Genetic identity of grey chiffchaffs trapped in the Netherlands in autumns of 2009-11

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Three subspecies of Common Chiffchaff *Phylloscopus collybita* are currently on the Dutch list: nominate *P c collybita* (hereafter nominate *collybita*), Scandinavian Chiffchaff *P c abietinus* (hereafter *abietinus*) and Siberian Chiffchaff *P c tristis* (hereafter *tristis*). Nominate *collybita* is a very common breeding bird in most of western Europe including the Netherlands, where it is also a migrant reaching south to southern Morocco in winter (eg, Speek & Speek 1984) and a winterer in small numbers (Bijlsma et al 2001). *Abietinus* breeds in northern Scandinavia and eastern Europe east to the Urals, Caucasus, Transcaucasia and northern Iran (Clements 2000). In the Netherlands, it has been mentioned as a migrant and winterer (Bijlsma et al 2001), although there is no publication describing its identification and occurrence in any detail. *Tristis* breeds in Siberia, east of the range of *abietinus*, and in the Netherlands it is regarded as a rarity, after being temporarily dropped in 1992-93 from the list of taxa to be considered by the Dutch rarities committee (CDNA) (van den Berg & Bosman 2001). Up to and including 2011, 39 *tristis* were accepted by the CDNA (Ebels 2009, Ovaa et al 2012), mainly concerning singing or calling birds from October to April. There has been much debate about the identification of Common Chiffchaff taxa and the occurrence of *tristis* in western Europe (eg, van den Berg & The Sound Approach 2009, Dean et al

528 Siberian Chiffchaff / Siberische Tjiftjaf *Phylloscopus collybita tristis* (right; same bird as in plate 529), with Common Chiffchaff / Tjiftjaf *Phylloscopus collybita collybita*, Wassenaar, Zuid-Holland, 31 October 2009 *(Vincent van der Spek/Vrs Meijendel)*.

2010). This paper reveals the first findings based on DNA-analyses of feather material collected from 41 chiffchaffs, more than half of which were initially identified as *abietinus*, at ringing stations in the west of the Netherlands in 2009-11.

Background

In western Europe, *tristis* is regarded the rarest of the three taxa. Song (eg, Martens & Meincke 1989) and calls (eg, Constantine & The Sound Approach 2006, van den Berg & The Sound Approach 2009) are nowadays regarded as diagnostic and sufficiently distinct to separate it from the two other taxa (Constantine & The Sound Approach 2012). But what about non-vocal birds?

Plumage characters to distinguish *tristis* from nominate *collybita* are described by, eg, Svensson (1992), Dean & Svensson (2005), Svensson et al (2009), van den Berg & The Sound Approach (2009) and Ebels (2009). *Tristis* is regarded as the subspecies with the least amount of green and yellow in the plumage. Typical birds are brown and many birds are grey, without yellow or green tones on upper- and underparts. Nominate *collybita* has the most green and yellow tones in its plumage. *Abietinus* is often considered to be intermediate between the two by Dutch ringers but in fact Svensson (1992) explains that *abietinus* and *collybita* generally are impossible to separate when handling single birds (see also Cramp 1992). He mentions that, on average, *collybita* is 'very slightly more green and less grey above' and that 'underparts are said to be more yellow and buff than in *abietinus*' but adds that he finds it difficult to confirm these differences and that 'individual variation seems to be just as pronounced as geographical'. However, both Cramp (1992) and Svensson (1992) mention that *abietinus* is 'a trifle' larger than both *collybita* and *tristis* but, because of extensive overlap, this does not present much help in identifying *abietinus* by ringers either. As far as the current knowledge goes, only completely brown or grey (non-vocal) chiffchaffs can be safely identified as *tristis*. In practice, however, many *tristis* do have some green on the upperside or yellow on the supercilium, underparts and bare parts (eg, van den Berg & The Sound Approach 2009). But, because of its alleged rareness, 'safety first' is the widely accepted view on the identification of *tristis*. For example, the CDNA does not accept records of non-vocal chiffchaffs that have green or yellow in their plumage, despite the plumage analyses in van den Berg & The Sound Approach (2009) of birds trapped and photographed by Arend Wassink in Kazakhstan.

