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Complete mitochondrial genome of *Otis tarda* (Gruiformes: Otididae) and phylogeny of Gruiformes inferred from mitochondrial DNA sequences

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Abstract The complete nucleotide sequence of mitochondrial genome of the Great bustard (*Otis tarda*) was determined by using polymerase chain reaction (PCR) method. The genome is 16,849 bp in size, containing 13 protein-coding, 2 ribosomal and 22 transfer RNA genes. Sequences of the tRNA genes can be folded into canonical cloverleaf secondary structure except for tRNA-Cys and tRNA-Ser (AGY), which lose "DHU" arm. Sequence analysis showed that the *O. tarda* mitochondrial control region (mtCR) contained many elements in common with other avian mtCRs. A microsatellite repeat was found in the 3′-peripheral domain of the *O. tarda* mtCR. Based on the mitochondrial DNA sequences of 12S rRNA, 16S rRNA and tRNA-Val, a phylogenetic study of Gruiformes was performed. The result showed that Otididae was a sister group to "core Gruiformes" and Charadriiformes with strong support (97% posterior probability values) in Bayesian analysis. The taxonomic status of Rhynochetidae, Mesitornithidae, Pedionomidae and Turnicidae that traditionally belonged to Gruiformes was also discussed in this paper.

Keywords *Otis tarda* · Mitochondrial genome · Phylogeny · Gruiformes

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Introduction

The animal mitochondrial genome is a closed, maternally inherited circular molecule typically composed of 37 genes coding for 13 proteins, 2 ribosomal RNAs (rRNAs) and 22 transfer RNAs (tRNAs) [40, 57]. Mitochondrial DNA (mtDNA) sequence data are important for the studies of taxonomy and phylogeny [5], genetic structure [7], biological identification [21] and conservation genetics [54] as mtDNA evolves more rapidly than the nuclear genome [4]. Recently, complete mtDNA sequence and gene arrangement comparisons have been employed as powerful new tools for resolving phylogenetic relationships [2, 15, 20, 30]. Up to May 2008, the complete mtDNA sequence of 80 avian species, none for bustards (Gruiformes: Otididae), are available in GenBank. The Great Bustard (*Otis tarda*) is listed as a vulnerable species with estimated global population of 31,000–37,000 individuals [1]. To obtain the complete mtDNA sequence of *O. tarda* will be valuable to know more about this precious species.

The classification of the order Gruiformes has been very unstable in the taxonomic history of birds. Traditionally, Gruiformes was classified as 12 families including Otididae (Bustards), Aramidae (Limpkin), Cariamidae (Seriemas), Eurypygidae (Sunbittern), Gruidae (Cranes), Heliornithidae (Sungrebes), Mesitornithidae (Mesites), Pedionomidae (Plains-wanderer), Psophiidae (Trumpeters), Rallidae (Rails), Rhynochetidae (Kagu), and Turnicidae (Button-quails) [22, 56]. Sibley and Monroe [46], however, recognised only nine families according to DNA hybridization. Dickinson [10] just removed Pedionomidae from the traditional Gruiformes and recognised 11 families.

Recently, Fain et al. [13] studied the phylogeny of "core Gruiformes" including Gruidae, Aramidae, Psophiidae, Heliornithidae, and Rallidae. In this study, by including

five more Gruiformes families, we aimed to discuss the phylogenetic relationship and classification of Gruiformes based on sequences of three mtDNA genes (12S rRNA, 16S rRNA and tRNA-Val).

Materials and methods

Samples and DNA extraction

Otis tarda sample (Sample No. AV01065) was obtained from the Animal Conservation Biology Laboratory, College of Life Sciences of Anhui Normal University. The mtDNA was extracted from the muscle tissue stored at −80°C using GENMED mtDNA Extraction Kit.

Primer design, PCR amplification and sequencing

The total length of *O. tarda* mtDNA was amplified by polymerase chain reaction (PCR) method. Since there was no relative complete mtDNA sequence available in Otididae family, the primer design took five steps. Firstly, some universal primers from published literatures (Table 1) were tried for PCR. Some fragments were gained and then sequenced. Secondly, based on the sequences obtained in the first step, some primers were designed using Primer 5.00 (PREMIER Biosoft International). Thirdly, based on the alignment of complete mtDNA sequences of five avian species (*Gallus gallus*, NC_001323; *Arenaria interpres*, NC_003712; *Larus dominicanus*, NC_007006; *Falco sparverius*, NC_008547; *Ninox novaeseelandiae*, AY309457), which are far from the Otididae family in phylogeny, some primers were designed in the conservative region using Oligo 6.0 [41]. Fourthly, the primers designed in the second and third steps were paired for PCR. Fifthly, after the former four steps, most of the mtDNA sequences had been obtained, but there still left some gaps. Thus some primers were designed based on the obtained sequences using Primer 5.00 for PCR to fill the gaps. The PCR products were expected less than 1,400 bp and each segment overlapped the next by 60–250 bp. Altogether, 21 pairs of primers (Table 1) were employed for amplifying and sequencing the complete mtDNA sequence of *O. tarda.*

