The LINNEAN SOCIETY of London BIOLOGICAL Journal of the Linnean Society

Biological Journal of the Linnean Society, 2016, **119**, 904–918. With 4 figures.

Phylogeny, biogeography, and diversification of barn owls (Aves: Strigiformes)

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Received 16 February 2016; revised 26 April 2016; accepted for publication 26 April 2016

The existence of substantial morphological variation has resulted in the description of numerous subspecies of the cosmopolitan barn owl, *Tyto alba*. However, preliminary studies have revealed a high degree of genetic variation between Old and New World barn owls, suggesting that the *T. alba* complex may consist of several species. We present a comprehensive study of its taxonomy and propose a spatiotemporal framework to explain the origin and patterns of dispersal and diversification within these cosmopolitan owls. We used a Bayesian relaxed molecular clock approach to assess the timing of diversification. To evaluate the biogeographical pattern, we considered dispersal in addition to temporal connectivity between areas. Finally, we used ecological niche modelling to evaluate their ecological niches. Our phylogenetic analyses suggest that barn owls of the Old and New World show a high degree of genetic divergence, and the barn owls of South and South-east Asia (*Tyto alba stertens* and *Tyto alba javanica*) cluster with the Australian barn owl *Tyto delicatula*. We propose to treat the *T. alba* complex as three species: *T. alba* (Africa, Europe), *Tyto furcata* (New World), and *Tyto javanica* (Australasia). The dating analyses indicate that the early divergence among the species of the *T. alba* complex took place in the Middle Miocene and we hypothesize that a common ancestor of the *T. alba* complex lived in Africa. A potential scenario suggests that *T. alba* dispersed to Europe and south-western Asia during the interglacial periods of the Miocene/Pliocene, and dispersed into the New World either via an eastern Asian route or a western north Atlantic one. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **119**, 904–918.

KEYWORDS: climate change – dating analyses – ecological niche modelling – land bridges – miocene – strigiformes.

INTRODUCTION

The genus *Tyto* (barn owls and allies) belongs to the oldest surviving owls, the Tytonidae, which diverged from the Strigidae in the late Eocene. The genus *Tyto* has been dated at least to the Middle Miocene and appeared in South-east Asia and New Guinea where, today, the highest diversity of *Tyto* occurs (del Hoyo *et al.*, 1999; Wink *et al.*, 2009). The common barn owl *Tyto alba* is one of the most cosmopolitan species, with between 10 and 28 defined subspecies located on all continents except Antarctica, as well as on an array of oceanic islands (del Hoyo *et al.*, 1999; König, Weick & Becking, 2008). High geographical variation in plumage and body size cast doubt on the subspecific status of many subspecies (Roulin, Wink & Salamin, 2009) as confirmed by recent molecular studies (König *et al.*, 2008; Nijman & Aliabadian, 2013). Unfortunately, there is still no consensus on the phylogeny and taxonomic

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status within the *Tyto* genus, and the status of the different taxa in the *T. alba* complex remains to be resolved (del Hoyo *et al.*, 1999).

König *et al.* (2008) defined 25 species in the genus *Tyto*, partially based on the molecular work presented by Wink & Heidrich (2000) and Wink, Saur-Gurth & Fuchs (2004) and Wink *et al.* (2008). Within the *T. alba* complex, they identified three widely distributed species. The first of these is the common barn owl *T. alba*, with 10 subspecies (*Tyto alba affinis*, *Tyto alba alba*, *Tyto alba erlangeri*, *Tyto alba ernesti*, *Tyto alba gracilirostris*, *Tyto alba guttata*, *Tyto alba hypermetra*, *Tyto alba javanica*, *Tyto alba schmitzi*, and *Tyto alba stertens*), distributed throughout most of Africa, Eurasia, and parts of South-east Asia. The second is the American barn owl *Tyto furcata* with at least five subspecies (*Tyto furcata contempta*, *Tyto furcata furcata*, *Tyto furcata hellmayri*, *Tyto furcata pratincola*, and *Tyto furcata tuidara*) from North, Central, and South America. The third widely distributed species is the Australian barn owl *Tyto delicatula* with at least four subspecies (*Tyto delicatula delicatula*, *Tyto delicatula interposita*, *Tyto delicatula meeki*, and *Tyto delicatula sumbaensis*) restricted to the easternmost part of South-east Asia, Australia, New Zealand, and parts of Polynesia. These three wide-ranging species differ in appearance, including overall size, the power of tarsus and toes, the amount of feathering on the tarsus, the degree of dark-redness, and the number and size of black spots (König *et al.*, 2008). Resolving the phylogeny of the *Tyto alba* group is of importance because this is one of the few vertebrates that is cosmopolitan. Such a study would bring useful insights about the origin, colonization pattern, and genetic differentiation of a given bird that is distributed worldwide (Monti *et al.*, 2015).

In addition to these three wide-ranging species, König *et al.* (2008) identified six small-range, island, taxa (*Tyto alba bargei*, *Tyto alba crassirostris*, *Tyto alba deroepstorffi*, *Tyto alba detorta*, *Tyto alba punctatissima*, and *Tyto alba thomensis*) as a separate monotypic species. Several of these arrangements were not supported by the molecular analyses (Wink & Heidrich, 2000; Wink *et al.*, 2004, 2008), with, for example, *Tyto bargei* nested among *T. furcata* and low support for the split between Old and New World barn owls. A large number of species identified by König *et al.* (2008) have not been subjected to a molecular phylogenetic analysis, and their taxonomic and systematic status remains unclear. For example, it is unknown whether the barn owls from South and South-east Asia group with the Eurasian or Australian barn owls (Christidis & Boles, 2008; Gregory, 2010; Round, 2012).

The above synopsis of the literature shows that species limits in the *T. alba* complex are unresolved, systematic relationships are unclear, as is the timing of the different speciation events. In the present study, we used a dense taxon sampling to assess phylogenetic relationships among members of the *T. alba* complex based on three mitochondrial (*Cox1*, *Cytb*, *16s*) markers and one nuclear (*Rag-1*) marker. We used independent calibration points for mitochondrial and nuclear genes to estimate the divergence time among barn owl species and to identify its geographical origin. We examined the association between geographical isolation and climatic variation among species of the *T. alba* complex and tested the hypothesis of occurrence niche divergence among them.

MATERIAL AND METHODS

Sampling

We examined 40 samples belonging to ten taxa: *hellmayri (*Bonaire*)*, *tuidara (*Argentina*)*, *pratincola* (Louisiana, Florida, Texas, and California), *bargei* (Curaçao), *alba* (Greece), *erlangeri* (Iran), *guttata* (Netherlands), *javanica* (Indonesia), *affinis* (Ethiopia), and *delicatula* (Australia) (Table 1). The taxa studied, showing their localities, collection numbers of museums, and GenBank accession numbers, are presented in Table 1.

