REVIEW ARTICLE

Mitochondrial genomes of parasitic arthropods: implications for studies of population genetics and evolution

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(*Received 5 July 2006; revised 15 August 2006; accepted 15 August 2006; first published online 11 October 2006*)

SUMMARY

Over 39 000 species of arthropods parasitize humans, domestic animals and wildlife. Despite their medical, veterinary and economic importance, most aspects of the population genetics and evolution of the vast majority of parasitic arthropods are poorly understood. Mitochondrial genomes are a rich source of markers for studies of population genetics and evolution. These markers include (1) nucleotide sequences of each of the 37 mitochondrial genes and non-coding regions; (2) concatenated nucleotide sequences of 2 or more genes; and (3) genomic features, such as gene duplications, gene rearrangements, and changes in gene content and secondary structures of RNAs. To date, the mitochondrial genomes of over 700 species of multi-cellular animals have been sequenced entirely, however, only 24 of these species are parasitic arthropods. Of the mitochondrial genome markers, only the nucleotide sequences of 4 mitochondrial genes, *cox1*, *cob*, *rrnS* and *rrnL*, have been well explored in population genetic and evolutionary studies of parasitic arthropods whereas the sequences of the other 33 genes, and various genomic features have not. We review current knowledge of the mitochondrial genomes of parasitic arthropods, summarize applications of mitochondrial genes and genomic features in population genetic and evolutionary studies, and highlight prospects for future research.

Key words: mitochondrial genome, parasitic arthropod, population genetics, systematics, phylogenetics, gene order, gene rearrangement.

INTRODUCTION

Mitochondria are the organelles in eukaryotic cells that produce cellular energy (ATP). Over 1500 proteins are thought to be involved in the function of mitochondria (Taylor *et al*. 2003). The vast majority of these proteins are encoded by the nuclear genome, whereas 13 of these proteins are encoded by the organelle's own genome: the mitochondrial (mt) genome. Phylogenetic analyses have established that mitochondria evolved from free-living α-proteobacteria (Andersson *et al*. 1998; Gray *et al*. 1999; Rand *et al*. 2004), via endosymbiosis about 2 billion years ago (Dimauro and Davidzon, 2005). A common consequence of endosymbiosis for bacteria is reduction in the size of the genome of the bacteria, either by gene transfer from the bacteria to the host genome, or by gene loss (Gray *et al*. 1999). In multicellular animals (hereafter animals), this process has led to mt genomes that are about 16 kb long and encode, typically, 13 proteins, 2 rRNA subunits and 22 tRNAs. The mt genomes of animals are extremely compact: there is usually only 1 large non-coding region in each genome, which has been shown, in a few well-studied animals, to control genome replication and gene transcription (Goddard and Wolstenholme, 1980; Taanman, 1999; Saito *et al*. 2005); there are no introns and few non-coding nucleotides outside the large non-coding region (Wolstenholme, 1992). Although encoding only a small fraction of the proteins needed for the function of mitochondria, mt genomes play a critical role in cellular activities. Indeed, mutations in mt genomes are known to be associated with at least 13 types of disorders in humans (Wallace, 2002; Dimauro and Davidzon, 2005). Mt genomes are also a rich source of information about the evolution of eukaryotes. Due to their small size, abundance in cells, and a simple mode of inheritance, mt genomes caught the attention of evolutionary biologists soon after they were discovered in the 1960s (Nass and Nass, 1962; Brown *et al*. 1979; Gray, 1989; Wolstenholme, 1992). Indeed, data from mt genomes have been used in thousands of studies and have greatly advanced our understanding of many evolutionary phenomena (e.g. Boore *et al*. 1998; Lang *et al*. 1999; Ingman *et al*. 2000).

Arthropods are an ancient lineage that originated about 600 million years (MY) ago (Bergstrom,

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Parasitology (2007), **134**, 153–167.
doi:10.1017/S0031182006001429 Printed in the United Kingdom

Table 1. Numbers of described species of parasitic arthropods

Subphylum Common names	Class, order (family/subfamily)	Hosts	Approximate number of species	References
Chelicerata				
Ticks	Acari, Ixodida	Reptiles, mammals, birds	900	Barker and Murrell (2004)
Mites	Acari, Mesostigmata, Trombidiformes, Sarcoptiformes	Mammals, birds, reptiles, amphibians, arthropods	20 000	David Walter, personal communication
Hexapoda				
Bed bugs	Insecta, Hemiptera (Cimicidae)	Mammals, birds	91	Ryckman *et al.* (1981)
Kissing bugs	Insecta, Hemiptera (Triatominae)	Humans	130	Beard (2005)
Lice	Insecta, Phthiraptera	Mammals, birds	4900	Durden and Musser (1994); Price *et al.* (2003)
Fleas	Insecta, Siphonaptera	Mammals, birds	2380	Lewis and Lewis (1985)
Mosquitoes	Insecta, Diptera (Culicidae)	Mammals, birds	3500	Eldridge (2005)
Biting midges	Insecta, Diptera (Ceratopogonidae)	Vertebrates	1565	Borkent (2005)
Black flies	Insecta, Diptera (Simuliidae)	Mammals, birds	90	Adler (2005)
Sand flies	Insecta, Diptera (Phlebotominae)	Mammals, birds	836	Munstermann (2005)
Tsetse flies	Insecta, Diptera (Glossinidae)	Mammals	34	Jordan (1993)
Bot flies, warble flies	Insecta, Diptera (Oestridae)	Mammals, birds	151	Azeredo-Espin *et al.* (2006)
Blow flies, screwworm flies	Insecta, Diptera (Calliphoridae)	Mammals, birds	80	Azeredo-Espin *et al.* (2006)
Horn flies & relatives	Insecta, Diptera (Muscidae)	Mammals, birds	52	Colless and McAlpine (1991); Dudaniec and Kleindorfer (2006)
Crustacea				
Sea 'lice', fish 'lice'	Branchiura	Fishes	130	[A]
Parasitic copepods	Copepoda	Fishes, invertebrates	3592	[B]
Parasitic isopods	Malacostraca, Isopoda (Epicaridea, Flabellifera)	Other crustaceans	709	[C]
'Tongue worms'	Pentastomida	Vertebrates	130	Almeida and Christoffersen (1999)
Total number of species			39 270	