Polymerase chain reaction mixtures contained 100 ng template DNA, 3 μl of 10× reaction buffer, 2 μl of 25 mmol/l $MgCl_2$, 2 μl of 2 mmol/l dNTPs, 1 μl of 10 μmol/l each primer, 1 unit of Taq DNA polymerase (Promega), and sterile double distilled water to make up a final volume of 30 μl. PCR reactions were performed in the MJ Model PTC-200 thermal cycler, consisting of an initial denaturation at 94°C for 5 min, 32 cycles of denaturation at 94°C for 50 s plus annealing at 52–56°C for 1 min and extension at 72°C for 55 s, and a final extension at 72°C for 7 min. The resultant PCR products were electrophoresed on 1% agarose gels, and then were purified by PCR cleanup Kit (V-gene) for sequencing on automatic DNA sequencer (Applied Biosystems, 3730).

Sequence analysis

Nucleotide sequences were edited using the program DNAstar (DNAstar Inc.) and aligned by ClustalX [52]. Protein-coding genes were identified using Sequin 5.35 and improved manually. The tRNA genes were identified using software tRNA Scan-SE 1.21 (http://lowelab.ucsc.edu/tRNA-SE) and their clover leaf secondary structure and anticodon sequences were identified using DNASIS 2.5 (Hitachi Software Engineering Inc.). Two rRNA, tRNA-Cys and tRNA-Ser (AGY) genes were determined by comparison with the known complete mtDNA sequences of *Porphyrio hochstetteri* (Gruiformes: Rallidae) (EF532934) and *Gallus gallus* (NC_001323). The complete mitochondrial genome sequence of *O. tarda* has been deposited in GenBank under accession number FJ751803.

Phylogenetic analysis

We sampled 10 families ever included in the order Gruiformes. Eurypygidae and Cariamidae were not included in this study for the lack of corresponding sequence data. For Charadriiformes has generally been thought to be the closest order to Gruiformes [27, 47] and recent studies indicated that Pedionomidae and Turnicidae should be placed in Charadriiformes [12, 33], thus representatives of five families widely accepted as members of Charadriiformes were included in this study. *Gallus gallus* (Aves: Galliformes) and *Anser albifrons* (Aves: Anseriformes) were designated as the root. The sequences of *O. tarda* were obtained in this study, and the other related sequences were obtained from GenBank (Table 2).

Gblock 0.91b [6] was used to delete gaps within certain regions of the 12s and 16s rRNA to avoid the alignment difficulties introduced by indels. Phylogenetic analysis was performed using maximum parsimony (MP) and maximum likelihood (ML) algorithms implemented in PAUP*4.0b10 [50]. For the MP analysis, a heuristic search, with 1,000 replicates of random addition sequences and tree bisection reconnection (TBR) branch swapping, was executed to obtain the MP tree. The robustness of the MP tree was assessed with 1,000 bootstrap replicates.

The Program Modeltest 3.6 [36] was used to choose an appropriate substitution model for the ML analysis. The model (GTR + I + G) was subsequently used in PAUP*4.0b10 to search the ML tree using a heuristic search with 1,000 replicates of random addition sequences and TBR branch

