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SHORT COMMUNICATION

Comparative mitochondrial genetics of North American and Eurasian mergansers with an emphasis on the endangered scaly-sided merganser (*Mergus squamatus*)

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Abstract The scaly-sided merganser, *Mergus squamatus*, is considered one of the most threatened sea duck species in the Palearctic with limited breeding and wintering distribution in China and Russia. To provide information for future conservation efforts, we sequenced a portion of the mitochondrial (mt) DNA control region in four species of mergansers and three additional sea duck taxa to characterize the evolutionary history of the scaly-sided merganser, infer population trends that may have led to its limited geographic distribution, and to compare indices of genetic diversity among species of mergansers. Scaly-sided mergansers exhibit substantially lower levels of mtDNA genetic diversity ($h = 0.292$, $\pi = 0.0007$) than other closely related sea ducks and many other avian taxa. The four haplotypes observed differed by a single base pair suggesting that the species has not experienced a recent population decline but has instead been at a low population level for some time. A phylogenetic analysis placed the scaly-sided merganser basal to North American and European forms of the common merganser, *M. merganser*. Our inclusion of a small number of male samples doubled the number of mtDNA haplotypes observed, suggesting that additional genetic variation likely exists within the global population if there is immigration of males from unsampled breeding areas.

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Introduction

Surveys of genetic diversity are an attractive first step toward inferring biological parameters, especially for species that have been little studied, have small or threatened population distributions, and are of conservation concern (Kuro-o et al. 2010; Miller et al. 2010). In particular, single marker studies offer a quick and inexpensive method to detect genetic patterns that can direct subsequent research efforts of species and populations via molecular and field research. The scaly-sided merganser (*Mergus squamatus*) breeds in the south–east Russia, north–east China and in North Korea (Fig. 1) and is considered one of the most threatened sea duck species in the Palearctic resulting from it's small population size and declining status from habitat loss, hunting, and disturbance (Liu et al. 2010). It is listed as 'endangered' by the International Union on the Conservation of Nature (IUCN 2008), 'rare' in the Red Data Book of Russian Federation (Iliashenko and Iliashenko 2000), and in the first rank category of the List of the Protected Wildlife of National Importance in China (Collar et al. 1994). World population estimates vary from 2,400 to 10,000 individuals (He et al. 2002; Kear 2005; Solovieva et al. 2006) and approximately 90% of the breeding population is thought to nest in Russia (Solovieva et al. 2006; Liu et al. 2010). Females use cavities for nesting, typically in older and decaying trees along rivers within the temperate conifer-broadleaf forest zone (Zhao et al. 1995).

The scaly-sided merganser is thought to interbreed with sympatric nesting congener, such as the goosander (*Mergus merganser* Surmach and Zaykin 1994). Little is known

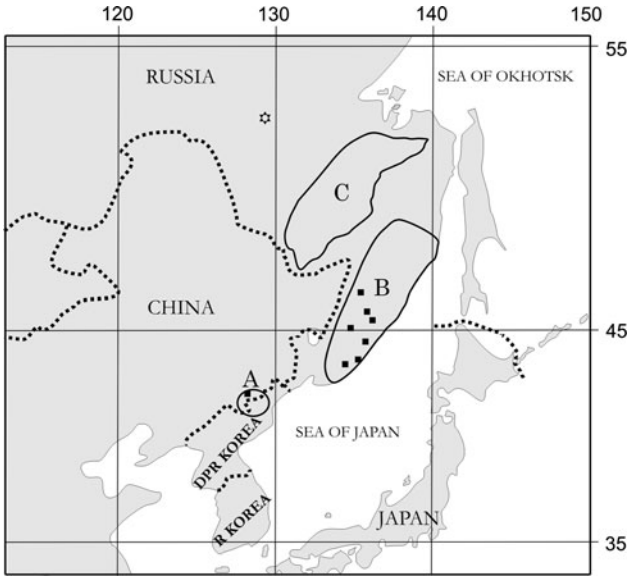

Fig. 1 Distribution of known breeding areas (solid lines) of the scaly-sided merganser (*Mergus squamatus*) and DNA sampling locations in East Asia (black squares). Breeding regions mentioned in the text include **a** Changbai Mountains, China, **b** Sikhote-Alin Range, Russia, and **c** Zeya-Bureya, Russia. The *star* indicates a previously known breeding location

about molecular evolutionary relationships between *M. squamatus* and *M. merganser*. Substantial genetic divergence exists between North American and European forms of *M. merganser* for nuclear and mtDNA (Hefti-Gautschi et al. 2009) and Pearce et al. (2009b) noted that wintering samples from the Russian Far East exhibited genetic characteristics of both forms. In a cladistic analysis of morphological characters, Livezey (1995) found that *M. squamatus* was more closely related to the ground nesting red-breasted merganser (*Mergus serrator*) than to the cavity nesting *M. merganser*. However, our study of male courtship displays and breeding behavior suggests that *M. squamatus* is closer to *M. merganser*, differing significantly from *M. serrator* (D.V. Solovyeva unpubl. data). In this paper, we provide the first genetic examination of the scaly-sided merganser by sequencing a portion of the mitochondrial (mt) DNA control region for samples collected in Russia and China. We compared mtDNA diversity values in the scaly-sided merganser to closely related sea ducks, examined haplotype frequencies from male and female samples as an index of dispersal tendencies, and used phylogenetic methods to assess evolutionary relationships among mergansers.

