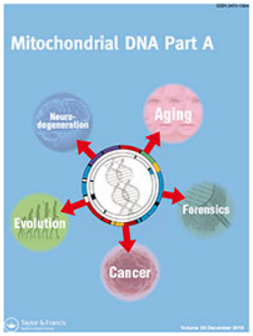

Taylor & Francis
Taylor & Francis Group

Mitochondrial DNA Part A

DNA Mapping, Sequencing, and Analysis

ISSN: 2470-1394 (Print) 2470-1408 (Online) Journal homepage: https://www.tandfonline.com/loi/imdn21

A new insight into the classification of dusky thrush complex: bearings on the phylogenetic relationships within the Turdidae

Yuanqiu Dong, Bo Li & Lizhi Zhou

To cite this article: Yuanqiu Dong, Bo Li & Lizhi Zhou (2018) A new insight into the classification of dusky thrush complex: bearings on the phylogenetic relationships within the Turdidae, Mitochondrial DNA Part A, 29:8, 1245-1252, DOI: 10.1080/24701394.2018.1439026

To link to this article: https://doi.org/10.1080/24701394.2018.1439026

Published online: 18 Feb 2018.

MITOCHONDRIAL DNA PART A
2018, VOL. 29, NO. 8, 1245–1252
https://doi.org/10.1080/24701394.2018.1439026

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

A new insight into the classification of dusky thrush complex: bearings on the phylogenetic relationships within the Turdidae

Yuanqiu Dong[a,b], Bo Li[a,b] and Lizhi Zhou[a,b]

[a]School of Resources and Envirodnmental Engineering, Anhui University, Hefei, China; [b]Anhui Biodiversity Information Center, Hefei, Anhui, China

ABSTRACT

Dusky thrush complex comprises of two sister species breeding in SC Siberia, which is the member of thrush *Turdus* from Turdidae. The phylogenetic resolution of Dusky thrush complex remains controversial, and a detailed research is still necessary. In this research, we determined the complete mtDNAs of both species, and estimated phylogenetic trees based on the mtDNA alignment of these and 21 other Turdidae species, to clarify the taxa status of the Dusky thrush complex. The squenced lengths of these three mitochondrial genomes were 16,737, 16,788 and 16,750 bp. The mtDNAs are circular molecules, containing the 37 typical genes, with an identical gene order and arrangement as those of other Turdidae. The ATG and TAA, respectively, are observed the most commonly start and stop codon. Most of the tRNA could be folded into the canonical cloverleaf secondary structure except for $tRNA^{Ser}$ (AGY) and $tRNA^{Leu}$ (CUN), which lose 'DHU' arm. The control region presented a higher A + T content than the average value for the whole mitogenome. The phylogenetic trees reconstructed by the concatenated nucleotide sequences of mtDNA genes (Cyt b, ND2 and COI) indicate the Dusky thrush complex cannot be divided into two species, but the relationships between Dusky thrush subspecies still need additional study. This study improves our understanding of mitogenomic structure and evolution of the Dusky thrush complex, which can provide further insights into our understanding of phylogeny and taxonomy in Turdidae.

ARTICLE HISTORY
Received 29 November 2017
Accepted 1 February 2018

KEYWORDS
Dusky thrush complex; turdidae; complete mitochondrial genome; phylogenetic relationship

Introduction

The widespread avian family Turdidae is a large group of passerine genera, with more than 340 species (Monroe and Sibley 1993). *Turdus* is by far the largest genus in Turdidae, comprising 65 species, with considerable disagreement between authors regarding the taxonomic status of certain taxa (Sibley and Monroe 1990; Kratter 2014). In addition, there is a controversial question about the taxonomic status of Dusky Thrush complex, which is a member of *Turdus*. Dusky Thrush complex composes of two subspecies: *Turdus naumanni naumanni* and *Turdus naumanni eunomus* (Zheng 1994). Recent studies about relationships among Turdidae species are based mostly on morphological and ecological characters. They showed differences in the pattern and/or coloration of head, upperparts, breast, tail, bill and legs (Cramp 1988; Clement 1999), as also in habitat. Accordingly, some authors believe *Turdus naumanni* and *Turdus eunomus* are best treated as two distinct species (Sangster et al. 2007). However, this view has been challenged recently, in particular, the taxonomy and systematic relationships within the Dusky Thrush complex remain problematic. The existence of intermediate specimens suggested they combined in a single species. However, there are no detailed studies of the interactions of *Turdus naumanni* and *Turdus eunomus,* and there is no evidence to suggest that these taxa are merging into a single population (Sangster et al. 2009). A recent study approved the former view, based on the non-overlapping breeding ranges and relatively rare hybridization between them (Sangster et al. 2009). A record of *Turdus naumanni* was published that compared with *Turdus eunomus* metrically by four of skeletal element. The measurements show significant similarities (Milchev and Boev 2014). But so far, *Turdus eunomus* is now recognized as a separate species, which is still in doubt.

