Molecular Phylogenetics and Evolution 48 (2008) 1145–1154

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Phylogenetic relationships, biogeography and speciation in the avian genus *Saxicola*

Juan Carlos Illera [a,d,*], David S. Richardson [a], Barbara Helm [b], Juan Carlos Atienza [c], Brent C. Emerson [a]

[a] Centre for Ecology, Evolution and Conservation, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK
[b] Max Planck Institute for Ornithology, D-82346 Andechs, Germany
[c] Spanish Ornitological Society (SEO/BirdLife), C/Melquiades Biencinto, 34, E-28053 Madrid, Spain
[d] Island Ecology and Evolution Research Group, IPNA, CSIC, C/Astrofísico Francisco Sánchez 3, La Laguna, Tenerife, 38206 Canary Islands, Spain

ARTICLE INFO

Article history:
Received 24 January 2008
Revised 19 April 2008
Accepted 12 May 2008
Available online 20 May 2008

Keywords:
Biogeography
Cryptic speciation
Phylogeny
Saxicola

ABSTRACT

The avian genus *Saxicola* is distributed throughout Africa, Asia, Europe and various islands across Oceania. Despite the fact that the group has great potential as a model to test evolutionary hypotheses due to the extensive variability in life history patterns recorded between and within species, the phylogenetic relationships among species and subspecies of this genus are poorly understood. We undertook a systematic investigation of the relationships within this genus with three main objectives in mind, (1) to test the monophyly of the genus; (2) to ascertain geographical origin and dispersal sequence; and (3) to test for monophyly within the most morphologically diverse species, *S. torquata* and *S. caprata*. We studied sequence data from the mitochondrial cytochrome *b* gene from 11 of the 12 recognized species and 15 of the 45 described subspecies. Four clades, two exclusively Asian, one Eurasian, and the fourth encompassing Eurasia and Africa, were identified. Based on our analyses, monophyly of the genus *Saxicola* is not supported and an Asian origin for the genus can be inferred. Results from DIVA analyses, tree topology and nodal age estimates suggest independent colonisation events from Asia to Africa and from Asia to the Western Palearctic, with the Sahara desert acting as a natural barrier for *S. torquata*. Subspecies and populations of *S. torquata* are not monophyletic due to *S. tectes*, *S. dacotiae* and *S. leucura* grouping within this complex. Subspecies and populations of *S. caprata* are monophyletic. Importantly, within *S. torquata* and *S. caprata*, slight morphological traits and plumage colour pattern differences used to recognize subspecies are indicative of the greater cryptic diversification that has occurred within this genus.

1. Introduction

Resolving phylogenetic relationships within and among species has become essential for the testing of evolutionary and biogeographical hypotheses, such as postglacial range expansion, colonisation, dispersal and speciation processes (e.g. Voelker, 1999; Hewiit, 2000; Emerson, 2002; Filardi and Moyle, 2005). Phylogenetic information is also important as it allows the identification of phylogenetically independent data required for meaningful ecological comparisons between species (Harvey and Pagel, 1991; Freckleton et al., 2002; Hansen and Orzack, 2005). Ecological approaches performed in the absence of such phylogenetic information may result in seriously biased interpretations (Freckleton, 2000; Duncan et al., 2007).

Stonechats (genus *Saxicola*) are small insectivorous birds inhabiting mainly open habitats dominated by woody shrubs, anywhere from the coast to mountainous alpine environments (Urquhart, 2002; Collar, 2005). This avian genus, with its array of different life history parameters across subspecies with disjunct distributions, is often used as a model for testing hypotheses relating to migration, physiology and breeding behaviour, such as photoperiodic responsiveness and migratory restlessness (e.g. Gwinner et al., 1983; Goymann et al., 2006; Helm et al., 2005; Helm, 2006; Helm and Gwinner, 2006; Wikelski et al., 2003). However, these questions require clear understanding of the phylogenetic relationships both between and within *Saxicola* taxa, something that is presently lacking. For example, although previous studies have investigated the phylogenetic relationships of parts of the *Saxicola* group (Wittmann et al., 1995; Wink et al., 2002) the evolutionary relationships among the Asian species of stonechats remains unknown. Furthermore, the genus itself may not be monophyletic as the placement of two taxa, *S. bifasciata* and *S. gutturalis*, has been called into question by some authors. Thus, for more than one century *S. bifasciata* has been a difficult taxa due to the similarity of some specific characteristics such as morphological design, colour pattern and behaviour with species from different genus (see Urquhart, 2002 and references there in; Collar, 2005). *Saxicola gutturalis*, an endemic of Timor, Roti and Semau islands, also presents an interesting taxonomic issue due to its atypical preference for dry deciduous forest

* Corresponding author. Address: Island Ecology and Evolution Research Group, IPNA, CSIC, C/Astrofísico Francisco Sánchez 3, La Laguna, Tenerife, 38206 Canary Islands, Spain. Fax: +34 922 260135.
E-mail addresses: jcillera@ipna.csic.es, jcillera3@hotmail.com (J.C. Illera).

1055-7903/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2008.05.016

and woodland in comparison with other *Saxicola* species that select non-forested open habitats. The morphology, foraging behaviour and above ground nesting location of *S. gutturalis* suggest it may be closer to a flycatcher than a *Saxicola* (Urquhart, 2002). Overall the lack of detailed and accurate knowledge of the relationships within this group has hampered the ability of investigator's to determine whether observed differences in behaviours, such as breeding or migration, are due to local adaptation or shared evolutionary history.

Other questions relating to the origin of the *Saxicola* group also remain unanswered. Stonechats are widely distributed across Africa, Europe and Asia, but it is unclear as to where, among these three continents, the genus may have originated. Indeed, in most avian phylogenetic studies of genera distributed on multiple continents, the hypothesis that the group arises in a small area (i.e. "center of origin" hypothesis) followed by dispersal to other areas has generally not been supported (e.g. Voelker, 1999; Voelker et al., 2007). A simple consideration of the number of species inhabiting each continent would suggest an Asian origin, since the majority of species inhabit this continent. However, the exclusively African wintering distribution of *S. rubetra*, the dramatic diversification of *S. torquata* in Africa with more than 15 subspecies, and the presence of three African endemics (*S. dacotiae*, *S. tectes* and *S. bifasciata*) could also be taken as evidence for an African origin.

Interestingly, two species within the genus, *S. torquata* and *S. caprata*, have undergone dramatic diversification resulting in more than 25 and 16 described subspecies, respectively. These are distributed across Europe, Asia and Africa (*S. torquata*), and Asia plus some islands of Oceania (*S. caprata*). Recently, it has been suggested that the genetic distances observed among three subspecies of *S. torquata* are sufficient to consider these to be distinct species (Wittmann et al., 1995; Wink et al., 2002). The findings of Wittmann et al. (1995) and Wink et al. (2002) suggest that the subtle morphological and colour pattern variation recorded within species could indicate substantial genetic structure between populations, or even the possibility of cryptic speciation.

The broad aim of this paper is to reconstruct the phylogenetic relationships of the genus *Saxicola* using the mitochondrial cytochrome *b* gene (cyt *b*). The specific aims are as follows: (1) to test if the genus *Saxicola* is monophyletic. For this purpose we analysed 11 of the 12 recognized species and 15 of the 45 described subspecies, including the extinct population of the Canary Island Stonechat (*S. dacotiae murielae*); (2) to reconstruct the ancestral area of the genus and to establish a sequence of dispersal. Due to the high number of species and subspecies inhabiting Africa and Asia (Urquhart, 2002) we predict either an Asian or African origin; (3) to test if the two taxonomically most diverse species, *S. torquata* and *S. caprata*, are both monophyletic and determine if the plethora of forms that each species exhibit is related to genetic divergence or phenotypic plasticity. Within *S. torquata* 14 morphologically diverse and/or geographically disjunct populations (nine subspecies) were analysed. Within *S. caprata* four populations that are morphologically diverse and/or geographically disjunct (three subspecies) were analysed.

