Phylogeny of the Oenanthe lugens complex (Aves, Muscicapidae: Saxicolinae): Paraphyly of a morphologically cohesive group within a recent radiation of open-habitat chats

ARTICLE in MOLECULAR PHYLOGENETICS AND EVOLUTION · AUGUST 2013
Impact Factor: 3.92 · DOI: 10.1016/j.ympev.2013.08.010 · Source: PubMed

CITATIONS
3

READS
119

2 AUTHORS, INCLUDING:

Manuel Schweizer
Natural History Museum Bern
48 PUBLICATIONS 241 CITATIONS

Available from: Manuel Schweizer
Retrieved on: 26 December 2015
Phylogeny of the *Oenanthe lugens* complex (Aves, Muscicapidae: Saxicolinae): Paraphyly of a morphologically cohesive group within a recent radiation of open-habitat chats

Manuel Schweizer\(^{a,\ast}\), Hadoram Shirihai\(^b\)

\(^a\) Naturhistorisches Museum der Burgergemeinde Bern, Bernastrasse 15, CH 3005 Bern, Switzerland
\(^b\) c/o Ausserdorfstrasse 6, CH 8052 Zürich, Switzerland

**A R T I C L E  I N F O**

Article history:
Received 26 April 2013
Revised 9 August 2013
Accepted 16 August 2013
Available online 28 August 2013

**Keywords:**
Molecular phylogeny
Taxonomy
Saxicolinae
Oenanthe
Biogeography
Pleistocene

**A B S T R A C T**

The morphologically inferred *Oenanthe lugens* complex comprises nine taxa of open-habitat chats which occur in rocky and/or mountainous areas adjacent to the Sahara-Sindian desert belt. It has traditionally been divided into the *lugubris* group of north-east Africa, the *lugentoides* group of the southern part of the Arabian Peninsula and the *lugens* group of North Africa and the Middle East. Previous molecular phylogenetic studies have shown that the *O. lugens* complex might not be monophyletic. However, it remained unclear how this result might have been affected by incomplete taxon sampling, as the *lugentoides* group and two out of three taxa of the *lugubris* group have not been analyzed so far. In this study, we present a phylogenetic hypothesis of the *O. lugens* complex based on two mitochondrial genes and one nuclear intron using, for the first time, a complete taxon sampling. The application of a multispecies coalescent approach allowed us to simultaneously estimate the sequence and timing of speciation events. The *O. lugens* complex was consistently revealed as a polyphyletic assemblage and the traditionally recognized groups should be treated as at least three different species: *O. lugens*, *Oenanthe lugubris*, and *Oenanthe lugentoides*. While *O. lugubris* and *O. lugentoides* were revealed to be sister groups, *O. lugens* was found to be closely related to the species pair *Oenanthe chrysopygia*/*Oenanthe xanthophryna*. The latter differ quite strongly in morphology and have traditionally not been associated with members of the *lugens* complex. We thus corroborate the results of previous studies, which demonstrated that morphology seems to be a poor predictor of phylogenetic relationships in *Oenanthe*. In contrast to the mtDNA markers analyzed, it was revealed that differences among taxa were not fixed in the nuclear intron. In the case of the taxa perica of the *lugens* group, an influence of introgression in autosomal markers cannot be excluded and deserves further study. The three species *O. lugens*, *O. lugubris*, and *O. lugentoides* and their associated taxa comprise a comparatively young radiation, which started to diversify in the Pliocene with major diversification events during the Pleistocene. The different taxa seem to have evolved during periods of increased aridity in isolation in rocky mountainous areas adjacent to hyper arid regions.

\(\ast\) Corresponding author. Fax: +41 31 350 74 99.

E-mail address: manuel.schweizer@nmbe.ch (M. Schweizer).

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Arid biomes are widespread on earth today. In most regions, however, an expansion of open habitats only began in the middle Miocene with an increased stepwise drying up since the Pliocene (Byrne et al., 2008; DeMenocal, 2004; Douady et al., 2003; Flower and Kennett, 1994; Jacobs, 2004). This aridification not only led to a fragmentation of mesic biomes and associated species, but also triggered the evolution of arid-adapted open-habitat taxa from mesic-adapted ancestors (cf. Byrne et al., 2008). Speciation also occurred within arid biomes, as the most arid parts of deserts can even act as dispersal barriers and drivers of vicariance to arid-adapted species (e.g. Ben Faleh et al., 2012; Guillaumet et al., 2008). Moreover, phases of increased temperatures in the early Pliocene were associated with a loss of Savanna connectivity in Africa, leading to vicariant speciation among species adapted to open habitats (e.g. Outlaw et al., 2007; Voelker, 2002; Voelker et al., 2012). A group characteristic of the arid and rocky habitats of Africa and Eurasia are the open-habitat chats of the traditionally recognized genera *Campicolaoides*, *Cercomela*, *Myrmecocichla*, *Oenanthe* and *Thamnolaena*. Several recently published molecular studies have shed light on their phylogenetic relationships (Aliabadian et al., 2012, 2007b; Förtschler et al., 2010; Outlaw et al., 2010; Sangster et al., 2010; Voelker et al., 2012). Most interestingly, the genera *Cercomela*, *Myrmecocichla* and *Oenanthe* were revealed to be polyphyletic assemblages suggesting the convergent evolution of morphological and ecological features among the different clades (cf.
Despite this progress in our understanding of the evolution of open-habitat chats, the phylogenetic relationships of some groups within *Oenanthe s. l.* are still not resolved, not least because of the lack of complete taxon sampling in previous studies. One example is the mourning wheatear *Oenanthe lugens* complex (*lugens* complex hereafter), which has provided a taxonomic conundrum for decades. The taxa belonging to the *lugens* complex occur in rocky and/or mountainous areas adjacent to the Saharo-Sindian desert belt (Fig. 1). On the basis of morphological characters, it has traditionally been considered to comprise eight to nine subspecies divided into three groups (Collar, 2005; Shirihai et al., 2011; Tye, 1989) (Table 1).

The eight to nine taxa of the *lugens* complex have variously been treated as belonging to a single species, *O. lugens* (Collar, 2005; Dean et al., 1992; Tye, 1989), as conspecific with *O. finschii* (Dementieff et al., 1968) or divided into different species assemblages (e.g. Dickinson, 2003; Panov, 2005) (Table 2). The taxon *halophila* of the *lugens* complex has additionally been considered as a distinct species by some authors (e.g. Svensson et al., 2009), and Redman et al. (2009) proposed species status for the taxon *schalowi*. For simplification, the taxa of the *lugens* complex are hereafter referred to by their subspecies name only.