Mitochondrial (mt) DNA is a double-stranded maternal inheritance genome, lack of recombination and had accelerated nucleotide substitution rates (Ingman et al. 2000; Liu et al. 2014). Usually, mtDNA is covalently closed circular molecule ranged from 15 to 17 kb in length and possesses a remarkably conserved set of 37 genes: 13 protein-coding genes, two rRNA genes, 22 putative tRNAs and one non-coding A+T-rich region (Saccone et al. 1991; Wolstenholme 1992; Boore 1999; Yuan et al. 2012). Compared with nuclear genes, mtDNA is conservative in gene content, abundant in cells, and intronless, but contains much important phylogenetic information. For these advantages, the mtDNA has become a major tool of phylogenetics and population genetic studies (Gissi et al. 2008; Rheindt et al. 2013). Under the rapid development of molecular biology, a few mitochondrial loci,

CONTACT Zhou Lizhi zhoulz@ahu.edu.cn Institute of Wetland Ecology and Biodiversity, School of Resources and Environmental Engineering, Anhui University, 111 Jiulong Road, Hefei 230601, China

Table 1. Primer sequences used in this study.

Primer no.	Amplifying area	Upper primer sequence (5′–3′)	Lower primer sequence (5′–3′)
1	73–2128	AGACTTAGTCCTAACCTTAC	GACCTCGTTTAGCCATTCATAC
2	1897–3439	CGACAGAGGAGCGTCCATTA	AGGTCGAATGGGGCTCGGTT
3	3171–4977	CTMGCAGTRTACTCCATCCT	TGTTTTATGTGGTTKGTDGT
4	4814–6561	TTCCTBCCNAAATGAYTAAT	TAGTAGGTGTCGTGTAGGGC
5	6431–8067	GGRGGMACAATCAAATGAGA	GTTCATGGTCAGGTTCAGGGGG
6	7861–9402	TTTTAAGCTAGAGAAAGAGG	TATCATGCTGCTGCTTCRAAGCC
7	9282–11908	GCCATRGARTACTACGAAGC	CATTACTTTCACTTGGATTTGCA
8	11692–13581	CCYATATGCAAGTATAGTTT	CAGGAGRGGTCRATTAAGTGGGA
9	13040–14773	CTAGCCCTAATAGGAACSCC	TGTTCDACTGGTTGGCTTCC
10	14613–16215	CTAGCCCTAGCCGCYTCHGT	AGGAACCAGWGGCGCAAAAG
11	16105–190	ACCCATTCATCATCATTGGC	GCCTGATGCCCGCTCCTATC

including Cyt b, ND2 and COI sequence become the represent gene for phylogenetics research (Klicka et al. 2005). Combinations of *Cyt b*, *ND2*, and *COI* gene sequences have been applied to phylogenetic problems at a variety of levels, ranging from related species to genera and families; they have been especially valuable for clarifying phylogenetic relationships within some controversial animal groups, especially that of some bird (Wilson 1991; Donnegoussé et al. 2002; Near et al. 2003; Gonzalez et al. 2009). As adequate animal mitochondrial genomes were sequenced, morphological characters are no longer sufficient for phylogenetic studies, establishing a phylogeny for Turdidae will facilitate study of Dusky Thrush complex evolutionary interest.

In this study, we have three groups of samples which were recognized as *Turdus naumanni* (T-1), the intermediate specimen (T-2) and *Turdus eunomus* (T-3). This gave us a tremendous opportunity to work on this taxonomic controversial issue. We sequenced the complete mitochondrial genome of Dusky Thrush complex, and analyzed the mitochondrial sequence data to investigate the distinction between three subspecies of Dusky Thrush complex. Furthermore, we used published sequences of mtDNA genes (*Cyt b*, *ND2* and *COI*) in GenBank from 21 Turdidae species to address the phylogenetic relationship of Dusky Thrush complex. The results may shed increased light on the phylogenetic status of three subspecies in Dusky Thrush complex and be used as a basis for further study of some Turdidae species.

Materials and methods

Ethical statement

No specific permits were required for the specimens collected for this study. The specimens were collected from the dead birds illegally hunted and transported, which were confiscated by Huangpu Mountain Forest Police Station, Chuzhou County, Anhui Province, China on 7 May 2013. Our experimental procedures complied with the current laws on animal welfare.

Sample collection

The samples were collected from the dead birds. The samples are field-stored at −20 °C at the Institute of Biodiversity and Wetland Ecology, School of Resources and Environmental Engineering, Anhui University.

DNA extraction, primer design and PCR amplification

Total genomic DNA was extracted by an over-night incubation at 37 °C in lysis buffer (10 mmol/L Tris (pH 7.5), 25 mmol/L EDTA, 75 mmol/L NaCl, 1% sodium dodecyl sulphate (SDS) and 1 mg of Proteinase K (TransGen, Beijing, China) and standard phenol–chloroform procedures (Sambrook et al. 2001). Extracted DNA was examined on a 1.0% agarose/TBE gel and stored at −20 °C as templates for PCR.

Based on alignment of complete mtDNA sequences of *Cyanoptila cyanomelana* (NC_015232), *Ficedula zanthopygia* (NC_015802) and *Luscinia calliope* (NC_015074), we designed eleven primer pairs using Primer 5.0 (Table 1). These primers were used to amplify and sequence the complete mtDNAs of T-1, T-2 and T-3. All generated sequences were less than 2000 bp each, with each segment overlapping the next by 80–150 bp.

PCR amplifications were carried out in 50-μl volumes containing 100 ng template DNA, 5 μl of 10 × reaction Buffer, 2 μl of 25 mM $MgCl_2$, 4 μl of 2 mM dNTPs, 1 μl of each 10 mM primer, 0.5 U Taq DNA polymerase (Trans Taq-T DNA Polymerase, Beijing, China), and sterile doubly distilled water to final volume. PCR amplification conditions were as follows: denaturation for 5 min at 94 °C, followed by 30 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 49–55 °C (depending on primer combinations), and elongation for 2 min at 72 °C, and a final extension step for 10 min at 72 °C. PCR products were purified and bidirectionally sequenced by Sangon Biotech Co., Ltd. (Shanghai, China).

