# Yellow-bellied or white-bellied? Identity of Arabian house bats (Vespertilionidae: *Scotophilus*) revealed from mitochondrial DNA and morphology

Peter Vallo[1*], Petr Benda[2,3] & Antonín Reiter[4]

[1]*Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, v.v.i., Květná 8, 603 65 Brno, Czech Republic*

[2]*Department of Zoology, National Museum (Natural History), Václavské nám. 68, 115 79 Praha 1, Czech Republic*

[3]*Department of Zoology, Faculty of Science, Charles University, Viničná 7, 128 44 Praha 2, Czech Republic*

[4]*South Moravian Museum in Znojmo, Přemyslovů 8, 669 45 Znojmo, Czech Republic*

Received 3 January 2011. Accepted 30 August 2011

**The yellow-bellied *Scotophilus dinganii* is the only African house bat species reported to occur in the Arabian Peninsula. Formerly, the Arabian house bats were referred to similar-looking white-bellied *S. leucogaster,* which differs from *S. dinganii* mainly by the colour of ventral pelage. We reassessed the taxonomic status of house bats from southwestern Yemen using genetic and morphological analyses. The Yemeni specimens clustered within two distantly related mitochondrial lineages of African *Scotophilus*: East African *S.* aff. *dinganii,* which is a paraphyletic group to *S. dinganii s.str.* from South Africa, and West African *S. leucogaster.* This taxonomic assignation was based on published sequences of reference museum specimens. Differences in external and cranial measurements also indicated the presence of two distinct taxa in Yemen. The Yemeni and comparative Ethiopian populations of *S.* aff. *dinganii* showed close morphological similarity to the type specimen of *S. nigrita colias* from Kenya. Because the Yemeni and Ethiopian yellow-bellied house bats cannot be synonymized with *S. dinganii,* the designation *S. colias* is tentatively suggested for this particular East African and Yemeni lineage of the *S. dinganii* complex. However, final correspondence of this name with the respective populations or applicability of some of other available names must yet be explored. Based on environmental differences of the Yemeni localities of origin, *S. colias* appears to be ecologically delimited to mountainous habitats, while *S. leucogaster* to harsh lowland deserts. This is consistent with known habitats of African populations of both species.**

**Key words**: **cytochrome *b*, phylogeny, cranial morphometrics, *Scotophilus dinganii, Scotophilus leucogaster*.**

## INTRODUCTION

The house bats of the genus *Scotophilus* Leach, 1821 (Vespertilionidae) include 15 recognized species inhabiting tropical regions of the Old World, seven of which occur in Africa (Simmons 2005; Goodman *et al.* 2005, 2006; Trujillo *et al.* 2009). Based on morphological and genetic evidence, the genus forms a monophyletic group but relationships among species are obscure (Robbins *et al.* 1985; Taylor 2000; Simmons 2005; Trujillo *et al.* 2009). The African house bat species look rather similar in external appearance, and differ mainly in size and pelage colour (Hayman & Hill 1971; Robbins *et al.* 1985; Taylor 2000; Jacobs *et al.* 2006). Due to their morphological similarity, and large distribution ranges of several species over most of sub-Saharan Africa, the systematics of *Scotophilus* has for years been confused and controversial, and taxa rather loosely defined (e.g. Hayman & Hill 1971; *cf.* Robbins *et al.* 1985).

According to Robbins *et al.* (1985) and Simmons (2005), medium-sized savanna forms of house bats with forearm length ranging from 48 to 56 mm are generally separated into two species, namely the yellow-bellied house bat *S. dinganii* (Smith, 1833) and the white-bellied house bat *S. leucogaster* (Cretzschmar, 1830). The species' names thus reflect, to a large degree, the colouration of their venters, as rigorously proved by Robbins *et al.* (1985). The authors examined more than 1600 specimens of both species from throughout Africa and claimed a lack of yellow on the ventrum in *S. leucogaster,* but a pale to reddish yellow venter colouration in *S. dinganii.* Despite this apparent external trait, concerns have been raised about

*Author for correspondence. E-mail: vallo@ivb.cz

the consistency of ventral colouration in the two species (e.g. Goodman *et al.* 2005). Confusion about consistent colour differences between these two taxa possibly has its origin in historical taxonomical rearrangements, as the names *S. dinganii* and *S. leucogaster* have been assigned rather intuitively to various geographical forms of house bats (*cf.* Robbins *et al.* 1985). This inconsistency in naming was further complicated by inappropriate use of the name *S. nigrita* (Schreber, 1774) for several African forms including currently recognized species *S. dinganii* and *S. leucogaster* (e.g. by Koopman 1975), although this name properly denotes the species of giant house bat with a junior synonym *Scotophilus gigas* Dobson, 1875 (Robbins 1978).

Only one species of African *Scotophilus* has been reported to occur in the Arabian Peninsula, the yellow-bellied house bat *S. dinganii* (Simmons 2005; Benda *et al.* 2011). Arabian house bats have been previously assigned to either *S. leucogaster* or *S. nigrita* (Harrison 1964; Harrison & Bates 1991; Al-Safadi 1991; Gaucher 1993; Al-Jumaily 1998). *S. dinganii* has long been considered as the most widely distributed taxon among the African representatives of the genus, inhabiting the whole sub-Saharan Africa from Senegal and Sierra Leone in the west to Eritrea and Somalia in the east and to Namibia and Natal in the south (Hayman & Hill 1971; Robbins *et al.* 1985; Taylor 2000; Simmons 2005; Monadjem *et al.* 2010). In Arabia, *S. dinganii* can be found only in the southwestern corner of the Peninsula, with confirmed records known from extreme southwestern Saudi Arabia (Harrison & Bates 1991; Gaucher 1993) and western Yemen (Harrison & Bates 1991; Al-Safadi 1991; Wranik *et al.* 1991; Al-Jumaily 2004; Benda *et al.* 2011).