Previous molecular phylogenetic studies have revealed a different pattern, however, as the *lugens* complex was found to be polyphyletic. The taxon *persica*, was revealed to be more closely related to the species-pair *O. xanthopyrnan*–*O. chrysoptera* than to *lugens* or *halophila* (Aliabadian et al., 2012; Förschler et al., 2010). Moreover, *lugubris* seemed to be more closely related to *O. leucopygna* and *O. albonigra* than to the other members of the *lugens* complex (Aliabadian et al., 2012). However, this result may have been influenced by incomplete taxon sampling, as the other members of the *lugubris* group (i.e. *schalowi* and *vauriei*) as well as the taxa belonging to the *lugentoides* group have never been included in molecular phylogenetic studies. A comparison of the results of the different molecular phylogenetic studies on open-habitat chats indeed demonstrate the importance of a robust taxon sampling in such a challenging group (cf. Aliabadian et al., 2012; Outlaw et al., 2010).

The aim of the present study was therefore to infer the relationships of the taxa traditionally assigned to the *lugens* complex for the first time using a complete taxon sampling based on mitochondrial and nuclear markers. There was no fresh tissue available for some of the taxa which occur in politically sensitive areas and hence we had to rely on toe-pad samples from museum specimens for them. We moreover included all taxa which clustered in the same clade as the taxa of the *lugens* complex analyzed so far in previous studies. Current molecular studies on open-habitat chats have relied on traditional phylogenetic tree reconstruction methods based on concatenated datasets of several genes (‘genetree analyses’). Especially in apparently rather young taxa such as the species within *Oenanthe* however, there is a huge potential for discrepancies between gene and species trees. We thus implemented a Bayesian multispecies coalescent approach (Heled and Drummond, 2010) to infer the relationship among the members of the *lugens* complex and associated taxa. This method also allowed us to estimate divergence time between species and to identify potential drivers of diversification.

2. Materials and methods

2.1. Species sampling and laboratory protocols

We sampled several individuals of all taxa traditionally included in the *lugens* complex (Table 3). We used toe-pad samples from museum specimens for several taxa for which no fresh tissue or blood samples were available (cf. Table 3). The identification of
sampled specimens in the wild and from museum collections was carefully checked by one of the authors based on morphological features (HS). Our dataset was additionally completed with sequences taken from GenBank for all potentially associated taxa according to the results of Aliabadian et al. (2012) (Table 3). Cercro-mela fusca and Oenanthe picata were used as outgroups in phyloge-netic analyses throughout the study (cf. Aliabadian et al., 2012).

Total genomic DNA from blood samples was isolated with the Wizard® SV Genomic DNA Purification System (Promega) following the manufacturer’s instructions. For the isolation of DNA from toe-pad samples of museum skins, the <i>seadex®</i> forensic kit (LGC Genomics) was used. The isolation of DNA and the preparation of PCR reactions for these toe-pad samples took place in a separate laboratory with different lab equipment than for the fresh blood samples to avoid potential contamination. In addition, laboratory equipment used for the handling of these samples was treated with DNA Away™ ( Molecular BioProducts) solution and/or irradiated with ultraviolet light prior to use.

Partial sequences of the two mitochondrial genes COI (cytochrome oxidase subunit I) and ND2 (NADH, NADH dehydrogenase subunit II) and the nuclear ODC (ornithine decarboxylase) Intron 6 with flanking coding regions were amplified with polymerase chain reaction using published primers and primers specifically designed for this study (Table 4). Primers were designed with Primer3 0.4.0 (Rozen and Skaletsky, 2000) as implemented in Geneious Pro 5.6 (Drummond et al., 2011). Three overlapping fragments with a length of 199–284 bp were amplified in the two mtDNA genes for DNA extracted from toe-pad samples. Negative controls were used to check for potential contamination. Total PCR reaction volumes were 25 μl containing 12.5 μl GoTaq® Hot Start Green Master Mix (Promega), 2 μl genomic DNA, 2 μl of each primer with a concentration of 10 μM and 6.5 μl ddH₂O, or 50 μl containing 25 μl QIAGEN Multiplex PCR Kit (Qiajen), 3 μl genomic DNA, 1 μl of each primer with a concentration of 10 μM and 20 μl ddH₂O. PCR was performed on a Techne TC-512 thermo-cycler or a SensoQuest Labcycler. When using the GoTaq® Hot Start Green Master Mix, amplifications were performed with an initial denauration at 94 °C for three minutes, followed by 35 or 40 cycles of denaturation at 95 °C for 30 s, annealing for 30 s at 57 °C for COI and 58 °C for ND2, and extension at 72 °C for one minute, with a final extension at 72 °C for seven minutes. For the QIAGEN Multiplex PCR Kit, we applied an initial denaturation at 95 °C for 15 min followed by 40 cycles of denaturation at 94 °C for 30 s, annealing for 1.5 min at 57 °C, and extension at 72 °C for 1.5 min, with a final extension at 72 °C for 10 min. PCR products were examined by gel electrophoresis to confirm amplification of the target fragments and then excised from gels and cleaned using the Wizard® SV Gel and PCR Clean-UP System (Promega). The products of two independent PCR runs were usually placed together before cleaning to increase the quantity of DNA. Sequencing was carried out by LGC Genomics (Berlin, Germany) or Macrogen Inc. (Seoul, South Korea).

### 2.2 Phylogenetic analyses

Sequences were edited with Geneious Pro. Individual sequences were checked by searching for apparent stop codons after the translation of sequences into amino acids. We moreover analyzed each fragment sequenced from toe-pad samples separately to check for potential contamination and the inclusion of chimeric sequences as a consequence (cf. Moyle et al., 2013).

For ODC, the genotype of individuals with multiple heterozygous positions was inferred with PHASE 2.1 (Stephens and Donnelly, 2003). Input files for PHASE were generated with SeqPHASE (Flot, 2010). We used a threshold of 70% for phase probabilities and compared the results among five independent runs. Phase ambiguities were coded with IUPAC codes.