Data analysis

Nucleotide sequences were edited using the program DNAstar (DNAstar Inc., Madison, WI) and aligned by Clustal X, protein-coding genes were identified using Sequin 5.35 and improved manually and comparing with the corresponding known complete mtDNA sequence of Turdidae. The 22 tRNA genes were identified using software tRNA Scan-SE 1.21 (http://lowelab.ucsc.edu/tRNAscan-SE) and their cloverleaf secondary structure and anticodon sequences were identified using DNASIS (Ver.2.5, Hitachi Software Engineering, Shiga, Japan). The complete mitochondrial genome sequence of *Turdus naumanni* and *Turdus eunomus* had been deposited in GenBank under accession nos. KJ834096 and KM015261.

To further discuss the phylogenetic relationships among the Turdidae, we got part of mtDNA sequences of 21 other Turdidae species from GenBank (Table 2), and phylogenetic

Table 2. The mitochondrial genomes used in this study.

Species	COI	Cytb	ND2	Complete
Catharus bicknelli	DQ433447.1	AY049490.1	AY049520.1	
Catharus dryas	FJ027323.1	AY049491.1	AY049516.1	
Cercomela melanura	JX255939.1	HM633267.1	JX256073.1	
Chaimarrornis leucocephalus	HM633274.1	JQ174395.1	KJ455369.1	
Copsychus malabaricus	JF498845.1	HM633277.1	DQ466859.1	
Copsychus saularis	KC354899.1	HM633278.1	KJ455390.1	
Ixoreus naevius	JN850755.1	AY049485.1	AY049512.1	
Monticola solitarius	AB842947.1	KJ456346.1	KJ455506.1	
Myadestes townsendi	EF484236.1	AF295082.1	DQ469609.1	
Oenanthe oenanthe	GU571510.1	GU055482.1	GU358816.1	
Saxicola ferrea	JQ176181.1	KJ456455.1	KJ455632.1	
Saxicola torquata	AB843133.1	KJ456456.1	FJ705788.1	
Sialia mexicana	DQ434074.1	DQ463684.1	AY752359.1	
Turdus hortulorum				NC_024552.1
Sialia sialis	EU525498.1	HM633380.1	AY049510.1	
Thamnolaea cinnamomeiventris	JX255950.1	HM633392.1	GU237124.1	
Turdus chrysolaus	GQ482817.1	DQ910940.1	DQ911070.1	
Turdus naumanni				KJ834096.1
Turdus eunomus				NC_028273.1
Turdus intermediate form				
Passer montanus	–	–	–	JX486030.1

analysis was performed using neighbour-joining (NJ) and maximum-parsimony (MP) algorithms implemented in MEGA 4.0 (MEGA Inc., Ocheyedan, IA) based on the concatenated sequences of mtDNA genes (*Cyt b*, *ND2* and *COI*) published in GenBank. *Passer montanus* was used as the out-group. The confidence values of both trees were evaluated by bootstrap analysis with 1000 replicates. *Passer montanus* is selected as outgroup.

Results

Genome structure and base composition

The complete sizes of the mitochondrial genome obtained from T-1, T-2 and T-3 are, respectively, 16,737, 16,788 and 16,750 bp. The completely sequenced mitochondrial genomes contained the D-loop, 13 genes encoding respiration-related proteins; 22 tRNA molecules; and 2 rRNA molecules (Table 3). Nine of the 37 genes, *ND6*, $tRNA^{Gln}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$, $tRNA^{Tyr}$, $tRNA^{Ser}$, $tRNA^{Glu}$, and $tRNA^{Pro}$, were found to be encoded by the light strand of mitochondrial DNA. AT-skew and GC-skew are a measure of compositional asymmetry. AT-skew values were always positive, while GC-skew values were negative. The AT-skew values of T-1, T-2 and T-3 were 0.115, 0.115 and 0.116, respectively. The GC-skew values were of T-1, T-2 and T-3 were −0.370, −0.368 and −0.365, respectively. Gene arrangement was highly conserved within the Turdus, without showing any structural rearrangement.

Protein-coding genes

Of these 13 PCGs, the *ND5* gene was the longest, which was 1818 bp in length, and the ATP8 was the smallest PCG having a size of 168 bp. Most genes are started with the methionine codon (ATG and ATA), with the exception of ND2 and COI, which presented the alternative starting codon GTG. In regard to stop codons, T-1 had most genes ending with the TAN codon (ND2, COII, ATPase8, ATPase6, ND3, ND4L, Cyt b and ND6) and the T- codon (COIII and ND4). Two genes ended with AGG (ND1, COI) and another with AGA mitochondrial-specific stop codons (ND5). One gene ended with different stop codons in T-2 and T-3 mitogenome: ND1 with an AGA instead of AGG.

Ribosomal, transfer RNA and non-coding region

The arrangement of the 12S rRNA and 16S rRNA are located between the $tRNA^{Phe}$ and $tRNA^{Leu}$ genes, and separated by the $tRNA^{Val}$. T-1 and T-3 have the same size of 16 S rRNA (1600 bp), which is less than T-2 (1608 bp). The size of 12 S rRNA is the same in T-1, T-2 and T-3. The mitochondrial genomes of T-1, T-2 and T-3 both encode 22 tRNA genes, ranging from 64 to 75 bp in length. These genes of the sequence range from 64 to 77 bp in length, most of them could be folded into the canonical cloverleaf secondary structure except for $tRNA^{Ser}$ (AGY) and $tRNA^{Leu}$ (CUN), which lose 'DHU' arm.