Laboratory procedures

We extracted DNA from samples of blood, muscles tissue or feathers obtained from either live animals trapped in the field or museum specimens. Total genomic DNAs was extracted using a standard salt extraction method (Bruford *et al.*, 1992), incubated overnight at 55 °C immersed in an extraction buffer (2% sodium dodecyl sulphate, 0.5 mg/mL proteinase K). Polymerase chain reaction (PCR) conditions, amplification procedures, and primers used in the present study are described in Irestedt *et al.* (2001) for the nuclear gene *Rag-1*, and, for the three mitochondrial genes, in Vences *et al.* (2000) for *16s*, Hebert, Guelph & Sl (2003) for *Cox1*, and Johansson *et al.* (2002) for *Cytb*. PCR products were purified using the QIA quick PCR purification Kit (Qiagen) in accordance with the manufacturer's instructions. The purified PCR products were sequenced using dye-labelled dideoxy terminator cycle sequencing with Big Dye, version 3.1 (Applied Biosystems, Inc). We analyzed 620 bp of *Cytb*, 660 bp of *Cox1*, 568 bp of *16s*, and 990 bp of *Rag-1*.

Table 1. Specimens of the barn owl *Tyto alba* complex: collection and GenBank accession numbers for the four genes sampled from birds in Africa, Asia, Europe, North America and Australia

Taxon	GenBank accession numbers				Collection numbers	Locality
	Cytb	*Cox1*	*16s*	*Rag-1*		
Tyto alba guttata	–	FJ465382	FJ465288	–	ZMA58235	Netherlands
Tyto alba guttata	–	FJ465383	FJ465289	–	ZMA58237	Netherlands
Tyto alba guttata	KX440453	KF432220	KX440413	KX440475	ZMA58962	Netherlands
Tyto alba guttata	KX440454	KF432219	KX440414	KX440476	ZMA58963	Netherlands
Tyto alba guttata	KX440455	KF432218	KX440415	KX440477	ZMA58964	Netherlands
Tyto alba guttata	KX440456	KF432221	KX440416	–	ZMA58965	Netherlands
Tyto alba guttata	KX440457	KF946918	KX440417	–	ZMA58843	Netherlands
Tyto alba guttata	KX440458	KF946919	KX440418	–	ZMA58844	Netherlands
Tyto alba alba	KX440449	KF432226	KX440409	KX440471	NHMC80.4.108.8	Greece
Tyto alba alba	KX440450	KF432223	KX440410	KX440472	NHMC80.4.108.9	Greece
Tyto alba alba	KX440451	KF432225	KX440411	KX440473	NHMC80.4.108.7	Greece
Tyto alba alba	KX440452	KF432224	KX440412	KX440474	NHMC80.4.108.6	Greece
Tyto alba affinis	–	–	KX440425	–	ZMA19883	Ethiopia
Tyto alba javanica	KX440459	KX440429	KX440419	–	ZMA334	Indonesia
Tyto alba javanica	KX440460	KX440430	KX440420	–	ZMA335	Indonesia
Tyto alba erlangeri	–	KF432228	KX440406	KX440468	MFUM800001	Iran
Tyto alba erlangeri	KX440447	KF432227	KX440407	KX440469	MFUM800002	Iran
Tyto alba erlangeri	KX440448	KX440428	KX440408	KX440470	MFUM800003	Iran
Tyto alba bargei	KX440432	KX440426	KX440394	–	ZMA55930	Netherlands Antilles
Tyto alba bargei	KX440433	FJ465378	FJ465284	–	ZMA55939	Netherlands Antilles
Tyto alba bargei	KX440434	FJ465379	FJ465285	–	ZMA55941	Netherlands Antilles
Tyto alba bargei	KX440435	FJ465380	FJ465286	–	ZMA55942	Netherlands Antilles
Tyto alba bargei	KX440436	KF432207	KX440395	–	ZMA58966	Netherlands Antilles
Tyto alba hellmayri	KX440437	FJ465375	FJ465281	–	ZMA55945	Netherlands Antilles
Tyto alba hellmayri	–	FJ465376	FJ465282	–	ZMA58257	Netherlands Antilles
Tyto alba hellmayri	KX440438	FJ465377	FJ465283	–	ZMA58259	Netherlands Antilles
Tyto alba hellmayri	–	KX440427	–	–	ZMA58253	Netherlands Antilles
Tyto alba pratincola	KX440439	KF432212	KX440396	KX440461	LSUMZ16306	USA
Tyto alba pratincola	–	KF432217	KX440397	KX440462	LSUMZ44989	USA
Tyto alba pratincola	KX440440	KF432213	KX440398	–	LSUMZ20610	USA
Tyto alba pratincola	KX440441	KF432210	KX440399	KX440463	LSUMZ20485	USA
Tyto alba pratincola	KX440442	KF432215	KX440400	KX440464	LSUMZ49512	USA
Tyto alba pratincola	KX440443	KF432215	KX440401	–	LSUMZ49511	USA
Tyto alba pratincola	–	KF432208	KX440402	KX440465	LSUMZ49510	USA
Tyto alba pratincola	KX440444	KF432209	KX440403	KX440466	LSUMZ49509	USA
Tyto alba pratincola	KX440445	KF432211	KX440404	KX440467	LSUMZ21784	USA
Tyto alba pratincola	KX440446	KF432216	KX440405	–	LSUMZ29566	USA
Tyto alba delicatula	–	KX440431	KX440421	–	ZMA21.978	Australia
Tyto alba delicatula	–	–	KX440422	–	ZMA21.979	Australia
Tyto alba tuidara	–	–	KX440423	–	ZMA22.100	Argentina
Tyto alba tuidara	–	–	KX440424	–	ZMA22.101	Argentina

Phylogenetic analysis

We performed separate phylogenetic analyses for each gene, the combined mitochondrial genes and for a combination of all genes (mtDNA+*Rag-1*) using maximum likelihood (ML) and Bayesian inference (BI), as implemented in RAXML, version 7.0.4 (Stamatakis, 2006) and MrBayes, version 3.2 (Huelsenbeck & Ronquist, 2001), respectively.

The best-fit nucleotide substitution models were determined with MRMODELTEST, version 2.3 (Nylander, 2004), in conjunction with PAUP* (Swofford, 2003) based on Akaike information criterion values (Ellegren, 2007). Gene partitions were analyzed both separately and in concatenation. The estimated models were used in a subsequent ML heuristic tree and Bayesian analyses (Table 2).

Table 2. Molecular properties per gene and partitions

Gene region	Alignment length	Model
16s	568	K80 + G
Cox1	660	HKY+G
Cox1 1st	220	K80
Cox1 2nd	220	F81
Cox1 3rd	220	HKY
Cytb	620	HKY+G
Cytb 1st	208	HKY
Cytb 2nd	206	K80+G
Cytb 3rd	206	F81
Rag-1	990	K80+G
Combined mtDNA genes	1848	GTR+I+G
Complete combined dataset	2838	TVM+I+G

Four independent Metropolis-coupled Markov chains were run in each analysis of MrBayes in which one cold chain and three heated chains were defined. Nucleotide substitution models were unlinked across partitions. For these chains, we performed 30 million iterations with sampling every 10 000 generations. All trees obtained before convergence as burn-in (25% trees) were discarded, and the log-likelihood values ($-\ln L$) and posterior probabilities for nodes were calculated from the remaining trees. We used TRACER, version 1.5 (Rambaut & Drummond, 2007) to ensure that our sampling of the posterior distribution had reached a sufficiently effective sample size (> 200) for meaningful parameter estimation.

ML searches were conducted with RAXML, version 7.0.3, on the web server with 100 rapid bootstrap inferences. All free model parameters (substitution rates, gamma shape parameter, base frequencies) were estimated using the best parameter settings found by MrBayes. We considered clades with bootstrap values ≥ 70 (Hillis & Bull, 1993) and BI ≥ 0.95 (Huelsenbeck & Ronquist, 2001) to be significantly supported.

