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Newly developed microsatellite markers for the Eurasian Sparrowhawk, *Accipiter nisus* (Linnaeus, 1758), with a preliminary assessment of its genetic variation

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ABSTRACT We report on the development of a set of microsatellite markers for the Eurasian Sparrowhawk, *Accipiter nisus* (Linnaeus, 1758), using an enrichment / high-throughput sequencing approach. Out of 9328 potential microsatellites identified, we established 24 tetrameric markers and assessed allelic variation based on samples from continental Europe and the Macaronesian archipelagos. Along with sequences of the mitochondrial *cox1* gene from across the species' range, we use the new markers for a preliminary assessment of genetic variation of *A. nisus*. We find a low mitochondrial variation with only four *cox1* haplotypes, one of which present in all five subspecies studied. Microsatellite analyses suggested a single, panmictic population, with a very low indication for differentiation between the European *A. nisus nisus* and the Macaronesian *A. nisus granti.* However, given the relatively few samples included in this study, our results require confirmation from more in-depth analyses with comprehensive sampling. The newly established microsatellites provide a tool for conservation assessments, conservation breeding and paternity analysis in this widespread raptor.

KEY WORDS Aves; Accipitridae; microsatellite genotyping; DNA barcoding; subspecies; phylogeography.

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INTRODUCTION

The Eurasian Sparrowhawk, *Accipiter nisus* (Linnaeus, 1758), is a rather small bird of prey characterized by distinct sexual dimorphism in size and coloration (Mebs, 2002), and a barred pattern on feathers of chest and belly as typical for species in the genus *Accipiter* Brisson, 1760 (Ortlieb, 1987; Mebs, 2002). Sparrowhaks breed in most of Europe and across Asia to the Pacific coast, plus Northern Africa and Macaronesia (Ortlieb, 1987; Mebs, 2002; Ferguson-Lees & Christie, 2009).

In general, *Accipiter nisus* is considered to be polytypic, with six to nine subspecies usually accepted which differ from each other mostly in body size, details of coloration and distribution. Besides the nominal subspecies occurring from continental Europe to southwestern Siberia and Central Asia, this includes at least *A. nisus granti* from Macaronesia (including Madeira and the Canary Islands), *A. nisus melaschistos* from eastern

Afghanistan to southwestern China, *A. nisus nisosimilis* from northwestern Siberia to northern China and Japan, and *A. nisus wolterstorffi* from Sardinia and Corsica, *A. nisus punicus* from northwestern Africa (Ortlieb, 1987; Gill et al., 2020), and in some taxonomic schemes also *A. nisus dementjevi* from Pamir-Alai to Tien Shan Mountains in Central Asia. Overall, the Eurasian Sparrowhawk is considered as Least Concern according to IUCN criteria (BirdLife International, 2016), but some subspecies are subject of particular conservation actions, e.g., the Macaronesian form *A. nisus granti.* Setting regional conservation priorities for this species requires understanding whether the currently distinguished subspecies represent genetically divergent management units. However, the genetic differentiation of the Eurasian Sparrowhawk remains poorly studied. Kerr et al. (2009), Johnsen et al. (2010), Breman et al. (2013), Aliabadian et al. (2013) and Saitoh et al. (2015) together provided a total of 36 DNA barcodes, i.e., partial DNA sequences of the mitochondrial gene for cytochrome oxidase subunit I (*cox1*), for *A. nisus* specimens from various sites across its range, revealing only very limited differences. No highly variable nuclear-encoded molecular markers, such as microsatellites, have been specifically developed for this species, although markers exist for related species such as the Northern Goshawk, *Accipiter gentilis* (developed by Topinka & May, 2004), and the phylogeographic structure of the Eurasian Sparrowhawk has not yet been studied from the perspective of the nuclear genome.

As a basis for such studies, we here present 24 newly developed microsatellite markers for the Eurasian Sparrowhawk, and test these in a preliminary assessment of genetic differentiation in the species, along with an additional 26 *cox1* barcode sequences.

MATERIAL AND METHODS

We extracted genomic DNA from muscle tissue samples of four specimens of *A. nisus* from Tenerife, Germany, Finland and Georgia (Table 1) and sent the pooled DNA to the Sequencing Genotyping Facility, Cornell Life Sciences Core Laboratory Center (CLC), U.S.A., for development of a microsatellite library. Digestion of DNA took place in three separate reactions with the restriction enzymes AluI, RsaI, and Hpy166II, and products were combined in equal amounts after heat inactivation of the restriction enzymes. The blunt ends were adenylated (+A) with Klenow (exo) and dATP, and after heat inactivation of the Klenow (exo-), the reactions were supplemented with ATP to 1 mM and an Illumina Y-adaptor was ligated with T4 DNA ligase. Enrichment of the fragments for microsatellites took place by hybridisation to and magnetic capture of biotinylated repeat probes (representing two unique dimers, five unique trimers, seven unique tetramers and two unique pentamers), followed by amplification and barcoding by PCR, and sequencing on an Illumina MiSeq instrument (2×250 bp paired reads). SeqMan NGen (version 11) was used for raw read assembly, and the program msatcommander 1.0.8_beta (for Mac OSX) was employed to scan the assembly for microsatellite loci and automatically design primer pairs. The constructed library contained 9328 proposed microsatellite markers with minimum consecutive perfect repeat lengths of at least six (12 bp) for any dimer and at least five for any trimer, tetramer, or pentamer and PCR product size of 150–450 bp, and is available as supplementary information (Supplementary Table S1 and from Figshare under DOI 10.6084/m9.figshare.14604537. Out of this library, we chose 24 loci based on following criteria (Perl et al. 2018): (i) tetrameric, (ii) repeat motif between 10 and 15, (iii) less than 1000 reads, as deep coverage could indicate multiple copies and (iv) GC content of 50 (Table 2), and tested these loci for successful amplification and for yielding unambiguously scorable and polymorphic PCR products.