The non-coding regions include a control region (D-loop) and a few intergenic spacers. The control region presented a higher A + T content than the average value for the whole mitogenome. The sizes of the control region in T-1, T-2 and T-3 mitochondrial genome are, respectively, 1253, 1296 and 1267 bp in length. Among the intergenic spacers, the longest one in T-1 and T-3 is 10 bp in length between $tRNA^{Ser}$ and COII, while 47 bp in T-2 between $rRNA^{Lus}$ and $tRNA^{Leu}$.

Phylogenetic tree

The NJ and MP phylogenetic trees, which are based on mtDNA sequences of Cyt b and D-loop genes among 21 Turdidae species, have the same topology (Figure 1). These species could be divided into four groups: *Turdus eunomus*, the intermediate, *Turdus naumanni*, *Thurdus chrysolaus* and *Thurdus hortulorum* form the first group (group A); *Ixoreus naevius*, *Catharus bicknelli* and *Catharus dryas* form the second group (group B); *Myadestes townsendi*, *Sialia mexicana* and *Sialia sialis* forms the third group (group C); another evolutionary line is more complex, including five groups

Table 3. Organization of the mitochondrial genome of *Turdus naumanni*.

Gene	Direction	Nucleotide no	Size	Spacer (+) or Overlap (−)	Start codon	Stop codon
$tRNA^{Phe}$	F	1–68	68	0		
rRNA-Ssu	F	69–1053	985	−8		
$tRNA^{Val}$	F	1046–1115	70	0		
rRNA-Lus	F	1116–2715	1600	−2		
$tRNA^{Leu}$	F	2714–2788	75	4		
ND1	F	2793–3770	978	6	ATG	AGA
$tRNA^{Ile}$	F	3777–3848	72	6		
$tRNA^{Gln}$	R	3855–3925	71	−1		
$tRNA^{Met}$	F	3925–3993	69	0		
ND2	F	3994–5034	1041	−1	GTG	TAA
$tRNA^{Trp}$	F	5034–5103	70	1		
$tRNA^{Ala}$	R	5105–5173	69	4		
$tRNA^{Asn}$	R	5178–5252	75	0		
$tRNA^{Cys}$	R	5253–5319	67	−1		
$tRNA^{Tyr}$	R	5319–5389	71	1		
COI	F	5391–6941	1551	−11	GTG	AGG
$tRNA^{Ser}$	R	6933–7005	73	3		
$tRNA^{Asp}$	F	7009–7078	70	10		
COII	F	7089–7772	684	0	ATG	TAA
$tRNA^{Lys}$	F	7773–7840	68	2		
ATP8	F	7843–8010	168	−10	ATG	TAA
ATP6	F	8001–8684	684	5	ATG	TAG
COIII	F	8690–9473	784	0	ATG	T-
$tRNA^{Gly}$	F	9474–9542	69	0		
ND3	F	9543–9893	351	1	ATG	TAA
$tRNA^{Arg}$	F	9895–9964	70	1		
ND4L	F	9966–10262	297	−7	ATG	TAA
ND4	F	10256–11633	1378	0	ATG	T-
$tRNA^{His}$	F	11634–11703	70	−3		
$tRNA^{Ser}$	F	11701–11766	66	2		
$tRNA^{Leu}$	F	11769–11839	71	0		
ND5	F	11840–13657	1818	8	ATG	AGA
Cytb	F	13666–14808	1143	1	ATG	TAA
$tRNA^{Thr}$	F	14810–14878	69	6		
$tRNA^{Pro}$	R	14885–14954	70	9		
ND6	R	14964–15482	519	1	ATG	TAG
$tRNA^{Glu}$	R	15484–15555	72	0		
Control region	F	15556–16737	1182	0		

