 14 |
| trnK tRNA-Arg(tcg) | 13518 | 13563 | + | 46 | 8 |
| trnE tRNA-Glu(ttc) | 13572 | 13635 | + | 62 | 0 |
| CR control region | 13634 | 14068 | + | 435 | 0 |
| trnS1 tRNA-Ser(gct) | 14069 | 14125 | + | 57 | 3 |
| trnL1 tRNA-Leu(tgg) | 14129 | 14132 | + | 61 | 3 |

Discussion

**General aspects of the new acariform mitogenome.** The mitogenome of *P. leontonychus* is the second published complete oribatid mite mitogenome so far and differs clearly from the previously described one from *S. magnus*, not only because of differences in the gene arrangement (Fig. 3) but also in the number of identified tRNAs (for details see below). As in other acariform mites, extensive gene order rearrangement became evident in *P. leontonychus* (Figs 3 and 5). However, compared with other sarcoptiform mites, it has the second least rearranged mitogenome compared to *L. polyphemus*, the hypothetical ancestor, according to the number of breakpoints calculated via CREx analysis (Supplementary Table S3). The least rearranged genome appears to be that of the second oribatid species, *S. magnus* (Fig. 5). Concerning PCGs, *P. leontonychus* has the same gene arrangement as *S. magnus*, with the exception of nad1 and nad2, which changed position and strand (Figs 3 and 5). All other species are not closely related to our study species and show multiple rearrangements of gene order and placement. However, there is one consistent gene arrangement within all studied Sarcoptiformes, namely cox1-cox2- tRNAAsp- atp8- atp6- cox3- tRNAAsp- only in Astigmata - nad3 (Fig. 5), indicating a potential ancestral pattern within this order. Also interesting is the comparison of used start/stop codons between the two Oribatida: For atp6 and nad5, both species use the same start and stop codons; otherwise there are only four further PCGs for which either the same start or stop codon is used in *P. leontonychus* and *S. magnus* (nad3 the start codon ATT; cox3, nad4L and nad6 the stop codon TAA).

In Acariformes, the nucleotide composition of the (+)-strand is generally biased towards A and T, with an average A + T-content of about 75%35. This is also true for *P. leontonychus* where there is a clear excess of A + T against G + C nucleotides. However, compared to the other described mite mitogenomes, our study species has - with 64.4% - the lowest A + T content 35, 46. In general, metazoan species show a clear strand asymmetry in...
nucleotide composition: the leading strand is biased in favor of A and C and consequently, the lagging strand in favor of T and G. In *P. leontonychus*, the genome has a positive AT-skew and a negative GC-skew of the leading strand, which is similar to other arthropods. This is particularly true for Acari, with the mitogenomes of both Acariformes and Parasitiformes usually having negative GC-skews. As there is no general trend in strand composition in acariform mites, it is not surprising that it differs also between *S. magnus* and *P. leontonychus*. There, both AT- and GC-skews are negative on the leading strand, indicating a reverse strand-compositional bias of the genome, i.e., meaning an excess of (i) T relative to A and (ii) G relative to C nucleotides.

**New insights into tRNA evolution and the pitfalls of their annotation.** tRNAs are characterized by their conserved secondary structure with the characteristic cloverleaf layout with a 7bp acceptor stem, a 5bp anticodon stem and a D- and a T-arm. This conserved structure is the basis of several available prediction tools including tRNAscan-SE, ARWEN and MiTFi, where the latter two were specifically developed for predicting tRNAs in mitochondrial genomes. Prediction is very reliable for tRNAs corresponding to the cloverleaf structure. However, identification of tRNAs lacking one or both arms or containing mismatches in the stems is challenging. Either such tRNAs are missed during prediction or predicted with an implausible secondary structure. In addition, multiple tRNAs with different anticodons at almost the same genomic position are predicted (either on the same or opposite strand). In the current study, we could identify 18 tRNAs using prediction tools and further 2 by manual annotation. Predictions of the different tools were in some cases contradictory, which had to be resolved by calculating the unconstrained and constrained MFEs as a proxy for the stability and selecting the sequence with the smaller MFE. For *S. magnus* - the only Oribatida mitogenome available up to now - only 6

