

Characterization of the Complete Mitochondrial Genome of Sea Duck *Mergus serrator* and Comparison with other Anseriformes Species

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ABSTRACT

As a key group in the Anatidae, the Mergini tribe (sea ducks) is strongly structured, with clear genetic assignments and phylogenetic relationships. The tribes also differ in terms of breeding strategy, longevity, and bionomic strategy. The mitochondrial genome (mitogenome) of *Mergus serrator* was 16,627 bp in length, and its structure was circular. The structure and gene arrangement of the genome were basically the same as those of Anseriformes species. The mean base composition of the mitogenome of Anseriformes was T (22.31 ± 0.51%), C (32.63 ± 0.64%), A (29.36 ± 0.64%), and G (15.71 ± 0.52%), indicating a slight specific bias towards A and C. AT content ranged of the mitogenome was from 50.27% to 55.31%, with an average value of 51.67 ± 1.10%, higher than the GC content and similar to that of birds in general (50.5% to 57.7%). In addition, the start and stop codons, the mitogenome consists of 3662 codons. The most commonly used amino acid was leucine (13.63%) in the use of *M. serrator*. The analyses indicated that the Anseriformes include the families Anseranatidae, Anhimidae, and Anatidae. Furthermore, Anatinae is composed of Aythyini, Anatini, Somaterini, and Mergini. *M. serrator* was sister to *M. merganser* and *M. squamatus*, and this group belongs to Mergini. The ω value of the ND3 gene in the Mergini tribe is lower than those for other tribes. The phylogenetic relationships were analyzed and *M. serrator* was sister to *M. merganser* and *M. squamatus*, and formed a closely evolved Mergini clade. Different evolutionary rates between the Mergini tribe and other tribes were found.

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Authors' Contribution

CP and LW conceived and designed the study. LW performed the statistical analysis, under supervision of LJ, LH, and ZJ. CP wrote the first draft of the manuscript, and the final version included edits from all authors. QL, CP and WL revised this manuscript.

Key words

Anseriformes, Mitochondrial DNA, Phylogenetic construction, Seabird, *Mergus serrator*

INTRODUCTION

Mitochondrial genome (mitogenome) harbors useful molecular information that can be used to infer phylogenetic relationships among Anseriformes different taxa (Livezey, 1997; Donne-Goussé *et al.*, 2002; Jiang *et al.*, 2009; Sun *et al.*, 2017), one of the best-studied groups

of birds (Livezey, 1986). Anseriformes includes 3 families, 8 tribes, and more than 150 species (Johnsgard, 2010; Ringelman *et al.*, 2015). Due to gene rearrangement, genetic introgression, gene flow, and hybridization, the taxonomic statuses and phylogenetic placements within the order remain in dispute (Scribner *et al.*, 2001; Gonzalez *et al.*, 2009; Lavretsky *et al.*, 2021). The Mergini tribe includes 20 species of which two are extinct (Johnsgard, 1966). As a key group in the Anatidae, the Mergini tribe (sea ducks) is strongly structured, with clear genetic assignments and phylogenetic relationships (Johnsgard, 2010). Whereas, the relationships among the Mergini tribe species are not certain based on multiple approaches and different genes so far. For example, the phylogenetic status of *Mergus serrator* is inconsistent based on different genes (Liu *et al.*, 2012, 2014; Lavretsky *et al.*, 2021). There is a remarkable difference in the life history and behavioral

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traits among different tribes in the Anseriformes (Peters *et al.*, 2012). Most tribes tend to live in inland streams, rivers, or ponds, such as the Anatini, Aythyini, and Anserini tribes (Debela *et al.*, 2021; Ponomarenko *et al.*, 2021). In contrast, the Mergini tribe roosts and forages in sea habitats (Johnsgard, 2010). The tribes also differ in terms of breeding strategy, longevity, and bionomic strategy (Savard *et al.*, 1998; Johnsgard, 2010). The Mergini tribe as an excellent tribe to investigate molecular adaptations to elevate metabolic burden associated with other life-history characteristics. Phylogenetic analyses can help investigate the relationships between mitochondrial DNA (mtDNA) molecular evolution and metabolic performance (Sruoga *et al.*, 2008; Sonsthagen *et al.*, 2011).

MtDNA is typically double-stranded and maternally (Wolstenholme, 1992; Roques *et al.*, 2004), it is valuable for studies of avian taxonomy and phylogeny (Shen *et al.*, 2009; Jetz *et al.*, 2012; Ren *et al.*, 2014), genetic structure (Ruan *et al.*, 2018), biological identification (Lecroy and Barker, 2006; Zhu *et al.*, 2021), and conservation genetics (Allendorf *et al.*, 2010), as it is a neutral molecular marker (Torroni *et al.*, 2001; Nabholz *et al.*, 2008). As the main energy suppliers of the cell, mitochondria produce approximately 90% of cellular ATP reserves through the highly efficient chemical osmotic coupling of electron and proton transfer to ATP synthesis (Saraste, 1999; Starkov, 2008). Evaluating the selective pressure of environmental temperature and oxygen availability on changes in mtDNA molecular could provide key insights for the adaptive evolution of mitogenomes (Luo *et al.*, 2013). The key to successful migration to a new habitat is whether it can adapt to different energetic demands. Energetic demand is essentially related to the ability of mitochondrial to generate energy through the process of oxidative phosphorylation, such as in certain positions encoded by the NADH dehydrogenase genes (ND4 and ND5) (Janssen *et al.*, 2004; Liu *et al.*, 2018). However, single or combined genes have obvious shortcomings in phylogenetic analysis, such as insufficient information (Liu *et al.*, 2012). These are overcome when using complete sequences of mitochondria, and it is becoming the first-choice marker for resolving controversial species relationships (García *et al.*, 2014; Peng *et al.*, 2017).

