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ORIGINAL ARTICLE

DNA barcoding and evolutionary relationships in *Accipiter* Brisson, 1760 (Aves, Falconiformes: Accipitridae) with a focus on African and Eurasian representatives

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Abstract We obtained full (647 bp) or mini (291 bp) DNA barcodes of 140 mostly African and European specimens of 25 *Accipiter* (Aves: Accipitridae) species. Kimura two-parameter (K2P) distances were calculated between barcodes to determine the thresholds of intra- and interspecific species boundaries. Thresholds were comparable to or higher than those in previous studies and ranged from 2.8 to 3.0 % (best compromise threshold based on cumulative intra- and interspecific K2P distances) and from 3.9 to 5.3 % (ten times the average intraspecific K2P distance). Identification success was determined using the best match and best close-match criteria and ranged between 84 % (mini barcodes) and 90 % (full barcodes). Incorrectly or ambiguously identified specimens belonged to two species that were represented by single sequences in the database (*A. madagascariensis* and *A. trivirgatus*) and three species pairs that shared at least one haplotype: viz. *A. nisus* and *A. rufiventris*, *A. gularis* and *A. virgatus*, and *A. cooperii* and *A. gundlachi*. The other 19 species were unambiguously identified using the full DNA barcodes. The studied species belong to eight traditional superspecies, of which three ([*gentilis*], [*cooperii*], and [*tachiro*]) were well supported. In one superspecies, [*badius*], species pairs were supported but not the superspecies.

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Zusammenfassung

DNA-Barcoding und evolutionäre Beziehungen innerhalb der Gattung *Accipiter* Brisson, 1760 (Aves, Falconiformes: Accipitridae), mit besonderem Schwerpunkt auf deren afrikanischen und eurasischen Vertretern

Wir verwendeten vollständige DNA-Barcodes (647 bp) oder Mini-Barcodes (291 bp) von 140 Individuen (hauptsächlich afrikanischer und europäischer Herkunft) aus 25 Accipiter-Arten (Aves: Accipitridae). Um die Schwellenwerte für die intra- und interspezifischen Artgrenzen zu ermitteln, berechneten wir die Kimura-2-Parameter-Distanzen (K2P) zwischen den Barcodes. Die Schwellenwerte waren vergleichbar oder höher als die aus früheren Studien und lagen zwischen 2,8 und 3,0 % (BCTh-Schwellenwert (Best Compromise Threshold) auf der Grundlage kumulierter intra- und interspezifischer K2P-Distanzen) beziehungsweise zwischen 3,9 und 5,3 % (zehnfacher Durchschnitt der intraspezifischen K2P-Distanz). Der Erfolg der Zuordnung wurde anhand von Best-Match- und Best-Close-Match-Kriterien bestimmt und reichte von 84 % (Mini-Barcodes) bis 90 % (vollständige

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Barcodes). Nicht korrekt oder nicht eindeutig bestimmte Individuen stammten von zwei Arten, die in der Datenbank jeweils nur durch einzelne Sequenzen vertreten waren, sowie von drei Artenpaaren, welche mindestens einen gemeinsamen Haplotyp aufwiesen, nämlich: *A. nisus* – *A. rufiventris, A. gularis* – *A. virgatus* und *A. cooperii* – *A. gundlachi*. Die übrigen 19 Arten konnten anhand der vollständigen DNA-Barcodes eindeutig zugeordnet werden. Die untersuchten Arten gehören zu acht traditionell gebräuchlichen Superspezies, von denen drei ([gentilis], [cooperii] und [tachiro]) gut bestätigt wurden. Für eine Superspezies ([badius]), konnten zwar Artenpaare bestätigt werden, die Superspezies jedoch nicht.

Keywords *Accipiter* · Archival DNA · COI · DNA barcoding · Molecular phylogeny · Taxonomy

Introduction

Members of the raptor genus *Accipiter* Brisson, 1760 have a near-cosmopolitan distribution and occur from the Arctic Circle to the humid tropics, and from sea level to high altitudes (Thiollay 1994). After some early attempts to do so (see Stresemann 1923 and references therein), the taxonomy of the genus was substantially revised by Wattel (1973), and he was the first to discuss the infrageneric division of the genus (see Fig. 1). Since then, eight taxa have been elevated to the species level and one species ("*Accipiter*" *buergersi*) has been redirected to the genus *Erythrotriorchis* (Thiollay 1994). Some of the *Accipiter* species, and many of the subspecies, have been defined based on subtle morphological differences (e.g., Swann 1922; Thiollay 1994). Because adaptive convergence in *Accipiter* may have resulted in similar plumage patterns in distantly related taxa (Riegner 2008), the infrageneric relationships of the genus *Accipiter* have proven difficult to disentangle (Ferguson-Lees and Christie 2001). To acknowledge the apparent strong relationship among several of the *Accipiter* species, Stresemann and Amadon (1979) grouped several species into superspecies (see Fig. 1), viz. "a monophyletic taxon of very closely related and largely, or entirely, allopatric species, too different to be included in a particular species" (Hawksworth 2010). This was based on similarities in morphology (e.g., plumage pattern, claw and toe size, wing shape and size, tail length), behavior (e.g., hunting behavior), and distribution. In an often used handbook, 51 species are currently recognised in the genus *Accipiter*, of which 43 are grouped into 13 superspecies (Thiollay 1994, Fig. 1).

Recently, molecular markers have shed light on the higher phylogenetic relationships of the Accipitridae (Mayr et al. 2003; Wink and Sauer-Gürth 2000, 2004; Griffiths et al. 2007; Lerner and Mindell 2005; Lerner et al. 2008; Lerner 2007), but a lower infrageneric phylogenetic analysis of the genus *Accipiter* is currently lacking. Here, we studied the infrageneric phylogenetic relationships in the genus *Accipiter*, in particular that of the African and Eurasian representatives, using the mitochondrial cytochrome *c* oxidase subunit I (COI) gene. This gene has previously been successfully used to disentangle the evolutionary relationships of closely related bird species of the Neotropics, the northern Atlantic region (e.g., Kerr et al. 2009a, b; Johnsen et al. 2010), and the Philippines (Ong et al. 2011). Moreover, a 694 bp region of the COI gene is now, by convention, used as the standard genetic marker to assist in identifying animal species (i.e., DNA barcoding; Hebert et al. 2003, 2004; Ward et al. 2008; Yassin et al. 2009; Pagès et al. 2010). A first DNA barcoding study of 25 % of the bird species of North America (Hebert et al. 2004) showed that the COI sequence variation within species was on average 20 times smaller than that between species, and that there was a clear gap (the so-called barcoding gap) between intra- and interspecific Kimura two-parameter (K2P) distance distributions. Hebert et al. (2004) proposed a standard sequence threshold of ten times the mean intraspecific variation for birds (=2.7 % sequence divergence) to define species boundaries and to flag up potentially new species. Subsequent studies have confirmed the existence of a barcoding gap in birds with a K2P distance threshold of 2.4–2.7 % and with an identification success rate of >94 %. This also holds for the family Accipitridae, which is a large group (~ 240 species) of diurnal birds of prey that includes hawks, kites, harriers, Old World vultures, and eagles. About 31 % of the species in that group have already been barcoded (Hebert et al. 2004; Yoo et al. 2006; Kerr et al. 2007; Cai et al. 2010; Johnsen et al. 2010; Ong et al. 2011). These studies have shown that accipitrid species have distinct COI sequences and that the average interspecific distance (6.6 %) is ten times higher than the mean intraspecific distance (0.66 %; Ong et al. 2011). Hence, distance-based DNA barcoding seems to provide sufficient information to identify and delineate a large majority of bird species, including Accipitridae, in pairwise comparisons (Yoo et al. 2006; Kerr et al. 2007; Tavares and Baker 2008; Aliabadian et al. 2009; Johnsen et al. 2010).

Because specimens of several *Accipiter* species are difficult to obtain (e.g., because they are rare or live in remote areas or on small islands), one has to rely in part on museum specimens. Unfortunately, such archival DNA is often strongly degraded and only short fragments may be sequenced successfully (Chelomina 2006). Therefore, we also retrieved mini barcodes (i.e., short COI sequences of 100–400 bp), since these have been successfully used in the identification of a variety of animal taxa (e.g., Hajibabei et al. 2006; Meusnier et al. 2008), including birds (Sonet et al. 2011).

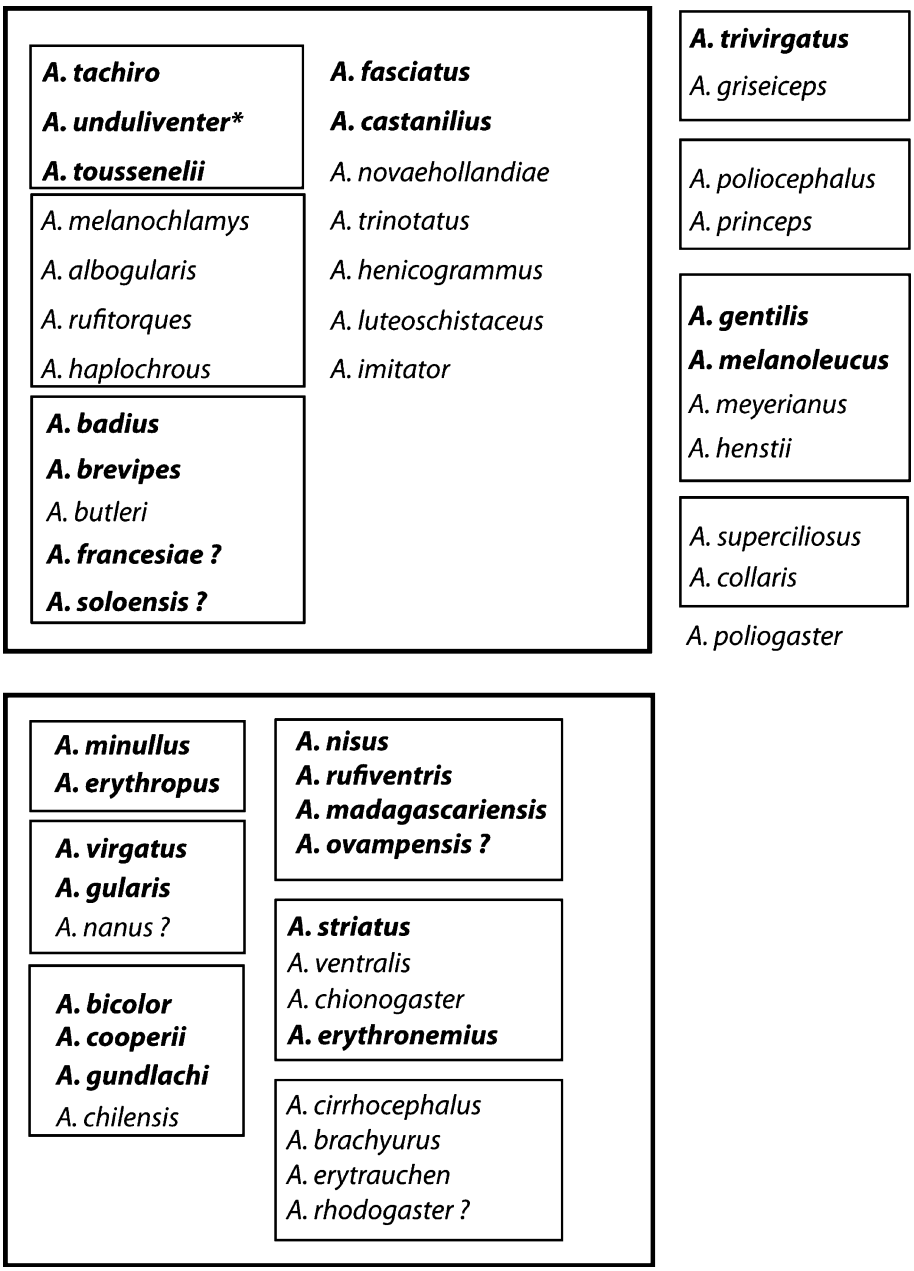


Fig. 1 Current status of the taxonomic relationships in the genus *Accipiter*. Figure adjusted from Wattel (1973). *Asterisk*: treated as a species by Louette (2003). *Boxes* group the traditional superspecies (see Thiollay 1994). The two larger *boxes in bold* represent higher level relationships among superspecies (see Wattel 1973). Species names in bold are species included in this study

The aims of this study were (1) to test whether DNA (mini) barcodes allow the identification of *Accipiter* species, (2) to resolve the molecular phylogenetic relationships in (part of) the genus *Accipiter*, focusing in particular on Eurasian and African representatives, and (3) to compare these results with the current morphology-based taxonomy.