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**Figure 2.** Predicted secondary structures of the 20 mt tRNAs of *P. leontonychus.*
tRNAs have been annotated in the initial publication. Klimg and O'Connor could identify another two tRNAs (tRNA^Ala^ and tRNA^Lys^) and re-annotate three of the previously described ones (tRNA^Phe^ > tRNA^Thr^, tRNA^Thr^ > tRNA^Val^) based on MFE values (Fig. 3). In our study, we could confirm the prediction of these tRNAs and could add four additional ones, mainly based on predictions by MITOS.

Nevertheless, our data suggest that besides three other sarcoptiform species, the two available oribatid mt genomes have a reduced set of tRNAs. Both oribatid species have lost tRNA^Ala^ and in addition, S. magnus lacks eight further tRNAs (tRNA^Ala^, tRNA^Lys^, tRNA^Glu^, tRNA^His^, tRNA^Thr^, tRNA^Ile^, tRNA^Met^, tRNA^Gly^) as well as tRNAs and could add four additional ones, mainly based on predictions by MITOS.

During acariform evolution, tRNAs appear to have gradually lost either the D- or T-arm or both, leaving tRNA^Ala^ with the typical cloverleaf structure in all currently known mitogenomes. This is especially true for the Astigmata, which retain only tRNA^Ala^ with the typical cloverleaf structure. Paraleius leontonychus, as a member of the Oribatida, has five tRNAs with both arms present. Whereas all other sarcoptiform mites analyzed so far have the lowest number of cloverleaf-like mt-tRNAs among mites, the number of tRNAs with two arms is much higher in P. leontonychus and in the range typical for trombidiform mites. Hence, the apparent increased reduction of tRNA arms in sarcoptiform mites appears to be at least in part due to a taxon sampling bias. In this context, we got another unexpected result concerning the secondary structure of the tRNA for cysteine. While tRNA^Phe^ lacks the T-arm in all known Acariformes species, it has the typical cloverleaf structure in both oribatids, P. leontonychus and S. magnus (here it is the only intact tRNA). Whether this is an ancestral feature in Oribatida, in general, remains questionable as both taxa do not represent basal species of this suborder. However, our result indicates that acariform mites might have lost the T-arm in tRNA^Phe^ multiple times independently, contradicting the hypothesis of Xue et al. that the T-arm loss in tRNA^Phe^ is likely ancestral in acariform mites. The lack of the T-arm in the tRNA^Phe^ of P. leontonychus is congruent with the pattern in other Acariformes, which supports Xue et al.'s hypothesis that truncation of these two tRNAs occurred once in the most recent common ancestor of Acariformes.

Besides the atypical secondary structure of tRNAs, there are further interesting phenomena which compli- cate a straightforward tRNA annotation. One well-known characteristic throughout metazoans is that many mt-tRNA genes overlap with other genes. This is particularly true for arthropods and velvet worms. Within the Acari, examples for such aberrant acceptor stems can be found in acariform mites, as in the genera Dermatophagoides, Leptotrombidium and Paraleius. The spider mite genus Tetranychus. To allow these tRNAs to function, a posttranscriptional RNA editing process, which restores the truncated acceptor stem in mt-tRNAs, has been previously shown to exist. Additionally, Yokobori and Pääbo showed that RNA editing further occurs when there is an overlap of tRNA acceptor stem and PCG encoded on the same strand. Both cases, mismatches in the acceptor stem and
overlap with a PCG were also found in three species of the Habronattus spider\textsuperscript{60,61} and it was postulated that a similar RNA editing mechanism could exist there too. Whether similar processes play a role in \textit{P. leontonychus}, remains to be seen. In nematodes it was previously shown that tRNAs with an extreme truncated structure are still functional because of a gene duplication of the elongation factor EF-Tu. For example, in \textit{Caenorhabditis elegans} nuclear DNA encodes two elongation factor EF-Tu homologs, EF-Tu1 and EF-Tu2, whereof EF-Tu1 binds to T-arm-lacking and EF-Tu2 to D-arm-lacking tRNAs only\textsuperscript{62,63}.