Microsatellites were amplified following the nested protocol of Schuelke (2000), modified to use rather than a M13 sequence the Illumina sequencing primer sequence (ACACTCTTTCCCTACACGACGCTCTTCCGATCT) as linker, i.e., this sequence preceded all forward primers and was included as a FAM-, NED- or HEX-labelled linker in the PCR. The amplification protocol consisted of 15 min of initial denaturation at 94°C, 30 cycles of 94°C (30 s), 60°C (45 s), 72°C (45 s), followed by 8 cycles of 94°C (30 s), 53°C (45 s), 72°C (45 s), and a final elongation step of 10 min at 72°C. PCR products were diluted once with 15 µl of RNasefree water, 15 µl of Genescan 500–ROX size standard (Applied Biosystems) added to 1 µl of each diluted product, and fragment analysis was performed on an ABI 3130xl Genetic Analyzer. Three markers of different product sizes and labelled with FAM, NED and HEX were combined in each run. We called alleles with GeneMapper® (SoftGenetics, State College, PA, U.S.A); ambiguous calls were either excluded if poor quality, or rounded up to the next unambiguous allele size. We tested for Hardy-Weinberg equilibrium and linkage disequilibrium in Arlequin (Excoffier et al., 2005) under Bonferroni correction (Rice, 1989).

We analysed population structure with the software STRUCTURE version 2.3.4 (Pritchard et al. 2000) under the assumption of an admixture model with correlated allele frequencies and locprior. We compared the number of clusters (K) with 1 million Markov Chain Monte Carlo (MCMC) iterations and a burn-in of 100,000, repeating each assessment of K ten times. To assess the optimal number of clusters we followed the ΔK method by Evanno et al. (2005) using STRUCTURE HARVESTER (Earl & von Holdt 2012). Principal Component Analyses (PCA) of microsatellite allele data was carried out with the packages *diveRsity* v. 1.9.9 (Keenan et al. 2013) and *adegenet* v. 2.1.3 (Jombart 2008) in the R environment (R Core Team 2020), following Jombart et al. (2009).

We sequenced the DNA barcode region of the mitochondrial gene for cytochrome oxidase subunit 1 (*cox1*) with the primer pairs Vert-F1 and Vert-R1 (Ward et al., 2005), and dgLCO1490 and dgHCO2198 (Meyer et al., 2005), using standard PCR protocols. PCR products were sequenced on automated capillary sequencers at LGC Genomics (Berlin, Germany), sequences quality-checked in CodonCode Aligner (CodonCode Corporation), aligned and trimmed to equal length in MEGA7 (Kumar et al., 2016). All newly determined sequences were submitted to GenBank (accession numbers MZ208929–MZ208954. New sequences were aligned with sequences available from Genbank (Table 1), with a matrix of 586 bp kept for analysis. To reconstruct a haplotype network we first inferred a Maximum Likelihood tree with the Jukes-Cantor substitution model in MEGA7 (Kumar et al., 2016), and used this tree together with the alignment as input for Haploviewer (written by G. B. Ewing; http: //www.cibiv.at/~greg/haploviewer), a software that implements the methodological approach of Salzburger et al. (2011).

RESULTS

The 32 samples available for microsatellite genotyping represented two *A. nisus* subspecies: the nominal subspecies *A. nisus nisus* from different parts of Europe, and *A. nisus granti* from Madeira and Tenerife (samples marked "New" in Table 1). We tested 24 microsatellite markers, all with tetranucleotide repeats (Table 2); two of these turned out to be monomorphic for the samples analyzed (An2789 and An14372) and were excluded from all subsequent analyses, but are reported here as they may become useful in a different context in future studies (e.g., Hailer et al., 2005; Nazareno & dos Reis, 2011). The remaining 22 markers had between 2 and 10 alleles (average 4.5), with an allele size range (including primers and linker) between 166 and 414 nucleotides. In two markers (An1107 and An22524) the amount of missing data was high (43.8% and 59.4%); for the remaining markers, missing data ranged from 3.1–28.1% (Table 2). Amplification failure amounted to 20.0% on average, with three samples >50% missing data, and the remaining samples having 0–45% (average 14.5%) missing data.

Significant differences between expected and observed heterozygosity were found in four loci (An3169, An8617, An13004 and An23385) suggesting they may not be in Hardy-Weinberg equilibrium; however, in separate analyses for ad-hoc geographical groups (Macaronesia, Iberian Peninsula, Central Europe, Eastern Europe, Scandinavia), no significant differences between expected and observed heterozygosity were found for these and other markers. To explore the presence of possible genetic clusters in the data, we ran STRUCTURE with the allele matrix of all except the two monomorphic markers, alternatively using (i) subspecies assignment (*granti* and *nisus*) or (ii) ad-hoc geographical groups as locprior; and then repeated both analyses (iii-iv) after also excluding the markers with excessive missing data and potential deviation from Hardy Weinberg equilibrium. In all four analyses, the highest likelihood (and smallest standard deviation among replicate runs) was for K = 1, i.e., the assumption