Bayesian inference (BI) was conducted using MrBayes 3.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003; Ronquist et al., 2012). We analyzed two datasets: both mtDNA genes together and a concatenated dataset consisting of both mtDNA genes and the nuclear Intron ODC (mtDNA-nuclear dataset). Only one sequence per individual for ODC could be analyzed in this approach and heterozygous positions among the
two alleles were coded with IUPAC ambiguity codes. Due to incomplete lineage sorting among species in ODC, we analyzed this marker separately with only an unrooted allele network (see below).

We used PartitionFinder 1.0.1 (Lanfear et al., 2012) to select the best-fitting partitioning schemes and models of nuclear evolution using the greedy algorithm with unlinked branch lengths for the mtDNA and the mtDNA-nuclear dataset. The BIC (Bayesian information criterion) was used as the optimality criterion for model selection.

In MrBayes, we then performed two independent runs of Metropolis-coupled Markov chain Monte Carlo analyses for the two datasets, with each run consisting of one cold chain and three heated chains with a default temperature of 0.2. The chains were run for 20 million generations with sampling every 100 generations. We checked that the average standard deviation of split frequencies converged towards zero and estimated the length of the burn-in by visually inspecting trace files with TRACER 1.5 (Rambaut and Drummond, 2007) and by monitoring the change in cumulative split frequencies using AWTY (Nylander et al., 2008; Wilgenbusch et al., 2004). The first 25% of samples were then discarded as burn-in well after the chains had reached stationarity. Additionally, we compared likelihoods and posterior probabilities of all parameters and splits to assess convergence between the two independent runs using TRACER and AWTY.

Furthermore, we employed a maximum-likelihood (ML) search using RAxML 7.0.4 (Stamatakis, 2006). The software was run on the web server with 100 rapid bootstrap inferences (Stamatakis et al., 2003) with all free model parameters estimated by the software (substitution rates, gamma shape parameter and base frequencies) using the best partitioning scheme as obtained with PartitionFinder.

Clades were considered to be supported by our analyses when bootstrap values for ML search were ≥70% (Hillis and Bull, 1993) and clade credibility values for the BI ≥0.95 (Huelsenbeck and Ronquist, 2001).

To graphically illustrate the relationships between the different ODC alleles as inferred with PHASE, we built an unrooted median-joining haplotype network using the software Networks 4.6.1 at default parameters (Bandelt et al., 1999).

We further used the multispecies coalescent algorithm ‘BEAST’ as implemented in Beast 1.7.4 (Drummond and Rambaut, 2007; Heled and Drummond, 2010) to infer a species tree from the three markers analyzed. The different taxa as inferred from morphological designation were defined a priori as ‘species’. As ‘BEAST can handle different numbers of individuals/gene copies for the different markers analyzed, we included both alleles of the nuclear intron for each individual in the analyses. We used unlinked clock and substitution models for the two mtDNA markers together versus the nuclear intron. Substitution models were selected in MrModeltest 2.2 (Nylander, 2004). A Yule process on species trees was implemented in all analyses. For the mtDNA, we implemented a normal prior for the substitution rate (ucld.mean parameter) using the robust overall divergence rate of 2.1 ± 0.1% per million year (Ma) (0.0105 ± 0.0005 substitution/site/Ma) as estimated across 12 orders of birds in a time period which is relevant for our study (Weir and Schluter, 2008). Although this rate was calculated for cytochrome b, we assume that the implemented range is wide enough to cover the variability found among different mtDNA markers. For the nuclear intron, we used a mean rate of 0.00135 ± 0.00045 substitution/site/Ma (Ellegren, 2007; Smith et al., 2013) again implemented as a normal prior. A relaxed uncorrelated lognormal distribution for the rates was used in preliminary runs to check if a strict molecular clock was appropriate for one of the datasets. The posterior distribution of the standard deviation of the substitution rate parameter was revealed to include zero for the nuclear intron. We therefore implemented a strict clock for this dataset in the final analyses. The MCMC analysis was run three times independently for 50 million generations with sampling every 1000 generations. We used TRACER to confirm appropriate burn-in and the adequate effective sample sizes (ESS) of the posterior distribution. We compared likelihoods and posterior probabilities of all parameters to assess convergence among the three independent runs using TRACER. The resulting maximum clade credibility tree and the 95% highest posterior density (HPD) distributions of each estimated node was calculated with TREEANNOTATOR 1.7.4 (Drummond and Rambaut, 2007) and visualized using FIGTREE 1.2. (Rambaut, 2008).

3. Results

3.1. Sequence characteristics

The final alignment consisted of 514 bp for COI, 544 bp for ND2 and 219 bp for ODC. Hence, the mtDNA dataset was 1058 bp in length and the mtDNA-nuclear dataset 1277 bp in length. The aligned sequences contained no indels and the translation of the two mtDNA genes into amino acids did not reveal any unexpected stop codons.

3.2. Phylogenetic analyses

PartitionFinder identified HKY + I + G as the best-fitting substitution model for both the mtDNA-nuclear and the mtDNA dataset. In the BI with MrBayes of the mtDNA-nuclear dataset (Fig. 2), the lugens complex was consistently revealed to be a polyphyletic assemblage. O. finschii was revealed to be the sister lineage of a clade consisting of the members of the lugens group as well as O. xanthophymma and O. chrysoejuga (clade 1, Fig. 2). Even the lugens group turned out not to be monophyletic. The taxon persica was found to be more closely related to a cluster consisting of the taxa O. chrysoejuga and O. xanthophymma than to the remaining members of the lugens group, although the cluster of the two persica samples was not robustly supported.

Halophila was found to be the sister group of a clade comprising lugens and warriae. The samples of the latter were nested within lugens. Within lugens, two clusters were robustly supported. However, they could not be associated with a particular locality or region and revealed no obvious geographic structure.

The lugubris and lugentoides groups were revealed to be each other’s closest relatives and both were found to be reciprocally monophyletic (clade 2, Fig. 2). However, the position of this clade was not robustly resolved. Lugentoides and boscaweni within the lugentoides group were not found to be monophyletic.

Within the lugubris group, the samples of vauriei and schalowi formed two well supported clades. However, the clade consisting of vauriei and schalowi and its position within lugubris was not robustly supported. Hence, lugubris was not monophyletic relative to vauriei and schalowi. Both samples of lugubris from Eritrea seemed to be more closely related to vauriei and schalowi than the other lugubris samples. However, the position of the different lineages within lugubris was not supported. The position of O. leucopyga, O. leucura and O. albignera could not be robustly resolved.