Materials and methods

Samples

Tissues and toe pads from *Accipiter* specimens deposited at the Royal Museum for Central Africa (RMCA) ($n = 83$), the Royal Belgian Institute of Natural Sciences (RBINS) ($n = 9$), and other museums ($n = 16$), as well as blood samples from field-caught individuals ($n = 11$) were used for molecular analysis. In addition, *Accipiter* COI sequences from GenBank ($n = 28$) and from the Barcode of Life Database (that were not in GenBank, $n = 2$; BOLD; http://www.boldsystems.org; Ratnasingham and Hebert 2007) were included (see "Appendix 1") if these comprised the fragment that we sequenced and if these contained ≤ 2 ambiguous positions. For instance, there are other sequences from *Accipiter* in GenBank and BOLD that are too short or lack up to the first 10–15 bp of the (mini) barcode region. Altogether, 148 specimens from 25 *Accipiter* species were included.

DNA extraction, PCR, and sequencing

Total genomic DNA was extracted from feathers or blood, but for most taxa it was extracted from toe pad cuts from museum specimens (collected as far back as 1852; see "Appendix 1"). DNA was extracted with the NucleoSpin tissue kit (Macherey–Nagel, Düren, Germany). The standard DNA barcode fragment for birds (Hebert et al. 2004) was amplified in two PCRs with an overlap of 41 bp. A fragment of 298 bp was amplified with the primers Bird1Fd (TCAACCAACCACAAAGAYATYGGYAC) (or an M13 tailed version: BirdF1dt) modified from Hebert et al. (2004) and BirdH_351d_370d (CCTGCTCCWGCT TCTAYDGT) (Sonet et al. 2011). This fragment corresponds to the first part of the standard DNA barcode region (Hebert et al. 2004). A 437 bp fragment of the second part of the DNA barcode region was amplified using the primer pair Aves_L288_310 (CGCATAAACAACATAAGCTTC TG) (Louette et al. 2011) and the M13-tailed birdR1dt (M13R-ACGTGGGAGATGATTCCGAAKCCKGG) modified from Hebert et al. (2004). Both fragments were concatenated and the data set was trimmed to 647 bp. This was necessary to allow the inclusion of *A. cooperii* and *A. trivirgatus* sequences from BOLD and GenBank. This dataset will be referred to as the barcode dataset. For all samples we sequenced at least the first 298 bp. Of these, we removed the first 7 bp, resulting in a dataset of 291 bp that will be referred to as the "mini barcode dataset".

PCR was carried out in a total volume of 30 µl, containing 2–4 µl of genomic DNA, 1× PCR buffer, 0.2 mM of each dNTP, 0.8 µM of each primer, 2.0 mM $MgCl_2$, 0.5 U of Platinum Taq DNA polymerase (Invitrogen), and mQ-H_2O. The PCR profile was 4 min at 94 °C followed by 35–40 cycles of 30 s at 94 °C, 30 s at 50 °C, and 45 s at 72 °C, with a final extension of 7 min at 72 °C. PCR products were purified using NucleoFast 96 PCR plates (Macherey–Nagel, Düren, Germany) and bidirectionally sequenced using the BigDye Terminator v1.1 kit on an ABI 3130xl automated capillary DNA sequencer (Life Technologies, Carlsbad, CA, USA). Sequences were visually inspected and aligned in SeqScape v2.5 (Life Technologies).

DNA barcoding analysis

Three datasets were analyzed: the mini barcode dataset (dataset A: 291 bp, 25 species, 148 sequences); the barcode dataset (dataset B: 647 bp, 18 species, 100 sequences), and a dataset that had the same species coverage as dataset B but was trimmed to the fragment size of the mini barcode dataset (dataset C: 291 bp, 18 species, 100 sequences). Comparison of the results based on these three datasets allowed us to disentangle the possible effects of species coverage and sequence length on the identification success.

The Kimura two-parameter (K2P) distance model (Kimura 1980) was used to calculate sequence divergences between and within species, and to construct neighbor-joining (NJ) (Saitou and Nei 1987) trees and histograms of intra- and interspecific distance frequencies. Distance tables and NJ trees were created in MEGA v4.10 (Tamura et al. 2007) and histograms were calculated using R v2.9.2. (R Development Core Team 2009) with the APE package v2.7-1 (Paradis et al. 2004). When calculating the mean intraspecific sequence divergence within the genus, species that were represented by a single sequence were omitted.

NJ trees provide a visual representation of the relationships among sequences and the divergence between specimens. However, criteria based on NJ trees (e.g., monophyly of species) perform badly (as compared to other methods) for various methodological reasons, and are therefore not preferentially used to determine the identification success (for a full discussion see Meier et al. 2006 and Virgilio et al. 2010). Therefore, the proportion of correctly identified specimens was estimated with the program Species Identifier using the best match (BM) and best close-match (BCM) criteria of Meier et al. (2006) When using BM, each query was assigned the species name of its best-matching sequence, regardless of how similar the query and reference sequences were. Identification was considered correct when both sequences were from the same species, incorrect if the query species differed from the closest reference species, or ambiguous if multiple species were the BM of the query species. BCM relies on an optimal distance threshold value of sequence similarity. Because the 10× intraspecific K2P distance-based threshold proposed by Hebert et al. (2003) may not be universally applicable (e.g., Meier and Paulay 2005), Meier et al. (2006) proposed adapting the distance threshold to the particular reference library used. Because the 10× threshold seems to work well in birds (Hebert et al. 2004; Kerr et al. 2009a, b; Johnsen et al. 2010; Ong et al. 2011) we used this threshold together with the "best compromise threshold" (BCTh) based on cumulative distribution curves of intra- and interspecific K2P distances (Lefébure et al. 2006). Note that species that are only represented by a single sequence in the dataset will generate incorrect identifications under the BM criterion, and incorrect (if there is a match closer than the BCTh) or impossible (if there is no match closer than the BCTh) identifications under the BCM criterion (Ross et al. 2008). Finally, for closely related species, alignment files were viewed in MEGA v4.10 to identify nucleotide characters that are diagnostic for a species ("pure characteristic attributes", sensu Rach et al. 2008).

Phylogenetic analysis

Maximum parsimony (MP) and maximum likelihood (ML) trees were estimated in PAUP* v4.0b10 (Swofford 2002) using a heuristic search with the tree-bisection-reconnection branch-swapping algorithm and random addition of taxa. Relative branch support was evaluated with 5,000 bootstrap replicates (Felsenstein 1985) for the NJ tree, 1,000 bootstrap replicates for the MP tree, and 200 for the ML tree. Phylogenetic trees for ML and Bayesian analyses were inferred with the nucleotide substitution model selected using jMODELTEST v0.1.1 (Posada 2008). The best model for the 291 bp data set was TVM + I. The best model for the 647 bp data set was TIM2 + I. A Bayesian tree was calculated using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). Four simultaneous chains (one cold, three heated) were run for ten million generations, and trees were sampled every 1,000 generations. To check the convergence and stability of the parameter estimates and to determine the burn-in value, Tracer v1.5 (Rambaut and Drummond 2009) was used to explore the log files. Initial trees generated in the burn-in phase (i.e., before establishing stable estimates of parameters) were discarded (burn-in value = 1,000, 10 % of the trees). The remaining trees were used to estimate tree topology, branch lengths, and substitution parameters. Phylogenetic relationships were inferred with *Buteo buteo* (GenBank accession number GQ481408) as the outgroup.

Results

Barcoding analysis

Frequency distributions of intra- and interspecific K2P distances are given in Fig. 2. There is no DNA barcoding gap in any of the three datasets. Average interspecific and intraspecific distances for each dataset are given in "Appendix 2". The NJ tree for each dataset is given in the Electronic supplementary material (ESM). The identification success for each dataset is summarized in Table 1. The 10× intraspecific sequence divergence threshold values were 4.8 % (dataset A), 5.3 % (dataset B), and 3.9 % (dataset C). The BCTh values were 3.0 % (dataset A), 2.8 % (dataset B), and 3.0 % (dataset C) (Fig. 3). For both methods the identification success was 84.35 % for dataset A and 90 % for the other two datasets. Six species were only represented by a single sequence. These were categorized as incorrect using the BM criterion. Under the BCM criterion, four of these (*A. cooperii*, *A. fasciatus*, *A. gundlachi*, and *A. melanoleucus*) were categorized as

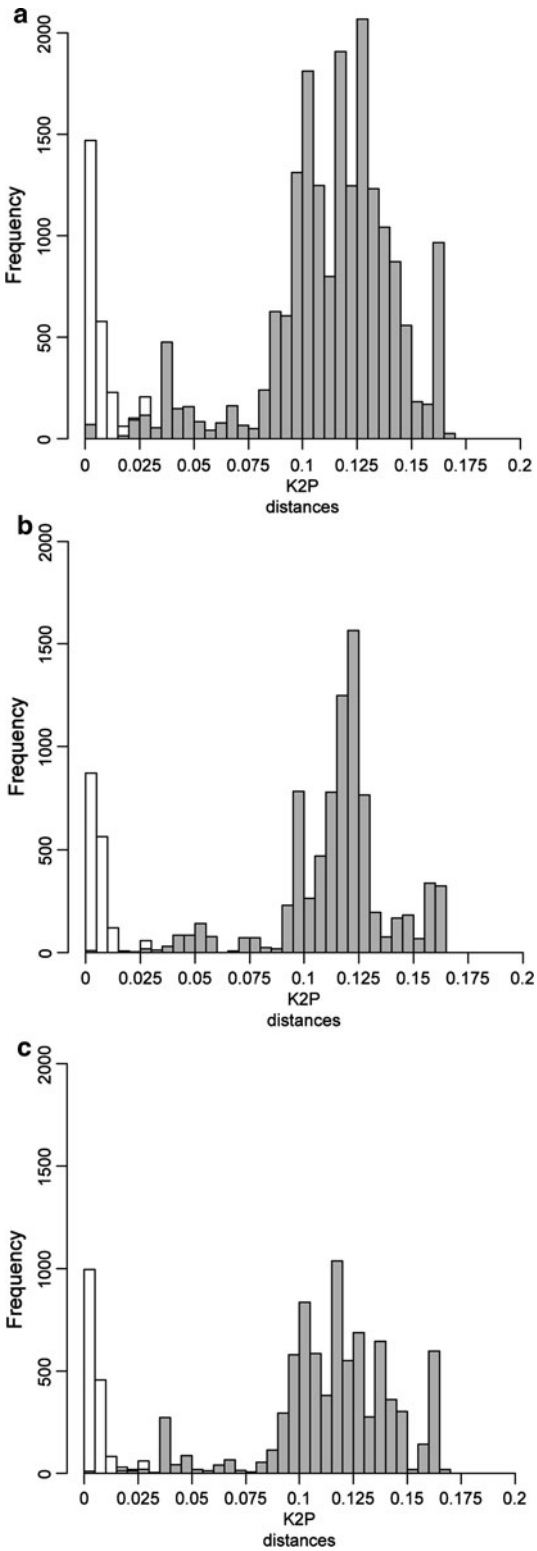


Fig. 2 Pairwise distance (K2P) distributions of intra- and interspecific sequence divergences for **a** the mini barcode fragment (291 bp), **b** the barcode fragment (647 bp), and **c** the mini barcode fragment (291 bp) but with the same species coverage as **b**