**Phylogenetic relationship of Sarcoptiformes.** Inferring “true” phylogenetic affinities and classification within the Acariformes has been a longstanding challenge. For example, the paraphyly of Trombidiformes (also supported by our phylogeny) and its consequences has been recently discussed in the course of mitogenomic studies\textsuperscript{28,64}. Consistent with several previous studies, our phylogenetic reconstruction based on the 13 mt-PCGs inferred the origin of Astigmata within Oribatida. In general, the origin of the Astigmata is a particularly widely discussed topic and several authors tried to answer this question by employing a variety of approaches\textsuperscript{28,65–67}. General historical concepts of relationships between Trombidiformes, Oribatida and Astigmata have been
Among the various different concepts put forward in the past, there are two widely established hypotheses: the first considers that both Oribatida and Astigmata are monophyletic sister groups, and the second assumes that a lineage within Oribatida is the sister group of Astigmata. In the classification by Lindquist et al., the acariform order Sarcoptiformes is divided into the two suborders Endeostigmata and Oribatida, whereof the latter one comprises five supercohorts: the most primitive Palaeosomatides, the early-derived Enarthronotides and Parhyposomatides and the middle-to-highly derived Mixonomatides and Desmonomatides. Our results are congruent with the findings of Dabert et al., who investigated the molecular phylogeny of acariform mites using sequences of the nuclear small subunit rRNA gene (18S rDNA) and COI amino acid data and found that Astigmata evolved within the Desmonomatides. Other molecular genetic studies either suggested a within-Oribatida origin for Astigmata or rejected it. Moreover, investigations based on different morphological traits as well as on the chemical composition of opisthonotal gland secretions provided an indication of an astigmatan evolution within the Oribatida. A recent study, however, inferred based on sequences of the small and large subunits of nuclear rDNA that Astigmata and “traditional” Desmonomatides are most likely reciprocally monophyletic sister groups. The logical next steps will be the integration of more sarcoptiform mitogenomes including species from each of the five supercohorts. We suppose that especially species of the basal desmonomatan Nothrina, plus basal Brachypylina (e.g. Hermannielloidea, Neoliodoidea) might be helpful to get a clearer picture of the within-Oribatida evolution of Astigmata. In addition, nuclear multilocus, and in particular genome scale data, would even further increase the resolution of ambiguous relationships and provide a robust phylogenetic framework of acariform mite relationships for comparative phylogenetic analyses in the hopefully near future.

Conclusions
The newly sequenced mitogenome of the oribatid mite P. leontonychus has important ramifications for our understanding of mitogenome evolution in sarcoptiform mites. It appears that throughout the acariform tree tRNAs have gradually lost either D- or T-arm or both. The previously reported extreme paucity of complete cloverleaf-like tRNAs in sarcoptiform as compared to trombidiform mites might be helpful to get a clearer picture of the within-Oribatida evolution of Astigmata. In addition, nuclear multilocus, and in particular genome scale data, would even further increase the resolution of ambiguous relationships and provide a robust phylogenetic framework of acariform mite relationships for comparative phylogenetic analyses in the hopefully near future.

**Figure 5.** Neighbor joining (NJ) tree based on distances calculated from a CREx gene rearrangement analysis. Genes are drawn in their original order; intergenic distances are not included and sizes of genes are not to scale. Protein-coding genes are colored in yellow, rRNAs in light grey and control regions in dark grey. All abbreviations are the same as in Fig. 1 and Table 1. Genes are transcribed from left to right excepting the underlined ones, which are located on the (−) strand. *Gene annotation of the present study was used for this analysis.
range typical for the Trombidiformes. Phylogenetic mitogenomic analyses suggest paraphyly of the Oribatida with respect to the Astigmata. However, as the mitochondrial genome is essentially just one single locus, potentially impacted by (ancient) incomplete lineage sorting, nuclear multilocus data will be necessary, together with an increased taxon sampling, to confirm these relationships within the Sarcoptiformes and provide a robust phylogenetic framework for the acariform mites.