[A] http://www.ucmp.berkeley.edu/arthropoda/crustacea/maxillopoda/branchiura.html; [B] http://www.ucmp.berkeley.edu/arthropoda/crustacea/maxillopoda/copepodasy.html, http://www.nmnh.si.edu/iz/copepod/; [C] http://tolweb.org/Isopoda, http://www.nmnh.si.edu/iz/isopod/

1979). The Arthropoda is the largest animal phylum: it has over 80% of the one million or so described species of animals (May, 1990). Arthropods have adapted to every habitat, from oceans, lakes, rivers to the land and sky. Given the old age of the arthropod lineage and its abundance on earth, it is not surprising that arthropods have evolved many different life-styles, including parasitism. Parasitism, where one animal (the parasite) lives on (ectoparasite) or in (endoparasite) the body of another animal (the host) at the expense of its host, has evolved many times in arthropods. For example, the habit of blood feeding evolved at least 21 times, independently, in arthropods (Black and Kondratieff, 2005). Of the ~800 000 described species of arthropods, 39 270 (~5%) are parasites by our definition above. Ticks and mites of the subphylum Chelicerata account for ~53% of the described species of parasitic arthropods, followed by parasitic insects (~35%); the rest (~12%) are parasitic crustaceans (Table 1). Parasitic arthropods eat the blood, skin, flesh or feathers of their hosts, which range from non-parasitic arthropods to fishes, reptiles, birds and mammals, including domestic animals and humans. Parasitic arthropods may be a nuisance or cause injury directly through their sucking and chewing, which may lead to inflammation and/or toxic effects. The body parts and excreta of parasitic

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Table 2. The 27 species of parasitic arthropods whose mitochondrial genomes have been sequenced entirely

Subphylum Species	Common name	Genome size (bp)	Accession number	Reference
Chelicerata				
Amblyomma triguttatum	Ornate kangaroo tick	14740	AB113317	Unpublished
Carios capensis	Soft tick	14418	AB075953	Unpublished
Haemaphysalis flava	Hard tick	14686	AB075954	Shao *et al*. (2004)
Ixodes hexagonus	Hedgehog tick	14539	AF081828	Black and Roehrdanz (1998)
Ixodes holocyclus	Paralysis tick	15007	AB075955	Shao *et al*. (2005*a*)
Ixodes persulcatus	Taiga tick	14539	AB073725	Shao *et al*. (2005*a*)
Ixodes uriae	Common seabird tick	15053	AB087746	Shao *et al*. (2005*a*)
Leptotrombidium akamushi	Chigger mite	13698	AB194045	Shao *et al*. (2006)
Leptotrombidium deliense	Chigger mite	13731	AB194044	Shao *et al*. (2006)
Leptotrombidium pallidum	Chigger mite	16779	AB180098	Shao *et al*. (2005*b*)
Ornithodoros moubata	Soft tick	14398	AB073679	Shao *et al*. (2004)
Ornithodoros porcinus	Soft tick	14378	AB105451	Mitani *et al*. (2004)
Rhipicephalus sanguineus	Brown dog tick	14710	AF081829	Black and Roehrdanz (1998)
Varroa destructor	Honeybee mite	15277, 16477	AY163547, AJ493124	Evans and Lopez (2002), Navajas *et al*. (2002)
Hexapoda				
Aedes albopictus	Asian tiger mosquito	16665	AY072044	Unpublished
Anopheles gambiae	African malaria mosquito	15363	L20934	Beard *et al*. (1993)
Anopheles quadrimaculatus	Common malaria mosquito	15455	L04272	Cockburn *et al*. (1990)
Campanulotes bidentatus	Small pigeon louse	14804	AY968672	Covacin *et al*. (2006)
Chrysomya putoria	African blow fly	15837	AF352790	Junqueira *et al*. (2004)
Cochliomyia hominivorax	Screwworm fly	16022	AF260826	Lessinger *et al*. (2000)
Dermatobia hominis	Human botfly	16360	AY463155	Unpublished
Haematobia irritans	Horn fly	16078	DQ029097	Unpublished
Heterodoxus macropus	Wallaby louse	14670	AF270939	Shao *et al*. (2001)
Triatoma dimidiata	Kissing bug	17019	AF301594	Dotson and Beard (2001)
Crustacea				
Argulus americanus	Fish 'louse'	15102	AY456187	Lavrov *et al*. (2004)
Armillifer armillatus	'Tongue worm'	16747	AY456186	As above
Lepeophtheirus salmonis	Salmon 'louse'	15445	AY625897	Tjensvoll *et al*. (2005)

arthropods may be antigens and can cause allergic diseases in their hosts. Most importantly, however, many parasitic arthropods are vectors of organisms that cause life-threatening diseases, like malaria, plague, typhus, trench fever, relapsing fever, leishmaniasis, yellow fever, and dengue fever. Malaria, alone, causes about a million human deaths each year (http://malaria.who.int/). Parasitic arthropods also cause substantial losses in agriculture and fisheries. For example, the cost for the control of ticks and tick-borne diseases was estimated to be US$ 7 billion globally (McCosker, 1979), and the control of the salmon 'louse', *Lepeophtheirus salmonis*, costs a farmer, on average, C$350 000 per crop in eastern Canada (Mustafa *et al*. 2001).

Despite their medical, veterinary and economic importance, most aspects of the population genetics and evolution of the vast majority of the parasitic arthropods are poorly understood, partly because of the lack of suitable markers. Mt genomes have been instructive in studies of the population genetics and evolution of both vertebrates and invertebrates (Avise, 2004), including parasitic nematodes and flatworms (Le *et al*. 2002; Hu and Gasser, 2006). For studies of parasitic arthropods, however, only the nucleotide (nt) sequences of a few mt genes have been used; many other markers from mt genomes have not been explored. Here, we review current knowledge on the mt genomes of parasitic arthropods, summarize applications of mt genes and genomic features in population genetic and evolutionary studies, and highlight prospects for future research.

