BMC Zoology

RESEARCH Open Access

A case of successful brood parasitism by Mandarin Duck (*Aix galericulata*) on Scaly-sided Merganser (*Mergus squamatus*)

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Abstract

Brood parasitism is a significant reproductive strategy among birds. Both the Scaly-sided Merganser (*Mergus squamatus*) and the Mandarin Duck (*Aix galericulata*) are tree-cavity-nesting waterbirds with overlapping ecological niches. Although field observations have previously suggested that Mandarin Ducks may parasitize the nests of Scaly-sided Mergansers, direct evidence of successful hatching of parasitic eggs has been lacking. During the 2024 breeding season in the Manjiang region of Changbai Mountain, Jilin Province, field monitoring documented a case where a Mandarin Duck laid an egg in an artificial nest box intended for Scaly-sided Mergansers. Using eggshell membranes collected after hatching, we performed species identification for all ducklings based on the mitochondrial 12S rRNA gene as a genetic marker. Combined with morphological observations, we confirmed that one duckling was a Mandarin Duck, while the rest were Scaly-sided Mergansers. Analyses of the mitochondrial D-loop region and eight microsatellite loci indicated that all Scaly-sided Merganser ducklings were full-sibling offspring from the same parental pair. This supports the genetic monogamy in this nest and the absence of intraspecific brood parasitism in this instance. Our study provides molecular-level evidence for a case of successful interspecific brood parasitism by a Mandarin Duck on a Scaly-sided Merganser, enriching our understanding of interspecific interactions among tree-cavity-nesting birds. The findings also offer insights for the conservation of endangered species like the Scaly-sided Merganser. Furthermore, we identified a sequence discrepancy in the Scaly-sided Merganser's 12S rRNA compared to existing reference sequences, suggesting a need for re-sequencing its mitochondrial genome.

Keywords DNA identification, interspecific brood parasitism, Scaly-sided Merganser, Mandarin Duck

Brood parasitism, a unique and complex reproductive strategy in birds where the parasite lays its eggs in the host's nest, shifting the costs of incubation and chick-rearing to the host, has garnered significant attention in the field of behavioral ecology. Based on whether the species builds its own nest, brood parasitism can be categorized as obligate or facultative [1, 2]. Obligate brood parasitism represents an extreme strategy adopted by species such as the Common Cuckoo (*Cuculus canorus*) and black-headed duck (*Heteronetta atricapilla*) [1, 3]. These species completely forgo nest-building, incubation, and parental care, relying entirely on hosts to perform all parental duties from incubation to fledging. In contrast, facultative brood parasitism occurs when individuals of a species, which are capable of building their own nests and breeding independently, occasionally lay eggs in the nests of conspecifics or heterospecifics. For facultative

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parasites, this behavior is often considered an adaptive tactic in response to ecological pressures such as nest-site limitation, predation pressure, or temporal mismatches in breeding. By transferring the task of incubation to a host, facultative parasitic individuals may potentially increase their overall fitness. Within cavity-nesting bird communities, the nest cavity entrance can act as a physical barrier to some extent against obligate brood parasites [4]. Consequently, recorded incidents of both intraspecific and interspecific brood parasitism are far less common among cavity-nesting birds compared to open-nesting species. This scarcity is primarily due to the high concealment of natural cavities, which makes direct observation challenging, with most existing research relying on monitoring using artificial nest boxes [5]. Therefore, documenting cases of brood parasitism holds significant importance for revealing the diversity of reproductive behaviors and the intricacies of interspecific interactions among cavity-nesting birds.

The Scaly-sided Merganser (*Mergus squamatus*) is a rare and endemic species in China, classified as a First-Grade State Key Protected Animal. It belongs to the family Anatidae within the order Anseriformes and is listed as Endangered on the IUCN Red List of Threatened Species [6, 7]. They possessed long, narrow bills and exhibited brownish coloration from the crown to the nape. They inhabit fast-flowing rivers in forested areas and nest in cavities of large living broad-leaved trees [8], typically more than 10 m above the ground. The clutch size ranges from approximately 7 to 18 eggs [9]. In recent years, due to the limited availability of natural tree cavities, the breeding success of Scaly-sided Mergansers has been constrained, making artificial nest boxes an important conservation tool. Similar to other cavity-nesting ducks, intraspecific brood parasitism is relatively common in this species. In a study conducted in the Primorsky Krai region of the Russian Far East, Solovyeva et al. successfully identified intraspecific parasitic eggs by assessing the consistency of embryonic development stages among eggs within the same clutch [9]. The results showed that parasitic eggs accounted for an average of approximately 4.1% (range 3%–4.7%) of all eggs with known developmental stages, and since parasitism during the host's own laying period is difficult to detect, the actual rate may be even higher. Generally, intraspecific parasitism has little impact on the overall reproductive success of the population [9, 10]. Consequently, only one case of nest abandonment attributable to intraspecific parasitism was recorded throughout the observation period [9]. Additionally, in 2019, Liu Dongping et al. observed in the Changbai Mountain area an incubating parent fiercely attacking a female that had intruded and settled in its nest [10]. Subsequently, the host did not exhibit obvious egg-rejection behavior during the incubation period, suggesting the occurrence of intraspecific brood parasitism in Scaly-sided Mergansers. Regarding interspecific brood parasitism, although Solovyeva et al. speculated that individual abandoned mixed clutches of Mandarin Duck and Scaly-sided Merganser eggs might have resulted from egg-laying by Scaly-sided Mergansers, this remained an indirect inference based solely on the nest site [9]. Beyond this, no confirmed successful cases of interspecific parasitism, whether with the Scaly-sided Merganser as the parasite or as the host, have been reported.