2. Materials and methods

2.1. Sampling effort and laboratory procedures

Although recent molecular genetic work has suggested that *Saxicola torquata* may be split into three different species (*S. torquata*, *S. axillaris* and *S. maura*; Wittmann et al., 1995; Wink et al., 2002), for our purposes we follow Sibley and Monroe's (1993) classification based on DNA–DNA hybridization. A combination of blood samples collected from live birds and tissue samples from *S. tectes* and museum specimens (muscle and liver preserved in ethanol, and toe pads from skins) were obtained (Table 1). For polytypic species we used only breeding, or apparently resident individuals (i.e. those subspecies known to inhabit those areas), so as to avoid including migrants in our analyses. Subspecies were assigned names according to their origin (i.e. according to localities where individuals were ringed) following information given in Urquhart (2002). Additionally, we obtained a blood sample from *Oenanthe oenanthe* and cyt *b* sequences from all genera belonging to the tribe Saxicolini (Voelker and Spellman, 2004) available from GenBank (*Pogonocichla*, *Swynnertonia*, *Stiphrornis*, *Sheppardia*, *Erithacus*, *Tarsiger*, *Cossypha*, *Phoenicurus*, *Chaimarrornis*, *Rhyacornis*, *Enicurus*, *Oenanthe*, *Thamnolaea*, *Alethe*, *Myiophonus*, *Monticola*, *Ficedula*) to identify the closest taxa for use as outgroups and test whether any of these species could fall within the *Saxicola* group. DNA was extracted from blood samples and tissues preserved in ethanol using a standard salt-extraction method (Sunnucks and Hales, 1996; Aljanabi and Martinez, 1997), and from museum toe pad samples using the Qiagen dneasy tissue kit according to the manufacturer's instructions.

A region of the cytochrome *b* gene was amplified using primers MT-A3 and MT-F2 (Wink et al., 2002) for all fresh samples. The museum toe pad samples were amplified in two (*S. gutturalis*) or three fragments (*S. dacotiae murielae*) using primers *Sax*G1F, *Sax*G1R, *Sax*G2F, *Sax*G2R, *Sax*G3R, SDM_F1, SDM_R1 and SDM_F2 (Appendix A). Polymerase chain reactions (PCR) were set up in 10 μl total volume including 5 μl of 2× ReddyMix™ PCR Master Mix (ABGENE), 0.5 μl (10 mM) of each primer and 1.5 μl of genomic DNA (25 ng/μl). Polymerase chain reactions were performed with a Tetrad 2 thermocycler under the following conditions: initial denaturation at 94 °C for 3 min followed by 35 cycles of denaturation at 94 °C for 30 s, with an annealing temperature of 50 °C for 30 s, and extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. Sequencing of the PCR products was performed using the Perkin Elmer BigDye terminator (v. 3.1) reaction mix in a volume of 10 μl using 1 μl of PCR product and the primers MT-A3, *Sax*G1F, *Sax*G2F, SDM_F1, SDM_F2, *Sax*Seq1 and *Sax*Seq2 (Appendix A). The following conditions were used: initial denaturation at 94 °C for 2 min followed by 25 cycles of denaturation at 94 °C for 30 s, with an annealing temperature of 50 or 52 °C for 30 s, and extension at 60 °C for 2 min and a final extension at 60 °C for 1 min. The final product was analysed on a Perkin Elmer ABI 3700 automated sequencer. Sequences were aligned by eye using BioEdit (version 7.01; Hall, 1999).

2.2. Phylogenetic analyses

A preliminary neighbour joining (NJ) analysis with other genera from the tribe Saxicolini (data not shown) identified *Oenanthe* and *Thamnolaea* as most closely related to the *Saxicola*, with all other genera being more distantly related. Using these two genera as outgroups we explored whether *S. bifasciata* and *S. gutturalis* species could be defined as members of the *Saxicola* using NJ analysis. Phylogenetic relationships for the ingroup were then inferred using three methods: maximum parsimony (MP) and maximum likelihood (ML) as implemented in PAUP* version 4.0 b10 (Swofford, 2002), and Bayesian inference (BI) using Mr. Bayes version 3.1 (Huelsenbeck and Ronquist, 2001). Maximum parsimony analysis was performed using a heuristic search strategy, equally weighted searches using 100 random stepwise addition sequence replicates and a tree bisection reconnection (TBR) branch-swapping algorithm. The most appropriate substitution model for ML and Bayesian analyses was inferred using MODELTEST 3.7 (Posada and Crandall, 1998). This program enables the comparison of 56 models of DNA substitution in a hierarchical hypothesis testing

Table 1
Taxa, sampling date, sample tissue, localities, museum voucher specimen and GenBank accession numbers of each taxa used

Species	Year/tissue	Locality (country)	M. catalogue	GenBank
S. dacotiae dacotiae	2001–03/blood	Fuerteventura (CI, Spain)		EU421077–78
S. dacotiae murielae	1913/toe pad	Alegranza (CI, Spain)	AMNH 581785/NHM 10.22.104	EU421079
S. torquata rubicola	2002/blood	Iberian Peninsula (Spain)		EU421080–84; EU421089–92
S. torquata rubicola	2006/muscle	Austria		EU421104–05
S. torquata rubicola	2006/muscle	Germany		EU421106–07
S. torquata rubicola	2002/blood	Ceuta (Spain)		EU421086–87
S. torquata rubicola	2005/blood	Morocco		EU421085
S. torquata indica	2002/blood	Shuklaphanta (Nepal)		EU421088
S. torquata maura	2006/muscle	Kazakhstan		EU421096–98; EU421101–02
S. torquata axillaris	2006/muscle	Mt Meru and the Ngorogoro Crater highlands (Tanzania)		EU421093–95; EU421100
S. torquata axillaris	2006/muscle	Nakuru-Naivasha region (Kenya)		EU421099
S. torquata promiscua	2001/blood	Mbeya (Tanzania)		EU421108
S. torquata hibernans	2006/blood	Ireland		EU421103; EU421111
S. torquata salax	2005/blood	Obudu (Nigeria)		EU421109
S. torquata voeltzkowi	2000/blood	Grande Comore Island (Comoros)		EU421110
S. torquata stonei	2006/blood	Fochville (South Africa)		EU421112–14
S. tectes	2005/muscle	Réunion Island		EU421119
S. jerdoni	2002/blood	Shuklaphanta (Nepal)		EU427504
S. rubetra	2002/blood	Iberian Peninsula (Spain)		EU421115–16
S. insignis	2002/blood	Shuklaphanta (Nepal)		EU421117
S. leucura	2002/blood	Shuklaphanta (Nepal)		EU421118
S. ferrea	2002/blood	Shuklaphanta (Nepal)		EU421127
S. caprata bicolor	2002/blood	Shuklaphanta (Nepal)		EU421120
S. caprata pyrrhonota	1990/liver/muscle	W-Timor Island (Indonesia)	WAM B23877;B24023–25	EU421122–23
S. caprata fruticola	1988/liver	Moyo Island, (Indonesia)	WAM B22216	EU421121
S. caprata fruticola	1989/liver	Lembata Island, (Indonesia)	WAM B22703	EU421124
S. bifasciata	2005/blood	Lydenburg (South Africa)		EU421126
S. gutturalis gutturalis	1911/toe pad	Timor Island	ZS 11.2284	EU421125
Oenanthe oenanthe seebohmi	2006/blood	Ifrain (Morocco)		EU421128
Thamnolaea cinnamomeiventris	Genebank	Voelker and Spellman (2004)		AY329476

CI, Canary Islands; AMNH, American Museum of Natural History; NHM, Natural History Museum; WAM, Western Australian Museum; ZS, Zoologische Staatssammlung.

framework. In the Bayesian analyses four independent MCMC chains were simultaneously run for 2,000,000 replicates, sampling one tree per 100 replicates. Convergence of the chains to a stationary distribution was assessed with TRACER v. 1.4 (Rambaut and Drummond, 2007), and on the basis of this we discarded trees from the first 200,000 generations. We used the remaining trees to obtain a 50% majority rule consensus tree. Three independent runs were performed to ensure the posterior probabilities were stable. Node support in ML and MP analyses was assessed with 100 and 1000 bootstrap replicates, respectively. Posterior probability values were used to assess nodal support for the Bayesian analysis.