MITOCHONDRIAL GENOMES OF PARASITIC ARTHROPODS

Of the over 700 species of animals whose mt genomes have been sequenced entirely, 24 are parasitic arthropods: 14 from the subphylum Chelicerata (ticks, mites, spiders and kin), 10 from the Hexapoda (insects and kin), and 3 from the Crustacea (crabs, shrimp and kin; Table 2). All these genomes are circular, and have the 37 genes that are typical of the

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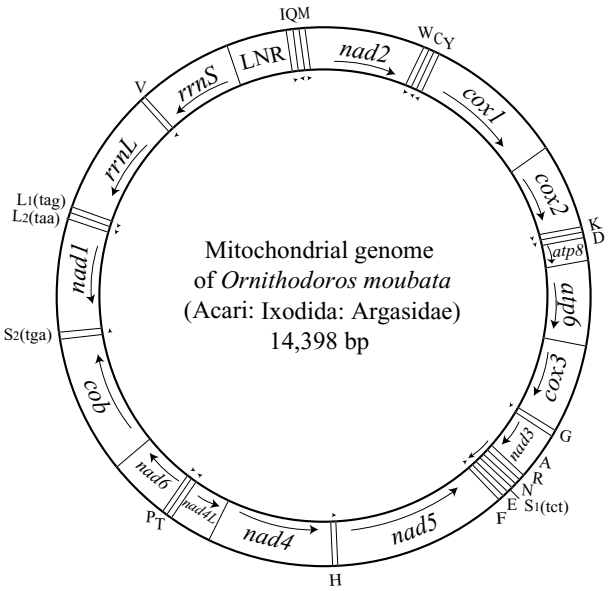

Fig. 1. The mitochondrial genome of the soft tick, *Ornithodoros moubata*. DNA strands are shown as 2 circles. Genes are represented as boxes and were drawn approximately to scale. Arrows inside and outside boxes (adjacent to the inner circle) indicate the orientation of transcription. Protein-coding and rRNA genes are abbreviated as *atp6* and *atp8* (for ATP synthase subunits 6 and 8), *cox1–3* (for cytochrome c oxidase subunits 1–3), *cob* (for cytochrome b), *nad1–6* and *4L* (for NADH dehydrogenase subunits 1–6 and 4L), and *rrnL* and *rrnS* (for large and small rRNA subunits). The transfer RNA (tRNA) genes are shown with the single-letter abbreviations of their corresponding amino acids. The 2 tRNA genes for leucine are L_1 (anti-codon sequence uag) and L_2(uaa), and those for serine are S_1(ucu) and S_2(uga). *LNR* is the abbreviation for the large non-coding region. The gene content and gene arrangement of *O. moubata* is identical to those inferred for the hypothetical ancestor of the arthropods (Staton *et al*. 1997).

mt genomes of animals: 13 for proteins, 2 for rRNAs and 22 for tRNAs, as exemplified by the mt genome of a soft tick, *Ornithodoros moubata* (Fig. 1). Most of the 27 parasitic arthropods whose mt genomes have been sequenced entirely have a single copy of each of the 37 genes, but there are 4 exceptions: (1) the chigger mite, *Leptotrombidium pallidum*, has 2 *rrnL* genes; (2) the African blow fly, *Chrysomya putoria*, has 2 *trnI* genes; (3) the human bot fly, *Dermatobia hominis*, has 2 *trnV* genes; and (4) the salmon 'louse', *Lepeophtheirus salmonis*, has 2 *trnK* genes (Fig. 2). In addition, *L. pallidum* has a pseudo-gene for small rRNA subunit, *PrrnS*, which is only half as long as the functional *rrnS* (Shao *et al*. 2005*b*). Non-coding regions are present in the mt genomes of all the 27 parasitic arthropods, but the size and number of non-coding regions vary. The non-coding regions of the mt genomes of the wallaby louse and the small pigeon louse are less than 100 bp long whereas the non-coding region of the kissing bug has over 2100 bp (Dotson and Beard, 2001; Shao *et al*. 2001; Covacin *et al*. 2006). For other parasitic arthropods, the non-coding regions range from 400 to 1000 bp, which is similar to those of most other animals. Eighteen of the 27 parasitic arthropods whose mt genomes have been sequenced entirely have a single copy of non-coding region in their mt genome, whereas the 2 ticks from the Australasian *Ixodes* lineage (sensu Barker and Murrell, 2004), the 4 metastriate ticks and 2 of the 3 chigger mites, have two non-coding regions that have near-identical nt sequences. The other chigger mite, *L. pallidum*, has 4 non-coding regions with near-identical nt sequences (Fig. 2).

The arrangement of mt genes varies among the 27 species of parasitic arthropods that have been sequenced. Of the 14 parasitic chelicerates sequenced, the non-Australasian *Ixodes* ticks and the soft ticks have an arrangement that is inferred to be ancestral for arthropods (Staton *et al*. 1997); thus, this gene arrangement has remained unchanged for over 400 MY in these two lineages (Fig. 2; Shao *et al*. 2004). The Australasian *Ixodes* ticks have the same gene arrangement as the non-Australasian *Ixodes* ticks and the soft ticks but have duplicate non-coding regions that have evolved in concert (Shao *et al*. 2005*a*). Several genes or blocks of genes changed positions and/or orientation-of-transcription in the mt genomes of the metastriate ticks and the honeybee mite (Fig. 2). The most rearranged mt genomes among the parasitic chelicerates are those of the chigger mites, in which over two thirds of the 37 genes have changed positions and/or orientation-of-transcription relative to the hypothetical ancestor of the arthropods (Shao *et al*. 2005*b*). Of the 13 species of parasitic insects and crustaceans whose mt genomes have been sequenced, the kissing bug, the screwworm fly, and the horn fly have a gene arrangement that is most similar to the inferred ancestral arrangement of the arthropods: the only difference is the arrangement of $trnL_2$ gene. This tRNA gene is between *nad1* and *rrnL* in the hypothetical ancestor of the arthropods but is between *cox1* and *cox2* in the kissing bug, the screwworm fly and the horn fly (Fig. 2). The rearrangement of $trnL_2$ is apparently a shared-derived character that unites the Hexapoda and the Crustacea to the exclusion of all other arthropods (Boore *et al*. 1998). Thus, the kissing bug, the screwworm fly and the horn fly have retained the ancestral gene arrangement of the Hexapoda and the Crustacea. Mt genomes with rearranged $trnL_2$ are also present in (1) the African blow fly and the human bot fly, which differ from the hypothetical ancestor of the Hexapoda and the Crustacea by having duplicated *trnI* and *trnV*, respectively; and (2) the 3 mosquitoes, the fish 'louse' and the 'tongue worm', which have the rearrangement of several other genes (Fig. 2). The $trnL_2$ has been further