The Mandarin Duck (*Aix galericulata*) is also a secondary cavity-nesting bird and overlaps ecologically with the Scaly-sided Merganser, as both species prefer forested environments adjacent to water bodies. The clutch size ranges from approximately 9 to 12 eggs [11]. Research by Cui et al. found that Mandarin Ducks exhibit a high frequency of intraspecific brood parasitism, with parasitism rates as high as 75%, and that their parasitic strategy is closely related to nest-site characteristics [12]. Gong et al. discovered that Mandarin Ducks, due to their strong nest-site preferences, may occupy the nests of other species, subsequently remove the host species' eggs, and incubate their own clutch [13]. Solovyeva et al. clearly documented instances of Mandarin Ducks laying eggs in Scaly-sided Merganser nests, although successful hatching was not observed, potentially because the parasitism led to nest abandonment by the merganser [9].

This study reports a case of interspecific brood parasitism by a Mandarin Duck on a Scaly-sided Merganser, discovered in an artificial nest box. By employing morphological and molecular biological methods (mitochondrial DNA and microsatellite markers), we confirm the identity of the parasitic species and describe the kinship relationships within the host's brood. Our findings provide direct evidence for the successful hatching of a parasitically laid egg, offering a new perspective for understanding the reproductive strategies and interspecific relationships among cavity-nesting Anatidae.

Methods

Study area and field monitoring

The study area is located in Manjiang Town (127°01′–128°06′E, 41°42′–42°49′N), southeast of Fusong County, Baishan City, Jilin Province, China. Situated on the western foothills of the Changbai Mountains, the area features a temperate forest ecosystem with a well-developed river network. The vegetation is dominated by tall trees, including Populus ussuriensis, Betula platyphylla, and Ulmus pumila, making it an important breeding ground for both the Scaly-sided Merganser and the Mandarin Duck.

In April 2024, monitoring was conducted in areas with a historically high concentration of Scaly-sided

Merganser nest sites. The interior of nest boxes was regularly inspected using cameras mounted on support poles. Following confirmation of Scaly-sided Merganser occupancy, on-site monitoring was carried out every five days to document egg-laying, incubation, and parental behavior. Two days after hatching was completed, eggshells were collected once the parent birds had left the nest with their brood. One individual sample was defined as an eggshell with a complete apex. In total, 6 post-hatching eggshells and 2 unhatched eggs were collected. The collected eggs were compared morphologically, and their species identity was determined based on characteristics including color, texture, size and shape.

Sample collection and DNA extraction

Samples were collected using non-invasive methods. Upon observing the successful fledging of ducklings, eggshell remains from hatched eggs were promptly collected, individually packaged and labeled in sealed bags, and stored at -20 °C for later use.

The eggshell membrane (including the chorioallantoic membrane) was aseptically removed using sterile scissors and forceps, cut into fragments, and placed in a centrifuge tube. An appropriate volume of distilled water was added. The sample was then centrifuged at 12,000 rpm for 1 min, and the turbid supernatant was discarded. This washing step was repeated until the liquid became clear. Subsequently, absolute ethanol was added, and the sample was centrifuged again to remove floating pigments before being air-dried. Then, 2 mL of DNA lysis buffer (containing 10% SDS, EDTA·2Na, pH 8.0) and 100 µL of Proteinase K (20 mg/mL) were added. The tube was sealed, secured on a shaking incubator, and incubated at 56 °C with agitation at 220 rpm for 12–14 h. Finally, genomic DNA was extracted using a Column-based Animal Tissue Genomic DNA Extraction Kit (manufactured by Beijing Zhuangmeng International Bio-gene Technology Co., Ltd.).