In this study, we sequenced the mitogenome of a typical sea duck, *M. serrator*, to analyze the genetic structure of and phylogenetic relationships among the Mergini tribe. In recent years, an increasing number of mitogenomes of the Mergini tribe have been sequenced. This study aims to shed increased light on the phylogenetic status of *M. serrator* in the Mergini tribe and to elucidate the evolutionary rates and molecular signatures of natural selection for all 13 mtDNA protein coding genes (PCGs)

in sea ducks.

MATERIALS AND METHODS

Ethics approval and consent to participate

The sample collection was strictly conducted under national ethical guidelines (Regulations for Administration of Affairs Concerning Experimental Animals, China, 1988) for animal husbandry and humane treatment.

Sample collection and genome sequencing

We collected a dead specimen of *M. serrator* from Dalian (38°51'52.22"N 121°39'39.11"E; Liaoning, China). A muscle sample was transferred to a 5 mL centrifuge tube, 1 mL 75% ethyl alcohol was added, and the sample was stored in a refrigerator at −20°C. The sample (specimen voucher: NIES20210320HXQSYS01) was deposited in the Museum of Laboratory of Biodiversity Investigation of Nanjing Institute of Environment Science.

Total genomic DNA was extracted from muscle tissue using the Easy Pure genomic DNA kit (Trans Gen Biotech Co, Beijing, China). The original sequence data (15.65 G) was deposited in NCBI's Sequence Read Archive (SRA; accession: SRR13516389), and complete mitogenome sequencing (15.62 G) was performed on an Illumina NovaSeq6000 platform (Novogene Bioinformatics Technology Co. Ltd., Tianjin, China) (Tang *et al.*, 2021). The mitogenome was assembled using the program NOVO Plasty 3.7 (Dierckx *et al.*, 2017) and then adjusted manually. The annotated using the MITOS Web Server (Bernt *et al.*, 2013). The assembled mitogenome sequences have been deposited in GenBank under accession numbers MZ365040.

Data acquisition and analysis

All sequences were aligned on Clustal X using the default options (Thompson *et al.*, 1997). PCGs were identified by comparison with the corresponding known complete mtDNA sequence of *M. merganser* and *M. squamatus* using Sequin 11.0. The 22 tRNA genes of cloverleaf secondary structures and anticodon sequences were identified from MITOS WebServer (<http://mitos.bioinf.uni-leipzig.de/index.py>). The control region (CR) was identified by sequence homology analyses (Cadahia *et al.*, 2009; Barker *et al.*, 2012). The start and stop codons of 13 PCGs were identified in the *M. serrator* mitogenome by ORF Finder (<https://www.ncbi.nlm.nih.gov/orffinder/>) and use the CGView Server software to generate a graphical map of the mitogenome and modified it manually (Table I). The codon number and relative synonymous codon usage (RSCU) of three species of *Mergus* mitochondrial PCGs were obtained using MEGA X and PhyloSuite.

Table I. Organization of the complete mtDNAs of *Mergus serrator*.

Gene	Location	Length (bp)	Start codon	End codon	Spacer (+) or overlap (-)	Strand
tRNA ^{Phe}	1-72	72			-1	H
12S rRNA	72-1066	995			0	H
tRNA ^{Val}	1067-1137	71			0	H
16S rRNA	1138-2737	1600			0	H
tRNA ^{Leu(UUR)}	2738-2811	74			6	H
ND1	2818-3795	978	ATA	AGG	-2	H
tRNA ^{Ile}	3794-3866	73			7	H
tRNA ^{Gln}	3874-3944	71			-1	L
tRNA ^{Met}	3944-4012	69			0	H
ND2	4013-5051	1039	ATG	T--	0	H
tRNA ^{Trp}	5052-5127	76			3	H
tRNA ^{Ala}	5131-5199	69			1	L
tRNA ^{Asn}	5201-5273	73			0	L
tRNA ^{Cys}	5274-5337	64			0	L
tRNA ^{Tyr}	5338-5408	71			1	L
COI	5410-6960	1551	GTG	AGG	-9	H
tRNA ^{Ser(UCN)}	6952-7024	73			2	L
tRNA ^{Asp}	7027-7095	69			1	H
CO II	7097-7783	687	GTG	TAA	1	H
tRNA ^{Lys}	7785-7853	69			1	H
ATP8	7855-8022	168	ATG	TAA	-10	H
ATP6	8013-8696	684	ATG	TAA	-1	H
COIII	8696-9479	784	ATG	T--	0	H
tRNA ^{Gly}	9480-9548	69			0	H
ND3	9549-9722	174	ATG	TAG	1	H
	9724-9900	177			1	H
tRNA ^{Arg}	9902-9971	70			0	H
ND4L	9972-10268	297	ATG	TAA	-7	H
ND4	10262-11639	1378	ATG	T--	0	H
tRNA ^{His}	11640-11708	69			0	H
tRNA ^{Ser(AGY)}	11709-11774	66			-1	H
tRNA ^{Leu(CUN)}	11774-11844	71			0	H
ND5	11845-13668	1824	GTG	TAA	-1	H
CYTB	13668-14810	1143	ATG	TAA	2	H
tRNA ^{Thr}	14813-14880	68			10	H
tRNA ^{Pro}	14891-14960	70			10	L
ND6	14971-15492	522	ATG	TAG	0	L
tRNA ^{Glu}	15493-15560	68			0	L
CR	15561-16627	1067			0	H