Subspecies	Accession number *cox1*	Voucher or sample number	Source paper	Location	Country
A. n. nisosimilis	AB842505	BJNSM317-10	Saitoh et al. 2015	Abashiri, Hokkaido,	Japan
A. n. nisosimilis	AB842506	BJNSM515-10	Saitoh et al. 2015	Tokachi, Hokkaido	Japan
A. n. nisosimilis	AB842507	BJNSM107-10	Saitoh et al. 2015	Obihiro-shi, Hokkaido	Japan
A. n. nisosimilis	AB842508	BJNSM275-10	Saitoh et al. 2015	Nemuro, Hokkaido	Japan
A. n. nisosimilis	AB842509	BJNSM815-11	Saitoh et al. 2015	Nemuro, Hokkaido	Japan
A. n. nisosimilis	AB843328	YIO123-10	Saitoh et al. 2015	Chiba, Honshu	Japan
A. n. nisosimilis	AB843329	YIO439-10	Saitoh et al. 2015	Tokyo, Honshu	Japan
A. n. nisosimilis	AB843330	YIO462-10	Saitoh et al. 2015	Hokkaido, Hokkaido	Japan
A. n. nisosimilis	EF515769	KRIBB1307	Yoo et al. 2006	NA	South Korea
A. n. nisosimilis	GQ481247	UWBM_51182	Kerr et al. 2009	Bukhta Melkovodnaya	Russia
A. n. nisosimilis	GQ481248	UWBM_66742	Kerr et al. 2009	Erzinsky District, Tuwa	Russia
A. n. nisosimilis	GQ481249	UWBM_64662	Kerr et al. 2009	Krasnodar	Russia
A. n. nisosimilis	GQ481250	UWBM_59777	Kerr et al. 2009	Choibalsan	Mongolia
A. n. nisosimilis	GQ481251	UWBM_46858	Kerr et al. 2009	Oblast Magadan	Russia
A. n. melaschistos	GQ922642	A055	Cai et al. 2010	Sichuan	China
A. n. nisus	GU571209	NHMO-BC6	Johnson et al.. 2010	Oslo	Norway
A. n. nisus	GU571210	NHMO-BC37	Johnson et al.. 2011	Jamfruland	Norway
A. n. nisus	GU571689	BISE-Aves450	Johnson et al.. 2012	Jakobsberg	Sweden
A. n. nisus	GU571690	BISE-Aves41	Johnson et al.. 2013	Kungälv	Sweden
A. nisus punicus	JF312087	RBINS_4846	Breman et al. 2013	NA	NA
A. nisus wolterstorffi	JF312085	RBINS_11136	Breman et al. 2013	Sardinia	Italy
A. nisus wolterstorffi	JF312161	RMCA_A20090104	Breman et al. 2013	Sardinia	Italy
A. nisus	JF312190	RMCA_Acc232	Breman et al. 2013	NA	Israel
A. nisus	JF312191	RMCA_Acc233	Breman et al. 2013	NA	Israel
A. n. nisus	JF312194	RMCA_JEMU042004	Breman et al. 2013	Tervuren	Belgium
A. n. nisus	KF946553	ZMA58243	Aliabadian et al. 2013	Malden	Netherlands
A. n. nisus	KF946554	ZMA58245	Aliabadian et al. 2013	Helden	Netherlands
A. n. nisus	KF946555	ZMA58246	Aliabadian et al. 2013	Reuver	Netherlands
A. n. nisus	KF946556	ZMA58247	Aliabadian et al. 2013	Culemborg	Netherlands
A. n. nisus	KF946557	ZMA58248	Aliabadian et al. 2013	Amsterdam	Netherlands
A. n. nisus	KF946558	ZMA58741	Aliabadian et al. 2013	Amsterdam	Netherlands
A. n. nisus	KF946559	ZMA58742	Aliabadian et al. 2013	Montfort	Netherlands
A. n. nisus	KF946560	ZMA58743	Aliabadian et al. 2013	Belfeld	Netherlands
A. n. nisus	KF946561	ZMA58744	Aliabadian et al. 2013	Laren	Netherlands
A. n. nisus	KF946562	ZMA58745	Aliabadian et al. 2013	Ammere	Netherlands
A. n. nisus	KF946563	ZMA58746	Aliabadian et al. 2013	Venlo	Netherlands
NA	KM360148	NA	Zang et al. 2014	NA	NA
NA	MN122826	NA	Margaryan 2019	NA	NA
A. n. nisus	MZ208929	MW43070	New	Treysa	Germany
A. n. nisus	MZ208930	MW43081	New	Treysa	Germany
A. n. nisus	MZ208931	MW62434*	New	Ottobrunn	Germany
A. n. nisus	MZ208932	MW62443	New	Ottobrunn	Germany
A. n. nisus	MZ208933	MW7991	New	Brunswick	Germany