Table 1 Primer sequence used in this study

Primer	Primer sequence[a] (5′ → 3′)	References	Product size (bp)
Ot1-F	TCACGTGAAATCAGCAACCC	[55]	345
Ot1-R	AAACACTTGAAACCGTCTCAT	[55]	
Ot2-F	ACTAGCTTCAGGCCCATTCTTTCC	[34]	938
Ot2-R	AGGCGTCTTGGGCTACTACT	This study	
Ot3-F	ACAAAGCATGGCACTGAAGATG	[8]	1,009
Ot3-R	CTTTCAGGTGTAAGCTGAATGC	[8]	
Ot4-F	TATGAAACTACCCCTGGAAG	This study	1,295
Ot4-R	CACAGGGTCTTCTCGTCTTA	This study	
Ot5-F	GGTGATGCCTGCCCAATG	[48]	689
Ot5-R	GCTAGGGAGAGGATTTGAACCTC	[48]	
Ot6-F	GCAATCCAGGTCGGTTTCTATC	[48]	1,341
Ot6-R	GGGGTATGGGCCCGATAGC	[48]	
Ot7-F	CTTCCTCCCACTAACACT	This study	1,329
Ot7-R	CTCTTATTTAAGGCTTTGAAGGC	[48]	
Ot8-F	CAGGAACTCACTAAACAAGA	This study	755
Ot8-R	GAAGAAGATTATTACGAAGGC	This study	
Ot9-F	TTCTCCAACCACAAAGACATTGGCAC	[21]	728
Ot9-R	ACGTGGGAGATAATTCCAAATCCTG	[21]	
Ot10-F	GACAGACCGAAACCTAAACAC	This study	1,089
Ot10-R	ACMATTAGGGCRTGGTCGTG	[48]	
Ot11-F	GCCGCATCAACCACTCA	This study	1307
Ot11-R	TGTAAGAAGGGTGGCGAAT	This study	
Ot12-F	AACCTGACCATGAACCTAA	This study	912
Ot12-R	TGGGCTCATGTKACDGTDACTCC	[48]	
Ot13-F	TTTGTCCTCCTACTAAGCC	This study	968
Ot13-R	GGGTCRAAKCCRCATTCRTANGG	[48]	
Ot14-F	GCCTGATACTGACACTTTG	This study	388
Ot14-R	AGGGTTGGGATTARGGTKGC	This study	
Ot15-F	GATTCTTCCTCAGTAGCC	This study	1,168
Ot15-R	GGWGCYTCTACRTGDGCTTTDGG	[48]	
Ot16-F	GCTCCCTCCACCTAACAACAC	This study	1,131
Ot16-R	AGTGCGGGTGATGATGAG	This study	
Ot17-F	GGTGCAAATCCAAGTAAAAG	This study	911
Ot17-R	TTGGCTGGAAGTGGAGAAGGC	This study	
Ot18-F	TACTCCACTCCAGCACHATAG	This study	1282
Ot18-R	GCGTGTAGATTTCGGATTAGTC	This study	
Ot19-F	GGAATCTGCCTAATAACAC	This study	887
Ot19-R	CAATGATGATGAATGGGT	This study	
Ot20-F	CCTCCATAAATCCAAACAACG	This study	814
Ot20-R	GGGTGTGGGTGRTTGTTGAGTTT	This study	
Ot21-F	TACCCCACAACTCGCCAATCC	This study	1,085
Ot21-R	AAAGAATGGGCCTGAAGCTAG	[28]	

[a] Codes for degenerate sites are as follows: D, AGT; H, ACT; K, GT; M, A C; N, ACGT; R, AG; W, AT; Y, CT

swapping. Reliability of the phylogenetic relationships was evaluated by performing 1,000 replicates of bootstrap analysis.

In addition, Bayesian inference of phylogeny was performed using the program Mrbayes 3.0 [23]. The substitution model selected by Modeltest 3.6 was likewise used

Table 2 Species in phylogenetic analysis

Higher taxon	Species	GenBank accession numbers	
		12S + Val	16S
Galliformes	*Gallus gallus*	NC_001323	NC_001323
Anseriformes	*Anser albifrons*	NC_004539	NC_004539
Gruiformes			
Aramidae	*Aramus guarauna*	DQ485816	DQ485854
Gruidae	*Anthropoides paradisea*	DQ485804	DQ485842
	Anthropoides virgo	DQ485805	DQ485843
	Balearica pavonina	DQ485802	DQ485840
	Balearica regulorum	DQ485801	DQ485839
	Bugeranus carunculatus	DQ485803	DQ485841
	Grus americana	DQ485808	DQ485846
	Grus japonensis	DQ485807	DQ485845
	Grus nigricollis	DQ485811	DQ485849
Heliornithidae	*Heliornis fulica*	DQ485819	DQ485857
Mesitornithidae	*Mesitornis unicolor*	DQ674557	DQ674596
Otididae	*Afrotis afra*	DQ674591	DQ674629
	Ardeotis kori	DQ674590	DQ674628
	Eupodotis senegalensis	DQ674592	DQ674630
	Tetrax tetrax	DQ674589	DQ674627
	Otis tarda	FJ751803	FJ751803
Pedionomidae	*Pedionomus torquatus*	DQ674586	DQ674624
Psophiidae	*Psophia crepitans*	DQ485817	DQ485855
	Psophia viridis	DQ485818	DQ485856
Rallidae	*Porphyrio hochstetteri*	EF532934	EF532934
	Fulica americana	DQ485827	DQ485863
	Gallinula chloropus	DQ485828	DQ485864
	Gallirallus philippensis	DQ485824	DQ485860
	Laterallus melanophaius	DQ485823	DQ485859
	Porphyrio porphyrio	DQ485822	DQ485858
	Porzana carolina	DQ485826	DQ485862
	Rallus longirostris	DQ485825	DQ485861
Rhynochetidae	*Rhynochetos jubatus*	EF532933	EF532933
Turnicidae	*Turnix sylvatica*	AF173588	AF173588
	Turnix varia	DQ674575	DQ674613
Charadriiformes	*Charadrius vociferus*	DQ485792	DQ485830
	Recurvirostra americana	DQ485793	DQ485831
	Uria aalge	DQ485794	DQ485832
	Larus atricilla	DQ485795	DQ485833
	Jacana spinosa	DQ485796	DQ485834

Taxonomic denomination followed [22]

in the Bayesian method. The Bayesian analysis started with randomly generated trees; four Markov chains under default heating values were run for 4 million generations and sampled every 100 generations. The "burn-in" was determined by checking for the likelihood of being stationary.

