Lingzhi Mitochondrial Genome

4

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Abstract

The mitochondrial genome is the second genome for eukaryotes. It has essential functions in many cellular processes, including producing energy, signaling, aging, and programmed cell death. Up to now, ten mitochondrial genomes from seven *Ganoderma* species had been released on the public database. The mitochondrial genomes of each species were described and compared here, and some intra-specific differences were also issued. The mitochondrial phylogenomic tree of Polyporales, which *Ganoderma* is belonged to in taxonomy, was constructed, and the potential DNA barcodes from the mitochondrial genome for this genus were discussed.

4.1 General Introduction

4.1.1 Concept

Mitochondria: the mitochondrion (plural mitochondria) is a highly specialized organelle with double membranes, existing in the cytoplasm of almost all eukaryotic cells (cells with clearly defined nuclei). As the cell's powerhouse, it produces large quantities of energy stored in adenosine triphosphate (ATP) through oxidative phosphorylation (Chinnery and Hudson 2013; van der Giezen and Tovar 2005). The number of mitochondria in a cell can vary significantly by organism and tissue, and each mitochondrion contains multiple mitochondrial DNA molecules (Basse 2010).

Mitochondrial genome (Mitogenome): mitochondria have their DNA, known as mitochondrial DNA or mtDNA. mtDNA was first discovered in animal cells (Nass and Nass 1963). mtDNA consists of a heavy (H) and a light (L) strand. The heavy strand/light strand terminology dates back to the 1970s and refers to the relative CsCl banding of the two single strands of the mitochondrial DNA. These strands have a G/C asymmetry leading to the sense strand being "light" and the reverse complement being "heavy" in their relative molecular weights. The sense strand (L) encodes 13 of the more than 90 subunits of the electron-transfer chain and 22 tRNAs and 2 rRNAs in humans (*Homo sapiens*). Humans' mitochondrial genome is a closed circular duplex DNA of estimated 16,500 nucleotide pairs (Brown et al. 1979). Then, the first complete sequence of a mitochondrial genome was reported from humans, and its exact size of 16.569 base pairs (bp) was presented (Anderson et al. 1981). In fungi, the first complete mitochondrial genome was sequenced from the yeast

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Saccharomyces cerevisiae in 1998, with the size of 85,779 bp (Foury et al. 1998).

4.1.2　Principle

4.1.2.1　Mitochondrial Origin

The current theory as to the origin of eukaryotic cells is endosymbiosis. Based on the endosymbiotic theory, mitochondria are the descendants of once free-living α-proteobacteria. The proteobacteria became endosymbiotic organelles of eukaryotes two billion years ago (Andersson et al. 1998; Gray et al. 1999). It is believed that mitochondria and chloroplasts began as prokaryotic organisms engulfed by the larger cells, either as food or parasites. That is why they are enclosed in double membranes. At some point, the relationship became mutually beneficial, and the mitochondria and chloroplasts evolved into permanent members in the cells as cellular machinery.

4.1.2.2　Mitochondrial Structure

Mitochondria are typically oval, spherical, or rod-shaped. Its size ranges from 0.5 to 1.0 μm in diameter. Double membranes enclose them. The membranes are lipid bilayers with proteins embedded within the layers. The region between the two membranes is the intermembrane space. The inner membrane is folded to form cristae. This folding increases the surface area of the membrane and maximizes cellular respiration output. Inside the inner membrane is the mitochondrial matrix, and within the matrix, there are ribosomes, enzymes, and mitochondrial DNA. The mitochondrion can reproduce and synthesize proteins independently. It contains the enzymes necessary for transcription and the transfer of RNAs and ribosomes required for translation and protein formation. Recent research revealed that, instead of small, oblong, and independent entities in the eukaryotic cytoplasm, mitochondria form dynamic networks of long tubular structures undergoing fusions and separations during the cell cycle or after changes of growth conditions (Fig. 4.1; Dujon 2020; Lackner 2014).

4.1.2.3　Mitochondrial Genetics

Mitochondrial DNA (mtDNA) is typically a small circular double-stranded DNA molecule that encodes many proteins and RNA involved primarily in cellular respiration. In some fungi and protists, mtDNA can be linear (van de Vossenberg et al. 2018). Mitochondrial DNA is well conserved within taxa.

Unlike nuclear DNA passed on from both parents, mitochondrial DNA is generally uniparentally inherited (with some notable exceptions). In animals, mtDNA is passed on maternally through the egg. In plants, mtDNA may be passed on maternally, paternally, or biparentally. There is also evidence for paternal leakage of mtDNA, where the offspring inherits most of their mtDNA from their mother and receives a small amount from their father. Uniparental inheritance of mitochondria is a common phenomenon in sexual eukaryotes. It leads to little opportunity for genetic recombination between different lineages of mitochondria.

4.1.2.4　Mitochondrial Evolution

Researches on mitochondrion evolution began with animal mitochondrial DNA. There is a widely accepted generalization concerning molecular evolution rates: the more important the function of a gene or protein, the more slowly it undergoes an evolutionary change in the primary structure. As mitochondria have essential cellular functions, the life of animals is crucially dependent on these functions. Mitochondrial evolution would be expected to be highly constrained. Besides, animal mitochondria genome is small in size and relatively uniform in structure among vertebrate and invertebrate animals. The implication is strong that this genome was reduced at an early stage of animal evolution to the minimum size compatible with function.