Table 1 Identification success with threshold values for the BCTh (a, b) and 10× (c, d) methods as determined via the BM (a, c) and BCM (b, d) criteria

Dataset	Threshold (BCTh) (%)	Correct	Ambiguous	Incorrect	No match closer than threshold
(a) BM					
A	3.00	124 (84.35 %)	19 (12.92 %)	4 (2.72 %)	–
B	2.80	90 (90.0 %)	6 (6.0 %)	4 (4.0 %)	–
C	3.00	90 (90.0 %)	5 (5.0 %)	5 (5.0 %)	–
Dataset	Threshold (BCTh) (%)	Correct	Ambiguous	Incorrect	No match closer than threshold
(b) BCM					
A	3.00	124 (84.35 %)	18 (12.24 %)	3 (2.04 %)	2 (1.36 %)
B	2.80	90 (90.0 %)	5 (5.0 %)	4 (4.0 %)	1 (1.0 %)
C	3.00	90 (90.0 %)	5 (5.0 %)	4 (4.0 %)	1 (1.0 %)
Dataset	Threshold (10×) (%)	Correct	Ambiguous	Incorrect	No match closer than threshold
(c) BM					
A	4.80	124 (84.35 %)	19 (12.92 %)	4 (2.72 %)	–
B	5.30	90 (90.0 %)	5 (5.0 %)	5 (5.0 %)	–
C	3.90	90 (90.0 %)	6 (6.0 %)	4 (4.0 %)	–
Dataset	Threshold (10×) (%)	Correct	Ambiguous	Incorrect	No match closer than threshold
(d) BCM					
A	4.80	124 (84.35 %)	18 (12.24 %)	3 (2.04 %)	2 (1.36 %)
B	5.30	90 (90.0 %)	5 (5.0 %)	4 (4.0 %)	1 (1.0 %)
C	3.90	90 (90.0 %)	5 (5.0 %)	4 (4.0 %)	1 (1.0 %)

incorrect, whereas *A. madagascariensis* and *A. trivirgatus* were categorized as not identified (i.e., there was no match closer than the BCTh). In three species pairs, both species shared haplotypes: viz. *A. nisus* and *A. rufiventris* (for dataset A only), *A. gularis* and *A. virgatus*, and *A. cooperii* and *A. gundlachi*.

Apart from the three species pairs in which the two species shared a haplotype, we identified nucleotide characters that were diagnostic for each species. Some examples are given in "Appendix 3". The number of diagnostic characters varied between one (*A. unduliventer* vs. *A. tachiro*/*A. toussenelii*) and 13 (*A. minullus* vs. *A. erythropus*).

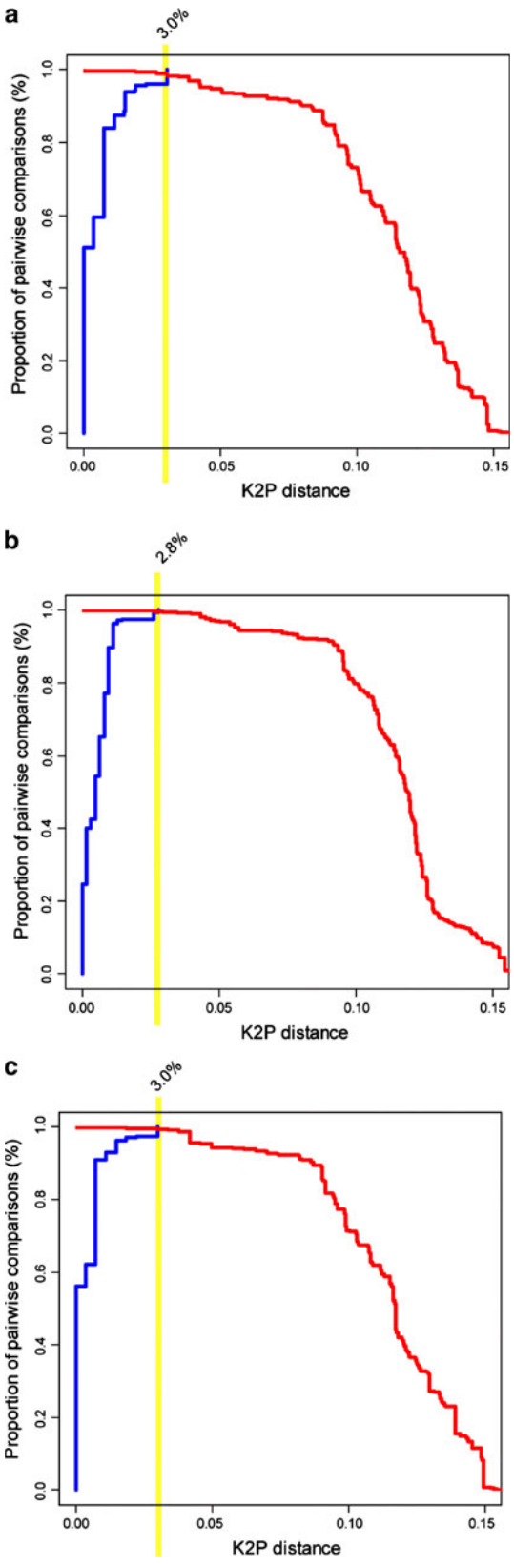
Phylogenetic relationships

Dataset A had 89 variable sites, of which 82 were parsimony-informative, and comprised 44 haplotypes. The phylogenetic relationships among these haplotypes are shown in Fig. 4. Dataset B yielded 200 variable sites, of which 174 were parsimony-informative, and comprised 42 haplotypes. The phylogenetic relationships among these haplotypes are shown in Fig. 5. The results of both datasets were largely congruent. A clade was considered well supported if posterior probabilities were ≥0.95 (Ranker et al. 2003) and values for NJ, ML, and MP were ≥70 (Hillis and Huelsenbeck 1992).

The monophyly of the traditional superspecies [gentilis], [cooperii], and [tachiro] was well supported. Within the superspecies [tachiro], the clades of *A. tachiro* and *A. toussenelii* were monophyletic. There was no support for the monophyly of the traditional superspecies [badius], but this superspecies consisted of two diverged clades, viz. *A. soloensis* + *A. francesiae* and *A. badius* + *A. brevipes* (Fig. 4). There was no support for the monophyly of the traditional superspecies [nisus]. Rather, *Accipiter ovampensis* and *A. madagascariensis* formed a well-supported clade that was strongly diverged from the other [nisus] species (*A. striatus* + *A. rufiventris* + *A. nisus*), which were monophyletic as well (Fig. 5). Dataset B showed that the members of the traditional superspecies [virgatus] formed a monophyletic group with *A. fasciatus*. Finally, *A. trivirgatus* was well differentiated from all other superspecies. The deeper phylogenetic relationships among the traditional superspecies were not resolved.

Discussion

In this study, we provided new (sometimes partial) DNA barcodes for 119 specimens of 19 *Accipiter* species, meaning that 25 species of the 51 currently known



◀ **Fig. 3** Optimum threshold (*yellow*) defined by the intersection between the cumulative frequency distribution curves of the intraspecific (in *blue*) and the interspecific (in *red*) pairwise distances for datasets A–C (**a**–**c**, respectively) (color figure online)

Accipiter species are now barcoded. The identification success rate was approximately 90 %, which is very similar to the success rate reported in other DNA barcoding studies in birds (Yoo et al. 2006; Kerr et al. 2007; Tavares and Baker 2008; Aliabadian et al. 2009; Johnsen et al. 2010). Our data suggest that the 10× intraspecific K2P distance threshold for species delimitation proposed by Hebert et al. (2004) would be 5.3 % for *Accipiter* (Table 1), which is higher than the 2.4–2.7 % thresholds for birds reported so far. This is because two species (*A. gentilis* and *A. badius*) showed large intraspecific divergences (up to 2.82 %; see also Johnsen et al. 2010 and Cai et al. 2010 and below).

The use of a single interspecific threshold that would be applicable to all taxonomic groups, however, has been questioned because patterns of intra- versus interspecific sequence divergence may vary across taxa, so the distance threshold should be adapted to each particular dataset (e.g., DeSalle et al. 2005; Meier et al. 2006). For instance, Meier and Paulay (2005) showed that thresholds of 3.2× to 6.8× were more suitable for the identification of marine gastropods than any single threshold. We therefore also calculated an alternative threshold value (i.e., "best compromise threshold," BCTh), as proposed by Lefébure et al. (2006). The BCTh for the *Accipiter* data set (2.8 % for the barcode dataset; 3 % for the mini barcode dataset) was much lower than the 10× intraspecific variation threshold (5.3 %). Nevertheless, the identification success obtained using both threshold values was 90 %. It therefore seems that the majority of the *Accipiter* species can be reliably identified using DNA barcoding with a threshold of 3 %. That said, although 76 % (i.e., 19 species) of the *Accipiter* species showed unique barcode clusters, the remaining 24 % (i.e., six species) had only one, or overlapping, barcode(s). Three species pairs could not be distinguished by DNA barcoding, viz. *A. nisus* and *A. rufiventris*; *A cooperii* and *A. gundlachi*, and *A. gularis* and *A. virgatus*. In all three cases, the species in question were closely related (see below). Unfortunately, we could not obtain full barcodes for all of the *Accipiter* specimens because the DNA of old museum material seemed to be partly degraded. We therefore also focused on a 298 bp mini barcode fragment that was amplified with more success. Our results showed that shorter sequence lengths do not impair species identification, since the identification success of the standard barcode (dataset B) was the same as that of the mini barcode (dataset C) (note that both datasets had the same species coverage). This supports the conclusion of Sonet et al. (2011) that DNA barcoding with shorter fragments than the standard barcode region works well for birds. However, we observed an effect of species diversity on the identification success using mini barcodes. Indeed, the