Taxonomic confusion about African *Scotophilus* was recently moderated by a molecular study done by Trujillo *et al.* (2009), who confirmed paraphyly within the yellow-bellied morphospecies *S. dinganii* and showed three independent evolutionary units to occur in West, East and South Africa, respectively. Unfortunately, their results provided limited information on African *Scotophilus* in Arabia. Since Arabian house bats were neglected also in the comprehensive revision by Robbins *et al.* (1985), the taxonomic affinity of Arabian *Scotophilus* apparently has not yet been resolved. Given that Arabian house bats reportedly vary in their ventral colouration from white to yellow (Harrison 1964; Harrison & Bates 1991; Gaucher 1993), the veracity of pelage colouration as a diagnostic character for Arabian *Scotophilus* also remains unclear.

In this study, we explore the taxonomic status of house bats collected in several localities of southwestern Yemen. Although some variance in colouration was apparent in captured bats, after a brief morphological examination these were tentatively identified as *S. dinganii* by Benda *et al.* (2011) in accordance with the current taxonomic opinion by Simmons (2005). Using sequences of the mitochondrial cytochrome *b* gene, we infer genetic structure of Arabian *Scotophilus* and their phylogenetic position within a spectrum of African congeneric forms. We further assess morphometric variation using standard external and cranial dimensions and link it to genetic variation. Based on combined genetic and morphological analysis, we address the taxonomic status of Arabian *Scotophilus* and partially state suitability of pelage colouration for house bat identification.

## MATERIALS & METHODS

*Sampling and morphological analysis*

Bats were netted in 2005 and 2007 in southwestern Yemen (Fig. 1, Table 1; *cf.* Benda *et al.* 2011). Captured specimens ($n$ = 39) were collected as vouchers and deposited at the National Museum in Prague (NMP), Czech Republic, and in the Biological Collection of the Sana'a University (BCSU) in Sana'a, Republic of Yemen. Prior to fixation in alcohol, all specimens were sexed, weighed and were measured at five basic external dimensions for general size comparison: body length (LC), tail length (LCd), forearm length (LAt), ear length (LA), and tragus length (LTr).

Skulls of 27 NMP specimens were extracted for detailed morphological analysis and measured at 14 dimensions using mechanical callipers with a precision of 0.02 mm: greatest length of skull (LCr), condylobasal length (LCb), zygomatic width (LaZ), width of interorbital constriction (LaI), neurocranium width (LaN), mastoidal width (LaM), height of neurocranium (ANc), largest horizontal length of tympanic bulla (LBT), width across upper canines at crowns (CC), width across third upper molars ($M^3M^3$), length of upper tooth-row from front of canine to back of third molar ($CM^3$), condylar length of mandible (LMd), height of coronoid process (ACo), length of lower tooth-row from front of canine to back of third molar ($CM_3$). Analysis of variance, principal component analysis and canonical discriminant analysis using

