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Central-West Siberian-breeding Bar-tailed Godwits (*Limosa lapponica*) segregate in two morphologically distinct flyway populations

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Long-distance migratory species often include multiple breeding populations, with distinct migration routes, wintering areas and annual-cycle timing. Detailed knowledge on population structure and migratory connectivity provides the basis for studies on the evolution of migration strategies and for species conservation. Currently, five subspecies of Bar-tailed Godwits *Limosa lapponica* have been described. However, with two apparently separate breeding and wintering areas, the taxonomic status of the subspecies *L. l. taymyrensis* remains unclear. Here we compare *taymyrensis* Bar-tailed Godwits wintering in the Middle East and West Africa, respectively, with respect to migration behaviour, breeding area, morphology and population genetic differentation in mitochondrial DNA. By tracking 52 individuals from wintering and staging areas over multiple years, we show that Bar-tailed Godwits wintering in the Middle East bred on the northern West-Siberian Plain (n = 19), while birds from West Africa bred further east, mostly on the Taimyr Peninsula (n = 12). The two groups differed significantly in body size and shape, and also in the timing of both northward and southward migrations. However, they were not genetically differentiated, indicating that the phenotypic (i.e. geographical, morphological and phenological) differences arose either very recently or without current reproductive isolation. We conclude that the *taymyrensis* taxon consists of two distinct

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populations with mostly non-overlapping flyways, which warrant treatment as separate taxonomic units. We propose to distinguish a more narrowly defined *taymyrensis* subspecies (i.e. the Bar-tailed Godwits wintering in West Africa and breeding on Taimyr), from a new subspecies (i.e. the birds wintering in the Middle East and breeding on the northern West-Siberian Plain).

Keywords: conservation, genetic population structure, shorebirds, subspeciation, migration, body size, body shape.

Migratory bird species often comprise different flyway populations, in which different groups of individuals of the same species are spatially and temporally isolated for at least part of the year (Newton 2008). The evolutionary mechanisms maintaining migratory routes have been extensively investigated (e.g. Bensch *et al.* 1999, Delmore & Irwin 2014), but are still far from understood (Piersma 2011). In general it is thought that flyway populations arise from divergent selection, which can lead to reproductive isolation, for instance because populations are separated in space or in time (Bearhop *et al.* 2005, Turbek *et al.* 2018). However, an increasing number of studies show that flyway populations can diverge phenotypically without reproductive isolation, as indicated by low neutral genetic differentiation (Buehler & Baker 2005, Marthinsen *et al.* 2007, Sokolovskis *et al.* 2019, Delmore *et al.* 2020). In addition, studies on migrants that travel in groups show that migration routes can be socially learned (Mueller *et al.* 2013, Flack *et al.* 2018). These findings challenge the assumption that genetic variation and reproductive barriers are a prerequisite for the evolution and maintenance of differences in migration routines.

Populations, including flyway populations, are evolutionarily significant units (Crandall *et al.* 2000), and are considered to be the basic units for the development of conservation management strategies (Delany *et al.* 2009). Populations can be delineated based on both phenotypic and genetic information (Crandall *et al.* 2000). Yet, many flyway populations remain undescribed because geographical linkages were notoriously difficult to establish before the advent of tracking techniques (Tomkovich 2010a) and genetic material from remote breeding sites is difficult to obtain. In particular, little is known about flyway population structure of shorebirds in Central Asia (Pearce-Higgins *et al.* 2017).

Bar-tailed Godwits *Limosa lapponica* are an iconic migratory shorebird species, renowned for their extremely long non-stop flights (Gill *et al.* 2009). Bar-tailed Godwits breed discontinuously across the Arctic and sub-Arctic in tundra, marshes and boreal forests from Scandinavia to Russia and Alaska. The northern hemisphere winter is spent in coastal habitats in northwest Europe, West, South and East Africa, the Middle East, Australia and New Zealand (Barter 1989, Delany *et al.* 2009). Based on geographical variation in morphology, five subspecies are currently recognized (Engelmoer & Roselaar 1998, Tomkovich & Serra 1999, Tomkovich 2008). The pattern of geographical linkages of these five subspecies (i.e. flyway populations) was established on the basis of ringing programmes (Wymenga *et al.* 1990, Atkinson 1996, Wilson *et al.* 2007, Tomkovich 2008, Duijns *et al.* 2012) and satellite tracking (Battley *et al.* 2012). However, as indicated by Tomkovich (2008), the status of the *taymyrensis* subspecies remains to be resolved. *Limosa l. taymyrensis* is considered to winter in the Middle East, East Africa and West Africa and to breed in Russia on the northern West-Siberian Plain, and on the Taimyr Peninsula (Delany *et al.* 2009). Based on sparse ring-recovery data and geographical surveys in the breeding area, Tomkovich (2008) suggested that *L. l. taymyrensis* probably comprises two distinct (flyway) populations, perhaps two subspecies, with spatially segregated flyways: one wintering in the Middle East, West Asia and East Africa and breeding on the northern West-Siberian Plain, and the other wintering in West Africa and breeding on the Taimyr Peninsula and surroundings.

Here we examine whether *L. l. taymyrensis* indeed consists of two phenotypically different and spatially segregated flyway populations. Using satellite-tracking, we describe the migration routes, breeding destinations and annual-cycle timing of Bar-tailed Godwits using wintering areas in the Middle East (Oman) and West Africa (Mauritania and Guinea-Bissau). To further

understand the extent to which the two groups are phenotypically different and reproductively isolated, we also examine differences in morphology and neutral genetic variation (in mitochondrial DNA) in the context of global variation across recognized subspecies. We discuss the implications of our study for the taxonomy of the species and the recognition of conservation management units.

METHODS

Study system

Five subspecies of Bar-tailed Godwits are currently recognized (Engelmoer & Roselaar 1998, Tomkovich 2008). These subspecies have distinct migratory routes and timing of migration but overlap in body size measurements:

1 *Limosa l. lapponica* breeds in Northern Fennoscandia and on the Kanin Peninsula (Russia) and winters in northwest Europe. This subspecies is of intermediate size compared with the other subspecies with respect to wing, bill and tarsus morphology.
2 *Limosa l. taymyrensis* breeds from the Yamal Peninsula to the lower Anabar River (central Siberia, Russia) and winters in West Africa (mainly Mauritania, Guinea-Bissau), South and East Africa (South Africa, Mozambique), the Middle East (Oman) and Asia (Iran, Pakistan, west India). The birds that winter in West Africa stage in northwest Europe during both northward and southward migrations, and so leapfrog the *lapponica* population (Duijns *et al.* 2012). This is the smallest subspecies in all measurements.
3 *Limosa l. menzbieri* breeds in central and Eastern Siberia (Russia) from about the Yana River east to Chaunskaya Bay, and winters predominately in northwest Australia. This subspecies is of intermediate size between *L. l. taymyrensis* and *L. l. baueri*.
4 *Limosa l. anadyrensis* breeds in the Anadyr River basin, and wintering areas are yet to be described. The morphological variation of *L. l. anadyrensis* is not fully described, but it appears to be intermediate between *menzbieri* and *baueri* in body size (Tomkovich 2010b).
5 *Limosa l. baueri* breeds in Alaska and winters in eastern Australia and New Zealand (Conklin *et al.* 2011). *Limosa l. baueri* is the largest subspecies.