However, evidence from restriction endonuclease cleavage maps implies that mtDNA evolves 5–10 times faster than single-copy nuclear DNA (Brown et al. 1979). The mean substitution rate in the three studied mitochondrial tRNA genes is at least 100 times higher than that in nuclear tRNA genes (Brown et al. 1982). The base substitution
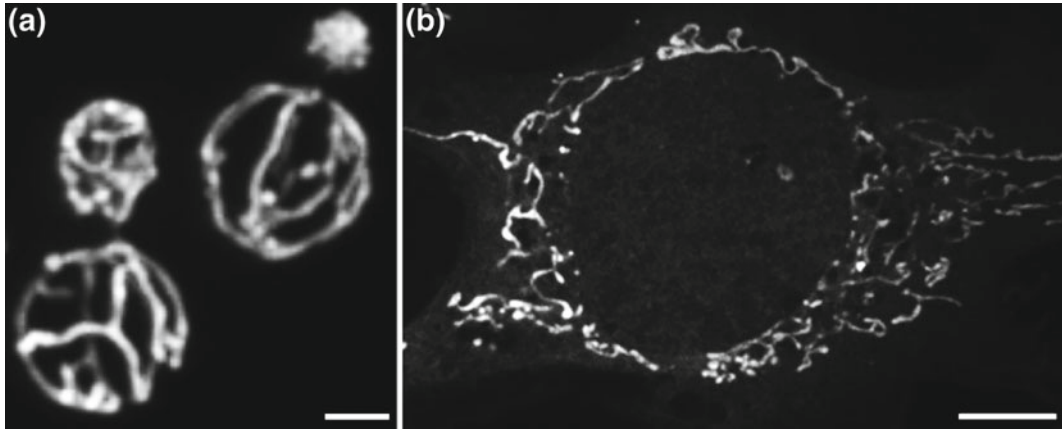
Fig. 4.1 Networks of mitochondria. **a** Mitochondria were forming connected, tubular networks. **b** Mitochondria were creating well-distributed tubular networks. Reprinted from Fig. 1 of Lackner (2014) with permissions

rate of mammalian mtDNA has been estimated to be 0.5–1.0% per lineage per 10^6 years (Brown 1980). For this reason, mitochondrial DNA is commonly used to study evolutionary relationships and population genetics in animals. Plant mtDNA evolves reasonably slowly and is less commonly used in evolutionary studies.

4.1.2.5 Mitochondrial Function

Mitochondria host the tricarboxylic acid (TCA) cycle and oxidative phosphorylation, breaking down sugars and fats into energy through aerobic respiration (cellular respiration). This metabolic process creates ATP, the energy source of a cell, through a series of steps that require oxygen. Beyond their well-known function as energy-producing entities, mitochondria are involved in a multitude of cellular processes, including the production of iron-sulfur clusters, calcium homeostasis, calcium signaling, haem synthesis, steroid synthesis, cellular differentiation, aging, programmed cell death (apoptosis), as well as maintaining the control of the cell cycle and cell growth (Malina et al. 2018).

The role of mitochondria in disease has been expanded beyond the respiratory chain, as defects in additional mitochondrial functions and behaviors have been linked to cancer, metabolic disorders, and neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's disease. Mutations in mitochondrial DNA can result in many human genetic disorders. Examples include diabetes, heart disease, myoclonic epilepsy, and Kearns-Sayre neuromuscular syndrome (Friedman and Nunnari 2014).

4.1.3 Research Methods For Mitochondrial Genome Analysis

4.1.3.1 Molecular Experiments

DNA Extraction

Genomic DNA could be extracted from fruiting bodies using Tiangen DNA Extraction Kit (DP305) or a fungal DNA kit (Cat. #D3390–00, Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions.

Next-Generation Sequencing

Whole genomic sequencing was conducted on an Illumina HiSeq 2500 Platform (Illumina, San Diego, CA, USA). Two 300 bp genomic DNA libraries were constructed for two *Ganoderma* species. For *G. meredithiae*, the produced paired-end reads were 100 bp long, while the paired-end reads of *G. applanatum* were 125 bp long.

4.1.3.2 Data Analyses

Mitochondrial Genome Assembly and Annotation

De novo assembly of the mitogenomes was performed using ABySS-pe version 1.5.2 (Simpson et al. 2009) for *G. meredithiae*, CLC Genomics Workbench Version 7.5.1 (CLC Bio, Aarhus, Denmark) for *G. applanatum*, and SPAdes 3.9.0 (Bankevich et al. 2012) for the five ones published in 2019. The assembled scaffolds were compared to the reference sequence using BLASTN version 2.2.23 (Altschul et al. 1990). The screened scaffolds were compared with the reference using Gepard version 1.30 (Krumsiek et al. 2007) and then assembled into a circular mitogenome sequence by manual editing. The coverage was examined using Tablet version 1.14.10.20 (Milne et al. 2013).

The mitogenome was annotated using MFannot (Lang et al. 2014) or MITOS (Bernt et al. 2013). The Sequence Manipulation Suite Version 2 (SMS2, http://www.bioinformatics.org/sms2/) was used to identify the hypothetical proteins. tRNA genes were initially identified using tRNAscan-SE (Lowe and Chan 2016). DNASTAR Lasergene (http://www.dnastar.com/) was used to analyze the base composition of the mitogenomes. The synonymous substitution rate (Ks) and the nonsynonymous substitution rate (Ka) for all PCGs in each of the mitogenomes were calculated using DnaSP (Rozas et al. 2017). Mitochondrial genome maps were generated by OGDRAW (Lohse et al. 2007).

Phylogenomic Analysis

The concatenated amino acid sequences were extracted from the GenBank files of the mitogenomes of Polyporales. They were then aligned using MAFFT v7.221 (Katoh et al. 2002) or MUSCLE version 3.6 (Edgar 2004). The neighbor-Joining analysis was conducted using MEGA version 6.06 (Tamura et al. 2013). Bootstrap values were calculated from 1,000 replicates.

4.2 Progress of Lingzhi Mitochondrial Genome

Up to date (October 2020), ten mitochondrial genomes of *Ganoderma* species were released from the NCBI database, seven of them recognized by the RefSeq database. Their information is summarized in Table 4.1. All of them were submitted by Chinese researchers.

