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Extensive and Evolutionarily Persistent Mitochondrial tRNA Editing in Velvet Worms (Phylum Onychophora)

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Abstract

Mitochondrial genomes of onychophorans (velvet worms) present an interesting problem: Some previous studies reported them lacking several transfer RNA (tRNA) genes, whereas others found that all their tRNA genes were present but severely reduced. To resolve this discrepancy, we determined complete mitochondrial DNA (mtDNA) sequences of the onychophorans *Oroperipatus* sp. and *Peripatoides sympatrica* as well as cDNA sequences from 14 and 10 of their tRNAs, respectively. We show that tRNA genes in these genomes are indeed highly reduced and encode truncated molecules, which are restored to more conventional structures by extensive tRNA editing. During this editing process, up to 34 nucleotides are added to the tRNA sequences encoded in *Oroperipatus* sp. mtDNA, rebuilding the aminoacyl acceptor stem, the TΨC arm, and in some extreme cases, the variable arm and even a part of the anticodon stem. The editing is less extreme in *P. sympatrica* in which at least a part of the TΨC arm is always encoded in mtDNA. When the entire TΨC arm is added de novo in *Oroperipatus* sp., the sequence of this arm is either identical or similar among different tRNA species, yet the sequences show substantial variation for each tRNA. These observations suggest that the arm is rebuilt, at least in part, by a template-independent mechanism and argue against the alternative possibility that tRNA genes or their parts are imported from the nucleus. By contrast, the 3′ end of the aminoacyl acceptor stem is likely restored by a template-dependent mechanism. The extreme tRNA editing reported here has been preserved for >140 My as it was found in both extant families of onychophorans. Furthermore, a similar type of tRNA editing may be present in several other groups of arthropods, which show a high degree of tRNA gene reduction in their mtDNA.

Key words: onychophora, mtDNA, tRNA editing.

Introduction

Mitochondrial DNA (mtDNA) of bilaterian animals is typically a circular molecule ~16 kbp in size with a well-conserved gene content of 37 genes: 2 for the small and large subunit ribosomal RNAs (rRNAs; *rns* and *rnl*), 22 for transfer RNAs (tRNAs), and 13 for protein subunits of complexes I, III, IV, and V involved in oxidative phosphorylation (NADH dehydrogenase subunits 1–6, *nad1-6*; cytochrome b, *cob*; cytochrome c oxidase subunits I-III, *cox1-3*; and ATP synthase subunits 6 and 8, *atp6* and *atp8*) (Lavrov 2007). Recently, complete mtDNA sequences of the onychophorans (velvet worms) *Epiperipatus biolleyi* (Podsiadlowski et al. 2008) and *Opisthopatus cinctipes* (Braband, Cameron, et al. 2010) were published and inferred to lack several tRNA genes. For *E. biolleyi*, the authors asserted that all nine missing tRNA genes translated four-fold degenerate codons and suggested that these genes are replaced by their nuclear counterparts via tRNA import from the cytosol into the mitochondria. Earlier, one of us had determined the complete mitochondrial genome from another specimen of *E. biolleyi* and annotated the genes differently (Lavrov 2001; Rota-Stabelli et al. 2010). In particular, 21 tRNA genes were found in the genome in a different arrangement to that inferred by Podsiadlowski et al. (2008) (fig. 1). These discrepancies between the results of the two studies led us to question mt-tRNA annotations in onychophoran mtDNA and encouraged us to look more closely at onychophoran mitochondrial tRNA biology.

The majority of the tRNA genes annotated in our previous study of *E. biolleyi* mtDNA were inferred to encode unusual tRNA structures often lacking a portion of the TΨC arms and the 3′ side of the aminoacyl acceptor stems (here and thereafter, we name tRNA arms by their standard names, without implying that the DHU arm actually contains a dihydrouridine and that the TΨC arm contains the TΨC sequence). Because a well-paired acceptor stem is essential for tRNA recognition by its cognate aminoacyl–tRNA synthetase (Hou and Schimmel 1988; McClain 1995), we suspected that tRNA structures are restored by RNA editing (Lavrov 2001). RNA editing has been defined as a programmed alteration of RNA primary structures that generates a sequence that could have been directly encoded at the DNA level (Price and Gray 1999). First discovered in mitochondrial mRNA of trypanosomatid protozoa (Benne et al. 1986), RNA editing has been subsequently found in tRNA, rRNA, mRNA, and microRNA molecules from a variety of eukaryotes (Grosjean 2005) as well as tRNA molecules in Archaea (Randau et al. 2009). tRNAs undergo the largest number of mechanistically different editing events in comparison with other RNAs within a cell (Alfonzo 2008). These include C-to-U editing in marsupials (Janke and Pääbo 1993; Borner et al. 1996), trypanosomes (Alfonzo et al.

