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Green Warbler *Phylloscopus nitidus* ringed at Blåvand: molecular confirmation of a Danish first and European eighth vagrant record

Kaukasisk lundsångare Phylloscopus nitidus *ringmärkt i Blåvand: Danmarks första och Europas åttonde raritetsfynd bekräftas molekylärt*

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Abstract

On 27 May 2015, a Green Warbler *Phylloscopus nitidus* was caught and ringed at Blåvand Bird Observatory, the westernmost point in Denmark. The species is challenging to identify on morphological basis alone, and the bird's identity was confirmed with phylogenetic analyses of mitochondrial DNA sequences. This constitutes the first record of Green Warbler in Denmark, the third record in Fennoscandia – following records on Öland, Sweden, on 29 May 2003, and Åland, Finland, on 20 May 2012 – and the eighth vagrant record in Europe outside the species' very restricted peripheral breeding range.

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Introduction

Green Warbler *Phylloscopus nitidus* breeds from northern Turkey eastwards to Iran, and winters in India and southeast Asia (del Hoyo et al. 2006). It reaches marginally into Europe in southern Russia and northern Georgia (del Hoyo et al. 2006). East of its breeding distribution the Greenish Warbler *Ph. trochiloides* and the Two-barred Warbler *Ph. plumbeitarsus* display a continuous distribution along a broken ring around the Tibetan Plateau, with several discernible subspecies/populations (Irwin et al. 2001).

The relationship between Green Warbler, Greenish Warbler, and Two-barred Warbler is complex (Irwin et al. 2001) and the Green Warbler has traditionally been regarded as a subspecies of Greenish Warbler. It was raised to species status by the British Ornithologists' Union in 2008 (Knox et al. 2008, BOU 2009), and by the Clements Checklist of Birds of the World in 2009 (Clements et al. 2009), while the International Ornithological Union regarded it as a species already in its first edition (Gill & Wright 2006). The Two-barred Warbler was given species status later (Gill & Wright 2006, Clements et al. 2014).

Before this first Danish record of Green Warbler there were two records from Fennoscandia and another five from remaining Europe. Two new records were added in 2016, making the total until and including 2016 ten records outside the restricted European breeding range (Table 1). Furthermore, there are seven accepted records in Israel (Slack 2009, IRDC 2016).

Description

On Wednesday 27 May 2015, the daily standardized ringing at Blåvand Bird Observatory could not be done because of strong wind (NW 12 m/s) and rain. Around noon, the wind decreased to 5 m/s the rain stopped, and the sun began to shine. Some of the mist nets were opened and after about one hour, one bird was caught. Surprisingly, this turned out to be a Greenish Warbler type. However, this bird clearly differed from the Greenish Warblers that are caught almost every year at Blåvand. The date was also quite early for Greenish Warbler at Blåvand, as most records of Greenish Warbler at Blåvand are from June. Because of the colouration, the bird was

Table 1. European records outside the peripheral breeding grounds of Green Warbler *Phylloscopus nitidus*.
Europeiska fynd utanför det perifera häckningsområdet av kaukasisk lundsångare Phylloscopus nitidus.

*Treatment/decision by national rarities committee. *Behandling/beslut av nationell raritetskommitté.*
**Another record, made 2000-09-27 in Greece, was listed by Hudson (2010), but this has been rejected by the Hellenic Rarities Committee (Nikos Probonas *in litt*.). *Ytterligare ett fynd i Grekland 2000-09-27 rapporterades av Hudson (2010), men detta fynd har underkänts av den grekiska raritetskommittén (Nikos Probonas* in litt*.).*

Area, country	**Site**	**Date (YMD)**	**RC treatment***	**Sequenced**	**Reference**
Område, land	*Lokal*	*Datum (ÅMD)*	*Rk-behandling**	*Sekvenserad*	*Referens*
Fennoscandia					
Sweden	Ottenby, Öland	2003-05-29	accepted	yes	Irwin & Hellström 2007
Finland	Lågskär, Åland	2012-05-20	accepted	no	Väisänen et al. 2015
Denmark	Blåvand, Jylland	2015-05-27	pending	yes	this study
Remaining NW Europe					
Germany	Helgoland	1867-10-11	accepted	no	Gätkhe 1900
UK	St Mary, Isles of Scilly	1983-09-26	accepted	no	Hudson 2010
Faroe Islands	Nolsoy	1997-06-08	accepted	yes	Sørensen & Jensen 2001
UK	Foula, Shetland	2014-05-31	accepted	no	Pennington *in litt.*
UK	Unst, Shetland	2016-05-12	accepted	yes	Pennington 2016
SE Europe					
Greece**	Antikythira Island	1998-09-18	accepted	no	Hellenic Rarities Committee 2006 & 2009
Romania	Chituk	2016-10-05	pending	no	Milvus Group 2016