Mitochondrial D-loop region amplification and analysis

Primers were designed with reference to the complete mitochondrial genome of the Scaly-sided Merganser (NCBI Accession No. NC_016723.1) as follows: MS-HVR-F: 5′-GGACACTACCCGAGACCTAC-3′; MS-HVR-R: 5′-GTGTGAGGGCATTTACACTG-3′. These primers amplify a D-loop fragment of approximately 1042 bp. The PCR amplification was performed in a 50 µL reaction mixture containing 50ng/µL of DNA template, 25 µL of 2× Utaq PCR Mix, 1 µL each of forward and reverse primers (10 µmol/L), and ddH_2O added to a final volume of 50 µL. The thermal cycling protocol consisted of an initial denaturation at 94 °C for 5 min; followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 51 °C for 1 min, and extension at 72 °C for 1 min; a final extension at 72 °C for 10 min; and a hold at 4 °C. A 5 µL aliquot of the PCR product was analyzed by 1% agarose gel electrophoresis to confirm the presence of the target band. The successful amplification products were then sent to Sangon Biotech (Shanghai) Co., Ltd. for bidirectional Sanger sequencing. The obtained sequences were subjected to similarity analysis using the BLAST tool on the NCBI platform to confirm the target fragment. Using the complete mitochondrial genome of the Scaly-sided Merganser (NC_016723.1) as the reference sequence, haplotype analysis was performed with DNA SP 5.10.

Amplification of microsatellite loci and kinship analysis

The inherent limitation of mitochondrial DNA is its inability to provide paternal information; D-loop results alone cannot fully define the mating system for this breeding event. This issue was resolved by the analysis of microsatellite (STR) markers, which are biparentally inherited nuclear DNA sequences [14]. Eight polymorphic microsatellite loci (Table 1) were selected from the literature on closely related species [15–22]. Universal primers were synthesized by Shanghai Bioengineering Technology Service Co., Ltd. For initial validation, PCR was performed on a randomly selected set of seven Scaly-sided Merganser DNA samples. The PCR amplification was performed in a total volume of 15 µL, containing 50ng/µL of DNA template, 12.5 µL of 2×Utaq PCR Mix, 0.5 µL of each forward and reverse primer (10 µmol/L), and ddH_2O added to a final volume of 15 µL. The amplification protocol was as follows: pre-denaturation at 94 ℃ for 5 min; 30 cycles of denaturation at 94 ℃ for 30 s, annealing for 30 s, and extension at 72 ℃ for 30 s; a final extension at 72 ℃ for 10 min; and holding at 4 ℃. The PCR products were preliminarily screened using 1% agarose gel electrophoresis and 8% non-denaturing polyacrylamide gel electrophoresis. Furthermore, the amplified products were verified by sequencing.

We used two methods, ML-Relate (https://www.montana.edu/kalinowski/software/ml-relate/index.html) and Colony v2 (http://www.zsl.org/science/research-projects/software/), to assign chicks from the same nest to relationship categories of full-sibling (FS), half-sibling (HS), or unrelated (UR) [23]. First, ML-Relate was applied with 10,000 random simulated genotypes to generate maximum likelihood (ML) kinship hypotheses. The significance of these hypotheses was tested by calculating the ratio of their probabilities to that of the full-sibling probability, the latter being based on the expected relatedness under genetic monogamy [24]. Colony was used as a second, consistency-based tool to reconstruct complete pedigrees [25]. Runs were performed with parameters as described in Lopes et al. [23]. The relationships inferred by Colony were compared with those from ML-Relate.

Table 1 Microsatellite primers used in this study

Locus	Repeat sequence	Primer sequence/5′-3′	Ta/℃	References
Smo4	$(AG)_{18}A(AAGG)_{10}$	F: ACTTTCCACAGCCTCTTTCACAA	51	[20]
		R: GACAGTGTTTGTCAATGGATTTT		
Bisi11	$(TCTA)_{15}$	F: CACGTATTAAAGCAGAAACACTGG	54	[22]
		R: CCTGGCACAAAGGGAGATAA		
Bisi15	$(TATC)_{11}$	F: TTGTGCCAGAGGGTAGTATGAA	54	[22]
		R: TGTGCTCAAGCCATCCATT		
Bisi16	$(ATAG)_{11}$	F: TTTGTCGTGCCAATAGAGTGTC	55	[22]
		R: ACGTGTTAGGGCGTTTTAGTTC		
APT008	$(GATA)_{12}$	F: CAAAGAAATCCTAGAACATCATTCAAAT	50	[21]
		R: TCTTCTGGCTTTTCACCTTAGTTTAGTA		
CmAAT35	$(AAT)_{9}$	F: TCCAGGTCACGTAGTTTTTAAGTA	52	[16]
		R: GACCTAAGGCCAACCCTATATC		
MM01	$(CA)_{17}$	F: GGTGTCCACAAAAGGTACGG	57	[19]
		R: CACAAGCATGGCTCAGAGG		
MM05	$(AC)_{23}$	F: CCAAATCTGACCACCAGGAG	57	[19]
		R: GCCGTCAGGCAAATAGGAAC		