Alternative phylogenetic hypotheses obtained enforcing the monophyly of selected species were compared using the Shimodaira–Hasegawa test. In addition, using PAUP, the combination of the four genes *Cytb*, *Cox1*, *16s*, and *Rag-1* and the combined tree of mitochondrial *Cytb* and the nuclear *Rag-1* genes of Wink *et al.* (2008) were compared using the Shimodaira–Hasegawa test (Shimodaira & Hasegawa, 1999) under full optimization (one-tailed test) and 1000 replicates (Stamatakis, 2006).

Finally, we made use of available sequences on GenBank to present complementary Bayesian analysis based on selected mitochondrial and nuclear genes. All available sequences for *Cytb* and *Rag-1* were downloaded and these were combined with our newly created sequences (see Supporting information, Table S1). Haplotype diversity in the *Cytb* gene (1044 bp) of barn owls was analyzed by the method of probability of parsimony using the TCS (Clement, Posada & Crandall, 2000).

Dating analysis

Molecular dating was carried out with BEAST, version 1.8 (Drummond & Rambaut, 2007). The tree was rooted with *Phodilus badius* and *Tyto novaehollandiae* (Australia), *Tyto castanops* (Tasmania), and *Tyto longimembris* (Taiwan).

The most appropriate models of nucleotide substitution, determined with MRMODELTEST, version 2.3 (Nylander, 2004), were used (HKY+I model for *Rag-1*, HKY for *Cox1*, TN93+G model for *16s* and HKY+G for *Cytb*). We used published substitution rates for each mitochondrial and nuclear genes, separately, from Lerner *et al.* (2011): for *16s*, a rate of 0.005 substitutions/site/Myr was used as mean for a normal prior with a rather high SD of 0.002 to account for differences between stem and loop regions and 0.016 substitutions/site/Myr for *Cox1*. Because *Rag-1* and *Cytb* were not included in Lerner *et al.* (2011), rates of 0.00135 substitutions/site/Myr (Ellegren, 2007; Smith *et al.*, 2013) and 0.0105 substitutions/site/Myr (Weir & Schluter, 2008; Schweizer & Shirihai, 2013) were used, respectively. These rates were implemented as means of a normal prior of the ucld.mean parameter with SDs of 0.005 for the mitochondrial and 0.0005 for the nuclear markers, respectively.

A Yule process on species trees and a log-normal relaxed clock model (uncorrelated) were implemented in all analyses. Furthermore, the alternative coalescence approaches (coalescent: expansion growth, constant size, logistic growth) were tested using Bayes factors implemented in TRACER, version 1.5 (Rambaut & Drummond, 2007). The default prior distributions were chosen for the other parameters. Four dependent Markov Chain Monte Carlo (MCMC) were run, each comprising 25 million generations that were sampled every 1000th generation. The output files were analyzed using TRACER, version 1.5 to determine the effective sample size of the posterior probability for all parameters and to confirm appropriate burn-in and convergence. The three independent runs were combined using LOG COMBINER, version 1.7.5 (Drummond & Rambaut, 2007) by considering 10% of the first trees as burn-in to produce the final results. Then, the highest clade credibility and the 95% highest posterior density (HPD) distributions for all nodes were estimated with TREE

ANNOTATOR, version 1.8.0 (Drummond & Rambaut, 2007). The phylogeny was visualized using FIGTREE, version 1.4.0 (Rambaut, 2008).

Establishing the ancestral area

We used LAGRANGE (http://www-reelab.net/lagrange; Ree *et al.*, 2005; Ree & Smith, 2008) to study ancestral distributions of three groups (i.e. *alba* from the Africa and Eurasia, *furcata* from the New World, and *delicatula*/*javanica* from Australasia). Ancestral reconstruction was performed according to information about divergence times (maximum clade credibility tree obtained from the best-fitting model in BEAST) and any available geological information (data matrix of species ranges coded as binary presence–absence values). This approach is a type of continuous-time model that considers models of dispersal, extinction, and cladogenesis (DEC) (Ree *et al.*, 2005; Ree & Smith, 2008). The DEC models considered not only the evolution along branches and during lineage splitting (cladogenesis), but also dispersal and extinction on single branches over time. The geographical range shared by close species is also modelled and the areas with the highest likelihood are mostly inherited (Ree *et al.*, 2005). According to current distribution, five geographical areas were assigned: the Nearctic-Neotropics, and the Palearctic, Ethiopian, Oriental, and Australian regions. An adjacency matrix was defined to consider which areas are connected to each other. By default, all possible ranges and all combinations of areas were allowed. In addition, the same rate of extinction and dispersal across areas and across all branches of the phylogenetic tree was assumed.

Ecological niche modelling

We conducted an ecological niche modelling analysis to test whether the three above-mentioned groups have their own ecological niche. A maximum entropy method (MAXENT, version 2.0) (Phillips & Dudík, 2008) was used to predict the potential distribution range based on point-occurrence data (only presence species records) and the environmental conditions across the study area (Thorn *et al.*, 2009; Warren, Glor & Turelli, 2010; Warren & Seifert, 2011). Georeferenced geographical records for three groups were collected from our field investigations and from collections linked to the Global Biodiversity Information Facility (http://www.gbif.org/) and Ornis (http://www.ornisnet.org/) databases. The bioclimatic variables, which are biologically related to temperature, precipitation, slope, and altitude (Hijmans, Cameron & Parra, 2005), were obtained from the WorldClim database, version 1.4, with a resolution of 2.5 min (http://www.worldclim.org). In total, 21 bioclimatic variables were extracted, comprising Annual Mean Temperature, Mean Diurnal Range, Isothermality, Temperature Seasonality, Maximum Temperature of Warmest Month, Minimum Temperature of Coldest Month, Temperature Annual Range, Mean Temperature of Wettest Quarter, Mean Temperature of Driest Quarter, Mean Temperature of Warmest Quarter, Mean Temperature of Coldest Quarter, Annual Precipitation, Precipitation of Wettest Month, Precipitation of Driest Month, Precipitation Seasonality, Precipitation of Wettest Quarter, Precipitation of Driest Quarter, Precipitation of Warmest Quarter, Precipitation of Coldest Quarter, Altitude, and Slope.

To control for colinearity among environmental variables and over-fitting problems, 1000 random geographical points within the study area were defined in ARCMAP (ESRI, 2011). The pairwise Pearson's correlation coefficients between all 21 climatic variables were calculated. Correlation coefficients larger than 0.75 have been considered as highly correlated variables (Rissler & Apodaca, 2007). The correlated variables were eliminated and, ultimately, only 11 environmental variables were extracted for the niche modelling analyses (Table 3).

Table 3. Eleven selected uncorrelated variables and the amount of estimation of relative contributions of each these environmental variables to the MAXENT model for three wide-ranging barn owl taxa

Code	Definition of layers	*Tyto alba*	*Tyto furcata*	*Tyto javanica*
BIO2	Mean diurnal range	3.8	8.2	2.3
BIO7	Temperature annual range	**49.5**	**37.9**	0.1
BIO8	Mean temperature of wettest quarter	5.0	**9.1**	5.5
BIO9	Mean temperature of driest quarter	3.9	6.4	1.9
BIO10	Mean temperature of warmest quarter	3.9	7.3	0.9
BIO15	Precipitation seasonality	5.6	3.3	0.1
BIO17	Precipitation of driest quarter	**5.9**	3.7	**9.0**
BIO18	Precipitation of warmest quarter	1.7	3.0	2.6
BIO19	Precipitation of coldest quarter	1.3	**11.5**	**16.4**
	Altitude	7.9	7.7	3.9
	Slope	**11.5**	1.9	**8.3**

The bold values in each column indicate the three variables with the most important contribution.