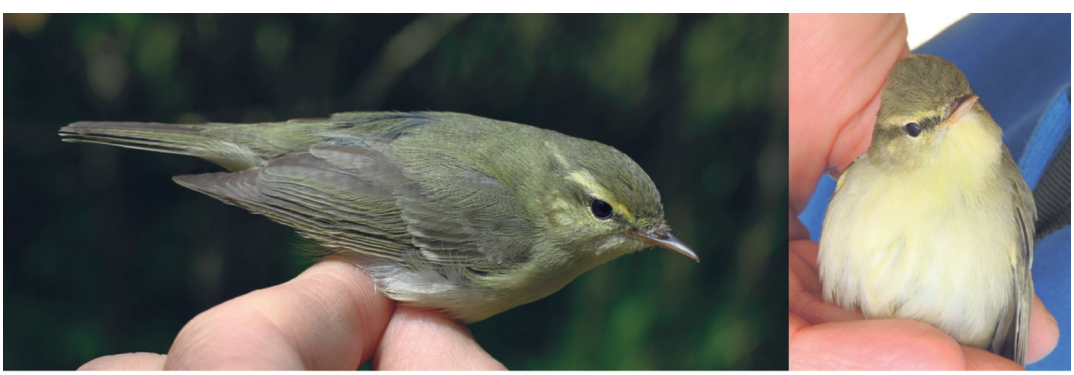

Figure 1. Green Warbler *Phylloscopus nitidus*, caught on 27 May 2015, in Blåvand, Denmark. Note rather green upperparts, one clearly visible wing-bar on the greater coverts, and yellowish breast, which are characters that distinguish it from Greenish Warbler *Ph. trochiloides* and Two-barred Warbler *Ph. plumbeitarsus*. Photos: Henrik Knudsen (left) and Morten Bentzon Hansen (right).
Kaukasisk lundsångare Phylloscopus nitidus*, fångad 27 maj 2015 i Blåvand, Danmark. Notera tämligen grön översida, endast ett synligt vingband längs de större armtäckarna samt gultonat bröst, vilka är karaktärer som skiljer den från lundsångare* Ph. trochiloides *och sibirisk lundsångare* Ph. plumbeitarsus*. Foton: Henrik Knudsen (vänster) och Morten Bentzon Hansen (höger).*

immediately suspected of being a probable Green Warbler.

The bird was measured and photographed (Figure 1). The wing length (maximum chord; Svensson 1992) was recorded at 61.5 mm, primary 3–4 formed the wing tip, and the tip of the second primary fell at the tip of primary 7. During the process, the bird shed two downs which were collected for DNA typing, as the species determination was not conclusive. After five hours, the bird was relocated and seen clearly in the field. The next day, however, the bird could not be found.

The colouration of the bird was quite different compared to a Greenish Warbler. The upperparts were bright green without the greyish wash, which is typical of Greenish Warbler. The supercilium was yellow, not white, as in Greenish Warbler. The throat and upper breast were yellow contrasting to the white lower breast and belly. The wing bar on the tip of the greater secondary coverts was prominent and broader compared to Greenish Warblers seen in the spring (Figure 1). In the hand, the size and shape of the bird was not perceived as different from Greenish Warbler, but on some photos the head looks larger and more robust than that of Greenish Warbler. The bird was not heard, but when a recording of Green Warbler song was played, the bird duly appeared. Regrettably, no recording of Greenish Warbler was played, so the potential response to that species is unknown.

Methods

The bases of the collected downs were cut off and digested for three hours at 56 °C in 100 µl lysis buffer (0.1 M Tris, 0.005 EDTA, 0.2% SDS, 0.2 M NaCl, pH 8.5) with 1.5 µl proteinase K (10 mg/ml) and then precipitated with ethanol and eluted in 20 µl ddH_2O. For typing of the mitochondrial cytochrome *b* (cyt*b*) gene, we used the Qiagen Multiplex PCR Kit (Qiagen Inc.), with amplification reactions containing 5 µl Qiagen Multiplex PCR Master Mix, 0.2 µl each of 10 µM primers ND5-Syl (Stervander et al. 2015) and mtF-NP (Fregin et al. 2009), 2 µl template DNA (non-diluted elution), and 2.6 µl water. We ran the PCR reactions for activation at 95 °C for 15 min. Then followed 40 three-step cycles with denaturation at 94 °C for 30 s; annealing for 20 cycles in a touchdown profile decreasing by 0.5°C per cycle from 55°C, followed by 20 cycles at 45°C for 90 s; and extension at 72 °C for 90 s. Finally, we allowed extension at 72 °C for 10 min. PCR products were checked on a 1% agarose gel, precipitated with NH4Ac and ethanol, and then dissolved in 25 µl water. We used 2 µl for sequencing with the internal sequencing primer Cytb_seq_H15541 (Stervander et al.2015) with the BigDye sequencing kit (Applied Biosystems) in an ABI Prism 3100 capillary sequencer (Applied Biosystems).

The cyt*b* sequence was manually inspected and edited in Geneious v. 10 (Biomatters). GenBank sequences of >900 bp were downloaded for all *Phylloscopus/Seicercus/Abrornis* (different genus names used for one monophyletic clade) and outgroups (*Cettia/Horornis, Aegithalos, Acrocephalus, Cisticola, Sylvia*), and aligned with the MAFFT v7.222 (Katoh et al. 2002) Geneious plugin. The 394 reference sequences included in this study are specified in Appendix 1.

Substitution models were evaluated with jModelTest v. 2.1.4 (Guindon & Gascuel 2003, Darriba et al. 2012), selecting from 88 available models allowing for rate heterogeneity according to four gamma categories and for a proportion of invariable sites. Model selection was performed per the Bayesian Information Criterion (BIC; Schwarz 1978).

Cyt*b* gene trees were computed within a Bayesian inference (BI) framework with BEAST v. 2.4.4 (Bouckaert et al. 2014), using a Yule tree prior, and a strict molecular clock with a rate of 0.0105 substitution/site/lineage/million years, based on overall cyt*b* substitution rates for a wide range of avian species (Weir & Schluter 2008). We applied four discrete categories over the gamma distribution, and estimated the frequency of invariant sites as well as the base frequencies. We performed two replicate runs, and sampled trees every 1,000 generations, over 30 million generations, of which the first 10% were discarded as burn-in. The results were inspected using Tracer v. 1.6 (Rambaut et al. 2013), ensuring stationarity and effective sample sizes (ESS) of >200, and ascertaining congruence between replicate runs.

The maximum clade credibility tree was calculated with TreeAnnotator (Bouckaert et al. 2014), using average node heights, and excluding 10% as burn-in. This tree was visualized and annotated in FigTree v.1.4.3 (Rambaut 2016) and Mega 7 (Kumar et al. 2016).