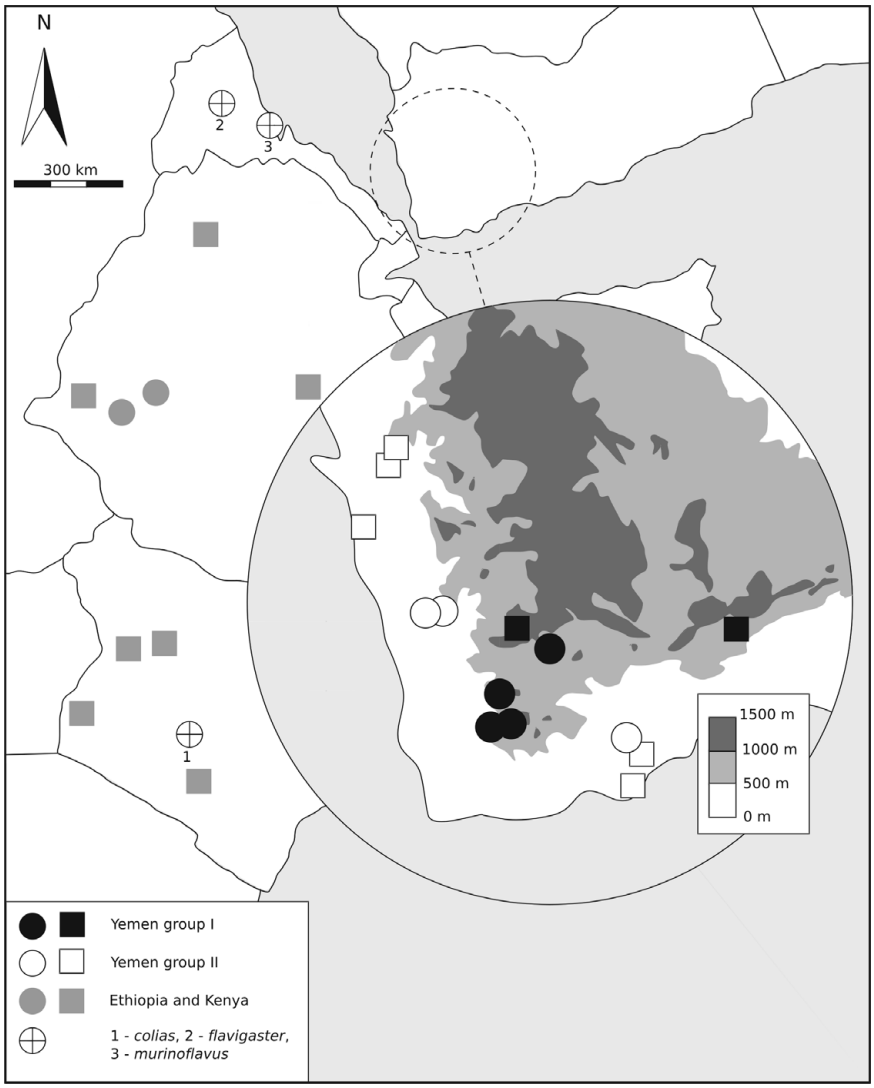


**Fig. 1**. Distribution of *Scotophilus* in Yemen, including the known or suspected taxonomic affinity of recorded specimens. Circles represent new records, squares represent previously published records (for details see Trujillo *et al.* (2009) and Benda *et al.* (2011)).

raw data were employed to reveal morphological differences. Statistical analyses were performed using Statistica 6.0 software (StatSoft, Tulsa, OK, U.S.A.). The Yemeni specimens were compared with a set of *S.* aff. *dinganii* from Ethiopia (NMP; $n = 12$ for external data and $n = 9$ for skull comparisons) and with type specimen of *S. nigrita colias* Thomas, 1904 (Natural History Museum, London, BMNH 2.7.6.11, collected at Fort Hall [= Murang'a], Kenya, 1255 m a.s.l.; Thomas 1904), which are considered to represent the East African subspecies *S. dinganii colias* (Simmons 2005).

*DNA processing and phylogenetic analysis*

Total genomic DNA was extracted from ethanol-preserved tissue (muscle or patagium) with JetQuick Spin Tissue Kit (Genomed, Löhne, Germany) according to the manufacturer's protocol. Complete mitochondrial gene for cytochrome *b* (cyt*b*) was amplified via polymerase chain reaction (PCR) using primers F1 (modified; 5′-CCACGACCAATGACAYGAAAA-3′) and R1 (5′-CCTTTTCTGGTTTACAAGACCAG-3′) by Sakai *et al.* (2003) in 25 $\mu$l reaction volume containing 12.5 $\mu$l Combi PPP Master Mix (Top-Bio,

**Table 1**. List of *Scotophilus* specimens from Yemen and Ethiopia used in phylogenetic analyses. Specimen pb3599 was kept intact and thus not included in the morphological analysis.

| NMP no. | Country | Locality | Coordinates | Haplotype | Group | Accession no. |
|---|---|---|---|---|---|---|
| pb3065 | Yemen | Suq ad Dabab (Taizz province) | 13°30′N, 43°57′E | Yem1 | I | HQ694502 |
| pb3066 | Yemen | Suq ad Dabab (Taizz province) | 13°30′N, 43°57′E | Yem1 | I | |
| pb3067 | Yemen | Suq ad Dabab (Taizz province) | 13°30′N, 43°57′E | Yem1 | I | |
| pb3068 | Yemen | Suq ad Dabab (Taizz province) | 13°30′N, 43°57′E | Yem1 | I | |
| pb3069 | Yemen | Suq ad Dabab (Taizz province) | 13°30′N, 43°57′E | Yem1 | I | |
| pb3070 | Yemen | Suq ad Dabab (Taizz province) | 13°30′N, 43°57′E | Yem1 | I | |
| pb3085 | Yemen | Najd an Nashamah (Taizz province) | 13°22′N, 44°01′E | Yem1 | I | |
| pb3086 | Yemen | Najd an Nashamah (Taizz province) | 13°22′N, 44°01′E | Yem1 | I | |
| pb3087 | Yemen | Najd an Nashamah (Taizz province) | 13°22′N, 44°01′E | Yem1 | I | |
| pb3088 | Yemen | Najd an Nashamah (Taizz province) | 13°22′N, 44°01′E | Yem1 | I | |
| pb3092 | Yemen | Wadi Maytam (Ibb province) | 13°52′N, 44°18′E | Yem1 | I | |
| pb3093 | Yemen | Wadi Maytam (Ibb province) | 13°52′N, 44°18′E | Yem1 | I | |
| pb3599 | Yemen | Kadamat al 'Abali (Wadi Tuban) | 13°08′N, 44°51′E | Yem2 | II | HQ694503 |
| pb3641 | Yemen | Assala, Mashgab (Taizz province) | 13°21′N, 43°57′E | Yem1 | I | |
| pb3643 | Yemen | Assala, Mashgab (Taizz province) | 13°21′N, 43°57′E | Yem1 | I | |
| pb3647 | Yemen | Assala, Mashgab (Taizz province) | 13°21′N, 43°57′E | Yem1 | I | |
| pb3648 | Yemen | Assala, Mashgab (Taizz province) | 13°21′N, 43°57′E | Yem1 | I | |
| pb3649 | Yemen | Assala, Mashgab (Taizz province) | 13°21′N, 43°57′E | Yem1 | I | |
| pb3651 | Yemen | Assala, Mashgab (Taizz province) | 13°21′N, 43°57′E | Yem1 | I | |
| pb3652 | Yemen | Assala, Mashgab (Taizz province) | 13°21′N, 43°57′E | Yem1 | I | |
| pb3676 | Yemen | Al Mawkir 1 (Wadi Zabid) | 14°09′N, 43°31′E | Yem3 | II | HQ694504 |
| pb3677 | Yemen | Al Mawkir 1 (Wadi Zabid) | 14°09′N, 43°31′E | Yem4 | II | HQ694505 |
| pb3718 | Yemen | Al Mawkir 2 (Wadi Zabid) | 14°10′N, 43°30′E | Yem3 | II | |
| pb3719 | Yemen | Al Mawkir 2 (Wadi Zabid) | 14°10′N, 43°30′E | Yem3 | II | |
| pb3721 | Yemen | Al Mawkir 2 (Wadi Zabid) | 14°10′N, 43°30′E | Yem3 | II | |
| pb3722 | Yemen | Al Mawkir 2 (Wadi Zabid) | 14°10′N, 43°30′E | Yem3 | I | |
| pb3725 | Yemen | Al Mawkir 2 (Wadi Zabid) | 14°10′N, 43°30′E | Yem5 | II | HQ694506 |
| pb2554 | Ethiopia | Metu (Oromia region) | 8°17′N, 35°35′E | Eth1 | – | HQ694507 |
| pb2557 | Ethiopia | Metu (Oromia region) | 8°17′N, 35°35′E | Eth2 | – | HQ694508 |
| pb2596 | Ethiopia | Bedele (Oromia region) | 8°24′N, 36°17′E | Eth3 | – | HQ694509 |