4.2.1 Structure of Lingzhi Mitochondrial Genome

As listed in Table 4.1, three mitochondrial genomes of '*Ganoderma lucidum* (Curtis) P. Karst.' was released online. The identity of this species is *G. sichuanense* J.D. Zhao and XQ. Zhang, as detailed in the previous taxonomic researches (Wang et al. 2012; Thawthong et al. 2017; Yao et al. 2020, 2013).

Strain CGMCC 5.26 was isolated from the fruiting body collected in China and then deposited to China General Microbiological Culture Collection Center (CGMCC, Beijing, China) in 1963 for medicinal usage. Its mitogenome (HF570115) was first reported in this genus *Ganoderma* (Li et al. 2013). This mitogenome is a typical circular DNA molecule of 60,630 bp with 26.67% GC content. About 62.69% of the mitochondrial genome contains 50 genes encoding two ribosomal RNAs, 26 transfer RNAs, one ribosomal protein gene *rps3*, 14 genes involved in respiratory chain complexes, four ORFs in the intron of other genes (ip1–4), and two ORFs in the intergenic regions (*orf1* and *orf2*). These genes were in the same orientation, except those genes encoding for *trnW-CCA* and three ORFs. In total, thirteen introns were found in the protein-coding genes *cox1*, *cox2*, *cox3*, *nad4*, *nad5*, and *rnl* and *rns* genes. All introns were the group I introns, except for the single type II intron *cox1 i6*.

The other released mitogenome (KC763799 = NC_021750) of the same strain was submitted by Qian, but it was not published. It has only five base pairs more than the one

Table 4.1. Information on the released *Ganoderma* mitochondrial genomes

Taxon	Strain	Accession	Length	GC content (%)	References	Submitted date
G. applanatum	CGMCC 5.249	KR109212 NC_027188	119,803 bp	26.66	Wang et al. (2016a)	2015.04.13
G. calidophilum	s136	MH252535 NC_037938	124,588 bp	25.43	Li et al. (2019)	2018.04.20
G. leucocontextum	s116	MH252534 NC_037937	88,194 bp	27.08	Li et al. (2019)	2018.04.20
G. lucidum	CGMCC 5.26	HF570115	60,630 bp	26.67	Li et al. (2013)	2012.12.18
G. lucidum	CGMCC 5.26	KC763799 NC_021750	60,635 bp	26.67	Qian unpublished	2013.05.10
G. lucidum	s26	MH252532	57,932 bp	26.75	Li et al. (2019)	2018.04.20
G. meredithiae	CGMCC 5.766	KP410262 NC_026782	78,447 bp	26.14	Wang et al. (2016b)	2015.01.09
G. sinense	CGMCC 5.69	KF673550 NC_022933	86,451 bp	26.78	Qian unpublished	2013.09.14
G. sp.	s8	MH252531	57,232 bp	26.46	Li et al. (2019)	2018.04.20
G. tsugae	s90	MH252533 NC_037936	92,511 bp	26.67	Li et al. (2019)	2018.04.20

(HF570115) reported by Li et al. (Li et al. 2013), while the GC content is almost the same. The gene order is the same between them, as listed in Table 4.2. But the introns reported in *rnl* and *rns* of HF570115 were not identified, so only 11 introns exist in the mitogenome KC763799. An ideogram of the mitogenome organization and gene classification is shown in Fig. 4.2. The two mitogenomes were from the same strain and assembled from the same raw data. Thus, the differences do not reflect the genetic variation of the same species. These differences need to be further validated using Sanger sequencing.

The more recently released mitogenome (MH252532) of *G. lucidum* s26 was sequenced from the strain isolated from a fruiting body collected from Tai'an City, Shandong Province, China. A comparison between this one and KC763799 was described (Li et al. 2019). The nucleotide sequence similarity between the two mitogenomes was 95.18%. Furthermore, most protein-coding genes and tRNA genes were conserved between them. *Ganoderma* CGMCC 5.26 has longer intergenic regions than *G.*

lucidum s26 does. In addition, single nucleotide polymorphisms were frequently observed in the intergenic region of the two mitogenomes. Besides, *G. lucidum* s26 had one more intron (12 introns in total, one more in *rns*) than *G. lucidum* CGMCC 5.26, indicating that the mitochondrial genomes of *G. lucidum* from different origin have been differentiated.

4.2.2 Evolution of Mitochondrial Genome in Genus *Ganoderma*

Besides the three mitogenomes of *G. lucidum*, there are still seven ones released in this genus (Table 4.1). A mount of inter-specific variance has been observed (Table 4.2).

4.2.2.1 Mitochondrial Genome of *Ganoderma meredithiae*

The strain CGMCC 5.766 equals CBS 271.88. It is the holotype culture isolated from the holotype