2.3. Ancestral area

We used the program DIVA v. 1.1 (Ronquist, 1996) to reconstruct the most probable ancestral area and sequence of dispersal. This method estimates ancestral distributions taking account the possibility of vicariance, dispersal and extinction events (Ronquist, 1997). We used the MP topology to estimate the most ancestral distribution at each node. All species are assigned to one or several unit biogeographic areas depending upon their current distribution. In order to compare this study to others we followed geographical regions defined in Fig. 1 of Outlaw et al. (2007) which have also been used with other avian lineages with similar distributions (e.g. Voelker, 1999, 2002). The eastern plus south-eastern Asia region which was divided into two areas (A and E). Thus, the ten regions used in this study are: (A) eastern Asia; (B) central Asian arid; (C) Himalayas; (D) south-western Asia plus Indian subcontinent; (E) Tropical and subtropical south-eastern Asia; (F) western Palearctic; (G) North African arid plus Saudi Peninsula; (H) African savannah; (I) South African arid; and (J) Madagascar plus Comoros and Réunion island. We first performed the DIVA analysis without restricting the number of ancestral areas. However, because this could result in a tendency for most areas descending from the root node (Ronquist, 1996), we performed a second analysis constraining the maximum number of unit areas in ancestral distributions to an intermediate value of five.

2.4. Estimation of time to the most recent common ancestor (TMRCA)

In order to estimate divergence times between and within clades the program BEAST (v. 1.4.6; Drummond and Rambaut, 2007) was used. The time of the most recent common ancestor (TMRCA) was estimated using the range of rate estimates for cytochrome *b* available for songbird species (Lovette, 2004; Päckert et al., 2007). We used an uncorrelated lognormal relaxed clock (Drummond et al., 2006) and defined the rate prior to have a normal distribution with a mean of 0.01 and standard deviation of 0.0075 substitutions per site per million years, corresponding to sequence divergence rate between 0.5% and 3.5% per million years. The most appropriate nucleotide substitution model inferred from MODELTEST, and the values obtained there, were used in the BEAST analyses. A Yule tree prior was used, following the recommendation of Drummond et al. (2007), for species level phylogenies. Two independent MCMC analyses of 10,000,000 steps, each with a burn-in of 1,000,000 steps were performed. Convergence of the chains to a stationary distribution was assessed with TRACER v. 1.4. After checking that both chains had converged, files were combined and then parameters of interest were estimated with TRACER.

3. Results

We obtained a total of 69 sequences of 958 base pairs (bp) from all subspecies with the exception of the museum samples of *S. gutturalis* and the extinct subspecies of the Canary Islands stonechat (*S. d. murielae*). For these two taxa shorter fragments of 328 and 363 bp for *S. gutturalis* and *S. d. murielae*, respectively, were obtained. Sequences have been deposited in the NCBI gene bank database (see Table 1 for accession numbers). All sequences were translated to amino acids according to the vertebrate mitochondrial genetic code and no unexpected start or stop codons were detected. Of the 958 sites, 296 were variable (31%), and of these 218 (23%) were parsimony informative. Nucleotide composition (T = 25.7%; C = 34.4%; A = 26.2%; G = 13.7%) was in accordance with expectations from other species within the same family (e.g. Voelker and Spellman, 2004; Voelker et al., 2007). Uncorrected pairwise distances among species and subspecies ranged from 0.3% to 12.5%. Only four subspecies pairs exhibit relatively low pairwise values (*S. d. dacotiae/S. d. murielae; S. t. hibernans/S. t. rubicola*; *S. t. promiscua/S. t. axillaris* and *S. c. fruticola/S. c. pyrrhonota*) and after their exclusion the lowest pairwise value is 2.7%.

3.1. Phylogenetic position of S. bifasciata and S. gutturalis

An initial NJ analysis (not shown) using 958 bp sequences determined that *S. bifasciata* is phylogenetically closer to the two outgroups than the remainder of species included in the genus *Saxicola* (98% bootstrap support). Thus, for subsequent analyses *S. bifasciata* was used as outgroup. A NJ analysis (not shown) was also performed using a 328 bp alignment so that *S. gutturalis* could be included. Results suggested that this species, although divergent, is monophyletic within the *Saxicola* (85% bootstrap support). Uncorrected pairwise sequence divergences between *Saxicola* species (with the exclusion of *S. bifasciata*) ranged from 2.7% to 11.3%.

3.2. Monophyly, origin and genetic relationships

The best fit model selected under the Akaike information criterion (AIC) in MODELTEST was the general time-reversible model including invariable sites and rate variation among sites model (GTR+I+G). High bootstrap support is found for the monophyly of the genus *Saxicola* (after excluding *S. bifasciata*) using MP (96%) and ML (100%) analyses but not with BI (Fig. 1, node A). Similar results were obtained when *S. gutturalis* and *S. d. murielae* are included in the short fragment analyses; monophyly of genus *Saxicola* is supported with ML (92%) and MP (85%), while BI fails to provide support for monophyly (Fig. 2, node A).

The three analyses (i.e. MP, ML and BI) all reveal similar tree topologies, but with some differences in relative nodal support. Importantly, all Asian species are consistently placed basally (Figs. 1 and 2). Phylogenetic relationships can be described by the identification of four main clades (nodes B, C, D and E) based on the geographic affinity of species and the genetic distances between and within species (Fig. 1). All trees support these clades with high nodal support, with the exception of Asian clade 1 (node D). The Eurasia-African clade (node E) has the widest geographic distribution including Asia, Africa and Europe. However, it only contains four recognised species *S. torquata*, *S. dacotiae*, *S. leucura* and *S. tectes*, although *S. torquata* is represented by a number of subspecies and populations. Our results did not support the monophyly of *S. torquata* due to *S. dacotiae*, *S. leucura* and *S. tectes* species grouping within this complex. Within this clade three further sub-clades (nodes F, G, H) can be described according to tree topology and geographical distribution of taxa. All analyses place *S. leucura*, and the most oriental specimens of *S. torquata* included in this study, at the base of this clade (Asian sub-clade) with high support values (node F). All individuals of the subspecies *S. torquata rubicola* (which breeds in western Palearctic) are grouped together (node I), except two Iberian *S. torquata rubicola* samples that are included with the most oriental specimen of *S. torquata* and *S. leucura* (node F). The African sub-clade includes all sub-Saharan African taxa (node G), but monophyly of this diverse group receives low nodal support. Populations of the Western Palaearctic sub-clade (i.e. individuals from Kazakhstan, Europe and North Africa) are grouped together with high nodal support (node H). Finally, all European populations, plus two North African populations of *S. torquata* (node I) and the Canary species (*S. dacotiae*), are sister lineages with high nodal support (node J). The Asian clade 1 (node D) groups together all subspecies and populations of *S. caprata* plus *S. insignis*. In all three analyses the mainland subspecies of *S. caprata* included in this study (*S. c. bicolor*) is always placed divergently at the base of this clade with high nodal support (node K). Indonesian island forms of *S. caprata* (*S. c. fruticola* and *S. c. pyrrhonota*) also group together (node L). However, only the individual of *S. c. fruticola* on Moyo Island receives high nodal support (ML: 99%; MP: 100%) to suggest differentiation from *S. c. pyrrhonota* and from the other *S. c. fruticola* individual from Lembata island. The Eurasian clade (node C) contains only one species, *S. rubetra*. This species is distributed throughout Europe and Western Asia during the breeding period but has an exclusively sub-Saharan Africa distribution during the winter. Finally, the Asian clade 2 (node B) describes a sister species relationship between *S. ferrea* and *S. jerdoni* that is supported by high nodal support in all three analyses.