Prague, Czech Republic), 200 $\mu$M of each primer, and 1.5–3 $\mu$l of extracted DNA. Initial denaturation at 94°C for 3 min was followed by 35 cycles of denaturation for 40 s at 94°C, annealing for 40 s at 50°C, and extension for 90 s at 65°C, with final extension at 65°C for 5 min. The resulting PCR products were purified with JetQuick PCR Purification Kit (Genomed) and sequenced commercially (Macrogen, Seoul, Korea) using BigDye Terminator sequencing chemistry (Applied Biosystems, Foster City, CA, U.S.A.) on ABI 3730xl sequencer. Primers for sequencing cyt*b* were the same as for PCR amplification, just for several samples newly designed internal *Scotophilus*-specific primer scot_iF518 (5′-GGCTTCTCCGTTGAYAAAGC-3′) was used to obtain clear readings for the 3′ half of the PCR product. Sequences were assembled and edited in Sequencher 4.6 (Gene Codes, Ann Arbor, MI, U.S.A.) and Contig Assembly Program (CAP; Huang 1992) implented in BioEdit 7.0 (Hall 1999). Sequences were submitted to GenBank with accession numbers HQ694502–HQ694509.

Sequences were aligned in BioEdit 7.0 (Hall 1999) and checked by eye. Polymorphism in Yemeni house bats was assessed using DnaSP 4.0 (Rozas *et al.* 2003). For phylogenetic comparison with Yemeni samples, we included additional representative samples ($n$ = 3) of Ethiopian *S.* aff. *dinganii* (Table 1), and selected GenBank sequences of Asian *S. kuhlii* (EU750915) and African *Scotophilus* published by Trujillo *et al.* (2009): *S. nux* (EU750933, EU750937), *S. nigritellus* (EU750971, EU750974), *S. viridis* (EU750949, EU750952), *S. dinganii* s.str. (EU750995, EU750997, EU750998), *S.* aff. *dinganii* from Ghana and westernmost Kenya (EU750979, EU750982), *S.* aff. *dinganii* from

Ethiopia (EU750954, EU750958, EU750960) and Kenya (EU750959, EU750962, EU750964), and *S. leucogaster* (EU750940).

Phylogenetic trees were computed in PAUP* 4.10b (Sinauer Associates, Sunderland, MA, U.S.A.) using maximum parsimony (MP). Tree space was heuristically searched with tree bisection-reconnection swapping algorithm on 100 random sequence additions. Reliability of branching pattern was assessed by bootstrapping using 1000 pseudoreplicates. Phylogeny was further estimated using Bayesian inference in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) under the Hasegawa-Kishino-Yano evolutionary model with gamma-distributed among-site rate variation (HKY85Γ; Hasegawa *et al.* 1985; Yang 1993). This model was suggested by the program MrModeltest 3.7 (Nylander 2000). We used two independent simultaneous Metropolis-coupled MCMC runs of four chains running for $10^6$ generations, sampled every 100th generation, starting from random trees. The first 2500 sampled trees were discarded as burn-in. A 50% majority rule consensus tree was constructed from the remaining trees with posterior probabilities representing confidence estimates of topology. Sequence divergences were expressed as pairwise Kimura two-parameter genetic distances (K2P; Kimura 1980) to allow comparison with other bat groups (Baker & Bradley 2006).

## RESULTS

Twenty-seven specimens of Yemeni house bats were sequenced for the complete mitochondrial cyt*b* gene (1140 bp). Sequences exhibited marked variation, which indicated a deeper structure. Based on sequence similarity, two groups could be distinguished in Yemeni house bats (Table 1). Group I contained 19 specimens, which were represented by only one unique haplotype (Yem1). Group II contained eight specimens represented by four unique haplotypes (Yem2–Yem5). The genetic difference between groups I and II ranged from 12.8% to 13.1%. Haplotype diversity in the group II was moderate (H = 0.484, S.D. = 0.104) and nucleotide diversity low ($\pi$ = 0.05008, S.D. = 0.0087).