As a result, many Dutch ringers until recently identified birds less green and yellow than nominate *collybita* but with some yellow on the underparts and/or supercilium and/or green on the mantle as *abietinus*, simply because the amount of yellow and green supposedly ruled out *tristis*. Levering & Keijl (2008), for instance, list for 1970-2006 79 *abietinus* trapped and ringed at Castricum, Noord-Holland, and 26 birds 'showing features' of *tristis* (not a single one being accepted). Likewise, Blom et al (2011) list 16 *abietinus* and five *tristis* trapped and ringed at Westenschouwen, Zeeland, in 1959-2009. However, recent publications led some ringers to challenge these results and to investigate newly trapped birds by using DNA analyses.

Methods

We designed a pilot project using a pragmatic research protocol. During the autumn migration periods of 2009-11, loose feathers that dropped during handling of trapped chiffchaffs were collected from 41 individuals, mostly from birds which did not look like typical nominate *collybita* or from very large birds (suggesting *abietinus*). This was done at five Dutch ringing stations: Vrg De Grauwe Gans (Almere, Flevoland), Vrs Castricum (Castricum, Noord-Holland), Vrs Meijendel (Wassenaar, Zuid-Holland), Vrs Van Lennep (Bloemendaal, Noord-Holland) and Vrs Schiermonnikoog (Schiermonnikoog, Friesland) (see www.trektellen.nl for more information about these ringing stations). From these feathers, DNA was extracted and the sequence of a fragment of 939 basepairs of the mitochondrial cytochrome B gene was obtained. To these results, we added orthologous (equivalent) sequences from individuals of: *collybita* (n=1; GenBank Z73487, from Lake Constance, southern Germany (Helbig et al 1996)), *abietinus* (n=8; seven sequences provided by Staffan Bensch from north-eastern Sweden and one sequence, GenBank Z73479, from Eilat, Israel (Helbig et al 1996)), and *tristis* (n=8; seven sequences provided by Staffan Bensch from Kazakhstan and one sequence, GenBank Z73482 from Mirnoye biological station, Yenisei, Siberia (Helbig et al 1996)) sequenced by others and deposited in the GenBank sequence database (www.ncbi.nlm.nih.gov/GenBank) or provided directly to us. Before the DNA-based identification, all ringers provided their 'tentative' identification based on plumage and biometry.

Results

We compared the cytochrome B sequences of all 41 sampled birds with the available data from 17

529 Siberian Chiffchaff / Siberische Tjiftjaf *Phylloscopus collybita tristis*, Wassenaar, Zuid-Holland, 31 October 2009 *(Vincent van der Spek/Vrs Meijendel)*. The bird (same as in plate 528), the very first in the project, was identified as *abietinus/tristis* by the ringers. Although not prominent, note green feathers on mantle. **530** Siberian Chiffchaff / Siberische Tjiftjaf *Phylloscopus collybita tristis*, Castricum, Noord-Holland, 28 October 2011 *(Luc Knijnsberg)*. Identified as *abietinus* by the ringers. Note amount of green on upperparts. **531** Siberian Chiffchaff / Siberische Tjiftjaf *Phylloscopus collybita tristis*, Castricum, Noord-Holland, 8 October 2010 *(Luc Knijnsberg)*. Identified as *abietinus* by the ringers. **532** Siberian Chiffchaff / Siberische Tjiftjaf *Phylloscopus collybita tristis*, Wassenaar, Zuid-Holland, 7 November 2010 *(Vincent van der Spek/Vrs Meijendel)*. The bird had many green feathers on upperparts and yellow feathers on head and, most strikingly, supercilium. Identified as *abietinus* by the ringers.

other birds representing the three subspecies. This resulted in three very distinct groups of sequences, corresponding to the subspecies *collybita*, *abietinus* and *tristis* (figure 1). The majority of the sequences (n=30) grouped with the *tristis* sequence types. The remaining sequences (n=11) grouped with the single *collybita* sequence. So, none of the sampled 41 birds showed a sequence typical of *abietinus*. In terms of sequence variation, each of the three chiffchaff taxa analysed here showed distinct sequence types. This confirms the previous results from Helbig et al (1996) and Bensch et al (2006). When we compared the molecular (mtDNA) identification and the in-hand (visual) identification, we saw a clear trend (table 1). All birds identified as *collybita* (n=9) were also *collybita* based on mtDNA. All birds identified as *tristis* (n=5) were also confirmed as such. Importantly, however, all birds identified as *abietinus* in the hand (n=23), were identified as *tristis* based on mtDNA, and not a single *abietinus* was identified based on mtDNA. We also compared the plumage variation of all birds that were mtDNA identified as *tristis*. For this, we distinguished 'greyish'