Negative values indicate overlapping; H, heavy strand; L, light strand.

Table II. Nucleotide composition of the mitochondrial genomes of 53 Anseriformes species.

Family/Genus	Species	T	C	A	G	AT skew	GC skew	A+T	G+C	Total	Accession number
Anatidae											
<i>Aix</i>	<i>Aix galericulata</i>	22.35	32.77	29.21	15.66	0.13	-0.35	51.57	48.43	16605	NC023969
<i>Anas</i>	<i>Anas acuta</i>	22.12	32.86	28.78	16.24	0.13	-0.34	50.90	49.10	16599	NC024631
	<i>A. crecca</i>	22.35	32.64	29.05	15.96	0.13	-0.34	51.40	48.60	16601	NC022452
<i>Anser</i>	<i>A. platyrhynchos</i>	22.21	32.81	29.20	15.78	0.14	-0.35	51.41	48.59	16604	NC009684
	<i>A. poecilorhyncha</i>	22.21	32.82	29.17	15.81	0.14	-0.35	51.37	48.63	16608	NC022418
	<i>Anser albifrons</i>	22.63	32.05	30.15	15.18	0.14	-0.36	52.78	47.22	16737	NC004539
	<i>A. anser</i>	22.58	32.14	30.18	15.10	0.14	-0.36	52.77	47.23	16738	NC011196
	<i>A. cygnoides</i>	22.49	32.24	30.21	15.06	0.15	-0.36	52.70	47.30	16739	NC023832
	<i>A. fabalis</i>	22.73	31.84	30.07	15.36	0.14	-0.35	52.80	47.20	16688	NC016922
	<i>A. indicus</i>	22.50	32.23	30.17	15.10	0.15	-0.36	52.67	47.33	16730	NC025654
<i>Asarcornis</i>	<i>Asarcornis scutulata</i>	21.77	33.22	28.88	16.13	0.14	-0.35	50.64	49.36	16539	NC052827
<i>Aythya</i>	<i>Aythya americana</i>	22.24	32.75	29.39	15.62	0.14	-0.35	51.62	48.38	16616	NC000877
	<i>A. ferina</i>	22.19	32.83	29.42	15.56	0.14	-0.36	51.61	48.39	16616	NC024602
	<i>A. fuligula</i>	22.18	32.87	29.42	15.52	0.14	-0.36	51.61	48.39	16616	NC024595
	<i>A. nyroca</i>	22.28	32.71	29.66	15.35	0.14	-0.36	51.94	48.06	16623	MW287344
<i>Branta</i>	<i>Branta bernicla</i>	22.68	31.99	29.91	15.42	0.14	-0.35	52.59	47.41	16747	KJ680301
	<i>B. canadensis</i>	22.60	32.07	30.18	15.14	0.14	-0.36	52.79	47.21	16760	NC007011
<i>Bucephala</i>	<i>Bucephala albeola</i>	21.82	33.36	28.52	16.30	0.13	-0.34	50.34	49.66	16614	MW849286
	<i>B. clangula</i>	21.65	33.46	28.68	16.21	0.14	-0.35	50.33	49.67	16625	MW849283
	<i>B. islandica</i>	21.65	33.43	28.68	16.24	0.14	-0.35	50.32	49.68	16627	MW849281
<i>Cairina</i>	<i>Cairina moschata</i>	21.93	32.95	29.01	16.12	0.14	-0.34	50.93	49.07	16610	NC010965
<i>Clangula</i>	<i>Clangula hyemalis</i>	21.83	33.31	29.18	15.68	0.14	-0.36	51.01	48.99	16610	MW849278
<i>Cygnus</i>	<i>Cygnus atratus</i>	22.20	32.55	29.52	15.73	0.14	-0.35	51.72	48.28	16748	NC012843
	<i>C. columbianus</i>	22.79	31.89	30.09	15.23	0.14	-0.35	52.88	47.12	16728	NC007691
	<i>C. cygnus</i>	22.79	31.97	29.97	15.27	0.14	-0.35	52.76	47.24	16724	NC027095
	<i>C. olor</i>	22.59	32.12	29.64	15.65	0.13	-0.34	52.23	47.77	16739	NC027096
<i>Dendrocygna</i>	<i>Dendrocygna javanica</i>	23.67	30.44	30.44	15.45	0.13	-0.33	54.11	45.89	16753	NC012844
<i>Heteronetta</i>	<i>Heteronetta atricapilla</i>	23.00	31.93	30.01	15.06	0.13	-0.36	53.00	47.00	16723	CM021836
<i>Histrionicus</i>	<i>Histrionicus histrionicus</i>	21.79	33.29	29.32	15.61	0.15	-0.36	51.11	48.89	16634	MW849288
<i>Lophodytes</i>	<i>Lophodytes cucullatus</i>	21.96	33.02	29.01	16.01	0.14	-0.35	50.97	49.03	16549	MW849287
<i>Mareca</i>	<i>Mareca falcata</i>	22.35	32.65	28.88	16.11	0.13	-0.34	51.24	48.76	16601	NC023352
	<i>M. penelope</i>	22.29	32.71	28.89	16.11	0.13	-0.34	51.18	48.82	16596	NC050973
	<i>M. strepera</i>	22.19	32.81	28.84	16.16	0.13	-0.34	51.03	48.97	16600	NC045373
<i>Melanitta</i>	<i>Melanitta deglandi</i>	21.58	33.45	28.74	16.22	0.14	-0.35	50.33	49.67	16452	MW849279
	<i>M. nigra</i>	21.77	33.32	28.83	16.08	0.14	-0.35	50.60	49.40	16552	MW849282
	<i>M. perspicillata</i>	21.61	33.49	28.66	16.24	0.14	-0.35	50.27	49.73	16499	MW849280
<i>Mergus</i>	<i>Mergus merganser</i>	21.88	33.24	28.73	16.16	0.14	-0.35	50.60	49.40	16630	NC040986
	<i>M. serrator</i>	21.72	33.38	28.77	16.13	0.14	-0.35	50.49	49.51	16627	MZ365040
	<i>M. squamatus</i>	22.26	32.76	29.03	15.95	0.13	-0.35	51.29	48.71	16595	NC016723