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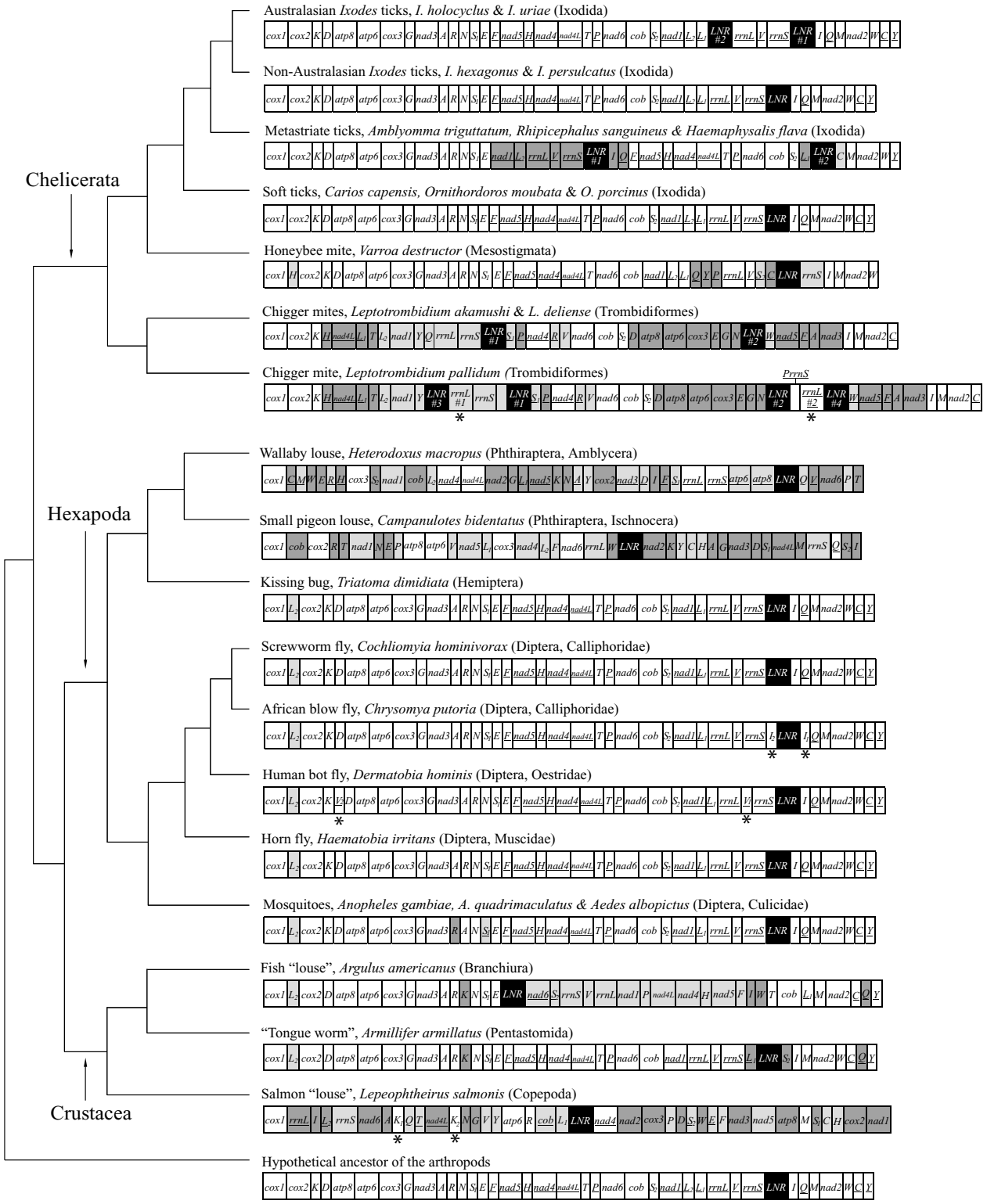

Fig. 2. Evolution of the arrangement of genes in the mitochondrial genomes of parasitic arthropods. The phylogenetic tree is from the Tree of Life Web Project (http://tolweb.org). Only the species whose mitochondrial genomes have been sequenced entirely are shown. For the purpose of illustration, the circular mitochondrial genomes are linearized arbitrarily at the 5′ end of *cox1*. Genes and large non-coding regions (*LNR*) are shown as boxes but were not drawn to scale. The abbreviations of the names of genes are defined in the legend of Fig. 1. Genes are transcribed from left to right except those genes whose names are underlined, which are transcribed from right to left. Asterisks indicate gene duplications. Large non-coding regions are highlighted in black. Dark, grey, shaded-boxes indicate genes that changed position in these genomes relative to the hypothetical ancestor of the arthropods. Pale, grey, shaded-boxes indicate genes that changed both position and the orientation-of-transcription, relative to the hypothetical ancestor of the arthropods.