Results and discussion

Characteristics of the *O. tarda* mitochondrial genome

The total length of the *O. tarda* mtDNA is 16,849 bp. The arrangement of the mitochondrial genome is the same with

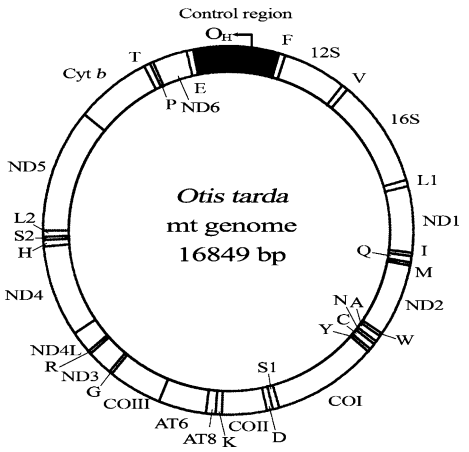

Fig. 1 Mitochondrial genome of *Otis tarda*. Genes encoded by the heavy strand are shown *outside of the circle*, whereas those encoded by the light strand are shown *inside the circle*. Gene abbreviations used are 12S, 12S rRNA; 16S, 16S rRNA; ND1-6, NADH dehydrogenase subunits 1–6; COI-III, cytochrome oxidase subunits I–III; AT6 and AT8, ATPase subunits 6 and 8; Cyt *b*, cytochrome *b*; and one-letter codes of amino acids, tRNA genes specifying them

typical avian mtDNA [15] and is shown in Fig. 1. The genome contains 13 protein-coding genes (ATP6, ATP8, COI-III, ND1-6, ND4L, and Cyt *b*), 2 ribosomal RNAs (12S rRNA and 16S rRNA), 22 transfer RNAs and a putative control region (Table 3). ND6 gene and 8 tRNA genes are encoded by the L-strand, whereas the other genes are encoded by the H-strand. The overall base composition of the L-strand (A = 30.5%, T = 24.2%, C = 31.6%, G = 13.7%) is similar to those of other avian species. The A + T content of 54.7% is within the range (51.6–55.7%) for avian mitochondrial genomes [18].

Protein-coding genes

Among the 13 protein-coding genes, the longest one is ND5 gene (1,815 bp), whereas the shortest one is ATPase8 gene (168 bp). The most common start codon is ATG found in eight genes. Nonstandard start codons are found in the COI and ND5 genes (GTG), ND2 gene (ATA) and ND3 gene (ATT). As in the mtDNA of the other birds, TAA is the most frequent stop codon in *O. tarda*. TAG and AGG are used twice, respectively, and AGA is found in ND5 gene. In COIII and ND4, a terminal T probably serves as the stop signal after it is completed to UAA by posttranscriptional polyadenylation [31]. In ND3 gene of *O. tarda* mtDNA, a base is not translated. It is similar to many birds and one turtle species for unknown frameshift mechanism [29].

tRNA genes

The tRNA genes range in size from 66 to 74 bp. Sequences of the tRNA genes can be folded into a canonical cloverleaf secondary structure except for tRNA-Cys and tRNA-Ser (AGY), which loses "DHU" arm (not shown in this paper). It is common that tRNA-Ser (AGY) cannot be folded into the canonical cloverleaf secondary structure in many vertebrate mtDNA [45, 58, 59].

A check in the GenBank shows that, as found in *O. tarda*, tRNA-Cys of few avian mtDNA cannot form a canonical cloverleaf secondary structure. This is also found in *Gekko gecko* (Reptilia: Gekkonidae) [17].

Spacers, overlaps, WANCY region and O_L

Similar to other vertebrate mtDNA, spacers and overlaps are also found in mtDNA of *O. tarda* (Table 2). The total overlaps and spacers are 33 bp and 107 bp, respectively. ATPase8 gene and ATPase6 gene share the longest overlap of 10 bp. The longest spacer of 41 bp is located between tRNA-Thr gene and ND6 gene. In this spacer, base C is much abundant (58.5%), while base G is absent.