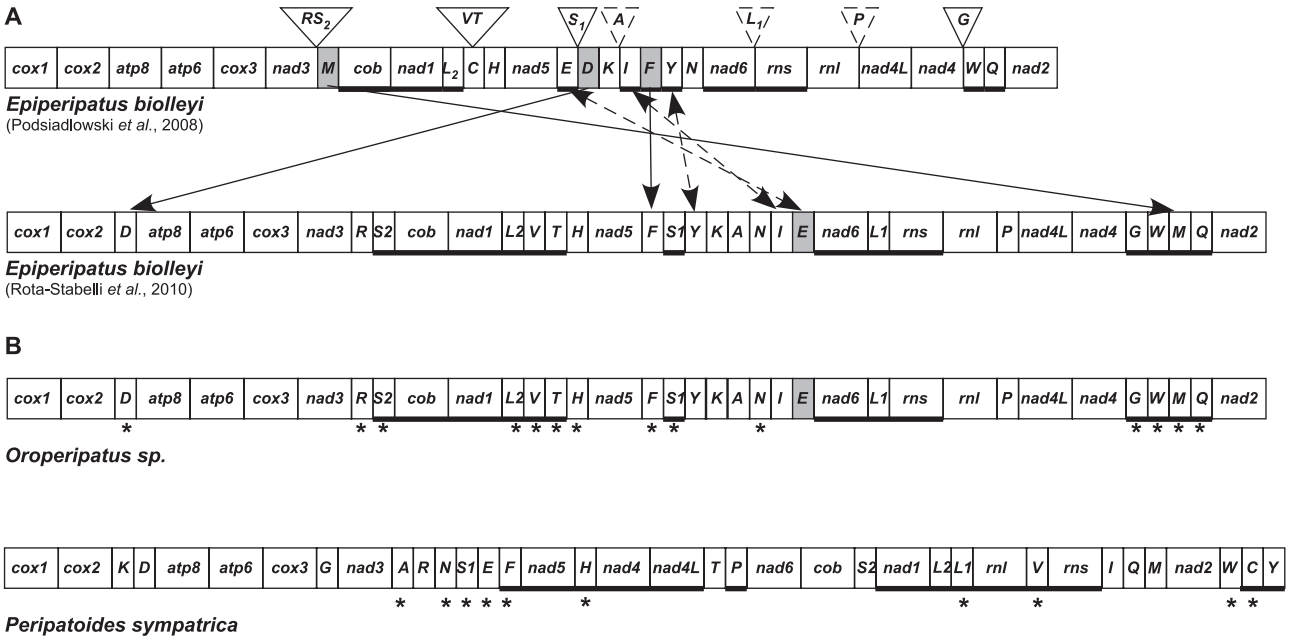

FIG. 1. Mitochondrial gene orders in Onychophora. (*A*) Comparison of mitochondrial gene arrangements in *Epiperipatus biolleyi* inferred by Podsiadlowski et al. (2008) and in our earlier study (Rota-Stabelli et al. 2010). (*B*) Inferred mitochondrial gene order in *Oroperipatus* sp. and *Peripatoides sympatrica* investigated for this study. Underlined genes are transcribed from right to left; other genes are transcribed in the opposite direction. Arrows indicate incongruence in tRNA gene annotations. Triangles indicate genes that were reported missing by Podsiadlowski et al. (2008) but found by Rota-Stabelli et al. (2010). Solid triangles and solid arrow lines indicate inferences supported by the present study. Asterisks under tRNA genes denote tRNAs for which cDNA sequences were amplified and sequenced.

1999), plants (Maréchal-Drouard et al. 1993; Fey et al. 2002), and the archaeon *Methanopyrus kandleri* (Randau et al. 2009); insertion editing in slime molds (Antes et al. 1998); 5′-end editing in amoebozoans (Lonergan and Gray 1993a, 1993b) and lower fungi (Laforest et al. 1997); 3′-end editing in bilaterian animals (Yokobori and Paabo 1995; Tomita et al. 1996; Yokobori and Pääbo 1997; Reichert et al. 1998; Lavrov et al. 2000) and a jacobid flagellate (Leigh and Lang 2004); or a combination of these (Gott et al. 2010). Interestingly, most of the cases of tRNA editing, at least in animals, appear to be recent acquisitions that arose independently in various lineages (Brennicke et al. 1999). The term "tRNA editing" usually (and subjectively, in our view) does not include the addition of the trinucleotide CCA during maturation of tRNAs (Chen et al. 1992). Furthermore, we do not include the adenosine to inosine (Holley, Apgar, et al. 1965; Holley, Everett, et al. 1965) and cytidine to lysidine (Muramatsu et al. 1988) conversions under the definition used in this article. If these activities were included, tRNA editing would need to be redefined as a ubiquitous process present in all domains of life. Nevertheless, in all known cases, relatively few nucleotides are edited in tRNA sequences.