Results

The trimmed sequence from the Blåvand bird was 681 bp, of which 633 bp covered parts of the cyt*b* gene from its start, preceded by the last 40 bp of the ND5 gene and 8 intergenic bp. The cyt*b* sequence

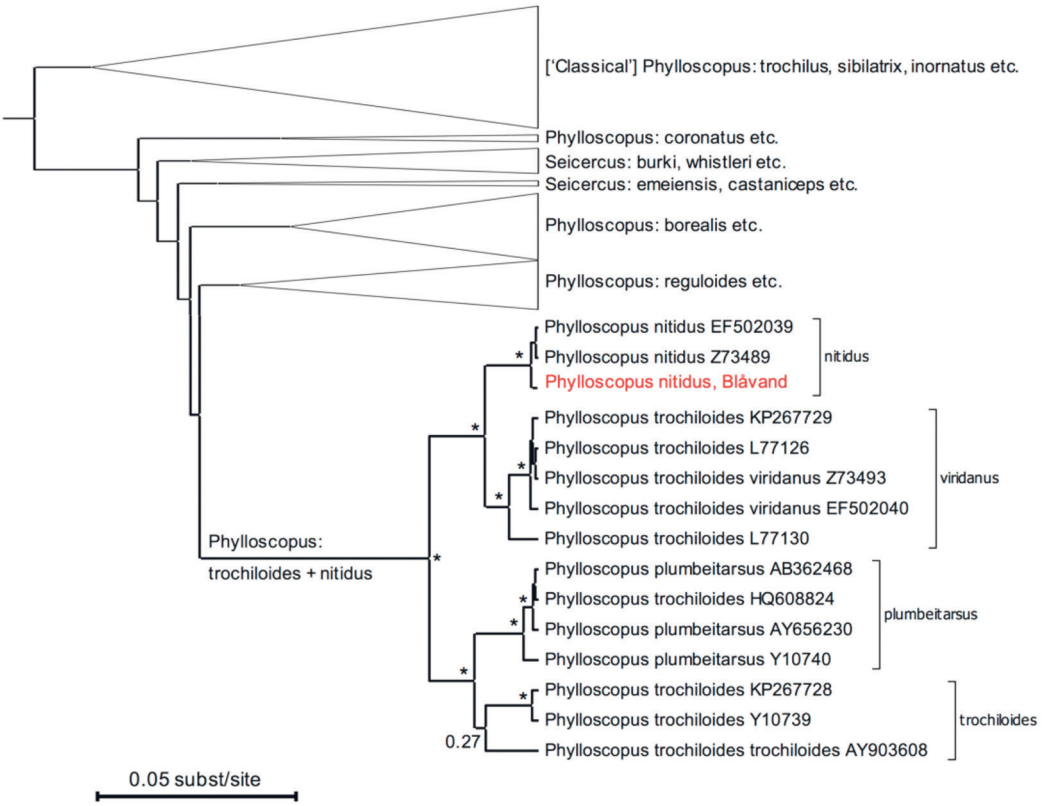

Figure 2. Phylogeny of *Seicercus* and *Phylloscopus* warblers, based on Baeysian inference of 394 cytochrome *b* sequences, which places the Blåvand bird (highlighted in red font) in the Green Warbler *Ph. nitidus* clade with maximal support. Sequences from the complex containing Green Warbler and Greenish Warbler *Ph. trochiloides* are shown with their taxon names as submitted to Genbank, with bracket annotations stating which clade they correspond to. Posterior probability (PP; 0.0–1.0) is indicated for nodes in this clade: * indicates PP = 1.0, PP otherwise stated with a number at the node. All other clades have been collapsed, with the height of the triangle corresponding to number of sequences, and labels including taxa representative of the clade. For a full list of included taxa, and all Genbank accession numbers, see Appendix 1. Note that the traditional use of the genus names *Seicercus* and *Phylloscopus* does not reflect monophyletic groups. The outgroups have been removed for clarity.
Fylogeni över sångare inom släktena Seicercus *och* Phylloscopus*, baserad på bayesiansk inferens av 394 cytokrom* b*-sekvenser, vilken placerar fågeln från Blåvand (markerad med röd text) i kladen med kaukasisk lundsångare* Ph. nitidus *med maximalt statistiskt stöd. Sekvenser från komplexet som omfattar kaukasisk lundsångare och lundsångare* Ph. trochiloides *visas namngivna såsom de skickats in till Genbank, med klamrar som indikerar vilken klad de tillhör. Posteriorisannolikhet (PP; 0,0–1,0) visas för noder inom denna klad: * indikerar PP = 1,0, PP visas annars med en siffra vid noden. Alla andra klader har minimerats och triangelns höjd motsvarar antalet sekvenser som ingår i kladen. Representativa taxa har listats bredvid triangeln. För den fulla listan över inkluderade taxa, samt deras Genbank-nummer, se Appendix 1. Notera att den traditionella användningen av släktesnamnen* Seicercus *och* Phylloscopus *inte motsvarar monofyletiska grupper. Utgrupperna har uteslutits från figuren för tydlighet.*

aligned with no gaps to cyt*b* sequences of related species, and is available at Genbank with the accession number MF188243.

The best estimated substitution model was HKY (Hasegawa et al. 1985) with rate variation following a discrete gamma distribution with four rate categories (G) and with an estimated fraction of invariant sites (I).

The computed cyt*b* phylogeny groups the Blåvand bird with full statistical support (posterior probability 1.0) together with two sequences of Green Warblers in a *nitidus* clade, that is sister to the *viridanus* clade (Figure 2). Those two clades, in turn, make up as a sister clade to the clade containing the sisters *trochiloides* and *plumbeitarsus* (Figure 2).

Discussion

Green Warbler may be an overlooked species in western and northern Europe because it is rather similar to Greenish Warbler. Even with good views the two species may be difficult to distinguish, and good views may be hard to achieve owing to the restless nature of the species and their habit of foraging high above the ground. However, very few Green Warblers have been caught in Europe compared to the number of Greenish Warblers, so the species is probably a quite rare vagrant in Europe.

The phylogenetic analysis clearly confirms that the Blåvand bird is a Green Warbler (Figure 2). It further highlights the special case that the Green/Greenish/Two-barred Warbler complex constitutes. Under a phylogenetic species concept, a species must be monophyletic, i.e. all of its subspecies/populations/individuals must be most closely related to each other, and together form a clade that contains no other taxa. However, the cyt*b* phylogeny shows that the two Greenish Warbler clades (*trochiloides* and *viridanus*; Figure 2) are not each other's closest relatives, but instead make up sister clades with Two-barred Warbler (*plumbeitarsus*) and Green Warbler (*nitidus*), respectively. Thus, judging from the cyt*b* phylogeny alone, Greenish Warbler is paraphyletic, and either all taxa within the complex represent one species, or all should be regarded separate species.