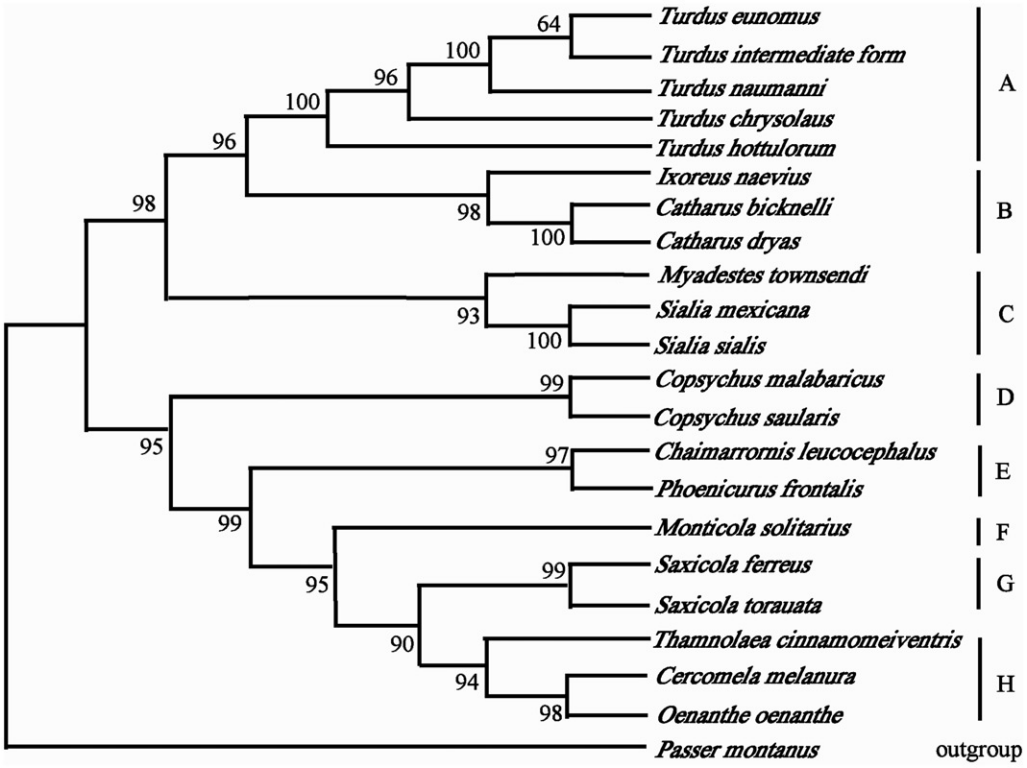

Figure 1. The phylogenetic relationship of 21 Turdidae species reconstructed with Cyt b, ND2 and COI gene dataset using MP analysis. The numbers on the branches are bootstrap values.

(group D-H); *Passer montanus* is in the base of each tree as the outgroup.

Discussion

Analysis of the complete mitochondrial genome of Dusky thrush complex

Comparing the mitochondrial genomes of some Turdidae species, we find that the length of those sequences is similar, which is well within the ranges for avian mitochondrial genomes and with the same situation found in other birds (Tong et al. 2006; Ke et al. 2010; Yang et al. 2010; Tu et al. 2011), and gene rearrangements are not observed in this new genome (Desjardins 1990; Härlid et al. 1998; Slack et al. 2003; Harrison et al. 2004). The complete sizes of the mitochondrial genome obtained from T-1, T-2 and T-3 are, respectively, 16,737, 16,788 and 16,750 bp. Compared with the mtDNA of birds, the overall base composition of Dusky thrush complex mtDNA is very steady (Desjardins 1990; Gibb et al. 2007). The overall nucleotide base composition was A, 29.38%; C, 32.40%; G, 14.91% and T, 23.31% for T-1, A, 29.34%; C, 32.41%; G, 14.97%, T, 23.28% for T-2, and A, 29.45%; C, 32.23%; G, 14.98% and T, 23.34% for T-3. The relative abundance of nucleotides is C > A > T > G, reflecting the strong AT bias (Liu et al. 2012, 2013). Guanine is the rarest nucleotide. The A + T content is higher than C + G content, which is within the range for avian mitochondrial genomes (51.6–55.7%) (Haring et al. 2001). Most animal mtDNA usually has a significant strand bias in nucleotide composition which can be measured as AT-skew and GC-skew (Quinn 1993). GC-skew values are negative, while AT- skew are positive. The average of AT-skew value is 0.115, the average of GC-skew value is −0.368 and all of them accordance these principles. D-loop is the only major non-coding segment of mtDNA, with higher variability, three to five times faster evolution, than other vertebrate mtDNA (Boore 2004). The D-loop locates between the $tRNA^{Phe}$ and $tRNA^{Glu}$ genes, according to its length and content of bases, as is quiet different from the mammals, but is very similar to the other Aves species (Ramirez et al. 1993; Taanman 1999).

Comparing protein-coding genes

We compared predicted initiation and termination codons of the 13 mitochondrial protein-coding genes (ATP8, ATP6, COI, COII, COIII, ND1, ND2, ND3, ND4L, ND4, ND5, ND6, Cytb) among the three Turdidae species, and found that most of protein coding genes used ATG as start codons, only a few species started with GTG. Stop codons also have the similar phenomena, TAA, TAG and T- occurred very commonly. Among the 13 protein-coding genes, ND6 initiation is ATG and termination is TAG; ND1 starts with ATG, and ends with AGG except for *Anser albifrons* which ends with TAA (Slack et al. 2003); ND2 starts with ATG and ends with TAG except *Anseranas semipalmata* and *Cygnus atratus* which ends with TAA (Harrison et al. 2004; Jiang et al. 2010). COI use GTG as the start codon and stops with AGG in all these species.

Phylogenetic analyses

Mitochondrial DNA has been used as a molecular marker for resovling phylogenetic relationships. In the absence of the complete mtDNA gene, the use of Cyt b, ND2 and COI genes are a workable solution for reconstructing phyogenetic relationships (Liu et al. 2013). The phylogenetic topologies of the Dusky thrush complex are primarily identical in NJ and MP. Phylogenetic trees with same topology and similar bootstrap values are based on mtDNA sequences of Cyt b, ND2 and COI genes among 21 species chosen in Turdidae (Figure 1). As presently recognized, the Dusky thrush complex consists of two species, *Turdus eunomus* and *Turdus naumanni* (Zheng 2005). There are eight clades in Turdidae based on two phylogenetic trees. *Turdus eunomus*, *Turdus* intermediate, *Turdus naumanni*, *Thurdus chrysolaus*, *Thurdus hortulorum* form the first group, Group A; Group B include *Ixoreus naevius*, *Catharus bicknelli* and *Catharus dryas*; *Myadestes townsendi*, *Sialia mexicana* and *Sialia sialis* forms the third group, Group C. The three groups above composed a big evolutionary line. Another evolutionary line is more complicated include five groups (groups D–H). Group D is composed with two sibling species: *Copsychus malabaricus* and *Copsychus saularis*. *Chaimarrornis leucocephalus* and *Phoenicurus frontalis* forming Group E; *Monticola solitarius* forms the sixth group, Group F; *Saxicola ferrea* and *Saxicola torquata* form the seventh group, Group G; The last group, Group H is composed with *Thamnolaea cinnamomeiventris*, *Cercomela melanura* and *Oenanthe oenanthe*.