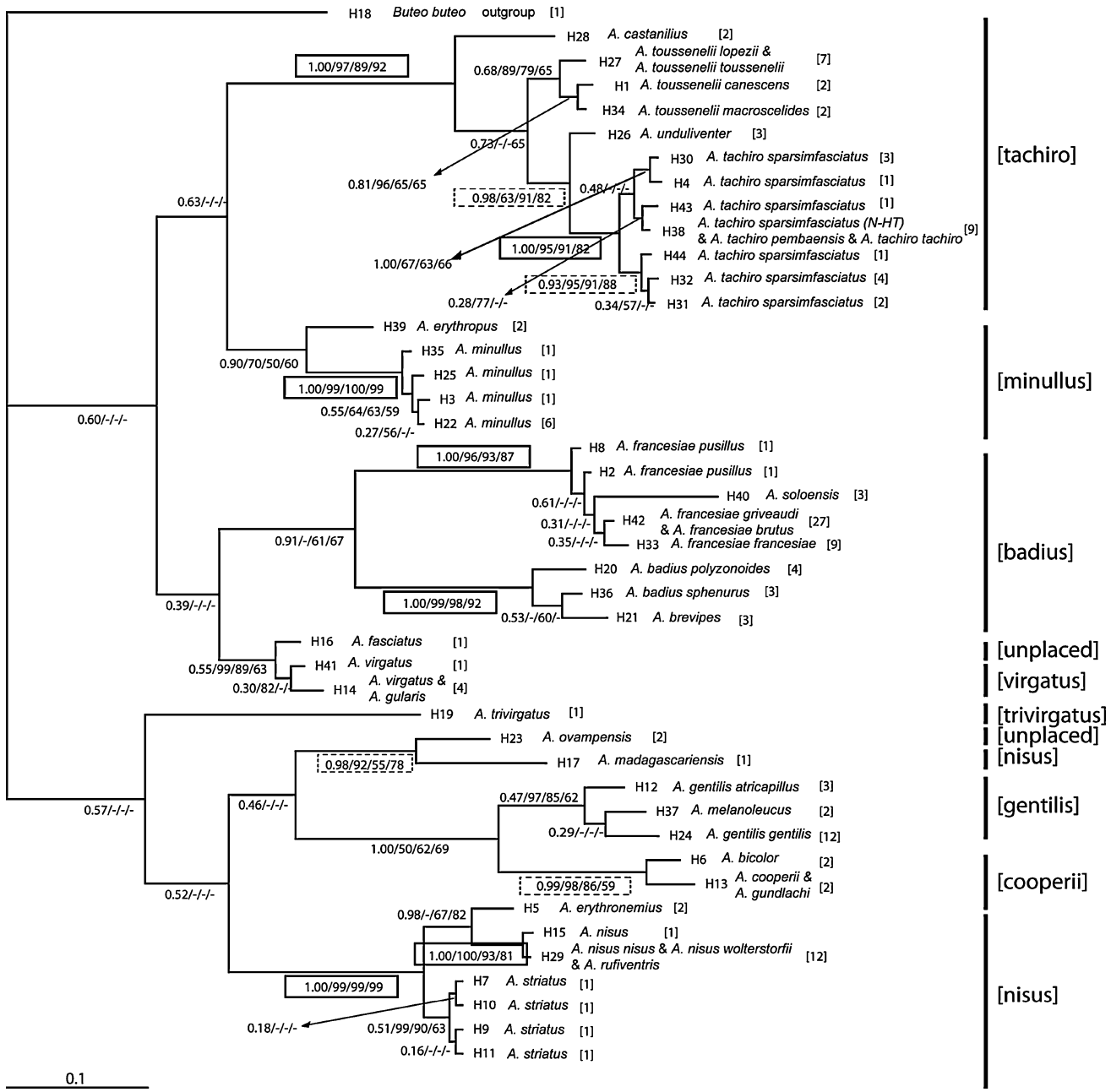


Fig. 4 Phylogenetic relationships in the genus *Accipiter* with *Buteo buteo* as the outgroup for dataset A. The scale indicates base substitutions per site. For each clade, Bayesian probabilities (BI) and bootstrap values for the neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) analyses are given as BI/NJ/MP/ML. When a clade is supported by all four methods, it is indicated by a *solid box*; *boxes with a dashed line* represent nodes that were supported by three of the analyses. Values are plotted on the consensus tree of the Bayesian analysis. The numbers in *square brackets* indicate the number of specimens for this haplotype

identification success of the more species-rich mini barcode dataset A (25 species) was almost 6 % lower than that of the less species-rich mini barcode dataset C (18 species). This lower identification success is caused by the inclusion of species that show high intraspecific variation (e.g., *A. badius*) or low interspecific divergence (e.g., *A. rufiventris* and *A. nisus*). Hence, the value of DNA barcoding for identifying and delimiting *Accipiter* species can only be determined after the remaining species have been barcoded and when each species is represented by a reasonable number of samples.

Taxonomy of *Accipiter* based on COI sequence data

Raptors are a well-studied group of birds, but many aspects of their taxonomy and evolution have remained unclear or

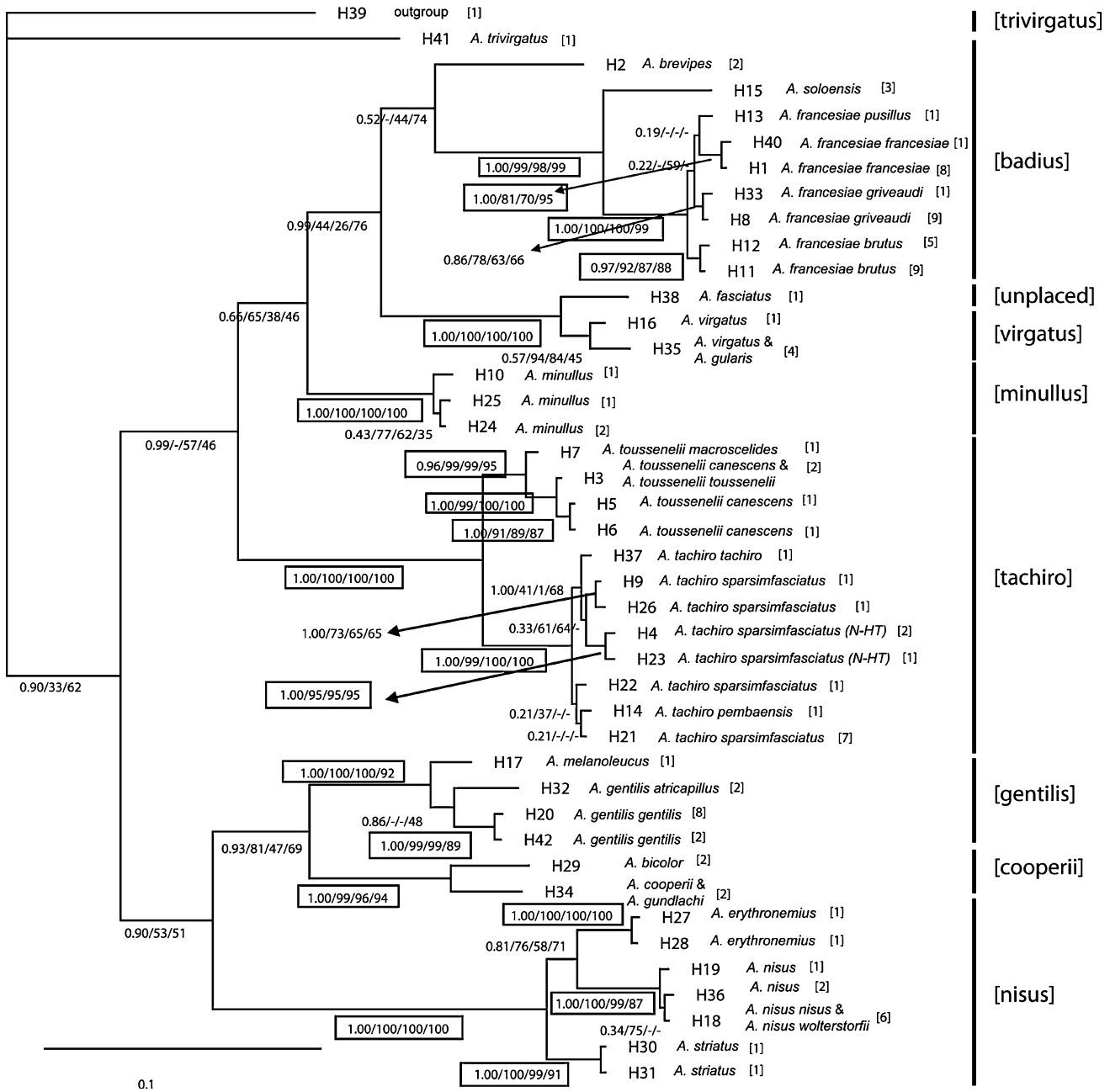


Fig. 5 Phylogenetic relationships in the genus *Accipiter* with *Buteo buteo* as the outgroup for dataset B. The scale indicates base substitutions per site. For each clade, Bayesian probabilities (BI) and bootstrap values for the neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) analyses are given as BI/NJ/MP/ML. When a clade is supported by all four methods, it is indicated by a *solid box*. Values are plotted on the consensus tree of the Bayesian analysis. The numbers in *square brackets* indicate the number of specimens for this haplotype

ambiguous. Here, we provide the first molecular phylogenetic analysis for *Accipiter* using the COI gene. The taxonomy of the genus *Accipiter* is traditionally based on plumage morphology, morphometrics (claw and toe size, wing shape and sizes, tail length), and behavior (hunting methods, main prey) (Wattel 1973; Thiollay 1994 and references in both). The use of a limited number of species and specimens and the use of a single mitochondrial gene to reconstruct the phylogeny of a taxon may limit the taxonomic value of the dataset. Yet, our dataset on *Accipiter* is the most exhaustive obtained so far, so we now use our results to—tentatively—suggest some future directions for taxonomic revisions within the genus. These directions are outlined below for each of the superspecies shown in Fig. 1.

