Neumann’s Warbler *Hemitesia neumanni* (Sylvioidae): the sole African member of a Palaeotropical Miocene avifauna

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We present molecular evidence that Neumann’s Warbler *Hemitesia neumanni* is deeply nested within the Cettiidae. The species’ distribution in the Albertine Rift of East Africa is intriguing, as the family Cettiidae is principally an Asian radiation. This disjunct distribution could be a result of colonization of Africa by long-distance dispersal, or the Cettiidae may at some point in the past have had a much larger geographical distribution that also covered parts of Africa.

**Keywords:** Africa, Albertine Rift, Asia, biogeography, Cettiidae, dispersal, phylogeny, vicariance, Warbler.

Neumann’s Warbler *Hemitesia neumanni* is a small passerine of uncertain affinities that has a restricted distribution in mountain forests in the Albertine Rift, East Africa. The short tail, relatively large head with a prominent whitish supercilium, black stripe through the eye, black lateral crown-stripe and a dark greenish grey central crown-stripe make it a very distinctive species. It is mostly found on or close to the ground (Bairlein et al. 2006). Rothschild (1908) originally described Neumann’s Warbler as *Sylvietta neumanni*. However, except for its short tail, it shows no particular resemblance to the genus *Sylvietta* (crombecs) and Rothschild’s decision to place it within this genus was probably influenced by a shared African distribution. Several external morphological differences between Neumann’s Warbler and the genus *Sylvietta*, and the observation that Neumann’s Warbler has many morphological features in common with the Asian warbler genus *Tesia*, led Chapin (1948) to place Neumann’s Warbler in the monotypic genus *Hemitesia*, in which it has been retained in subsequent classifications (e.g. Watson et al. 1986, Sibley & Monroe 1990, Bairlein et al. 2006).

The combination of being a restricted-range species in Africa and having a potentially close relationship with Asian warblers makes an investigation of the affinities of Neumann’s Warbler interesting, as it may improve the understanding of the timing, frequency and direction of historical avifaunal exchanges between Africa and Asia. In this study, we examine the phylogenetic relationships of Neumann’s Warbler and estimate divergence times for a diverse taxon sampling of Asian and African warblers by analysing nuclear and mitochondrial DNA sequences.

**METHODS**

**Taxon sampling, PCR amplification and sequencing**

We examined the phylogenetic relationships of *Hemitesia* by analysing DNA sequences from the mitochondrial cytochrome *b* gene and from three
nuclear loci, myoglobin intron 2, ornithine decarboxylase introns 6–7 (ODC) and glyceraldehyde-3-phosphodehydrogenase intron 11 (GAPDH). The taxon sampling includes a broad selection of African and Asian warblers, including all major clades in the Cettiidae identified by Alström et al. (2006) and the genera Sylvietta and Tesia, to which Hemitesia has been associated. We have also included representatives from a selected number of other major oscine lineages, some suboscines and the Rifleman Acanthisitta chloris. The trees were rooted with a parrot, as parrots have been suggested to be the closest relatives to passerine birds (Hackett et al. 2008). Voucher and GenBank accession numbers are provided in Table 1.

For extractions, PCR amplification and sequencing procedures from study skin samples, we followed the procedures described in Irestedt et al. (2006). Two specimens of Hemitesia, one in the Swedish Museum of Natural History, Stockholm, Sweden, and one in The Natural History Museum, Tring, UK, were examined.

**Phylogenetic analyses and estimation of divergence times**

We used Bayesian inference to estimate phylogenetic relationships. The models of nucleotide substitution were selected for each gene individually using the Akaike information criterion implemented in the program MRMODELTEST 2.2 (Nylander 2004) in conjunction with PAUP* (Swofford 2002). Due to a rather low number of insertions in the non-coding nuclear loci, the sequences could be aligned easily by eye. All gaps were treated as missing data.

Posterior probabilities of nodes and parameters in the substitution models were approximated with MCMC and Metropolis-coupling using the program MRBAYES 3.1.1 (Ronquist & Huelsenbeck 2003). Analyses were performed for both the individual genes (10 million generations), nuclear (10 million generations) and a concatenated dataset (50 million generations), with trees sampled every 1000 generations. The program AWTY (Nylander et al. 2008b) was used to estimate when the chains had reached their apparent target distributions, and trees sampled during the burn-in phase were discarded. To evaluate further statistical support for the topology, maximum likelihood bootstrapping (1000 replicates) was performed on the concatenated sequences in TREEFINDER (Jobb et al. 2004, Jobb 2008) using default settings and the best-fit model proposed by TREEFINDER.