Table 4.2 Gene contents in *Ganoderma* mitochondrial genomes

Gene order[#]	*G. lucidum*	*G. meredithiae*	*G. applanatum*	*G. sinense*	*G. tsugae*	*G. calidophilum*	*G. leucocontextum*	Gene order 2[#]
1	*rnl*	*rnl* (1i[*])	*rnl* (6i)	*rnl* (3i)	*rnl* (1i)	*rnl* (4i)	*rnl* (2i)	1
2	trnS-GCU	trnS-GCU						
3	atp6	atp6	atp6	atp6	atp6	atp6	atp6	3
2'					trnS-GCU			
37'		trnR-UCG						
4	trnM-CAU	trnM-CAU	trnM-CAU	trnM-CAU	trnM-CAU	trnM-CAU	*cox1* (9i)	15
5	trnH-GUG	trnH-GUG	trnH-GUG	trnH-GUG	trnH-GUG	trnH-GUG	*nad4* (1i)	14
6	trnS-UGA	trnS-UGA	trnS-UGA	trnS-UGA	trnS-UGA	trnS-UGA	rps3	35
7	trnM-CAU	trnM-CAU	trnM-CAU	trnM-CAU	trnM-CAU	trnM-CAU	nad6	34
8	trnY-GUA	trnY-GUA	trnY-GUA	trnY-GUA	trnY-GUA	trnY-GUA	atp8	33
9	trnK-UUU	trnK-UUU	trnK-UUU	trnK-UUU	trnK-UUU	trnK-UUU	trnS-GCU	32
10	trnQ-UUG	trnQ-UUG	trnQ-UUG	trnQ-UUG	trnQ-UUG	trnQ-UUG	nad2	31
11	trnT-UGU	trnT-UGU	trnT-UGU	trnT-UGU	trnT-UGU	trnT-UGU	*nad3* (1i)	30
12	trnF-GAA	trnF-GAA	trnF-GAA	trnF-GAA	trnF-GAA	trnF-GAA	trnG-UCC	29
13	trnA-UGC	trnA-UGC	trnA-UGC	trnA-UGC	trnA-UGC	trnA-UGC	trnW-CCA	28
14	*nad4* (1i)	*nad4* (1i)	*nad4* (2i)	*nad4* (1i)	*nad4* (1i)	*nad4* (1i)	trnD-GUC	27
15	*cox1* (6i)	*cox1* (10i)	*cox1* (14i)	*cox1* (12i)	*cox1* (11i)	*cox1* (14i)	atp9	26
16	trnE-UUC	trnE-UUC	trnE-UUC	trnE-UUC	trnE-UUC	trnE-UUC		
17	trnM-CAU	trnM-CAU	trnM-CAU	trnM-CAU	trnM-CAU	trnM-CAU	trnR-UCU	24
18	trnL-UAA	trnL-UAA	trnL-UAA	trnL-UAA	trnL-UAA	trnL-UAA	nad1	23
19	trnN-GUU	trnN-GUU	trnN-GUU	trnN-GUU	trnN-GUU	trnN-GUU	trnI-GAU	22
20	trnP-UGG	trnP-UGG	trnP-UGG	trnP-UGG	trnP-UGG	trnP-UGG	*cob* (4i)	21
37'				trnR-UCG			trnP-UGG	20
21	cob	*cob* (3i)	*cob* (6i)	*cob* (5i)	*cob* (4i)	*cob* (6i)	trnN-GUU	19

(continued)

Table 4.2 (continued)

Gene order[#]	G. lucidum	G. meredithiae	G. applanatum	G. sinense	G. tsugae	G. calidophilum	G. leucocontextum	Gene order 2[#]
22	trnI-GAU	trnI-GAU	trnI-GAU	trnI-GAU	trnI-GAU	trnI-GAU	trnL-UAA	18
23	nad1	nad1	nad1	nad1	*nad1* (2i)	*nad1* (1i)	trnM-CAU	17
24	trnR-UCU	trnR-UCU	trnR-UCU	trnR-UCU	trnR-UCU		trnE-UUC	16
25	trnC-GCA	trnC-GCA		trnC-GCA		trnC-GCA	trnS-GCU	2
26	atp9	atp9	atp9	atp9	atp9	atp9	trnM-CAU	4
27	trnD-GUC	trnD-GUC	trnD-GUC	trnD-GUC	trnD-GUC	trnD-GUC	trnH-GUG	5
28	trnW-CCA	trnW-CCA	trnW-CCA	trnW-CCA	trnW-CCA	trnW-CCA	trnS-UGA	6
29	trnG-UCC	trnG-UCC	trnG-UCC	trnG-UCC	trnG-UCC	trnG-UCC	trnM-CAU	7
30	nad3	nad3	nad3	nad3	nad3	nad3	trnY-GUA	8
31	nad2	nad2	nad2	nad2	nad2	nad2	trnK-UUU	9
32	trnS-GCU	trnS-GCU	trnS-GCU	trnS-GCU	trnS-GCU	nad6	trnQ-UUG	10
33	atp8	atp8	atp8	atp8	atp8	rps3	trnT-UGU	11
34	nad6	nad6	nad6	nad6	nad6	**trnS-GCU**	trnF-GAA	12
35	rps3	rps3	rps3	rps3	rps3	**atp8**	trnA-UGC	13
36	*cox2* (1i)	*cox2* (1i)	*cox2* (1i)	*cox2* (3i)	cox2	cox2	cox2	36
45		trnI-UAU						
44'		trnR-UCU						
37	trnR-UCG		trnR-UCG	trnR-UCG				
38	trnL-UAG	trnL-UAG	trnL-UAG	trnL-UAG	trnL-UAG	trnL-UAG	trnL-UAG	38
39	trnV-UAC	trnV-UAC	trnV-UAC	trnV-UAC	trnV-UAC	trnV-UAC	trnV-UAC	39
40	rns	rns	rns	*rns* (2i)	rns	rns	rns	40
37'					trnR-UCG	trnR-UCG	trnR-UCG	37'
37"				trnR-UCG[$]				
41	*nad5* (2i)	*nad5* (2i)	*nad5* (5i)	*nad5* (2i)	*nad5* (4i)	*nad5* (4i)	*nad5* (2i)	41
42	nad4L	nad4L	nad4L	nad4L	nad4L	nad4L	nad4L	42
43	*cox3* (1i)	cox3	*cox3* (1i)	*cox3* (2i)	*cox3* (1i)	*cox3* (1i)	cox3	43

(continued)

Table 4.2 (continued)

Gene order[#]	G. lucidum	G. meredithiae	G. applanatum	G. sinense	G. tsugae	G. calidophilum	G. leucocontextum	Gene order 2[#]
44			trnR-UCU	trnR-UCU		trnR-UCU		44
25"		trnC-GCA[$]						

The first column [#] Gene order is for the former six species; gene order 2 is for the last one, *G. leucocontextum*, showing the mitogenome's large rearrangements