**Methods**

**Sampling and DNA-extraction.** *Paraleius leontonychus* was collected from a bark sample of *Picea abies* infested by different bark beetle species in Paldau (Styria, Austria; 46°55′53.0″N 15°45′54.0″E), in autumn 2015. Specimens were extracted alive with a Berlese-Tullgren funnel and preserved in 100% ethanol for further investigation.

Whole genomic DNA was extracted from a single mite individual using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer’s protocol. Purified DNA was eluted in a single step in 50 µl HPLC water. After DNA extraction, the sclerotized body remnants were mounted on permanent slides as voucher.

**Library preparation and sequencing.** Total genomic DNA was quantified using the Quantifluor® dsDNA Dye on a Quantus™ Fluorometer (Promega, Mannheim, Germany). For library preparation with the NEBNext® Ultra II DNA Library Prep Kit for Illumina® (New England BioLabs, Frankfurt, Germany) with the NEBNext® Multiplex Oligos for Illumina® (Index Primers Set 1) according to manufacturer’s instructions 1 ng total DNA was randomly fragmented by ultrasonication in a microTUBE on a M220 Focused-ultrasonicator (Covaris, Woburn, MA, USA) according to Thannesberger et al.75. End repair and adapter ligation were performed according to manufacturer’s instructions and size selection and PCR amplification with 12 cycles according to Thannesberger et al.75. The library was purified and eluted in 30 µl 1 x TE pH 8.0 and the quality was examined on an Agilent BioAnalyzer High Sensitivity DNA chip (Agilent Technologies, CA, USA) and again quantified on a Quantus™ Fluorometer (Promega, Mannheim, Germany). The final library was sequenced at 8 pM with 5% PhiX with v3 600 cycles chemistry on an Illumina MiSeq desktop sequencer in paired end mode. FastQ raw data were used for sequence analysis.

**De novo assembly and annotation.** Raw sequences were quality controlled with fastQC76. Filtering and assembly was performed in CLC Genomics Workbench (version 6.5.2, CLC bio, Aarhus, Denmark). The contig representing the mitogenome was identified with a BLAST+ search of the *S. magnus* mitogenome against all contigs in the assembly. A missing sequence stretch between 16S and 12S rRNAs (13739 .. 13894) was identified by mapping the raw reads against the draft mt genome. This gap was closed with Sanger sequences of the ampiclon generated with three primer pairs (Supplementary Table S4). The assembled genome was annotated using the MITOS Webserver under the mitochondrial genetic code for invertebrates (revision 656; http://mitos.bioinf.uni-leipzig.de). The resulting annotation was curated manually. As the MITOS prediction for the 16S rRNA comprised only 534 bps, we extracted the 16S sequences from the available Sarcoptiformes mitogenomes (Supplementary Table S5) which included only those species with a complete set of 13 PCGs. First, sequences of *Paraleius leontonychus* were contiguated and annotated. Then, the single alignments were concatenated into one data set, which included only those species with a complete set of 13 PCGs. This set was used as input for the phylogenetic analyses included only those species with a complete set of 13 PCGs. First, sequences of each PCG were aligned separately using the TranslatorX server (http://translatorx.co.uk), where MAFFT85 is used to build the protein alignment. Translation was done under the invertebrate mt genetic code. Additionally, poorly aligned sites were excluded using the alignment cleaning program GBlocks (implemented within TranslatorX) under the default parameters86. Finally, the single alignments were concatenated into one data set, with a final length of 6,879 bp, using DAMBE 5.5.2487.