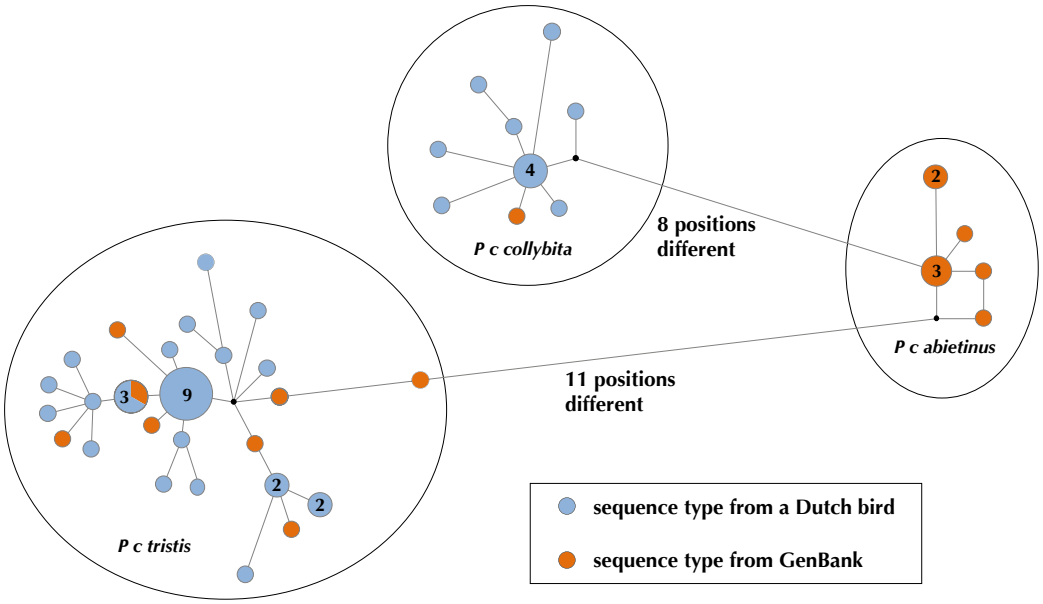

FIGURE 1 Network of mtDNA cytochrome B sequences representing three Common Chiffchaff *Phylloscopus collybita* subspecies. Each coloured circle represents a unique haplotype (sequence). Circles without a number are only seen once, otherwise the number indicates the number of times this sequence type was observed. The length of the lines connecting the circles corresponds with the number of sequence differences between the sequence types. The short line connecting most of the sequence types represents a single position (out of 939) being different.

(with some green) and 'brownish' *tristis* but we were unable to detect a clear DNA difference between these two *tristis* plumage types. Two birds from Meijendel, Wassenaar, with long wings (67 mm) turned out to be *collybita* based on mtDNA.

Discussion

Our results suggest that *tristis* is a scarce but regular migrant in the Netherlands and that it is much more common than its current status suggests, even when taking into account the statement by some ringers that autumn 2010 appeared to be an unusually good year for 'suspicious' chiffchaffs. It also shows that plumage variation in *tristis* is larger than typically acknowledged (cf Svensson 1992), which confirms Arend Wassink's contribution in van den Berg & The Sound Approach (2009). It is also noteworthy that not a single *abietinus* was identified by mtDNA. The two birds with long wings (67 mm) turned out to be *collybita* based on mtDNA. According to Svensson (1992), a wing length above 64 mm is out of range for both *tristis* and *collybita* but within the range of *abietinus*. This suggests that wing length may not be diagnostic for *abietinus*. It appeared that the ringers of the five ringing stations were quite able

TABLE 1 Common Chiffchaff *Phylloscopus collybita* subspecies identification by ringers compared with identification using mtDNA

identification by ringers	**identification by mtDNA**			
	P c collybita	*P c abietinus*	*P c tristis*	**Total**
P c collybita	9	0	0	9
P c collybita/abietinus	2	0	0	2
P c abietinus	0	0	23	23
P c abietinus/tristis	0	0	2	2
P c tristis	0	0	5	5
Total	**11**	**0**	**30**	**41**

533 Siberian Chiffchaff / Siberische Tjiftjaf *Phylloscopus collybita tristis*, Almere, Flevoland, 26 October 2010 *(Ton Eggenhuizen)*. Identified as *tristis* by the ringers due to lack of yellow and green on, eg, head, supercilium and upperparts.