Here, we describe the complete mitochondrial genomes of the onychophorans *Oroperipatus* sp. and *Peripatoides sympatrica* (Trewick 1998) and report the presence of a novel and more extensive type of RNA editing that restores the 3′ half of the aminoacyl acceptor stem, the TΨC arm, and in a few cases, even variable arms and anticodon stems of most mt-tRNAs. This extreme tRNA editing has likely been preserved for >140 My since the divergence between two extant families of onychophorans sampled for this study.

Materials and Methods

Specimen Collection and Preservation

The specimen of *P. sympatrica* was collected near Huntly town in the Waikato region, North Island, New Zealand. The specimen of *Oroperipatus* sp. was collected at the Belize Foundation for Research and Environmental Education in the Toledo district of Belize, bordering the Bladen Nature Reserve. The *P. sympatrica* was stored at −80°c and the *Oroperipatus* sp. in 80% ethanol at −20°C.

Nucleic Acid Extraction and tRNA Circularization

Total RNA was prepared from each specimen using TRIZOL Reagent (Invitrogen). The DNA was prepared from the same individuals using the 2× hexadecyltrimethylammonium bromide buffer and phenol–chloroform extraction (Saghai-Maroof et al. 1984). To circularize tRNA molecules, total RNA was ligated using T4 RNA ligase (Fermentas) as previously described (Price and Gray 1999).

Sequencing of mtDNA

Regions of *rns* and *cox3* (*P. sympatrica*) and *cox1* and *nad5* (*Oroperipatus* sp.) were amplified using animal-specific primers for these genes (Burger et al. 2007) and used to design species-specific primers. Complete mtDNA from both species was amplified in two overlapping fragments using the TaKaRa LA-PCR kit and sheared into overlapping fragments as described in (Burger et al. 2007). For *P. sympatrica*, these fragments were barcoded and used for the GS FLX Titanium library preparation (454 Life Sciences) along with other samples. Pyrosequencing

was carried out on a Genome Sequencer FLX Instrument (454 Life Sciences) at the University of Indiana Center for Genomics and Bioinformatics. For *Oroperipatus* sp., sheared fragments were processed using Invitrogen TOPO Shotgun Subcloning Kit following the manufacturer's protocol. Clones were sequenced on an Applied Biosystems 3730xl DNA Analyzer at Iowa State University DNA Facility. All sequences were assembled using the STADEN package v. 1.6.0. Gaps and uncertainties in the assembly were filled/resolved by primer walking using conventional Sanger sequencing (Staden 1996). The assembled genomes were annotated as previously described (Lavrov et al. 2004) and deposited to GenBank under accession numbers JF800075 and JF800076.

cDNA Synthesis, PCR Amplification, and Cloning of tRNA

Primers used for reverse transcription and subsequent polymerase chain reaction (PCR) amplification were designed based on complete mitochondrial sequences (supplementary table S1, Supplementary Material online). The nine 5′ nucleotides in some primers were added to create a restriction site for HindIII or BamHI plus three terminal nucleotides. Reverse transcription was performed using SuperScript III Reverse Transcriptase (Invitrogen) under conditions described by the manufacturer. PCR amplification of cDNA products was performed with recombinant *Taq* DNA polymerase (Invitrogen). PCR products were cloned using TOPO TA Cloning Kit for Sequencing (Invitrogen). At least ten clones for each tRNA have been sequenced at Iowa State University DNA Facility.

Results

Onychophoran mt Genomes Contain a Complete Set of tRNA Genes

The newly determined mitochondrial genomes of *Oroperipatus* sp. and *P. sympatrica* are ~14.8 and >14.2 kb in size and contain a conserved set of genes for 13 proteins and 2 rRNA typical for animal mtDNA (fig. 1). In addition, we identified all 22 expected tRNA genes in *P. sympatrica* mtDNA and 21 putative tRNA genes in *Oroperipatus* sp. (all except *trnC(gca)*). The mitochondrial genome of *Oroperipatus* sp. has a gene arrangement identical to that of *E. biolleyi*, reported in our previous study (Rota-Stabelli et al. 2010), with the tRNA sequences in the two genomes being ~80% identical (range 61–100%). The mitochondrial genome of *P. sympatrica* also has an identical gene order to that of *Peripatoides* sp. (Rota-Stabelli et al. 2010) and their tRNA sequences are ~85% identical (range 66–100%). When compared between *Oroperipatus* sp. and *P. sympatrica*, tRNA sequences were only ~56% identical. Previous reports (Podsiadlowski et al. 2008; Braband, Cameron, et al. 2010; Braband, Podsiadlowski, et al. 2010; Rota-Stabelli et al. 2010) described several interesting features of onychophoran mitochondrial genomes. Yet, their most outstanding feature—the severe reduction of nearly all tRNA genes—remained unrecognized and/or unreported.