Satellite tracking

Capture and deployment

We used solar-powered 4.5-g Argos Platform Terminal Transmitters (PTTs, Microwave Telemetry, Inc., Columbia, MD, USA) to track Bar-tailed Godwits. PTTs applied in 2015–18 were programmed to operate on a duty cycle of 10 h 'on' for transmitting locations, followed by 25 h 'off' for charging of batteries; PTTs applied in 2019 were programmed to operate continuously when sufficiently charged. During the 'on' phase and when sufficiently charged, PTTs transmitted signals to Argos satellites every 60 s. When signals were received by a satellite, the perceived Doppler shift in signal frequency of successive transmissions was used to estimate the position of the transmitter (CLS 2016).

We tagged Bar-tailed Godwits in Barr Al Hikman (Sultanate of Oman), Banc d'Arguin (Mauritania), the Bijagós Archipelago (Guinea-Bissau) and the Wadden Sea (The Netherlands) (Table 1). The first three sites were wintering sites and the birds at the Wadden Sea were caught during northward migration at a time when both *taymyrensis* and the nominate subspecies co-occur in the area (Duijns *et al.* 2009). Satellite tracks from eight of the 16 birds caught in Mauritania and The Netherlands in 2016 were previously published in Rakhimberdiev *et al.* (2018).

Bar-tailed Godwits are sexually dimorphic, with males being smaller than females and having a much brighter rusty-red ventral plumage during the breeding season (Piersma & Jukema 1990, Piersma *et al.* 2001). To minimize tag effects, we mostly selected the larger individuals, in this case the females, identified in the field based on bill size (birds with a bill larger than 84 mm were considered females; Piersma & Jukema 1990). We also only tagged adults, because young birds often spend their first summer at the wintering areas (Piersma *et al.* 1996); birds were identified as either first-year birds or older on the basis of plumage characteristics (Prater *et al.* 1977).

Transmitters were deployed using a leg-loop harness made of 0.075-inch (1.9-mm) tubular Teflon tape, weighing approximately 1.5 g; the tags plus harness weighed approximately 6 g. This attachment represented less than 2.7% of the body

Table 1. Details of capture sites and periods, number of tracking data and tagged birds and mean ± SD of bird mass and bill length.

	Middle East birds	West Africa birds		
	Oman	Mauritania	Guinea-Bissau	The Netherlands
Location coordinates	20.6°N; 58.6°E	19.8°N; 16.3°W	11.2°N; 16.0°W	53.4°N; 5.8°E and 52.5°N; 4.6°E
Catching technique	Mist-net	Mist-net	Mist-net and cannon net	Wilster net
Catch year	2015	2015, 2017, 2018	2018, 2019	2016, 2019
No. of tags deployed	10	8	26	8
No. of females	10	6	22	8
Tagging season	Winter	Winter	Winter	Spring
No. of individuals with more than one migratory flight	10	7	17	6
No. of individuals breeding	9	2	6	3
No. of breeding locations	19	3	6	3
No. of possible breeding locations	4	2	2	2
Bird mass (g)	268.8 ± 13.5	377.1 ± 43.8	272.8 ± 41.8	372.4 ± 68.2
Bill length (mm)	93.4 ± 13.5	90.9 ± 9.2	94.6 ± 9.1	96.1 ± 4.1

mass of all tagged birds (Table 1). The tagging work was carried out under several permits, which are listed in the Acknowledgements. To make sure that tags were applied in the same way, tagging was standardized and carried out by R.A.B., J.tH. and A.R. Nevertheless, we observed a difference in tracking success between the Middle East and the West Africa birds (Table 1). Most notable was the likelihood of tracking at least one migratory flight, which was considerably higher in the Middle East birds (Table 1). This may be related to differences in winter survival or a group-specific difference in tag acceptance, but we lack a clear explanation for the difference. This may require further research, but we assume that the difference in tracking success does not affect the outcomes of this study on timing and the definition of breeding areas.

Analysis of tracking data

To objectively classify the location of, and the arrival at and departure from, breeding, staging and wintering locations, we followed the procedures developed by Chan *et al.* (2019). With this method: (1) tracking data are filtered out for implausible locations, (2) locations are grouped into discrete sites and (3) arrival and departure times are calculated for each site.

With the Argos tracking system, location filtering is needed because locations are estimated using Doppler geolocations, which induce location errors that range from a few tens of metres up to hundreds of kilometres (Douglas *et al.* 2012, CLS 2016). For this reason, following Douglas *et al.* (2012), we retained all standard locations (location classes 3, 2 and 1) and filtered out all auxiliary locations (location classes 0, A, B and Z) by setting filtering-out parameters at 120 km/h for the maximum sustainable rate of movement (Chan *et al.* 2019). We then calculated the Great Circle distance, time difference and rate of movement between all successive locations. All analyses were performed in the R computing environment (R Development Core Team 2020). The Great Circle distance was calculated using the spDistsN1 function in the R package 'sp' (Pebesma & Bivand 2005).

To classify sites as wintering, staging or breeding sites, we grouped locations into discrete sites using clustering methods. As we only used 'stationary' locations for site clustering, we first classified each location either as 'stationary', 'flight' or 'undefined'. 'Stationary' locations were defined as locations where speed of movement between consecutive locations was less than 20 km/h and distance was less than 50 km. Locations where speed of movement between the previous and the next location was more than 20 km/h were defined as 'flight'. Locations with a distance greater than 50 km to the previous and next locations and with a speed below 20 km/h were

defined as 'undefined'. 'Stationary' locations were grouped into distinct sites using hierarchical clustering analysis based on a distance matrix, with the R function distm in the geosphere package (Hijmans *et al.* 2017). The distance matrix was used as an input to create hierarchical clusters using the NbClust R package (Charrad *et al.* 2014). The NbClust function determines the optimal number of clusters from the hierarchical clustering by looking at the number of clusters that received the most support from 30 different indices based on the distance matrix, within and between clusters (indices detailed in Charrad *et al.* 2014). We used the 'Complete' aggregation method and the silhouette index to determine the optimal number of clusters, which maximized distances between sites and minimized distance between locations within a site (Charrad *et al.* 2014).

To estimate arrival time at a location, we first identified the first 'stationary' point at a site. If the previous point was classified as 'flight', the arrival time was calculated by extrapolating the average speed of a non-stop flight over the intervening Great Circle route between the first 'stationary' point and the previous 'flight' point. Flight speed was assumed to be 57 km/h (Piersma & Jukema 1990, Chan *et al.* 2019). If the previous point was calculated as 'stationary', we assumed that the flight from the previous site to the subsequent one occurred at the midway of the time interval between the two and hence the arrival time was calculated as the midway of the time interval between the two points minus half of the estimated flight time between them. If the previous point was calculated as 'undefined', arrival time was simply assumed to be the midway of the time interval between the two points. Departure time was estimated in the same way.

All locations were classified as wintering, spring staging, Siberian spring staging, breeding, Siberian autumn staging or autumn staging, based on the spatial and temporal criteria listed in Table 2. Attributes of locations/sites were based on current knowledge on the natural history of the species (Piersma *et al.* 1996). Locations with latitude below 20°N were assigned as winter locations (all tracked birds spent the winter below this latitude). Breeding takes place in Siberia (latitude above 60°N) between late May and the end of June and both sexes incubate. The full incubation period lasts 20–21 days (Piersma *et al.* 1996) and we assume a probable breeding attempt if a bird stayed in the same area (within Siberia and between late May and the end of June) for 19 days or more (Table 2). Staging takes place in between the wintering and breeding sites. If birds were tracked over multiple years, each year was evaluated separately.