534 Siberian Chiffchaff / Siberische Tjiftjaf *Phylloscopus collybita tristis*, Castricum, Noord-Holland, 22 November 2010 *(Richard Reijnders)*. Identified as *tristis* by the ringer.

to pick out chiffchaffs which looked like another subspecies than *collybita* on the basis of plumage characters but they appeared to mistake them for *abietinus*. They often did consider the possibility of a 'greyish' *tristis*, but in many cases ruled out this taxon based on the CDNA criteria for acceptance. It means that they were not able to correctly identify many *tristis* by plumage, despite having much experience with chiffchaffs in the hand.

All this obviously raises the question how to identify *abietinus* without DNA, and suggests that in the past many trapped *tristis* were erroneously identified as *abietinus*. Clearly, if any morphological criteria were used in the past, they should be considered inconclusive or even erroneous.

As all 'atypical' Common Chiffchaffs in this sample were identified as *tristis* based on mtDNA, *tristis* is obviously much less rare than previously assumed.

535 Common Chiffchaff / Tjiftjaf *Phylloscopus collybita collybita*, Wassenaar, Zuid-Holland, 8 October 2010 *(Vincent van der Spek/Vrs Meijendel)*. Identified as *collybita/abietinus* by the ringers; *abietinus* was suggested based on its wing length of 67 mm.

Future research

All afore-mentioned ringing stations are planning to co-operate in a continuation of this research on a larger and more systematic scale during the next autumns. Combined with plumage details and biometry and properly documented with photographs and, if possible, sound-recordings this will hopefully result in a large sample that will allow us to shed more light on the status and identification criteria of *collybita*, *tristis* and, especially, *abietinus*.

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Samenvatting

GENETISCHE IDENTITEIT VAN GRIJZE TJIFTJAFFEN GEVANGEN IN NEDERLAND IN HET NAJAAR VAN 2009-11 Op de Nederlandse lijst staan drie ondersoorten van Tjiftjaf *Phylloscopus collybita*: nominaat *P c collybita* (hierna nominaat *collybita*), Scandinavische Tjiftjaf *P c abietinus* (hierna *abietinus*) en Siberische Tjiftjaf *P c tristis* (hierna *tristis*). Nominaat *collybita* is een zeer algemene broedvogel, doortrekker en schaarse wintergast. *Abietinus* wordt als doortrekker en wintergast beschouwd hoewel in geen enkele bron het voorkomen in detail wordt beschreven. *Tristis* is een zeldzaamheid die beoordeeld wordt door de CDNA, met tot en met 2011 39 aanvaarde exemplaren. Het gaat bij *tristis* met name om zingende of roepende vogels laat in het najaar en in de winter. Op zang en roep zijn ze goed te onderscheiden van de andere taxa. Maar hoe om te gaan met vogels die geen geluid maken, zoals vaak op ringbanen?

Nominaat *collybita* heeft het meeste geel en groen in het kleed, *tristis* het minst. *Abietinus* wordt vaak als intermediair beschouwd maar verschilt in feite qua uiterlijk niet of nauwelijks van nominaat *collybita*, behalve dat hij gemiddeld ietsje groter is dan zowel nominaat *collybita* als *tristis*. Een klassieke *tristis* is bruin op de bovendelen, maar veel vogels geven eerder een grijze indruk. Het is bekend dat *tristis* meer geel- of groentinten op de kop, bovendelen of onderdelen kan hebben dan vroeger werd gedacht. De CDNA hanteert echter de stelregel dat niet-roepende vogels met geel en/of groen in de wenkbrauwstreep, op de kop en op de boven- en onderdelen niet als *tristis* aanvaard kunnen worden omdat atypische *abietinus* of nominaat *collybita* niet kunnen worden uitgesloten. Door zo nauwkeurig naar de verdeling van groenen geeltinten te kijken, is bij met name veel ringers ten onrechte het idee ontstaan dat vogels met wat geel of groen op 'onwenselijke' plekken per definitie geen *tristis* zijn, en dus altijd *abietinus* betreffen.

Bij enkele ringers rees de vraag of DNA-analyses licht op de zaak konden werpen. Daartoe werd een pragmatisch onderzoeksprotocol opgezet. In 2009-11 zijn op vijf Nederlandse ringstations uit vier provincies veertjes verzameld die tijdens het hanteren van gevangen vogels waren losgekomen, in totaal van 41 Tjiftjaffen (met name van vogels waarvan verwacht werd dat het geen *collybita* betrof) waarvan de determinatie van de ringers werd genoteerd. Uit deze veren is DNA geïsoleerd en van een fragment van 939 baseparen van het mitochondriale cytochroom-B-gen werd de basepaarvolgorde bepaald ('sequencen'). De verkregen sequenties werden vergeleken met 17 sequenties van de drie taxa in de internationale sequentiedatabank GenBank en van Staffan Bensch (Universiteit Lund, Zweden), afkomstig van vogels uit de broedgebieden (één nominaat *collybita*, acht *abietinus* en acht *tristis*).