1. The traditional superspecies [tachiro] consists of *A. castanilius* and members of the *A. tachiro* complex, and is limited to Africa. The superspecies is well supported by our phylogenetic analyses. Based on plumage characteristics and habitat preferences, Louette (2007a) recognized three paraspecies within *A. tachiro*, viz. a generally more cryptically (especially in the female) plumaged woodland taxon (*A. tachiro*), and a generally more colorful (especially in the female, which shows the masculine plumage type) forest taxon (*A. toussenelii*). The subspecies *A. unduliventer* is a geographically isolated, rather colorful form (Louette 2003) from the Ethiopian highlands that is intermediate in this respect and may be considered as a third paraspecies (Louette 2007b). The three taxa show similar sequence divergences and are best considered to be at the same taxonomic level. Given that the sequence divergence (and also the morphological divergence) between the three taxa is comparable to that of other *Accipiter* species (3.6 % for the mini barcode dataset; we could not obtain the entire barcode fragment for *A. unduliventer*) and above the BCTh of 3 %, they could be considered different species (Jordaens et al., unpublished). *Accipiter castanilius* is a sister species to the *A. tachiro* complex. The taxa are also morphologically closely related, but *A. castanilius* is smaller (Wattel 1973; Thiollay 1994).
2. The traditional superspecies [cooperii] comprises four species, viz. *A. bicolor*, *A. cooperii*, *A. gundlachi*, and *A. chilensis*. This clade is also well supported in our phylogeny (although we have no molecular data for *A. chilensis*). *Accipiter cooperii* is widespread in North (N) and Central (C) America, whereas *A. bicolor* is widespread in South and Central America. The threatened species *Accipiter gundlachi* only occurs in Cuba, and is intermediate in plumage between *A. cooperii* and *A. bicolor*. At present, there is no indication of which of these two species is the ancestor of *A. gundlachi* (e.g., Reynard et al. 1987). Given that *A. gundlachi* and *A. cooperii* share the same haplotype, our data indicate that (1) the ancestral species of *A. gundlachi* is *A. cooperii*; (2) *A. gundlachi* and *A. cooperii* are one and the same species; or (3) the *A. gundlachi* specimen is a hybrid with *A. cooperii* as the mother. The first scenario is not unlikely, since *Accipiter cooperii* is migratory from North America to Central America, whereas there is no evidence of *A. bicolor* being migratory. Moreover, *A. cooperii* is sometimes seen in Cuba during migration (Rodríguez-Santana 2010). The data also suggest recent colonization of Cuba (see also Bildstein 2004) or old colonization with ongoig hybridization (Reynard et al. 1987). Hence, more data are needed to select the scenario which is the most likely.
3. The traditional superspecies [gentilis] with a nearly worldwide distribution comprises four species (*A. henstii*, *A. meyerianus*, *A. gentilis*, and *A. melanoleucus*), but we only have data for the latter two. Within *A. gentilis* we find two strongly diverged haplotypes that correspond to the subspecies *A. g. atricapillus* from N America and W Mexico and to *A. g. gentilis* that occurs in Europe, Asia, and extreme NW Africa. Such strong divergence has already been noted by Johnsen et al. (2010) and Cai et al. (2010). *Accipiter melanoleucus* has an Afrotropical distribution, and adults have very different plumage from *A. gentilis* (Wattel 1973). The three taxa show comparable K2P sequence divergences (2.6 %), and this may warrant assigning them an equal taxonomic rank.
4. Even though the taxa of the traditional Old World superspecies [badius] (i.e., *A. badius*, *A. brevipes*, *A. soloensis*, *A. francesiae*, and *A. butleri*) clustered together in our phylogenetic analyses (we have no molecular data for *A. butleri*), a close relationship within this clade is not supported. Rather, we observed a strong support for two separate clades. One clade comprises *A. francesiae* and *A. soloensis*, while the other clade comprises *A. badius* and *A. brevipes*. *Accipiter soloensis* from Asia is the closest relative of *A. francesiae* that occurs in Madagascar and on the Comoro islands. It is thus possible that (the ancestor of) this *Accipiter* made an overshoot migration to Madagascar in the distant past (Thiollay 1994; Ferguson-Lees and Christie 2001), and may have settled in the Malagasy region after losing its migratory behavior (Louette et al. 2011; see also Louette and Herremans 1985). A comparable situation is found in the genus *Buteo*, where *B. brachypterus* occurs in Madagascar whereas its closest relatives, viz. *B. vulpinus* and *B. japonicus*, have a Palearctic distribution (Kruckenhauser et al. 2004). *Accipiter brevipes* and *A. badius* were formerly considered conspecific by some authors, but the majority considered both to be distinct species (Vaurie 1961 and references therein). Ananian et al. (2010) have shown that both species breed sympatrically in Armenia, and Yosef et al. (2001) showed that they even occasionally hybridize. A close relationship between *A. brevipes*, which breeds in SE Europe and SW Asia, and which winters in northern sub-Saharan Africa, and the two African subspecies of *A. badius* (viz. *polyzonoides* and *sphenurus*) is also apparent from our data. The three taxa show similar sequence divergences and hence it would be best to give them the same taxonomic rank. This superspecies merits further taxonomic revision, since it was previously also suggested that *A. brevipes* was closely related to *A. soloensis* (Thiollay 1994), which is contradicted by

our data. Such revision should include the SE Asian subspecies of *A. badius* (viz. *dussumieri*, *badius*, and *poliopsis*).

5. The two smallest African accipiters, *A. minullus* and *A. erythropus*, are traditionally grouped into the superspecies [minullus], and are sometimes considered conspecific (e.g., Wattel 1973; Snow 1978; see also Louette 2002). Morphologically, they share a unique pattern of a white rump and broken white upper tail-bars, but *A. minullus* is less rufous than *A. erythropus*. The K2P sequence divergence between both species was 5.1 %, and is in the range of what is observed between other *Accipiter* species (i.e., above the 10× and BC thresholds). Hence, both species seem well differentiated but closely related, and may still hybridize (Louette 2002).
6. The traditional superspecies [nisus] is a complex group of *Accipiter* species with a worldwide distribution. *Accipiter nisus* is sometimes considered to be conspecific with *A. rufiventris*. This combination was included in a superspecies with *A. madagascariensis*, *A. ovampensis*, and perhaps the species of the *A. striatus* complex (Thiollay 1994). *Accipiter striatus* itself forms a superspecies with *A. chionogaster*, *A. ventralis*, and *A. erythronemius*. Our results support neither the monophyly of the superspecies [nisus] nor that of the superspecies [striatus]. Rather, *A. ovampensis* and *A. madagascariensis* are a well-diverged clade from *A. nisus*, whereas *A. nisus* forms a clade with *A. striatus* and *A. erythronemius*. Moreover, the single haplotype of *A. rufiventris* is the same as one of the haplotypes of *A. nisus*, so that its status as a separate species can be strongly questioned.
7. The Asian *Accipiter virgatus* has been considered to include *A. gularis*, with which it forms the superspecies [gularis]. The full species status of the latter is now generally agreed upon (Thiollay 1994 and references therein). Yet, the single haplotype of *A. gularis* studied by us is the same as one of the haplotypes of *A. virgatus*. This would suggest that both taxa are either the same species, or that the split between *A. gularis* and *A. virgatus* is a very recent one. However, both *A. virgatus* sequences were retrieved from BOLD and originate from specimens collected in Mongolia, which is outside the distribution range of the species (Thiollay 1994). Hence, both specimens could have been mistaken for the closely related *A. gularis* which is common in that region. If this is true, then our data suggest that both species would probably be identifiable using DNA barcodes.

   Interestingly, this clade also contains the Australian *A. fasciatus*, which is believed to be unrelated to the superspecies [gularis]. The sequence of *A. fasciatus* was retrieved from BOLD and derived from DNA extracted from the skull of a juvenile. Hence, the identification of this *A. fasciatus* specimen is at least doubtful. Indeed, we recently received material from the small North Australian *A. cirrhocephalus*, and preliminary sequence data suggest that the *A. fasciatus* specimen is very similar to *A. cirrhocephalus*. *Accipiter cirrhocephalus* is also strikingly similar in the morphology of the toes, claws, wings, and tail to members of the superspecies [gularis] (Wattel 1973). Thus, the juvenile *A. fasciatus* most likely represents an (adult) *A. cirrhocephalus* specimen.
8. Finally, the Asian *Accipiter trivirgatus* is well diverged from all others in our phylogenetic analysis, and its status as a separate entity seems justified. Its only close relative seems to be *A. griceiceps* (for which we do not have molecular data) (Mayr 1949).

In conclusion, although not all traditional (super)species were included, our study shows that the standardized DNA barcoding approach is effective for identifying most *Accipiter* species that have been studied so far. Further, this approach also highlights taxa for taxonomic review because some species show exceptionally large intraspecific divergences or because some species pairs show identical DNA barcodes. The results of the phylogenetic analyses performed in the current study are largely congruent with the current morphology-based taxonomy. However, additional nuclear and/or mitochondrial markers will be necessary to resolve deeper phylogenetic relationships within the genus.

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Appendix 1

See Table 2.

Table 2 List of specimens and GenBank and BOLD accession numbers used in this study

Species name	Voucher code/field collection code	Sequence length (bp)	GenBank/ BOLD	Locality	Latitude/longitude	Collection year
Accipiter badius polyzonoides	RBINS15866	298	JF312106	DR Congo		1939 (T)
Accipiter badius polyzonoides	RBINS20641	298	JF312108	DR Congo		1940 (T)
Accipiter badius polyzonoides	RMCA125526	298	JF312136	DR Congo (Tanganyika region)		1971 (T)
Accipiter badius polyzonoides	RMCA101060	298	JF312119	DR Congo (Kwango region)	05° 41′00″S/19° 17′00″E[a]	1959 (T)
Accipiter badius sphenurus	LIV57107466	298	JF312105	Tanzania (16 km S of Same)	04°13′80″S/ 37°45′00″E	1950 (T)
Accipiter badius sphenurus	RMCA113719	298	JF312090	DR Congo (Ubangi region)	03°45′00″N/19° 3′ 0″E[a]	1965 (T)
Accipiter badius sphenurus	RMCA95922	298	JF312159	DR Congo (Ubangi region)	3° 30′ 0″N/20° 31′ 0″E[a]	1958 (T)
Accipiter bicolor			FJ027015	Argentina (Corrientes)		
Accipiter bicolor			FJ027014	Argentina (Corrientes)		
Accipiter brevipes	RMCAB110A3	694	JF312109	Israel	Bird migrating	2000 (B)
Accipiter brevipes	RMCA77865	298	JF312152	DR Congo (District Gangala-na-Bodio)	3°42′34.20″N/ 29°17′14.20″E[a]	1955 (T)
Accipiter brevipes	RMCAB110A4	694	JF312192	Israel	Bird migrating	2000 (B)
Accipiter castanilius	RMCA118890	298	JF312132	DR Congo (Kivu)	02°46′59″S/ 28°25′00.00″E[a]	1969 (T)
Accipiter castanilius	RMCA7622A41	298	JF312150	DR Congo (Kivu)	03°07′59″S/ 28°18′00.00″E[a]	1975 (T)
Accipiter cooperii			AY666285	Canada (Ontario)		
Accipiter erythronemius			FJ027018	Argentina (Corrientes)		
Accipiter erythronemius			FJ027017	Argentina (Corrientes)		
Accipiter erythropus zenkeri	RMCA7731A3	298	JF312092	DR Congo (Bas-Congo, Ngongo)	05°30′00.00″S/ 14°41′00.00″E[a]	1977 (T)
Accipiter erythropus zenkeri	RBINS20640	298	JF312107	DR Congo		1974 (T)
Accipiter fasciatus			ROM157413	Australia		
Accipiter francesiae brutus	RMCAA817A1	694	JF312162	Mayotte (Comoros)	12°49′30″S/ 45°08′00″E	1995 (F)
Accipiter francesiae brutus	RMCAA817A10	694	JF312163	Mayotte (Comoros)	12°49′30″S/ 45°08′00″E	1995 (F)
Accipiter francesiae brutus	RMCAA817A11	694	JF312164	Mayotte (Comoros)	12°49′30″S/ 45°08′00″E	1995 (F)
Accipiter francesiae brutus	RMCAA817A13	694	JF312165	Mayotte (Comoros)	12°49′30″S/ 45°08′00″E	1995 (F)
Accipiter francesiae brutus	RMCAA817A14	694	JF312166	Mayotte (Comoros)	12°49′30″S/ 45°08′00″E	1995 (F)
Accipiter francesiae brutus	RMCAA817A15	694	JF312167	Mayotte (Comoros)	12°49′30″S/ 45°08′00″E	1995 (F)
Accipiter francesiae brutus	RMCAA817A16	694	JF312168	Mayotte (Comoros)	12°49′30″S/ 45°08′00″E	1995 (F)
Accipiter francesiae brutus	RMCAA817A17	588	JF312169	Mayotte (Comoros)	12°49′30″S/ 45°08′00″E	1995 (F)
Accipiter francesiae brutus	RMCAA817A18	694	JF312170	Mayotte (Comoros)	12°49′30″S/ 45°08′00″E	1995 (F)