When birds lose their tags or die, the PTTs may continue transmitting and this makes it difficult to classify the last-used site. Because of this ambiguity, staging or wintering sites with no subsequent movements were omitted from further analysis. Because the core interest of this work was to determine the breeding sites, we still included breeding sites with no subsequent movements ($n = 10$), but classified these sites as 'possible breeding'.

Table 2. Information on the different sites used in this study. The second left and middle columns indicate spatial and temporal criteria used to classify any location. The two right columns present the observed arrival (normal font) and departure (italic font) dates at a site. Dates given are the average dates and the range is given in parentheses.

Site	Spatial criterion on latitude	Temporal criterion on arrival date *and duration*	Middle East arrival and *departure* date	West Africa arrival and *departure* date
Wintering	<21°N	None	10 Aug (20 Jul – 7 Sep) *18 Apr (13 Apr – 30 Apr)*	2 Sep (14 Aug – 8 Oct) *29 Apr (21 Apr – 15 May)*
Spring staging	>21°N and <60°N	>1 Apr and <1 Jun	20 Apr (1 Apr– 2 May) *24 May (18 May – 11 Jun)*	3 May (24 Apr – 20 May) *4 Jun (28 May – 16 Jun)*
Siberian spring staging	>60°N	>1 May and <8 Jun	24 May (21 May – 29 May) *30 May (26 May – 5 Jun)*	30 May (26 May – 4 Jun) *4 Jun (31 May – 10 Jun)*
Breeding	>60°N	>23 May and <23 Jun; *duration > 18 days*	1 Jun (25 May – 14 Jun) *10 July (22 Jun – 4 Aug)*	6 Jun (1 Jun – 19 Jun) *11 Jul (30 Jun – 31 Jul)*
Siberian autumn staging	>60°N	>8 Jun and <31 Sep	7 Jul (13 Jun – 27 Jul) *20 Jul (1 Jul – 13 Aug)*	10 Jul (10 Jun – 1 Aug) *31 Jul (16 Jul – 20 Aug)*
Autumn staging	>21°N and <60°N	>8 Jun and <31 Oct	19 Jul (1 Jul – 17 Aug) *9 Aug (18 Jul – 6 Sep)*	3 Aug (19 Jul – 23 Aug) *26 Aug (8 Aug – 4 Oct)*

Body size and body shape

We obtained morphological and molecular-sexing data from Bar-tailed Godwits caught in Oman (n = 76), Mauritania (n = 288), Guinea-Bissau (n = 33) and The Netherlands (n = 2) (see Table 1 for details on site coordinates and means of capture). In the analyses, Mauritania and Guinea-Bissau data are grouped as West Africa birds, including two birds captured in The Netherlands that were later observed in Mauritania. No molecular-sexing data were taken from the satellite-tagged birds, so these were not part of the current analyses.

All captured birds received a metal ring and a unique combination of colour rings and flag(s) (see Spaans *et al.* 2011), and we measured bill (exposed culmen), total head and (diagonal) tarsus length to the nearest 0.1 mm, and flattened and straightened wing chord to the nearest 1 mm. Due to flight-feather moult, wing measurements could not be taken from three Middle East birds and 64 West Africa birds. Tarsus length was not taken from one Middle East bird and 46 West Africa birds. From all 76 Middle East birds and 318 West Africa birds we acquired a 20–60 μL blood sample from the brachial vein for molecular analysis. These samples were stored in 96% ethanol. In five cases from West Africa a feather sample was taken for DNA extraction for molecular sexing (see Genetic analysis section).

To enable testing for morphological differences between the Middle East and West Africa birds in the context of global variation in Bar-tailed Godwits, we used morphological data of all known subspecies, with the exception of *L. l. anadyrensis*, for which too few samples were available for meaningful comparisons. For the nominate subspecies *L. l. lapponica* we used data collected in The Netherlands. To separate *L. l. lapponica* from *L. l. taymyrensis*, we followed the criteria of Duijns *et al.* (2012): birds were classified as *L. l. lapponica* if they were caught or observed in The Netherlands during the winter months (November–March) or if they showed active wing moult in autumn (August–October). In this dataset, sex was visually determined in the hand based on morphological and plumage characteristics (Duijns *et al.* 2012). For *L. l. menzbieri* we used data collected in Roebuck Bay, Australia, where sex had been based on molecular sexing (unpublished data). For *L. l. baueri* we used morphological data from Conklin *et al.* (2011), which presented information for 1807 birds collected at several sites in New Zealand. Sex was determined based on a combination of morphology (bill >99 mm indicates female, <90 mm indicates male) and supplemental plumage (when present (January–October), greater extent and richer red colour indicate male) (Conklin *et al.* 2011). The sex of birds in or near the overlap zone in bill lengths was confirmed or determined based on plumage characteristics at banding or subsequent sightings; this allowed unambiguous sexing of 96% of individuals. We assume that the exclusion of 77 birds (4%) that could not be confidently sexed will have a negligible impact on our conclusions.

We tested for morphological differences between the populations of Bar-tailed Godwits wintering in the Middle East and in West Africa and the other populations by means of one-way ANOVAs and Tukey's honest significant difference tests. To compare body shape for both groups we performed principal component analysis (PCA) using body dimensions (bill length, total head, tarsus and wing) as continuous variables and sex and subspecies/group as categorical variables. To show the relative loading of each categorical variable we constructed the biplot by the first and second principal components (PCs), in which 88% of the variation was accumulated. The first component (on the x-axis, PC1) is generally interpreted as size, the second (on the y-axis, PC2) as shape (Somers 1986). The PCA was performed using the R function prcomp.

Genetic analysis

DNA extraction

Depending on sample type, storage medium and type of enquiry (molecular sexing and/or mitochondrial DNA (mtDNA)), we used various methods for DNA extraction. For all samples stored in 95% ethanol, a subsample was dried at 55°C before DNA extraction to ensure the evaporation of ethanol. For all extraction methods, extract quality and/or polymerase chain reaction (PCR) product (including negative controls) were assessed by electrophoresis through a 2% agarose gel.

Among blood samples stored in ethanol, we extracted DNA from 152 samples (for sexing only) using a rapid alkaline (NaOH) extraction method, lysing blood cells with 0.2 M NaOH at 75°C for 20 min and neutralizing the solution with 0.04 M

Tris–HCl (pH 7.5) (Rudbeck & Dissing 1998, Malagó *et al.* 2002). For 30 additional samples (sexing only), we used the ammonium acetate extraction method (Richardson *et al.* 2001), lysing blood in soapy buffer with proteinase K, followed by a clean-up with ammonium acetate and ethanol perspiration. These two methods were verified in several bird species to give the same results (Y. I. Verkuil, unpubl. data). For the remaining blood and organ tissue samples stored in ethanol (sexing only, $n = 185$; sexing/mtDNA, $n = 60$; mtDNA only, $n = 34$), we used the DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD, USA) following the manufacturer's instructions for tissue.

For feather samples ($n = 12$, sexing only), we used a modified version of the tissue method of the DNeasy Blood and Tissue Kit. After chopping the feather base, it was lysed in 80 μL buffer ATL and 25 μL proteinase K solution at 56°C for two nights. This was followed by a second lysis step, for which we added 180 μL buffer AL and incubated at 70°C for 10 min. The column binding and wash steps followed the manufacturer's protocol. To increase the yield at final elution, the AE buffer was preheated at 70°C and the same 50 μL lysis buffer was applied to the filter column twice and incubated at room temperature for 5 min each time.