Table continues on next page.....

Family/Genus	Species	T	C	A	G	AT skew	GC skew	A+T	G+C	Total	Accession number
<i>Netta</i>	<i>Netta rufina</i>	22.44	32.59	29.61	15.37	0.14	-0.36	52.04	47.96	16625	NC024922
<i>Nettapus</i>	<i>Nettapus auritus</i>	22.83	32.07	29.79	15.31	0.13	-0.35	52.62	47.38	16643	CM021833
<i>Oxyura</i>	<i>Oxyura jamaicensis</i>	22.94	32.30	28.81	15.94	0.11	-0.34	51.75	48.25	15914	CM021834
<i>Polysticta</i>	<i>Polysticta stelleri</i>	21.89	33.04	28.79	16.28	0.14	-0.34	50.68	49.32	16612	MW849289
<i>Sibirionetta</i>	<i>Sibirionetta formosa</i>	22.51	32.44	29.52	15.53	0.13	-0.35	52.03	47.97	16592	NC015482
<i>Somateria</i>	<i>Somateria fischeri</i>	21.90	33.12	28.74	16.25	0.14	-0.34	50.64	49.36	16601	MW849290
	<i>S. mollissima</i>	21.97	33.02	28.62	16.39	0.13	-0.34	50.59	49.41	16626	MW849292
	<i>S. spectabilis</i>	21.93	33.07	28.71	16.29	0.13	-0.34	50.65	49.35	16627	MW849291
<i>Spatula</i>	<i>Spatula clypeata</i>	22.47	32.49	29.39	15.65	0.13	-0.35	51.86	48.14	16599	NC028346
<i>Stictonetta</i>	<i>Stictonetta naevosa</i>	22.39	32.80	28.45	16.35	0.12	-0.33	50.84	49.16	16778	CM021835
<i>Tadorna</i>	<i>Tadorna ferruginea</i>	21.99	32.96	29.57	15.48	0.15	-0.36	51.56	48.44	16639	NC024640
	<i>T. tadorna</i>	22.21	32.82	29.20	15.78	0.14	-0.35	51.40	48.60	16604	NC024750
Anhimidae											
<i>Chauna</i>	<i>Chauna torquata</i>	24.01	30.82	31.31	13.87	0.13	-0.38	55.31	44.69	16766	NC052807
Anseranatidae											
<i>Anseranas</i>	<i>Anseranas semipalmata</i>	23.49	31.38	30.92	14.20	0.14	-0.38	54.41	45.59	16868	NC005933
Averages		22.31	32.63	29.36	15.71	0.14	-0.35	51.67	48.33	16632	
Standard deviation		0.51	0.64	0.64	0.52	0.01	0.01	1.10	1.10	127.12	

Retrieved and downloaded the complete mitogenomes of 53 Anseriformes species from GenBank, and including 2 families and 46 genera (Table II). We used MEGA5.0 (Tamura *et al.*, 2011) and DNASTAR (Burland, 2000) to compare the sequences of these birds and to analyze their base content. The calculation for the skewing of nucleotide composition was AT skew = $(A - T) / (A + T)$, GC skew = $(G - C) / (G + C)$ (Perna and Kocher, 1995).