Table 2 continued

Species name	Voucher code/field collection code	Sequence length (bp)	GenBank/ BOLD	Locality	Latitude/longitude	Collection year
Accipiter francesiae brutus	RMCAA817A19	694	JF312171	Mayotte (Comoros)	12°49′30″S/ 45°08′00″E	1995 (F)
Accipiter francesiae brutus	RMCAA817A2	694	JF312172	Mayotte (Comoros)	12°49′30″S/ 45°08′00″E	1995 (F)
Accipiter francesiae brutus	RMCAA817A20	694	JF312173	Mayotte (Comoros)	12°49′30″S/ 45°08′00″E	1995 (F)
Accipiter francesiae brutus	RMCAA817A3	694	JF312174	Mayotte (Comoros)	12°49′30″S/ 45°08′00″E	1995 (F)
Accipiter francesiae brutus	RMCAA817A5	585	JF312175	Mayotte (Comoros)	12°49′30″S/ 45°08′00″E	1995 (F)
Accipiter francesiae brutus	RMCAA817A7	694	JF312177	Mayotte (Comoros)	12°49′30″S/ 45°08′00″E	1995 (F)
Accipiter francesiae brutus	RMCAA817A8	694	JF312178	Mayotte (Comoros)	12°49′30″S/ 45°08′00″E	1995 (F)
Accipiter francesiae brutus	RMCAA817A9	572	JF312179	Mayotte (Comoros)	12°49′30″S/ 45°08′00″E	1995 (F)
Accipiter francesiae francesiae	RMCAA90380001	697	JF792344	Madagascar	14°19′42″S/ 48°34′54″E	2009 (B)
Accipiter francesiae francesiae	RMCAA90380002	697	JF792345	Madagascar	15°28′42″S/ 48°28′00″E	2009 (B)
Accipiter francesiae francesiae	RMCAA90380003	697	JF792346	Madagascar	15°28′42″S/ 48°28′00″E	2009 (B)
Accipiter francesiae francesiae	RMCAA90380004	697	JF792347	Madagascar	15°59′00″S/ 47°56′18″E	2009 (B)
Accipiter francesiae francesiae	RMCAA90380005	697	JF792348	Madagascar	15°37′48″S/ 49°58′30″E	2009 (B)
Accipiter francesiae francesiae	RMCAA90380006	697	JF792349	Madagascar	18°15′06″S/ 49°17′54″E	2009 (B)
Accipiter francesiae francesiae	RMCAA90380007	697	JF792350	Madagascar	18°13′24″S/ 49°18′48″E	2009 (B)
Accipiter francesiae francesiae	RBINS35416	694	JF312086	Madagascar	18°55′S/47°33′E[a]	1930 (T)
Accipiter francesiae francesiae	RBINS35415	694	JF312104	Madagascar	18°55′S/47°33′E[a]	1930 (T)
Accipiter francesiae griveaudi	RMCA8343A199	694	JF312093	Grand Comoro (Comoros)	11°39′17″S/ 43°19′13″E	1983 (T)
Accipiter francesiae griveaudi	RMCAAcc006	694	JF312096	Grand Comoro (Comoros)	11°48′S/43°16′E	2000 (F)
Accipiter francesiae griveaudi	RMCAAcc007	694	JF312097	Grand Comoro (Comoros)	11°48′S/43°16′E	2000 (F)
Accipiter francesiae griveaudi	RMCAAcc008	654	JF312098	Grand Comoro (Comoros)	11°48′S/43°16′E	2000 (F)
Accipiter francesiae griveaudi	RMCAAcc010	694	JF312099	Grand Comoro (Comoros)	11°48′S/43°16′E	2000 (F)
Accipiter francesiae griveaudi	RMCAAccfran006	694	JF312100	Grand Comoro (Comoros)	11°48′S/43°16′E	2000 (F)
Accipiter francesiae griveaudi	RMCAAccfran007	694	JF312101	Grand Comoro (Comoros)	11°48′S/43°16′E	2000 (F)
Accipiter francesiae griveaudi	RMCAAccfran008	654	JF312102	Grand Comoro (Comoros)	11°48′S/43°16′E	2000 (F)
Accipiter francesiae griveaudi	RMCAAccfran009	694	JF312103	Grand Comoro (Comoros)	11°48′S/43°16′E	2000 (F)
Accipiter francesiae griveaudi	RMCAAcc011	694	JF312189	Grand Comoro (Comoros)	11°48′S/43°16′E	2000 (F)

Table 2 continued

Species name	Voucher code/field collection code	Sequence length (bp)	GenBank/ BOLD	Locality	Latitude/longitude	Collection year
Accipiter francesiae pusillus	RMCA8343A358	694	JF312094	Anjouan (Comoros)	12°12′49″S/ 44°26′13″E	1983 (T)
Accipiter francesiae pusillus	A9023A2/LI1941/662	298	JF312095	Anjouan (Comoros)	12°12′49″S/ 44°26′13″E	1941 (T)
Accipiter gentilis	RMCAA9037A0002	694	JF792342	South Korea (Gwanmaedo island)	34°41′20.88″N/ 125°12′15.77″E	2009 (F)
Accipiter gentilis arrigonii	RBINS11135	298	JF312084	Italy (Sardinia)	40° 7′15.15″N/9° 0′46.41″E	1931 (T)
Accipiter gentilis atricapillus			DQ433279	Canada (Yukon)		
Accipiter gentilis atricapillus			AY666498	North America		
Accipiter gentilis atricapillus		636	DQ433276	Canada (Yukon)		
Accipiter gentilis gentilis		699	GQ922622	China		
Accipiter gentilis gentilis		699	GQ922623	China		
Accipiter gentilis gentilis		699	GQ922624	China		
Accipiter gentilis gentilis		699	GQ922625	China		
Accipiter gentilis gentilis		721	GU571207	Norway (Åkershus)		
Accipiter gentilis gentilis		730	GU571208	Norway (Åkershus)		
Accipiter gentilis gentilis		648	GU571687	Sweden (Jamtland)		
Accipiter gentilis gentilis		648	GU571688	Sweden (Jamtland)		
Accipiter gentilis gentilis	RMCAJEMU17042006	694	JF312195	Belgium (Tervuren)	50°49′51.17″N/ 04°31′06.34″E	2006 (F)
Accipiter gularis	RMCAA9037A0003	694	JF792343	South Korea (Gwanmaedo Island)	34°14′17.03″N/ 126° 3′16.66″E	2009 (F)
Accipiter gularis	RMCAA9037A0001	694	JF792341	South Korea (Gwanmaedo Island)	34°41′20.88″N/ 125°12′15.77″E	2009 (F)
Accipiter gundlachi	RMCABO005A0001	694	JF792337	Cuba (Camagüey)	21°23′57.00″N/ 77°54′29.01″W	2010 (F)
Accipiter madagascariensis	JEMU23	298	JF792339	Madagascar		? (B)
Accipiter melanoleucus	RMCA7636A1	298	JF312091	DR Congo (Boende)	00°46′33.63″S/21° 8′3.34″E	1976 (T)
Accipiter melanoleucus	RMCAA9011A0001b	694	JF312188	DR Congo (Kizunu Byama)	02°31′49.44″S/ 28°50′59.64″E	2009 (F)
Accipiter minullus	RBINS63545	298	JF312116	Burundi (Bujumbura)	3°23′S/29°18′E	1980 (T)
Accipiter minullus	RMCA109210	298	JF312123	Ethiopia (Iowaka)	10°15′N/39°45′E	1945 (T)
Accipiter minullus	RMCA119482	298	JF312133	DR Congo (Katanga)	11°33′36″S/27° 28′ 12″E[a]	1969 (T)
Accipiter minullus	RMCA1500	298	JF312137	DR Congo (Baraka)	04°05′34.60″S/ 29°05′09.18″E	1910 (T)
Accipiter minullus	RMCA69605	298	JF312147	Rwanda (Manyaga)	1°56′00″S/ 30°03′00″E	1953 (T)
Accipiter minullus	RMCA88394	694	JF312158	DR Congo (Kitega)	3°25′48″S/29°57′E[a]	1957 (T)

Table 2 continued

Species name	Voucher code/field collection code	Sequence length (bp)	GenBank/ BOLD	Locality	Latitude/longitude	Collection year
Accipiter minullus	UCZM138894	694	JF312207	Tanzania (Ndundulu)	7°48′0.00″S/ 36°30′0.00″E	2007 (B)
Accipiter minullus	UCZM140336	665	JF312212	Tanzania (Ndundulu)	7°48′0.00″S/ 36°30′0.00″E	2007 (B)
Accipiter minullus	UCZM140524	672	JF312213	Tanzania (Ndundulu)	7°48′0.00″S/ 36°30′0.00″E	2007 (B)
Accipiter nisus	RMCAB110A1	694	JF312190	Israel	Bird migrating	2000 (B)
Accipiter nisus	RMCAB110A2	694	JF312191	Israel	Bird migrating	2000 (B)
Accipiter nisus nisus	RMCAJEMU042004	694	JF312194	Belgium (Tervuren)	50°49′51.17″N/ 04°31′06.34″E	2004 (F)
Accipiter nisus nisus			GQ481251	Russia (Magadanskaya)		
Accipiter nisus nisus			GQ481250	Mongolia (Aymag)		
Accipiter nisus nisus			GQ481249	Russia (Arkhipo Osipovka)		
Accipiter nisus nisus			GQ481248	Russia (Ozero)		
Accipiter nisus nisus			GQ481247	Russia (Melkovodnoe)		
Accipiter nisus wolterstorffi	RBINS11136	298	JF312085	Italy (Sardinia)	40° 7′15.15″N/9° 0′46.41″E	1930 (T)
Accipiter nisus wolterstorfii	RMCAA20090104	677	JF312161	Italy (Sardinia, Santa Guistia)	39°48′15.36″N/ 08°34′22.45″E	2009 (F)
Accipiter ovampensis	RMCA104539	298	JF312121	DR Congo (Mt Mukuene)	11°40′12.00″S/ 27°29′09.35″E	1959 (T)
Accipiter ovampensis	RMCA106223	298	JF312122	DR Congo (Musoko Kandele)	05°05′16,98″S/ 16°31′08.74″E	1960 (T)
Accipiter rufiventris	RMCA115655	298	JF312131	DR Congo (Memba, Kivu prov.)	10°37′56.75″S/ 27°08′01.65″E	1967 (T)
Accipiter rufiventris	RMCA46470	298	JF312144	Rwanda (near Kisenyi)	0°15′30.24″S/ 29°53′19.33″E	1949 (T)
Accipiter rufiventris rufiventris	RBINS53865	298	JF312112	South Africa	29°00′S/24°00′E[a]	1972 (T)
Accipiter soloensis	RMCAA90082	694	JF312182	South Korea	34°41′47.66″N/ 125°11′54.10″E	2008 (F)
Accipiter soloensis	RMCAA90084	694	JF312183	South Korea	34°41′47.66″N/ 125°11′54.10″E	2007 (F)
Accipiter soloensis	RMCAA90085	694	JF312184	South Korea	37°26′42.37″N/ 127°16′34.39″E	2008 (F)
Accipiter striatus			DQ434244	Canada (Ontario)		
Accipiter striatus			DQ434243	Canada (Ontario)		
Accipiter striatus			DQ433281	Canada (Ontario)		
Accipiter striatus			DQ433280	Canada (Ontario)		
Accipiter tachiro pembaensis	RMCAA84A1	694	JF312180	Tanzania (Pemba Island)	05°01′50″S/ 39°46′20″E	2008 (F)
Accipiter tachiro sparsimfasciatus	RMCA7444A157	298	JF312140	DR Congo (Kivu)	02°20′00″S/ 28°47′00″E[a]	1943 (T)
Accipiter tachiro sparsimfasciatus	RMCAA20081213	694	JF312142	Uganda (Kampala)	00°18′51.37″N/ 32°34′22.34″E	2008 (F)
Accipiter tachiro sparsimfasciatus	RMCA8972	298	JF792340	Kenya (Londiani)	00°10′0.00″S/ 35°36′0.00″E	1914 (T)
Accipiter tachiro sparsimfasciatus	RMCA125524	298	JF312135	DR Congo (Katanga)	07°09′00″S/ 29°37′00″E[a]	1971 (T)
Accipiter tachiro sparsimfasciatus	RMCA118800	298	JF312138	Kenya	04°13′00″S/ 39°25′00″E[a]	1965 (T)
Accipiter tachiro sparsimfasciatus	RMCA8004A01	298	JF312153	DR Congo (Bikara, Kivu)	00°15′26.00″S/ 29°11′48.00E	1980 (T)