Analyses performed with the short fragment sequences to include *S. gutturalis* and *S. dacotiae murielae* result in similar topologies for all clades (except node F of Eurasia-African clade) but with lower nodal support (Fig. 2). The extinct population of the Canary Islands stonechat (*S. dacotiae murielae*) groups with the extant population of this species inhabiting the island of Fuerteventura as was expected (node R). In contrast, the basal position and long branch length of the Asian species *S. gutturalis* suggests that this species could be considered as a distinct lineage within the genus *Saxicola*, although its phylogenetic relationship to the other Asian species remains unresolved.

3.3. Historical biogeography

Unconstrained DIVA analysis inferred 18 dispersal events and a root node including all areas except North Africa plus Saudi Peninsula, African savannah and South African (data not shown). The optimal area reconstruction from the constrained search to no more than five areas inferred 21 dispersal events and indicated that the ancestral area of the genus occurred in a distribution covering either arid central Asian or south-western Asia plus Indian subcontinent. The inferred ancestral area of *S. torquata* resulted in 26 equally parsimonious reconstructions only excluding regions of African mainland and tropical and subtropical south-eastern Asia (Fig. 3).

3.4. Estimates of TMRCAs

Table 2 shows the mean and 95% highest posterior densities of the time of most recent common ancestor (TMRCA) for the main nodes. The age estimate for node A, the MRCA for the genus *Saxicola*, suggests diversification began during the late Miocene, approximately 8.2 million years (Mya), with the Asian clade 2 (Fig. 1, node B) being the first distinct lineage to emerge. Shortly after this, approximately 8.1 Mya, the Eurasian clade diverged from what would later become the Asian clade 1 and the Eurasia-African clade. These latter two clades are estimated to have diverged approximately 7.1 Mya (node N). Within the Eurasia-African clade

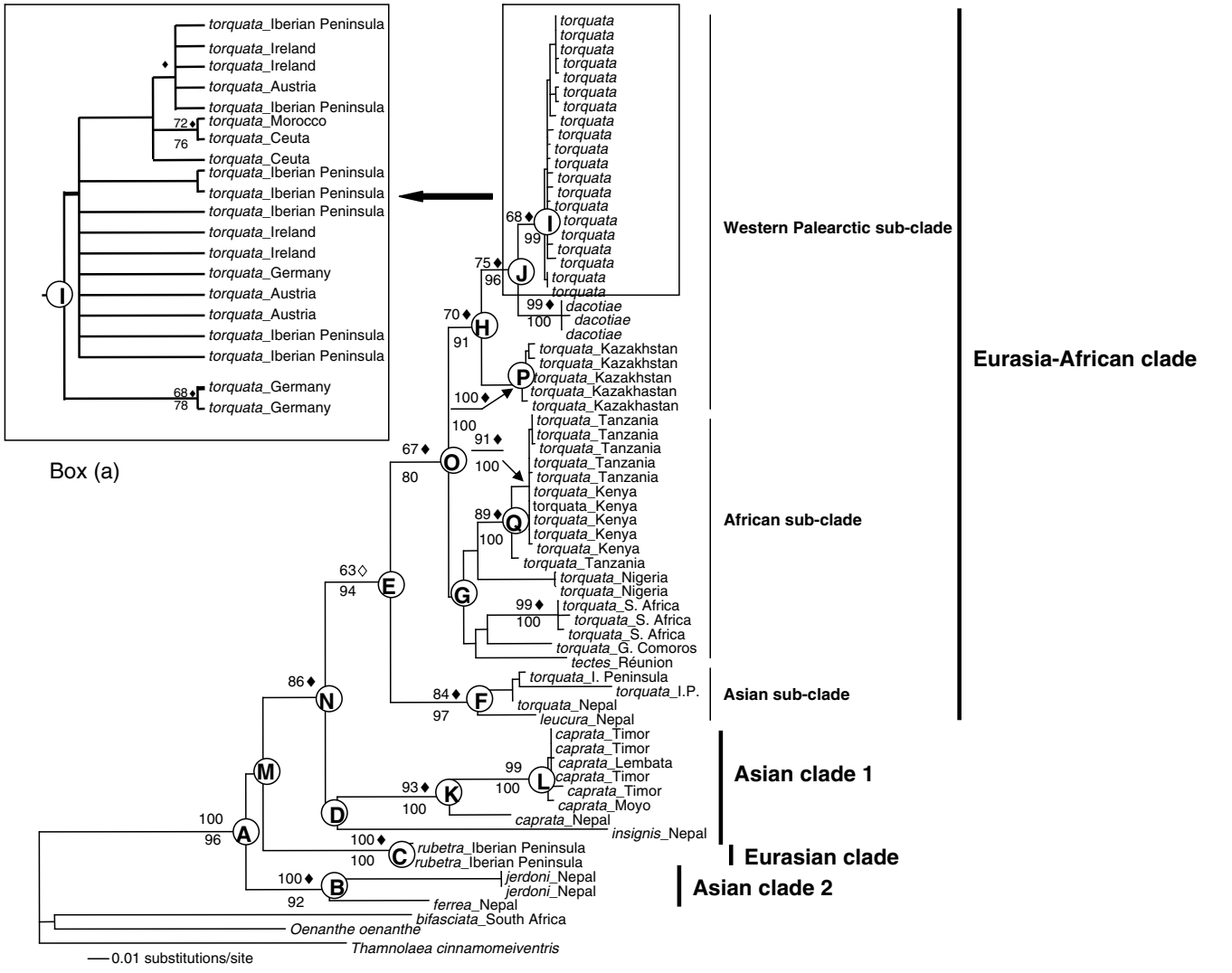

Fig. 1. Maximum likelihood tree (958 bp mtDNA cyt *b*) for *Saxicola* based on the GTR+I+G model of evolution. Numbers above nodes show the ML bootstrap support (>60%). Numbers below nodes indicate MP bootstrap support (>60%). Closed diamonds indicate Bayesian posterior probability support ⩾0.97 and open diamonds indicate posterior probability support ⩾0.93. Letters show nodes discussed in the text. The cladogram in the box (a) shows specific details on phylogenetic relationships and nodal support within node I (see text).

the Asian sub-clade, and the lineage that would ultimately give rise to the Western Palearctic and African sub-clades, are estimated to have diverged approximately 5.2 Mya ago (node E). The African sub-clade appears to have split from the Western Palearctic around 3.7 Mya (node O), while divergence within the Western Palearctic taxa is estimated to have commenced approximately 2.5 Mya (node H). Finally, the Canary Islands species (*S. dacotiae*) and the European and North African populations of *S. torquata* appear to have diverged approximately 1.6 Mya (Table 2).

4. Discussion

4.1. Systematic review

We sampled 11 of the 12 species (92%) recognized by Sibley and Monroe (1993) as being in the genus *Saxicola*, plus 15 of the 45 subspecies (33%) described for the polytypic species (Urquhart, 2002). The topology and nodal support of the resulting trees obtained in this study have revealed a high level of differentiation between species and subspecies, providing new insights into the phylogenetic relationships and patterns of diversification within this genus. Furthermore, our results do not support the placement of *S. bifasciata* within the genus *Saxicola*. The taxonomic position of this species has been much discussed and it has been placed in no fewer than five genera (see Urquhart, 2002, for an extensive review on this topic). Further molecular studies, including more taxa within the subfamily Muscicapinae, will be necessary to correctly ascertain the taxonomic affiliation of this species. With regard to *S. gutturalis*, we found support for its inclusion within the genus *Saxicola* with our evidence demonstrating it to be a divergent lineage (Fig. 2, node S). However, the limited sequence data we were able to obtain for this taxonomic group does not allow for any clear assessment of its taxonomic affinity within the genus.