Analysis of phylogeny

We collected the complete mitogenome of 53 Anseriformes species for phylogenetic analysis (Table II). Corresponding *Gallus gallus*, *Hydrophasianus chirurgus*, and *Porzana fusca* sequence were used as outgroups. Phylogenetic trees were estimated using ML and BI methods. The optimal parameter model selected by MrModel Test 3.06 (Nylander *et al.*, 2004) and PAUP 4.0b10 software (Swofford, 2003). The GTR + I + G model that was selected as the best fit model for nucleotide phylogenetic analysis. The BI trees of Anseriformes birds were constructed using MrBayes 3.1.2 software (Ronquist *et al.*, 2012). The ML tree was constructed using the MEGA5.0 software (Tamura *et al.*, 2011), as follows: run the files in Phy format, select the best model using the Mr Model Test 3.06 software filter, set the ML in bootstrap mode, repeat bootstraps 50 times, and run the whole process 10,000 times to obtain the ML tree. Finally,

Treeview32 (Saldanha, 2004) software was used to create and annotate the evolutionary tree diagram.

Analysis of adaptive molecular evolution

In order to test the possible influence of topology on the inference of selected locations, estimated ω (dN/dS) rate using the topology inferred from genomic data (Nadeau *et al.*, 2007; Stoletzki and Eyre-Walker, 2011). dN is the non-synonymous substitution rate, or the rate at which changes in nucleotide sites lead to changes in new amino acid chains. Things are the opposite for dS (synonymous substitution). The dN/dS ratio provides evidence for selective restraint and pressures acting on PCGs (Shen *et al.*, 2009; Botero-Castro *et al.*, 2017). If natural selection is favoring amino acid changes, $\omega > 1$ indicates positive selection. A ratio below 1 indicates purification selection, and a ratio at 1 indicates neutral evolution.

The phylogenetic tree has many branches, and test the role of selection on the Mergini branch. We employed CodeML branch models that estimate the selective pressure between Mergini tribes and other tribes, which allow the estimation of dN/dS of specific branches and clades of interest related to other clades and other parts of the tree (Zhang *et al.*, 2005). In the dual scale and all branch site models described below, we label these species in the Mergini tribes as the foreground branches in a separate analysis. Use branch-site analysis to determine whether a

portion of sites was subject to positive selection. To further evaluate these sites, we performed a positive selection analysis using the site model in PAML4. Selection Test was carried out using likelihood-ratio tests (LRT). The model was compared with a null model, in which the ω of the foreground partition class of the selected location was restricted to 1 (Zhang *et al.*, 2005). LRT was used to identify the significance of selective pressure between different pedigrees.

RESULTS AND DISCUSSION

Genome organization and composition

M. serrator is a typical sea duck in the Mergini tribe and its complete mitogenome was found to be 16,627 bp (Table I). So far, the length of the mitogenome of birds that has been sequenced is from 16,300 bp to 23,500 bp (Gao *et al.*, 2009; Lei, 2015). Among Anseriformes species, the shortest mitogenome belongs to *Oxyura jamaicensis* (15,914 bp) and the longest is that of *Anseranas semipalmata* (16,868 bp). In the Anseriformes taxa, the length and gene arrangement of the mitogenome are quite conserved. The genomes of *M. serrator* contained a typical set of 37 genes (Fig. 1), including 13 PCGs, 2 ribosomal RNAs, 22 transfer RNAs, and a non-coding putative CR (Table I, Fig. 2). The structure and gene arrangement were basically the same as most birds (Gao *et al.*, 2009; Lei, 2015). An ND6 gene and eight tRNA genes (tRNA^{Gln}, tRNA^{Ala}, tRNA^{Asn}, tRNA^{Cys}, tRNA^{Tyr}, tRNA^{Ser}(UCN), tRNA^{Pro}, and tRNA^{Glu}) were encoded by the L-strand, whereas other genes were encoded by the H-strand (Table I).

The overall information about the mitogenome of *M. serrator* was as follows: T: 21.72%, C: 33.38%, A: 28.77%, and G: 16.13% and the content of GC is 49.51% with A+T-rich feature, same as in other species of Anseriformes. The mean base composition of Anseriformes is T (22.31 ± 0.51%), C (32.63 ± 0.64%), A (29.36 ± 0.64%), and G (15.71 ± 0.52%). Additionally, in our study, the sequence of base richness was mainly C > A > T > G, and only *Chauna torquata* had base contents of A > C > T > G. The AT content range is 50.27% ~ 55.31%, with an average value is 51.67 ± 1.10%, which is higher than the GC content and similar to that of birds in general (50.5% to 57.7%) (Gao *et al.*, 2009).