A. n. nisus	MZ208934	MW9891	New	Westfalen	Germany
A. n. nisus	MZ208935	MW9897	New	South Argyllshire	UK
A. n. nisus	MZ208936	MW9898	New	South Argyllshire	UK
A. n. nisus	MZ208937	MW9899	New	South Argyllshire	UK
A. n. nisus	MZ208938	MW7482 *	New	Hamina	Finland
A. n. nisus	MZ208939	MW7483	New	Parikkala	Finland
A. n. nisus	MZ208940	MW7484	New	Pälkäne	Finland
A. n. nisus	MZ208941	MW66072*	New	Batum	Georgia
A. n. nisus	MZ208942	MW66073	New	Batum	Georgia
A. n. nisus	MZ208943	MW66074	New	Batum	Georgia
A. n. nisus	MZ208944	MW2594	New	Skaland	Norway
A. n. nisus	MZ208945	MW2620	New	Bleikvasslia	Norway
A. n. nisus	MZ208947	MW2821	New	Igis	Switzerland
A. n. nisus	MZ208948	MW2826	New	Malans	Switzerland
A. n. nisus	MZ208949	MW59	New	Bonaduz	Switzerland
A. n. nisus	MZ208950	MW9900	New	NA	Switzerland
A. n. nisus	MZ208951	MW9901	New	NA	Switzerland
A. n. nisus	MZ208952	MW21692	New	Lleida	Spain
A. n. nisus	NA	MW63905	NA	Frielendorf	Germany
A. n. nisus	NA	MW2588	NA	Skaland	Norway
A. n. nisus	NA	MW2589	NA	Oksfjordhamn	Norway
A. n. granti	MZ208946	MW9893	New	Madeira	Portugal
A. n. granti	MZ208953	MW19750*	New	Tenerife	Spain
A. n. granti	MZ208954	MW19753	New	Tenerife	Spain
A. n. granti	NA	MW19751	NA	Tenerife	Spain
A. n. granti	NA	MW19752	NA	Tenerife	Spain
A. n. granti	NA	MW19754	NA	Tenerife	Spain

Table 1. DNA sequences of the cox1 gene used for haplotype network reconstruction, and additional samples used for microsatellite genotyping, with sample numbers, GenBank accession numbers (will be added upon manuscript acceptance for new samples) and locality information. Samples with MW numbers (tissue collection of Michael Wink) were newly sequenced for this study for cox1 and genotyped for microsatellites. NA, information not available (samples with NA instead of accession number were not sequenced for cox1 but genotyped for microsatellites only). Asterisks mark the four samples that were used for microsatellite development.

of a single panmictic cluster (Figs. 1–4). The highest ΔK corresponded to K = 2 in analyses (i) and (ii), and to K = 4 in analyses (iii) and (iv); this method however cannot assess K = 1. Plots of cluster assignment of individual samples provided no evidence for actual clusters: each individual had similar assignment probabilities to the two or four clusters specified, with perhaps a minimal tendency in some runs to differentiate the Macaronesian samples (subspecies *granti*) by slightly different cluster assignment probabilities (Figs. 1–8). PCAs of the microsatellite data, with the 22-marker or 16-marker data sets, supported the results of the clustering analyses and revealed no apparent geographical or subspecific groupings. Samples of *A. nisus granti* had their group centroid slightly shifted compared to those of *A. nisus nisus,* but both groups were widely overlapping along both axes of PC1 and PC2 (Figs. 5, 10).

Analysis of the altogether 64 *cox1* sequences of *A. nisus* from across its range which, based on location, represent six currently accepted subspecies, revealed a very low differentiation in this mitochondrial gene. After exclusion of the single sample of *A. nisus. punicus* (accession number JF312087) for which only a very short DNA fragment was available, four haplotypes were recognized in the 586 bp alignment, differing from each other by a maximum of two mutational steps (Figs. 9, 10). The central haplotype contained 44 samples