For blood samples preserved in Queen's lysis buffer ($n = 41$, mtDNA only), we used the NucleoSpin Blood QuickPure Kit (Macherey-Nagel, Düren, Germany), following the manufacturer's protocol.

Molecular sexing

For molecular sexing, we used the primers 2602F/2669R and PCR protocols of van der Velde *et al.* (2017). We performed several error checks. Of the 439 individual samples, 51 were randomly picked to repeat the PCR step, and seven of those samples were repeated a third time. Also, three of the 12 feather samples were repeated; for one individual we used a second feather, which was extracted with the same Qiagen DNeasy feather protocol, and for two we used a blood sample from the same individual, extracted with the NaOH method. In all cases, the assigned sexes were 100% consistent.

Population structure

To investigate potential genetic population structure, we assembled 135 DNA samples representing five recognized or hypothesized breeding populations within the global range of Bar-tailed Godwits (Table S1): *L. l. lapponica* ($n = 12$), *L. l. taymyrensis* (Middle East; $n = 30$), *L. l. taymyrensis* (West Africa; $n = 33$), *L. l. menzbieri* ($n = 30$) and *L. l. baueri* ($n = 30$). We used samples collected in known breeding areas, or from non-breeding Bar-tailed Godwits that could be confidently assigned to a breeding population based on known individual or population movements (e.g. through mark–recapture/resight, remote tracking or long-term population study). All blood or muscle-tissue samples were acquired from museum collections, or collected by colleagues in the field under requisite permits appropriate to their respective countries and institutions.

We developed species-specific primers for the mtDNA control region (CR), using a D-loop sequence of *L. lapponica* published in GenBank, accession number AY524807.1 (https://www.ncbi.nlm.nih.gov/nuccore/AY524807.1). We started with the mtDNA control region primers for *Calidris* sandpipers L98 and H772 (Wenink *et al.* 1993), of which H772 is commonly used in shorebirds (the numbers refer to the approximate position in the control region, and L and H refer to the light and heavy strands). However, these primers did not match with the DNA sequence of *L. lapponica*. The modified primer combination L89 (5′-ACATCGATCATGTGGTGG-3′, $T_m = 54.5$) and H773 (5′-TGTTGGTATGATTCCCCG-3′, $T_m = 53.9$) accounted for a difference with L98 at 11 nucleotide positions and with H772 at nine positions. Initial tests confirmed that primers L89 and H773 worked in *L. lapponica* samples from Australia, Oman and The Netherlands, but not in *L. limosa* or *Calidris alba* (for *L. limosa* primers see Zhu *et al.* 2021). The PCR product covered approximately 680 nucleotides of the 5′ end of the CR (domains I and II, partly). The PCR profile consisted of an initial denaturation of 2 min at 94°C, followed by 25 cycles of 30 s at 94°C, 30 s annealing at 54°C and an extension of 2 min at 72°C. The final concentrations in the 10-μL PCR (including 1 μL DNA template) were 1 μM of each primer, 1× *Taq* DNA polymerase buffer, 3.2 mM dNTPs and 0.03 U/μL *Taq* DNA polymerase (Invitrogen, Inc., Carlsbad, CA, USA). PCR products were enzymatically cleaned (following Werle *et al.* 1994), sequenced in both directions using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Inc., Waltham, MA, USA) according to the manufacturer's instructions, and

analysed on an ABI 3730 DNA Analyzer (Applied Biosystems).

Sequences were aligned and edited in Geneious Pro ver. 8.0.0 (Biomatters Ltd, Auckland, New Zealand). We obtained unambiguous consensus sequences of 556 base pairs of the mtDNA CR for all 135 samples: (GenBank accession numbers OK557805–OK557939), and identified 27 segregating sites. Nucleotide diversity was low ($\pi = 0.0041$ overall, range 0.0031–0.0044 per population), and haplotype diversity was 0.923 overall (range 0.843–0.931 per population). We detected no significant deviations from mutation-drift equilibrium (Tajima's $D = -0.74$ to -1.60 per population, all $P > 0.05$).

We used Arlequin ver. 3.5.1 (Excoffier *et al.* 2005) to compare the proportion of within- and among-population genetic variation (analysis of molecular variation) and to estimate degree of pairwise population differentiation (F_{ST}) based on genetic distance (Tajima & Nei 1984), with P values calculated from 1000 permutations. We estimated and visualized the haplotype network in the R package *pegas* (Paradis 2010).

RESULTS

Year-round spatial and temporal segregation

The Bar-tailed Godwits tracked from the Middle East and West Africa wintering areas segregated in the breeding areas with no overlap (Fig. 1). The segregated breeding areas largely matched the two known Central-West Siberian breeding areas. The breeding sites of the Middle East birds were on the northern West-Siberia Plain and the breeding sites of the West Africa birds were centred on the Taimyr Peninsula, north-central Siberia.

All the Bar-tailed Godwits from wintering areas in the Middle East staged in the Caspian and Aral Seas, during both northward and southward migrations (Fig. 1). Some of these birds also staged for short periods at sites in the United Arab Emirates, Iran and India during both migrations. Both before and after the breeding season, birds staged south of their breeding sites on the West-Siberia Plain. After leaving the breeding sites, some birds moved 800–1000 km north to stage in high Arctic coastal Siberia and Belyy Island before embarking on southward migration.

All Bar-tailed Godwits tracked from wintering sites in West Africa staged in the Wadden Sea, during both northward and southward migration (Fig. 1). During northward migration, some birds stopped in Spain, Portugal or France for a few days, but always continued on to the Wadden Sea. From there, most West Africa birds flew directly to the northern West-Siberian Plain, i.e. near or in the breeding area of the Middle East birds, and then continued on to breeding sites farther east on the Taimyr Peninsula. Upon leaving their Taimyr breeding sites, all birds routinely moved north before leaving for the Wadden Sea. Some West Africa Bar-tailed Godwits staged at Belyy Island

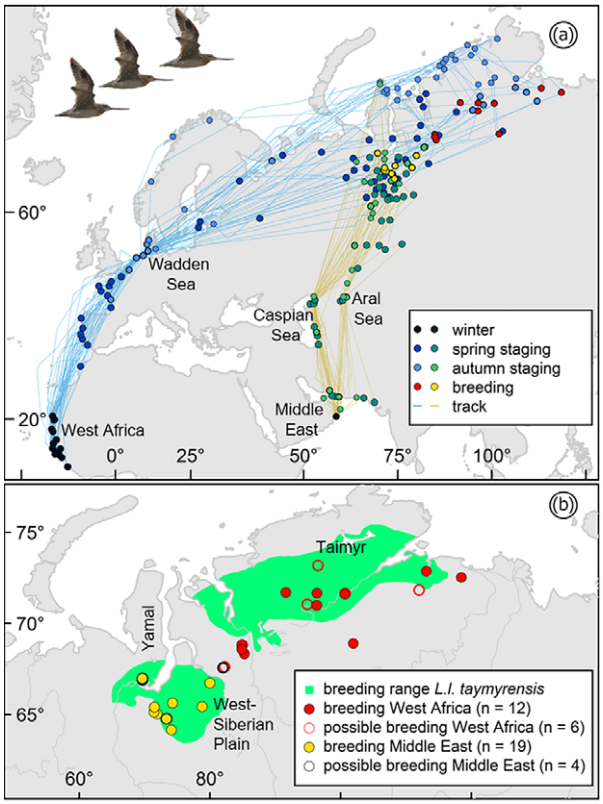

Figure 1. (a) Timing of migratory movements in Bar-tailed Godwits wintering in West Africa (blue lines and blue and red circles) and the Middle East (yellow lines and green and yellow circles). Note that autumn sites are plotted on top of spring sites. For visualization purposes, Siberian staging sites are not indicated by a separate colour, but they can be deduced from the latitude. Map is in Mercator projection. (b) Breeding sites derived from tracking data compared with the known breeding range based on Lappo *et al.* (2012). See Methods section for how sites were classified. [Colour figure can be viewed at wileyonlinelibrary.com]

just north of the Yamal Peninsula, i.e. using the same areas as the Middle East birds.