Vertebrate mtDNA usually shows significant chain bias in nucleotide composition, and this chain bias can be measured as AT and GC skew. We measured the AT skew (0.14) and GC skew (-0.35) of the H-strand in the *M. serrator* mitogenome. The mean AT skew was 0.14 ± 0.01, and the GC skew was -0.35 ± 0.01. It shows that the nucleotide composition of the complete mitogenome of Anseriformes has a slight specific bias against A and C.

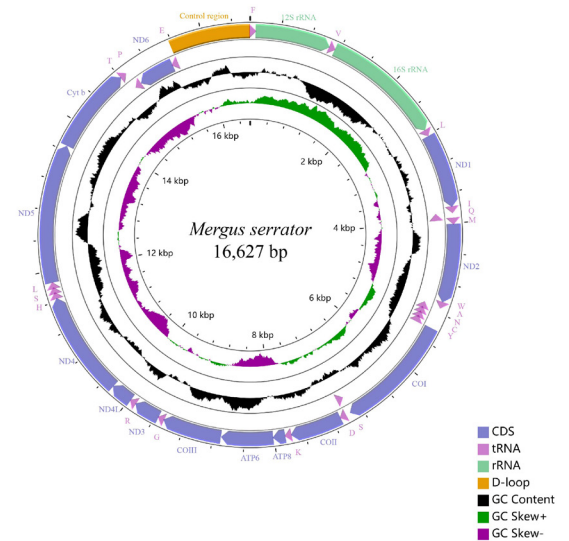


Fig. 1. Mitochondrial genomes of *M. serrator*. Arrows indicate the orientation of gene transcription. PCGs are shown as blue arrows, rRNA as light green arrows, control region as orange arrows, and tRNA genes as purple arrows. tRNA genes are designated by single-letter amino acid abbreviations. Labeling from the outside to the inside circle: genes encoded on the heavy strand, genes encoded on the light strand. The black circle demonstrates the GC content (the peaks outside/inside the circle indicate values higher or lower than average GC content). The purple-green circle demonstrates GC skew, purple (between 0 and 1), and green (between -1 and 0).

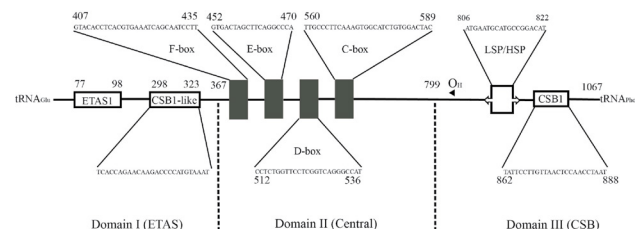


Fig. 2. General structure of the mtDNA control region of *M. serrator*.

Analysis of PCGs and codon usage

The genome of *M. serrator* had 13 PCGs, and 12 PCGs were encoded on the H-strand except ND6. The overall length of 13 PCGs was 11,229 bp, which accounted for 67.53% of the whole mitogenome (Table I). The longest was the ND5 gene (1,824 bp) and the shortest was the ATP8 gene (168 bp). Among the 13 PCGs, the start codon of 9 PCGs was ATG, and the nonstandard start codon GTG was found in COI, COII, and ND5 genes. In addition, the start codon of ND1 gene was ATA (Table I). The most common stop codon was TAA, which has been used six

times. TAG and AGG were used twice as stop codons, and the terminal T probably served as the stop signal in the ND2, ND4, and COIII genes. In the ND3 gene, the base A of the stop codon was not translated.

The RSCU values of 13 PCGs are shown in Figure 3. In addition, the start and stop codons, the mitogenome consists of 3662 codons. The most commonly used amino acid was leucine (13.63%) in the use of 13 PCGs of *M. serrator*, followed by proline (11.47%), serine (9.59%), and threonine (8.06%). In three Mergini species, eight amino acids (Pro, Thr, Leu1, Arg, Ala, Ser1, Val, and Gly) were encoded by four different codons, and the others were encoded by two different codons. The overall AT-skew and GC-skew of the 13 PCGs was 0.06 and -0.36, respectively. The base composition of PCGs has a higher A + C contents, and a preferred to use A or C nucleotides in the first codon position (Fig. 3). This phenomenon also occurs in other species of Mergini, especially *M. squamatus* and *M. merganser*.