**Phylogenetic and gene rearrangement analyses.** To infer the phylogenetic position of *P. leontonychus* within the Acariformes, we generated a data set of 37 mite taxa (six Parasitiformes and 31 Acariformes, Supplementary Table S5) which included only those species with a complete set of 13 PCGs. First, sequences of each PCG were aligned separately using the TranslatorX server (http://translatorx.co.uk), where MAFFT85 is used to build the protein alignment. Translation was done under the invertebrate mt genetic code. Additionally, poorly aligned sites were excluded using the alignment cleaning program GBlocks (implemented within TranslatorX) under the default parameters86. Finally, the single alignments were concatenated into one data set, with a final length of 6,879 bp, using DAMBE 5.5.2487.

The datasets generated and/or analyzed in the current study are available from the corresponding author on request.

All analyses were performed using data sets of both nucleotide (ND) and amino acid (AAD) sequences of the PCGs, which were partitioned by genes and by codon positions. To select the best-fitting partitioning scheme...
and models of evolution, we used PartitionFinder v2\textsuperscript{88,89} with the settings: (i) unlink branch lengths and (ii) use the corrected Akaike information criterion (AICc) for model selection. ML and BI analyses were performed using the RAxML web-server (http://emnet.vital-it.ch/raxml-bb/index.php)\textsuperscript{86} and MrBayes v3.2.4\textsuperscript{87} under the best substitution models and partition scheme identified (Supplementary Table S6). Bayesian analyses were run with the settings nst = 6 and rates = invgamma for the ND and aamodelpr = fixed(wag) for the AAD. Posterior probabilities were obtained from a Metropolis-coupled Markov chain Monte Carlo simulation conducting two runs simultaneously, each with four chains (one cold, three heated) for 5,000,000 (ND) or 2,000,000 (AAD) generations. Trees were sampled every 1,000 generations and the first 10% were discarded as burn-in. Mixing and convergence of the parameters to stationary distributions were evaluated in Tracer v1.6\textsuperscript{92}. All estimated parameters showed ESS values above 200.

To explore the potential of mitogenomic rearrangements for answering phylogenetic questions, we applied a pairwise comparison approach of the mt gene order of the same mitie species as in the phylogenetic analyses. Only the Chilean predatory mite Phytoseiulus persimilis was excluded from this analyses because of its extremely reshuffled gene order (35 genes changed position) compared with L. polyphemus\textsuperscript{83}. The analysis was performed using CREx with default parameters. For a hierarchical grouping of the taxa, the distances were imported into PAST\textsuperscript{87} and analyzed using the NJ clustering method with Euclidean distance as the similarity index.