The sibling species in Groups B, C, D and G are well known to be independent species, with more than 99 bootstrap values. They can be the scale plate for Turdidae independent species judgement. *Turdus eunomus* and *Turdus* intermediate in group A are sister groups, then they grouped together with *Turdus naumanni*, which suggested that *Turdus naumanni* might be the primitive taxon among two species(-subspecies) and shows strong support in NJ and MP trees (100% bootstrap values, respectively), *Turdus eunomus* and *Turdus* intermediate form are closer in genetic relationship than *Turdus naumanni*.

The genetic distance show more detailed conclusion (Table 4). The genetic distances between sister species are change from 0.076 to 0.135. We can know the smallest genetic distance is between *Turdus hortulorum* and *Turdus chrysolaus*, which is 0.076. Dusky thrush complex subspecies (*Turdus eunomus*, the intermediate, *Turdus naumanni*) are significantly lower (0.001–0.007), as most likely indicated they are not independent species. Hebert (2004) determined COI barcodes for 260 species of North American birds and found that COI sequence differences between closely related species were far higher than differences within species (18-fold higher), which is quite similar to ours.

The results are also supported by the neighbour-joining tree which constructed based on protein-coding genes Cyt b, ND2 and COI genes (Figure 2). The NJ tree supports that *Turdus eunomus* and the intermediate have closer genetic relationship than *Turdus naumanni*, but the Dusky thrush complex probably cannot divided into two species. We suggest that *Turdus eunomus* and *Turdus naumanni* should be

Table 4. Genetic distances between 22 species of passerines computed by Kimura Two-parameter model.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1																					
2	0.113																				
3	0.166	0.179																			
4	0.178	0.182	0.129																		
5	0.179	0.179	0.160	0.152																	
6	0.183	0.177	0.155	0.159	0.135																
7	0.136	0.143	0.173	0.168	0.167	0.171															
8	0.173	0.183	0.132	0.131	0.147	0.158	0.169														
9	0.162	0.177	0.165	0.164	0.180	0.179	0.159	0.179													
10	0.174	0.173	0.104	0.128	0.154	0.151	0.175	0.128	0.171												
11	0.169	0.179	0.131	0.119	0.147	0.160	0.160	0.128	0.173	0.137											
12	0.173	0.182	0.126	0.138	0.160	0.158	0.168	0.122	0.182	0.122	0.136										
13	0.184	0.190	0.142	0.155	0.166	0.165	0.171	0.139	0.182	0.132	0.153	0.119									
14	0.174	0.185	0.186	0.176	0.180	0.177	0.178	0.188	0.167	0.186	0.186	0.189	0.191								
15	0.164	0.164	0.187	0.169	0.185	0.176	0.148	0.178	0.165	0.188	0.179	0.185	0.195	0.180							
16	0.177	0.184	0.191	0.192	0.192	0.191	0.181	0.197	0.183	0.194	0.191	0.194	0.203	0.082	0.190						
17	0.178	0.185	0.127	0.137	0.164	0.165	0.166	0.130	0.183	0.124	0.141	0.129	0.149	0.204	0.188	0.207					
18	0.155	0.167	0.185	0.180	0.185	0.173	0.151	0.175	0.168	0.187	0.174	0.176	0.186	0.179	0.076	0.196	0.182				
19	0.158	0.159	0.187	0.174	0.188	0.170	0.149	0.176	0.168	0.188	0.176	0.169	0.177	0.178	0.074	0.194	0.186	0.065			
20	0.159	0.160	0.188	0.174	0.188	0.170	0.149	0.177	0.168	0.187	0.177	0.171	0.177	0.178	0.075	0.196	0.187	0.066	0.001		
21	0.163	0.165	0.194	0.182	0.195	0.176	0.155	0.183	0.175	0.195	0.182	0.175	0.183	0.184	0.080	0.199	0.191	0.070	0.007	0.007	
22	0.212	0.208	0.196	0.201	0.198	0.197	0.210	0.192	0.206	0.186	0.194	0.195	0.203	0.217	0.215	0.218	0.198	0.215	0.211	0.212	0.215

(1) *Catharus bicknelli*, (2) *Catharus dryas*, (3) *Cercomela melanura*, (4) *Chaimarrornis leucocephalus*, (5) *Copsychus malabaricus*, (6) *Copsychus saularis*, (7) *Ixoreus naevius*, (8) *Monticola solitarius*, (9) *Myadestes townsendi*, (10) *Oenanthe oenanthe*, (11) *Phoenicurus frontalis*, (12) *Saxicola ferreus*, (13) *Saxicola torquata*, (1). *Sialia mexicana*, (15) *Turdus hortulorum*, (16) *Sialia sialis*, (17) *Thamnolaea cinnamomeiventris*, (18) *Turdus chrysolaus*, (19) *Turdus naumanni*, (20) *Turdus eunomus*, (21) *Turdus* intermediate form and (22) *Passer montanus*.