Methods

A total of 38 samples from unique scaly-sided merganser were examined in this study with most ($n = 33$; Table 1)

Table 1 Mitochondrial (mt) DNA haplotypes, location of variable sites, and numbers of each haplotype observed among female and male scaly-sided mergansers in Russia and China

Haplotype	Variable site	Location				Total
		Russia		China		
	011 826 521	Female	Male	Female	Male	
1	CTA	24	5	1	1	31
2	TTA	3	1		1	5
3	CTG		1			1
4	CCA		1			1

collected in the Sikhote-Alin region of Russia (Fig. 1) in spring and summer between 2003 and 2008. Sampling locales included the Avvakumovka, Iman, Kievka, Margaritovka, and Malinovka rivers (Fig. 1). Samples were obtained by capturing birds with mist nets placed over rivers (all males and four females), capturing hens on nests (20 females), and by collecting feathers from nest bowls in tree cavities (three samples). All captured birds were marked with metal tarsus bands on one leg and a yellow plastic band engraved with alphabetic-numeric codes on the other leg. Feather samples from nest bowls were used for DNA only when we were certain that the sample was unique and not from a bird captured elsewhere on the study area. Two additional Russian samples of scaly-sided merganser came from museum specimens (Burke Museum 72195 and 74828) that were collected in August 2001 on the Dal'nyaya River. Three scaly-sided merganser samples from China came from the Changbai mountain region (Fig. 1) and were collected between 2007 and 2009.

Methods of DNA extraction from feathers and tissues and molecular sexing of samples were performed as mentioned in Pearce et al. (2009a). For scaly-sided merganser samples, we obtained sequence information from a 405 bp fragment of the control region (domain I) of mtDNA by using primers MMCRL F and MMCRL R. These PCR primers were designed for *M. merganser* in Europe (Hefti-Gautschi et al. 2009). For interspecific comparisons of genetic diversity, we used these same PCR primers to generate sequencing data for two other mergansers species, the red-breasted merganser and the common merganser (or goosander), which occur in Eurasia as well as North America. Sample collection and mtDNA sequence analysis for the red-breasted merganser and the common merganser are described in Pearce et al. (2009a, b), but levels of mtDNA diversity for these species were not previously examined. Final sequences for all merganser samples were aligned with the program AlignIR version 2.0 (LI-COR) and organized into unique haplotypes using the COLLAPSE function of the software FaBox (Villesen 2007).

Table 2 Comparative indices of mitochondrial DNA control region sequence diversity among three species of merganser sampled in Russia, China, North America, and Europe

Species and sampling area	*N*	No. of base pairs sequenced	No. of haplotypes	Nucleotide diversity (π)	Haplotype diversity (h)	Polymorphic sites
Scaly-sided merganser						
Russia and China	38	405	4	0.0007	0.292	3
Common merganser						
Beringia	27	436	6	0.0029	0.661	8
Alaska and British Columbia, Canada	36	436	9	0.0033	0.858	8
Washington	23	436	5	0.0025	0.747	4
Western Ontario	8	436	4	0.0049	0.642	8
Eastern North America	25	436	7	0.0054	0.760	13
Red-breasted merganser						
Alaska	38	425	12	0.0046	0.840	14
Eastern North America	8	425	5	0.0060	0.857	8
Europe	15	425	10	0.0060	0.952	11

We calculated a number of different indices of mtDNA genetic diversity in the scaly-sided merganser for comparison to common and red-breasted mergansers. We used the program ARLEQUIN version 3.11 (Excoffier et al. 2005) to calculate haplotype (h) and nucleotide (π) diversity and assessed patterns of historic range expansion by calculating Fu's F_s (Fu 1997) and Tajima's D (Tajima 1989). Significantly negative values, reflecting an excess of polymorphisms at low frequency, can be interpreted as evidence of population expansion (Fu 1997) and or purifying selection. We also examined the mismatch distribution for the observed number of differences between all pairs of haplotypes (Rogers and Harpending 1992). This analysis assumes that signatures in the distribution of pairwise nucleotide differences result from episodes of population growth and decline, although we acknowledge that different processes, such as population structure, may produce similar mismatch patterns.