MAXENT models were retained for niche modelling analyses. In MAXENT, we set the convergence threshold to $1.0e^{-5}$ with 1000 iterations and the regularization value to $1.0e^{-4}$. The logistic output format was chosen. Evaluation of the predictive ability of the MAXENT models was based on both a 70 : 30 split of locality sample sizes into training and test data partitions, and five replicate tests of random data partitions. The resultant ASCII file was depicted using ARCGIS, version 9.1 (ESRI, 2011). Moreover, we used ENMTOOLS (Warren & Seifert, 2011) for additional statistical analyses of niche modelling. Niche overlap among three groups, *alba* from the Africa and Eurasia, *furcata* from the New World, and *delicatula*/*javanica* from Australasia, was evaluated using the Schoener's *D* and *I* statistics. In addition, to evaluate the level of divergence in the ecological niches obtained of all three species, identity tests were performed in ENMTOOLS. We used the identity test to determine whether the niche models of species were identical or exhibit a statistically significant difference.

RESULTS

Sequence characteristics

The final alignment comprised 2838 bp (*16s*: 568 bp; *Cox1*: 660 bp; *Cytb*: 620 bp; *Rag*-1: 990 bp) (Table 4). The mitochondrial (mt)DNA sequences consisted of 1848 bp in length.

Phylogenetic analysis

For the main Bayesian and ML analyses performed on the concatenated dataset, ML: –ln 10620.6162 and BI harmonic mean −10927.29. When considering the 50% majority-rule consensus trees resulting from each of the four different genes separately, the concatenated mtDNA alignment or the combined dataset comprising mitochondrial and nuclear genes, all were topologically congruent. In addition, all provided well-supported clades with posterior probabilities > 0.95. The topology of the ML trees was completely congruent with the BI topologies, and all of the main nodes were well supported (bootstrap values > 70). Three main clades were detected in all trees. The *furcata* clade contains the subspecies *T. a. pratincola*, *T. a. hellmayri* and *T. a. tuidara* from mainland North and South America, as well as *T. a. bargei* from Curaçao. The *alba* clade contains all samples from *T. a. erlangeri*, *T. a. alba*, *T. a. guttata* and *T. a. affinis*. *Tyto a. javanica* from Indonesia and *T. a. stertens* from India are not part of the *alba* clade and are grouped with *T. a. delicatula* from Australia (Fig. 1). High genetic divergence was estimated among all of the three clades. The nuclear gene (*Rag-1*) showed a relatively low genetic divergence between the *alba* and *furcata* clades (Table 5).

Restricting the analysis to the *Cytb* and *Rag-1* genes, we find equally strong support for the monophyly of these three clades, with significant amounts of genetic divergence between them. Barn owls from the eastern Indonesian island of Sumba are basal to a clade containing *delicatula*, *javanica*, and *stertens*. Different from the concatenated dataset, we no longer find support for *alba* and *furcata* being sister taxa; instead, *delicatula* and *furcata* are sister taxa (see Supporting information, Figure S1). Finally, our haplotype analysis of *Cytb* clearly shows these same three taxa, although the single sample of *sumbaensis* now no longer forms part of the haplotypes containing *delicatula*, *javanica*, and *stertens* (see Supporting information, Figure S2).

Combining these data gives strong support for three distinct monophyletic taxa that are probably best considered as separate species, i.e. *T. alba*, *T. furcata* (including *bargei*), and *T. javanica* (including *delicatula* and *stertens*). Because *javanica* was first described by Gmelin in 1788 and then

Table 4. Length of the alignment and DNA substitution in barn owl genes

Locus	*Cox1*	*Cytb*	*16s*	Concentrate mtDNA	*Rag-1*
Length (bp)	660	621	569	1448	990
Number of individuals (haplotypes)	32 (9)	30 (9)	40 (10)	43	17 (10)
BI harmonic mean	−1884.89	−1860.76	−1573.42	−5458.10	−1733.83
ML score	1885.7485	1688.5380	1401.2760	5112.1416	1652.9974
Phylogenetic informative sites	189	341	76	–	40
Transition/transversion ratio	7.11	2.00	2.38	–	4.39
Number of parsimony informative bases (%)	26	27	19	–	7
Number of variable bases (%)	46	49	74	–	48

BI, Bayesian inference; ML, maximum likelihood.

