

## Distribution of *Asellia tridens* (Chiroptera: Hipposideridae) lineages including representatives from Saudi Arabia

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The genus *Asellia* was recently revised, giving an insight into the genetic and morphological characteristics of a group whose distribution spans from North Africa into Asia. We studied the genus further by considering additional mitochondrial markers and sampling localities. The deepest previously identified split in *A. tridens* is supported (Middle East/North Africa), along with the association of North and West African lineages. Central Saudi Arabia is found to represent the easternmost extent of a North African haplogroup when considering concatenated fragments of Cytochrome-*b*, NADH Dehydrogenase 2, and Cytochrome Oxidase 1 genes. New distribution data further emphasises the diversity found in the Middle East and suggests some mixing of haplotypes over long distances.

**Keywords:** *Asellia tridens*; phylogeography; mtDNA; Arabia

### Introduction

There has been a recent taxonomic revision of the genus *Asellia* (Benda, Vallo, & Reiter, 2011) employing both molecular and morphological data. The association with arid conditions of *A. tridens* (and closely related species) sees its range extend from northern Africa to the western edge of the Asian subcontinent. Benda et al. (2011) made a thorough treatment of the diversity of the group, demonstrating that there appeared to be some clustering of morphological characters according to region of origin. This study adds additional locations and mitochondrial gene regions to the sample map for *Asellia tridens*. We attempt to ascertain whether there are clear phylogenetic delineations within this group, as hinted at by previous work, and whether they reflect geographical origins.

### Material and Methods

**Sampling.** *Asellia* were taken from those available specimens from Benda et al. (2011) (n=46), including individuals for which no genetic data was used previously (n=24). In Saudi Arabia individuals were caught at a single roosting site (n=17) in the Qasim Region. Samples included individuals from *A. arabica* and *A. italosomalia* which were used as outgroups to *A. tridens*. Bats were captured using harp traps and mist nets. Euthanasia was performed by briefly sedating the animal using xylazine hydrochloride (RompunH, 20 mg/ml, Bayer, Leverkusen, Germany) and then followed by intraperitoneal injection of Euthatal (Pentobarbital Sodium 200 mg/ml, Merial, Essex, UK). Animal death was inferred from absence of respiratory movement and heart beat, and when there was loss of colour in mucous membranes.

**Molecular data collection.** DNA extraction was performed from pectoral muscle tissue or wing membrane punches using Qiagen extraction reagents and separate spin columns (Epoch Life

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Science). Three mitochondrial fragments were amplified within the gene regions of cytochrome b (cytb), NADH dehydrogenase 2 (ND2), and Cytochrome Oxidase subunit I (COI) with the following primer combinations; Molcit/MVZ16 (Racey, Barratt, Burland, Deaville, & Gotelli 2007), ERL4/ERH4 (He et al., 2012), and LCOI490/HCO2198 (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994) respectively. For all fragments PCR was run for 40 cycles at 48°C annealing temperature.

*Phylogenetic analysis.* Sequence data was cleaned and aligned in BIOEDIT v7.0.5 (Hall, 2005). The most appropriate nucleotide substitution model to apply for the subsequent analyses was determined according to the Akaike Information Criterion (AIC; Akaike, 1974) using JMODELTEST2 (Posada, 2008). Nucleotide substitutions and translation into amino acids was performed through MEGA5 (Tamura et al., 2011), which was also used to construct a Neighbour joining phylogeny for the cytochrome b gene only. A maximum parsimony haplotype network was calculated for all three mtDNA fragments concatenated into a single sequence of 2245 bp using the TCS (Clement, Posada, & Crandall, 2000) application. A three-gene phylogeny was constructed through the MRBAYES 3.1 (Ronquist & Huelsenbeck, 2003) was used. We used the default models for our gene partitions, and set to infer coding bias assuming that only variable characters can be observed for both (“CODING = VARIABLE”). Bayesian analysis was undertaken using a random starting tree and  $1 \times 10^6$  generations with one cold and three heated chains, sampling trees every 100 generations.

## Results

A total of 2245 bases of sequence data were generated and aligned across three mitochondrial genes for 39 individuals from *A. arabica* Benda, Vallo and Reiter, 2011, *A. italosomalica* De Beux, 1931, and *A. tridens* (Geoffroy, 1813) (GENBANK accession numbers in table S1). Within *A. tridens* the numbers of variable sites were as follows; 41/749 bp of cytb, 84/875 bp of ND2, and 12/621 bp of COI. The cytb gene demonstrated the best fit to the HKY+G substitution model.