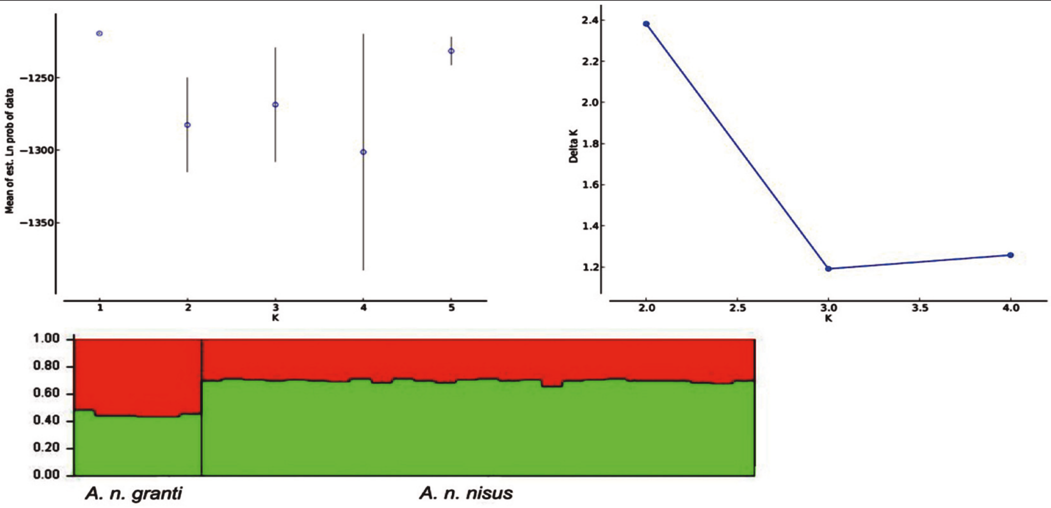
Marker	Repeat motif	Primer sequence (5'-3')	N samples genotyped	N alleles	Allele size range	HO	HE	Missing data
An2789#	(AAAC)6	Fwd: CTCTGCAAGCAAATCCCGTAG Rev: CCAGTAACAAGGGCAAGGAAC	24	1	347	NA	NA	25.0%
An14372#	(AAAC)7	Fwd: TAAAGAGATGGGAGCAGTTGG Rev: GTGCAGGGTATGATCACTTTGG	25	1	433	NA	NA	21.9%
An22524##	(AAAG)11	Fwd: CACAGCACCATCACTCCTTTC Rev: CGGAGGAACACATGCATACAG	13	4	240-272	0.385	0.351	59.4%
An1107##	(AAAC)8	Fwd: ACATGCTAACTCTGCTCCAG Rev: AGTTACCCACGACTTGCAAAG	18	2	348-364	0.111	0.110	43.8%
An13004##	(ACCT)6	Fwd: TAGCCTGCTTTGTAAGTGGG Rev: AAATTCGATCACAGGAGCCAC	25	3	194-214	0.320*	0.536	21.9%
An377##	(ACTC)15	Fwd: GTGACAGAGTGACTTGGCATG Rev: AAGGATTCTGGAAGGTGGACC	28	7	177-197	0.793	0.759	9.4%
An3169##	(ATCC)15	Fwd: AGGACAACACATCTCCCAGTC Rev: CCACACGTCTTTCCATCTGAC	24	6	177-231	0.542*	0.807	25.0%
An8617##	(ATCC)7	Fwd: TGAGGAGTCAGGTGAAAGAAGG Rev: TGCCTTGAGATTCATGTGGAC	26	4	197-209	0.077*	0.281	18.8%
An23385##	(ATCC)10	Fwd: TTCAGGGATATGCTGGATGGG Rev: CTCCTGTCCATCCATGTCAATG	24	6	245-273	0.667*	0.864	25.0%
An2088	(AACC)7	Fwd: ATAGGATGCAGAAGAGGACCC Rev: GAGGTAAGGGACAGCTGAAATC	26	8	241-273	0.692	0.825	18.8%
An2977	(ACAG)8	Fwd: TACATTGGCCGAGATCTGCAG Rev: CACAGTCAAGCATTTCCCTCC	24	5	264-280	0.417	0.480	25.0%
An3053	(AAAC)8	Fwd: ACCCTGATTGTAGCAGTAGTCC Rev: AGACTGCATGGGATTCCTAGAC	26	3	326-334	0.346	0.298	18.8%
An3611	(ATCC)7	Fwd: GGACTTCAGCGGGTTATTCAC Rev: AGCTATCTCCTGTCCATCCATG	27	9	202-234	0.815	0.861	15.6%
An3738	(AAAC)9	Fwd: CTGACCTACATGCTGCAACAC Rev: CCAAACAGTCTAACCCACAACC	31	4	171-191	0.786	0.766	12.5%
An7105	(AAGG)12	Fwd: AACTCCATTCCAACCAGACCC Rev: CAATCCCTTTGTCTTCCTCCC	26	10	203-251	0.654	0.865	18.8%
An17888	(AGCC)9	Fwd: CTGCCATGTGAGAAGTGGAAC Rev: ACTATGCCGTCTATTCCCACC	29	2	172-180	0.104	0.164	9.4%
An18968	(AAAC)6	Fwd: GCATCTGACCTCGTTTGTGTC Rev: TCCTAATGAGACCTGAGCACC	28	3	406-414	0.407	0.427	15.6%
An31006	(AAAC)6	Fwd: TTAAGAGCACCCTAGTACGGC Rev: AGGACGTGTGGTAGTCATAGC	29	3	325-333	0.517	0.482	9.4%
An31349	(AAAC)6	Fwd: TGTGGCCAGCATTATTGACAC Rev: AATTGCCCACAGTACAGCATG	27	3	379-387	0.259	0.338	15.6%
An31977	(AACC)7	Fwd: GCAGATAAGGAGGAAGGAACAC Rev: CGGCATTACTGAGATACAAGCC	26	2	315-319	0.040	0.040	21.9%
An40639	(ACAT)7	Fwd: ATTATCCCTCAACCTGCCCTC Rev: GTGGAGAATGTCAAGCCCATG	28	5	195-211	0.581	0.651	12.5%
An50077	(AAGC)7	Fwd: CACATTCCACTCCTTGCTCTG Rev: AGTGGGATGAGCGTTGTCTTC	31	2	189-193	0.065	0.064	3.1%
An82902	(AGGG)6	Fwd: TACGGTACCAGAATCTTGCCC Rev: AACTCAATGTGACAGTTGGCC	27	3	208-216	0.259	0.289	15.6%
An108380	(AGAT)15	Fwd: CTCCAGTGTTTGCTAGTTGGC Rev: CTAACACTAACACCCGAAAGCC	23	6	166-194	0.69565	0.78357	28.1%

Table 2. List of forward (Fwd) and reverse (Rev) primers for 24 newly established microsatellite markers for *Accipiter nisus.* Repeat counts are from the initial library; numbers and length ranges of alleles, as well as percent of missing data, refer to the entire set of 32 samples. Length range (inferred bp) include primers and linker. NA, not applicable; # monomorphic marker, excluded from descriptive statistics and analyses; ## marker with either excessive amount of missing data or significant deviation from Hardy-Weinberg equilibrium (marked with an asterisk), excluded from some analyses. HO, observed heterozygosity; HE, expected heterozygosity.