[*]Number in the blankets denotes how many introns exist in the gene

[$]The gene is one more duplicate

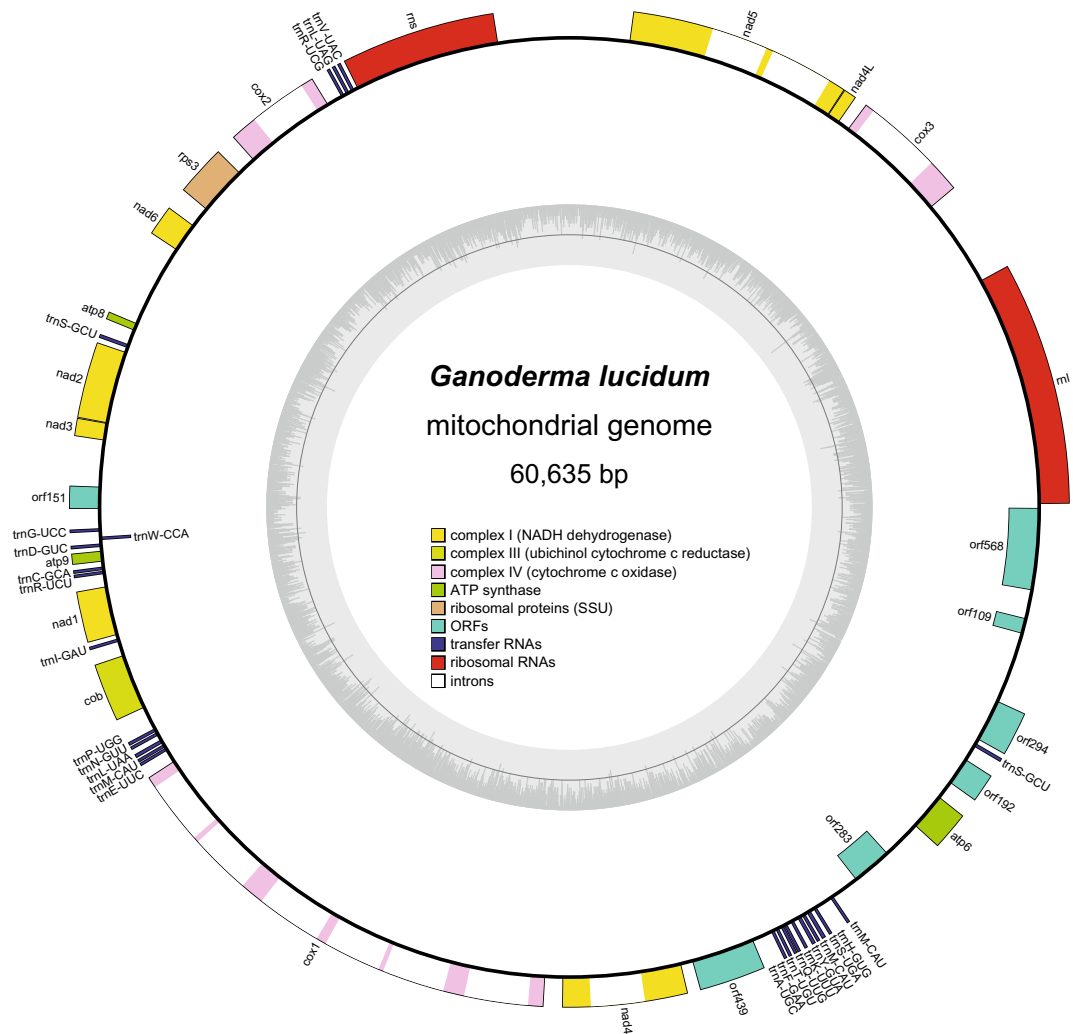Gene in bold of *G. calidophilum* denotes a rearrangement

Fig. 4.2 Mitochondrial genome map of *G. lucidum* CGMCC 5.26 (NC_021750)

specimen of *G. meredithiae* Adask. & Gilb. (JEA 345). JEA 345 is deposited in US National Fungus Collections (BPI, Beltsville, Maryland). The mitogenome of this species was reported by Wang et al. (2016b).

The 100 bp paired-end reads were assembled using ABySS-pe. The mitogenome (HF570115) of *G. lucidum* CGMCC 5.26 served as a reference sequence. A total of 1.35 GB clean data of 100 bp paired-end reads were produced. After assembly, 14,054 contigs were obtained. All the contigs were aligned with the reference sequence. And two contigs were identified as a mitochondrial origin. Contig 133 was 46,593 bp long, and contig 223 was 31,145 bp long. The two contigs shared a 390 bp long repeat sequence at both ends. The two contigs were assembled according to the reference sequence. Four gaps were detected in the preliminary mitochondrial genome sequence. They were then filled by comparing the assembly results produced by AbySS-pe software with parameters of k-mer 25 and k-mer 64. The authors mapped reads onto this genome sequence to validate this mitogenome's assembly and check its coverage. A total of 546,887 reads were mapped, accounting for 4.0% of all the reads produced (546,887/13,512,066). The average coverage depth was 693.5, and the maximum coverage depth was 1,552.

This mitogenome is a circular mtDNA with a total length of 78,447 bp and a GC content of 26.14%. It encodes a set of mitochondrial protein and RNA genes, including 15 conserved proteins, 29 tRNAs, large and small ribosomal RNAs, and 18 homing endonucleases. All structural genes are located on the same strand except *trnW-CCA* (Fig. 4.3). The gene order is listed in Table 4.2. The tRNA genes code for all 20 standard amino acids. Most amino acids are coded by only one tRNA gene; however, two *trnC-GCA*, two *trnI* (*trnI-GAU* and *trnI-UAU*), two *trnL* (*trnL-UAA* and *trnL-UAG*), three *trnM-CAU*, three *trnR* (one *trnR-UCG* and two *trnR-UCU*), and three *trnS* (one *trnS-UGA* and two *trnS-GCU*) are found in this mitogenome. Eighteen introns are detected in six genes: *cob* (3),

cox1 (10), *cox2* (1), *nad4* (1), *nad5* (2), and *rnl* (1). For each intron, there is one homing endonuclease. Three of the 18 endonucleases are GIY-YIG type (ip4, ip10, and ip17), and the others are LAGLIDADG type.