Reconstruction of phylogenetic trees was based on the five newly obtained unique sequences from Yemen and three from Ethiopia (Eth1–Eth3), and 19 relevant representative sequences of *Scotophilus* from Genbank. Trees were rooted with the basal taxon of the genus, *S. kuhlii*. MP analysis recovered four most parsimonious trees (726 steps), which differed in minute rearrangements of terminal nodes within well-supported clades (Fig. 2). The topology of the Bayesian consensus tree was basically identical to that of the MP trees, including support for the clades representing the respective taxa. Two clades contained the newly obtained Arabian haplotypes from the Yemeni specimens. The one haplotype representing the group I fell within East African *S.* aff. *dinganii* clade, which also included the new Ethiopian haplotypes. Four other haplotypes belonging to group II clustered with the only available sequence of *S. leucogaster*. Pairwise sequence divergence between the two clades comprising Yemeni haplotypes ranged from 12.4% to 13.6%. Within the *S. leucogaster* clade, the sequence divergence was very low, ranging from 0.4% to 0.6% between the haplotypes from Yemen and the haplotype from Ghana. Sequence divergence within the East African *S.* aff. *dinganii* clade reached values of 0.3–5.6%; the Yemeni haplotype differed by 1.4–2.3% from the closely related haplotypes from Ethiopia and by 3.8–4.8% from the remaining haplotype from Ethiopia and haplotypes from Kenya.

Morphological comparison of the Yemeni specimens showed all of these bats to be very similar in their measurements (Table 2). However, when specimens were segregated into the mtDNA-based groups I and II, they could be distinguished by diagnostic morphological characters. The group II specimens had significantly larger forearms, smaller ears and tragi, and smaller skulls than the group I specimens (Fig. 3, Table 2). These two groups also differed in skull shape, mainly in the form of neurocranium. The group II specimens had relatively and also absolutely wider and higher neurocrania (Fig. 4), lower coronoid processes of mandibles, and larger tympanic bullae (Table 2) than the group I specimens. The most significant differences between these groups were found in the ear and tragus lengths, height of coronoid process and relative mastoidal width (Table 2). The group I bats from Yemen conformed in the relative size characters and skull shape with comparative samples from Ethiopia, as well as with the type specimen of *Scotophilus nigrita colias* Thomas, 1904 from Kenya. Morphological differences among the compared samples were confirmed also by the canonical analysis (resulting from stepwise discriminant analysis; not shown) as well as the principal component analysis of forearm length and skull measurements, which

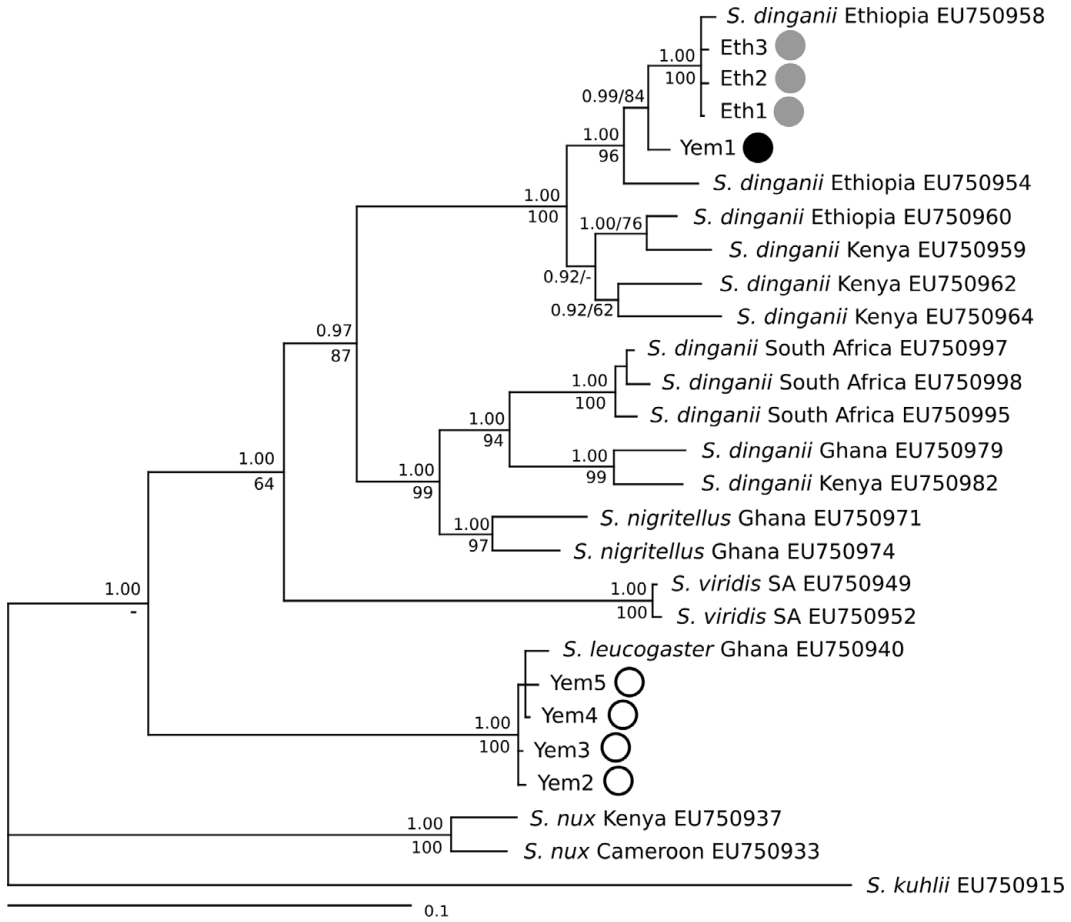


**Fig. 2.** Bayesian consensus tree showing position of newly obtained unique haplotypes from Yemen within the *Scotophilus* phylogeny. Symbols highlighting newly obtained haplotypes correspond with Fig. 1. Bayesian posterior probabilities are given above and MP bootstrap values below the branches.

clearly separated the group I specimens from Yemen and the specimens from Ethiopia including the *colias* type from the Yemeni specimens of group II (Fig. 5).