The results of the BI with MrBayes based on the mtDNA dataset revealed an almost identical tree topology to that of the mtDNA-nuclear dataset and there was no conflict between supported nodes (Fig. 2).

The resulting tree from the ML analyses of the mtDNA-nuclear dataset on the basis of a single substitution model was very similar to the BI with MrBayes with no conflict between supported nodes (Fig. 2). However, the clade of the two persica samples was robustly supported, while the clades within lugens gained no robust sup-
Table 3
Specimens sampled, collection locality, sample type, collection number and GenBank accession numbers for the three markers analyzed in this study. BMNH, Natural History Museum, Tring; IAR, Institute of Avian Research Wilhelmshaven, Vogelwarte Helgoland; MIUT, Museum of Isfahan University of Technology; NHMO, Natural History Museum, University of Oslo; NRM, Swedish Museum of Natural History; UMMZ, University of Michigan Museum of Zoology; ZMUC, Zoological Museum of the University of Copenhagen.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Locality</th>
<th>Sample Type</th>
<th>Museum Number</th>
<th>Field number</th>
<th>COI</th>
<th>ND2</th>
<th>ODC</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oenanthe lugens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oenanthe lugens halophila</td>
<td>IAR 103</td>
<td>Blood</td>
<td>ZMUC131966</td>
<td></td>
<td>KF512750</td>
<td>KF512787</td>
<td>KF512718</td>
<td></td>
</tr>
<tr>
<td>Oenanthe lugens warriae</td>
<td>NE Azraq, Jordan</td>
<td>Blood</td>
<td>NHMO 22655</td>
<td></td>
<td>HMO46858</td>
<td>JX256095</td>
<td>JX256179</td>
<td></td>
</tr>
<tr>
<td>Oenanthe lugens warriae</td>
<td>NE Azraq, Jordan</td>
<td>Blood</td>
<td>OWM001</td>
<td></td>
<td>KF512762</td>
<td>KF512799</td>
<td>KF512711</td>
<td></td>
</tr>
<tr>
<td>Oenanthe lugens warriae</td>
<td>NE Azraq, Jordan</td>
<td>Blood</td>
<td>A1006</td>
<td></td>
<td>KF512753</td>
<td>KF512790</td>
<td>KF512723</td>
<td></td>
</tr>
<tr>
<td>Oenanthe lugenoides boscavieni</td>
<td>Daun, Hadramaut</td>
<td>Toe pad</td>
<td>BMNH19345.14.12</td>
<td></td>
<td>KF512769</td>
<td>KF512806</td>
<td>KF512742</td>
<td></td>
</tr>
<tr>
<td>Oenanthe lugenoides boscavieni</td>
<td>Near Halaf, jabal Qara</td>
<td>Toe pad</td>
<td>BMNH197721.35</td>
<td></td>
<td>KF512778</td>
<td>KF512815</td>
<td>KF512743</td>
<td></td>
</tr>
<tr>
<td>Oenanthe lugenoides boscavieni</td>
<td>Dhala, 7000ft, Aden</td>
<td>Toe pad</td>
<td>BMNH1965.M.12141</td>
<td></td>
<td>KF512770</td>
<td>KF512807</td>
<td>KF512740</td>
<td></td>
</tr>
<tr>
<td>Oenanthe lugenoides boscavieni</td>
<td>Dhala, 4550ft, Aden</td>
<td>Toe pad</td>
<td>BMNH1965.M.12140</td>
<td></td>
<td>KF512772</td>
<td>KF512809</td>
<td>KF512741</td>
<td></td>
</tr>
<tr>
<td>Oenanthe lugenoides lugentoides</td>
<td>Barad, Taif, 6000, Saudi Arabia</td>
<td>Toe pad</td>
<td>BMNH1965.M.12146</td>
<td></td>
<td>KF512771</td>
<td>KF512808</td>
<td>KF512744</td>
<td></td>
</tr>
<tr>
<td>Oenanthe lugubris lugubris</td>
<td>Jemmu Valley, Ethiopia</td>
<td>Blood</td>
<td>A1109</td>
<td></td>
<td>KF512763</td>
<td>KF512800</td>
<td>KF512732</td>
<td>Male, black-bellied morph</td>
</tr>
<tr>
<td>Oenanthe lugubris lugubris</td>
<td>Jemmu Valley, Ethiopia</td>
<td>Blood</td>
<td>A1130</td>
<td></td>
<td>KF512764</td>
<td>KF512801</td>
<td>KF512735</td>
<td>Male, white-bellied morph</td>
</tr>
<tr>
<td>Oenanthe lugubris lugubris</td>
<td>Jemmu Valley, Ethiopia</td>
<td>Blood</td>
<td>A1132</td>
<td></td>
<td>KF512765</td>
<td>KF512802</td>
<td>KF512736</td>
<td>Female</td>
</tr>
<tr>
<td>Oenanthe lugubris lugubris</td>
<td>Jemmu Valley, Ethiopia</td>
<td>Blood</td>
<td>A1131</td>
<td></td>
<td>KF512766</td>
<td>KF512803</td>
<td>KF512734</td>
<td>Male, white-bellied morph</td>
</tr>
<tr>
<td>Oenanthe lugubris lugubris</td>
<td>170 miles S of lake Tana, &quot;Abyssinia&quot;</td>
<td>Toe pad</td>
<td>BMNH19271115.5313</td>
<td></td>
<td>KF512773</td>
<td>KF512810</td>
<td>-</td>
<td>Male, black-bellied morph</td>
</tr>
<tr>
<td>Oenanthe lugubris lugubris</td>
<td>Kuntaba, &quot;Abyssinia&quot;</td>
<td>Toe pad</td>
<td>BMNH1900.1.3.49</td>
<td></td>
<td>KF512774</td>
<td>KF512811</td>
<td>KF512739</td>
<td>Male, black-bellied morph</td>
</tr>
<tr>
<td>Oenanthe lugubris lugubris</td>
<td>Near Asmara, 6000ft, Ethria</td>
<td>Toe pad</td>
<td>BMNH195252.12</td>
<td></td>
<td>KF512775</td>
<td>KF512812</td>
<td>KF512733</td>
<td>Male, white-bellied morph</td>
</tr>
<tr>
<td>Oenanthe lugubris lugubris</td>
<td>Near Asmara, 8000ft, Ethria</td>
<td>Toe pad</td>
<td>BMNH19525211</td>
<td></td>
<td>KF512781</td>
<td>KF512818</td>
<td>KF512731</td>
<td>Male, black-bellied morph</td>
</tr>
<tr>
<td>Oenanthe lugubris schalowi</td>
<td>Mt Suswa, 5500ft, Naivasha schalowi</td>
<td>Toe pad</td>
<td>BMNH191612.1.1234</td>
<td></td>
<td>KF512779</td>
<td>KF512816</td>
<td>KF512737</td>
<td>Male, black-bellied morph</td>
</tr>
<tr>
<td>Oenanthe lugubris schalowi</td>
<td>Lake Nakuru, Kenya</td>
<td>Toe pad</td>
<td>BMNH1965.M.12161</td>
<td></td>
<td>KF512780</td>
<td>KF512817</td>
<td>KF512738</td>
<td>-</td>
</tr>
</tbody>
</table>
port. The *vauriei* samples were revealed to be the sister group to the *schalowi* and *lugubris* samples, although these relationships as well as the monophyly of all *lugubris* samples were not robustly supported. The clade consisting of two *lugentoides* samples was robustly supported. As in the analyses with ‘BEAST’ (see below), *O. albengira* was found to be the sister taxon of clade 1 and *O. leucura* that of clade 2. However, these relationships gained no robust support. In the ML analyses of the mtDNA dataset, the clade consisting of the *lugens* samples OLL104 and OLP002 gained robust support. Moreover, there was some robustly supported clustering within *lugubris* for the clades (BMNH1927.11.5.313 + BMNH1900.1.3.49 (A1129 + A1130A + 1131 + A1132)). Otherwise, there was no significant difference to the analysis of the mtDNA-nuclear dataset.