Mitochondrial Genes Encode Highly Unusual tRNA Molecules

The majority of tRNA genes identified in both mitochondrial genomes appeared to be truncated and encode incomplete tRNA structures (figs. 2 and 3; supplementary figs. S1 and S2, Supplementary Material online). The extent of reduction was more extreme in *Oroperipatus* sp. genes, all of which were inferred to encode tRNA molecules lacking the entire 3′ side of the acceptor stem, at least a part of the TΨC arm, and in some cases, the variable loop. Furthermore, in two cases (*trnT(ugu)* and *trnN(guu)*), the last two nucleotides of the predicted anticodon stem (positions 42 and 43 in a standard tRNA structure) were missing, with position 41 abutting the 5′ ends of the downstream tRNA genes (*trnV(uac)* and *trnI(gau)*, respectively). By contrast, tRNA genes in *P. sympatrica* mtDNA were less reduced, with the extent of reduction ranging from a few nucleotides to the entire 3′ side of the acceptor stem plus a part of the TΨC arm. None of the tRNAs encoded in *P. sympatrica* lacked the entire TΨC arm and one tRNA gene (*trnF(gaa)*) appeared to encode an intact tRNA molecule. When comparing the structural sequences of cognate tRNA genes between the two species, we found that the anticodon arms were generally well conserved, whereas DHU arms were poorly conserved. Moreover, four encoded tRNAs were inferred to form a D-replacement loop instead of a standard DHU arm. This feature was observed in the *trnS(ucu)* gene in both genomes (also characteristic to all bilaterian animals; Garey and Wolstenholme 1989; Chimnaronk et al. 2005) as well as *Oroperipatus* sp. *trnF(gaa)* and *P. sympatrica trnH(gug)*.

Mature tRNAs Have Conventional Structures

Although the finding—in both onychophoran mt genomes—of a complete or nearly complete set of expected tRNA gene–like sequences provided a strong indication that these genes are functional, a degenerate nature of the encoded tRNAs would make it impossible for them to participate in protein synthesis without extensive editing. To check for the presence of such editing, we investigated the structure of onychophoran tRNAs using the reverse transcription polymerase chain reaction approach. In all cases when we were able to amplify a mature tRNA (with the trinucleotide CCA added to the sequence; Chen et al. 1992), the 3′ end of the molecule was edited. On average, 26 nucleotides were added to the 3′ end among 14 successfully amplified tRNAs in *Oroperipatus* sp. (fig. 2), and 14 nucleotides were added to the 3′ end among 10 studied tRNAs in *P. sympatrica*. The addition of these nucleotides restored tRNA's potential to form a more standard structure with a well-formed TΨC arm, paired amino acid acceptor stem, a discriminatory nucleotide (always an A), and the 3′ terminal CCA trinucleotide. For a few tRNAs in each onychophoran species, we found an additional mismatch between mtDNA and cDNA sequences in parts other than the 3′ end of the molecule (figs. 2 and 3). Given their rarity and multiple possible sources (ribonucleotide modification,