The origin of L-strand replication (O_L) usually locates in a cluster of five tRNA genes: $tRNA^{Trp}$–$tRNA^{Ala}$–$tRNA^{Asn}$–$tRNA^{Cys}$–$tRNA^{Tyr}$ (WANCY) in many vertebrates. *O. tarda* lacks this O_L region, as is found in many birds [44]. This may indicate that avian mitochondrial genomes departed from their mammalian and amphibian counterparts during the course of evolution of vertebrate species [9]. Interestingly, resent studies showed that the O_L region is also absent in the crocodilian mtDNA [24, 26, 58].

Control region

The control region (CR) of mtDNA in *O. tarda* is 1,265 bp, which locates between tRNA-Glu and tRNA-Phe genes. The mitochondrial control region (mtCR) is responsible for transcription and replication of the mitochondrial genome [51]. The overall base composition of the *O. tarda* mtCR (L-strand) is A, 31.5%; T, 28.1%; G, 13.4%; C, 27.0%. These values show that there is an A + T (59.6%) to G + C (40.4%) asymmetry in this sequence. Three internal CR portions have been recognized: the 5′-peripheral domain, the central conserved domain and the 3′-peripheral domain ([42, 49]). In the avian CR, the 5′-peripheral domain contains the extended termination-associated sequence (ETAS), termination-associated sequence (TAS), the central conserved domain contains the F, E, D and C boxes, and the 3′-peripheral domain contains the origin of H-strand replication (O_H), conserved sequence block 1 (CSB1) and the H- and L-strand transcriptional promoter (HSP-LSP) sites [39].

After an alignment with described consensus counterpart mammalian and avian sequences [9, 37, 38, 49, 53], some conserved sequence boxes such as F, E, D, C and CSB1 are identified (highlighted in Fig. 2a) in *O. tarda* mtCR. A

Table 3 Organization of the mitochondrial genome of *Otis tarda*

Gene/region	Start position	End position	Size (bp)	Spacer (+) or Overlap (−)	Codon		Strand[a]
					Start	Stop	
Control region	1	1265	1,265	–	–	–	–
tRNA-Phe	1266	1333	68	–	–	–	H
12S rRNA	1334	2302	969	–	–	–	H
tRNA-Val	2303	2375	73	–	–	–	H
16S rRNA	2376	3971	1,596	–	–	–	H
tRNA-Leu (UUR)	3972	4045	74	+13	–	–	H
NADH1	4059	5036	978	–2	ATG	AGG	H
tRNA-Ile	5035	5106	72	+7	–	–	H
tRNA-Gln	5184	5114	71	–	–	–	L
tRNA-Met	5185	5253	69	–	–	–	H
NADH2	5254	6294	1,041	–2	ATA	TAG	H
tRNA-Trp	6293	6362	70	+1	–	–	H
tRNA-Ala	6432	6364	69	+2	–	–	L
tRNA-Asn	6507	6435	73	+2	–	–	L
tRNA-Cys	6576	6510	67	–1	–	–	L
tRNA-Tyr	6646	6576	71	+1	–	–	L
COI	6648	8198	1,551	–9	GTG	AGG	H
tRNA-Ser (UCN)	8262	8190	73	+2	–	–	L
tRNA-Asp	8265	8334	70	+1	–	–	H
COII	8336	9019	684	+1	GTG	TAA	H
tRNA-Lys	9021	9091	71	+1	–	–	H
ATPase8	9093	9260	168	–10	ATG	TAA	H
ATPase6	9251	9934	684	–1	ATG	TAA	H
COIII	9934	10717	784	–	ATG	T++	H
tRNA-Gly	10718	10786	69	–	–	–	H
NADH3[b]	10787	10960	351	+4	ATT	TAA	H
	10962	11138					
tRNA-Arg	11143	11210	68	+1	–	–	H
NADH4L	11212	11508	297	–7	ATG	TAA	H
NADH4	11502	12879	1,378	–	ATG	T++	H
tRNA-His	12880	12948	69	–	–	–	H
tRNA-Ser (AGY)	12949	13014	66	–1	–	–	H
tRNA-Leu (CUN)	13014	13084	71	–	–	–	H
NADH5	13085	14899	1,815	+11	GTG	AGA	H
Cyt *b*	14911	16053	1,143	+3	ATG	TAA	H
tRNA-Thr	16057	16125	69	+12	–	–	H
tRNA-Pro	16208	16138	71	+41	–	–	L
NADH6	16771	16250	522	+4	ATG	TAG	L
tRNA-Glu	16849	16776	74	–	–	–	L

[a] *H* heavy strand; *L* light strand

[b] Join (10787–10960, 10962–11138), frameshift mechanism unknown [29]

putative ETAS sequence block (ETAS1, highlighted in Fig. 2a) is located, which has 66.1% similarity to the mammalian consensus ETAS1 sequence [43]. A putative TAS sequence (boxed in Fig. 2a) that is described by Foran et al. [14] and consensus in many avian species [38] is found inside ETAS1. HSP and LSP sites are located according to alignment with conserved sequence described by L'Abbé et al. [25]. According to the assumption that a poly-C sequence upstream of CSB1 represents the origin of H-strand replication [53], the position of O_H is located (Fig. 2a).