We used a relaxed clock model implemented in BEAST 1.5.3 (Drummond et al. 2006) to estimate divergence times between phylogenetic lineages based on the concatenated dataset. As a calibration point we used the split between Acanthisitta and all other passerines, as this has been linked to the geological separation between New Zealand and Antarctica (Barker et al. 2002, Ericson et al. 2002). The dating of this split has often been assumed to be between 85 and 82 million years ago (Mya), but this timing has recently been suggested to be less certain, 85–65 Mya (McLoughlin 2001, Ladiges & Cantrill 2007). To account for this uncertainty we used a normally distributed tree prior with a median at 76 Mya and a standard deviation of 8 Mya (quintiles 2.5% = 60.3 Mya; 5% = 62.8 Mya; 95% = 89.2 Mya; 97.5% = 91.7 Mya). As for the other priors, we used default settings with the exception of the tree prior that was set to reflect a Yule process and an uncorrelated log-normal distribution was used for the molecular clock model. We used the locus-specific models of nucleotide substitution and ran MCMC chains for 25 million generations. The program TRACER 1.4.1 (Rambaut & Drummond 2007) was used to evaluate the run to help ensure that adequate effective sample sizes and mixing had occurred for parameter and dating estimation.

**RESULTS**

**Variation in the molecular dataset and model selection**

Taking into account the absence of a few short fragments for some taxa, the alignments analysed are 420 bp for GAPDH, 720 bp for ODC, 742 bp for myoglobin and 900 bp for cytochrome b. Some indels in more variable regions were found to be autapomorphic, but most other indels were congruent with the phylogenetic tree obtained from the analysis of the combined dataset.

The prior selection of substitution models supported the GTR+I+Γ model for cytochrome b and ODC, and the GTR+Γ for GAPDH and myoglobin; for the maximum likelihood bootstrap, the GTR+I+Γ model was used. After discarding the burn-in phase, the final inference was based on a total of 9000–9500 samples from the posterior for the individual loci and 49 000 samples from the
Table 1. Specimen data and GenBank accession numbers for samples used in the study.

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concatenated dataset. For the phylogenetic inference of the concatenated dataset of all genes, the mode of the posterior distribution of topologies is presented as a 50% majority-rule consensus tree (Fig. 1).

Phylogenetic relationships and divergence time estimates

The four single-locus gene trees are variously but generally well resolved; the myoglobin tree shows the most structure and the GAPDH tree the least structure. Although there are multiple incongruences among the gene trees, very few of these are strongly supported in two or more trees (Supporting Information Figs S1–S5). The tree based on the concatenated multilocus dataset (Fig. 1) is fairly well resolved and mostly well supported. Hemitesia is nested within Cettiidae (sensu Alström et al. 2006) and sister to the Asian Stubtail Urosphena squameiceps, with strong support. This placement within the Cettiidae is recovered in all single-locus analyses, and the sister relationship with U. squameiceps is inferred in three of the gene trees. The maximum likelihood bootstrapping of the concatenated multilocus dataset also strongly supported this position for Hemitesia (bootstrap support values are shown in Fig. 1). The Chestnut-capped Flycatcher Erythrocercus mccallii is recovered as sister to the Cettiidae with high posterior probability (1.0).

The chronogram (Fig. 2) agrees well in topology with the phylogram (Fig. 1), at least with respect to the strongly supported nodes. The deepest split within Cettiidae is inferred to have taken place c. 23 Mya, while the Hemitesia–Urosphena squameiceps split is estimated at c. 17 Mya. The separation between Erythrocercus mccallii and Cettiidae was considerably earlier, c. 30 Mya.

DISCUSSION

The position of Hemitesia as deeply nested within Cettiidae is strongly supported by both the individual loci and the concatenated dataset. There is also good support for a close relationship between Hemitesia and U. squameiceps, as all major clades in the Cettiidae identified by Alström et al. (2006) are represented in the present study. However, as c. 20 Cettiidae species are missing from the present study, a denser taxon sampling is needed to conclusively establish the exact position of Hemitesia.
Figure 1. The majority-rule consensus tree obtained from the Bayesian analysis of concatenated sequences (cytochrome b, myoglobin, ODC and GAPDH). Posterior probabilities (left) and maximum likelihood bootstrap support values (right) are indicated at nodes. Posterior probabilities of 1.0 and bootstrap support values of 100% are indicated with an asterisk. Shaded box: relative positions of *Hemitesia neumannii* within the family Cettidae (*sensu* Alström et al. 2006) in (a) the mitochondrial (cytochrome b), and (b) the nuclear tree (myoglobin, ODC and GAPDH). The complete mitochondrial and the individual nuclear gene trees are shown in Supporting Information Figures S1–S5.
Figure 2. Chronogram with divergence and confidence intervals (grey bars), estimated under a relaxed clock model implemented in BEAST 1.5.3 (Drummond et al. 2006). For calibration of the chronogram, the postulated separation of *Acanthisitta* from all other passerines in the phylogeny was used.
within Cettiidae. In terms of external morphology, Hemitesia shares more features with Urosphe\-na and Tesia than with the African genus Sylvietta, such as long legs with large feet, broad, flattened bills and plumage patterns on the head (Chapin 1948, Bairlein et al. 2006). In addition, Hemitesia has 10 rectrices, in common with other Cettiidae species (Alström et al. 2006) but unlike most other passerines.