Het bleek dat 30 van de 41 Nederlandse monsters correspondeerden met sequenties van *tristis*, 11 correspondeerden met nominaat *collybita* en geen enkel monster kwam overeen met *abietinus*. Wanneer de determinatie op basis van mtDNA werd vergeleken met die van de ringers (tabel 1), viel op dat alle als nominaat *collybita* gedetermineerde vogels (9) dat op basis van mtDNA ook waren. De vijf vogels die door de ringers als *tristis* werden genoteerd, waren dat ook op basis van mtDNA. Echter, alle vogels (n=23) die in de hand als *abietinus* waren gedetermineerd bleken op basis van mtDNA *tristis* te zijn.

Dit kleinschalige onderzoek op een beperkt aantal ringstations toont alleen in de jaren 2009-11 op basis van mtDNA al 30 exemplaren van *tristis* aan. Zelfs als wordt meegewogen dat 2010 volgens veel ringers een opmerkelijk goed jaar voor 'atypische' Tjiftjaffen was, wijzen de resultaten er toch op dat *tristis* eerder schaars dan zeldzaam is. De deelnemende ringers bleken goed in staat om 'verdachte' Tjiftjaffen op te merken maar waren, ondanks hun ervaring, in veel gevallen niet in staat om *abietinus* en *tristis* te onderscheiden. Goede criteria om *abietinus* zonder DNA te determineren lijken niet voorhanden, tenzij men de biometrie van een extreem groot exemplaar voldoende acht. Twee vogels uit deze steekproef met lange vleugels (67 mm), volgens de literatuur buiten de bandbreedte van *collybita* en *tristis* maar passend binnen *abietinus*, bleken op basis van mtDNA echter toch *collybita* te zijn. De vraag is daarmee in hoeverre biometrie bruikbaar is. Er komt een uitgebreid vervolgonderzoek naar Tjiftjaffen, dat zich met name zal toespitsen op *abietinus*.

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APPENDIX 1 Common Chiffchaffs *Phylloscopus collybita* trapped by five ringing groups in the Netherlands and identified as Siberian Chifchaff *P c tristis* based on mtDNA in 2009-11 (these reports have not yet been considered by the Dutch rarities committee (CDNA))

Locality	**Date**	**Ring number**
Wassenaar, Zuid-Holland	31 October 2009	Y87188
Wassenaar, Zuid-Holland	8 November 2009	Y87199
Almere, Flevoland	26 October 2010	AAC854
Castricum, Noord-Holland	30 October 2010	Y76947
Wassenaar, Zuid-Holland	7 November 2010	Y87756
Almere, Flevoland	7 November 2010	AAC858
Almere, Flevoland	7 November 2010	AAC860
Castricum, Noord-Holland	7 November 2010	Y78015
Castricum, Noord-Holland	7 November 2010	Y78019
Castricum, Noord-Holland	7 November 2010	Y78021
Castricum, Noord-Holland	7 November 2010	Y78022
Castricum, Noord-Holland	7 November 2010	Y78033
Castricum, Noord-Holland	7 November 2010	Y78043
Castricum, Noord-Holland	7 November 2010	Y78044
Castricum, Noord-Holland	7 November 2010	Y78055
Castricum, Noord-Holland	8 November 2010	Y78079
Wassenaar, Zuid-Holland	9 November 2010	Y87787
Castricum, Noord-Holland	10 November 2010	Y78092
Castricum, Noord-Holland	10 November 2010	Y78093
Castricum, Noord-Holland	14 November 2010	Y78096
Almere, Flevoland	17 November 2010	AAC885
Castricum, Noord-Holland	18 November 2010	Y79581
Castricum, Noord-Holland	19 November 2010	AT87347
Castricum, Noord-Holland	20 November 2010	Y78139
Castricum, Noord-Holland	20 November 2010	Y78140
Almere, Flevoland	20 November 2010	AAC899
Castricum, Noord-Holland	21 November 2010	Y78110
Almere, Flevoland	28 November 2010	AAC904
Schiermonnikoog, Friesland	26 October 2011	Y92990
Castricum, Noord-Holland	28 October 2011	Y79427