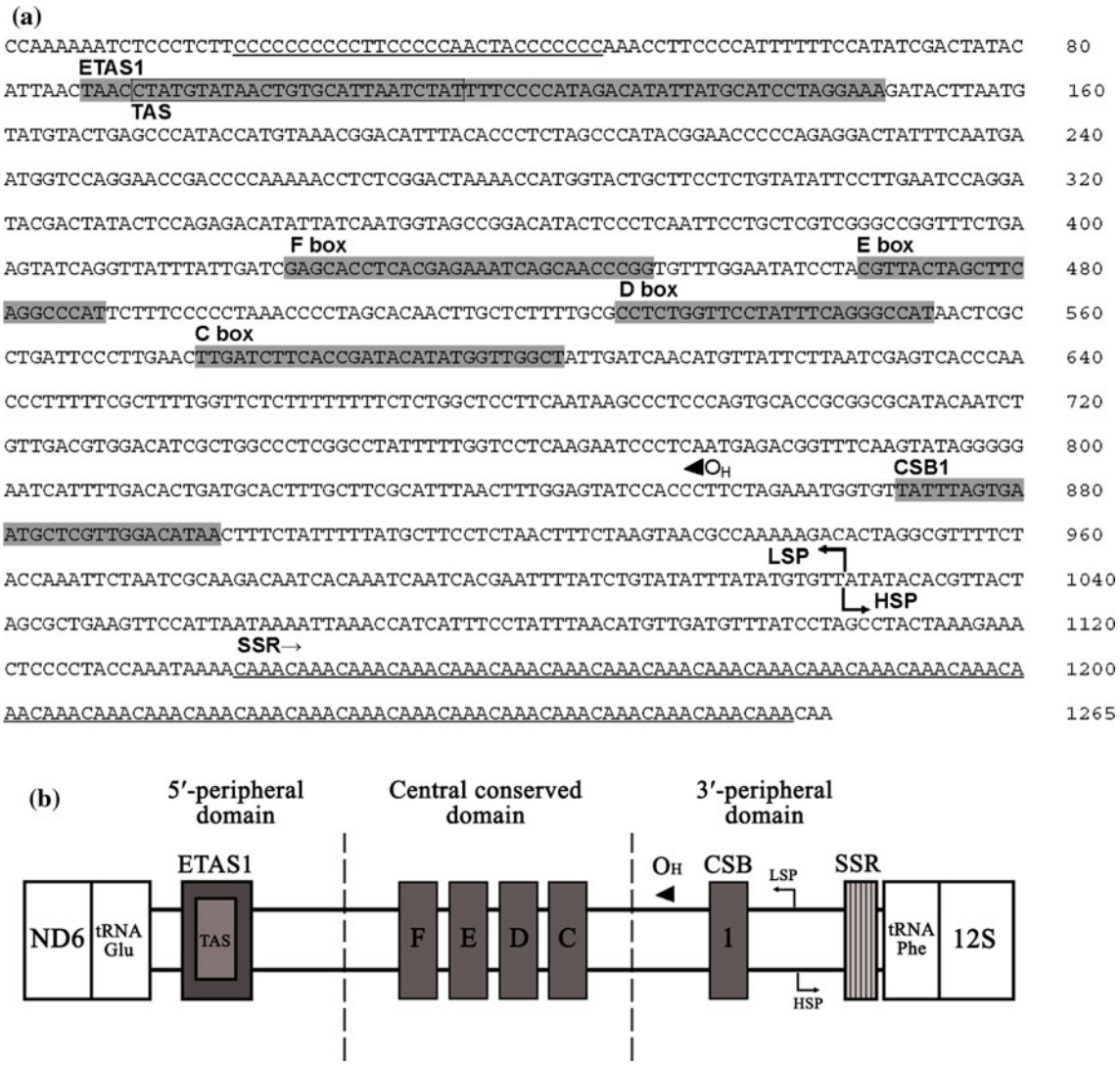

Fig. 2 The L-strand sequence and a schematic representation of the *Otis tarda* control region (CR). **a** The sequence of the CR. *Underlined at the front of the sequence* is the interrupted poly-C sequence. Also *underlined* is the simple sequence repeat (SSR), a tetranucleotide microsatellite (CAAA). *Highlighted* is the extended termination-associated sequence (ETAS)-1. The *boxed sequence* inside ETAS1 is the termination-associated sequence (TAS). Also *highlighted* are the F, E, D and C boxes and conserved sequence block (CSB)-1. The ◀ *symbol* represents origin of heavy strand replication (O_H). The ⮡ *symbols* represent the light (L) and heavy (H) strand promotor (LSP-HSP) sites. **b** A schematic representation of the CR, which shows the flanking genes and portions of the CR sequence represented in this figure

The 5′-peripheral domain contains an interrupted poly-C sequence (underlined in Fig. 2a). This structure is conserved across many avian species [37, 38]. Although it could potentially form a stable hairpin structure [37], its function has not been determined yet.