When the relationship suggested by Colony agreed with a significant relationship found by ML-Relate, that relationship was accepted; when the two programs did not agree, the relationship was left unassigned. When all chicks collected from the same nest were full-siblings, we assumed monogamy; when at least one chick was classified as a half-sibling, we assumed extra-pair paternity (EPP); when at least one chick was classified as unrelated to other chicks, we assumed conspecific brood parasitism (CBP).

Mitochondrial 12S rRNA gene amplification

Given the relatively conserved evolutionary rate of the 12S rRNA gene, we selected this marker for species identification. Based on an alignment of the mitochondrial genomes of the Scaly-sided Merganser (GenBank Accession No. NC_016723.1) and the Mandarin Duck (GenBank Accession No. KF437906.1), a highly divergent region within the 12S rRNA gene was selected for designing specific primers. The primer sequences were as follows: MergAix-12S-F: 5'-AAACCCACCTAGAG GAGCCTGTT-3'; MergAix-12S-R: 5'-GCCCGTCACC CTCCTCATAAG-3'. PCR amplification was performed in a total volume of 50 μL, consisting of 50ng/μL DNA template, 25 μL 2×Utaq PCR Mix, 1 μL each of forward and reverse primers (10 μmol/L), and ddH_2O added to a final volume of 50 μL. The amplification protocol was as follows: pre-denaturation at 94 ℃ for 5 min; 30 cycles of denaturation at 94 ℃ for 1 min, annealing at 51 ℃ for 1 min, and extension at 72 ℃ for 1 min; a final extension at 72 ℃ for 10 min; and holding at 4 ℃. Following PCR amplification, the products were verified by agarose gel electrophoresis and subsequently sent for sequencing. The obtained sequences were aligned against reference sequences using BLAST to calculate sequence similarity.

To investigate sequence differences in the 12S rRNA gene of Mergus squamatus, we additionally sequenced 18 individuals from this region during 2023–2025 for comparative analysis. Mitochondrial genome sequences of closely related species were retrieved from GenBank as reference sequences, including the Common Merganser (Mergus merganser, KU140667.1), Falcated Duck (Anas falcata, KC759527.1), Baikal Teal (Anas formosa, JF730435.1), and Mallard (Anas platyrhynchos, EU755253.1). These sequences were aligned with the reference genome of Mergus squamatus (NC_016723.1) to examine differences in the 12S rRNA gene.

Results

Field observation and morphological identification

In May 2024, an artificial nest box located by the Manjiang River was occupied by a pair of Scaly-sided Mergansers. Prior to incubation, a total of eight eggs were observed in the nest. Six of these eggs were uniform in size, while the remaining two were notably smaller and were preliminarily identified as likely belonging to a Mandarin Duck. On June 7th, the first hatched duckling displayed a short and broad bill and a yellowish-brown plumage, which are not characteristic of the Scaly-sided Merganser. This individual was therefore identified as a Mandarin Duck duckling (Fig. 1A). This duckling remained in the nest for one day after hatching before departing. On June 9th, ducklings with typical Scaly-sided Merganser features successfully hatched (Fig. 1B).

Comparative morphological analysis revealed that five hatched eggshells and one unhatched egg shared consistent traits: a grayish-white color and texture typical of Scaly-sided Merganser eggs. The remaining hatched eggshell and the other unhatched egg exhibited a distinct yellowish-cream color and were significantly smaller in