4.2. Origin, biogeography and speciation

DIVA analysis suggests that the *Saxicola* group originated within the Asian region around either arid central Asian or south-western Asia plus Indian subcontinent, with subsequent dispersal and diversification in four main directions (Figs. 1 and 3): (1) the Asian mainland (Asian clade 2, node B), (2) Europe and Western Asia (Eurasian clade, node C), (3) southern Asia plus the Asian islands

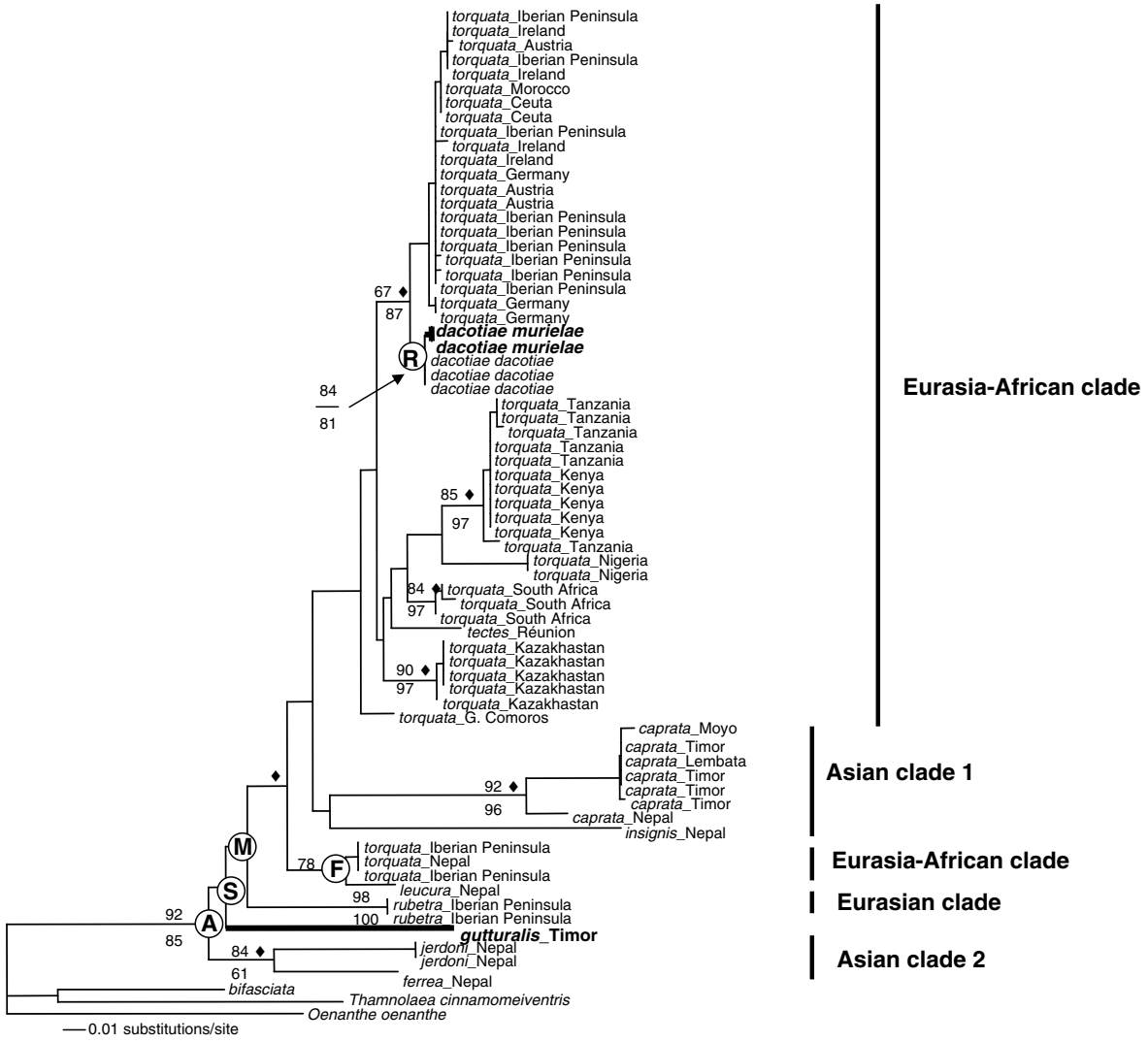

Fig. 2. Maximum likelihood tree (328 bp mtDNA cyt *b*) for *Saxicola* (including *S. gutturalis* and *S. d. murielae*, both emphasized) based on the GTR+I+G model of evolution. Numbers above nodes show the ML bootstrap support (>60%). Numbers below nodes indicate MP bootstrap support (>60%). Closed diamonds indicate Bayesian posterior probability support $\geqslant 0.98$. Letters show nodes discussed in the text.

(Asian clade 1, node D) and (4) Europe and Africa (Eurasia-African clade, node E). The Asian origin of the genus *Saxicola* is similar to other passerines distributed on multiple continents such as *Anthus* and *Motacilla* (Voelker, 1999, 2002), but different to *Monticola*, another genus of tribe Saxicolini which is suggested to have an ancestral area in the arid region of northern Africa plus Saudi Peninsula or the African savannah, or both (Outlaw et al., 2007). Interestingly, *Saxicola* genus shows evidence of an *in situ* speciation process within the Asian region more than speciation due to intercontinental dispersal events such as has been found in other widespread avian genus (Voelker, 1999, 2002). The only species that was not included in this study (*S. macrorhyncha*) is unlikely to affect this conclusion because its distribution is currently constrained to west–north India (with older records in Pakistan and Afghanistan; Urquhart, 2002). Based on this narrow distribution we would expect that this species would fall between, or within, clades B, C or D.

The most genetically diverse and geographically vast clade is the Eurasia-African clade (E), with three sub-clades distributed throughout Asia, Africa and Europe (nodes F, G, H) (Figs. 1 and 3). DIVA analyses suggest a number of possible reconstructions of the ancestral area of this clade. These all infer a Eurasian ancestral distribution with the exception of some alternatives that also include Madagascar plus Comoros and Réunion. However, the possibility that the ancestor of this clade occurred in such a widespread distribution seems unlikely. The estimated ages of Comoros and Réunion islands (0.5 and 2.1 Ma, respectively) postdate the estimated time of the most recent common ancestor of the clade descending from node E (5.16 Ma), and Madagascar has been separated from other land masses around 88 Ma (Warren et al., 2003), which would imply an over water dispersal movement from Madagascar to Africa. Therefore, it seems plausible to exclude Madagascar, Comoros and Réunion islands as ancestral areas (Voelker, 2002) and suggest a Eurasian origin (probably Asian) for *S. torquata*. DIVA analysis and tree topology suggests three or four plausible sequences of dispersal within this clade, initiated from Asia during the early Pliocene. The Asian origin of this clade is supported by the basal position of the most eastern population of *S. torquata* analysed in this study plus *S. leucura*, both inhabiting Nepal. The first plausible sequence of dispersal involves colonisation across Europe from Western Asia (node H) extending into North Africa (including the Canary Islands; node J). The second inferred colonisation is from Asia to sub-Saharan Africa (including the Western Indian Ocean archipelagos; node G). The third and