3.3. Network analysis of ODC

Despite apparent incomplete lineage sorting in the nuclear intron ODC, some basic patterns in the unrooted network were similar to the results of the phylogenetic analyses (Fig. 3). *Lugens*, *warriae* and *halophila* were basically found clustering together as were *lugubris*, *schalowi* and *vauriei* as well as *lugentoides* and *boscaveni*. Moreover, *persica* was found near *chrysopygia* and *xanthopyrma*. However, three alleles of *lugens* were found adjacent to *persica* and *xanthopyrmyrma*, clearly separate from the other alleles of this taxon. These comprised one allele of sample A1152 from *Oenanthe persica* and *Oenanthe xanthopyrma*. They were found to be separate from the remaining taxa of clade 1, which it was associated with in the phylogenetic analyses.

3.4. Species tree analyses using ‘BEAST’

The comparison of the three independent runs for the ‘BEAST’ analyses in TRACER revealed high convergence among the different parameters. They were then combined using LOGCOMBINER 1.7.4 (Drummond and Rambaut, 2007) with 10% burn-in for each, resulting in effective sample sizes >235 for all parameters.

The multispecies coalescence approach uncovered a similar pattern compared to the phylogenetic analyses (Fig. 4). Both clade 1 and clade 2 were revealed as well, however, within clade 1, *persica* was found to be the sister group of (*halophila* + (*lugens* + *warriae*) and not of (*chrysopygia* + *xanthopyrma*). Within the *lugubris* group, *vauriei* split first from *lugubris* and *schalowi*. *O. leucura* was inferred as the sister taxon of clade 2, while *O. leucopygia* was the sister taxon of clade 2 + *O. leucura*. *O. albengira* was found to be the sister group of clade 1. However, the positions of *O. leucura* and *O. leucopygia* were not supported.

The divergence between the two basal clades revealed in the species tree analyses occurred in the early Pliocene (Fig. 4). The first splits in clade 1 and 2 took place in the early Pleistocene or late Pliocene, but diversification within the *lugens* and the *lugubris* group most probably started only from the middle Pleistocene onwards.

4. Discussion

In this study, we present a phylogenetic hypothesis of the *Oenanthe lugens* complex based on molecular data using for the first time a complete taxon sampling. The morphologically inferred *lugens* complex was consistently revealed to be a polyphyletic assemblage. We thus corroborate the results of previous studies which demonstrated that morphology (especially plumage features) seems to be a poor predictor of phylogenetic relationships in *Oenanthe* and in open-habitat chats in general (cf. Aliabadian et al., 2012). As with previous studies (Aliabadian et al., 2012, 2007a), the basal relationship of the taxa analyzed here could not be robustly resolved and the resolution of the mtDNA and nuclear markers so far analyzed seems not to be appropriate for disentangling this apparently fast initial radiation. In contrast to the mtDNA markers analyzed, there were no fixed differences among taxa in the nuclear intron and several taxa shared alleles. This can be explained as the result of the generally longer coalescence times in nuclear compared to mtDNA markers (e.g. Zink and Barrowclough, 2008). However, an influence of introgression in autosomal markers cannot be excluded at least in the case of *persica* and *lugens*. The application of the recently developed multispecies coalescent approach (Heled and Drummond, 2010) allowed us to simultaneously estimate the sequence and timing of speciation events despite the lack of complete lineage sorting in the nuclear data. The results of this approach in general were congruent with the phylogenetic