References

1. Feldmeier, B., Hoffmeier, K. & Pfenniger, M. The complete mitochondrial genome of Radix balthica (Pulmonata, Basommatophora), obtained by low coverage shot gun next generation sequencing. Mol. Phylogenet. Evol. 57, 1329–1333 (2010).
2. Fischer, C. et al. Complete mitochondrial DNA sequences of the threadfin chilid (Teleostei: tetraodontidae) and the blunthead chilid (Teleostei: muraenidae) and patterns of mitochondrial genome evolution in cichlid fishes. PLoS One 8, e67048 (2013).
3. Besnard, G. et al. Fast assembly of the mitochondrial genome of a plant parasitic nematode (Meloidogyne graminicola) using next generation sequencing. C. R. Biol. 337, 295–301 (2014).
4. Hahn, C., Bachmann, L. & Chevreux, B. Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads—a baiting and iterative mapping approach. Nucleic Acids Res. 41, e129–e129 (2013).
5. Jex, A. R., Hall, R. S., Littlewood, D. T. & Gasser, R. B. An integrated pipeline for next-generation sequencing and annotation of mitochondrial genomes. Nucleic Acids Res. 38, 522–533 (2009).
6. Bernt, M. et al. MITOS: Improved de novo metazoan mitochondrial genome annotation. Mol. Phylogenet. Evol. 69, 313–319 (2013).
7. Simon, C. et al. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87, 651–701 (1994).
8. Boone, J. L. & Brown, W. M. Mitochondrial genomes of Galathealinum, Helobdella, and Platynereis: sequence and gene arrangement comparisons indicate that Pogonophora is not a phylum and Annelida and Arthropoda are not sister taxa. Mol. Biol. Evol. 17, 87–106 (2000).
9. Satoh, T. P., Miya, M., Mabuchi, K. & Nishida, M. Structure and variation of the mitochondrial genome of fishes. BMC Genomics 17, 719 (2016).
10. Shao, R. Mitochondrial genome analysis of the predatory mite Phytoseiulus persimilis and the blunthead chilid (Teleostei: muraenidae) and patterns of mitochondrial genome evolution in cichlid fishes. PLoS One 8, e67048 (2013).
11. Li, H. et al. Comparative phylogeography of the endemic Japanese weasel (Mustela itatsi) and the continental Siberian weasel (Mustela sibirica) revealed by complete mitochondrial genome sequences. Biol. J. Linn. Soc. 120, 333–348 (2016).
12. Simon, S. & Hadrys, H. A comparative analysis of complete mitochondrial genomes among Hexapoda. Mol. Phylogenet. Evol. 69, 393–403 (2013).
13. Kobilmiller, S. et al. Whole mitochondrial genomes illuminate ancient intercontinental dispersals of grey wolves (Canis lupus). J. Biogeogr. 43, 1728–1738 (2016).
14. Weigert, A. et al. Evolution of mitochondrial gene order in Annelida. Mol. Phylogenet. Evol. 94, 196–206 (2016).
15. Basso, A. et al. The highly rearranged mitochondrial genomes of the crabs Maja crispata and Maja squinado (Majidae) and gene order evolution in Brachyura. Sci. Rep. 7, 4096 (2017).
16. Harrisson, K. et al. Pleistocene divergence across a mountain range and the influence of selection on mitogenome evolution in threatened Australian freshwater cod species. Heredity 116, 506–515 (2015).
17. Li, H. et al. Episodic positive selection at mitochondrial genome in an introduced biological control agent. Mitochondrion 28, 67–72 (2016).
18. Wende, S. et al. Biological evidence for the world’s smallest tRNAs. Biochimie 100, 151–158 (2014).
19. Fleur, J. L. et al. Idiosyncrasies in decoding mitochondrial genomes. Biochimie 100, 95–106 (2014).
20. Schneider, A. Mitochondrial RNA import and its consequences for mitochondrial translation. Annu. Rev. Biochem. 80, 1033–1053 (2011).
21. Salinas-Giegé, T., Giegé, R. & Giegé, P. tRNA biology in mitochondria. Int. J. Mol. Sci. 16, 4518–4559 (2015).
22. Wolstenholme, D. R. Animal mitochondrial DNA: structure and evolution. Int. Rev. Cytol. 141, 173–216 (1992).
23. Höhling, F., Pütz, J., Florenz, C. & Stadler, P. F. Armless mitochondrial tRNAs in enoplea (nematoda). RNA Biol. 9, 1161–1166 (2012).
24. Masta, S. E. & Boore, J. L. Parallel evolution of truncated transfer RNA genes in arachnid mitochondrial genomes. Mol. Biol. Evol. 25, 949–959 (2008).
25. Shao, R. et al. The mitochondrial genomes of soft ticks have an arrangement of genes that has remained unchanged for over 400 million years. Insect Mol. Biol. 13, 219–224 (2004).
26. Dermauw, W., Vanholme, B., Leroy, L. & Van Leeuwen, T. Mitochondrial genome analysis of the predatory mite Phytoseiulus persimilis and a revisit of the Metaseiulus occidentalis mitochondrial genome. Genome 53, 285–301 (2010).