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Table 3. Comparison of the mitochondrial and nuclear genomes of the mosquito, *Anopheles gambiae*

Characteristic	Mitochondrial genome	Nuclear genome
Size	15363 bp	278244063 bp
Number of DNA molecules per cell	~1000	3 in haploid cells; 6 in diploid cells
Number of genes encoded	37	13683
Gene density	1 per 415 bp	1 per 20335 bp
Introns	No	42991864 bp (15% of the genome)
Coding sequence	14818 bp (96.5%)	19274180 bp (7%)
Mode of inheritance	Maternal inheritance	Mendelian inheritance for autosomes and X chromosome; paternal inheritance for Y chromosome
Genetic recombination	No evidence for presence	Yes

rearranged in the wallaby louse, and the small pigeon louse and the salmon 'louse'; all these 3 species have highly rearranged mt genomes, in which over four fifths of the 37 genes have changed locations and/or orientation-of-transcription (Fig. 2; Shao *et al*. 2001; Tjensvoll *et al*. 2005; Covacin *et al*. 2006).

MITOCHONDRIAL GENOMES ARE A RICH SOURCE OF MARKERS FOR STUDIES OF POPULATION GENETICS AND EVOLUTION

The mt genomes of extant organisms have been evolving for about 2 billion years, and thus contain much information about the evolution of these organisms. Two main types of markers from mt genomes can be used in population genetic and evolutionary studies: nt sequences, and genomic features. These 2 types of markers are complementary because they evolved by different mechanisms and at different rates (Lang *et al*. 1999), and can be further divided into (1) nt sequences of single genes, 2 or more genes, non-coding regions, all of the protein-coding genes, all of the tRNA genes, or the entire set of 37 genes, and (2) duplication of genes or non-coding regions, gene rearrangements, and variation in secondary structures of tRNAs and rRNAs, and variation in the number of repeats in non-coding regions.

Each of these markers has strengths and weaknesses, and their usefulness depends on the questions at hand, and the taxa studied. Nt sequences of mt genomes are a universal marker and are available, in theory, from all taxa. But the reliability of nt sequences in population genetic and evolutionary studies is affected by heterogeneity in evolutionary rate, bias in base composition and saturation of nt substitutions (Rokas and Holland, 2000). Genomic features do not have these problems but are not universal markers, and can only be useful in lineages where these features occur. Sequences of single genes are relatively easy to collect, and thus can be obtained for a large number of taxa in a single study. But sequences of single genes obviously contain a relatively small number of informative characters. Entire mt genomes, on the other hand, have many more informative characters than single genes but can be difficult to sequence.

ADVANTAGES OF MITOCHONDRIAL GENOMES OVER NUCLEAR GENOMES FOR STUDIES OF POPULATION GENETICS AND EVOLUTION

Mitochondrial genomes are dwarfed by nuclear genomes in many ways. The mosquito, *Anopheles gambiae*, is the only parasitic arthropod whose mt and nuclear genomes have been sequenced entirely (Beard *et al*. 1993; Holt *et al*. 2002). The nuclear genome of this mosquito is more than 18000 times larger, and has 370 times as many genes as the mt genome (Table 3). It is obvious that nuclear genomes contain many more informative characters and genomic features than mt genomes. Nuclear genomes, however, are not necessarily superior to mt genomes for studies of population genetics and evolution. Rather, mt genomes have several advantages over nuclear genomes for population genetic and evolutionary studies. First, with few exceptions, mt genomes are inherited only through maternal lineages. Thus, mt genome markers are more suitable and more powerful than nuclear genome markers for tracing the evolution of maternal lineages. Second, nt sequences of mt genes evolve faster than most nuclear genes, and thus are more suitable to address evolutionary questions at low taxonomic levels, eg. species, genera, or families. Third, mt genomes have a much higher proportion of coding sequence than nuclear genomes, and thus are better markers for studies that use coding sequences. For example, 96.5% of the mt genome of *A. gambiae* is coding sequence whereas only 7% of the nuclear genome of this mosquito is coding sequence (Table 3). Fourth, mt genomes are far easier to sequence and annotate than nuclear genomes, due to their small size and the abundance of mitochondria in cells.

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APPLICATIONS OF MITOCHONDRIAL GENOMES IN POPULATION GENETIC AND EVOLUTIONARY STUDIES

Nucleotide sequences of single genes

Sequences of single mt genes have been used commonly to study the population genetics and evolution of parasitic arthropods. Of the 37 mt genes, *cox1*, *cob*, *rrnS* and *rrnL* have been used the most (e.g. Black and Piesman, 1994; Norris *et al*. 1996; Mangold *et al*. 1998; Murrell *et al*. 1999; Beati and Keirans, 2001; Leo *et al*. 2002; Skerratt *et al*. 2002; Reed *et al*. 2004; Mirabello and Conn, 2006); *nad4* was used in only 1 study (Kittler *et al*. 2003). The other 10 protein-coding genes, the 22 tRNA genes and non-coding regions of the mt genome have not been explored in parasitic arthropods. Hu *et al*. (2003) compared the nt sequences of the mt genomes of 2 populations of the human hookworm, *Necator americanus*, and ranked the mt genes and non-coding regions by degree of sequence divergence as (1) non-coding regions > protein-coding genes > tRNA genes > rRNA genes; (2) between the 2 rRNA genes: *rrnL* > *rrnS*; and (3) among the 12 protein-coding genes (hookworms lack *atp8* gene): *nad1* > *nad3* > *cob* > *cox2* > *nad2* > *nad6* > *atp6* > *cox1* > *nad4* > *nad5* > *cox3* > *nad4L*. This ranking illustrates a simple but important fact: mt genes and non-coding regions evolve at different rates, and thus each is suitable for addressing evolutionary questions over a particular timescale. If we assume, for the sake of argument, that the ranking above applies to parasitic arthropods, then the fastest-evolving non-coding regions and genes (i.e. *nad1* and *nad3*) and the slowest-evolving protein-coding genes (i.e. *nad5*, *cox3* and *nad4L*) have not been explored in parasitic arthropods.