The eight Bar-tailed Godwits tagged in the Wadden Sea showed similar migrations to breeding sites in the Taimyr Peninsula as the birds tagged in West Africa. Two of the Wadden Sea birds were tracked to wintering areas in West Africa (Mauritania and Guinea-Bissau); the remainder of these tags stopped working before birds arrived in the wintering area. Based on these similarities we combined the Wadden Sea birds with the West Africa birds in all further analyses.

Hence, although the migration routes crossed each other, with overlap in pre- and post-breeding staging areas, the ranges of the Middle East and West Africa wintering populations were spatially segregated, almost year-round. In 2015, a Middle East bird possibly spent the breeding period within 12 km of a West Africa bird, but as these were the last reporting locations in both cases, it remains uncertain if these birds actually bred at these locations (birds may have moved on without a functional tag, or died; see Methods section).

The phenology, i.e. the northward migration, the arrival in the staging and breeding sites, and the southward migration, was earlier in the Middle East birds than the West Africa birds (Fig. 2, Table 2). There was some temporal overlap in the pre- and post-breeding staging areas in Siberia (Table 2).

Body size and shape

A comparison of Middle East and West Africa wintering Bar-tailed Godwits with the three other subspecies showed significant variation among all groups (Fig. 3, Table S2). The Middle East birds and West Africa birds differed significantly in total head (both sexes), bill length (males only) and wing length (males only). The Middle East birds had the smallest total head and bill lengths of all groups, but the West Africa birds had the smallest wing lengths. In general, morphological differences among the three western Palaearctic groups (*L. l. lapponica* and Middle East and West Africa birds) were relatively small compared with the variation within the combined Beringian subspecies (*L. l. baueri*,

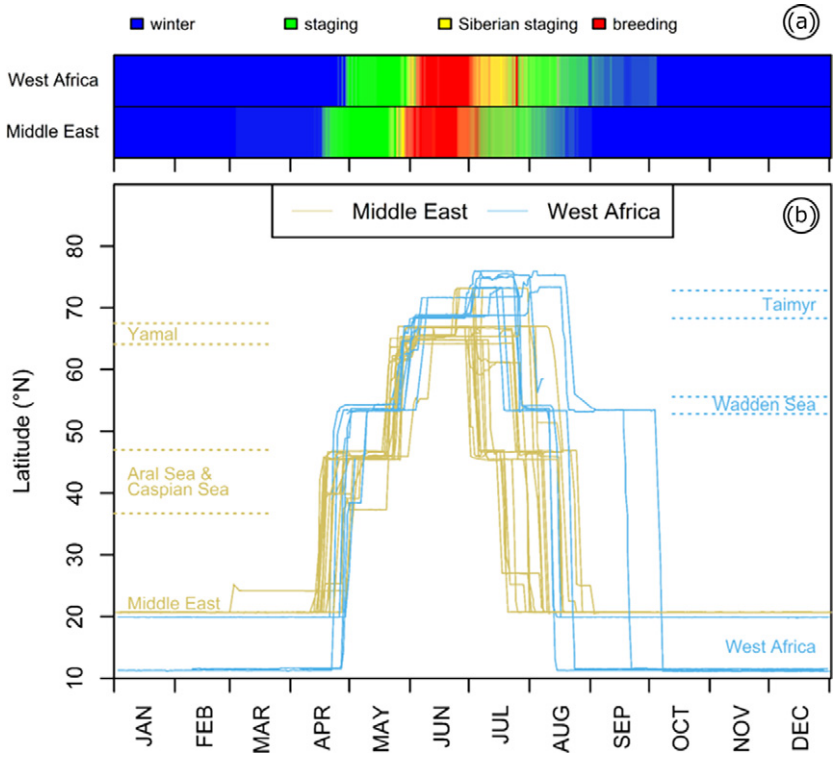

Figure 2. Phenology of Bar-tailed Godwits wintering in the Middle East and West Africa. Each colour is made slightly transparent to visualize variation between individuals. (b) Latitude against day of year. Both plots include data from individuals that were considered breeding (data from all years combined). [Colour figure can be viewed at wileyonlinelibrary.com]

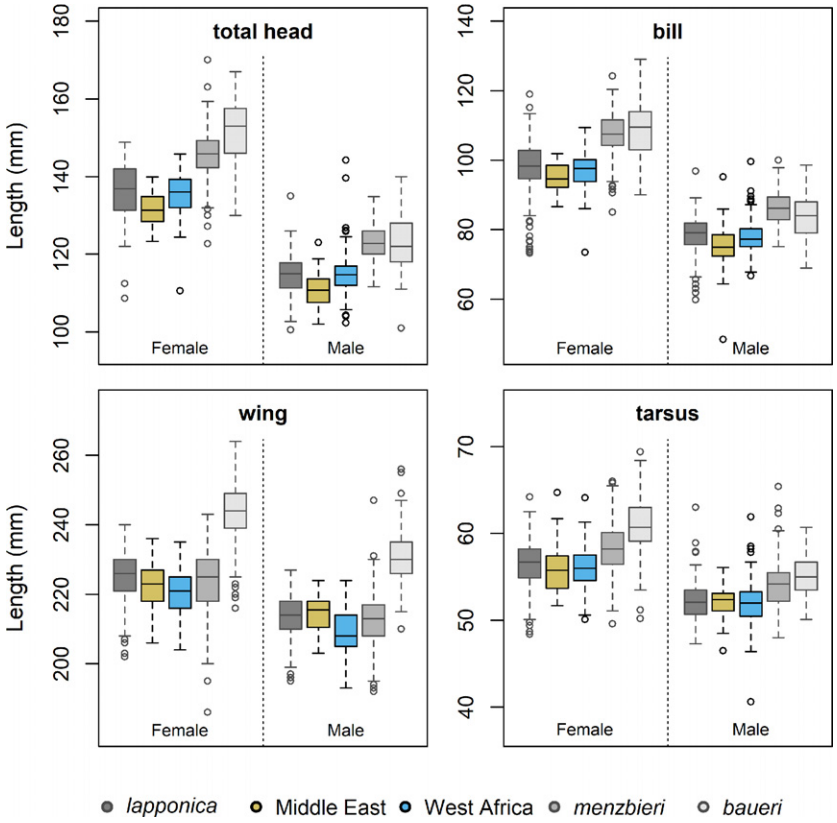

Figure 3. Boxplots showing length of total head, bill, wing and tarsus of female and male Bar-tailed Godwits from *Limosa lapponica lapponica* (The Netherlands), Middle East (Oman), West Africa (Mauritania and Guinea-Bissau), *Limosa lapponica menzbieri* (Australia) and *Limosa lapponica baueri* (New Zealand). Populations are ordered from west to east with respect to breeding range. Thick horizontal lines show medians, top and bottom lines of the box show the 25th and 75th centiles respectively, and whiskers show maximum and minimum values or 1.5 times the interquartile range (whichever is smaller). [Colour figure can be viewed at wileyonlinelibrary.com]

L. l. menzbieri). A PCA of the body dimensions showed that the first two principal components explained 92% of the variance (80.60% and 11.48% for PC1 and PC2, respectively) (Fig. 4). The projection of *L. l. baueri* on the far right of the *x*-axis (PC1; i.e. body size) indicated that this subspecies has the largest body size, followed by *L. l. menzbieri*. The three western Palearctic groups on the right of the *x*-axis are smallest in body size. Projection of the five populations on the *y*-axis (PC2) indicates that populations differ in body shape, with *L. l. lapponica* and the West Africa birds aligning on the same *y*-axis of body shape and hence only differing in body size.