Compared with the mitogenome of *G. lucidum* (NC_021750), *G. meredithiae* has larger genome content, lower GC content, three more tRNA genes (*trnC-GCA*, *trnI-UAU*, and *trnR-UCG*), and seven more introns in the protein-coding or rRNA genes.

4.2.2.2   Mitochondrial Genome of *Ganoderma applanatum*

The strain CGMCC 5.249 of *G. applanatum* (Pers.) Pat. was isolated from a basidiocarp collected from Changbai Mountain, Jilin Province, China. The mitogenome of this species was reported by Wang et al. (2016a).

The 125 bp pair-end reads were assembled using CLC Genomics Workbench (CLC Bio, version 7.5.1, Aarhus, Denmark). The authors used the mitogenome of *G. meredithiae* CGMCC 5.766 as the reference to identify the scaffolds and to determine their order. Manual comparison of the ends of the ordered scaffolds helped to assemble the scaffolds into a circular molecule.

A total of 1.54 GB clean data of 125 bp paired-end reads were produced. After assembly, 21,718 contigs were obtained. All the contigs were aligned with the reference sequence, and three contigs were identified as a mitochondrial origin. Contigs 21, 22, and 47 were 35,819 bp, 83,529 bp, and 304 bp long, respectively. The contigs were assembled in the order: 22-47-21-47. A 287 bp long repeat sequence was shared by the tail of contig 22 and the head of contig 47; a 21 bp long repeat sequence was shared by the tail of contig 47 and the heads of contigs 21 and 22; another 21 bp long repeat sequence was shared by the tail of contig 21 and the head of contig 47. No gap was detected in the assembled mitochondrial genome sequence. To validate the mitogenome assembly, the authors mapped reads onto this genome sequence. A total of 2,000,647 reads were mapped, accounting for 16.2% of all
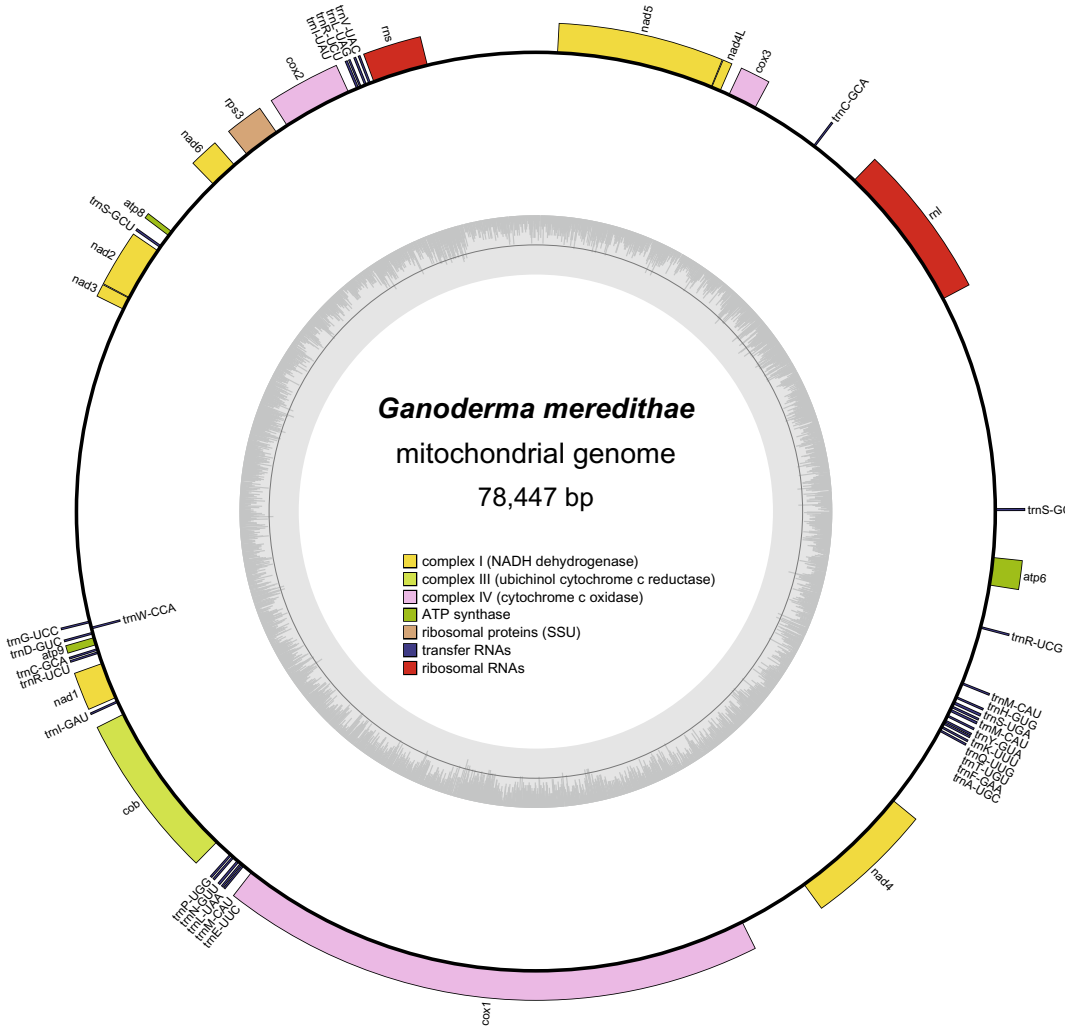
Fig. 4.3 Mitochondrial genome map of *G. meredithiae*

the reads produced. The average coverage depth was 2,086.9, and the maximum coverage depth was 3,458.