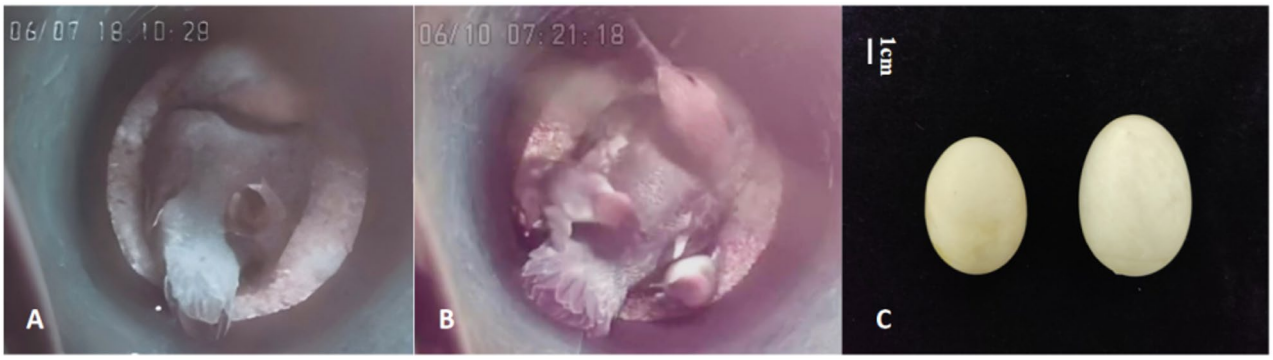

Fig. 1 The status of the Scaly-sided Merganser nest during the 2024 breeding season. **A**. A parasitic duckling hatched in the nest on June 7, 2024. **B**. Successful hatching of Scaly-sided Merganser ducklings in the same nest on June 10, 2024. **C**. Comparison, Left: parasitic egg; Right: host egg

size, matching the characteristic appearance of Mandarin Duck eggs. A comparison of the two unhatched eggs is shown in Fig. 1C; the egg on the left measured 5.4 cm in length and 3.8 cm in widest diameter, while the egg on the right measured 6.6 cm in length and 4.8 cm in widest diameter.

Based on these morphological characteristics, it was preliminarily concluded that the nest had been subjected to interspecific brood parasitism by a Mandarin Duck, and that one of the parasitic eggs was successfully incubated and hatched by the Scaly-sided Merganser host.

Molecular biological identification results

Based on 12S rRNA gene sequence alignment, the sequence of the parasitic duckling (A1) showed 100% similarity to the Mandarin Duck reference sequence (GenBank Accession No. KF437906.1), confirming its identity as a Mandarin Duck.We then examined the sequences of the co-nesting Scaly-sided Mergansers. These sequences showed 98% similarity to the species reference sequence (NC_016723.1). This difference was primarily due to a 14-bp insertion present in all our obtained sequences but absent in the reference sequence. This 14-bp insertion is located at position 1856 of the M. squamatus mitochondrial reference genome (NC_016723.1). The results from the 18 additionally sequenced Scaly-sided Merganser ducklings further confirmed this characteristic, all of which exhibited the same insertion.Combined with egg color, shape, size, and parental morphology, the nest was conclusively identified as that of Scaly-sided Mergansers (Fig. 2). Additionally, a comparison between the Scaly-sided Merganser and Mandarin Duck sequences revealed a diagnostic variation at position 1832: the Scaly-sided Merganser sequence has a thymine (T) deletion relative to the Mandarin Duck sequence in the 12S rRNA gene (labeled area in Fig. 2).

Subsequently, we compared our sequences against mitochondrial genome reference sequences of closely related species from GenBank, such as the Common Merganser (*Mergus merganser*, KU140667.1), Falcated Duck (*Anas falcata*, KC759527.1), Baikal Teal (*Anas formosa*, JF730435.1), and Mallard (*Anas platyrhynchos*, EU755253.1). The alignment revealed that this continuous 14-bp segment was not missing in the 12S rRNA genes of these related species (Fig. 3).

Kinship analysis of the host ducklings

Mitochondrial D-loop region sequences were successfully obtained for all five Scaly-sided Merganser ducklings. Haplotype analysis identified only one haplotype among the five individuals (see Table 2), indicating that all ducklings in this nest shared the same maternal lineage.

Analysis of the eight microsatellite loci showed that all pairwise comparisons between ducklings were consistently identified as full-sibling (FS) relationships by both the ML-Relate and Colony software (see Table 3).

Discussion

This study confirms, through field observations and molecular biological data, a case of interspecific brood parasitism by a Mandarin Duck (*Aix galericulata*) on a Scaly-sided Merganser (*Mergus squamatus*) nest, which resulted in successful hatching by the merganser host. It also elucidates the kinship relationships among the Scaly-sided Merganser ducklings in this nest. This discovery holds significant implications, as it broadens our understanding of the scope of non-obligate brood parasitism and provides critical insights into the reproductive strategies, interspecific interactions, and behavioral adaptations under ecological pressures among cavity-nesting Anatidae.