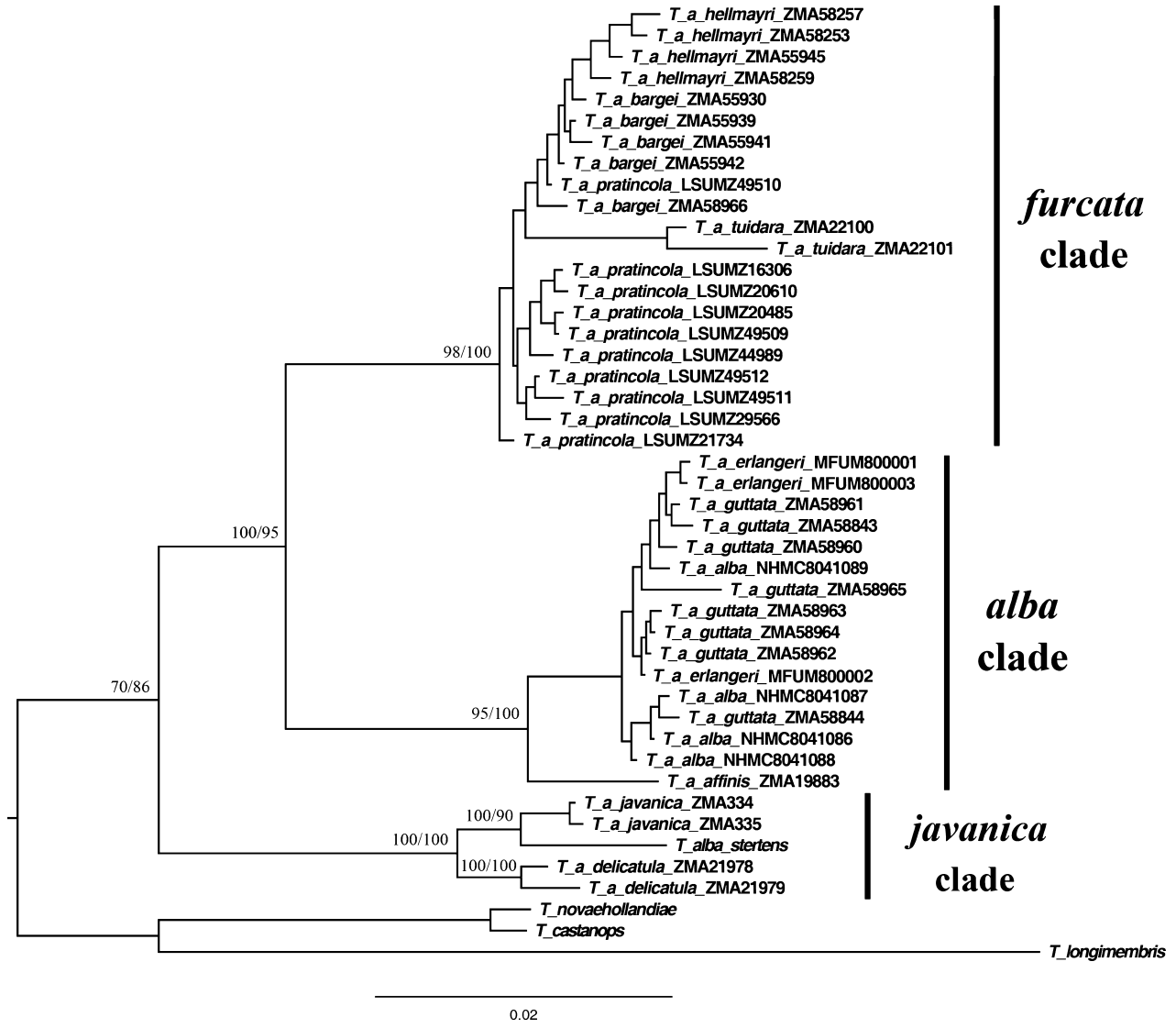

Figure 1. Fifty-percent majority-rule consensus tree sampled from the posterior distribution of the most-partitioned analysis based on 2838 bp of *16s*, *Cox1*, *Cytb*, and *Rag1*. Posterior probability values from the Bayesian analysis are indicated as the first number and the second number represents maximum likelihood bootstrap values.

delicatula by Gould in 1837, the name *javanica* has nomenclatural priority as the correct name for the Australasian clade and species.

Our results are corroborated by the Shimodaira–Hasegawa tests (Table 6) on alternative hypotheses about the monophyly of each of three clades *alba*, *furcata*, and *javanica*. The reciprocal monophyly of *T. alba*, *T. furcata*, and *T. javanica*, as defined above, was not significantly different from our combined tree of four genes (*Rag-1*, *Cytb*, *Cox1*, *16s*). In addition, the Shimodaira–Hasegawa test comparing our best tree with the combined tree of Wink *et al.* (2008) showed no significant difference (Table 6).

Dating analysis

All three independent runs of the BEAST analyses showed a high convergence among all parameters. Analysis of the combined file showed that effective sizes exceeded 355 for all parameters. The BEAST dating analysis produced a phylogenetic estimate that strongly agrees with that obtained from the concatenate Bayesian analysis (Fig. 2). Three main clades, *T. alba*, *T. furcata*, and *T. javanica*, were also detected in BEAST analysis trees. Similar to the *Cytb* and *Rag-1* analysis (see Supporting information, Figure S1) but different from our concatenated dataset (Fig. 1), in the tree obtained using BEAST, *T. furcata* was found to be a sister group

Table 5. Genetic distances between and within species of *Tyto* species were calculated in four genes *Cox1*, *Cytb*, *16s*, and *Rag-1*using the Kimura two-parameter as implemented in ExcaliBAR (Aliabadian *et al.*, 2014)

	Cytb	*Cox1*	*16s*	*Rag-1*
Genetic divergence within *furcata* clade	0.0041	0.0039	0.005	0.0024
Genetic divergence within *alba* clade	0.0162	0.0131	0.0169	0.0012
Genetic divergence within *bargei*	0.0066	0.000938	0.0009	–
Genetic divergence between *alba* and *furcata* clades	0.0721	0.0551	0.0376	0.0048
Genetic divergence between *bargei* and *furcata* clade	0.0091	0.0035	0.0026	–
Genetic divergence between *alba* clade and *javanica*	0.0954	0.0637	0.0447	–
Genetic divergence between *javanica* and *delicatula*	–	0.0064	0.0122	–
Genetic divergence between *alba* clade and *stertens*	0.088	0.067	–	–
Genetic divergence between *stertens* and *delicatula*	–	0.006	–	–
Genetic divergence between *stertens* and *javanica*	0.001	0.007	–	–

Table 6. Comparison of alternative phylogenetic hypotheses and combined tree *Cytb* and *Rag-1* genes of Wink *et al.* (2008) with our best tree using the Shimodaira–Hasegawa test performed in RAXML

Topology tested	Tree likelihood	Shimodaira–Hasegawa test
Best tree	−631.696143	Best
Monophyly of *alba* clade	−6317.710062	NS
Monophyly of *furcata* clade	−6317.699226	NS
Monophyly of *javanica* clade	−6317.702293	NS
Tree of Wink *et al.* (2008)	−6317.696143	NS

Tree likelihoods were calculated in PAUP* (Swofford 2002). NS, not significantly worse than the best topology (significant, $P < 0.05$).

of the *T. javanica* clade and not that of *T. alba*. The dating results indicate that the *T. alba* complex originated during the Middle Miocene. The initial split within this complex took place in the Middle Miocene, approximately 11 Mya (95% HPD: 3.67–19.69 Mya) when *T. alba* evolved first. The *T. furcate* and *T. javanica* separated from each other in the Early Miocene, approximately 8 Mya (95% HPD: 2.96–14.91 Mya). Diversification within the *T. javanica* clade started after 3.0 Mya, whereas, within the *T. furcata*, it began as early as 4.0 Ma.

Biogeographical reconstruction

The global ML at the root node obtained 15.18 with an extinction rate of 0.07996 and a dispersal rate of 0.07041. A total of nine nodes were defined (Fig. 3). The origin of the first clade (node 1) including the *T. alba* group, which occurred in the Old World, is also recovered as having occurred in the Palearctic/Afrotropical regions (EP) (> 0.93 CI). Regarding the origin of the *furcata* and *javanica* clades (node 3), this is less clear-cut. Our analysis suggests an origin in Australia/Nearctic and Neotropical regions (AN) (> 0.35 CI). The subsequent splits comprising the *furcata* (node 4) and *javanica* (node 5) clades suggest a Nearctic or Neotropical origin for the former and an Australian origin for the latter.

Ecological niche modelling

Ecological niche models for *T. alba*, *T. furcata*, and *T. javanica* were run based on 64, 276, and 42 point localities, respectively (Fig. 4). The values for the area under the curve, which shows the quality of the models with respect to discriminating between presence records and random background points, for each of these species, was high, ranging from 0.993 to 0.983. The ecological niche models of the three species showed strong clade-level patterns and, accordingly, each of these three species occupied different niches. The potential distribution of *T. alba* and *T. furcata*, is shown mainly in Africa and Eurasia and in South and North America, respectively, in line with their actual distribution (Fig. 4A, C). The potential distribution of *T. javanica* is depicted in Australia and other parts of its range in South and South-east Asia (Fig. 4B). The result of the analysis of variable contributions for each species demonstrated the most important climatic factors for defining the MAXENT models (Table 3).