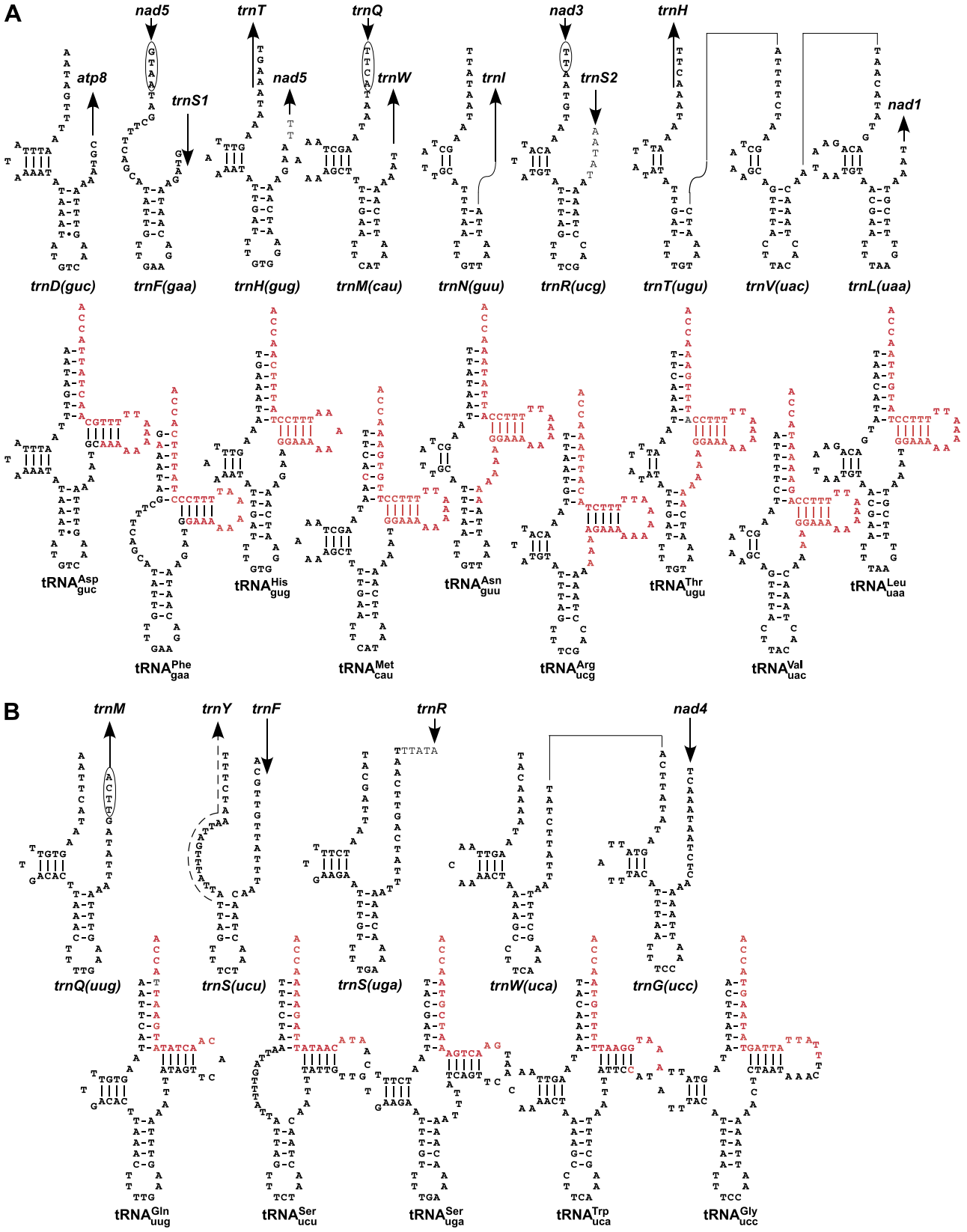

Fig. 2. Secondary structures of *Oroperipatus* sp. mt-tRNAs as inferred from the genomic sequence (above) and as modified at the RNA level (below). (*A*) tRNAs in which most of the TΨC arm is created de novo. (*B*) tRNAs in which at least a part of the TΨC loop is encoded in mtDNA. The downstream gene is shown for each tRNA gene; its transcriptional direction is indicated by an arrow. The upstream gene is shown only when it overlaps with the tRNA gene. If two overlapping genes have the same transcriptional direction, the shared nucleotides are circled. Edited nucleotides in cDNA sequences are marked by color. Intergenic nucleotides are italicized.