Molecular identification analysis

Our results successfully confirmed that the parasitic duckling was a Mandarin Duck. Sequence alignment revealed a difference at site 1832 of the mitochondrial

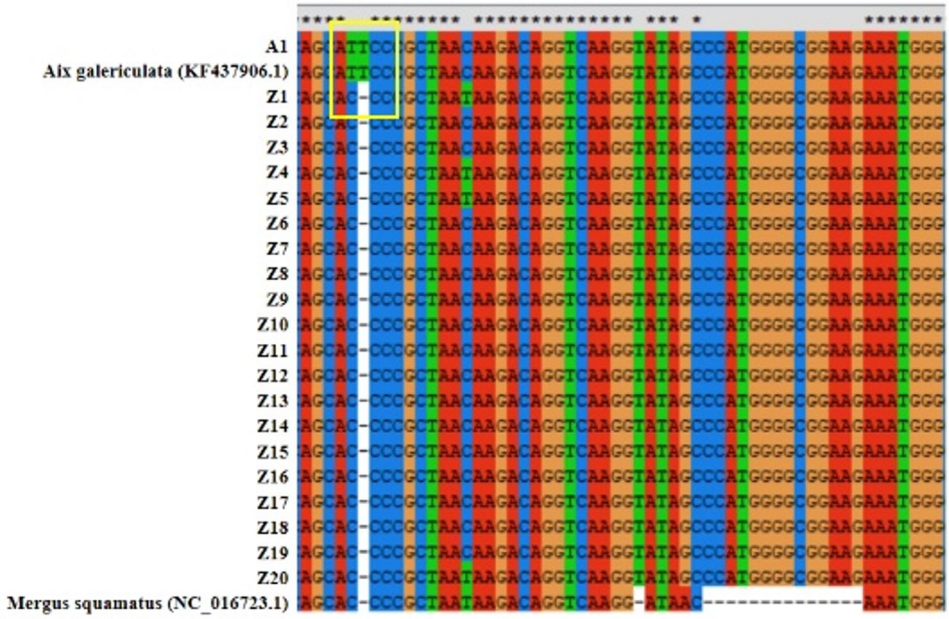

Fig. 2 Sequence alignment of the 12S rRNA gene from Scaly-sided Merganser and the parasitic duckling. A1: Sequence from the parasitic duckling; Z1–Z20: Sequences from Scaly-sided Merganser ducklings; Aix galericulata (KF437906.1): Reference sequence of the Mandarin Duck; Mergus squamatus (NC_016723.1): Reference sequence of the Scaly-sided Merganser

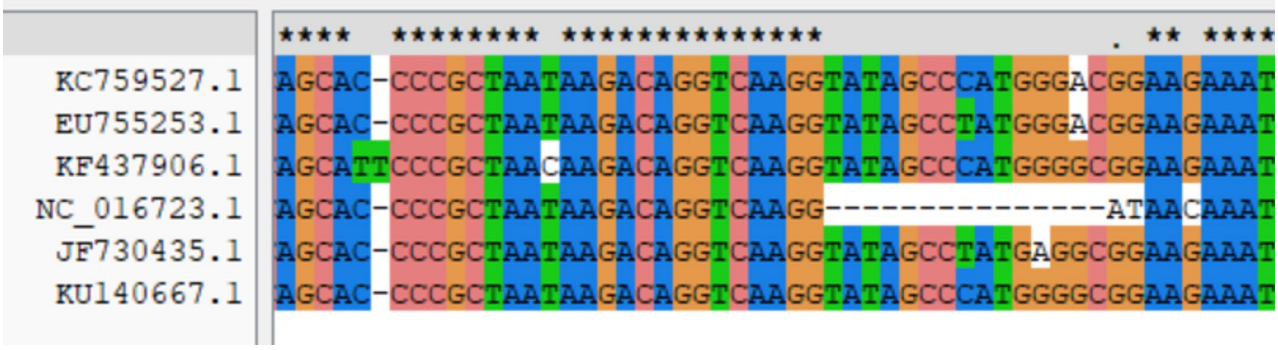

Fig. 3 Comparative alignment of mitochondrial 12S rRNA gene sequences among six waterfowl species. The aligned sequences are from the following species: Mergus squamatus (NC_016723.1), Aix galericulata (KF437906.1), Mergus merganser (KU140667.1), Anas falcata (KC759527.1), Anas formosa (JF730435.1), and Anas platyrhynchos (EU755253.1)

genome, which can serve as a genetic marker for distinguishing the two species at the molecular level.

A noteworthy detail meriting further discussion is that the 12S rRNA sequences obtained from the host Scaly-sided Merganser ducklings in the same nest differed from the database reference sequence annotated for this species (NC_016723.1). Specifically, our sequences contained an additional continuous 14-bp insertion. This discrepancy was consistent across all 20 sequences analyzed, including those from the initial two ducklings and an additional 18 sequences from other Scaly-sided Merganser ducklings sampled in the same region. No deletion of the consecutive 14-bp fragment in the 12S rRNA gene was observed in the five species closely related to Mergus squamatus. We posit two possible explanations for this finding: the apparent absence of the 14-bp segment in the current Scaly-sided Merganser reference sequence may could instead stem from technical artifacts during sequencing or genome assembly; alternatively, the individual used to generate the reference sequence (collected from the Nature Reserve Management Station in Anhui Province, China) might represent a genetic outlier, and its sequence may not accurately reflect the typical mitochondrial genome of the species. Therefore, we suggest that re-sequencing of the complete mitochondrial genome of the Scaly-sided Merganser should be performed, to better represent the species' genetic identity.