The result of estimating pairwise niche overlap among our three phylogroups is given in Table 7. As a general pattern, a lower amount of niche overlap

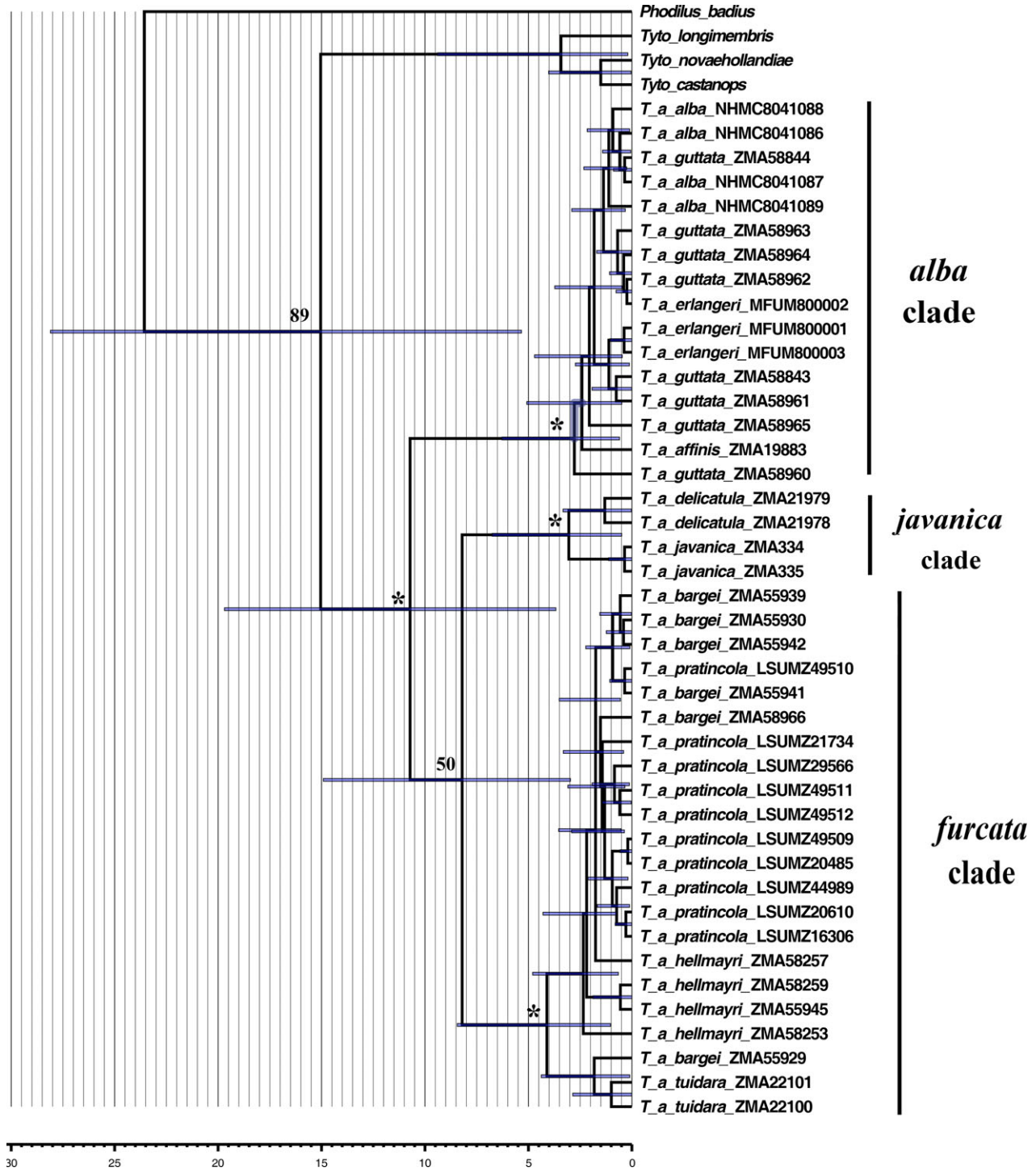

Figure 2. Time-calibrated maximum clade credibility tree of the BEAST analyses based on 2838 bp of *16s*, *Cox1*, *Cytb*, and *Rag1*. Grey bars represent the 95% highest posterior density distributions for the estimated divergence time at each node. Posterior probabilities at nodes ≥ 0.5 are indicated, and the star smark nodes with posterior probabilities ≥ 0.99.

was demonstrated among all of our three species ($D < 0.4$). The outcome amounts of the identity test rejected the first hypothesis of niche identity and demonstrated that the actual occurrences of our species are more different than expected by chance (see Supporting information, Figure S3).

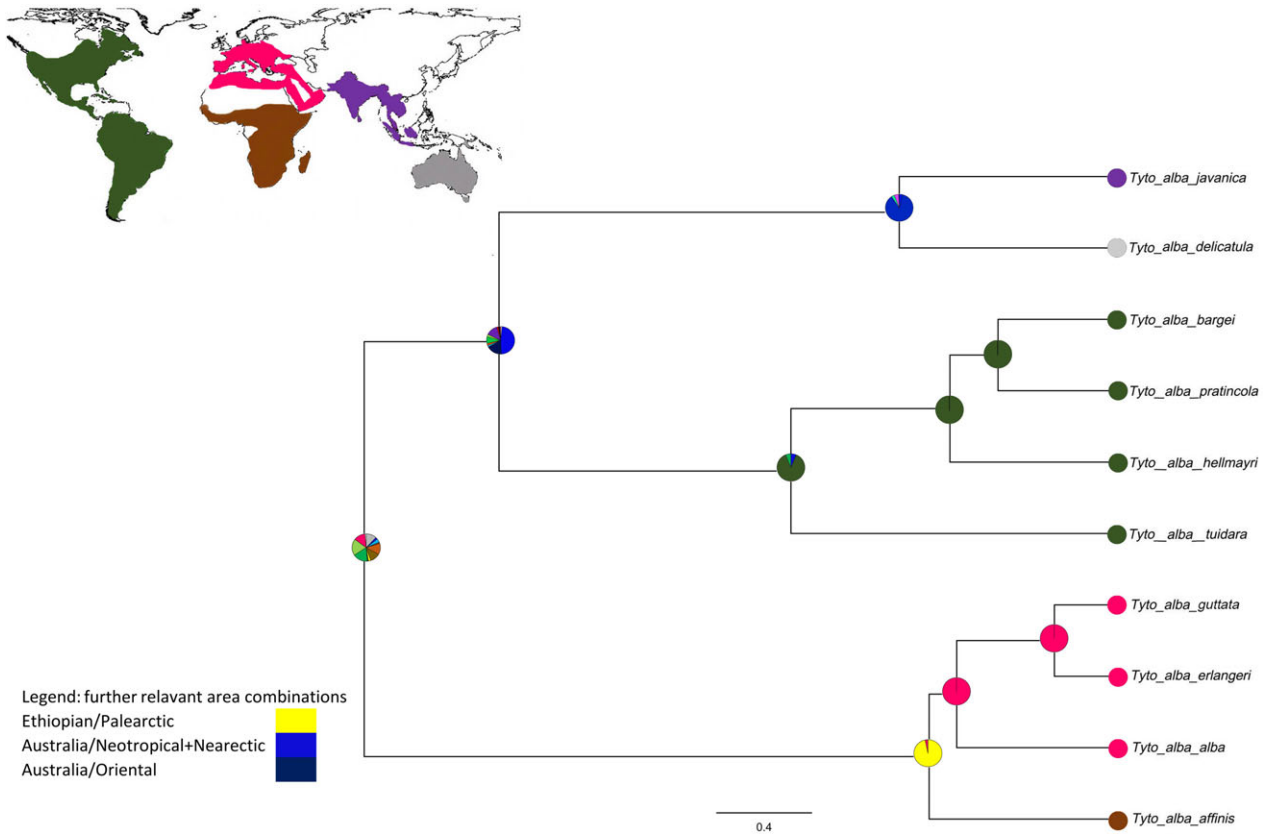

Figure 3. Biogeographical reconstruction obtained using LAGRANGE of the area splits at the various nodes of *Tyto alba* complex.