A haplotype network was constructed across 2245 bp of concatenated sequence data (Figure 1). There is a high level of genetic diversity in the Middle East within *A. tridens*, the southern tip of the Arabian peninsula being particularly diverse, as seen in the wide phylogenetic distribution of bats sampled in Oman. The cytb phylogeny included 58 individuals as well as the 22 haplotypes from Benda et al. (2011) for reference (Figure S1), showing the three Saudi Arabian haplotypes grouping with those from Egypt, Libya, and Sudan. The Bayesian phylogeny showed a single well supported node within *A. tridens* (Figure 2). Whilst there is a discontinuity between the African and Middle Eastern locations, there is a less consistent pattern emerging with the geographic versus the phylogenetic position of some of the West African samples. The Saudi Arabian haplotype fell into the ‘North Africa’ haplogroup (North west Africa; excluding Libya, Mauritania, and Morocco) rather than the most diverse ‘Middle East’ and smaller ‘West Africa’ haplogroups.

## Discussion

The addition of new locations and mitochondrial genes to the study of this species across its geographic range has failed to reinforce the lineage separation suggested in Benda et al. (2011). The geographical limits of some lineages can be seen to extend over wide areas; for example the North African haplogroup (as seen from the concatenated dataset) extends into Saudi Arabia, separate from the remaining Middle East lineages. There is also an interesting connection across the whole of North Africa into West Africa, but also demonstrating potential isolation between regions in Mauritania, as shown by the haplotype network (Figure 1).

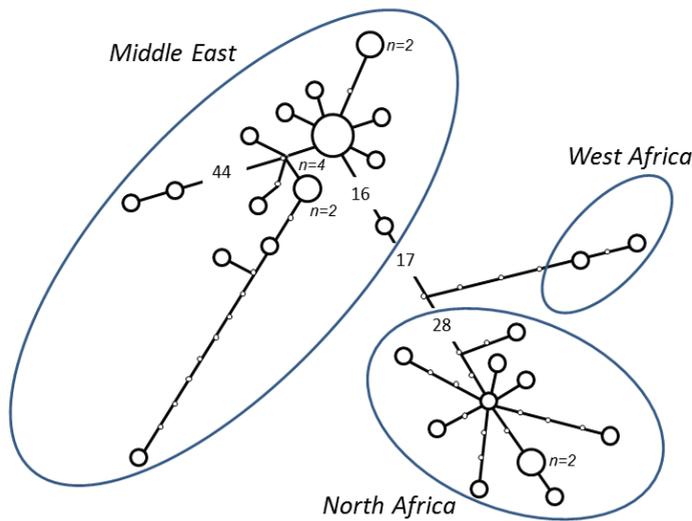


Figure 1. A haplotype network for the concatenated dataset of 2245 bp of mitochondrial sequence data of *Asellia tridens*. The Middle East group is those countries east of Egypt but also contains a haplotype from West Africa, the West Africa group is the remaining haplotypes from Mauritania, and the North Africa haplogroup is those locations west of (and including Egypt) as well as the sample from Saudi Arabia.

The cytochrome b gene demonstrated how all of the haplotypes compared with previously analysed locations. There are shared haplotypes between distant locations, which is suggestive of a degree of connectivity across large areas such as between Saudi Arabia and Morocco. It is also possible that this reflects retention of ancestral polymorphism. But it is unclear how this could maintain such discontinuities as seen in the 44 bp distance between haplotypes in the Middle East haplogroup (Figure 1). It is more likely that there do exist isolated populations in some areas of the range of this species. However, the *Asellia* haplotypes do not overlap in the Arabian peninsula; the Asian clade is distributed across Oman and UAE as well as in Mesopotamia, while the African clade extends into Saudi Arabia (and presumably SW Yemen). This is in general accordance with the *Rhinopoma hardwickii/cystops* geographical division, as well as the extension of African taxa like *Rhinolophus clivosus* or *Nycteris thebaica* in Arabia, both reaching central Saudi Arabia.