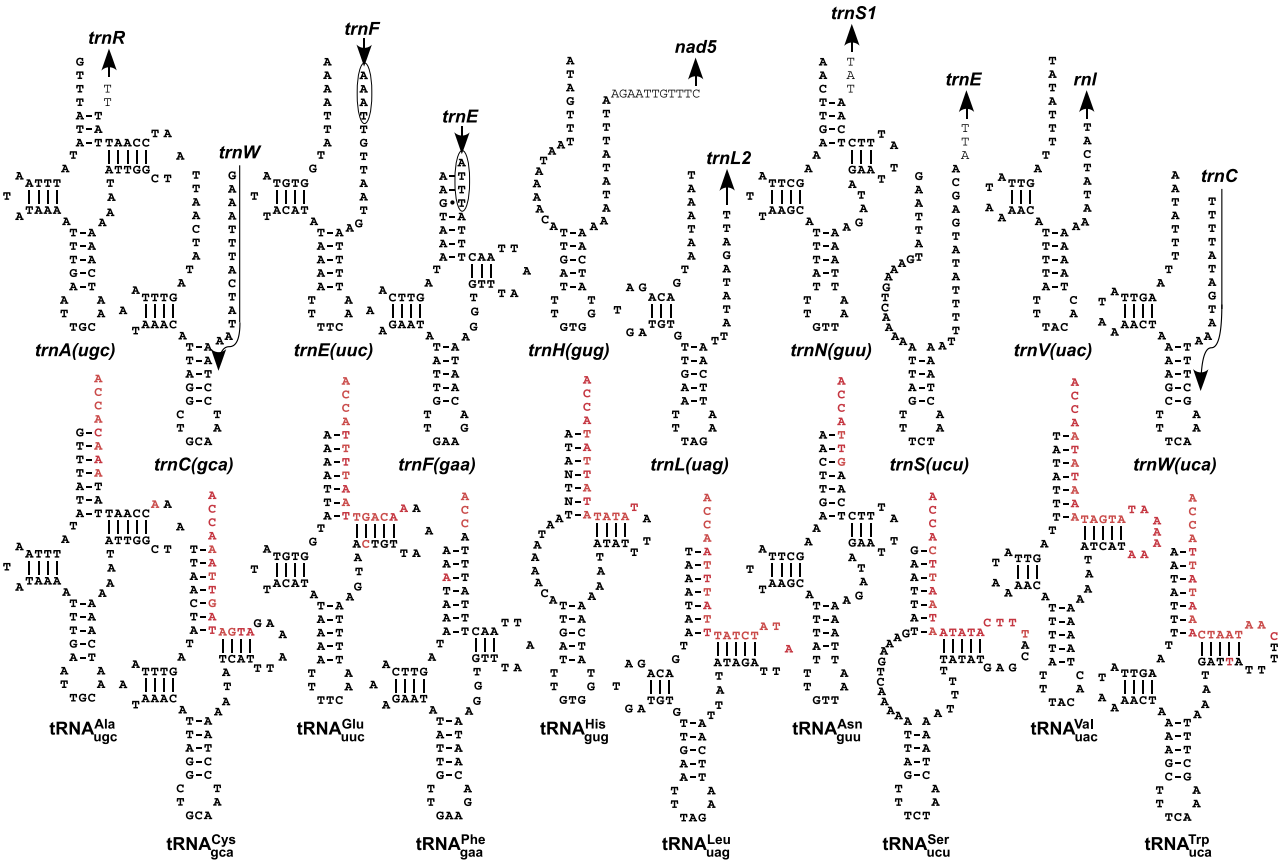

Fig. 3. Secondary structures of *Peripatoides sympatrica* mt-tRNAs as inferred from the genomic sequence (above) and as modified at the RNA level (below). The downstream gene is shown for each tRNA gene; its transcriptional direction is indicated by an arrow. The upstream gene is shown only when it overlaps with the tRNA gene. If two overlapping genes have the same transcriptional direction, the shared nucleotides are circled. Edited nucleotides in cDNA sequences are marked by color. Intergenic nucleotides are italicized.

cloning/PCR errors, natural polymorphism, exonuclease digestion, or additional editing), these mismatches were not investigated further in this study. Finally, we were unable to amplify mature tRNA sequences for the remaining seven inferred tRNA genes of *Oroperipatus* sp. (supplementary fig. 1, Supplementary Material online), potentially due to modifications of ribonucleotides in these tRNA that can interfere with proper primer annealing in cDNA synthesis. In general, the design of appropriate primers based on *Oroperipatus* sp. gene sequences was complicated by their reduced size and the AT-rich composition. Our annotation of these genes should remain provisional for now.

Conservation and Variation in the TΨC Arm

Among the inferred tRNA genes in *Oroperipatus* sp. at least seven appeared to encode molecules lacking the entire TΨC arm and two other encoded molecules lacking all but one or two nucleotides in this arm (fig. 2A). Because no template exists within a tRNA molecule to rebuild the TΨC arm, the editing of these tRNAs is particularly interesting. Among the nine tRNAs, most cDNA sequences corresponding to mature *Oroperipatus* sp. $tRNA^{Met}_{CAU}$, $tRNA^{Asn}_{GUU}$, $tRNA^{Thr}_{UGU}$, and $tRNA^{Val}_{UAC}$ had identical TΨC arm sequences, viz., 5′-GGA_8T_5CC-3′. The remaining five tRNAs contained highly similar TΨC arm sequences (typically AGA_8T_5CT in $tRNA^{Arg}_{UCG}$, GCA_8T_5GC in $tRNA^{Asp}_{GUC}$, GGA_8T_3CC in $tRNA^{His}_{GUG}$, and GGA_8T_4CC in $tRNA^{Phe}_{GAA}$, and $tRNA^{Leu}_{UAA}$, but see below) (fig. 2A). Interestingly, no such uniformity in TΨC arm sequences was found either among the remaining *Oroperipatus* sp. tRNAs (fig. 2B) or among the *P. sympatrica* tRNAs (fig. 3) in all of which at least a part of the TΨC loop was DNA encoded. Furthermore, substantial variation was observed among individual cDNA clones from the same tRNA in both species of onychophorans, mostly in the number of As and Ts within the TΨC loop. To investigate the extent of this variation, we determined sequences from additional clones of *Oroperipatus* sp. $tRNA^{Leu}_{UAA}$ (29 in total) and *P. sympatrica* $tRNA^{Val}_{UAC}$ (25 in total) (fig. 4). Among the $tRNA^{Leu}_{UAA}$ clones, the TΨC arm sequence GGA_8T_4CC was the most common (8/29 clones). For the remaining clones, the number of As in the TΨC arm varied from seven to ten, the number of Ts—from four to nine, and a few clones had a single C inserted among As or Ts. Furthermore, two clones had an aberrant GGA_9T_5CTT sequence of the TΨC arm and were missing the CCA triplet. Finally, a few clones had a two-nucleotide deletion at the 5′ end of tRNA. This deletion is adjacent to the 3′ end of the reverse primer used for reverse transcription and PCR, and we attribute it to a PCR error rather than natural variation. Among the *P. sympatrica* $tRNA^{Val}_{UAC}$ clones, the