To examine general evolutionary relationships among taxa, we used MEGA version 4.0.2 (Tamura et al. 2007) to generate neighbor-joining (NJ) trees with 10,000 bootstrap replicates. We confirmed levels of NJ bootstrap support using BEAST (Bayesian evolutionary analysis by sampling trees) version 1.5.3 (Drummond and Rambaut 2007). Haplotypes representative of major clades of the common merganser from Pearce et al. (2009b) and goosander from Hefti-Gautschi et al. (2009) were included as well as those from the hooded merganser (*Lophodytes cucullatus*; Pearce et al. 2008) and the red-breasted merganser (Pearce et al. 2009b). For outgroup sequences, we also used the MMCRL F and MMCRL R primers (mentioned above) to obtain data from single samples of the Barrow's goldeneye (*Bucephala islandica*), the common goldeneye (*B. clangula*), and the bufflehead (*B. albeola*). Samples for these species came from tissues collected during winter by hunters in North America as part of the US Fish and Wildlife Service Annual Parts Collection Survey. All mtDNA sequences summarized in this study have been deposited in DDBJ/EMBL/GenBank for the scaly-sided merganser (accession numbers HM639863–HM639866), the common merganser (FJ191173–FJ191234), the red-breasted merganser (FJ190670–FJ190784), the hooded merganser (EF486446–FJ486489), the common goldeneye (HM639867), the bufflehead (HM639868), and the Barrow's goldeneye (HM639869). Accession numbers for additional common merganser and red-breasted merganser sequences are given in Pearce et al. (2009b).

Results and discussion

We identified four mtDNA haplotypes in scaly-sided merganser samples that differed by a single base pair at each variable site (Table 1). Haplotype 1 was observed in all sampling locales in Russia and China. Two singleton haplotypes (#3 and #4) were observed in male samples from Russia and the only example of haplotype #2 in China was identified in a male sample. Levels of mtDNA genetic variability (nucleotide and haplotype diversity) were substantially lower for the scaly-sided merganser in comparison to those from other merganser populations, including those with much smaller sample sizes (Table 2). The mismatch distribution was unimodal (not shown) and did not differ from simulated distributions under models of sudden demographic ($P = 0.153$) or spatial ($P = 0.09$) expansion. Estimates of Fu's F_s (-2.18, $P = 0.02$) and

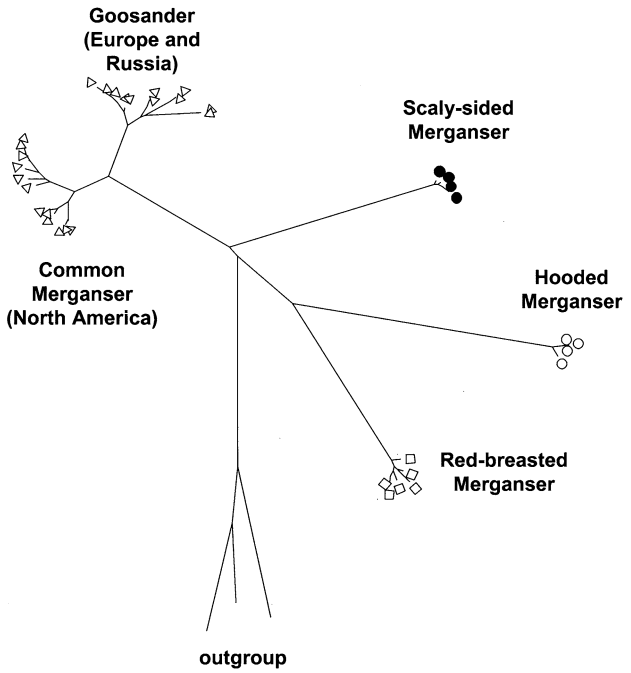

Fig. 2 Neighbor-joining phylogram of mtDNA control region sequences from four species of merganser. Bootstrap support values were >70% and Bayesian posterior probabilities were 1.0 for all major branches. Outgroup sequences include Barrow's goldeneye (*Bucephala islandica*), common goldeneye (*B. clangula*), and bufflehead (*B. albeola*) from North America (see "Methods")

Tajima's D (-1.27, $P = 0.08$) were negative suggesting historical population growth or purifying selection, but levels of statistical significance for these values were not consistent. This corresponds well with the recent increase of the Sikhote-Alin breeding population and possible increase of Chinese Changbai population (Solovieva et al. 2006; Liu et al. 2010). The phylogenetic arrangement of merganser species based on the control region mtDNA data was well defined (Fig. 2) with no differences between NJ and Bayesian topologies (not shown). There was no evidence for paraphyly among any of the taxa. Within the phylogram the scaly-sided merganser occurs as a sister group to the North American and Eurasian forms of *M. merganser*.