However, while mitochondrial markers such as cyt*b* are appropriate for taxon identification, taxonomic conclusions should not rest solely on such information. Mitochondrial genetic markers differ from nuclear genetic markers because of a faster molecular substitution rate, and because of maternal inheritance. When populations diverge, mitochondrial and nuclear genetic markers may leave different phylogenetic signatures because they sort at different rates. Also, if diverged populations later come into secondary contact, mitochondrial and nuclear genetic markers will be differentially prone to introgression depending on the stage of divergence (Rheindt & Edwards 2011). For that reason, interpretations of phylogenetic relationship should best be based on multi-locus analyses including nuclear genetic markers (e.g. Rheindt & Edwards 2011, Stervander et al. 2015).

Further, the Greenish/Green/Two-barred Warbler clade is unusually well studied, and illustrates some of the problems highlighted above. The Greenish and Two-barred Warbler occur in a broken ring around the Tibetan plateau. The Two-barred Warbler occupies the north-eastern part of the ring, and is isolated with a break in the ring to the south, while it meets the Greenish Warbler in a contact zone to the west. Despite the contact, the two species remain their integrity. The distribution of the Greenish warbler continues counter-clockwise around the Tibetan plateau until the gap the occurs east of the plateau, before reaching the distribution of the Two-barred Warbler. When Irwin at el. (2001) analysed divergence in song and mitochondrial DNA along the broken ring, they concluded that the complex had likely evolved along the ring. Neighbouring populations were only slightly differentiated, but the differentiation progressed on a gradient along the ring, and reproductive isolation was complete in the two end-points, represented by the Siberian populations of Greenish and Two-barred Warbler. However, when Alcaide et al. (2014) made use of "next generation sequencing" of massive amounts of nuclear DNA, they revealed that this interpretation was not correct. Instead they uncovered that there have been multiple historical breaks in gene flow, when populations have been isolated at several locations along the ring. Nevertheless, while this species complex may not represent a rare case of a "ring species", it illustrates a complex evolutionary history resulting in varying levels of genetic differentiation between subspecies and species.

Finally, it is worth to mention that the two genera *Phylloscopus* and *Seicercus*, which presently constitute the family Phylloscopidae, are paraphyletic (Figure 2; Olsson et al. 2004). The family will therefore likely be split into multiple genera that better reflect their evolutionary relationships (P. Alström *pers. comm.*, Gill & Donsker 2017).

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Author contributions

H. K. trapped the bird and wrote the description; A. B. K. researched previous records and contributed to the manuscript; M. S. performed the lab work and the phylogenetic analyses, and wrote the manuscript.

References

Alcaide, M., Scordato, E. S., Price, T. D. & Irwin, D. E. 2014. Genomic divergence in a ring species complex. *Nature* 511: 83–5.

BOU (British Ornithologists' Union) 2009. Records Committee: 37th Report. *Ibis* 151: 224–230.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C-H., Xie, D., Suchard, MA., Rambaut, A. & Drummond, A. J. 2014. BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. *PLoS Computational Biology* 10(4): e1003537.

Clements, J. F., Schulenberg, T. S., Iliff, M. J., Sullivan, B. L. & Wood C. L. 2009. *The eBird/Clements checklist of birds of the world: v2009*. URL: http://www.birds.cornell.edu/clementschecklist/updateindex/dec09overview/

Clements, J. F., Schulenberg, T. S., Iliff, M. J., Sullivan, B. L., Wood C. L., Fredericks, T. A. & Roberson, D. 2014. *The eBird/Clements checklist of birds of the world: v2014*. URL: http://www.birds.cornell.edu/clementschecklist/2014-overview/

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772–772.

Fregin, S., Haase, M., Olsson, U. & Alström, P. 2009. Multilocus phylogeny of the family Acrocephalidae (Aves: Passeriformes) – the traditional taxonomy overthrown. *Molecular Phylogenetics and Evolution* 52: 866–878.

Gill, F. & Donsker, D. (eds). 2017. *IOC World Bird List (v 7.2)*. DOI: 10.14344/IOC.ML.7.2.

Gill, F. & Wright, M. (eds). 2006. *Birds of the World: Recommended English Names*. Christopher Helm, London.

Guindon, S. & Gascuel, O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696–704.

Gätke, H. 1900. *Die Vogelwarte Helgoland*. 2. Aufl. Joh. Heinr. Meyer, Braunschweig.

Hasegawa, M., Kishino, H. & Yano, T.A. 1985. Dating of the Human Ape Splitting by a Molecular Clock of Mitochondrial-DNA. *Journal of Molecular Evolution* 22: 160–174.

Hellenic Rarities Committee 2006. *Annual Report – 2005*. URL: http://rarities.ornithologiki.gr/gr/eaop/annual_reports.htm.

Hellenic Rarities Committee 2009. *Annual Report – 2008*. URL: http://rarities.ornithologiki.gr/gr/eaop/annual_reports.htm.

Hudson, N. 2010. Green Warbler on Scilly: new to Britain. *British Birds* 103: 93–99.

del Hoyo, J., Elliott, A., Christie, D. A. (eds.) 2006. *Handbook of the Birds of the World. Vol. 11. Old World Flycatchers to Old World Warblers*. Lynx Edicions, Barcelona.

IRDC 2016. Israel Rarities and Distribution Committee. URL: http://www.israbirding.com/irdc/bulletins/bulletin_10/.

Irwin, D. E., Bensch, S. & Price, T. D. 2001. Speciation in a ring. *Nature* 409: 333–337.

Irwin, D. E. & Hellström, M. 2007. Green Warbler *Phylloscopus (trochiloides) nitidus* recorded at Ottenby, Öland: a first record for Scandinavia. *Ornis Svecica* 17: 75–80.

Katoh, K., Misawa, K., Kuma, K. & Miyata, T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059–3066.

Knox, A. G., Collinson, J. M., Parkin, D. T., Sangster, G. & Svensson, L. 2008. Taxonomic recommendations for British birds: Fifth report. *Ibis* 150: 833–835.