Table 2 Haplotype and its variation site

Haplotype	Variable sites														
	1	3	117	121	131	206	210	247	839	848	920	954	960	980	999	1026	1030
NC_016723.1	G	G	G	A	G	G	C	T	T	A	C	T	C	G	C	T	C
H1	-	-	-	-	-	T	T	.	C	C	.	.	T	.	A	C	G

Table 3 Inferred kinship for Mergus squamatus nestling-pairs

Pair	rQG	ML-Relate output	Colony output	Inferred kinship
1–2	0.481	FS	FS	FS
1–3	0.596	FS	FS	FS
1–4	0.301	FS	FS	FS
1–5	0.367	FS	FS	FS
2–3	0.896	FS	FS	FS
2–4	0.548	FS	FS	FS
2–5	0.239	FS	FS	FS
3–4	0.467	FS	FS	FS
3–5	0.153	FS	FS	FS
4–5	0.375	FS	FS	FS

Brood parasitism behavior and successful host incubation

The Mandarin Duck is a facultative parasite [12]. Research by Gong et al. revealed that Mandarin Ducks engage in nest usurpation, competing for nest sites with other coexisting bird species [13]. Furthermore, Solovyeva et al. recorded an incident of interspecific brood parasitism by a Mandarin Duck targeting a Scaly-sided Merganser nest, although it was not explored in depth [9]. Therefore, documented cases of clear interspecific brood parasitism in mandarin ducks are relatively scarce. Three possible explanations are discussed below.

In 2023, we observed snake intrusion into the nest that was later used in this experiment and was subject to interspecific brood parasitism. This observation suggests that nests in this area face a high risk of destruction. Accordingly, the original nest of the Mandarin Duck female that performed the interspecific brood parasitism in this experiment may have been destroyed. Failing to locate a suitable conspecific nest for intraspecific parasitism in the short term, and given that nest-boxes provide suitable opportunities, this female may have instead laid her eggs in a Scaly-sided Merganser nest-box. Therefore, this parasitic event by the Mandarin Duck can be interpreted as an example of interspecific nest parasitism adopted by a species under pressure of reproductive failure [26].

Furthermore, the study area constitutes a mature forest-river ecosystem. Despite abundant natural nesting trees and the provision of multiple artificial nest boxes within the forest, high-quality tree cavities suitable for nesting—characterized by appropriate height, entrance dimensions, and internal dryness—may still represent a limited resource [13]. Some artificial nest boxes are used alternately by Mandarin Ducks and Chinese mergansers, reflecting intense competition for high-quality nest sites [13, 27]. Given the partial overlap in their ecological niches and the potential for interspecific competition, it is plausible that this female Mandarin Duck may have lost its original nest after being outcompeted in contest over a cavity, subsequently depositing its egg in the nest of its competitor. Therefore, this parasitic act could also

be interpreted as a behavior triggered by competition for limited available nest-site resources.

Alternatively, accidental nest misidentification by the Mandarin Duck female could be given as an explanation, though it seems unlikely. Research by Cui et al. found that when selecting nest sites, Mandarin Ducks show a preference for nest boxes containing nest material, with the highest utilization rate observed for boxes containing nest material of the Eurasian Red Squirrel (*Sciurus vulgaris*) [12]. Many bird species possess the ability to accurately identify their own nests using cues such as olfaction, which is fundamental to their reproductive success [28]. Although both Mandarin Duck and Scaly-sided Merganser nests in the Manjiang area primarily consist of artificial nest boxes, subtle differences in species-specific olfactory cues or in the finer structural details of subsequent nest material arrangement may still exist.

The acceptance and successful incubation of a morphologically distinct foreign egg by the Scaly-sided Merganser host is similar to the response observed in some brood-parasitized bird species. For example, Yasukawa reported that a Red-winged Blackbird accepted and successfully hatched a morphologically distinct Yellow-billed Cuckoo egg, while Attisano et al. found that the fan-tailed gerygone accepted and successfully hatched non-mimetic eggs of the Shining Bronze Cuckoo [29, 30]. The acceptance of a Mandarin Duck egg by the Scaly-sided Merganser may be explained by assuming that the latter likely has not experienced significant historical brood parasitism pressure; consequently, they may not have evolved specific egg recognition/rejection abilities. Research by Robert et al. found that in the absence of parasitism pressure, the Village Weaver (*Ploceus cucullatus*) exhibits a greatly diminished ability to recognize foreign eggs [31]. Alternatively, insufficient light inside the nest may reduce the ability to discern egg color and pattern, making cavity-nesting birds more inclined to accept any object within the nest that conforms to the general shape of an egg [32]. Research by Attard et al. found that hosts using enclosed nests have greater difficulty recognizing parasitic eggs and exhibit lower rejection rates compared to those using open nests [33]. Therefore, it is plausible that the Scaly-sided Merganser in this instance simply failed to recognize the foreign egg in its nest.