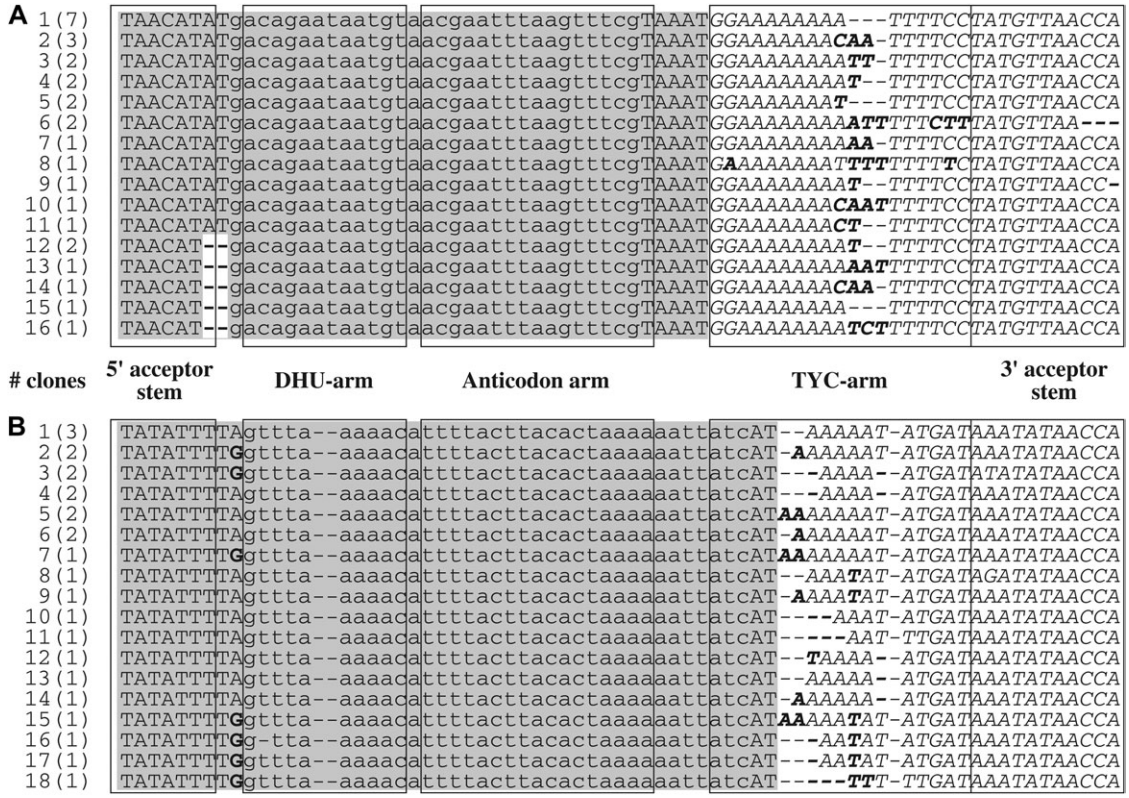

FIG. 4. cDNA sequence variation in edited tRNAs. (*A*) *Oroperipatus* sp. mt-tRNA$^{Leu}_{UAA}$. (*B*) *Peripatoides sympatrica* mt-tRNA$^{Val}_{UAC}$. Sequence encoded in the genome is shaded, whereas that added during editing is italicized. Region used to design primers is shown in lower case. Variable sites among the cDNA sequences are in bold.

most common sequences for the TΨC arm were $TAGTA_{5}$-TACTA and $TAGTATA_{6}TACTA$ (four clones each), but the number of As varied between 0 and 7 for the remaining clones. Furthermore, a few TΨC arm sequenced had a single T inserted within the run of As.

Discussion

The Most Extensive tRNA Editing Reported to Date

After the initial discovery in the amoeboid protozoon *Acanthamoeba castellanii* (Lonergan and Gray 1993a), tRNA editing has been reported in organelles of a variety of organisms (Janke and Pääbo 1993; Lonergan and Gray 1993b; Maréchal-Drouard et al. 1993; Borner et al. 1996; Tomita et al. 1996; Laforest et al. 1997; Yokobori and Pääbo 1997; Antes et al. 1998; Reichert et al. 1998; Alfonzo et al. 1999; Lavrov et al. 2000; Fey et al. 2002; Leigh and Lang 2004; Alfonzo 2008; Randau et al. 2009; Gott et al. 2010), where it has likely evolved independently multiple times and involved various mechanisms. However, in the majority of reported cases, tRNA editing was limited to between one and three nucleotides, usually at either the 3′ or the 5′ end of the acceptor stem. Previously, the most extensive tRNA editing (up to five nucleotides + the CCA trinucleotide) was found in mitochondrial tRNAs of the centipede *Lithobius forficatus* (Lavrov et al. 2000). tRNA editing in onychophoran mitochondria, reported in this study, is much more extensive and involves the addition of up to 34—or more than a half of the total—nucleotides present in mature tRNAs (including the CCA trinucleotide). The pattern of tRNA editing in both onychophorans studied here is similar, but the extent of editing is greater in *Oroperipatus* sp., where the entire TΨC arm is added at the RNA level in at least seven tRNAs. Furthermore, *Oroperipatus* sp. appears to have evolved an additional novel mechanism that rebuilds this arm de novo with a similar sequence of nucleotides.