There is a simple sequence repeat (SSR) in the end of 3′-peripheral domain (Fig. 2a). The SSRs comprise 31 perfect tetranucleotide microsatellite repeats consisting of $(dC\text{-}dA\text{-}dA\text{-}dA)_{31}\cdot(dG\text{-}dT\text{-}dT\text{-}dT)_{31}$. The same tetranucleotide microsatellite repeats has also been found in *Rhea americana* (Aves: Rheidae) [19] and *Pygoscelis adeliae* (Aves: Spheniscidae) [39]. Large repeat that is usually found in the 3′-peripheral domain in many avian CRs is absent in *O. tarda* mtCR.

Phylogeny

The result of phylogenetic analysis is shown in Fig. 3. As the resultant MP and ML trees have the same topology structure, thus only the ML tree is shown (the numbers above branches represent bootstrap support for MP/ML). Rhynochetidae has been classified as a member of Gruiformes for long time [10, 22, 46, 56]. But, in our study, Rhynochetidae is outside of traditional Gruiformes with

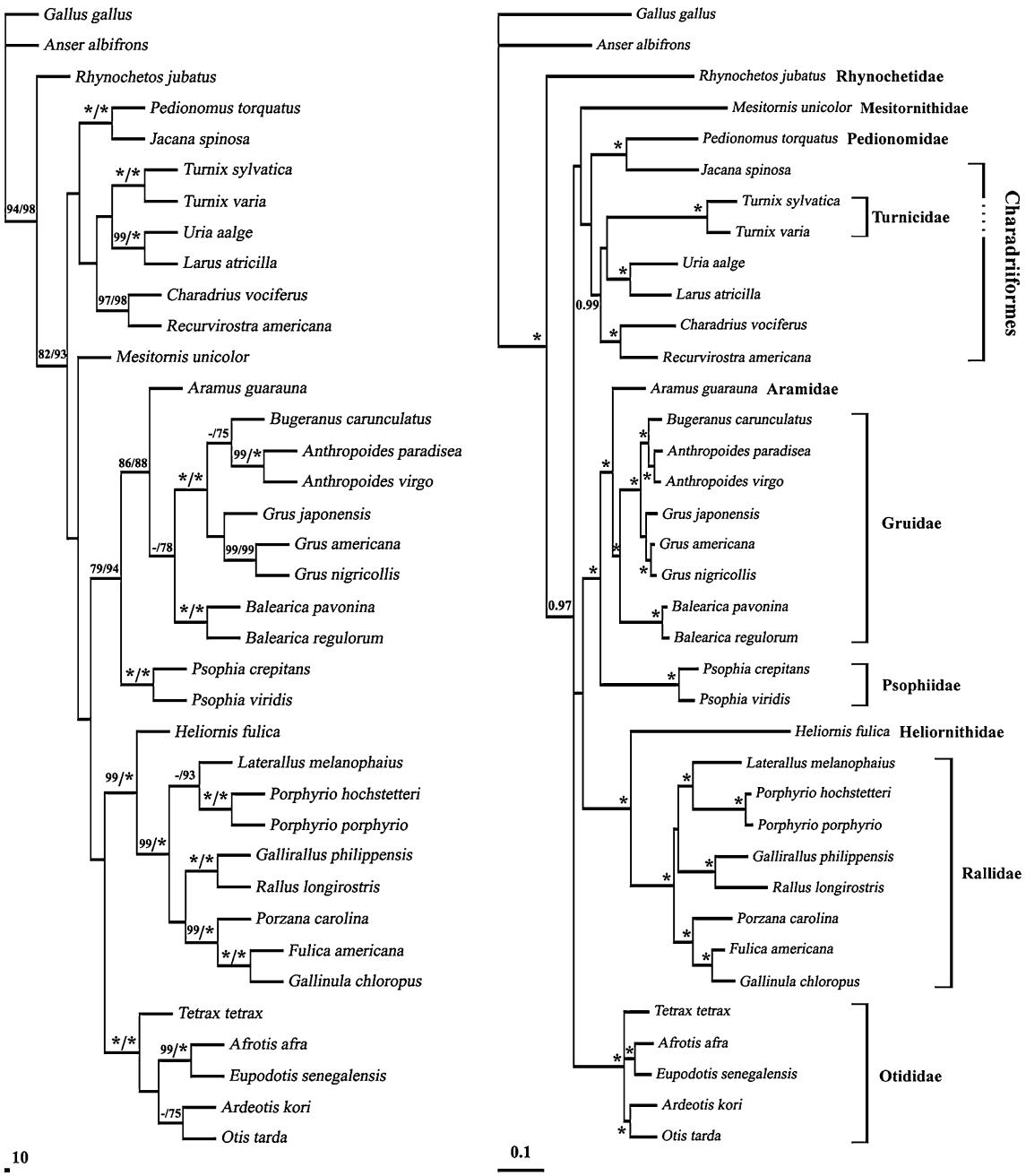

Fig. 3 Phylogenetic relationships of Gruiformes based on mitochondrial 12S, 16S and Val sequences. (*Left*) Trees obtained with maximum parsimony (CI = 0.3638, RI = 0.5126) and maximum likelihood (−LnL = 35142.11765) analysis. *Numbers* represent bootstrap values (MP/ML) and only those >70% are shown. *Asterisks* indicate bootstrap values of 100%. (*Right*) Tree obtained with Bayesian analysis (−LnL = 30320.417, Pinvar = 0.369941). *Numbers* represent posterior probabilities and only those >95% are shown. *Asterisks* indicate posterior probabilities of 100%

strong support in MP, ML and Bayesian (BA) trees (94% bootstrap values, 98% bootstrap values and 100% posterior probability values, respectively). Once Rhynochetidae was thought to be affiliated with the family Ardeidae (Aves: Ciconiiformes) for possessing powder down feathers [32]. Based on sequences of intron 7 of the nuclear encoded β-fibrinogen gene (FGB-int7), Fain and Houde [11] found that Rhynochetidae did not group with traditional Gruiformes, but instead with the proposed clade Metaves, which also include the doves, hoatzin, flamingos, tropicbirds, sandgrouse, grebes and some other birds. Hackett et al. [16], however, found that Rhynochetidae grouped with families from Caprimulgiformes and Apodiformes based on large sequence data representing 19 nuclear loci.

Thus, more phylogenetic studies are needed to confirm the taxonomic status of Rhynochetidae.