---

**Table 3 (continued)**

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Locality</th>
<th>Sample type</th>
<th>Museum Number</th>
<th>Field number</th>
<th>COI</th>
<th>ND2</th>
<th>ODC</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Oenanthe lugubris</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vauriei</td>
<td>Somaliland</td>
<td>Toe pad</td>
<td>BMNH1965.M.12150</td>
<td>KFS12776</td>
<td>KFS12813</td>
<td>KFS12730</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Oenanthe lugubris</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vauriei</td>
<td></td>
<td>Toe pad</td>
<td>BMNH1965.M.12152</td>
<td>KFS12782</td>
<td>KFS12819</td>
<td>KFS12729</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Oenanthe persica</em></td>
<td></td>
<td></td>
<td>ZMU1C137759</td>
<td>KFS12749</td>
<td>KFS12786</td>
<td>KFS12728</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Oenanthe persica</em></td>
<td></td>
<td></td>
<td>BMNH20052.7</td>
<td>JX256097</td>
<td>JX256181</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Oenanthe picata</em></td>
<td></td>
<td></td>
<td>NRM 20046664</td>
<td>DQ683497</td>
<td>JX256101</td>
<td>JX256187</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Oenanthe xanthopyrma</em></td>
<td></td>
<td></td>
<td>MIUT 2003.7(27)</td>
<td>DQ683509</td>
<td>JX255949</td>
<td>JX256106</td>
<td>JX256190</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NHMO 23723</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 4**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>COI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C010eF1</td>
<td>5'-TCTCAAAACCAACACACCACAGACATTG-3'</td>
<td></td>
</tr>
<tr>
<td>C010eF1_Neu</td>
<td>5'-GGCGGATAGCCCTACGCGGATTA-3'</td>
<td></td>
</tr>
<tr>
<td>C010eR1</td>
<td>5'-GGGCTGACTGACTGATGGAAAATGCT-3'</td>
<td></td>
</tr>
<tr>
<td>C010eF2</td>
<td>5'-GGGATGACTGACTGATGGAAAATGCT-3'</td>
<td></td>
</tr>
<tr>
<td>C010eR2</td>
<td>5'-GGGGCTGACTGACTGATGGAAAATGCT-3'</td>
<td></td>
</tr>
<tr>
<td>C010eF3</td>
<td>5'-GGGGCTGACTGACTGATGGAAAATGCT-3'</td>
<td></td>
</tr>
<tr>
<td>C010eR3</td>
<td>5'-GGGGCTGACTGACTGATGGAAAATGCT-3'</td>
<td></td>
</tr>
<tr>
<td>C010eR4</td>
<td>5'-GGGGCTGACTGACTGATGGAAAATGCT-3'</td>
<td></td>
</tr>
<tr>
<td>ND2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ND20eF1</td>
<td>5'-TTACGCTTCCTCCTAGGAAACTACA-3'</td>
<td></td>
</tr>
<tr>
<td>ND20eR1</td>
<td>5'-TGCGACTTCCTCCTAGGAAACTACA-3'</td>
<td></td>
</tr>
<tr>
<td>ND20eR2</td>
<td>5'-TCCACCCACCGCTTCCTCCTAC-3'</td>
<td></td>
</tr>
<tr>
<td>ND20eR3</td>
<td>5'-TCCACCCACCGCTTCCTCCTAC-3'</td>
<td></td>
</tr>
<tr>
<td>Meel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASNH</td>
<td>5'-GCCTACGGCTTGATGGAAAATGCT-3'</td>
<td></td>
</tr>
<tr>
<td>ODC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ODCF1</td>
<td>5'-ACGAGGCTTTCTCTGAGGAGG-3'</td>
<td></td>
</tr>
<tr>
<td>ODCR1</td>
<td>5'-TTCTGTTGACAAAGTTTCTGAGG-3'</td>
<td></td>
</tr>
</tbody>
</table>
analyses based on Bayesian inference and maximum likelihood, however, some conflicts were also uncovered.

4.1. Phylogenetic relationships

We revealed that the members of the *lugubris* group are monophyletic and form a robustly supported clade together with the *lugentoides* group (clade 2, Fig. 2). However, the position of this clade could not be robustly resolved. The position of *lugubris* was not robustly resolved in Aliabadian et al. (2012) either.

Within the *lugubris* group, *vauriei* and *schalowi* formed two well supported clades, however, the monophyly of the *lugubris* samples relative to *vauriei* and *schalowi* was not robustly supported. The breeding areas of *lugubris*, *schalowi* and *vauriei* are clearly allopatric and this pattern is unlikely to be the result of recent hybridization. *Lugubris* has a much larger range than do *schalowi* and *vauriei* and this pattern may instead just be caused by incomplete lineage sorting even in the mtDNA and reflect the recent separation of the three taxa. The males of *lugubris* occur in a white- and black-bellied morph (*Vaurie, 1950*). Both morphs were admixed in our samples and did not structure separately (cf. Table 2).

The species tree of the multispecies coalescent approach revealed that *vauriei* split first and formed the sister taxon to *lugubris* and *schalowi*. *Vauriei* shares plumage features with the *lugentoides* group and was even considered an intermediate taxon connecting the *lugentoides* group with *lugubris* and *schalowi* (*Vaurie, 1959*).

We found the members of the *lugens* group (i.e. *lugens*, *halophil-a*, *persica* and *warriae*) in a clade with *O. finschii* as well as with *O. chrysopygia* and *O. xanthopyrma* (clade 1) in congruence with Aliabadian et al. (2012) (Figs. 2 and 4). The sister group relationship of *O. finschii* to the other members of clade 1 is also concordant with the results of previous studies (Aliabadian et al., 2012, 2007b). *O. finschii* breeds from Turkey south and eastwards over the Levant, Iran, southern Central Asia and Afghanistan to western Pakistan (Collar, 2005). Its breeding range overlaps with that of *persica* in the Zagros mountains of Iran (Collar, 2005; Cornwallis, 1975). At least in south-west Iran, the breeding areas of *O. finschii* and *persica* seem to be partly separated by habitat in areas of sympathy (Cornwallis, 1975). Moreover, *O. finschii* breeds in sympathy with *O. xanthopyrma* in south-east Turkey and western Iran as well as with *O. chrysopygia* in Iran, southern Central Asia and Afghanistan (Collar, 2005; Cornwallis, 1975). In the zone of sympathy between *O. finschii*...
and O. chrysopygia, the breeding areas of the two are separated by altitude (Cornwallis, 1975). There is apparently no interbreeding between these taxa.

The taxon warriae has until recently been considered as a dark morph of lugens, and Förchler et al. (2010), using two mtDNA markers, found warriae not to be different from lugens. For both mtDNA markers and the nuclear intron, we also found warriae to be nested within lugens. However, despite this lack of genetic differentiation in the markers analyzed so far, Shirihai et al. (2011) demonstrated that this range restricted almost entirely black wheatear is not a morph of lugens and should be better treated as a taxon of its own.