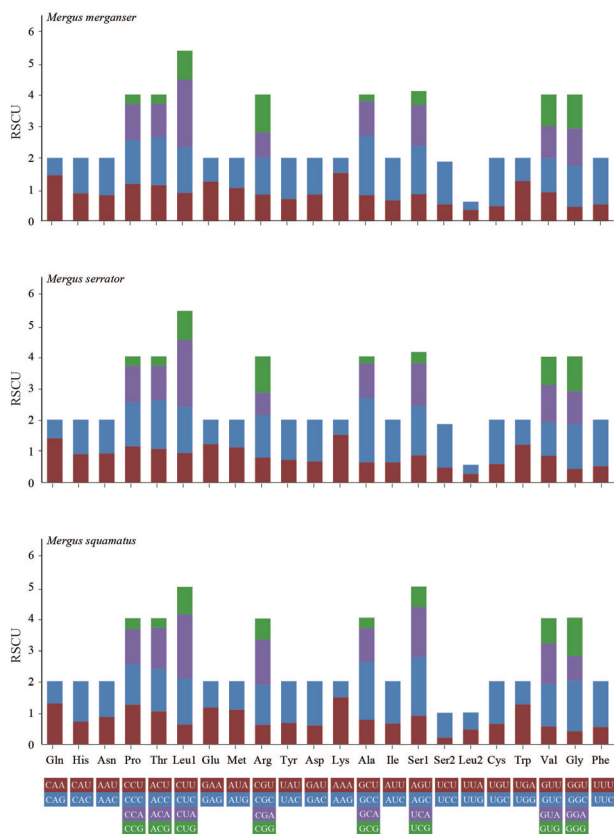


Fig. 3. Codon number and relative synonymous codon usage (RSCU) of mitochondrial PCGs of three species of *Mergus*.

Ribosomal RNAs, transfer RNAs, and CR

In the *M. serrator* mitogenome, ribosomal RNA genes include 12S rRNA and 16S rRNA, which are 995 bp and 1600 bp in length, respectively. They are located between the *tRNA^{Phe}* and *tRNA^{Leu}* genes, and separated by the *tRNA^{Val}*, a feature often found in the mitogenomes of birds (Cheng *et al.*, 2011; Jin *et al.*, 2012). Two ribosomal RNAs were identified on the H-strand. The A + T content of both rRNA genes were collectively 52.5% and they had a positive AT-skew (0.28).

A total of 22 tRNAs were found in the mitogenome, and they were scattered between rRNAs and PCGs. Their sizes is between 64 bp and 76 bp (Table I, Supplementary Fig. 1). Three tRNA clusters, namely, IQM (*tRNA^{Ile}* *tRNA^{Gln}* *tRNA^{Met}*), WANCY (*tRNA^{Trp}* *tRNA^{Ala}* *tRNA^{Asn}* *tRNA^{Cys}* *tRNA^{Tyr}*), and HSL (*tRNA^{His}* *tRNA^{Ser}* (AGY) *tRNA^{Leu}* (CUN)), were determined. Sequences of the tRNA genes could be folded into a canonical cloverleaf secondary structure.

The CR of mtDNAs of *M. serrator* was 1,067 bp, which was located between the *tRNA^{Glu}* and *tRNA^{Phe}* genes. The CR could be divided into three domains according to the base composition (5'-peripheral domain, Domain I; central conserved domain, Domain II; and 3'-peripheral domains, Domain III). In Domain I, extended termination-associated sequences 1 (ETAS1) was observed at position nt 77 - 98 (Fig. 2). A sequence block was also found in domain I (nt 298–323), which is similar to the conserved sequence block (CSB1-like). Four conserved sequence boxes (C, D, E, and F) were identified in Domain II. CBS1 (nt 407–589) and the H- and L-strand transcription promoter sites (nt 806–822) occurred in Domain III.

Phylogenetic analysis

The complete sequences should be suitable for the phylogenetic analysis and research of Anseriformes. Reconstruction of BI and ML trees using complete mitogenome sequences of 56 species. Use the best-fit model of GTR+G+I (-lnL = 258460.5951, AIC= 516937.2376) and calculated with the Modeltest and Mrmodeltest programs for analysis. Since the generated BI and ML trees show the same topological structure, only the BI tree is displayed (the numbers on the branches represent the bootstrap support for BI and ML trees).

Traditional morphological analysis divides Anatinae into Tadornini, Tachyerini, Cairinini, Anatini, Aythyini, Mergini, Oxyurini, and others. In the literature, Anseriformes species are relatively clearly classified at the level of the family but not the subfamily and genus (Liu *et al.*, 2012; García *et al.*, 2014; Hu *et al.*, 2017). The replacement rate and mutation rate of the mitochondrial sequence of Anseriformes species are high (Fain *et al.*, 2007; Yang *et al.*, 2010). The BI and ML trees showed

the complete mitogenome has the ability to distinguish 53 Anseriformes species. The phylogenetic analysis based on the complete mitogenome strongly supports the monophylicity of the order Anseriformes. The analyses indicated that the Anseriformes include the families Anseranatidae, Anhimidae, and Anatidae. *Anseranas semipalmata* was included in the branch of Anseranatidae, and *Chauna torquata* was included in the branch of Anhimidae, and other species belong to the branch of Anatidae (Fig. 4). The branch of Anatidae included Dendrocygninae, Oxyurinae, Stictonettinae, Anserinae, Tadorninae, and Anatinae. The Anatinae had the largest number (32) of species. The Anatinae included Aythyini, Anatini, Somaterini, and Mergini. The results suggest that *M. serrator* is sister to *M. merganser* and *M. squamatus*, which is slightly different from a previous study (Liu *et al.*, 2014). However, both studies suggest that this group belongs to the Mergini.