Population genetic structure

We found no evidence of genetic differentiation in mtDNA CR between Middle East and West Africa populations. Among the 45 unique haplotypes we detected globally (Fig. 5), the most common haplotypes were shared by both populations, and no haplotypes with a frequency greater than two were exclusive to either population. In general, the global haplotype network demonstrated a star-like branching pattern indicative of shallow, recent structure and unsorted lineages, with little evidence for fixation of population-specific haplotypes. Only seven haplotypes were shared by five or more individuals (range 1–29 samples per haplotype), and the numerous low-frequency haplotypes were separated by only one or two mutations (Fig. 5).

Accordingly, global population differentiation in mtDNA was generally low; analysis of molecular variation estimates of among- and within-population variation were 14.88% and 85.12%, respectively ($P < 0.001$). Population pairwise F_{ST}

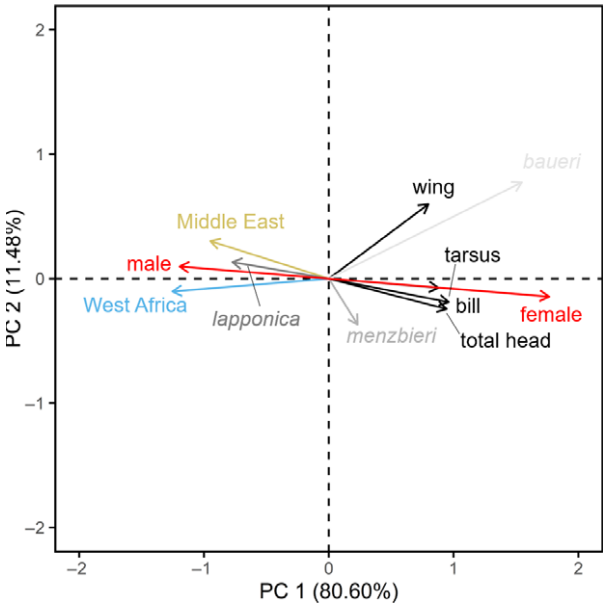

Figure 4. Principal component analysis correlation of linear dimensions (length of bill, total head, wing and tarsus) with sex and subspecies as explanatory variables. [Colour figure can be viewed at wileyonlinelibrary.com]

values ranged from –0.009 to 0.325 (Table 3), and differentiation between the Middle East and West Africa populations was close to zero (F_{ST} = 0.008, P = 0.29). Notably, none of the three western Palaearctic groups (*L. l. lapponica*, Middle East, West Africa) were significantly differentiated from each other (all $F_{ST} \leq 0.04$, $P \geq 0.09$; Table 3). By contrast, the Beringian subspecies *L. l. baueri* and *L. l. menzbieri* were distinguishable from one another and all other populations (all $F_{ST} > 0.06$, $P \leq 0.01$).

DISCUSSION

By describing individual migrations with satellite-tracking, we confirmed that the currently described *taymyrensis* taxon, as predicted by Tomkovich (2008), consists of two flyway populations that are spatially segregated nearly year-round. We found that the Bar-tailed Godwits wintering in the Middle East stage in the Caspian and Aral Sea and breed on the northern West-Siberian Plain, whereas the birds wintering in West Africa stage

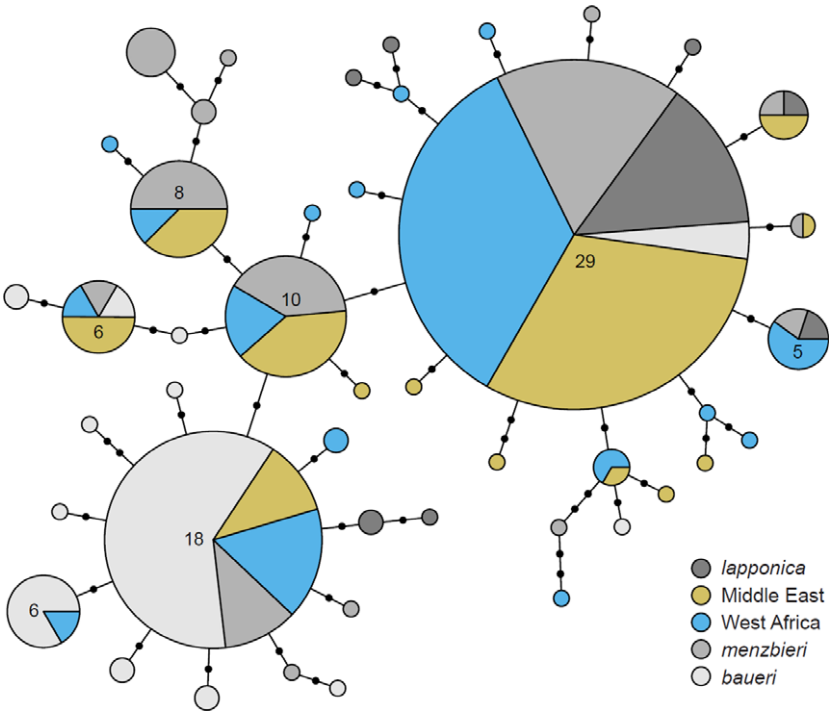

Figure 5. Distribution of 45 observed mtDNA haplotypes across Bar-tailed Godwit populations (n = 135 individuals). Numbers indicate total individuals sharing common haplotypes. Black dots indicate number of mutations separating haplotypes. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 3. Population genetic (mtDNA) differentiation among five Bar-tailed Godwit populations (ordered geographically west–east by breeding area). Below diagonal: population pairwise F_{ST} (distance method); above diagonal: *P* value based on 1000 permutations. Significant F_{ST} values are in bold.

	lapponica	Middle East	West Africa	*menzbieri*	*baueri*
lapponica	*	0.092	0.519	0.020	<0.001
Middle East	0.038	*	0.228	0.010	<0.001
West Africa	−0.009	0.008	*	0.005	<0.001
menzbieri	**0.090**	**0.057**	**0.068**	*	<0.001
baueri	**0.243**	**0.325**	**0.239**	**0.215**	*

in the Wadden Sea and breed on the Taimyr Peninsula and surroundings. The breeding locations of the two flyway populations were completely separated and, although the breeding locations in Taimyr extended beyond the borders of the known range for the *taymyrensis* subspecies, they generally corresponded to the two geographically isolated breeding areas described previously (Lappo *et al.* 2012; Fig. 1b). The only areas where the two flyway populations occasionally overlapped for brief periods were the pre- and post-breeding Siberian staging areas. Flyway populations also differed in the timing of migration, with Bar-tailed Godwits wintering in the Middle East running on a slightly earlier annual cycle, with earlier spring and autumn migration, than the birds wintering in West Africa. This difference further contributes to the nearly year-round spatial segregation.