Within the lugens group, sexes are virtually identical in lugens and persica and differ only slightly in warriae, whereas halophila shows a pronounced sexual dimorphism (Shirihai et al., 2011; Appendix 1). Moreover, lugens and persica are morphologically only subtly differentiated. Male halophila on the other hand, although similar, can be readily separated from both lugens and persica (Shirihai et al., 2011). Nevertheless, halophila and lugens together with warriae were consistently inferred as each other's closest relatives. The position of persica however, was not resolved unambiguously. Our phylogenetic analyses found persica to be more closely related to O. chrysopygia and O. xanthopyrma. In congruence with previous molecular phylogenetic studies (Aliaabadian et al., 2012, 2007b), the clade consisting of these three taxa formed the sister group to the remaining members of the lugens group. O. chrysopygia and O. xanthopyrma have traditionally not been associated with members of the lugens complex. They were instead considered to be closely related to O. moesta or even O. deserti (e.g. Tye, 1989), which were revealed to be phylogenetically more distantly related to the taxa analyzed here (cf. Aliaabadian et al., 2012). While O. chrysopygia breeds from southern Transcaucasia, northern Iran, southern Central Asia and Afghanistan to western Pakistan, the breeding area of O. xanthopyrma is restricted to south-east Turkey and western Iran (Ayé et al., 2012; Collar, 2005; Porter and Aspinall, 2010). Although the two differ quite sharply in plumage features, they have for a long time been considered conspecifics, not least because they supposedly hybridize where their areas meet in north-west Iran (Chamani et al., 2010; Vaurie, 1949). Hybridization between the two in their contact zone would not be surprising given their close genetic relationship. However, the characters of apparent hybrids (’cummingi’) are shown by some first-year O. xanthopyrma (Roselaar, 1995). More recently, they have been treated as separate species (Collar, 2005; Dickinson, 2003). Persica breeds in sympatry with O. xanthopyrma and O. chrysopygia in western Iran (Cornwallis, 1975; Porter and Aspinall, 2010). In areas where the breeding ranges between persica and O. chrysopygia overlap, however, the two are largely separated by different altitudinal ranges (Cornwallis, 1975). Persica is quite distinct in plumage features from O. xanthopyrma and O. chrysopygia and their apparent close relationship is hence rather unexpected. However, morphological features seem to be a poor predictor of phylogenetic relationships in Oenanthe in general (cf. Aliaabadian et al., 2012).

The multispecies coalescent approach revealed a different pattern, though. It placed persica with moderate support as the sister group of lugens and halophila. O. chrysopygia and O. xanthopyrma
instead were revealed to be the sister clade of the whole lugens group again with moderate support (Fig. 4). Surprisingly, three alleles of the nuclear intron ODC stemming from two individuals of lugens were found clustering with persica and O. xanthoprymna rather than with the other alleles of lugens. This could be due to the retention of an ancestral polymorphism but could also indicate (ancient) introgression between lugens and persica. No signs of introgression have been found in mtDNA so far however, pointing to the former explanation. Nevertheless, it should be taken into account that in the absence of selection, mtDNA is less likely to introgress than autosomal DNA, often resulting in a very low level of mtDNA introgression in hybrid zones of birds (Rheindt and Edwards, 2011). Interestingly, birds with morphological characters suggesting persica have been found in Syria, which is usually considered to be inhabited by lugens (Shirihai et al., 2011). Persica-like birds have also been found especially in winter in southern Israel, southern Jordan and in the Sinai, Egypt (Shirihai et al. 2011). Persica is migratory and these birds could be individuals away from their traditional wintering grounds in Eastern Arabia (cf. Shirihai et al., 2011). However, persica-like birds analyzed so far from Israel, Jordan and Syria displayed mtDNA haplotypes of lugens (Shirihai & Schweizer unpublished). Further studies are needed to infer whether the morphological variation in lugens is higher than hitherto appreciated or if there is indeed a zone of introgression between lugens and persica.

4.2. Notes on temporal diversification patterns and biogeography

The basal nodes in the Oenanthe clade analyzed here were not robustly resolved in our phylogenetic inference and, accordingly, the temporal estimates for the basal splits as revealed by the multispecies coalescence approach revealed rather large confidence intervals. Nonetheless, we have an indication that the initial divergence events among the ingroup species happened in the late Pliocene and that this group of wheatears thus comprises a comparatively young radiation. The diversification within clades 1 and 2 then started within the Pleistocene, although the distributions of the 95% highest posterior densities include the late Pliocene. Splits within the lugubris, lugentoides as well as the lugens group including O. xanthoprymna and O. chrysopygia seem to have occurred after 0.9 Mya, which broadly marked the start of an intensification of duration and amplitude of climatic cycles resulting in the dramatic late Pleistocene glacial cycles (e.g. Hewitt, 2000). In contrast to the pattern of temperate species, it has been hypothesized that bird taxa adapted to open habitats might have reached their maximum range extent during glacial periods when steppe-like communities expanded, while isolation and fragmentation occurred during the much shorter interglacial times (Garcia et al., 2011a,b). Steppe or arid-adapted taxa are thus expected to show low genetic structuring because of the short time of isolation (Garcia et al., 2011a,b). This prediction obviously does not fit the group.
of *Oenanthe* species analyzed here, whose taxa evolved during the late Pliocene. Episodes of increased aridity during glacial periods might have rendered the Saharo-Saharan desert belt an inhospitable area even for arid-adapted species like *Oenanthe* wheatears, and the contemporary taxa might have evolved in isolation in rocky mountainous areas adjacent to hyper-arid areas.

It is not clear whether a common ancestor of the *lugentoides* group colonized the Arabian Peninsula from the Horn of Africa or if the latter region was colonized from Arabia by an ancestor of the *lugubris* group. The former scenario seems to be more likely as the majority of the breeding landbirds (and most endemic species) of the southern and south-western part of the Arabian Peninsula are shared with the Afrotopical region (*Roselaar*, 2006). Moreover, an African origin is likely for the whole clade (including *O. leucura*), given that *O. leucopygia* as the putative sister group is widely distributed over the Sahara in North Africa with the extension of its breeding area into the Levant and the northern part of the Arabian Peninsula. In contrast, an Asiatic origin is likely for clade 2 as its sister taxon, *O. albomitra*, breeds from Iran eastwards over Iraq to Afghanistan and Pakistan (*Collar*, 2005). An ancestor of *halophila* might have then colonized the Maghreb from the Levant. However, unless the basal relationships of the *Oenanthe* taxa analyzed here have been robustly resolved using more samples per taxa and more molecular markers, these biogeographic considerations have to be considered as tentative.