Kumar, S., Stecher, G. & Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33(7): 1870–1874.

Milvus Group 2016. URL: http://milvus.ro/en/12-146-depasari-inelate-in-2016-pe-grindul-chituc/8640 (accessed on 23 November 2016).

Olsson U., Alström, P. & Sundberg, P. 2004. Non-monophyly of the avian genus *Seicercus* (Aves: Sylviidae) revealed by mitochondrial DNA. *Zoologica Scripta* 33(6): 501–510.

Pennington, M. 2016. Green Warbler at Baltasound, Shetland, 12–15 May 2016 – first record for Scotland. *Scottish Birds* 36: 264–268.

Rambaut, A., Suchard, M.A. & Drummond, A.J. 2013. *Tracer v. 1.6*, available from http://tree.bio.ed.ac.uk/software/tracer/.

Rambaut, A. 2016. *FigTree v. 1.4.3*, available from http://tree.bio.ed.ac.uk/software/figtree/.

Rheindt, F. E. & Edwards, S.V. 2011. Genetic introgression: an integral but neglected component of speciation in birds. *Auk* 128(4): 620–632.

Schwarz, G. 1978. Estimating Dimension of a Model. *Annals of Statistics* 6: 461–464.

Slack, R. 2009. *Rare Birds Where and When. An Analysis of Status and Distribution in Britain and Ireland*. Volume 1. Rare Bird Books, York.

Stervander, M., Illera, J. C., Kvist, L., Barbosa, P., Keehnen, N. P., Pruisscher, P., Bensch, S. & Hansson, B. 2015. Disentangling the complex evolutionary history of the Western Palearctic blue tits (*Cyanistes* spp.) – phylogenomic analyses suggest radiation by multiple colonisation events and subsequent isolation. *Molecular Ecology* 24: 2477–2494.

Svensson, L. 1992. *Identification Guide to European Passerines*. 4th revised and enlarged edition. L. Svensson, Stockholm.

Sørensen, S. & Jensen, J. K. 2001. Sjældne fugle på Færøerne i 1997–2000. Rapport nr. 30 fra Sjældenhedsudvalget. *DOFT* 95(2): 44–50.

Väisänen, R., Huhtinen, H. Lampila P., Lehikoinen, A., Lehikoinen, P., Normaja, J. & Velmala, W. 2015: Rariteettikomitean hyväksymät vuoden 2014 harvinaisuushavainnot (Summary: Rare birds in Finland in 2014.) *Linnut – vuosikirja* 2014: 78–93.

Weir, J. T. & Schluter, D. 2008. Calibrating the avian molecular clock. *Molecular Ecology* 17: 2321–2328.

Sammanfattning

Den 27 maj 2016 fångades och ringmärktes en lundsångarliknande fågel vid Blåvands fågelstation, som är belägen vid Danmarks västligaste punkt. Datumet är tidigare än tyngdpunkten för lundsångarfynd vid Blåvand, och fågeln avvek något i dräkt från lundsångare *Phylloscopus trochiloides*. Redan vid fångsten misstänktes att fågeln var en kaukasisk lundsångare *Ph. nitidus*, men dessa är

svåra att säkert bestämma enbart baserat på morfologi. Färgsättningen skilde sig jämfört med lundsångare på så sätt att den aktuella fågeln hade en tydligare grön ovansida, utan gråton (Figur 1). Dessutom var ögonbrynsstrecket gult snarare än vitt och strupe samt övre bröst var gula, tydligt kontrasterande mot vitt nedre bröst och mage (Figur 1). Vingbandet, format av de större armtäckarnas ljusa spetsar, var bredare och tydligare jämfört med lundsångare (Figur 1).

Vid hanteringen tappade fågeln två dun, vilka användes för utvinning av DNA. Med hjälp av PCR mångfaldigades en DNA-sekvens från den maternellt nedärvda mitokondriegenen cytokrom *b*. En fylogenetisk analys av denna sekvens, tillsammans med alla publikt tillgängliga cytokrom *b*-sekvenser från fåglar inom släktena *Phylloscopus/Seicercus/ Abrornis* (olika släktesnamn som används för en och samma monofyletiska klad) samt utgrupper (taxa från släktena *Cettia/Horornis, Aegithalos, Acrocephalus, Cisticola* och *Sylvia*) bekräftar att fågeln var en kaukasisk lundsångare (Figur 2).

Det fylogenetiska trädet (Figur 2) belyser ett par speciella förhållanden. *Phylloscopus* och *Seicercus* är båda parafyletiska släkten, dvs. de omfattar inte bara taxa som är närmast besläktade med varandra och bildar obrutna monofyletiska grupper. I stället förekommer arter som kallas *Phylloscopus* och *Seicercus* på olika ställen i fylogenin (Figur 2). Därmed är en taxonomisk revision att vänta för hela familjen Phylloscopidae. Dessutom uppvisar lundsångarkomplexet, med lundsångare, kaukasisk lundsångare och sibirisk lundsångare *Ph. plumbeitarsus*, ett osedvanligt komplicerat inbördes förhållande. Medan den kaukasiska lundsångaren har ett isolerat utbredningsområde huvudsakligen i Kaukasus och norra Iran återfinns lundsångaren och den sibiriska lundsångaren öster därom i en bruten ring kring Tibetanska högplatån. De två arterna möts, utan att blandas, i östra Sibirien, medan urskiljbara populationer av lundsångaren förekommer med en kontinuerlig utbredning motsols till platåns östra sida. Tidigare, med stöd av analyser av mitokondriellt DNA och sång, har detta komplex tolkats som representanter för en s.k. ringart, där en art spridits och differentierats kontinuerligt längs en ring, så att ändarna, där de möts, är fullgoda arter, medan populationerna dem emellan endast skiljer sig gradvis. Nyare studier, baserade på analyser av stora delar av cellkärnans arvsmassa hos flera av arterna, visar att det inte alls handlar om en ringart, utan att flera av populationerna runt ringen tidvis har varit isolerade. Hybridisering och introgression av genetiskt material mellan arterna har skapat ett komplext fylogenetiskt mönster, där det mitokondriella fylogenetiska trädet inte speglar artträdet (Figur 2).