The prospect of migration has been raised for this bat, for distances covering large parts of the species range (Harrison, 1957) or at least for short distances between summer and winter roosts (Gaisler, Madkour, Pelikán, 1972; Benda, Lučan, Shohdi, Porteš, & Horáček, 2014) although there are also suggestions discounting any migration in favour of winter hibernation (Amichai, Levin, Kronfeld-Schor, Roll, & Yom-Tov, 2013). If migration were to be the case in even some populations then it may explain the co-occurrence of widespread haplotypes. An alternative hypothesis is a recent origin of the compared populations, i.e. relatively modern spread of the species in both parts of the range from restricted refugia, see Benda et al. (2011). Only thorough examination of contemporary gene flow could answer such queries.

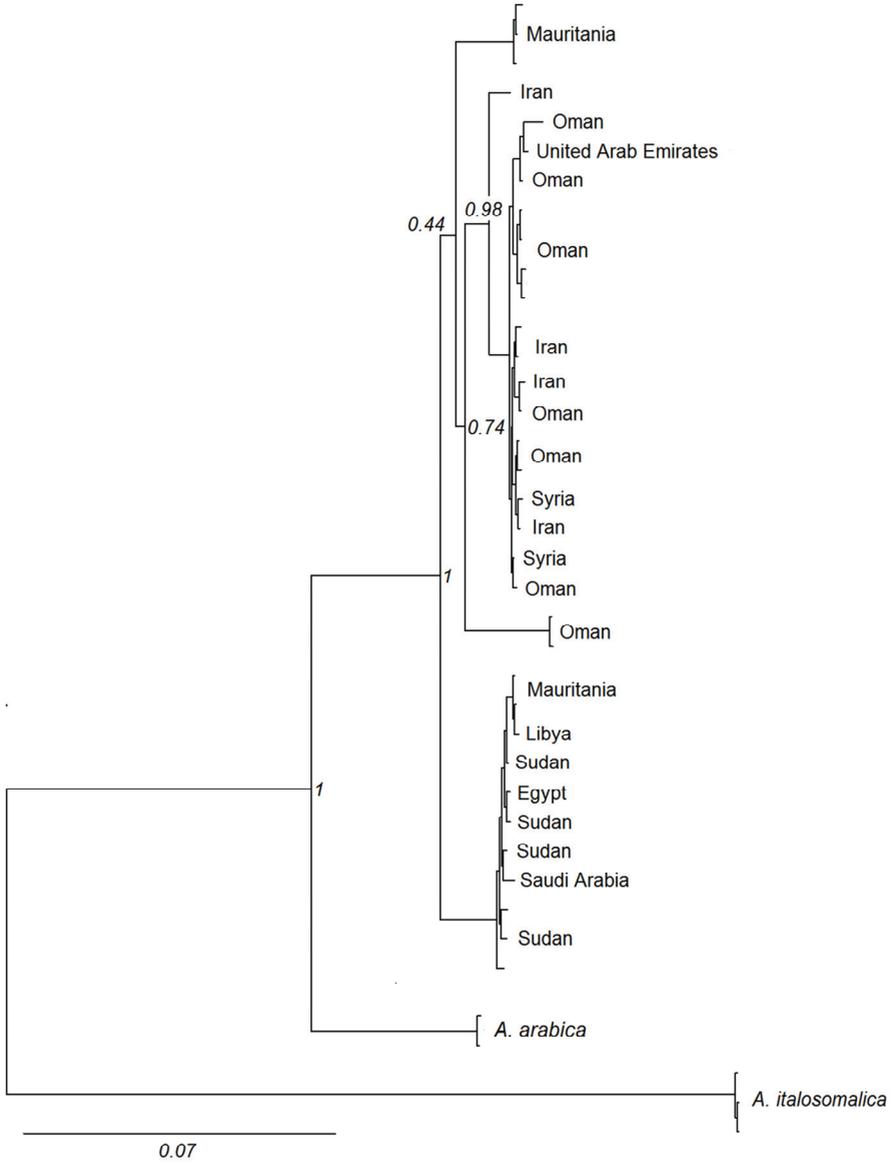


Figure 2. A three gene Bayesian phylogeny for *Asellia tridens* with country of origin for each tip.

**Supplementary Material**

The tables are given as a Supplementary Annex, which is available via the “Supplementary” tab on the article’s online page (<http://dx.doi.org/10.1080/09397140.2016.1250708>).

**Ethics Statement**

Handling and euthanasia were conducted humanely according to the Institutional Animal Care and Use Committee at the University of Arkansas according to protocol number 07002 (permission granted on 15 August 2006). Permission for sample collection was granted by the Saudi Wildlife Authority.

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### Disclosure Statement

No potential conflict of interest was reported by the authors.

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