DISCUSSION

Phylogenetic relationships

Tyto alba complex is a cosmopolitan species showing a high geographical phenotypic variation (Roulin *et al.*, 2009). Several molecular studies have been conducted to clarify intraspecific relationships (Wink *et al.*, 2004, 2008; Weick, 2006; Alaie Kakhki & Aliabadian, 2012; Nijman & Aliabadian, 2013; Colihueque *et al.*, 2015), although a comprehensive study that includes some morphologically divergent and geographically isolated species of this complex has yet to be conducted.

Our combined molecular dataset including three mitochondrial genes (*Cytb*, *Cox1*, *16s*) and one nuclear gene (*Rag-1*) demonstrated three main clades of *T. alba*, *T. furcata*, and *T. javanica* in both the Bayesian and ML phylogenetical analyses. The first two clades comprise an Old World clade (*T. alba*) containing *T. a. erlangeri* from the Middle-East, *T. a. alba* and *T. a. guttata* from Europe, *T. a. affinis* from Africa, and a New World clade (*T. furcata*), including *T. f. hellmayri*, *T. f. pratincola* and *T. f. tuidara* from South and North America, and *T. f. bargei* from Curaçao. The amount of variation between these two clades in all genes (except *Rag-1*) strongly suggests that these taxa are best considered as separate species. All our results confirmed the high amount of divergence between species with high levels of support (using *Cytb*: Alaie Kakhki & Aliabadian, 2012; using *Cox1*: Nijman & Aliabadian, 2013). Having sequenced *Cytb* and *Rag-1* genes, Wink *et al.* (2004) showed high levels of divergence between *alba* and *furcata*, although without having strong support for the split. The Shimodaira–Hasegawa test confirmed that these two clades form a monophyletic group.

Wink *et al.* (2008) considered *T. a. bargei* from Curaçao as a separate species; this interpretation is not confirmed in our analyses because *bargei* is nested within the *furcata* clade. Although not all bird species represent reciprocally monophyletic mtDNA groups (Funk & Omland, 2003; McKay & Zink, 2010; Ross, 2014) and non-monophyly does not falsify species rank if there are other lines of evidence (e.g. diagnosable plumages, reproductive isolation) supporting the existence of two evolutionarily

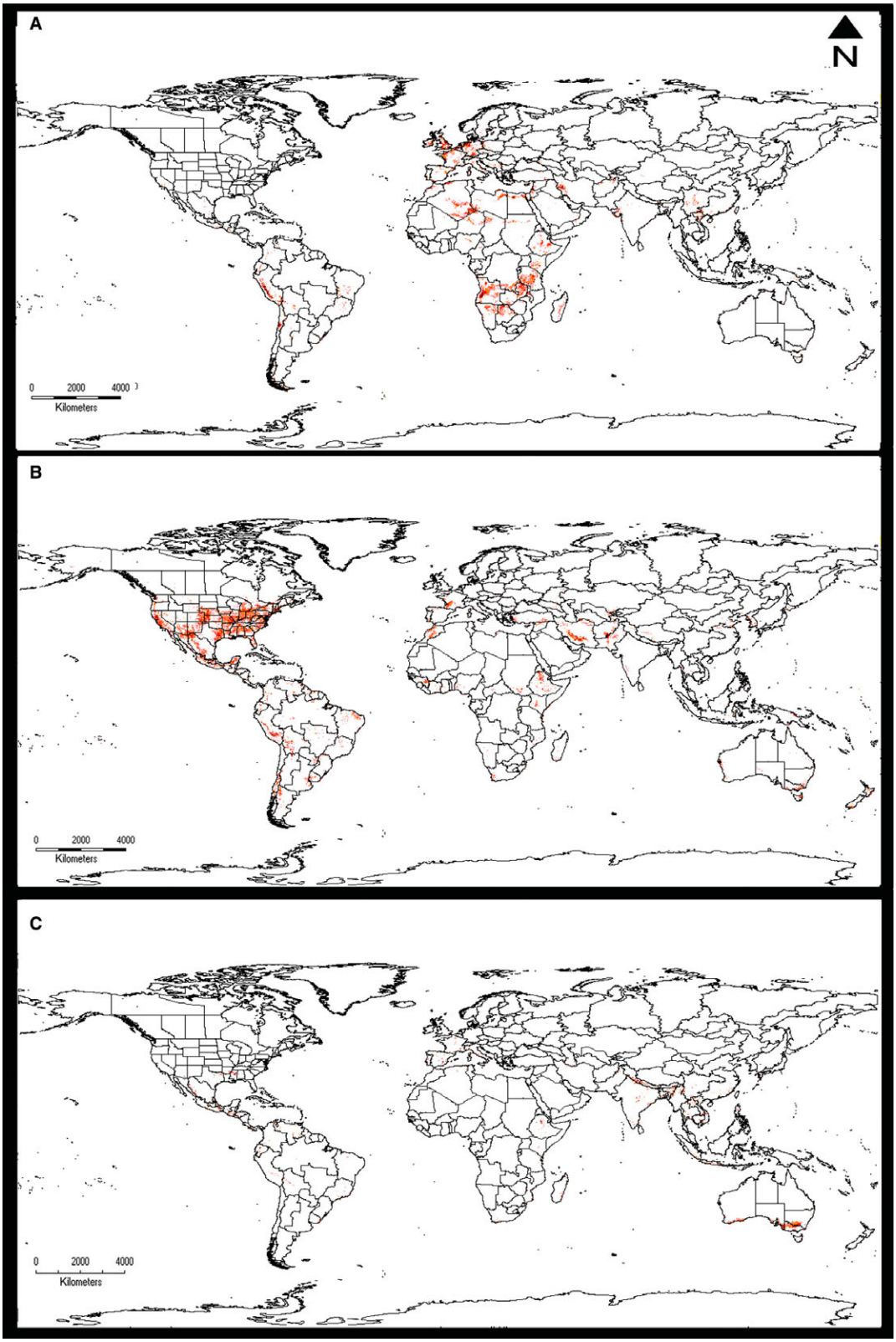

Figure 4. Ecological niche models for three species within the genus *Tyto* at a global level: *Tyto alba* (A); *Tyto furcata* (B) and *Tyto javanica* (C). Warmer colours imply higher probability of existence of species.

Table 7. Pairwise niche overlap values, Schoener's *D* and niche similarity (*I*) for three candidate species, *Tyto alba*, *Tyto furcata*, and *Tyto javanica*

	Tyto alba	*Tyto javanica*	*Tyto furcata*
Tyto alba	0	*D*: 0.305997056	*D*: 0.237114112
Tyto javanica	*I*: 0.538399866	0	*D*: 0.335910765
Tyto furcata	*I*: 0.478315723	*I*: 0.530758048	0

distinct taxa (De Queiroz, 2007; Sangster, 2014), the barn owls on Curaçao are best considered as an insular form of the American barn owl, which, in line with other species occurring on islands, is smaller than the mainland forms. The high level of divergence between the *alba* clade and the *javanica* and *stertens* was above the introduced species level threshold. As a result, *T. a. javanica* and *T. a. stertens* from south-west Asia are best not considered as part of the *alba* clade from the Old World. Our results rather showed that *T. a. javanica* and *T. a. stertens* are nested within the *delicatula* members. This result was also shown in a complimentary tree including our dataset of *Cytb* and *Rag-1* with the dataset of Wink *et al.* (2008, 2009) (see Supporting information, Figure S1).