Pelage colouration of the venter in the NMP Yemeni specimens was yellow with a buffy tint on the throat in all 19 bats of the group I. Also, all 12 NMP specimens from Ethiopia used in morphological comparison showed a similar appearance to the group I specimens. In seven bats of the Yemeni group II, ventral colouration was greyish white with a brownish tint on the throat. The remaining specimen NMP pb3599 of the group II (Yem2) had creamy yellowish white ventral colour with a brownish tint on the throat, rendering them similar in appearance to specimens of the group I. Bats of the two groups otherwise differed also in pigmentation of ears and muzzle, which was darker in group I and paler in group II specimens.

## DISCUSSION

In Arabia, the occurrence of *Scotophilus* is limited to the southwestern corner of the Peninsula (Harrison & Bates 1991; Benda *et al.* 2011). The taxonomic affinity of the Arabian house bat populations, as reported by various authors over the last 50 years, included three African taxa, namely *S. nigrita, S. leucogaster* and *S. dinganii.* Robbins (1978), however, showed that the name *S. nigrita* had been incorrectly used for medium-sized house bats (Robbins 1978), and most recently the Arabian house bats were assigned to *S. dinganii* (Simmons 2005). The recent inventory of Yemeni bats by Benda *et al.* (2011) presented new records of *Scotophilus* from several localities, additional to

**Table 2**. External and cranial dimensions, selected indices, and statistical comparison (ANOVA) of the Yemeni specimens of *Scotophilus*.

| | Group I | | | | | Group II | | | | | ANOVA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | M | min | max | S.D. | *n* | M | min | max | S.D. | d.f. | *F* | *P* |
| LC | 27 | 72.78 | 68.0 | 77.0 | 2.486 | 12 | 76.00 | 72.0 | 82.0 | 3.411 | 37 | 11.056 | 0.002 |
| LCd | 27 | 53.59 | 46.0 | 58.0 | 2.693 | 12 | 51.25 | 48.0 | 55.0 | 2.221 | 37 | 6.948 | 0.012 |
| LAt | 27 | 50.48 | 47.8 | 53.2 | 1.428 | 12 | 51.81 | 50.4 | 53.5 | 1.074 | 37 | 8.281 | 0.007 |
| LA | 27 | 19.06 | 18.0 | 20.9 | 0.723 | 12 | 17.63 | 17.2 | 18.0 | 0.284 | 37 | 42.899 | 0.000 |
| LTr | 27 | 8.50 | 7.8 | 9.5 | 0.489 | 12 | 7.80 | 7.4 | 8.3 | 0.266 | 37 | 21.777 | 0.000 |
| LCr | 15 | 19.18 | 18.12 | 19.93 | 0.447 | 6 | 18.78 | 18.11 | 19.46 | 0.465 | 19 | 3.412 | 0.080 |
| LCb | 15 | 17.97 | 17.35 | 18.53 | 0.300 | 6 | 17.61 | 17.14 | 17.88 | 0.306 | 19 | 6.345 | 0.021 |
| LaZ | 15 | 13.37 | 12.80 | 13.80 | 0.287 | 6 | 13.51 | 12.67 | 13.98 | 0.450 | 19 | 0.740 | 0.400 |
| LaI | 15 | 4.50 | 4.27 | 4.89 | 0.191 | 6 | 4.77 | 4.61 | 5.00 | 0.158 | 19 | 8.922 | 0.008 |
| LaN | 15 | 9.29 | 8.98 | 9.74 | 0.181 | 6 | 9.30 | 9.11 | 9.46 | 0.132 | 19 | 0.001 | 0.978 |
| LaM | 15 | 11.68 | 11.35 | 12.19 | 0.273 | 6 | 12.08 | 11.77 | 12.41 | 0.230 | 19 | 10.129 | 0.005 |
| ANc | 15 | 7.74 | 7.22 | 8.23 | 0.295 | 6 | 8.07 | 7.78 | 8.42 | 0.229 | 19 | 6.050 | 0.024 |
| LBT | 15 | 4.02 | 3.82 | 4.25 | 0.133 | 6 | 4.11 | 4.00 | 4.24 | 0.082 | 19 | 2.099 | 0.164 |
| CC | 15 | 6.81 | 6.62 | 6.98 | 0.130 | 6 | 6.71 | 6.18 | 7.19 | 0.351 | 19 | 0.868 | 0.363 |
| $M^3M^3$ | 15 | 8.79 | 8.57 | 8.98 | 0.125 | 6 | 8.51 | 8.17 | 8.74 | 0.217 | 19 | 14.004 | 0.001 |
| $CM^3$ | 15 | 6.82 | 6.59 | 7.03 | 0.134 | 6 | 6.74 | 6.42 | 6.85 | 0.167 | 19 | 1.544 | 0.229 |
| LMd | 15 | 14.05 | 13.66 | 14.50 | 0.248 | 6 | 13.78 | 13.56 | 14.14 | 0.210 | 19 | 5.732 | 0.027 |
| ACo | 15 | 5.98 | 5.57 | 6.22 | 0.174 | 6 | 5.48 | 5.28 | 5.75 | 0.211 | 19 | 31.424 | 0.000 |
| $CM_3$ | 15 | 7.68 | 7.36 | 8.01 | 0.184 | 6 | 7.72 | 7.36 | 7.91 | 0.199 | 19 | 0.211 | 0.652 |
| LaM/LCb | 15 | 0.650 | 0.632 | 0.670 | 0.011 | 6 | 0.686 | 0.674 | 0.708 | 0.012 | 19 | 43.024 | 0.000 |
| LaN/LCb | 15 | 0.517 | 0.498 | 0.531 | 0.010 | 6 | 0.528 | 0.513 | 0.536 | 0.009 | 19 | 5.099 | 0.036 |
| ANc/LCb | 15 | 0.430 | 0.408 | 0.457 | 0.014 | 6 | 0.458 | 0.444 | 0.484 | 0.015 | 19 | 16.351 | 0.001 |