In MP and ML trees, Otididae (Bustards) is embedded in the clade of traditional Gruiformes without strong support. While in BA tree, Gruidae, Aramidae, Psophiidae, Heliornithidae and Rallidae are grouped to a clade defined as "core Gruiformes" [13]. Otididae is a sister group to core Gruiformes and Charadriiformes with strong support (97% posterior probability values). Moreover, the study of Fain and Houde [11] and Hackett et al. [16] showed that Otididae was outside of core Gruiformes. Recently, Fain and Houde [12] included bustards in an order: "Otidiformes". It is supported by the result of Bayesian analysis in this study. As there were only five genera from Otididae sampled in this study, the trees just show part of the phylogeny of Otididae that has been studied by Pitra et al. [35] and Broders et al. [3].

Pedionomidae and Turnicidae are embedded in the Charadriiformes clade in all trees. The result is also supported by recent phylogenetic studies [12, 16, 33]. This indicates that Pedionomidae and Turnicidae correctly belong to Charadriiformes.

In MP and ML trees, Mesitornithidae is a sister group to the traditional Gruiformes clade with weak support. While in BA tree, Mesitornithidae is a sister group to the Charadriiformes clade with weak support. Fain and Houde [11] found that Mesitornithidae grouped with the proposed clade Metaves. The study of Hackett et al. [16] confirmed that the Mesitornithidae was a sister group of the doves. The lack of homologues sequence data in our analysis may be the reason that Mesitornithidae is unstable in the phylogenetic trees.

In contrast to the overall taxonomic uncertainty surrounding many lineages considered gruiform, a consensus has begun to emerge that there is a monophyletic "core" consisting of Gruidae, Aramidae, Psophiidae, Heliornithidae and Rallidae [11, 13]. It is supported by the result of Bayesian analysis in this study. The phylogenetic relationship of the core Gruiformes based on Bayesian analysis is shown in Fig. 4. It has the same topology with the study of Livezey [27] and Fain et al. [13]. In MP and ML trees of this study, by removing Otididae, the phylogenetic relationship of the five families is the same with Fig. 4.

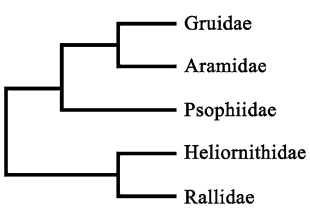

Fig. 4 Phylogenetic relationship of core Gruiformes

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