Fyndet i Blåvand utgör Danmarks första fynd av kaukasisk lundsångare, Fennoskandias tredje – efter fynd på Öland den 29 maj 2003 och Åland den 20 maj 2012 – och Europas åttonde fynd (Tabell 1) utanför ett begränsat och perifert europeiskt häckningsområde i södra Ryssland och norra Georgien.

Appendix 1

Taxa and GenBank accession number for cytochrome *b* sequences included in the phylogenetic analysis.
Taxa och GenBank-nummer för de cytokrom b*-sekvenser som inkluderats i den fylogenetiska analysen.*

Taxon ***Taxon***	**Accession no** ***Genbank-nr***
Abrornis chloronotus	KJ456391
Abrornis chloronotus simlaensis	DQ008504
Abrornis humei	KJ456394
Abrornis humei humei	Z73488
Abrornis inornata	AY635054
Abrornis inornata	DQ792799
Abrornis inornata	DQ792800
Abrornis inornata	KF742677
Abrornis inornata	L77135
Abrornis inornata	NC_024726
Abrornis inornata	Y10734
Abrornis maculipennis	HQ608828
Abrornis maculipennis	KJ456395
Abrornis maculipennis	Y10731
Abrornis maculipennis maculipennis	AY635055
Abrornis proregulus	AY635058
Abrornis proregulus	DQ792796
Abrornis proregulus	DQ792797
Abrornis proregulus	DQ792798
Abrornis proregulus	HQ608830
Abrornis proregulus	L77134
Abrornis proregulus	Y10733
Abrornis yunnanensis	HQ608833
Acrocephalus schoenobaenus	Z73475
Acrocephalus scirpaceus scirpaceus	Z73483
Aegithalos concinnus	JX398849
Aegithalos concinnus concinnus	KF951091
Cettia fortipes	L77122
Cisticola juncidis	Z73474
Horornis diphone	AB159194
Horornis diphone	AB159195
Horornis diphone	AB159196
Horornis diphone	AB159197
Horornis diphone	AB159198
Horornis diphone	AB694915
Horornis diphone	AB694916
Horornis diphone	HQ608838
Horornis seebohmi	AB281094
Horornis seebohmi	AB281095
Horornis seebohmi	AB281096
Phylloscopus affinis	DQ008503
Phylloscopus affinis	KJ456389
Phylloscopus affinis	L77128
Phylloscopus affinis	Y10730
Phylloscopus amoenus	AY887676
Phylloscopus armandii	HQ608831
Phylloscopus bonelli	AY944179
Phylloscopus bonelli	L77127
Phylloscopus bonelli bonelli	Z73485
Phylloscopus bonelli bonelli	Z73486

Taxon ***Taxon***	**Accession no** ***Genbank-nr***
Phylloscopus reguloides reguloides	AY656235
Phylloscopus reguloides reguloides	AY656236
Phylloscopus reguloides reguloides	AY656237
Phylloscopus reguloides reguloides	AY656238
Phylloscopus reguloides ticehursti	AY656205
Phylloscopus reguloides ticehursti	AY656206
Phylloscopus reguloides ticehursti	AY656207
Phylloscopus reguloides ticehursti	AY656233
Phylloscopus reguloides ticehursti	AY656234
Phylloscopus reguloides ticehursti	AY656242
Phylloscopus reguloides ticehursti	AY656243
Phylloscopus ricketti	AY656239
Phylloscopus ricketti	GU045622
Phylloscopus ricketti	GU045623
Phylloscopus ricketti	GU045624
Phylloscopus ricketti	GU045625
Phylloscopus ruficapilla minullus	AY635060
Phylloscopus sarasinorum sarasinorum	AY656240
Phylloscopus schwarzi	AY635061
Phylloscopus schwarzi	HQ608825
Phylloscopus schwarzi	Y10728
Phylloscopus sibilatrix	AY944178
Phylloscopus sibilatrix	L77123
Phylloscopus sibilatrix	Z73491
Phylloscopus sindianus	L77136
Phylloscopus sindianus lorenzii	HQ706180
Phylloscopus sindianus lorenzii	Z73478
Phylloscopus subviridis	KJ456398
Phylloscopus trivirgatus	L77145
Phylloscopus trivirgatus trivirgatus	AY656244
Phylloscopus trochiloides	HQ608824
Phylloscopus trochiloides	KP267728
Phylloscopus trochiloides	KP267729
Phylloscopus trochiloides	L77126
Phylloscopus trochiloides	L77130
Phylloscopus trochiloides	Y10739
Phylloscopus trochiloides trochiloides	AY903608
Phylloscopus trochiloides viridanus	EF502040
Phylloscopus trochiloides viridanus	Z73493
Phylloscopus trochilus	AJ004326
Phylloscopus trochilus	AY944177
Phylloscopus trochilus	DQ174582
Phylloscopus trochilus	DQ174583
Phylloscopus trochilus	DQ174584
Phylloscopus trochilus	DQ174585
Phylloscopus trochilus	DQ174586
Phylloscopus trochilus	DQ174587
Phylloscopus trochilus	DQ174588
Phylloscopus trochilus	DQ174589
Phylloscopus trochilus	DQ174590