### 4.3. Taxonomy

The wheatears of the genus *Oenanthe* comprise a comparatively young radiation with in some cases ongoing hybridization between traditionally recognized species as in *Oenanthe hispanica melanoleuca* and *Oenanthe pleschanka* (*Panov*, 2005). Morphologically distinct allopatric taxa with signs of pre-zygotic isolation (different reaction to heterospecific vs. conspecific vocal and visual stimuli) cannot even be distinguished using standard mtDNA markers as in the case of *Oenanthe cyprica* and *O. pleschanka* (*Randler* et al., 2011). The formulation and testing of species hypotheses is notoriously difficult in such cases based on genetic data and should thus rely on an integrative approach using a combination of genetic, phenotypic, behavioral and ecological data.

In the *lugens* complex, the nuclear intron in particular was revealed to be affected by incomplete lineage sorting among taxa. This may be a consequence of the long coalescence time associated taxa. It should be taken into account that paraphyletic gene trees are not rare even among allopatric or reproductively isolated taxa (*Funk* and *Omland*, 2003; cf. also *Tobias* et al., 2010). Especially in the case of *persica* and *lugens* however, it is not clear how much this pattern is caused by (ancient) hybridization or introgression. The multispecies coalescence approach applied here accounted for incomplete lineage sorting among taxa. However, we are aware that our results have to be confirmed with more markers and additional samples for some species. Nevertheless, they might be considered as an overall picture of the true genetic relationships among taxa.

All taxa of the *lugens* complex are clearly differentiated from each other by morphometrics and plumage features with the exception of *boscai*, which is only marginally different from *lugentoides* (*Dean* et al., 1992; *Shirihai* and *Svensson*, in prep.; Appendix 1). Hence, they could be considered to be diagnosably different and all would qualify as species under a phylogenetic species concept (cf. *Cracraft*, 1989). However, we base the following taxonomic interpretation on an integrative approach towards species delimitation (*Helbig* et al., 2002; *Padial* et al., 2010; *Tobias* et al., 2010; *Will* et al., 2005).

As the different groups within the *lugens* complex were shown not to form a monophyletic group, it can no longer be considered a single species or a superspecies. The *lugubris* and the *lugentoides* group were found to be well-supported reciprocally monophyletic clusters and have probably been isolated for a long time as indicated by their deep phylogenetic splits. This in combination with their diagnostically different plumages (cf. *Förschler* et al., 2010) makes it clear that the *lugubris* and *lugentoides* group (as well as the *lugens* group) should be treated as different species. The Arabian Wheatear *O. lugentoides* (including *O. lugentoides boscowi*) would thus be an additional endemic species of the Arabian Peninsula as already tentatively suggested by some authors (e.g. *Jennings*, 2010; *Porter* and *Aspinall*, 2010). The three allopatric taxa of the *lugubris* group would then be treated under *O. lugubris*. We acknowledge however, that the genetic differentiations of *schalowi* and *vauriei* from *lugubris* might be considered sufficient to treat them as separate species, although this might render *lugubris* paraphyletic in its mtDNA. This would lead to the recognition of the three species, namely Abyssinian Wheatear *O. lugubris*, Vaurie’s Wheatear *O. vauriei*, Schalow’s Wheatear *O. schalowi*. However, we refrain from proposing a split before vocal and behavioral differences between *lugubris*, *schalowi* and *vauriei* have been quantitatively assessed and conservatively propose to treat them as a polytypic species *O. lugubris*.

Förschler et al. (2010) proposed splitting *persica* as a monotypic species from *lugens* and *halophila* which would then be retained under *O. lugens*. However, until the exact status of the somewhat intermediate birds in Syria is clarified and the potential introgression between *persica* and *lugens* is investigated in more detail, we refrain from splitting *persica* from *lugens*. Such a taxonomic treatment renders *O. lugens* paraphyletic in its mtDNA. However, gene tree paraphyly does not necessarily disagree with an integrative approach towards species as the evolution of reproductive isolation might precede complete lineage sorting (cf *Tobias* et al., 2010). Moreover, by analyzing more samples of *persica* and *persica*-like birds from Syria, the potential influence of isolation by distance or even of introgression between *persica* and *O. chrysoptiga* or *O. xanthoprymna* on this pattern should be investigated.

Although *halophila* and *lugens* have only very recently separated, the former is unique among the taxa of the *lugens* group in showing strong sexual dimorphism. Moreover, males can be constantly separated by plumage characteristics from *lugens* and *persica* and *halophila* differs slightly in vocalizations from *lugens* (*Shirihai* et al., 2011; Shirihai and *Svensson*, in prep.). This could be taken as evidence that *halophila* might have reached reproductive isolation from *lugens* and should be considered a species on its own right, i.e. Maghreb Wheatear *Oenanthe halophila*. The taxon *warriae* is not separable from *lugens* by the genetic markers analyzed so far, although it differs not only distinctively in plumage features, but also in morphometrics, habitat choice and potentially also in song (*Shirihai* et al., 2011). Taking into account that other *Oenanthe* taxa which are not separable by mtDNA markers show signs of pre-zygotic isolation (*Randler* et al., 2011), *warriae* might well have developed incipient reproductive isolation from *lugens*. However, until especially vocalizations of the whole *lugens* group have been quantitatively analyzed, until indications of potential pre-zygotic isolation have been investigated (cf. *Randler* et al., 2011), and until the status of *persica* is clarified (see above), we conservatively propose maintaining *halophila* and *warriae* (as well as *persica*) as subspecies within *O. lugens*. This study thus serves as a baseline for further research using an integrative approach to disentangling the taxonomy of this complex radiation.

**Acknowledgements**

The two authors are grateful to the many people and institutions for kindly providing tissue and/or toe pad samples and/or for allowing access to their collections: J. Fjeldså and J.-B. Kristen-
sen (Zoological Museum, University of Copenhagen), A. Gamauf and H.M. Berg (Natural History Museum Wien), Martin Haase (Vogelwarte Hiddensee, Universität Greifswald), R. Prys-Jones and M. Adams (Natural History Museum, Tring), as well as D. Berkowicz (Zoological Museum, Tel Aviv University). C.G. Kirwan and S.T. Hertwig kindly commented on previous versions of the manuscript. H.S. thanks many people who were involved in the collection of samples for this study including various teams associated with his work at the ringing station in Eilat (under the wings of NRA & SPNI), the late A.J. Helbig, M. San Roman, Y. Lehnardt, Y. Ayé, R., Schweizer, M., Roth, T., 2012. Birds of Central Asia. Christopher Helm, London.