Evolutionary Conservation of tRNA Editing

In addition to its unprecedented extent, onychophoran tRNA editing is also unusual among animals in its phylogenetic persistence. In general, tRNA editing is regarded as a mechanism that allows animal mitochondria "to stay fit without sex" (Börner et al. 1997) in the face of accumulation of deleterious mutations driven by Muller's Ratchet (Muller 1964). However, the evolution of an editing mechanism, while providing a short-term solution to the fitness decline, relaxes selection on tRNA genes that leads to their further degeneration and makes tRNA editing a requirement for the survival of organisms (Lavrov et al. 2000). It might be hypothesized that the energy and time investment required for RNA editing makes an organism less fit in the long run and eventually leads to its demise. Supporting this type of reasoning, tRNA editing has been primarily observed in individual species rather than in clades of animals (by contrast, mRNA editing is widespread and likely

preserved among land plants; Malek et al. 1996). The apparent conservation of tRNA editing reported in this study provides an interesting exception to this observation. Onychophora is an ancient group of animals, possibly related to marine Lobopoda, known from the Early Cambrian (Bergström and Hou 2001; Monge-Nájera and Xianguang 2002). The two onychophorans used for this study belong to two recognized extant families, Peripatidae and Peripatopsidae, that probably diverged in the mid-Mesozoic, 175–140 Mya (Monge-Najera 1995). We suggest that tRNA editing was already present in the common ancestor of these two lineages and was retained thereafter. Such a long persistence of tRNA editing may be due either to some unknown benefits or, more likely, to the relaxed selection pressure on this niche-specific group of animals.

A Combination of Editing Mechanisms Is Required for tRNA Editing Observed in Onychophora

Previous cases of tRNA 3′- and 5′-editing can be explained by either a template-independent mechanism, where specific nucleotides (usually As or, more rarely, Cs) are added to the 3′ end of the molecule or by a template-dependent mechanism, where one part of the tRNA structure is used, either directly or indirectly, as a template for another (Schürer et al. 2001). The editing observed in onychophoran tRNAs appears to represent a combination of these two types. The editing of the aminoacyl acceptor stem is likely template dependent as it adds specific nucleotides to match the 5′ sequence of the stem. Yet, in the cases when the complete TΨC arm is created de novo, at least its 5′ half appears be generated by a template-independent mechanism. A specific pattern of the de novo generated TΨC arm sequences (GGA_xT_yCC) suggests that a combination of already known editing mechanisms may be involved, including polyadenylation (Yokobori and Pääbo 1997), the A→G editing for two nucleotides at the 5′ end of the arm (Price and Gray 1999), followed by template-dependent editing (e.g., Lavrov et al. 2000, #27547).

In principle, two alternative explanations can be invoked to explain the generation of the TΨC arm: editing using an external template, such as a guide RNA (gRNA) and ligation of several tRNA parts, but we argue against both of them. Although gRNAs do participate in mRNA editing in unicellular eukaryotes, particularly in trypanosomes (Benne et al. 1986), editing using gRNAs is usually limited to insertions and deletions of U residues and requires a template downstream of the editing site for the gRNA 5 anchor sequence to anneal to. Because most tRNA genes are followed directly by other genes (fig. 2), there should be neither the anchor sequence nor the sequence to be edited in their transcripts. Furthermore, the observation that the sequence of the rebuilt TΨC arm is either identical or similar among different tRNA species, yet variable for each tRNA argues against the possibility that tRNA molecules are combined from separate individually transcribed parts as found in the archaeal parasite *Nanoarchaeum equitans* (Randau et al. 2005). This is because the variation in the TΨC arm sequence among tRNAs, in which a part of the TΨC loop is mtDNA encoded (fig. 2*B*), suggests that there is no specific selection pressure to keep this sequence conserved among tRNAs in which the complete TΨC arm is rebuilt (fig. 2*A*) if they are nuclear encoded. Conversely, observed variation within the same tRNA (fig. 3) would require complicated explanations (such as multiple copies of genes) if 3′ ends are DNA encoded.

As a final point, we note that although the tRNA editing described in onychophoran mtDNA is undoubtedly extreme and bizarre, it might not be unique among animals. Recently, the presence of severely truncated tRNA genes has been reported in the mitochondrial genomes of arachnids (Masta and Boore 2008) and of gall midges (Beckenbach and Joy 2009). It would be very interesting to investigate whether tRNA genes are similarly edited in these animals and the molecular machinery involved in this process.

Supplementary Material

Supplementary table S1 and figures S1–S2 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

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