Phylloscopus borealoides	AB362459
Phylloscopus borealoides	AB362467
Phylloscopus borealoides	AY887677
Phylloscopus brehmi	AJ004325
Phylloscopus brehmi	Z73476
Phylloscopus calciatilis	GU045613
Phylloscopus calciatilis	GU045614
Phylloscopus calciatilis	GU045615
Phylloscopus calciatilis	GU045616
Phylloscopus calciatilis	GU045617
Phylloscopus calciatilis	GU045618
Phylloscopus calciatilis	GU045619
Phylloscopus calciatilis	GU045620
Phylloscopus calciatilis x ricketti	GU045621
Phylloscopus canariensis	Z73477
Phylloscopus cantator	KJ456390
Phylloscopus cantator cantator	AY656208
Phylloscopus cebuensis	JN827147
Phylloscopus cebuensis	L77144
Phylloscopus claudiae	AY656209
Phylloscopus claudiae	AY656210
Phylloscopus claudiae	AY656211
Phylloscopus claudiae	AY656212
Phylloscopus claudiae	AY656213
Phylloscopus collybita	AJ004324
Phylloscopus collybita	HQ608821
Phylloscopus collybita	L77125
Phylloscopus collybita abietinus	DQ174604
Phylloscopus collybita abietinus	DQ174605
Phylloscopus collybita abietinus	DQ174606
Phylloscopus collybita abietinus	DQ174607
Phylloscopus collybita abietinus	DQ174608
Phylloscopus collybita abietinus	HQ121526
Phylloscopus collybita abietinus	Z73479
Phylloscopus collybita brevirostris	Z73480
Phylloscopus collybita caucasicus	Z73481
Phylloscopus collybita collybita	Z73487
Phylloscopus collybita menzbieri	AF136374
Phylloscopus collybita tristis	HF562844
Phylloscopus collybita tristis	HF562845
Phylloscopus collybita tristis	HF562846
Phylloscopus collybita tristis	Z73482
Phylloscopus coronatus	AB362460
Phylloscopus coronatus	AY635053
Phylloscopus coronatus	HQ608834
Phylloscopus coronatus	L77139
Phylloscopus davisoni	AY656214
Phylloscopus davisoni disturbans	AY656217
Phylloscopus davisoni klossi	AY656215
Phylloscopus emeiensis	AY656218
Phylloscopus fuligiventer	KJ456392
Phylloscopus fuscatus	DQ119527
Phylloscopus trochilus	DQ174591
Phylloscopus trochilus	DQ174592
Phylloscopus trochilus	DQ174593
Phylloscopus trochilus	DQ174594
Phylloscopus trochilus	DQ174595
Phylloscopus trochilus	DQ174596
Phylloscopus trochilus	DQ174597
Phylloscopus trochilus	DQ174598
Phylloscopus trochilus	JX869889
Phylloscopus trochilus	L77124
Phylloscopus trochilus trochilus	DQ673875
Phylloscopus trochilus trochilus	Z73492
Phylloscopus tytleri	AY887679
Phylloscopus tytleri	L77132
Seicercus affinis	KJ456460
Seicercus affinis intermedius	AY635066
Seicercus affinis intermedius	AY635069
Seicercus affinis intermedius	AY635070
Seicercus affinis intermedius	AY635071
Seicercus affinis ocularis	AY635063
Seicercus borealis	HQ608832
Seicercus borealis	L77138
Seicercus borealis	L77143
Seicercus borealis borealis	AB362438
Seicercus borealis borealis	AB362439
Seicercus borealis borealis	AB362440
Seicercus borealis borealis	AB362441
Seicercus borealis borealis	AB362442
Seicercus borealis borealis	AB362443
Seicercus borealis borealis	AB362444
Seicercus borealis borealis	AB362445
Seicercus borealis borealis	AB362446
Seicercus borealis borealis	AB362462
Seicercus borealis borealis	AB530997
Seicercus borealis borealis	AB530998
Seicercus borealis borealis	AB530999
Seicercus borealis borealis	AB531000
Seicercus borealis borealis	AB531001
Seicercus borealis borealis	AB531002
Seicercus borealis borealis	AB531003
Seicercus borealis borealis	AY635052
Seicercus borealis borealis	HQ243658
Seicercus borealis borealis	HQ243659
Seicercus borealis borealis	HQ243660
Seicercus borealis borealis	KP245888
Seicercus borealis borealis	Z73484
Seicercus borealis kennicotti	AB362435
Seicercus borealis kennicotti	AB362436
Seicercus borealis kennicotti	AB362437
Seicercus borealis kennicotti	AB362461
Seicercus burkii	AY635064
Seicercus burkii	AY635065

Phylloscopus fuscatus	DQ174599
Phylloscopus fuscatus	DQ174600
Phylloscopus fuscatus	DQ174601
Phylloscopus fuscatus	DQ174602
Phylloscopus fuscatus	DQ174603
Phylloscopus fuscatus	HQ608823
Phylloscopus fuscatus	Y10729
Phylloscopus goodsoni fokiensis	AY656219
Phylloscopus goodsoni fokiensis	AY656220
Phylloscopus goodsoni fokiensis	AY656221
Phylloscopus goodsoni fokiensis	AY656222
Phylloscopus goodsoni fokiensis	AY656223
Phylloscopus goodsoni goodsoni	AY656225
Phylloscopus goodsoni goodsoni	AY656226
Phylloscopus goodsoni goodsoni	AY656227
Phylloscopus goodsoni goodsoni	AY656228
Phylloscopus griseolus	KJ456393
Phylloscopus hainanus	AY656229
Phylloscopus magnirostris	AY635056
Phylloscopus magnirostris	AY887681
Phylloscopus magnirostris	HQ608822
Phylloscopus magnirostris	L77129
Phylloscopus magnirostris	Y10737
Phylloscopus nigrorum benguetensis	AY656204
Phylloscopus nitidus	EF502039
Phylloscopus nitidus	Z73489
Phylloscopus occipitalis	AY635057
Phylloscopus occipitalis	EU372678
Phylloscopus occipitalis	L77131
Phylloscopus occipitalis	Y10735
Phylloscopus occisinensis	HQ608829
Phylloscopus ogilviegranti ogilviegranti	AY656216
Phylloscopus orientalis	AY887678
Phylloscopus orientalis	Z73490
Phylloscopus plumbeitarsus	AB362468
Phylloscopus plumbeitarsus	AY656230
Phylloscopus plumbeitarsus	Y10740
Phylloscopus poliocephalus giulianetti	AY656224
Phylloscopus pulcher	HQ608826
Phylloscopus pulcher	KJ456396
Phylloscopus pulcher	KJ567540
Phylloscopus pulcher	KJ567541
Phylloscopus pulcher	KJ567542
Phylloscopus pulcher	KJ567543
Phylloscopus pulcher	KJ567544
Phylloscopus pulcher	KJ567545
Phylloscopus pulcher	KJ567546
Phylloscopus pulcher	KJ567547
Phylloscopus pulcher	KJ567548
Phylloscopus pulcher	KJ567549
Phylloscopus pulcher	KJ567550
Phylloscopus pulcher	KJ567551
Seicercus burkii	HQ608856
Seicercus castaniceps	AY887680
Seicercus castaniceps	HQ608857
Seicercus castaniceps	KJ456461
Seicercus castaniceps castaniceps	AY635067
Seicercus examinandus	AB362424
Seicercus examinandus	AB362425
Seicercus examinandus	AB362426
Seicercus examinandus	AB362427
Seicercus examinandus	AB362428
Seicercus examinandus	AB362429
Seicercus examinandus	AB362430
Seicercus examinandus	AB362431
Seicercus examinandus	AB362432
Seicercus examinandus	AB362433
Seicercus examinandus	AB362434
Seicercus examinandus	AB362463
Seicercus examinandus	AB362464
Seicercus examinandus	KP245873
Seicercus examinandus	KP245874
Seicercus examinandus	KP245875
Seicercus examinandus	KP245876
Seicercus examinandus	KP245877
Seicercus examinandus	KP245878
Seicercus examinandus	KP245879
Seicercus examinandus	KP245880
Seicercus examinandus	KP245881
Seicercus examinandus	KP245882
Seicercus examinandus	KP245883
Seicercus examinandus	KP245884
Seicercus examinandus	KP245885
Seicercus examinandus	KP245886
Seicercus examinandus	KP245887
Seicercus examinandus	KP245895
Seicercus examinandus	KP245896
Seicercus examinandus	KP245897
Seicercus examinandus	KP245898
Seicercus grammiceps grammiceps	AY635068
Seicercus ijimae	L77141
Seicercus ijimae	Y10741
Seicercus montis montis	AY635073
Seicercus omeiensis	AY635078
Seicercus poliogenys	AY635079
Seicercus poliogenys	AY635080
Seicercus poliogenys	AY635081
Seicercus poliogenys	KJ456462
Seicercus soror	AY635082
Seicercus soror	AY635083
Seicercus soror	KM875497
Seicercus tenellipes	AY903607
Seicercus tenellipes	L77140
Seicercus tenellipes	Y10738