In addition to the nearly year-round spatial segregation and differences in migration timing (Fig. 2), we also found that the two flyway populations differ morphologically in total head (both sexes) and wing (males only) length, despite substantial variation within each group in all measured traits (Fig. 3). Geographical variation within wader species and subspecies is common (Engelmoer & Roselaar 1998) and is likely to result from divergent selection associated with ecological differences in one or more phases of the annual cycle (Rieseberg *et al.* 2002, Winker 2010). In waders, variation in body size (total head, bill and tarsus) may similarly reflect adjustments to climate and habitat differences at winter, staging or breeding sites (Barbosa & Moreno 1999, Nebel *et al.* 2005) whereas wing morphology is related to flight performance (Lockwood *et al.* 1998). In the subspecies *L. l. baueri*, bill and body size differences (of a similar magnitude as the differences in the Middle East and West African birds) occur in a latitudinal cline across the Alaskan breeding range (Conklin *et al.* 2011). These Bar-tailed Godwits share a flyway and mix at all non-breeding sites, suggesting that bill and body size differences are adaptations to differential ecological selection pressures in the breeding season. Similarly, the observed differences in head–bill body size may reflect adjustments to climate and habitat in the breeding areas, as most West African and Middle East Bar-tailed Godwits breed at different latitudes and in different habitats: mainly tundra versus mainly forest tundra and bogs of the boreal zone, respectively (Tomkovich 2008, Lappo *et al.* 2012), whereas they have rather similar winter habitats (intertidal mudflats) and winter prey types (Annelid worms) (Piersma & Engelmoer 1982, Lourenço *et al.* 2017, Bom *et al.* 2018). The observed differences in wing morphology remain unexplained. In general, birds with longer wings migrate over longer distances (Mönkkönen 1995, Conklin 2019). It is thus unexpected that the Bar-tailed Godwits wintering in the Middle East (with a 5000-km migration distance) have longer wings compared with their conspecifics wintering in West Africa (with a 10 000-km migration distance). Alternatively, differences in wing morphology could be the result of selection pressures in the breeding area, where males perform acrobatic display flights (see discussion in Zhu *et al.* 2020). In this context it is interesting that we found larger differences in wing length between the Middle East and West Africa males than in females.

We found no genetic differentiation in mtDNA between birds wintering in the Middle East and West Africa. In fact, all three western Palaearctic groups (including *L. l. lapponica* and *L. l. taymyrensis*) were genetically similar, despite spanning three migratory flyways. This suggests either that reproductive isolation was only recently

established, and so undetectable with a single population genetic marker, or that there is ongoing gene flow between the populations. The mitochondrial control region is fast-evolving and maternally inherited (i.e. haploid), and so relatively sensitive to recent population processes, compared with nuclear markers (Zink & Barrowclough 2008). However, a genome-wide approach using many markers (e.g. genotyping-by-sequencing; Narum *et al.* 2013) could yet reveal signals of subtle, perhaps very recent, isolation within *L. l. taymyrensis*. Alternatively, low levels of gene flow may be preventing any degree of genetic differentiation, as immigration of only a single individual per generation can be sufficient to homogenize populations (Slatkin 1985).

With presumably neutral genetic markers (such as mtDNA), we cannot discern the evolutionary processes behind the phenotypic variation we describe. However, there are two general scenarios that could explain phenotypic divergence (in this case, the maintenance of two flyway populations) without neutral genetic differentiation: (1) selection and divergence in one or a few isolated genomic regions (i.e. heterogeneous gene flow; Nosil *et al.* 2009), or (2) phenotypic plasticity with neither selection nor reproductive isolation (Crispo 2008). The first scenario has been described at so-called 'migratory divides', at which a strong selection gradient promotes divergent migratory phenotypes, often with inviable or sub-optimal hybrid phenotypes, as was described for Swainson's Thrush *Catharus ustulatus* (Delmore & Irwin 2014). This can occur with little or no reproductive isolation, and heritable genomic variation at only relevant functional loci (e.g. Delmore *et al.* 2020). In the second scenario, divergent migratory phenotypes have neither functional nor neutral genomic signals, because they arise from developmental plasticity or post-development phenotypic flexibility (Piersma & Drent 2003). This is most easily imagined in socially migrating birds in which behaviour and routes are culturally learned and maintained, as shown in several non-passerine birds (Mueller *et al.* 2013, Flack *et al.* 2018) and proposed in the closely-related Black-tailed Godwit *L. limosa* (Loonstra *et al.* 2020). When social groups overlap in space and time, such as the overlapping pre- and post-breeding Siberian staging areas in our study, exchanges of individuals may occur when birds from one flyway population join flocks of the other, as shown in White-fronted Geese *Anser albifrons* (Kölzsch *et al.* 2019). Despite substantial ringing and resighting efforts in the wintering areas of both populations of Bar-tailed Godwits, switching between flyways has never been shown. Nevertheless, an intriguing recovery exists from Barr Al Hikman (Oman) in 2008 of a Bar-tailed Godwit originally ringed in Langebaan (South Africa) in 1988 (Bom 2019). Langebaan is assumed to be used by Bar-tailed Godwits migrating in the East-Atlantic flyway (Delany *et al.* 2009), but the lack of ringing and resighting data from this site limits further speculation. For a better understanding of how migratory systems like that of the Bar-tailed Godwit have evolved and are maintained, we argue that it is important to ring and track birds from the breeding sites.

The differentiation of Bar-tailed Godwits from the Middle East and in West Africa with respect to migration behaviour, breeding areas, morphology and ecology warrants the recognition of separate populations (Crandall *et al.* 2000, Moritz 2002). As the subspecies of Bar-tailed Godwits and most other bird species are described on the basis of geographical and morphological differences, our results also warrant a taxonomic split of *L. l. taymyrensis* (Haig *et al.* 2006, Phillimore & Owens 2006). The *taymyrensis* holotype was described based on a specimen collected at the Taimyr Peninsula (Engelmoer & Roselaar 1998). Therefore, the population breeding on the northern West-Siberian Plain and wintering along the coasts of the Middle East and spreading also along the coasts of East Africa and in India should become known as a separate subspecies, new to science (see formal description below).

Current population estimates from surveys in the non-breeding areas for *L. l. taymyrensis* assume that this taxon consists of two populations, one mainly wintering in the Middle East (100 000–150 000 birds) and another in West Africa (600 000 birds) (Delany *et al.* 2009). Our study justifies this approach and adds that the two flyway populations also segregate on their breeding areas. It is important to establish population estimates and trends for the two populations, as currently only the status of the birds wintering in West Africa is known (and reported declining) (van Roomen *et al.* 2015). Additional monitoring of the two populations through satellite tracking can help to evaluate threats and better characterize important wintering, staging and breeding sites and habitats along the flyways of both populations.