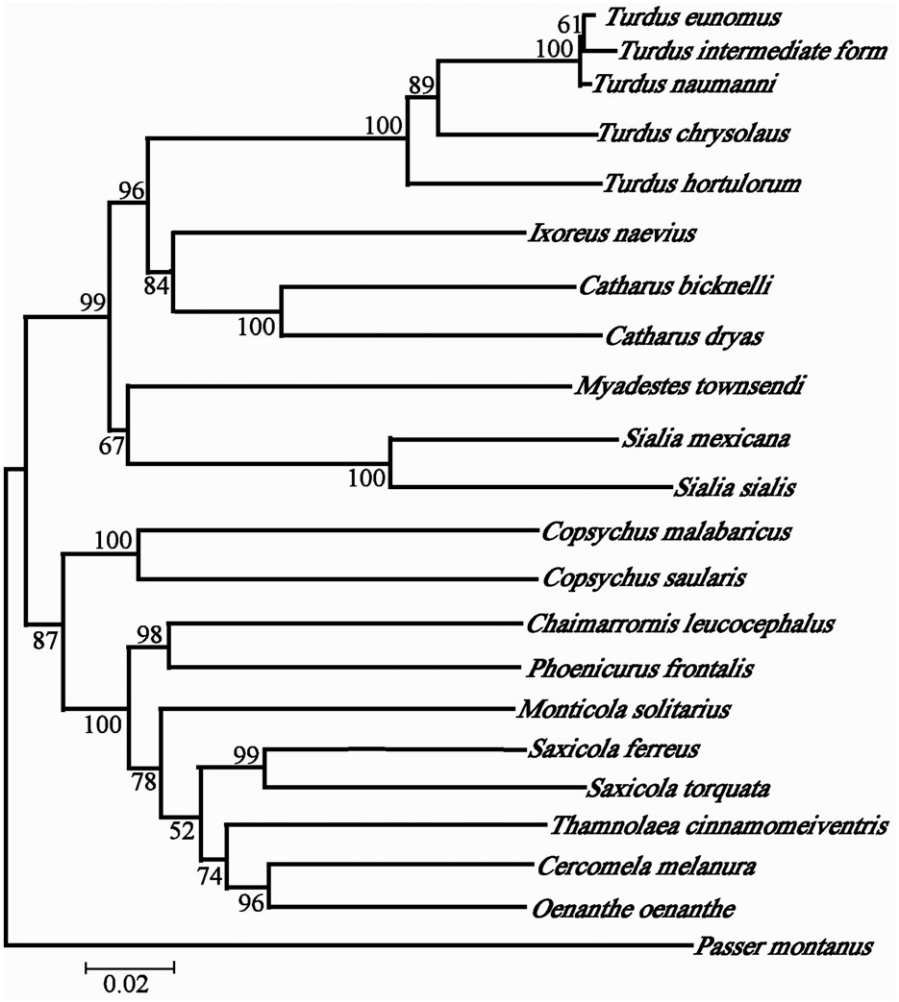

Figure 2. Neighbour-joining phylogeny tree of 21 Turdidae species with genetic distances. The numbers on the branches are bootstrap values.

treated as two subspecies that is *Turdus naumanni naumanni* and *Turdus naumanni eunomus*. The relationships among subspecies also need additional study to be well supported. In future, sequencing more mtDNA from various taxonomic levels will significantly improve our understanding of mtDNA evolution and phylogenetic relationships within the Turdidae.

Disclosure statement

The authors declare that the study was conducted in the absence of any commercial of financial relationships that could be constructed as potential conflicts of interest.

Funding

This work was supported by funding from the National Natural Science Foundation of China [No. 31772485] and the Graduate Student Innovation Research Projects of Anhui University [YQH100237].

References

Boore JL. 1999. Animal mitochondrial genomes. Nucleic Acids Res. 27:1767–1780.

Boore JL. 2004. Complete mitochondrial genome sequence of *Urechis caupo*, a representative of the phylum Echiura. BMC Genomics. 5:67.

Desjardins P. 1990. Sequence and gene organization of the chicken mitochondrial genome. A novel gene order in higher vertebrates. J Mol Biol. 212:599–634.

Donnegoussé C, Laudet V, Hänni C. 2002. A molecular phylogeny of anseriformes based on mitochondrial DNA analysis. Mol Phylogenet Evol. 23:339–356.

Gibb GC, Kardailsky O, Kimball RT, Braun EL, Penny D. 2007. Mitochondrial genomes and avian phylogeny: complex characters and resolvability without explosive radiations. Mol Biol Evol. 24:269.

Gissi C, Iannelli F, Pesole G. 2008. Evolution of the mitochondrial genome of Metazoa as exemplified by comparison of congeneric species. Heredity. 101:301–320.

Gonzalez J, Düttmann H, Wink M. 2009. Phylogenetic relationships based on two mitochondrial genes and hybridization patterns in Anatidae. J Zool. 279:310–318.

Härlid A, Janke A, Arnason U. 1998. The complete mitochondrial genome of *Rhea americana* and early avian divergences. J Mol Evol. 46:669.

Haring E, Kruckenhauser L, Gamauf A, Riesing MJ, Pinsker W. 2001. The complete sequence of the mitochondrial genome of *Buteo buteo* (Aves, Accipitridae) indicates an early split in the phylogeny of raptors. Mol Biol Evol. 18:1892–1904.