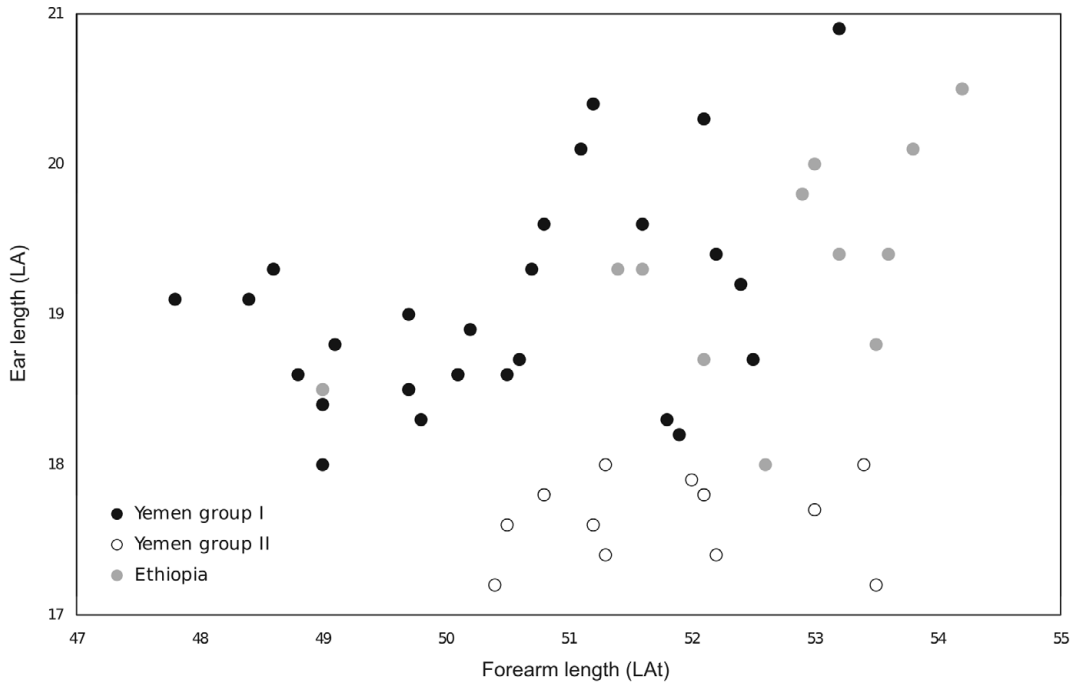


**Fig. 3**. Bivariate plot of *Scotophilus* samples: forearm length (LAt) *vs* ear length (LA). Use of symbols is consistent with previous figures, grey symbols indicate Ethiopian specimens.