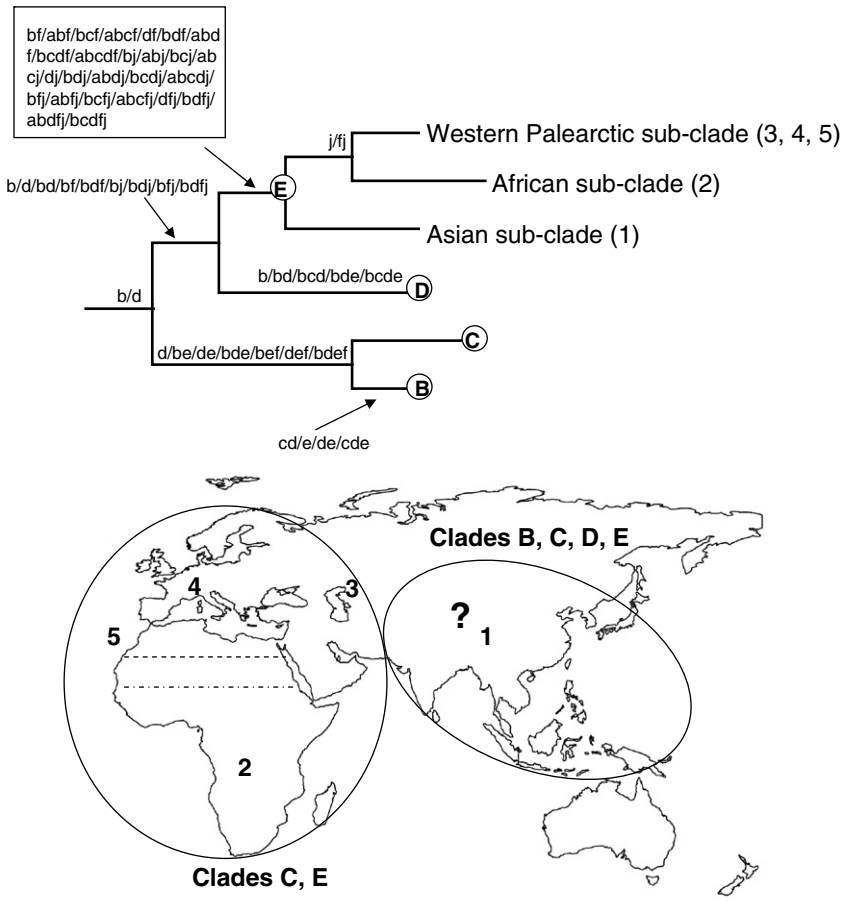

Fig. 3. Phylogram with ancestral distributions (lower case letters) of the main nodes obtained with DIVA and world distribution of the clades and sub-clades of genus *Saxicola*. (a) Eastern Asia; (b) central Asian arid; (c) Himalayas; (d) south-western Asia plus Indian subcontinent; (e) tropical and subtropical south-eastern Asia; (f) western Palearctic; (g) North African arid plus Saudi Peninsula; (h) African savannah; (i) South African arid; (j) Madagascar plus Comoros and Réunion island. For clade E results of DIVA analysis suggests the MRCA to be in Asia, with colonisation from here to Africa and the Western Palearctic (see text). Dashed lines show the approximate limits of the Sahara desert. Capital letters represent clades (B, Asian clade 2; C, Eurasian clade; D, Asian clade 1; E, Eurasia-African clade). Numbers within figure represent the geographical situation of sub-clades within Eurasia-African clade (see text).

fourth possible colonisation pathways could have involved a circular colonisation either from Asia to Africa, and then to the Palearctic or, from Asia to the Palearctic and then to Africa.

4.2.1. Eurasia-African clade

The monophyly of the Eurasia-African clade is supported by all three tree-building methods (Fig. 1, node E). The genetic divergence observed among subspecies of *S. torquata* in our study is consistent with previous studies that have revealed a high degree of differentiation between three subspecies of *Saxicola torquata* (*S. t. rubicola*, *S. t. axillaris* and *S. t. maura*; Wittmann et al., 1995; Wink et al., 2002). A similar high level of differentiation was found among the additional *S. torquata* subspecies studied here (uncorrected pairwise sequence divergence ranged from 3.8% to 5.4%), except for European *S. t rubicola*/*S. t. hibernans* and Tanzanian *S. t. axillaris*/*S. t. promiscua* subspecies pairs, where a lower level of differentiation was observed, 0.6% and 0.8%, respectively. Overall, relationships within the *S. torquata* species complex are inconsistent with a monophyletic assemblage because three other species recognized in the Sibley and Monroe's (1993) classification (*S. tectes*, *S. dacotiae* and *S. leucura*) grouping within this complex. Thus, *S. torquata* could be recognized as comprising a complex of species that are not each others closest relatives. The dramatic radiation of *S. torquata* across three continents is particularly interesting from a biogeographic point of view. The tree topology, and the high nodal support separating North African populations (included in Western Palearctic sub-clade; node H) from the Sub-Saharan subspecies of *S. torquata* (node G), suggest that that the Sahara desert is a natural barrier limiting the gene flow between the south and north of Afri-

Table 2
Estimated time of the most recent common ancestor

Node	Mean (Mya)	95% Highest posterior density	
		Lower (Mya)	Upper (Mya)
A	8.13	6.75	9.70
B	4.43	3.17	5.76
D	6.59	5.08	8.04
E	5.16	4.15	6.21
F	2.77	1.97	3.64
G	3.31	2.55	4.15
H	2.52	1.85	3.20
I	0.52	0.33	0.73
J	1.60	1.12	2.15
K	3.01	2.09	3.97
L	0.36	0.16	0.60
M	8.09	6.67	9.61
N	7.06	5.82	8.33
O	3.69	2.97	4.43
Q	0.78	0.37	1.09

Mean, lower and upper 95% highest posterior density values obtained in BEAST are shown. Dates are in million of years (Mya) before present. Nodes are shown in Fig. 1.

ca, favouring the development of different evolutionary lineages (Douady et al., 2003).

The close phylogenetic relationship between European *Saxicola* samples (node I) and those from Kazakhstan (node P) suggests that the origin of European populations could have been from western Asia rather than some sub-Saharan Africa. The extremely short branch lengths obtained below node I correspond to four European and two North African populations. This suggests there is little significant differentiation among these populations. However, without population level data we can not exclude contemporary gene flow between some of the populations which would also preclude stronger differentiation. Information obtained from ringing recoveries has demonstrated movements of *S. torquata* within Europe and between Europe and North Africa for wintering (Helm et al., 2006). These movements could enhance the opportunity for genetic exchange between populations if some migrant individuals do not come back to the breeding areas, a behaviour that has previously been observed (Helm et al., 2006). Either possibility could explain the relatively low levels of differentiation in morphological traits and colour pattern recorded within populations below node I (Urquhart, 2002), and the fact that we found limited genetic structure within and between the European and North African populations (Fig. 1, box a).

The tree topology also provides plausible evidence for the colonisation and speciation of the Canary Islands stonechat (*S. dacotiae*) from some North African or European population of *S. torquata* (node J). Our results support the divergence among these two lineages to have occurred during the Pleistocene period around 1.6 million of years ago.

Much has been discussed about the taxonomic validity of the extinct subspecies of *S. dacotiae* (*S. d. murielae*) on the Canary Islands (see for example, Urquhart, 2002) due to slight plumage colour patterns differences used to recognize this subspecies (Bannerman, 1913) and the variability in the plumage colour recorded within the extant population (*S. d. dacotiae*) on the island of Fuerteventura (Illera and Atienza, 2002). Our results show slight genetic differences (0.3%) between individuals of the extinct and extant populations analysed (Fig. 2). Unfortunately, the limited sequence data we have been able to gain for the extinct subspecies does not allow us to resolve this question unambiguously.