Table 2 continued

Species name	Voucher code/field collection code	Sequence length (bp)	GenBank/ BOLD	Locality	Latitude/longitude	Collection year
Accipiter tachiro sparsimfasciatus	RMCA8042A01	655	JF312156	DR Congo (Kasai, Tshibungu)	05°37′34.00″S/ 22°15′57.00″E	1980 (T)
Accipiter tachiro sparsimfasciatus	RMCA99562	298	JF312160	DR Congo (Katanga)	10°21′00″S/ 23°29′00″E[a]	1958 (T)
Accipiter tachiro sparsimfasciatus	UCZM132997	694	JF312199	Tanzania (Morogoro, Nguru South For. Res.)	06°09′00.00″S/ 37°29′00.00″E	2003 (B)
Accipiter tachiro sparsimfasciatus	UCZM134376	694	JF312200	Tanzania (Morogoro, Mvoro, Udaha Camp)	10°40′00.00″S/ 35°05′00.00″E	2003 (B)
Accipiter tachiro sparsimfasciatus	UCZM135334	694	JF312201	Tanzania (Kigoma)	07°12′00.00″S/ 37°08′00.00″E	2004 (B)
Accipiter tachiro sparsimfasciatus	UCZM136464	658	JF312202	Tanzania (Morogoro, Kilombero West For.)	07°53′53.00″S/ 36°30′00.00″E	1995 (B)
Accipiter tachiro sparsimfasciatus	UCZM136478	694	JF312203	Tanzania (Nambiga Forest, Mahenge)	08°40′57.20″S/ 36°43′00.91″E	1995 (B)
Accipiter tachiro sparsimfasciatus	UCZM137485	694	JF312204	Tanzania (Morogoro, Nguru South For. Res.)	06°09′00.00″S/ 37°29′00.00″E	2005 (B)
Accipiter tachiro sparsimfasciatus	UCZM137590	694	JF312205	Tanzania (Mt. Wara)	05°35′00.00″S/ 37°35′00.00″E	2005 (B)
Accipiter tachiro sparsimfasciatus	UCZM139158	683	JF312208	Tanzania (Udzungwa Mts., Ndundulu)	07°48′00.00″S/ 36°30′00.00″E	2007 (B)
Accipiter tachiro sparsimfasciatus	UCZM139220	674	JF312209	Tanzania (Udzungwa Mts., Ndundulu)	07°48′00.00″S/ 36°30′00.00″E	2007 (B)
Accipiter tachiro sparsimfasciatus	UCZM139239	668	JF312210	Tanzania (Udzungwa Mts., Ndundulu)	07°48′00.00″S/ 36°30′00.00″E	2007 (B)
Accipiter tachiro sparsimfasciatus	UCZM140577	664	JF312214	Tanzania (Rukwa)	07°58′1.6″S/ 31°37′1.0″E	2007 (B)
Accipiter tachiro tachiro	RBINS4948[a]	298	JF792338	South Africa (Cape of good hope)	34°21′00″S/ 18°28′E	1852 (T)
Accipiter tachiro/ toussenelii hybrid	RMCA64432	298	JF312141	DR Congo (Uele Ibembo)	02°38′.00″N/ 23°37′00″E	1952 (T)
Accipiter toussenelii canescens	RMCA114514	298	JF312128	DR Congo (Kivu)	02°16′00″S/ 28°03′00″E[a]	1966 (T)
Accipiter toussenelii canescens	RMCA115134	694	JF312130	DR Congo (Ubangi)	02°57′00″N/ 19°25′00″E[a]	1966 (T)
Accipiter toussenelii canescens	RMCA65704	694	JF312146	DR Congo (Uele)	00°27′00″N/ 27°17′00″E[a]	1953 (T)
Accipiter toussenelii canescens	RMCA70932	694	JF312148	DR Congo (Ituri)	00°55′00″N/ 28°39′00″E[a]	1954 (T)
Accipiter toussenelii canescens	RMCA80988	298	JF312157	DR Congo (Equator)	00°41′00″N/ 19°57′00″E[a]	1954 (T)
Accipiter toussenelii lopezi	RMCAA90220002	298	JF312082	Equatorial Guinea (Fernando Poo Island)	3°30′0.00N/ 8°42′0.00″E	2009 (F)
Accipiter toussenelii lopezi	RMCAA90220001	298	JF312083	Equatorial Guinea (Fernando Poo Island)	3°30′0.00N/ 8°42′0.00″E	2009 (F)
Accipiter toussenelii macroscelides	RMCA8036A153	298	JF312154	Liberia	06°45′00.00″N/ 08°45′00.00″W[a]	1980 (T)
Accipiter toussenelii macroscelides	RMCA8036A204	694	JF312155	Liberia	06°45′00.00″N/ 08°45′00.00″W[a]	1980 (T)
Accipiter toussenelii toussenelii	RMCA60351	694	JF312145	DR Congo (Bas-Congo, Banana)	06°00′00″S/ 12°24′00″E	1952 (T)
Accipiter trivirgatus			CMCPB058-10	Philippines		
Accipiter unduliventer	RMCA109246	298	JF312124	Ethiopia (Djem Djem Forest)	09°02′12.30″N/ 38°13′2.06E	1946 (T)

Table 2 continued

Species name	Voucher code/field collection code	Sequence length (bp)	GenBank/ BOLD	Locality	Latitude/longitude	Collection year
Accipiter unduliventer	RMCA109472	298	JF312125	Ethiopia (Djem Djem Forest)	09°02′12.30″N/ 38°13′2.06E	1946 (T)
Accipiter unduliventer	RMCA109473	298	JF312126	Ethiopia (Djem Djem Forest)	09°02′12.30″N/ 38°13′2.06E	1946 (T)
Accipiter virgatus	RMCAA9009A0001	694	JF312186	Taiwan (Taipei)	24°54′56.56″N/ 121°40′26.17″E	2008 (F)
Accipiter virgatus			GQ481253	Mongolia (Aymag)		
Accipiter virgatus			GQ481252	Mongolia (Aymag)		
Buteo buteo vulpinus			GQ481408	Russia (Romanova Kaliningradskaya)		

HT 291/647 refers to the haplotype in the analysis of the 291 bp or the 647 bp dataset, respectively. Acronyms: *RMCA* Royal Museum for Central Africa, *RBINS* Royal Belgian Institute of Natural Sciences, *UCZM* University of Copenhagen Zoological Museum, *LIV* World Museum Liverpool, *LI* Ober-Österreichische Landesmuseen. Codes that are *underlined* refer to samples that are not vouchered in the museums. Since several locality names were approximations, longitude and latitude data were estimated in some cases

[a] Denotes these estimates. The tissue source for the sequences is indicated with (B) for bloodsamples, (F) for a feather, and (T) for a toepad

Appendix 2

See Table 3.

Table 3 Mean intra- (diagonal and in bold) and interspecific pairwise K2P distances among 25 *Accipiter* species of (a) dataset A, and among 18 species of (b) dataset B and (c) dataset C

	Tous	*fran*	*badi*	*er_p*	*ovam*	*cast*	*undu*	*tach*	*minu*	*brev*	*solo*	*er_m*	*bico*
(a)													
A. tousseneli (tous)	**0.007**												
A. francesiae (fran)	0.115	**0.003**											
A. badius (badi)	0.118	0.097	**0.017**										
A. erythropus (er_p)	0.087	0.1	0.102	**0**									
A. ovampensis (ovam)	0.096	0.126	0.129	0.088	**0**								
A. castanilius (cast)	0.054	0.124	0.114	0.092	0.109	**0**							
A. unduliventer (undu)	0.033	0.13	0.128	0.096	0.1	0.038	**0**						
A. tachiro (tach)	0.046	0.139	0.128	0.099	0.116	0.062	0.03	**0.009**					
A. minullus (minu)	0.085	0.096	0.098	0.051	0.097	0.101	0.105	0.099	**0.002**				
A. brevipes (brev)	0.123	0.114	0.036	0.093	0.11	0.128	0.137	0.14	0.097	**0**			
A. soloensis (solo)	0.099	0.045	0.099	0.125	0.128	0.123	0.114	0.113	0.111	0.12	**0**		
A. erytrhonemius (er_m)	0.127	0.147	0.115	0.109	0.105	0.136	0.136	0.129	0.12	0.115	0.142	**0**	
A. bicolor (bico)	0.108	0.099	0.109	0.071	0.071	0.114	0.114	0.122	0.088	0.105	0.119	0.118	**0**
A. striatus (stri)	0.127	0.148	0.12	0.101	0.083	0.127	0.127	0.13	0.119	0.115	0.143	0.03	0.105
A. gentilis (gent)	0.114	0.119	0.109	0.093	0.096	0.136	0.122	0.131	0.106	0.096	0.118	0.092	0.065
A. cooperi (coop)	0.108	0.095	0.118	0.079	0.071	0.123	0.114	0.122	0.106	0.105	0.115	0.123	0.015
A. gularis (gula)	0.096	0.094	0.08	0.059	0.083	0.088	0.105	0.108	0.076	0.085	0.098	0.124	0.092
A. virgatus (virg)	0.094	0.092	0.079	0.059	0.086	0.089	0.104	0.106	0.076	0.083	0.096	0.122	0.092
A. rufiventris (rufi)	0.132	0.148	0.119	0.105	0.1	0.132	0.131	0.125	0.123	0.115	0.133	0.038	0.101
A. nisus (nisu)	0.132	0.148	0.119	0.105	0.1	0.132	0.131	0.125	0.123	0.115	0.133	0.038	0.101