König *et al.* (2008) noted that *Tyto alba sumbaensis* could be raised as a separated species. The haplotype network analyses and MrBayes tree for the complementary dataset demonstrated a high genetic divergence for the *delicatula* clade as a result on its own confirmed this high genetic divergence, although it remains unclear whether we should raise this owl as a species on its own (Supporting Information, Figure S2).

Ecological niche modelling

The results of our ecological niche modelling, which could be used to provide additional support to decide whether or not certain taxa that differ genetically and/or morphologically are indeed sufficiently different to warrant species status, strongly suggests that the three wide-ranging barn owls species (*T. alba*, *T. furcata*, and *T. javanica*) do indeed have distinct ecological niches. The difference among their ecological niches is clearly shown to be significant by the results of the identity test (see Supporting information, Figure S1). Moreover, among 11 climatic variables, temperature annual range could be considered as the most influential bioclimatic factor on the distribution pattern of all of three species, such that all of them preferred a temperate or warm climate and are absent from a cold climate (König *et al.*, 2008).

Biogeography and temporal diversification patterns

The dating analyses demonstrated that the initial divergence among species of *T. alba* complex took place in the Middle Miocene (period spanning from 11–16 Mya). The earliest split, which occurred approximately 11 Mya, separated the *alba* clade (an Old World clade, clade 1) from two remaining clades and, subsequently, the separation between two clades, *furcata* clade (a New World clade, clade 2) and *javanica* clade (the Australasian clade, clade 3), occurred almost 8 Mya ago.

The temporal and spatial patterns obtained suggest that a common ancestor of *T. alba* complex might have lived in Africa from which it subsequently spread and diversified. The pattern of the early diversification is congruent with the main periods of climate oscillation around this time. A considerable change in both climate and environment during in this time (e.g. Bezrukova *et al.*, 1999; White *et al.*, 1999; Demske, Mohr & Oberhansli, 2000). Indeed, during the warmer period of the Miocene, an early representative of this eastern branch reached Alaska and founded a lineage that spread all the way to Patagonia. The *alba* clade (node 1), including species of the Old World, has an African/European origin, whereas the origin of the two other clades including *furcata* and *delicatula* (node 3) is Australasia/America. Possibly, Africa had been occupied by ancestors of the *T. alba* complex and, essentially, this cosmopolitan complex and, all specie arises from these African ancestors. Most likely, *T. alba* spread towards Asia, South-east Asia and finally Oceania. Simultaneously, one branch of Australasian barn owls spread up to the Bering Strait to reach the Americas. This scenario must nevertheless be firmly demonstrated using extra genetic/genomic analyses. It could be proposed that barn owls dispersed from Africa in the Miocene/Pliocene during climatic and tectonic oscillations, and then reached Europe and some limited parts of South-west of Asia. The establishment of an Afro-Arabian-Eurasian land bridge from 15 Mya onwards (Bosworth, Huchon & McClay, 2005; Metallinou *et al.*, 2012), as a result of the collision of the Afro-Arabian and Eurasian plates in the Middle Miocene and the closing of the West Tethys seaway, might have facilitated dispersal between Africa and triggered important floral and faunal exchanges (Pook *et al.*, 2009; Metallinou *et al.*, 2012; Zhou *et al.*, 2012).

Palaeobotanic data from Iceland proposed that the North Atlantic land bridge, which is considered as one of the main Euro-American faunal exchange routes for several terrestrial organisms, including mammals (White *et al*., 1997; Gingerich, 2006), occurred during the Oligocene (30 Mya) and as late as the Miocene (10–6 Mya) (Denk, Grimssom & Zetter, 2010). Barn owls may have used the North Atlantic land bridge (Gingerich, 2006; Denk *et al.*, 2010) as a suitable corridor for reaching the Americas during the warmest period of the early Pliocene (Scotese, 1998; Cox & Moore, 2005). Furthermore, almost simultaneously during this time, they expanded their distribution range from South-west of Asia toward the Australian plate and, probably by passing the Oriental region, reached Australia and the offshore islands. This remains as an important issue to be established in the future because barn owl expansion in America via the North Atlantic land bridge would indicate that American barn owls originate from the African/European *T. alba*, whereas expansion via the Bering Strait would imply that American barn owls originate from *T. javanica*.

Taxonomic revision

Based on the results obtained, we propose redefining the taxonomic situation of some members of the barn owl complex:

1. Each of the Old World and New World groups (*alba* and *furcata* clades) form well-supported monophyletic groups with a high degree of genetic and niche divergence. They are best considered as distinct and separate species (i.e. the common barn owl *T. alba* from Afrotropical and Palearctic Region at least as far east as eastern Iran and the American barn owl *T. furcata* from Nearctic and Neotropical Regions, including at least part of the Caribbean).
2. The barn owls of Curaçao, sometimes recognized as an endemic species *T. bargei* (König *et al.*, 2008; Wink *et al.*, 2008, 2009), show a (very) low amount of genetic divergence with the *furcata* clade for all genes studied. Neither taxon (*bargei* or *furcata*) was reciprocally monophyletic in our analyses. We suggest that *bargei* is at best considered as a subspecies of *T. furcata* (i.e. *T. f. bargei*), although we recognize the need for additional genomic analyses that provide a better resolution with respect to genetic differentiation.
3. The taxa *javanica* and *T. a. stertens* were found to be part of the *delicatula* members. Because the name *javanica* has nomenclatural priority over *delicatula*, *T. javanica* (including *delicatula* and *stertens*) could therefore be considered as the correct name for eastern barn owl species.
4. *Tyto a. sumbaensis* from Sumba Islands shows high genetic divergence with *T. delicatula*, and might be represent a distinct species (*Tyto sumbaensis*) and not a subspecies of *T. delicatula* (cf. König*et al.*, 2008). However, as for *T. f. bargei*, complementary genomic analyses are needed.

ACKNOWLEDGEMENTS

We are grateful to the museums and their curators who provided some of the samples for the present study: Donna L. Dittmann (Museum of Natural Science, Louisiana State University); Kees Roselaar and Tineke G. Prins (Zoological Museum Amsterdam); and Christos Barboutis (Natural History Museum of Crete). The present study was partially supported by a grant from Ferdowsi University of Mashhad to NAK (P.1021). We thank four anonymous reviewers whose comments and suggestions improved our manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Fifty-percent majority-rule consensus tree sampled from the posterior distribution of the most-partitioned analysis for the combined dataset of Rag-1 and Cytb with Wink *et al.* (2008, 2009). Posterior probability values from the Bayesian analysis are indicated at the > 99% (**) > 95% (*) significance levels.

Figure S2. Haplotype networks of species of *Tyto*, based on 620 bp of combined dataset of the mitochondrial cytochrome *c* oxidase subunit I gene (*Cytb*) with Wink *et al.* (2008, 2009). The number written inside the haplotypes indicates the sample size.

Figure S3. The result of identity test and niche overlap analyses. The blue and red curves depict the identity test results with 15 replicates and the red and blue dots show the results of niche overlap analyses (I and D). Statistically significant intervals exist between calculated values of I and D and the values of the identity test.

Table S1. GenBank accession numbers of the samples of *Tyto alba* complex (Wink et al., 2008, 2009).