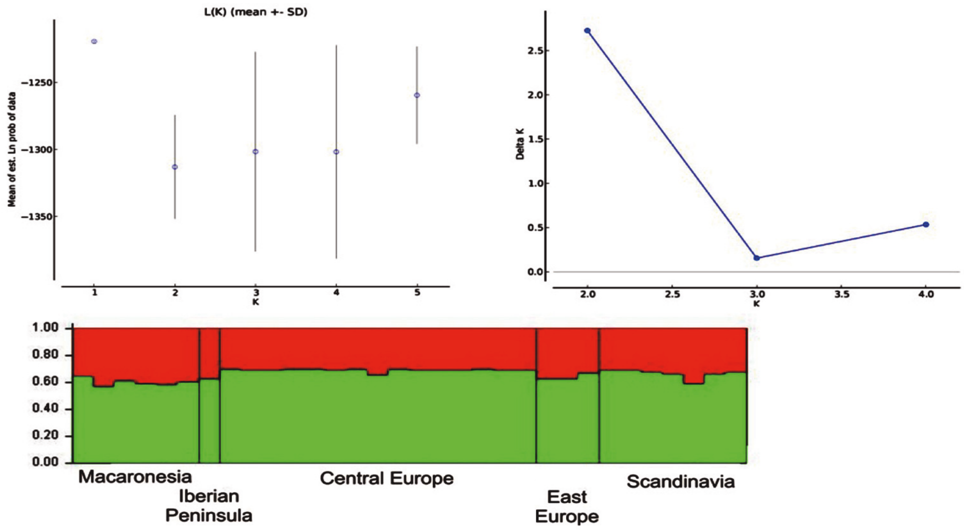
of all five subspecies included in the analysis, and differed by one mutational step each from the four other haplotypes: two singleton haplotypes containing one sample of *A. nisus nisosimilis* and one sample of unknown subspecies attribution; one haplotype representing three samples of *A. nisus nisus;* and one haplotype representing samples of *A. nisus nisus* and *A. nisus nisosimilis.* The island samples from Madeira, Tenerife and Sardinia (*A. nisus granti, A. nisus wolterstorffi*) did not show any sequence difference.

DISCUSSION

The primary goal of this study was mainly to establish novel markers, not to perform a thorough analysis of range-wide variation in the Eurasian Sparrowhawk which would have required a much larger number of samples and more geographic sampling. For instance, analyses such as STRUCTURE often perform poorly with data sets characterized by few samples and uneven distribution of samples across putative genetic populations. De-



1



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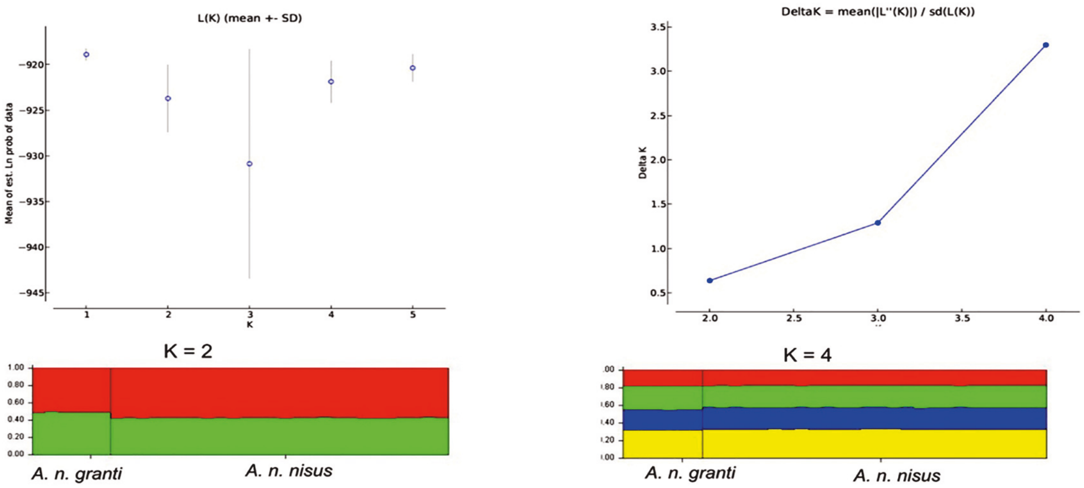
Figures 1, 2. Results of analyses with STRUCTURE of the full dataset of 22 polymorphic microsatellites for 32 samples of the Eurasian Sparrowhawk, *Accipiter nisus*. Each panel shows a graph with likelihood values for runs with different numbers of assumed clusters (K = 1 to 5), a second graph with delta-K values, and an exemplary plot of cluster membership for all individuals for K = 2. Fig. 1 shows analyses in which subspecies assignment was used as locprior (Macaronesia vs. Europe), whereas in Fig. 2, samples from continental Europe were divided into several ad-hoc geographical groups and these used as locprior.

spite these restrictions, it is worth reporting that mitochondrial DNA sequences and microsatellites both failed to indicate a relevant genetic differentiation of any geographical group or subspecies included in the respective data set. Only in the Macaronesian subspecies *A. nisus granti* did the microsatellite data indicate a possible, very weak differentiation that requires confirmation from future study with more comprehensive sampling. Because these birds are partial migrants, it cannot be excluded that a purely resident part of the Macaronesian subspecies may be genetically distinct but was not sampled for this study - although we consider this an unlikely hypothesis. For the North African subspecies *A. nisus punicus*, only a short *cox1* sequence was available from the study of Breman et al. (2013), but for this short stretch of the gene it does not show differences to the other subspecies, making a strong genetic divergence of this subspecies unlikely. For this and the remaining subspecies included in this study, only mtDNA information (i.e., *cox1* sequences) was available and