Kinship and mating system

Through analysis of mitochondrial D-loop haplotypes and nuclear genomic STRs, this study revealed the kinship structure within the Scaly-sided Merganser nest. The mitochondrial D-loop analysis showed that all ducklings shared an identical haplotype, indicating that all Scaly-sided Merganser ducklings in the nest were sired by the same female [34]. The genotypes of all Scaly-sided Merganser ducklings across eight polymorphic microsatellite loci were consistent with a full-sibling relationship. Concurrently, these results confirm the absence of intraspecific brood parasitism in the studied nest and support a monogamous genetic mating system for this Scaly-sided Merganser pair, in line with observations of social monogamy in this species [35]. Scaly-sided Mergansers exhibit behaviors associated with persistent pair bonds, such as reusing the same nest site over consecutive years and maintaining pair associations on the wintering grounds [35–37]. These behavioral traits likely facilitate their monogamous mating strategy.

Conclusion

This study confirms a case of successful interspecific brood parasitism, where a Mandarin Duck egg was laid in and subsequently hatched by a Scaly-sided Merganser host. This event was likely driven by competition for nest-site resources or individual reproductive pressures. Molecular identification established the parasitic egg's origin, while kinship analysis indicated strict genetic monogamy within this nest. The findings highlight the need to consider interspecific nest-site competition and potential parasitic interference in the conservation of endangered species. Furthermore, the discrepancy identified in the Scaly-sided Merganser's mitochondrial 12S rRNA sequence underscores the necessity of re-sequencing its complete mitochondrial genome, using samples spanning all of the species distribution range. This case also reinforces the importance of integrating morphological, ecological, and molecular data for accurate species identification and behavioral interpretation.

Acknowledgements

We would like to express our sincere gratitude to Mr. Li Guodong from Manjiang Town, Baishan City, Jilin Province, China, for his contribution to the material collection in this study.

Author contributions

Conceptualization: Shu Liu, Donghong Li. Formal analysis: Shu Liu, Donghong Li, Shiyu Zhang, Yongbin Zhao. Funding acquisition: Yongbin Zhao. Investigation: Shu Liu. Methodology: Donghong Li. Resources: Guodong Yi. Supervision: Donghong Li, Shiyu Zhang, Yongbin Zhao. Visualization: Shu Liu, Yongbin Zhao. Writing – original draft: Shu Liu. Writing – review & editing: Donghong Li, Shiyu Zhang, Yongbin Zhao, Guodong Yi.

Funding

This study is funded by the Jilin Provincial Department of Science and Technology (http://kjt.jl.gov.cn), under the program titled "Research on Reproductive Strategies for the Scaly-sided Merganser (Mergus squamatus) in the Changbai Mountain Range" (grant number: 20220101331JC).

Data availability

The data reported in this paper have been deposited in the GenBase [1] in National Genomics Data Center [2], Beijing Institute of Genomics, Chinese Academy of Sciences/China National Center for Bioinformation, under accession number: 1).Mergus squamatus mitochondrial 12S rRNA fragment: C_AA158845.1 to C_AA158864.1, 2).Aix galericulata Mitochondrial 12S rRNA fragment: C_AA159413.1, 3).Mergus squamatus mitochondrial D-loop fragmentthat: C_AA159408.1 to C_AA159412.1 that is publicly accessible at https://ngdc.cncb.ac.cn/genbase. References: [1] GenBase: A Nucleotide Sequence Database. Genomics Proteomics Bioinformatics 2024, qzae047

[PMID=38913867]. [2] Database Resources of the National Genomics Data Center, China National Center for Bioinformation in 2024. Nucleic Acids Res 2024, 52(D1):D18-D32 [PMID=38018256].

Declarations

Ethics approval and consent to participate

This study only involved manipulating the hatching eggshells of wild birds, without capturing, processing, or experimentally manipulating birds. All investigations comply with Chinese laws and regulations on wildlife conservation (the Wildlife Protection Law of the People's Republic of China). The observation and research of waterbirds in the study area have been approved by the Science and Technology Ethics Committee of Jilin Normal University.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 3 February 2026 / Accepted: 22 May 2026
Published online: 28 May 2026

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