Two individuals of *S. torquata* caught in the Iberian Peninsula (central Spain) were, unexpectedly, grouped together with individuals of *S. torquata* and *S. leucura* from Nepal in the basal position of the Eurasia-African clade (node F). There are four possible explanations for this puzzling result: (1) samples were mislabelled, (2) samples were contaminated, (3) the samples were taken from migratory birds from the Asian populations, or (4) the birds were born in Spain, but they come from a population derived from the previous settlement (at an unknown date) of birds from an unknown Asian population. We are sure samples were not mislabelled because they came from different ringing teams (Nepal and Spain) and they were received and carefully managed in the laboratory, without any relabelling, by the same person (JCI). In order to rule out the second explanation, we repeated the DNA extraction and sequencing of these individuals. The results remained exactly the same. The last two hypotheses are difficult to reject. There are plenty of records of vagrant and wintering Asian individuals (mainly assigned to *S. t. maura*) in Europe. However, due to high plumage variation in individuals of the two European subspecies (*S. t. rubicola* and *S. t. hibernans*) many of these are now suspected to be confused with sedentary European populations (Urquhart, 2002). Further molecular studies will be needed to ascertain how frequently Asian individuals arrive in Western Europe, and whether they breed successfully, or even whether any Asian population could be settled already in Europe.

The high genetic differentiation recorded among *S. torquata* subspecies (Fig. 1, nodes F, G, H, J) typically coincides with geographically disjunct populations. This result provides evidence for the presence of reproductive barriers (and limited gene flow) between subspecies, and suggests that these taxa have had long and independent evolutionary histories. However, differences in colour patterns and morphological traits between subspecies are not always clear due to the highly variable nature of these traits within subspecies (Urquhart, 2002; Collar, 2005). This variability can complicate the correct identification of subspecies in the field (which are barely distinguishable, see above) but provides an example of cryptic speciation within the taxa. The degree of genetic differentiation between *S. torquata* subspecies (except the pairs *S. t. hibernans*/*S. t. rubicola* and *S. t. axillaris*/*S. t. promiscua*) is no less than that of valid species recognized within the same family (e.g. Outlaw et al., 2007; Voelker et al., 2007). Therefore, it may well be that these *S. torquata* are distinct enough to be regarded as true species. Specifically, based on tree topology it is possible to infer that *S. torquata* individuals of the western Palearctic (from Europe and North Africa) are a sister species to *S. dacotiae*. Similarly individuals from Kazakhstan may represent a sister species to the clade of *S. dacotiae* and western Palearctic *S. torquata*. Other potential species level differences can be inferred for Tanzania and Kenya individuals, Nigerian samples, the individual from Grand Comoro, and samples from South Africa. Finally, there is a clade of three individuals that forms a sister lineage to *S. leucura*. However, species level inferences for this group are complicated by the disparate geographic origin of these samples. However, we caution against taxonomic revision until phylogenetic relationships of additional subspecies and populations of *S. torquata* (especially from Africa and Asia) are assessed, preferably with additional nuclear markers.

4.2.2. Asian clade 1

The position of *S. insignis* in the Asian clade appears to be supported by all three tree-building methods, but with low bootstrap and posterior probability support. *Saxicola insignis* could also be considered a distinct lineage within the radiation of *Saxicola*. All subspecies and populations of *S. caprata* are grouped together and their monophyletic origin is unambiguously supported with high bootstrap values and posterior probability (Fig. 1, node K). Estimated time for the most common ancestor of all *S. caprata* species suggests a diversification origin during the mid Pliocene (Table 2). Within the Indonesian island forms the ML and MP methods support, with high bootstrap values, differentiation between the Moyo island population and the other two island populations (Lembata and Timor islands; node L). The short branch length of this last group suggests a very recent divergence. Divergence within the Indonesian islands is estimated to have occurred around 360,000 years ago.

The tree topology suggests a degree of differentiation within *S. caprata* deeper than was previously thought to exist (Urquhart, 2002), clearly indicating that mainland and island taxa have an independent evolutionary history. The level of genetic differentiation between *S. caprata bicolor* and all the other Indonesian subspecies analysed in this study (uncorrected sequence divergence ranged from 4.4% to 4.5%) is similar to that seen between other valid avian species considered within the family (see, for instance, Outlaw et al., 2007; Voelker et al., 2007). This finding provides another example of cryptic speciation within this genus and argues for the taxonomic re-evaluation of subspecies and populations within *S. caprata*. Specifically, the tree topology suggests a Nepal species with a Moyo–Timor–Lembata sister species.

4.2.3. Eurasian clade and Asian clade 2

The Eurasian and Asian clades are placed as the basal members of the genus *Saxicola* in all our analyses. The Eurasian clade con-

tains only one species (*S. rubetra*) and appears as an isolated clade between the Asian clade 2 and the super-clade including Asian clade 1 and the Eurasia-African clade (Fig. 1, node N). Interestingly, the migratory behaviour of this species is atypical for the genus as it is the only one with a long distance trans-Saharan migration. The breeding distribution is exclusively Palearctic (all Europe and central and western Asia) but its wintering distribution is completely sub-Saharan, mainly restricted to the equatorial latitudes of east and west Africa (Urquhart, 2002). Contrary to findings for the *S. torquata* taxa, the Sahara desert is clearly not a barrier for this species.

Finally, the Asian clade 2 is the most basal lineage of the genus *Saxicola*. In all three analyses the species *S. ferrea* and *S. jerdoni* are clearly sister species, and there was little genetic differentiation within the two individuals of *S. jerdoni* analysed in this study (node B). Our results support an old origin for this clade within the early Pliocene (Table 2).

4.3. Conservation implications

This study has served to clarify the phylogenetic relationships within the genus *Saxicola* and allowed the formulation of plausible hypotheses concerning the origin (Asian) and timing of diversification within this genus. Our results have also shown that the high degree of diversification found within *S. torquata* and *S. caprata* species is deeper than was expected based on the slight morphological and plumage colour patterns differences recorded in the literature. These findings provide an example of cryptic speciation within this avian genus. As conservation strategies are usually based on the concept of preserving distinct species or evolutionary significant units, the findings also raise important implications regarding the protection of the genetically differentiated populations within both taxonomic groups. Unfortunately, information on the distribution and conservation status of many of these populations is either scarce or absent. Further molecular studies, focusing on improving our understanding of the genetic relationships within and between subspecies are required to provide the necessary information to tackle a taxonomic re-evaluation of the stonechats. Such studies should result in better knowledge of the biogeographic history and diversification process that have occurred in the stonechats, which is essential for preserving the biodiversity within this genus.

Acknowledgments

Dawie de Swardt (National Museum), Ursula Franke, Paul Sweet (American Museum of Natural History), Carol Attié, Ben Warren, Wolfgang Goymann, Ulf Ottosson, José Luis Tella, Kobie Raijmakers, David Serrano, Heiner Flinks, Josef Reichholf (Zoologische Staatssammlung), Mark Adams (Natural History Museum), Claire Stevenson (Western Australian Museum), MONTICOLA and CHAGRA Spanish ringing groups and Bird Conservation Nepal provided samples. The Spanish and Morocco governments gave permission to trap and ring birds. We thank Isabel Sanmartín for assisting with DIVA analyses and two anonymous referees for valuable comments on the manuscript. This work was supported by a postdoctoral fellowship from the Spanish Ministry of Education and Science (Ref.: EX2005-0585) and a travel grant from European Science Foundation to J.C.I., and by a NERC postdoctoral fellowship to D.S.R.

Appendix A

Sequencing mtDNA primers (cyt *b*) used in this study

Name	Sequence	Reference
MT-A3	5′ GCCCCATCCAACATCTCAGCATGATGAAACTTCG 3′	Wink et al. (2002)
MT-F2	5′ CTAAGAAGGGTGGAGTCTTCAGTTTTTGGTTTACAAGACCAATG 3′	Wink et al. (2002)
*Sax*G1F	5′ CTCAGCCATCCCATACATTG 3′	This study
*Sax*G1R	5′ GTGGGTTGTTTGAGCCTGTT 3′	This study
*Sax*G2F	5′ CCCATATATGCCGAAACGTA 3′	This study
*Sax*G2R	5′ CAATGTATGGGATGGCTGAG 3′	This study
*Sax*G3R	5′ AGGTTGGGGGAGAATAGGG 3′	This study
SDM_F1	5′ AAAGAGACCTGAAATGTCG 3′	This study
SDM_R1	5′ CTGTTTCGTGTAGGAATGTG 3′	This study
SDM_F2	5′ CTGAAATGTCGGAGTCATC 3′	This study
*Sax*Seq1	5′ CCACCCATACTACTCCACAAAAGA 3′	This study
*Sax*Seq2	5′ CTACACGAAACAGGCTCAAACAA 3′	This study

References

Aljanabi, S.M., Martinez, I., 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res. 25, 4692–4693.