Nucleotide sequences of non-coding regions

Mt non-coding regions evolve faster than coding regions and thus, generally, provide more resolution for recent evolutionary divergences than coding regions. Several studies have showed that mt non-coding regions are instructive for addressing phylogenetic relationships at low taxonomic levels (e.g. among populations, species and genera), but none of these studies was on parasitic arthropods. Liu *et al*. (2006) showed that a hypervariable segment of the mt non-coding region was instructive about the origins and phylogeographic history of domestic chickens. Aranishi and Okimoto (2005) studied polymorphisms in the nt sequence of a non-coding region of the mt genome of the Pacific oyster, *Crassostrea gigas*. These authors concluded that the nt sequence of this non-coding region was a suitable marker for resolving intra-specific relationships in the Pacific oyster and was more useful for this purpose than the less polymorphic coding regions. Drovetski (2002) compared 3 nuclear non-coding regions with an mt non-coding region of grouse (Aves: Tetraoninae), and showed that the fast-evolving mt non-coding region provided more resolution than the slow-evolving nuclear non-coding regions for relationships among 8 genera of grouse.

Concatenated nucleotide sequences of two or more genes

Sampling error is a major problem in population genetic and evolutionary studies that use sequences of single genes because different genes may have different evolutionary histories (Philippe *et al*. 2005). Further, the information in a single gene often cannot resolve phylogenetic relationships at a range of taxonomic levels. This problem may be overcome by using concatenated nt sequences of 2 or more genes. For example, in cnidarians, the commonly studied mt genes, *cox1* and *rrnL*, evolved slowly, and were not informative about the evolution of these invertebrates. McFadden *et al*. (2004) sequenced fragments of 3 fast-evolving mt protein-coding genes, *nad2*, *nad3* and *nad6*, to see if any of these genes contain sufficient variation to resolve phylogenetic relationships among genera of the anthozoan subclass Octocorallia. The relationships were poorly resolved when each gene was used alone, but there was much more resolution when concatenated nt sequence of the 3 genes was used. In 2 other studies, Hassanin (2006) showed that concatenated sequence of 6 mt protein-coding genes (*atp6, atp8, cox1–3* and *nad2*) was instructive about the relationships among subphyla of arthropods, and Macey *et al*. (1999) showed that concatenated sequence of 2 protein-coding genes (*nad1* and *cox1*) and 9 tRNA genes (*trnI*, *trnQ*, *trnM*, *trnW*, *trnA*, *trnN*, *trnC* and *trnY*) was able to resolve relationships among gekkonid lizards of the genus *Teratoscincus*.

Sequence of entire coding region

The nt sequence of the entire mt coding region (i.e. all 37 genes) has been used mainly in studies of the evolution of modern humans, *Homo sapiens* (e.g. Ingman and Gyllensten, 2003; Macaulay *et al*. 2005; Rajkumar *et al*. 2005; Thangaraj *et al*. 2005). These studies indicate that sequence of the entire coding region is more powerful than part of the coding region or non-coding region in population genetic studies. For example, by analysis of the nt sequence of the entire mt coding region, Rajkumar *et al*. (2005) identified 2 lineages (M30 and M31) in the human mt macrohaplogroup M, and led to the rejection of a hypothesis of 2 other lineages (M3 and M4) that have been previously defined, solely, from the non-coding region sequence.

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Concatenated sequence of the entire set of 13 protein-coding genes

There have been many studies that use concatenated nt sequences or inferred amino acid sequences of entire sets of mt protein-coding genes to test phylogenetic relationships inferred from morphological data and nuclear gene sequences, but none of these studies was of parasitic arthropods. Most of these studies confirmed the relationships proposed from morphological data or nuclear gene sequences, and often provided more resolution of relationships that were ambiguous in the morphological and nuclear analyses (e.g. Kumazawa and Nishida, 1999; Nikaido *et al*. 2000; Janke *et al*. 2001; Arnason *et al*. 2002; Elmerot *et al*. 2002; Bae *et al*. 2004; Davis *et al*. 2004; Lavrov *et al*. 2004; Macey *et al*. 2004; Reyes *et al*. 2004; San Mauro *et al*. 2004). There are also cases, however, in which concatenated sequences of mt protein-coding genes shed little light on controversial relationships inferred from morphology or nuclear genes (Brinkmann *et al*. 2004), or indicated relationships that contradicted those inferred from morphology or nuclear genes (Dong and Kumazawa, 2005; Mueller *et al*. 2004; Nardi *et al*. 2003; Scouras *et al*. 2004).

Concatenated nucleotide sequence of tRNA genes

tRNA genes are less popular than protein-coding and rRNA genes for population genetic and evolutionary studies, primarily because of their small size (~65 bp each) and the effort required to sequence the 22 tRNA genes that spread throughout mt genomes. Sequences of tRNA genes, however, are accumulating in databases as more and more mt genomes are sequenced. Concatenated nt sequences of mt tRNA genes have been explored in only a few studies so far; none of them was on parasitic arthropods. Kumazawa and Nishida (1993) compared the power of concatenated sequence of the entire set of mt tRNA genes with the sequences of *cox1* and *cob* to recover a well-established phylogeny of 7 representative animals that diverged 20–600 MY ago. The tRNA genes recovered the phylogeny with 100% bootstrap support whereas neither *cox1* nor *cob* recovered this phylogeny. In another study, Kumazawa and Nishida (1995) showed that concatenated sequence of 11 tRNA genes recovered a sister-group relationship between birds and crocodilians relative to mammals. Haring *et al*. (2001) also showed that concatenated sequence of the entire set of 22 tRNA genes, together with the 2 rRNA genes, gave better resolution and higher bootstrap support in a phylogeny among 5 orders of birds than did the protein-coding and rRNA genes.

Gene rearrangements

Nucleotide sequences of mt genomes may be powerful markers in population genetic and evolutionary studies but are not without problems. Different substitution rates among nucleotide sites and among lineages, saturation of substitutions in lineages, non-independent substitutions among sites, and functional constraints on nucleotide substitution may confound the inference of phylogeny with nt sequences (Rokas and Holland, 2000). Compared with nt substitutions, mt genomic features are larger-scale mutations and occur much less frequently (Boore and Brown, 1998; Rokas and Holland, 2000). Mt genomic features, thus offer another way to test the relationships inferred from nt sequences.