Phylloscopus pulcher	KJ567552
Phylloscopus pulcher	KJ567553
Phylloscopus pulcher	KJ567554
Phylloscopus pulcher	KJ567555
Phylloscopus pulcher	KJ567556
Phylloscopus pulcher	KJ567557
Phylloscopus pulcher	KJ567558
Phylloscopus pulcher	KJ567559
Phylloscopus pulcher	KJ567560
Phylloscopus pulcher	KJ567561
Phylloscopus pulcher	KJ567562
Phylloscopus pulcher	KJ567563
Phylloscopus pulcher	KJ567564
Phylloscopus pulcher	KJ567565
Phylloscopus pulcher	KJ567566
Phylloscopus pulcher	KJ567567
Phylloscopus pulcher	KJ567568
Phylloscopus pulcher	KJ567569
Phylloscopus pulcher	KJ567570
Phylloscopus pulcher	KJ567571
Phylloscopus pulcher	KJ567572
Phylloscopus pulcher	KJ567573
Phylloscopus pulcher	KJ567574
Phylloscopus pulcher	KJ567575
Phylloscopus pulcher	KJ567576
Phylloscopus pulcher	KJ567577
Phylloscopus pulcher	KJ567579
Phylloscopus pulcher	KJ567580
Phylloscopus pulcher	KJ567581
Phylloscopus pulcher	KJ567582
Phylloscopus pulcher	KJ567583
Phylloscopus pulcher	KJ567584
Phylloscopus pulcher	KJ567585
Phylloscopus pulcher	KJ567586
Phylloscopus pulcher	KJ567587
Phylloscopus pulcher	KJ567588
Phylloscopus pulcher	KJ567589
Phylloscopus pulcher	KJ567590
Phylloscopus pulcher	KJ567592
Phylloscopus pulcher	KJ567594
Phylloscopus pulcher	KJ567595
Phylloscopus pulcher	KJ567596
Phylloscopus pulcher	L77133
Phylloscopus pulcher	Y10732
Phylloscopus pulcher pulcher	AY635059
Phylloscopus reguloides	HQ608827
Phylloscopus reguloides	KJ456397
Phylloscopus reguloides	KM248527
Phylloscopus reguloides	KM248529
Phylloscopus reguloides	Y10736
Phylloscopus reguloides assamensis	AY656231
Phylloscopus reguloides assamensis	AY656232
Seicercus tephrocephalus	AY635084
Seicercus tephrocephalus	AY635085
Seicercus tephrocephalus	HQ706182
Seicercus umbrovirens mackenzianus	AY635062
Seicercus valentini latouchei	AY635072
Seicercus valentini latouchei	AY635086
Seicercus valentini latouchei	AY635088
Seicercus valentini valentini	AY635087
Seicercus valentini valentini	AY635089
Seicercus whistleri	KJ456463
Seicercus whistleri nemoralis	AY635074
Seicercus whistleri nemoralis	AY635076
Seicercus whistleri whistleri	AY635075
Seicercus whistleri whistleri	AY635077
Seicercus whistleri whistleri	AY635090
Seicercus xanthodryas	AB362447
Seicercus xanthodryas	AB362448
Seicercus xanthodryas	AB362449
Seicercus xanthodryas	AB362450
Seicercus xanthodryas	AB362451
Seicercus xanthodryas	AB362452
Seicercus xanthodryas	AB362453
Seicercus xanthodryas	AB362454
Seicercus xanthodryas	AB362455
Seicercus xanthodryas	AB362456
Seicercus xanthodryas	AB362457
Seicercus xanthodryas	AB362458
Seicercus xanthodryas	AB362465
Seicercus xanthodryas	AB362466
Seicercus xanthoschistos tephrodiras	AY656246
Seicercus xanthoschistos xanthoschistos	AY635091
Sylvia atricapilla atricapilla	Z73494
Sylvia melanocephala	L77121