27. Mans, B. J., de Klerk, D., Pienaar, R., de Castro, M. H. & Latif, A. A. The mitochondrial genomes of Nuttallitella namaqua (Ixodoidea: Nuttallitiidae) and Argas australiensis (Argasidae: Argasidae): estimation of divergence dates for the major tick lineages and reconstruction of ancestral blood-feeding characters. PLoS One 7, e49461 (2012).
28. Klimov, P. B. & O’Connor, B. M. Improved tRNA prediction in the American house dust mite reveals widespread occurrence of extremely short minimal tRNAs in acariform mites. BMC Genomics 10, 598 (2009).
29. Xue, X. F., Guo, J. F., Dong, Y., Hong, X. Y. & Shao, R. Mitochondrial genome evolution and tRNA truncation in Acariformes mites: new evidence from ecriphodyth mites. Sci. Rep. 6, 18920 (2016).
30. Domes, K., Maraun, M., Scheu, S. & Cameron, S. L. The complete mitochondrial genome of the sexual orbibatid mite Steganoacarus magnus: genome rearrangements and loss of tRNAs. BMC Genomics 9, 532 (2008).
31. Minelli, A., Boxshall, G. & Fucso, G. In Arthropod biology and evolution: molecules, development, morphology (Springer Science & Business Media, 2013).
32. Choi, E. H., Park, S. J., Jang, K. H. & Hwang, W. Complete mitochondrial genome of a Chinese scorpion Mesobuthus martensi (Chelicerata, Scorpiones, Buthidae) Full Length Research Paper. DNA Seq. 18, 461–473 (2007).
76. Andrews, S. FastQC: a quality control tool for high throughput sequence data. Available at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc (2010).
77. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. Basic local alignment search tool. *J. Mol. Biol.* **215**, 403–410 (1990).
78. Edgar, R. C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**, 1792–1797 (2004).
79. Zuker, M. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* **31**, 3406–3415 (2003).
80. Lorenz, R. et al. ViennaRNA Package 2.0. *Algorithms Mol. Biol.* **6**, 26–26 (2011).
81. Bernt, M. et al. CREx: inferring genomic rearrangements based on common intervals. *Bioinformatics* **23**, 2957–2958 (2007).
82. Grant, J. R. & Stothard, P. The CGView Server: a comparative genomics tool for circular genomes. *Nucleic Acids Res.* **36**, W181–W184 (2008).
83. Perna, N. T. & Kocher, T. D. Unequal base frequencies and the estimation of substitution rates. *Mol Biol Evol.* **12**, 359–361 (1995).
84. Abascal, F., Zardoya, R. & Telford, M. J. TranslatorX: multiple alignment of nucleotide sequences guided by amino acid translations. *Nucleic Acids Res.* **38**, W7–W13 (2010).
85. Katoh, K., Kuma, K., Toh, H. & Miyata, T. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res.* **33**, 511–518 (2004).
86. Talavera, G. & Castresana, J. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol.* **56**, 564–577 (2007).
87. Xia, X. DAMBE5: A comprehensive software package for data analysis in molecular biology and evolution. *Mol. Biol. Evol.* **30**, 1720–1728 (2013).
88. Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T. & Calcott, B. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Mol. Biol. Evol.* **34**, 772–773 (2016).
89. Guindon, S. et al. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst. Biol.* **59**, 307–321 (2010).
90. Stamatakis, A., Hoover, P. & Rougemont, J. A rapid bootstrap algorithm for the RAxML web servers. *Syst. Biol.* **57**, 758–771 (2008).
91. Ronquist, F. et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61**, 539–542 (2012).
92. Rambaut, A., Suchard, M., Xie, D. & Drummond, A. Tracer v1.6. Available from, http://beast.bio.ed.ac.uk/Tracer (2014).
93. Hammer, Ø., Harper, D. A. T. & Ryan, P. D. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4**, 1–9 (2001).

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**Author Contributions**

S.S. and G.G.T. conceived the study. G.G.T. performed the genome assembly and annotation and tRNA analyses and contributed in writing. S.S. performed Sanger sequencing, did the phylogenetic and rearrangement analyses, conceived and wrote the manuscript. S.K. contributed to discussions during the data analyses and helped in writing. I.K. conducted the Illumina library preparation and sequencing. S.K., I.K. and G.G.T. revised the manuscript.

**Additional Information**

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