Our analyses of samples from much of what is believed to be the current breeding distribution of the scaly-sided merganser in Russia and China revealed limited mtDNA diversity. Despite a similar number of haplotypes, levels of mtDNA diversity in the scaly-sided merganser were substantially lower than other merganser species examined here and previously reported for the hooded merganser by Pearce et al. (2008). The single base pair differences among scaly-sided merganser haplotypes suggest that the species has been at a low population level for some time. A greater number of more diverse haplotypes would be expected if the scaly-sided merganser had gone through a more recent population decline (e.g., Scribner et al. 2001; Kuro-o et al. 2010). Values of π and h for the scaly-sided merganser are some of the lowest observed among a number of avian species and within the range (0.25–0.55) documented across five other endangered bird taxa (reviewed in Kuro-o et al. 2010). Other measures of mtDNA diversity that suggest expansion (mismatch distribution and Fu's F_s) are likely the result of the few nucleotide substitutions observed among the four haplotypes that resemble an expanding population. We couch our conclusions about mtDNA diversity with the acknowledgment that a single, female-mediate marker system may not fully reflect genetic diversity of an entire species or population (McCusker and Bentzen 2010). Limited variability in the mtDNA control region does not always imply low levels of variation elsewhere in the genome, therefore further genetic assessments of scaly-sided mergansers should include a survey of nuclear loci for comparisons to the mtDNA information presented here.

The inclusion of male samples in this study doubled the total number of haplotypes observed and suggests that additional female genetic diversity exists elsewhere, such as unstudied Zeya-Bureya region (Fig. 1). The lesser and greater Xingan population in China does not exceed several pairs and thus can't be considered a significant source population (T Dahmer, J Harris, P Liu, pers. comm.). A similar finding of sex-biased genetic diversity was documented by Ruokonen et al. (2010) for the Fennoscandian lesser white-fronted goose (*Anser erythropus*) where haplotypic richness increased dramatically over time as a result of increased immigration by males. Because mtDNA is maternally transmitted and female waterfowl species may return to their natal site for subsequent breeding, dispersing males may offer an index of genetic diversity on a larger scale and are thus useful for population genetic surveys in species with sex-biased movement patterns. For example, in a genetic assessment of the threatened Steller's eider (*Polysticta stelleri*), Pearce et al. (2005) found that the level of population differentiation for mtDNA was higher with female samples in comparison to male samples, suggesting greater natal dispersal by males. Similar patterns of sex-biased dispersal were noted for the common mergansers in North America using both satellite telemetry (Pearce and Petersen 2009) and genetic data (Pearce et al. 2009a).

Future research efforts based on our genetic findings could include targeting a larger proportion of the scaly-sided merganser population for DNA sampling to better characterize molecular diversity and population dynamics. Congregations of molting and wintering waterfowl have been useful for sampling a broader section of the entire population for inferring natal origins (Pearce et al. 2009a), survival (Flint et al. 2000), and migratory patterns (Bollinger and Derksen 1996). However, based on recent stable

isotope and geolocator data (D. Solovyeva et al., unpubl. data), it appears that male scaly-sided mergansers molt in small flocks on river systems that are distant from breeding areas, whereas females molt during the brood rearing period on rivers within their breeding areas (D. Solovyeva et al., unpubl. data). Similarly, He et al. (2002) found that the scaly-sided merganser wintering in China are widespread and occur at low densities making capture of numerous individuals during winter difficult.

Phylogenetic placement of the scaly-sided merganser as basal to North American and European forms of *M. merganser* suggests that lower diversity values do not result from a recent evolutionary history and are instead the result of historic low population size. We found no evidence for paraphyly among sympatric merganser taxa, which is a pattern that is observed among some dabbling ducks (Peters et al. 2005). At first glance this suggests that hybridization may not be a threat to the endangered scaly-sided merganser in areas where it is sympatric with *M. merganser*, but further breeding ground samples, nuclear DNA analysis, and behavioral observations are necessary to fully evaluate the possibility and degree of interspecific matings. Similar to recent comparisons of molecular and morphological data in dabbling ducks (Peters et al. 2005) we also observed discordance between genetic and phenotypic assessments of the evolutionary relationships of mergansers and their close allies. Since our analysis is limited to a single molecular marker and does not include control region information for the smew, a broader taxonomic and genetic (nuclear and mtDNA) phylogeny of all sea duck species is the obvious next step for assessing the degree to which molecular patterns differ from the relationships observed by Livezey (1995) based on extensive morphological characterization. However, evolutionary relationships based on multiple genetic and phenotypic characters may still differ due to divergent responses to selective pressures and time scales (Zink and Remsen 1986; Peters et al. 2005).

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