In parasitic arthropods, 2 types of mt genomic features have been shown to be instructive to evolutionary studies: gene rearrangements, and duplications of non-coding region (see the section below). Mt gene rearrangements were initially used to address phylum-level relationships, due to the observation that the arrangements of mt genes were conserved at low taxonomic levels but varied at high levels (Boore and Brown, 1998). We now know, however, that mt gene arrangements also vary at low taxonomic levels, such as among genera and families. Several studies have shown that mt gene rearrangements are instructive markers to resolve relationships at low taxonomic levels. Covacin *et al*. (2006) reported that the mt genes between *cox1* and *cox3* have at least 4 different arrangements among 10 species of lice (Phthiraptera) from 6 families. Black and Roehrdanz (1998) and Campbell and Barker (1998) showed that the rearrangements of a tRNA gene, and a block of 7 genes are shared-derived characters that unite all known metastriate ticks (family Ixodidae). Further, in a study of whiteflies, Thao *et al*. (2004) showed that mt gene rearrangements are instructive for resolving phylogenetic relationships among genera of the family Aleyrodidae. Not all mt gene rearrangements, however, are informative. For example, two neighbouring genes, *trnK* and *trnD*, swapped positions independently in the locust and the honeybee (Crozier and Crozier, 1993; Flook *et al*. 1995), and 2 novel gene boundaries, *cox2-trnG* and *cox3-trnR*, evolved independently in the plague thrips (Thysanoptera) and a lepidopsocid species (Psocoptera; Shao and Barker, 2003). The chance of convergent evolution of novel gene arrangement, however, is low and such convergence can usually be identified by broadening the taxon sampling (Dowton *et al*. 2002).

Duplication of genes and non-coding regions

Mt genomes of animals typically have only 1 copy of each of the 37 genes and the non-coding region.

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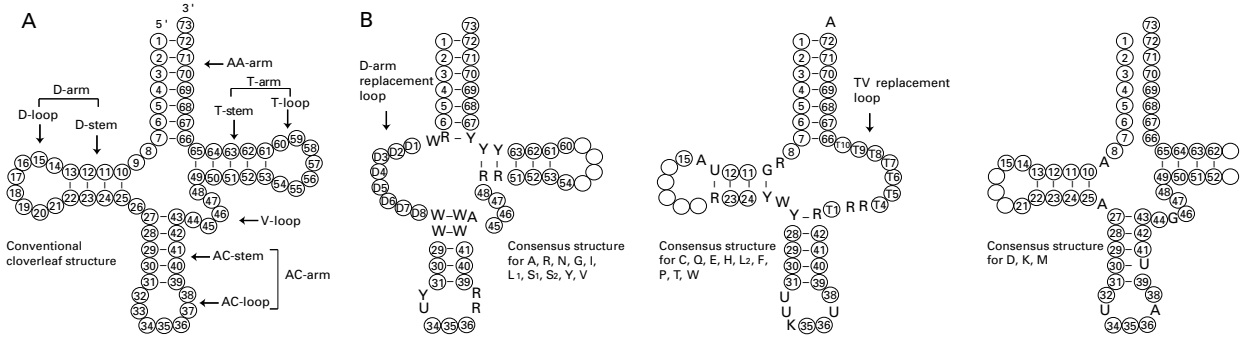

Fig. 3. The conventional cloverleaf secondary structure of tRNAs (A), and the consensus structures of the tRNAs of the chigger mite, *Leptotrombidium pallidum* (B). The numbering of nucleotides follows Sprinzl *et al*. (1989), except for those at D-arm replacement loop and TV replacement loop, which start with 'D' and 'T' respectively. Degenerate nucleotides are: K = G/U; R = A/G; Y = C/U; and W = A/U.

Duplicate genes, however, are present in 4 of the 27 parasitic arthropods whose mt genomes were sequenced entirely: the African blowfly (duplication of *trnI*), the human botfly (*trnV*), the salmon 'louse' (*trnK*), and the chigger mite, *L. pallidum* (*rrnL*, Fig. 2). Duplicate non-coding regions are present in the Australasian lineage of *Ixodes* ticks, the metastriate ticks and chigger mites. Further, the chigger mite, *L. pallidum*, has a pseudo-*rrnS* gene, in addition to the apparently functional *rrnS*. Lessinger *et al*. (2004) reported that duplicate *trnI* genes in the African blow fly, *Chrysomya putoria*, were also present in 2 other *Chrysomya* species, *C. megacephala* and *C. albiceps*, whose mt genomes had been sequenced partially. These authors suggested that duplicate *trnI* genes might be a shared-derived character for species of the genus *Chrysomya*. Shao *et al*. (2005*a*) showed that duplicate non-coding regions occurred in both Australasian *Ixodes* ticks (one of the 2 main lineages of *Ixodes* ticks) and metastriate ticks, and were a shared-derived character for each of these 2 lineages. Among the chigger mites, however, duplicate *rrnL* genes and the pseudo-*rrnS* gene occurred only in *L. pallidum*, not in the other 3 species of *Leptotrombidium* studied (Shao *et al*. 2006). Studies of more *Leptotrombidium* species and different populations of *L. pallidum* should reveal whether duplicate *rrnL* genes and the presence of pseudo-*rrnS* gene are instructive specific or intra-specific markers.