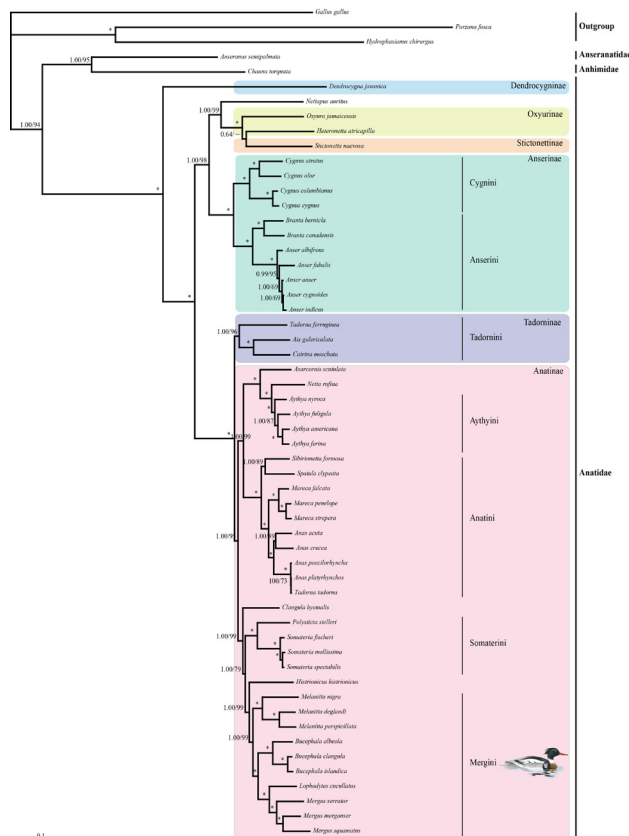


Fig. 4. BI and ML trees constructed based on complete mtDNA sequences of Anseriformes. Numbers represent bootstrap values (MP/ML) and only those >60% are shown. Asterisks indicate posterior probabilities of 100%.

Selection analysis

Most mitochondrial genes besides the ND6 gene are conserved and evolved under purifying selection (Fig. 5). The branch model detected significant signals of positive selection for the ND6 gene tested in all tribes of Anseriformes. It also fitted better for most PCGs when the Mergini tribe was labeled as a foreground branch (Fig. 5) (LRT p value < 0.05). In addition, the ND6 gene showed the highest ω values ($\omega > 1$) both for the foreground ($\omega = 4.59$) and background ($\omega = 3.28$) branches, compared to other genes. The ω value of the ND3 gene in the Mergini tribe is lower than those for other tribes. The ω values of other genes in the Mergini tribe are higher than those for other tribes. The free-ratio model fitting the data of 13 PCGs is significantly better than the null hypothesis (one-ratio model), indicating that there are different evolution rates among the tribes included in our data set.

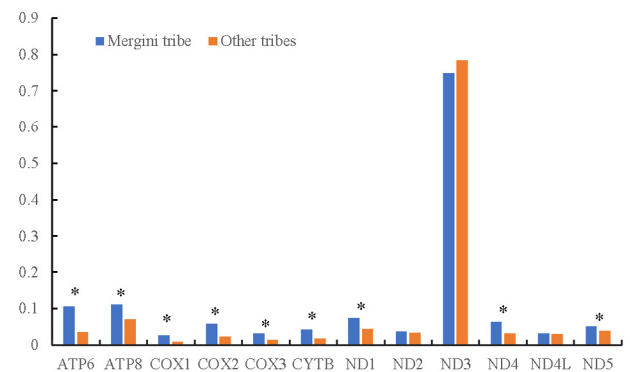


Fig. 5. Natural selection strength and the ratio of non-synonymous to synonymous substitutions (ω) calculated using the two-ratio model for 12 purifying selective protein-coding genes between the Mergini tribe and other tribes. Genes in which the two-ratio model fits better to the data (LRT $p < 0.05$) are marked with an asterisk.

CONCLUSION

The mitogenome (16,627 bp) was larger than those of most Anseriformes species. A positive AT skew consistent with other Anseriformes species was found, which implies a slightly specific bias towards A and C in the Anseriformes. PCGs accounted for 67.53% of the whole mitogenome and most of these genes were encoded by the L-strand. ATG and TAA were the most frequent start codon and stop codon, respectively. We summarized the RSCU values for the 13 PCGs and 3788 codons and high A+C contents were found, which also occurred in other species of Mergini. Two ribosomal RNA genes, 12S rRNA and 16S rRNA, were encoded on the H-strand and had a positive A-T skew. Sequences of 22 tRNA genes ranged from 64 bp to 76 bp and could be folded into a canonical cloverleaf

secondary structure. The phylogenetic relationships were analyzed and *M. serrator* was sister to *M. merganser* and *M. squamatus*, and formed a closely evolved Mergini clade. Compared to other species of Anseriformes, most PCGs evolved under purifying selection except for the ND6 gene. Different evolutionary rates between the Mergini tribe and other tribes were found.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: <https://dx.doi.org/10.17582/journal.pjz/20220314030351>

Conflict of interest statement

The authors have declared no conflict of interest.

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