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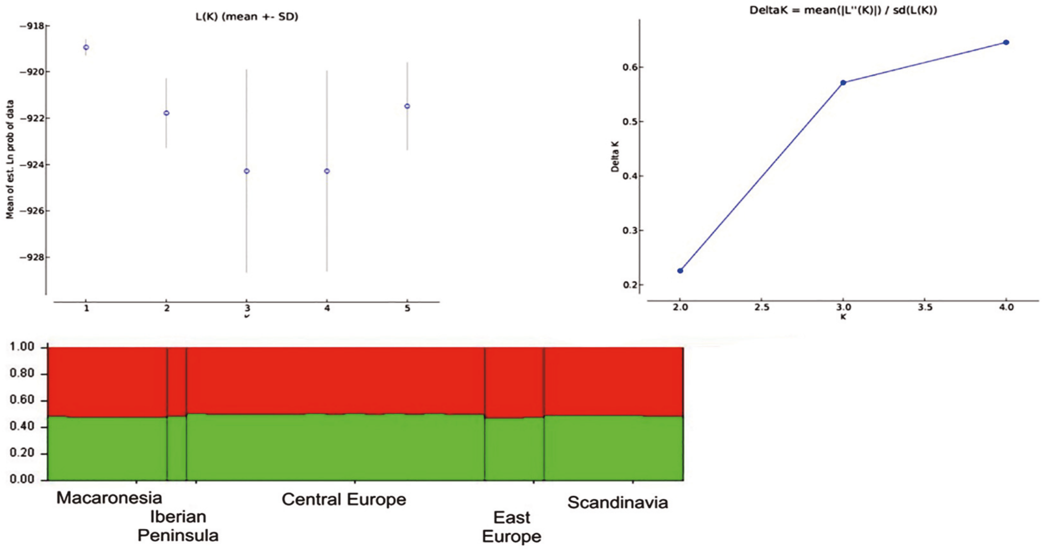

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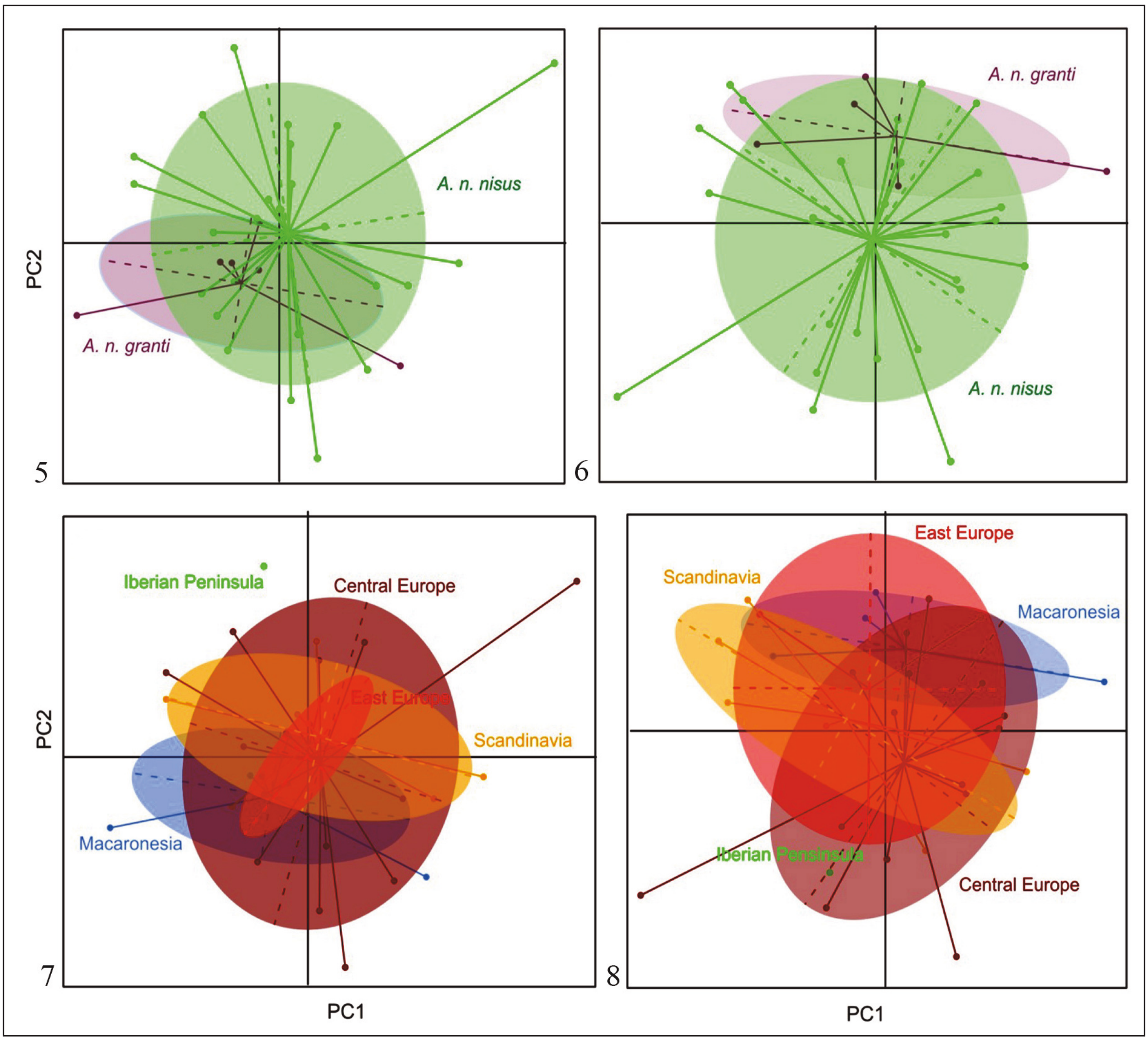
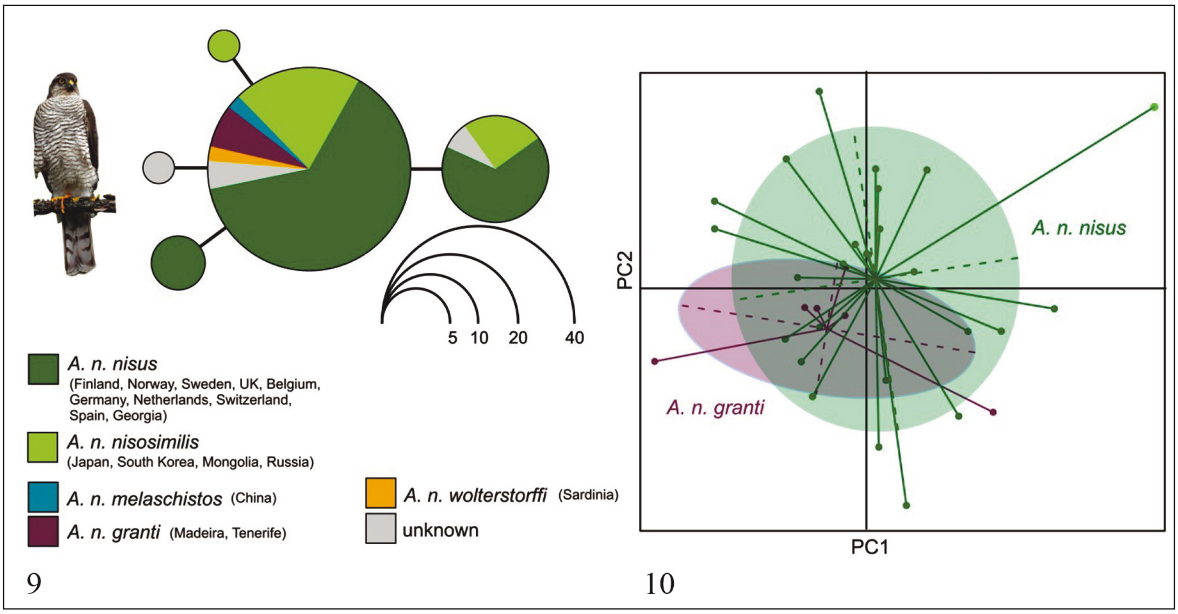
Figures 3, 4. Results of analyses with STRUCTURE of the reduced dataset of 16 polymorphic microsatellites for 32 samples of the Eurasian Sparrowhawk, *Accipiter nisus*, after excluding 6 markers with either excessive amounts of missing data or lack of Hardy-Weinberg equilibrium. Each panel shows a graph with likelihood values for runs with different numbers of assumed clusters (K = 1 to 5), a second graph with delta-K values, and an exemplary plot of cluster membership for all individuals for K = 2 (and K = 4 in Fig. 3). Fig. 3 shows analyses in which subspecies assignment was used as locprior (Macaronesia vs. Europe), whereas in Fig. 4, samples from continental Europe were divided into several ad-hoc geographical groups and these used as locprior.