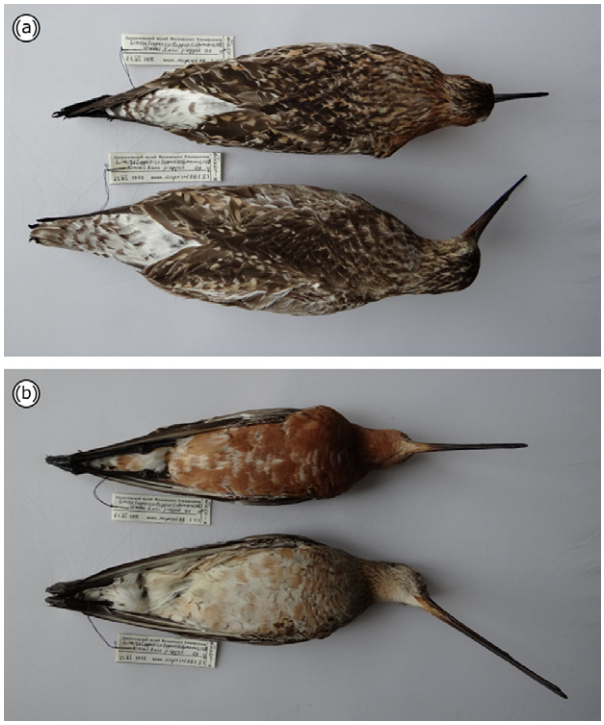

Figure 6. Paratype and holotype of *Limosa lapponica yamalensis* subsp. nov. (a) Upper dorsal aspects and (b) lower ventral aspects. The upper bird in both pictures is the holotype and the lower bird the paratype. [Colour figure can be viewed at wileyonlinelibrary.com]

Limosa lapponica yamalensis subsp. nov

Holotype

Specimen no. R-115010, Zoological Museum of Lomonosov Moscow State University preserved as a study skin. Adult male with large active brood patches collected on 21 June 1998 at Yun'yakha River mouth, Shchuchya River Valley, Yamalo-Nenets Autonomous Okrug, Russia (67.49°N, 68.41°E) by V. V. Morozov (Fig. 6). This bird is in full summer plumage.

Description of holotype

Colour coding references: *Naturalist's Color Guide* (Smithe 1975). Centres of feathers on forehead, crown, nape, eye stripe, ear cover, mantle, scapulars, tertials and greater primary coverts from dark greyish brown (20) to dusky brown (19); edges of these feathers and notches on scapulars and tertials tawny (38) and cinnamon (39); several winter feathers among scapulars and tertials olive-brown (28) at the base drab (28). Back, rump and upper tail coverts white with few army brown (219B) spots on the back, multiple on upper tail coverts and form bars on the longest coverts. Alteration of white and dark brownish olive (129) bars on the tail. Primaries and secondaries hair brown (119A) to olive-brown (28) with secondaries and inner tertials fringed white. Wing coverts olive-brown (28) with more worn and faded ones drab (27); centres of feathers olive-brown (28), shafts dusky brown (19). Axillaries white with olive-brown (28) shaft-streaks and/or bars. Chin sayal brown (223C). Throat, supercilium, foreneck, chest, breast, belly, vent and flanks tawny (38) to cinnamon (39). On flanks with few dark drab (119B) bars, chevrons and stripes, turning into central wedges on breast sides. Undertail coverts white with transition to subterminal tawny (38) spots and dark drub (119B) shaft-streaks, stripes and/or bars. Measurements of the freshly collected bird (in mm): total head 108.8, bill 73.0, tarsus 49.2, wing 203.

Paratype

Specimen no. R-115009, Zoological Museum of Lomonosov Moscow State University preserved as a study skin. Adult female collected on 21 June 1998 at Yun'yakha River mouth, Shchuchya River Valley, Yamalo-Nenets Autonomous Okrug, Russia (67.49°N, 68.41°E) by V. V. Morozov (Fig. 6). This bird is in full summer plumage (Fig. 6).

Description of the paratype

Centres of feathers on forehead, crown, nape, eye stripe, ear cover, mantle, scapulars, tertials and greater primary coverts from sepia (119) to hair brown (119A); edges of these feathers (most are worn off) and notches on scapulars from salmon colour (6) to pale horn colour (92). Back, rump, upper tail coverts white with few Prout's brown (121A) spots on the back, common subterminal chevrons on upper tail coverts and bars on the longest coverts. Tail hair brown (119A) notched with white. Primaries fuscous (21) to sepia (219), secondaries and inner tertials hair brown (119A) fringed white. Wing coverts drab (27) with olive-brown (28) centres and dusky brown (19) shafts. Axillaries white with drab (27) bars and subterminal chevrons. Chin and supercilium pale horn colour (92). Throat, foreneck, chest, breast, belly, vent and flanks salmon colour (6) with multiple hair brown (119A) streaks on foreneck and dull

chevrons decreasing in number from breast to belly; chevrons are brighter and numerous on flanks. White and salmon-coloured feathers mix in about equal proportion on belly and vent. Undertail coverts white with Prout's brown (121A) spots and subterminal chevrons. Measurements of the freshly collected bird (in mm): total head 131.7, bill 103.0, tarsus 54.5, wing 228.

Etymology

The subspecies name refers to the Yamal Peninsula, a core breeding area of the population and the place where the type specimens originate from in Western Siberia, Russia.

Diagnosis

The new taxon differs significantly in morphometrics from other subspecies, especially regarding total head and bill. Table S2 and Figure 3 give an overview of the morphometrics measurements of all subspecies, including *yamalensis* (referred to as Middle East).

Distribution

The new subspecies *yamalensis* breeds on the northern West-Siberian Plain including the Yamal Peninsula (Fig. 1). Birds of the subspecies follow the Central-Asian Flyway, with main stopover sites in the Caspian Sea and Aral Sea. It has confirmed wintering areas in Oman and connections with other wintering areas in the Middle East, Iran, Pakistan and West India (this study). Other wintering areas probably include East Africa (Delany *et al.* 2009). Two ring recoveries show that the subspecies can winter as far as South Africa (Underhill *et al.* 1999, Bom 2019).

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AUTHOR CONTRIBUTIONS

Roeland A. Bom: Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); software (equal); validation (equal); visualization (equal); writing–original draft (equal); writing–review and

editing (equal). **Jesse R. Conklin:** Conceptualization (equal); data curation (equal); formal analysis (equal); methodology (equal); writing–review and editing (equal). **Yvonne I. Verkuil:** Conceptualization (equal); data curation (equal); formal analysis (equal); methodology (equal); writing–review and editing (equal). **José A. Alves:** Conceptualization (equal); funding acquisition (equal). **Jimmy de Fouw:** Conceptualization (equal). **Anne Dekinga:** Data curation (equal). **Chris J. Hassell:** Data curation (equal). **Raymond H. G. Klaassen:** Conceptualization (equal). **Andy Y. Kwarteng:** Funding acquisition (equal); project administration (equal). **Eldar Rakhimberdiev:** Conceptualization (equal). **Afonso Rocha:** Data curation (equal); methodology (equal). **Job ten Horn:** Data curation (equal); investigation (equal). **T. Lee Tibbitts:** Conceptualization (equal); investigation (equal); methodology (equal). **Pavel S. Tomkovich:** Conceptualization (equal); collected DNA samples, and responsible for holotype description. **Reginald Victor:** Funding acquisition (equal); project administration (equal); supervision (equal). **Theunis Piersma:** Conceptualization (equal); data curation (equal); methodology (equal); supervision (equal); validation (equal).
[Correction added on 11 November 2021, after first online publication: the Author Contributions section has been modified in this version.]

Data availability statement

Morphological data can be found at https://dataportal.nioz.nl/doi/10.25850/nioz/7b.b.wc. The tracking data is archived at Movebank (https://www.movebank.org/cms/webapp?gwt_fragment=page=studies,path=study265875917 and https://www.movebank.org/cms/webapp?gwt_fragment=page=studies,path=study118428098). The genetic data are available in NCBI GenBank at https://www.ncbi.nlm.nih.gov/genbank, accession numbers OK557805-OK557939).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Sample information for mtDNA analysis of population structure in Bar-tailed Godwits.

Table S2. Morphometrics of birds from five populations of Bar-tailed Godwits.