Harrison GL, Mclenachan PA, Phillips MJ, Slack KE, Cooper A, Penny D. 2004. Four new avian mitochondrial genomes help get to basic evolutionary questions in the late cretaceous. Mol Biol Evol. 21:974.

Ingman M, Kaessmann H, Pääbo S, Gyllensten U. 2000. Mitochondrial genome variation and the origin of modern humans. Nature. 408:708.

Jiang F, Miao Y, Liang W, Ye H, Liu H, Liu B. 2010. The complete mitochondrial genomes of the whistling duck (*Dendrocygna javanica*) and black swan (*Cygnus atratus*): dating evolutionary divergence in Galloanserae. Mol Biol Rep. 37:3001.

Ke Y, Huang Y, Lei FM. 2010. Sequencing and analysis of the complete mitochondrial genome of *Podoces hendersoni* (Ave, Corvidae). Hereditas. 32:951.

Klicka J, Voelker G, Spellman GM. 2005. A molecular phylogenetic analysis of the "true thrushes (Aves: Turdinae)". Mol Phylogenet Evol. 34:486–500.

Kratter AW. 2014. The howard and moore complete checklist of the birds of the world. Auk. 122:712–714.

Liu G, Zhou L, Gu C. 2014. The complete mitochondrial genome of Brown wood owl *Strix leptogrammica* (Strigiformes: Strigidae). Mitochondrial DNA. 25:370.

Liu G, Zhou L, Zhang L, Luo Z, Xu W. 2013. The complete mitochondrial genome of bean goose (*Anser fabalis*) and implications for anseriformes taxonomy. PLoS ONE. 8:e63334.

Liu G, Zhou LZ, Gu CM. 2012. Complete sequence and gene organization of the mitochondrial genome of scaly-sided merganser (*Mergus squamatus*) and phylogeny of some Anatidae species. Mol Biol Rep. 39:2139–2145.

Milchev B, Boev Z. 2014. First record of the Naumann's Thrush (*Turdus naumanni* Temminck, 1820, sensu lato) in Bulgaria. Acta Zool Bulg. 66:559–561.

Monroe BL, Sibley CG. 1993. A world checklist of birds. New Haven: Yale University Press.

Near TJ, Pesavento JJ, Cheng CH. 2003. Mitochondrial DNA, morphology, and the phylogenetic relationships of Antarctic icefishes (Notothenioidei: Channichthyidae). Mol Phylogenet Evol. 28:87–98.

Quinn TW. 1993. Sequence evolution in and around the mitochondrial control region in birds. J Mol Evol. 37:417–425.

Ramirez V, Savoie P, Morais R. 1993. Molecular characterization and evolution of a duck mitochondrial genome. J Mol Evol. 37:296–310.

Rheindt FE, Cuervo AM, Brumfield RT. 2013. Rampant polyphyly indicates cryptic diversity in a clade of neotropical flycatchers (Aves: Tyrannidae). Biol J Linn Soc. 108:889–900.

Saccone C, Pesole G, Sbisá E. 1991. The main regulatory region of mammalian mitochondrial DNA: structure-function model and evolutionary pattern. J Mol Evol. 33:83–91.

Sambrook J, Fritsch EF, Maniatis T. 2001. Molecular cloning. A laboratory manual. Anal Biochem. 186:182–183.

Sangster G, van den Berg AB, van Loon AJ, Roselaar CS. 2009. Dutch avifaunal list: taxonomic changes in 2004–2008. Ardea. 97:373–381.

Sangster G, Collinson JM, Knox AG, Parkin DT, Svensson L. 2007. Taxonomic recommendations for British birds: fourth report. IBIS. 149:707–710.

Sibley CG, Monroe BL. 1990. Distribution and taxonomy of birds of the world. New Haven: Yale University Press.

Slack KE, Janke A, Penny D, Arnason U. 2003. Two new avian mitochondrial genomes (penguin and goose) and a summary of bird and reptile mitogenomic features. Gene. 302:43–52.

Taanman JW. 1999. The mitochondrial genome: structure, transcription, translation and replication. Biochim Biophys Acta. 1410:103.

Tong XM, Liang Y, Wang W, Xu SQ, Zheng XG, Wang J, Yu J. 2006. Complete sequence and gene organization of the Tibetan chicken mitochondrial genome. Hereditas. 28:769.

Tu JF, Huang YH, Liu SF, Li N. 2011. Complete sequence determination and analysis of Beijing duck mitochondrial genome. Chin J Zool. 44:28–33.

Wilson AC. 1991. Evolution of the cytochrome b gene of mammals. J Mol Evol. 32:128.

Wolstenholme DR. 1992. Animal mitochondrial DNA: structure and evolution. Int Rev Cytol. 141:173.

Yang R, Wu X, Yan P, Su X, Yang B. 2010. Complete mitochondrial genome of *Otis tarda* (Gruiformes: Otididae) and phylogeny of Gruiformes inferred from mitochondrial DNA sequences. Mol Biol Rep. 37: 3057–3066.

Yuan Y, Li Q, Yu H, Kong L. 2012. The complete mitochondrial genomes of six heterodont bivalves (Tellinoidea and Solenoidea): variable gene arrangements and phylogenetic implications. PLoS ONE. 7:e32353.

Zheng GM. 2005. A checklist on the classification and distribution of the birds of China. Beijing: Science Press.

Zheng ZX. 1994. A complete checklist of species and subspecies of Chinese birds. Beijing: Science Press.