Bannerman, D., 1913. Descriptions of *Saxicola dacotiae murielae* & *Acanthis cannabina harterti* subsp. n. from Canary Islands. Bull. Br. Ornithol. Club 33, 37–39.

Collar, N.J., 2005. Family Turdidae (Thrushes). In: Del Hoyo, J., Elliott, A., Christie, D.A. (Eds.), Handbook of the Birds of the World. Cuckoo-Shrikes to Thrushes, vol. 10. Lynx Editions, Barcelona, pp. 514–807.

Douady, C.J., Catzeflis, F., Raman, J., Springeri, M.S., Stanhope, M.J., 2003. The Sahara as a vicariant agent, and the role of Miocene climatic events, in the diversification of the mammalian order Macroscelidea (elephant shrews). Proc. Natl. Acad. Sci. USA 100, 8325–8330.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4, 0699–0710.

Drummond, A.J., Ho, S.Y.W., Rawlence, N., Rambaut, A., 2007. A rough guide to Beast 1.4. Available from: <http://beast-mcmc.googlecode.com/files/BEAST14_Manual_6July2007.pdf>.

Duncan, R.P., Forsyth, D.M., Hone, J., 2007. Testing the metabolic theory of ecology: allometric scaling exponents in mammals. Ecology 88, 324–333.

Emerson, B.C., 2002. Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. Mol. Ecol. 11, 951–966.

Filardi, C.E., Moyle, R.G., 2005. Single origin of a pan-Pacific bird group and upstream colonization of Australasia. Nature 438, 216–219.

Freckleton, R.P., 2000. Phylogenetic tests of ecological and evolutionary hypotheses: checking for phylogenetic independence. Funct. Ecol. 14, 129–134.

Freckleton, R.P., Harvey, P.H., Pagel, M., 2002. Phylogenetic analysis and comparative data: a test and review of evidence. Am. Nat. 160, 712–726.

Goymann, W., Geue, D., Schwabl, I., Flinks, H., Schmidl, D., Schwabl, H., Gwinner, E., 2006. Testosterone and corticosterone during the breeding cycle of equatorial and European stonechats (*Saxicola torquata axillaris* and *S. t. rubicola*). Horm. Behav. 50, 779–785.

Gwinner, E., Dittami, J., Gwinner, H., 1983. Postjuvenile moult in East African and Central European Stonechats (*Saxicola torquata axillaris*, *S. t. rubicola*) and its modification by photoperiod. Oecologia 60, 66–70.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Hansen, T.F., Orzack, S.H., 2005. Assessing current adaptation and phylogenetic inertia as explanations of trait evolution: the need for controlled comparisons. Evolution 59, 2063–2072.

Harvey, P.H., Pagel, M.D., 1991. The Comparative Method in Evolutionary Biology. Oxford University Press, Oxford.

Helm, B., 2006. *Zugunruhe* of migratory and non-migratory birds in a circannual context. J. Avian Biol. 37, 533–540.

Helm, B., Gwinner, W.E., 2006. Migratory restlessness in an equatorial non-migratory bird. PLoS Biol. 4, 611–614.

Helm, B., Gwinner, E., Trost, L., 2005. Flexible seasonal timing and migratory behavior: results from Stonechat breeding programs. Ann. NY Acad. Sci. 1046, 216–227.

Helm, B., Fiedler, E.W., Callion, J., 2006.Movements of European stonechats *Saxicola torquata* according to ringing recoveries. Ardea 94, 33–44.

Hewiit, G.M., 2000. The genetic legacy of the Quaternary ice ages. Nature 405, 907–913.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Illera, J.C., Atienza, J.C., 2002. Determinación del sexo y edad en la Tarabilla Canaria *Saxicola dacotiae* mediante el estudio de la muda. Ardeola 49, 273–281.

Lovette, I.J., 2004. Mitochondrial dating and mixed support for the 2% rule in birds. Auk 121, 1–6.

Outlaw, R.K., Voelker, G., Outlaw, D.C., 2007. Molecular systematics and historical biogeography of the rock-thrushes (Muscicapidae: Monticola). Auk 124, 561–577.

Päckert, M., Martens, J., Tietze, D.T., Dietzen, C., Wink, M., Kvist, L., 2007. Calibration of a molecular clock in tits (Paridae)—do nucleotide substitution rates of mitochondrial genes deviate from the 2% rule? Mol. Phylogenet. Evol. 44, 1–14.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Rambaut, A., Drummond, A.J., 2007. Tracer v1.4. Available from: <http://beast.bio.ed.ac.uk/Tracer>.

Ronquist, F., 1996. DIVA v. 1.1. Computer program for MacOs and Win32. Available from: <http://morphbank.ebc.uu.se/DIVA>.

Ronquist, F., 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. Syst. Biol. 46, 195–203.

Sibley, C.G., Monroe Jr., B.L., 1993. A Supplement to the Distribution and Taxonomy of Birds of the World. Yale University Press, New Haven, CT.

Sunnucks, P., Hales, D.F., 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase I–II in aphids of the Genus Sitobion (Hemiptera: Aphididae). Mol. Biol. Evol. 13, 51–524.

Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (* and other methods), version 4.0b10 for Unix. Sinauer Associates, Sunderland, MA.

Urquhart, E.D., 2002. Stonechats: Guide to the Genus *Saxicola*. Christopher Helm, London, UK.

Voelker, G., 1999. Dispersal, vicariance, and clocks: historical biogeography and speciation in a cosmopolitan passerine genus (*Anthus*: Motacillidae). Evolution 53, 1536–1552.

Voelker, G., 2002. Systematics and historical biogeography of wagtails: dispersal versus vicariance revisited. Condor 104, 725–739.

Voelker, G., Spellman, D.C., 2004. Nuclear and mitochondrial DNA evidence of polyphyly in the avian superfamily Muscicapoidea. Mol. Phylogenet. Evol. 30, 386–394.

Voelker, G., Rohwer, G.M.S., Bowie, R.C.K., Outlaw, D.C., 2007. Molecular systematics of a speciose, cosmopolitan songbird genus: defining the limits of, and relationships among, the *Turdus* thrushes. Mol. Phylogenet. Evol. 42, 422–434.

Warren, B.H., Bermingham, E., Bowie, R.C.K., Prys-Jones, R.P., Thébaud, C., 2003. Molecular phylogeography reveals island colonization history and diversification of western Indian Ocean sunbirds (Nectarinia: Nectariniidae). Mol. Phylogenet. Evol. 29, 67–85.

Wikelski, M., Spinney, L., Schelsky, W., Scheuerlein, A., Gwinner, E., 2003. Slow pace of life in tropical sedentary birds: a common-garden experiment on four stonechat populations from different latitudes. Proc. R. Soc. Lond. B 270, 2383–2388.

Wink, M., Sauer-Gürth, H., Heidrich, P., Witt, H-H.H.-H., Gwinner, E., 2002. A molecular phylogeny of stonechats and related turdids. In: Urquhart, E. (Ed.), Stonechats. Christopher Helm, London, pp. 22–30.

Wittmann, U., Heidrich, P., Wink, M., Gwinner, E., 1995. Speciation in the stonechat (*Saxicola torquata*) inferred from nucleotide sequences of the cytochrome-*b* gene. J. Zoo. Syst. Evol. Res. 33, 116–122.