The mitogenome of *G. applanatum* is 119,803 bp long, with the GC content being 26.66%. It encodes 15 conserved proteins, 25 tRNAs, and the large and small ribosomal RNAs. All structural genes are located on the same strand except *trnW-CCA* (Fig. 4.4). The gene order is listed in Table 4.2. The tRNA genes contain codons for all 20 standard amino acids except Cysteine. Most amino acids are represented by only one tRNA gene; however, two *trnL* (*trnL-UAA* and *trnL-UAG*), two *trnS* (*trnS-GCU* and *trnS-UGA*), three *trnM-CAU*, and three *trnR* (one *trnR-UCG* and two *trnR-UCU*) genes are found in this mitogenome. Thirty-five introns are detected in seven genes, i.e., *cob* (6), *cox1* (14), *cox2* (1), *cox3* (1), *nad4* (2), *nad5* (5), and *rnl* (6).

Compared with the mitogenome of *G. lucidum* (NC_021750), *G. applanatum* has larger genome content, almost the same GC content, one less tRNA gene, and 24 more introns in the protein-coding or rRNA genes.

Fig. 4.4 Mitochondrial genome map of *G. applanatum*

4.2.2.3 Mitochondrial Genome of *Ganoderma sinense*

The strain CGMCC 5.69 of *G. sinense* J.D. Zhao, L.W. Hsu, and X.Q. Zhang was isolated from a basidiocarp collected from Hainan Province, China. This mitogenome was submitted by J. Qian, Institute of Medicinal Plant Development, Beijing, China.

The mitogenome of *G. sinense* is 86,451 bp long, with the GC content being 26.78%. It encodes 15 conserved proteins, 27 tRNAs, and the large and small ribosomal RNAs. All of them are located on the same strand except *trnW-CCA*. The gene order is listed in Table 4.2. A total of 30 introns exist in this mitogenome. Compared with the mitogenome of *G. lucidum* (NC_021750), *G. sinense* has larger genome content, higher GC content, one more tRNA gene, and 19 more introns in the protein-coding or rRNA genes. In detail, *trnS-GCU* is missing; two *trnR-UCG* and one *trnR-UCU* are added; three more introns in *rnl*, six more in *cox1*, five more in *cob*, two more in *cox2* and *rns*, respectively, and one more in *cox3*.

4.2.2.4 Mitochondrial Genome of *Ganoderma tsugae*

The strain s90 of *G. tsugae* Murrill was isolated from a basidiocarp collected from Wangqing County, Jilin Province, China. This mitogenome was published by Li et al. (2019).

The mitogenome of *G. tsugae* is 92,511 bp long, with the GC content being 26.67%. It encodes 15 conserved proteins, 25 tRNAs, and the large and small ribosomal RNAs. All structural genes are on the same strand except *atp6*, *trnS-GCU*, and *trnW-CCA*. The gene order is listed in Table 4.2. A total of 24 introns exist in this mitogenome. Compared with the mitogenome of *G. lucidum* (NC_021750), *G. tsugae* has larger genome content, the same GC content, one less tRNA gene, and 13 more introns in the protein-coding or rRNA genes. That is, *trnC-GCA* is missing; the locations of *trnS-GCU* and *trnR-UCG* are changed; one more intron in *rnl*, five more in *cox1*, four more in *cob*, two more in *nad1* and *nad5*, respectively.

4.2.2.5 Mitochondrial Genome of *Ganoderma calidophilum*

The strain s136 of *G. calidophilum* J.D. Zhao, L. W. Hsu, and X.Q. Zhang was isolated from a basidiocarp collected from Qiongzhong County, Hainan Province, China. This mitogenome was published by Li et al. (2019).

The mitogenome of *G. calidophilum* is the largest one in this genus up to now. It is 124,588 bp long, with the GC content of 25.43%. It encodes 15 conserved proteins, 25 tRNAs, and the large and small ribosomal RNAs. All of them are on the same strand except *trnW-CCA*. The gene order is listed in Table 4.2. A total of 31 introns exist in this mitogenome. Compared with the mitogenome of *G. lucidum* (NC_021750), *G. calidophilum* has larger genome content, lower GC content, one less tRNA gene, and 20 more introns in the protein-coding or rRNA genes. Specifically, *trnS-GCU* is missing, and the locations of *trnR-UCU* and *trnR-UCG* are changed; *trnS-GCU* and *atp8* are shifted to the region between *rps3* and *cox2*. In particular, there are four more introns in *rnl*, eight more in *cox1*, six more in *cob*, one more in *nad1*, and two more in *nad5*.

4.2.2.6 Mitochondrial Genome of *Ganoderma leucocontextum*

The strain s116 of *G. leucocontextum* TH Li et al. was isolated from a basidiocarp collected from Huidong County, Sichuan Province, China. This mitogenome was published by Li et al. (2019).

The mitogenome of *G. leucocontextum* is 88,194 bp long, with the GC content being 27.08%. It encodes 15 conserved proteins, 25 tRNAs, and the large and small ribosomal RNAs. A total of 22 genes are on the same strand, and 20 genes are on the anti-sense strand. The gene order is listed in Table 4.2. A total of 19 introns exist in this mitogenome. Compared with the mitogenome of *G. lucidum* (NC_021750), the gene order of *G. leucocontextum* is much different, as shown in Table 4.2. The tRNA genes (gene order 4 to 13) are not located close to *atp6* (gene order 3); in contrast, they are shifted to the region before *cox2* (gene order 36). And there is a big reverse region including genes from order 16 to 35. This arrangement of mitochondrial genes has not been seen in other *Ganoderma* species. It has larger genome content, higher GC content, one less tRNA gene, and eight more introns in the protein-coding or rRNA genes. In detail, *trnC-GCA* is missing. There are two more introns in *rnl*, three more in *cox1*, one more in *nad3*, and four more in *cob*.