revealed a negligible amount of divergence; however, it is well-known that the purely maternally inherited, not recombining mtDNA can be affected by introgression phenomena, especially in cases of sex-biased dispersal, and therefore it will be worth testing in future studies whether especially the eastern subspecies (*A. nisus nisosimilis, A. nisus melaschistos*) may be divergent in their nuclear genome.

Although our study covers only a limited selection of samples, our preliminary study allows to draw the hypothesis that the Eurasian Sparrowhawk is characterized by only limited genetic variation across its range, in agreement with the pattern in numerous other raptors (e.g., Scheider et al. 2004; Sonsthagen et al., 2004). Many Eurasian bird taxa show such patterns of panmixia, probably reflecting that during the last 2 million years most parts of Eurasia have undergone a regular change from warm and cold period (ice ages), the last one ending only 12000 years ago. During ice ages, most Eurasia bird taxa had to move to refuge areas in southern



Figures 5-8. Results of Principal Component Analyses (first and second axis, respectively) from microsatellite data for the Eurasian Sparrowhawk, *Accipiter nisus*. Panels A and C show the results of a PCA based on all 22 polymorphic microsatellites; Figs. 7, 8. show results from a reduced set of 16 markers, after excluding markers with excessive missing data and deviation from Hardy-Weinberg equilibrium. In Fig. 5 and Fig. 6, samples are color-coded according to currently accepted subspecies assignment, in Fig. 7 and Fig. 8, additionally, samples of *A. n. nisus* from Europe are color-coded according to ad-hoc geographical groups. PC1 and PC2 are the two first principal components.



Figures 9, 10. Molecular evidence for low molecular differentiation among populations of the Eurasian Sparrowhawk (*Accipiter nisus*). Fig. 9: haplotype network based on 586 bp of the mitochondrial *cox1* gene for 63 samples from across the species' range. Fig. 10: Principal Component Analysis based on 22 microsatellite markers for 32 samples, showing very low divergence among *A. n. nisus* from various parts of Europe, and *A. n. granti* from the Macaronesian islands, Tenerife and Madeira (recolored version of Fig. 5). Assignment of samples to subspecies in both panels is based on their geographical occurrence. PC1 and PC2 are the two first principal components (explaining 9.3% and 8.8%. of the variation).

Europe, Africa or southern Asia, where lineages mixed. Even if a genetic differentiation existed before, it was probably lost during the times in refuge areas (Wang et al., 2017; Carneiro et al., 2019; Parau et al., 2019).

Besides phylogeography, the newly developed microsatellites presented here provide a genomic resource for future conservation genetics, conservation breeding, individual recognition and paternity analysis of Eurasian Sparrowhawks.

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