Table 3 continued

	Tous	*fran*	*badi*	*er_p*	*ovam*	*cast*	*undu*	*tach*	*minu*	*brev*	*solo*	*er_m*	*bico*
A. fasciatus (fasc)	0.091	0.098	0.076	0.059	0.088	0.083	0.101	0.101	0.076	0.072	0.102	0.115	0.088
A. madagscariensis (mada)	0.114	0.121	0.144	0.079	0.05	0.118	0.113	0.125	0.101	0.133	0.133	0.114	0.08
A. gundlachi (gund)	0.108	0.095	0.118	0.079	0.071	0.123	0.114	0.122	0.106	0.105	0.115	0.123	0.015
A. trivirgatus (triv)	0.151	0.137	0.134	0.133	0.1	0.141	0.15	0.161	0.138	0.119	0.144	0.123	0.096
A. melanoleucus (mela)	0.114	0.127	0.122	0.088	0.088	0.128	0.114	0.124	0.105	0.105	0.119	0.1	0.059

	stri	*gent*	*coop*	*gula*	*virg*	*rufi*	*nisu*	*fasc*	*mada*	*gund*	*triv*	*mela*
(a)												
A. tousseneli (tous)												
A. francesiae (fran)												
A. badius (badi)												
A. erythropus (er_p)												
A. ovampensis (ovam)												
A. castanilius (cast)												
A. unduliventer (undu)												
A. tachiro (tach)												
A. minullus (minu)												
A. brevipes (brev)												
A. soloensis (solo)												
A. erytrhonemius (er_m)												
A. bicolor (bico)												
A. striatus (stri)	**0**											
A. gentilis (gent)	0.084	**0.011**										
A. cooperi (coop)	0.11	0.065	–									
A. gularis (gula)	0.106	0.115	0.101	**0**								
A. virgatus (virg)	0.108	0.114	0.101	0.004	**0.008**							
A. rufiventris (rufi)	0.038	0.086	0.105	0.124	0.123	**0**						
A. nisus (nisu)	0.038	0.086	0.105	0.124	0.123	0	**0**					
A. fasciatus (fasc)	0.106	0.106	0.097	0.019	0.018	0.115	0.115	–				
A. madagscariensis (mada)	0.101	0.107	0.088	0.097	0.097	0.118	0.118	0.092	–			
A. gundlachi (gund)	0.11	0.065	0	0.101	0.101	0.105	0.105	0.097	0.088	–		
A. trivirgatus (triv)	0.11	0.115	0.101	0.134	0.132	0.119	0.119	0.124	0.109	0.101	–	
A. melanoleucus (mela)	0.097	0.029	0.059	0.106	0.104	0.092	0.092	0.097	0.105	0.059	0.11	**0**

	fran	*brev*	*tous*	*tach*	*minu*	*solo*	*virg*	*mela*	*nisu*
(b)									
A. francesiae (fran)	**0.006**								
A. brevipes (brev)	0.1	**0**							
A. toussenelii (tous)	0.127	0.119	**0.006**						
A. tachiro (tach)	0.125	0.123	0.045	**0.004**					
A. minullus (minu)	0.096	0.08	0.096	0.099	**0.003**				
A. soloensis (solo)	0.056	0.096	0.114	0.108	0.098	**0**			
A. virgatus (virg)	0.094	0.09	0.113	0.114	0.076	0.096	**0.009**		
A. melanoleucus (mela)	0.13	0.116	0.116	0.116	0.101	0.116	0.119	–	
A. nisus (nisu)	0.154	0.136	0.119	0.11	0.126	0.144	0.143	0.108	**0.001**
A. gentilis (gent)	0.121	0.111	0.115	0.117	0.105	0.119	0.122	0.027	0.108
A. erythronemius (er m)	0.15	0.134	0.127	0.121	0.115	0.144	0.145	0.113	0.045
A. bicolor (bico)	0.107	0.107	0.11	0.116	0.087	0.115	0.098	0.079	0.106
A. striatus (stri)	0.144	0.134	0.133	0.121	0.118	0.142	0.141	0.106	0.044

Table 3 continued

	fran	*brev*	*tous*	*tach*	*minu*	*solo*	*virg*	*mela*	*nisu*
A. cooperii (coop)	0.12	0.113	0.109	0.114	0.1	0.121	0.108	0.079	0.109
A. gularis (gula)	0.095	0.092	0.115	0.117	0.078	0.096	0.004	0.12	0.146
A. fasciatus (fasc)	0.097	0.086	0.117	0.11	0.074	0.104	0.028	0.112	0.142
A. gundlachi (gund)	0.12	0.113	0.109	0.114	0.1	0.121	0.108	0.079	0.109
A. trivirgatus (triv)	0.132	0.122	0.138	0.129	0.125	0.131	0.139	0.118	0.142

	gent	*er_m*	*bico*	*stri*	*coop*	*gula*	*fasc*	*gund*	*triv*
(b)									
A. francesiae (fran)									
A. brevipes (brev)									
A. toussenelii (tous)									
A. tachiro (tach)									
A. minullus (minu)									
A. soloensis (solo)									
A. virgatus (virg)									
A. melanoleucus (mela)									
A. nisus (nisu)									
A. gentilis (gent)	**0.008**								
A. erythronemius (er m)	0.108	**0.002**							
A. bicolor (bico)	0.073	0.113	**0**						
A. striatus (stri)	0.096	0.035	0.101	**0**					
A. cooperii (coop)	0.079	0.107	0.035	0.103	–				
A. gularis (gula)	0.124	0.148	0.099	0.142	0.109	**0**			
A. fasciatus (fasc)	0.117	0.14	0.095	0.14	0.109	0.029	–		
A. gundlachi (gund)	0.079	0.107	0.035	0.103	0	0.109	0.109	–	
A. trivirgatus (triv)	0.118	0.138	0.11	0.136	0.117	0.141	0.128	0.117	–

	fran	*brev*	*tous*	*tach*	*minu*	*solo*	*virg*	*mela*	*nisu*
(c)									
A. francesiae (fran)	**0.003**								
A. brevipes (brev)	0.116	**0**							
A. toussenelii (tous)	0.115	0.123	**0.004**						
A. tachiro (tach)	0.14	0.135	0.044	**0.007**					
A. minullus (minu)	0.095	0.099	0.09	0.101	**0.004**				
A. soloensis (solo)	0.044	0.123	0.1	0.115	0.11	**0**			
A. virgatus (virg)	0.09	0.086	0.097	0.106	0.074	0.094	**0.007**		
A. melanoleucus (mela)	0.124	0.108	0.113	0.124	0.103	0.117	0.102	–	
A. nisus (nisu)	0.15	0.112	0.131	0.12	0.125	0.135	0.125	0.094	**0**
A. gentilis (gent)	0.116	0.099	0.115	0.132	0.105	0.116	0.112	0.029	0.089
A. erythronemius (er_m)	0.149	0.113	0.126	0.124	0.122	0.144	0.124	0.103	0.038
A. bicolor (bico)	0.097	0.108	0.108	0.123	0.086	0.117	0.091	0.058	0.103
A. striatus (stri)	0.145	0.117	0.131	0.129	0.116	0.14	0.106	0.095	0.042
A. cooperii (coop)	0.097	0.103	0.104	0.119	0.108	0.117	0.104	0.062	0.103
A. gularis (gula)	0.092	0.087	0.098	0.108	0.074	0.096	0.004	0.104	0.126
A. fasciatus (fasc)	0.096	0.074	0.094	0.102	0.074	0.1	0.017	0.095	0.117
A. gundlachi (gund)	0.097	0.103	0.104	0.119	0.108	0.117	0.104	0.062	0.103
A. trivirgatus (triv)	0.138	0.117	0.15	0.155	0.139	0.146	0.134	0.112	0.116

Table 3 continued

	gent	*er_m*	*bico*	*stri*	*coop*	*gula*	*fasc*	*gund*	*triv*
(c)									
A. francesiae (fran)									
A. brevipes (brev)									
A. toussenelii (tous)									
A. tachiro (tach)									
A. minullus (minu)									
A. soloensis (solo)									
A. virgatus (virg)									
A. melanoleucus (mela)									
A. nisus (nisu)									
A. gentilis (gent)	**0.009**								
A. erythronemius (er_m)	0.094	**0**							
A. bicolor (bico)	0.065	0.121	**0**						
A. striatus (stri)	0.082	0.034	0.103	**0**					
A. cooperii (coop)	0.069	0.121	0.019	0.112	–				
A. gularis (gula)	0.114	0.126	0.091	0.104	0.104	**0**			
A. fasciatus (fasc)	0.105	0.117	0.086	0.104	0.099	0.019	–		
A. gundlachi (gund)	0.069	0.121	0.019	0.112	0	0.104	0.099	–	
A. trivirgatus (triv)	0.117	0.121	0.099	0.112	0.099	0.136	0.126	0.099	–

Appendix 3

See Fig. 6.

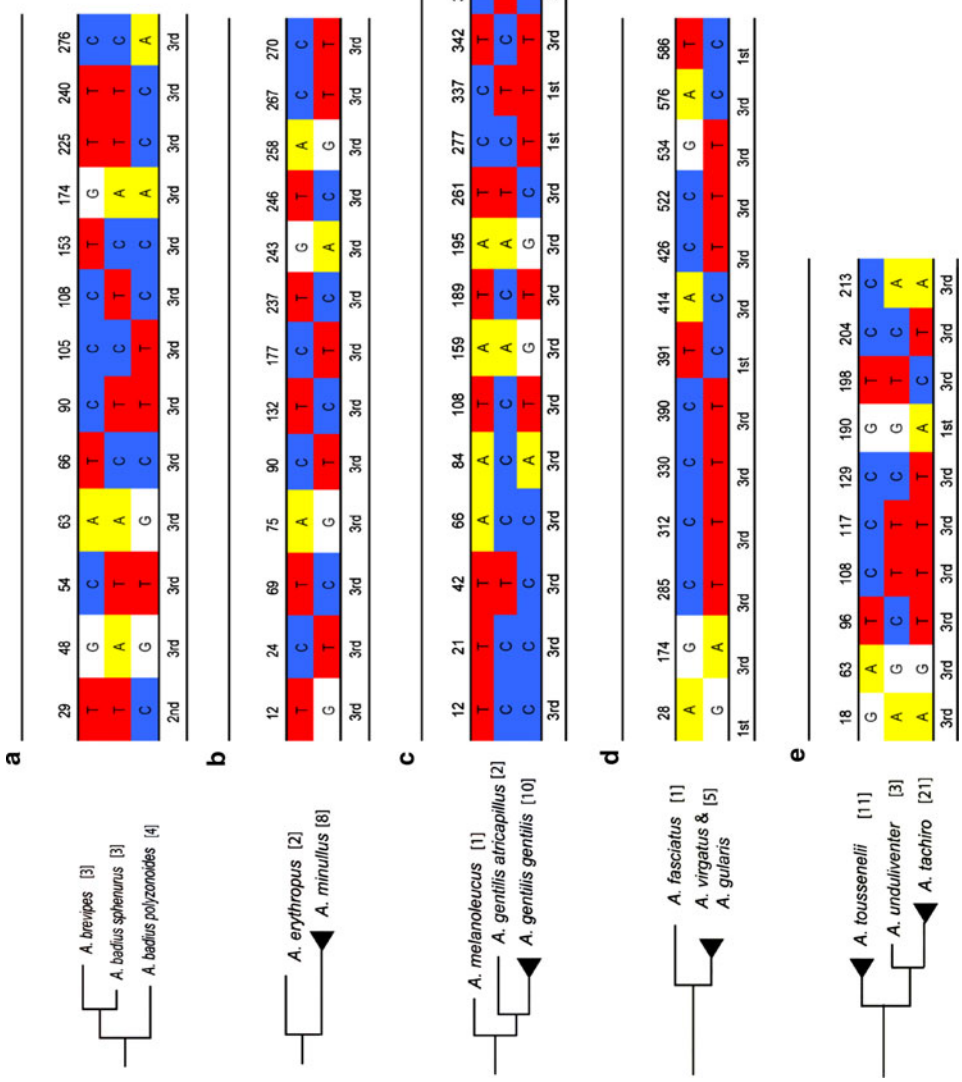

Fig. 6 Some examples of nucleotide character that were diagnostic for a species (sensu Rach et al. 2008). The *top row of numbers* gives the bp postions in the aligned 647 bp barcode fragment. The *bottom row* indicates whether the given position was located at the first, second, or third codon position

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