4.2.2.7 Mitochondrial Phylogenomics of *Ganoderma*

To investigate the mitochondrial phylogeny of *Ganoderma*, the accessible mitogenomes in Polyporales were all included: *Phlebia radiate* (NC_020148), *Taiwanofungus camphoratus* (NC_042771), *Trametes cingulata* (NC_013933), *Trametes hirsute* (NC_037239), and *Wolfiporia cocos* (NC_050681). Amino acid sequences of the 15 conserved protein-coding genes were extracted from the mitogenomes. The 15 proteins included subunits of the respiratory chain complexes (*cox1*, *cox2*, *cox3*, and *cob*), ATPase subunits (*atp6*, *atp8*,

and *atp9*), NADH:quinone reductase subunits (*nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, and *nad6*), and ribosomal protein S3 (*rps3*). The alignment of the concatenated 15 genes is 5837 amino acid long.

As shown in Fig. 4.5, the genus *Ganoderma* is monophyly and contains nine sequenced members. *Ganoderma lucidum* and *G.* sp. s8 clustered in the same branch with very short branch lengths and very strong support, hinting that they are the same species. *Ganoderma meredithae* has the closest relationship with *G. lucidum*. *Trametes* is the sister group of *Ganoderma*, consistent with the previous results (Wang et al. 2016a, b; Li et al. 2019).

Ganoderma calidophilum has the largest mitogenome among the *Ganoderma* members, while *G.* sp. s8 has the smallest one. The former is more than twice in mitogenome size as the latter (Table 4.1). Except *for G. lucidum* (including *G.* sp. s8), *G. meredithae* has the smallest mitogenome. This might suggest that the evolution of the mitochondrial genome of *Ganoderma* correlates with the phylogeny in some branch to some extent. For GC content, *G. leucocontextum*

has the highest value, while *G. calidophilum* has the lowest one. For the number of tRNAs, *G. meredithae* has the most genes (29), while *G. applanatum*, *G. calidophilum*, *G. leucocontextum*, and *G. tsugae* have the least genes (25). For the number of introns, *G. applanatum* has the most (35); in contrast, *G. lucidum* has the least (11–13). The mitogenomes of *Ganoderma* presented highly genetic diversity in terms of genome content, gene order and structure, and so on.

4.3 Future Perspective

4.3.1 Problems

Ganoderma species have high medicinal values (Wang et al. 2012). There are three *Ganoderma* species recorded in the Chinese pharmacopeia, i.e., *G. lucidum*, *G. sinense*, and *G. tsugae*. The mitogenomes of them had been published or recovered in the previous researches and compared here. Nevertheless, most of the mitogenomes of the species in this genus *Ganoderma*

Fig. 4.5 Mitochondrial phylogenomic tree of Polyporales
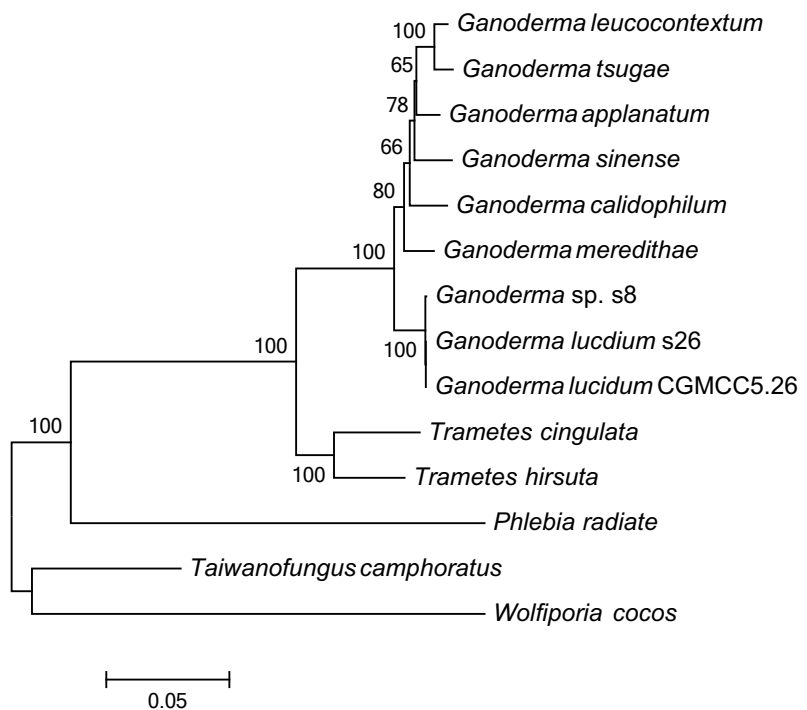
have not been studied. More than 80 phylogenetic species of *Ganoderma* were recognized in a meta-analysis of internal transcribed spacer (ITS) rDNA sequences (Fryssouli et al. 2020), and many of them have been proven to be with significant medicinal or ecological values. Only seven *Ganoderma* species (Table 4.1 and Fig. 4.5) were investigated for their mitochondrial genomes. More intense sampling is in need for the future mitogenome researches of *Ganoderma* at both inter- and intra-specific levels.

4.3.2   Topics of Interests

ITS has demonstrated high efficacy in resolving relationships amongst most of the *Ganoderma* taxa; however, it was not equally useful at elucidating species boundaries across the entire genus (Fryssouli et al. 2020). Mitochondrial small subunit (*mtSSU = rns*) gene was used to infer this genus's phylogeny (Hong and Jung 2004). However, the incongruence between mitochondrial and nuclear phylogenies of the genus was detected based on three mitochondrial genes and seven nuclear genes (Wang 2012).

On the other hand, phylogenetic analyses based on different single genes of mitogenome recovered incongruent tree topologies. But the BI phylogeny based on the *cox1* gene was mostly consistent with that generated using combined datasets, indicating this gene could be a potential molecular marker for phylogenetic analysis (Li et al. 2019). These findings suggest that selecting suitable molecular markers is essential for studying phylogenetic relationships in the *Ganoderma* genus.

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