Erythrocercus mccallii was also found to be sister to the Cettiidae by Johansson et al. (2008) based on three nuclear loci (ODC, myoglobin, β-fibrinogen intron 5), although the support was considerably lower than in the present study. This species has an exclusively African distribution. However, except for Cetti’s Warbler Cettia cetti, which ranges into Europe and North Africa, and Hemitesia, the family Cettiidae is principally an Asian radiation.

The current geographical distribution of Hemiesia may have been shaped by multiple historical biogeographical events, but the major competing scenarios to account for its present distribution would be based on either dispersal or vicariance in combination with local extinction. First, it is possible that the ancestor of Hemitesia colonized Africa by long-distance dispersal from Asia. A number of extant Cettiidae species are migratory, and some species (e.g. Palau Bush Warbler Cettia annae, Shade Bush Warbler Cettia parens, Fiji Bush Warbler Cettia ruficapilla) have been able to colonize islands in the Pacific (Bairlein et al. 2006, LeCroy & Barker 2006). Urosphe\-na consists of one migratory species breeding in northeast Asia and wintering in southeast Asia (U. squashameiceps), and two endemic species in the mountains of Borneo (Bornean Stubtail Urosphe\-na whiteheadi) and Timor (Timor Stubtail Urosphe\-na subulata). Long-distance dispersal between Africa and Asia has also been hypothesized to explain the current distribution of other passerine clades with complex distributions shared between these two continents (e.g. Fuchs et al. 2007, Jönsson et al. 2008, Nylander et al. 2008a, Voelker et al. 2009, Jönsson et al. 2010a).

Another possibility based on vicariance and local extinction is that an ancestral species in the clade that gave rise to Hemitesia and Urosphe\-na at some point in time had a much larger geographical distribution that also covered parts of Africa. The occurrence of land connections between Asia and northeast Africa (Vrielnyck et al. 1997, Rögl 1998, Harzhauser et al. 2002) and continuous forests from eastern Asia to central Africa (Mandaville 1977, Utescher et al. 2007) during the mid-Miocene could have made it feasible for organisms occupying forests or forest edge to have distributions that included both Africa and Asia. Whereas much of Asia has stayed largely forested, the forests of Africa, the Arabian Peninsula and southwestern Asia contracted and became fragmented during the mid- to late Miocene (Mandaville 1977, Retallack 1992, Vrba 1993, Flower & Kennett 1994 and references therein). The loss of humid forest environments in Africa may thus have led to extinctions or reduced relict distributions of surviving avian forest clades in Africa (Fjeldså & Bowie 2008), as opposed to Asia, where diverse forests continued to allow further diversification.

In recent avian phylogenetic literature there are numerous examples of avifaunal exchanges between Africa and Asia (e.g. Beresford et al. 2005, Moyle & Marks 2006, Nylander et al. 2008a, Fuchs et al. 2009), but as these include cases of various ages as well as clades adapted to different types of habitats (e.g. xeric and forested) it is not straightforward to find a pattern to support whether the dispersal or vicariance scenario is the most plausible explanation for the enigmatic distribution of Hemitesia. However, we suggest that the vicariance hypothesis may be supported by some recent phylogenetic studies, where occasional African forest species of a similar age have been found to be nested within large Asian forest clades, e.g. Illadopsis and Ptyrricus within Timaliidae (Gelang et al. 2009), African Pitta Pitta angolensis and Green-breasted Pitta Pitta reichenouai within the otherwise Asian–Australasian Pittidae (Irestedt et al. 2006, Moyle et al. 2006), two clades of African orioles nested within the Asian Oriolus (Jönsson et al. 2010b), and the occurrence of the Congo Peacock Afropavo congensis in central Africa (Crowe et al. 2006). We also suggest that past extinctions may have played an important role in forming the present distribution of avian clades. Additional phylogenetic studies with divergence time estimates of forest clades shared between Africa and Asia are warranted to test this hypothesis.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figures S1–S5. The majority-rule consensus trees obtained from the Bayesian analysis of the individual genes and the concatenated nuclear genes. S1: cytochrome b, S2: myoglobin intron 2 (myo), S3: ornithine decarboxylase introns 6–7 (ODC), S4: glyceraldehyde-3-phosphodehydrogenase intron 11 (GAPDH), and S5: the concatenated nuclear genes (myo, ODC and GAPDH).

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