Secondary structures of tRNAs

In most animals, all of the mt tRNAs can form clover-leaf-shaped secondary structures except tRNA-Ser (ncu) which lacks a D-arm and has a replacement loop instead (Fig. 3). The unusual structure of tRNA-Ser (ncu) was thought to be ancestral to all multi-cellular animals (Wolstenholme, 1992). In a number of animal species, however, several other tRNAs also lack a D-arm or a T-arm (Yamazaki *et al*. 1997; Macey *et al*. 2000; Lavrov and Brown, 2001; Masta and Boore, 2004). The most extensive changes in tRNA secondary structures occurred in nematodes of the class Secernentea, where all of the 22 tRNAs lack either a D-arm or a T-arm (Okimoto *et al*. 1992). Comparison between species of nematodes in this class with *Trichinella spiralis* indicates that 8 of the 20 modified secondary structures appear to be shared-derived characters for species of the class Secernentea (Lavrov and Brown, 2001). Unusual tRNA secondary structures that are likely to be derived characters have also been found in parasitic arthropods. In the honeybee mite and the metastriate ticks, tRNA-Cys lacks a D-arm (Black and Roehrdanz, 1998; Navajas *et al*. 2002); in the salmon 'louse', tRNA-Arg and tRNA-Ala lack D-arms whereas tRNA-Glu lacks the T-arm (Tjensvoll *et al*. 2005). In the chigger mite, *L. pallidum*, 9 of the 22 tRNAs lack a T-arm whereas 10 other tRNAs lack a D-arm (Fig. 3; Shao *et al*. 2005*b*). Another 3 chigger mites, *L. akamushi*, *L. deliense*, and *L. fletcheri*, share the truncated tRNAs with *L. pallidum* (Shao *et al*. 2006). Whether or not these modified tRNAs are instructive for population genetic and evolutionary studies of parasitic arthropods waits to be tested.

Nucleotide sequences combined with genomic features

The rapid advances in DNA sequencing technology have made it possible to sequence entire or near-entire mt genomes of a number of taxa relatively quickly and cheaply, and thus, to analyse nt sequences and genomic features simultaneously in a single study. This combined approach allows for reciprocal tests of the results obtained from different types of data. The power of this approach has been demonstrated in studies of phylogenetic relationships at the levels of phylum, subphylum and family (Boore and Brown, 2000; Morrison *et al*. 2002;

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Boore and Staton, 2002; Lavrov *et al*. 2004; Macey *et al*. 2004); 1 of these studies was on parasitic arthropods (Lavrov *et al*. 2004). We use 3 of these studies below to introduce this combined approach. Boore and Brown (2000) analysed the concatenated amino acid sequence of 8 mt proteins of 9 species from 5 animal phyla: Annelida, Arthropoda, Chordata, Mollusca, and Pogonophora. This study indicated that (1) pogonophorans ('beard worms') are a lineage within the phylum Annelida, rather than a sister lineage of the Annelida, and (2) annelids are more closely related to mollusks than to arthropods. Bootstrap support for these two relationships, however, was low (49% and 69% respectively). Boore and Brown (2000) then analysed the concatenated nt sequence of 12 mt tRNA genes, and the arrangement of over half of the total mt genes. These analyses provided more support for the two relationships above: for the first, the bootstrap value increased from 49% to 90%, and the second was supported by 2 shared-derived gene arrangements, *trnS-nad2-cox1*, and *trnC-trnM-rrnS*. Morrison *et al*. (2002) reported 4 gene rearrangements in hermit crabs (Anomura: Crustacea), and showed that 3 of these rearrangements corroborated relationships that were strongly supported by nt sequences of nuclear genes (*18S*, *28S*) and mt genes (*cox1*, *rrnL*) whereas one of the gene rearrangements helped resolve the phylogeny of 3 families that could not be resolved with the nt sequences. Lavrov *et al*. (2004) addressed the controversial phylogenetic position of pentastomids ('tongue worms'): a group of endoparasites that was thought to be related to arthropods. The analysis of concatenated amino acid sequence of 12 mt proteins (excluding ATP8) placed pentastomids in a well-supported group with arthropods and nematodes and, further, as the sister group of the nematodes. This sister-group relationship, however, was in doubt because the pentastomids and nematodes analysed have long branches in the phylogenetic tree. Thus, long-branch attraction may have confounded the analysis (Philippe and Laurent, 1998). The authors then analysed the mt gene arrangement and found that the pentastomid species had the gene arrangement, *cox1-trnL*(*uaa*)*-cox2*, which is a shared-derived character for Pancrustacea (Crustacea + Hexapoda). This gene arrangement strongly supports the view that pentastomids are arthropods and allows for the rejection of a sister-group relationship between pentastomids and nematodes. Further, the pentastomid species also shared 2 novel gene arrangements, *trnR-trnK-trnN* and *trnY-trnQ-trnC*, with 2 species of crustacea from the classes Cephalocarida and Maxillopoda. These 2 novel gene arrangements indicate strongly that pentastomids are crustaceans and are more closely related to cephalocarid and maxillopod crustaceans than to other crustaceans and hexapods.

CONCLUSION

Mt genomes are a rich source of markers for studies of population genetics and evolution. These markers include nt sequences of 3 types of genes (for proteins, rRNAs and tRNAs) and non-coding regions, and various genomic features. In parasitic arthropods, however, only the nt sequences of 4 of the 37 mt genes (*cox1*, *cob*, *rrnS* and *rrnL*) have been well explored. The sequences of 33 other mt genes and non-coding regions have not been explored; neither have most of the genomic features, such as gene duplications and novel gene arrangements. In addition, most population genetic and evolutionary studies of parasitic arthropods have relied on partial sequences of single mt genes whereas the more powerful approach of combing sequences of 2 or more genes together, or combining nt sequences with genomic features, has not received the attention it deserves. Thus, it is obvious to us that future studies on the mt genomes of parasitic arthropods should include (1) sequencing entire mt genomes of more species, especially from lineages that are currently not represented or are under-represented in databases (e.g. fleas, mites, parasitic crustaceans), and from lineages that have highly rearranged mt genomes (e.g. lice, salmon 'lice', chigger mites); (2) exploring the utility of genomic features, and nt sequences of non-coding regions and genes other than *cox1*, *cob*, *rrnS* and *rrnL*; and (3) applying approaches that combine sequences of 2 or more genes together, or combine nt sequences with genomic features in population genetic and evolutionary studies of parasitic arthropods.

We thank Professor Robin Gasser for initiating this review. We would also like to thank the editor, Professor Stephen Phillips, and the two anonymous reviewers for valuable comments on this manuscript. This work was supported by an Australian Postdoctoral Fellowship grant (DP0662755) to R.S. from the Australian Research Council.

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