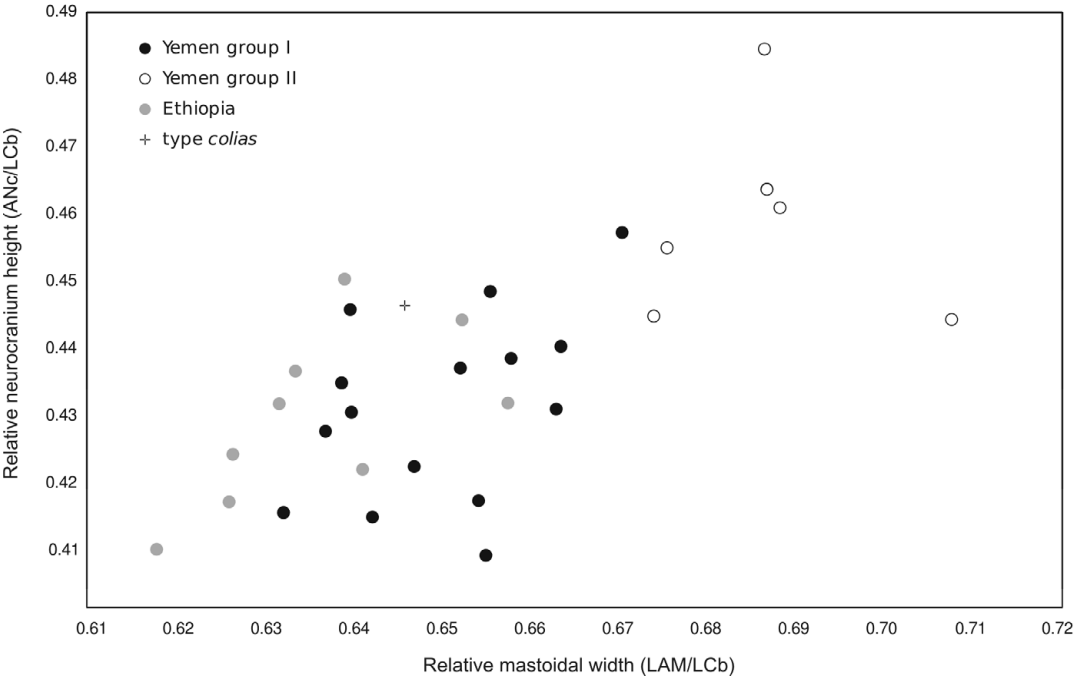


**Fig. 4**. Bivariate plot of *Scotophilus* samples: relative mastoidal width (LaM/LCb) *vs* relative neurocranium height (ANc/LCb). Use of symbols is consistent with previous figures; + = type specimen of *S. nigrita colias* Thomas, 1904.

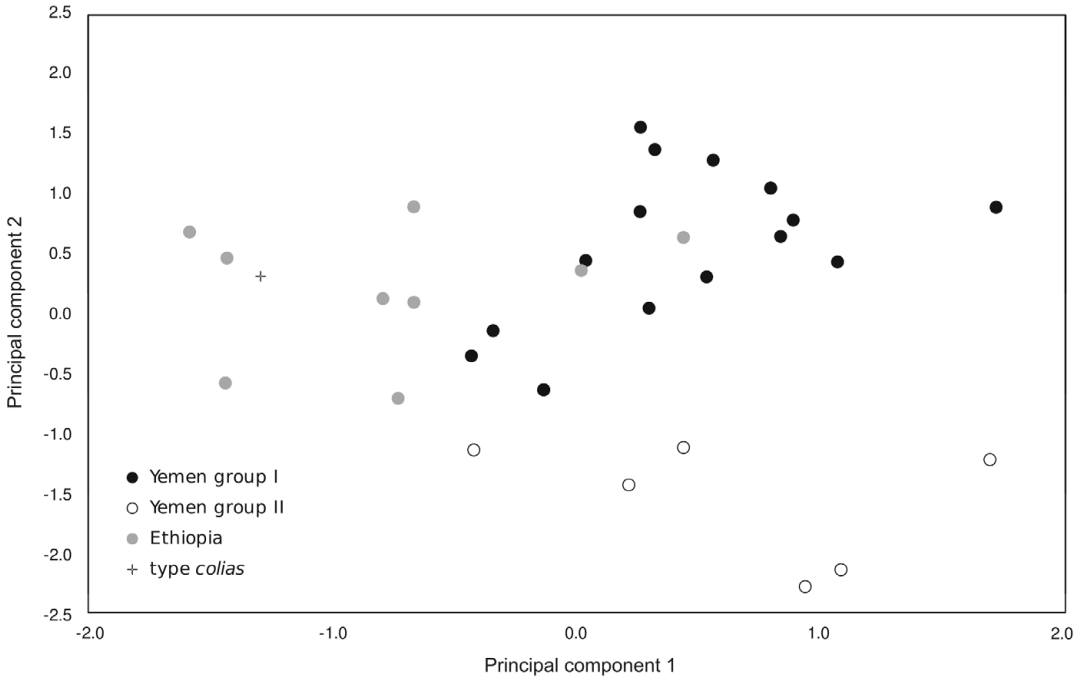


**Fig. 5**. Results of a principal component analysis of *Scotophilus* samples (PC1 = 51.49% of variance; PC2 = 13.71%) based on 14 skull dimensions and forearm length. Use of symbols is consistent with previous figures.

known records from the Reydah escarpment in Saudi Arabia (Harrison & Bates 1991; Gaucher 1993) and Yemeni records from Lahj (Harrison 1964) and Saber (Wranik *et al.* 1991) in the Aden area, Al Kadan and Wadi Qualaiah (Al-Safadi 1991), and Al Hudaydah (Harrison & Bates 1991) in the Al Hudaydah region, and from Ibb (Al-Jumaily 2004) (Fig. 1).

After being tentatively assigned to *S. dinganii,* the newly collected Yemeni specimens were analysed in detail using molecular genetic and morphological approaches to examine what we initially regarded as intraspecific variation in pelage colour and size. Surprisingly, these 27 specimens clustered into two well-differentiated groups of haplotypes differing by more than 12% in pairwise genetic distance. According to known values of cyt*b* sequence divergences between bat species (Baker & Bradley 2006), and particularly *Scotophilus* (Jacobs *et al.* 2006; Trujillo *et al.* 2009), this clearly indicates the presence of two distinct species in Yemen. Specific distinction between the two Yemeni groups is further endorsed by their phylogenetic positions within different lineages on the phylogenetic tree for African *Scotophilus*: haplotype Yem1 within *S.* aff. *dinganii* from Ethiopia and Kenya, and haplotypes Yem2–Yem5 with *S. leucogaster* from Ghana. Genetic variation within both clades containing the Yemeni haplotypes is in remarkable contrast to their respective geographical distributions. The geographically rather restricted East African *S.* aff. *dinganii* clade shows a rather deep internal structure and considerable sequence divergences up to 5.6%, values that fall within the range typifying sister species (Baker & Bradley 2006; Trujillo *et al.* 2009), thus suggesting the existence of cryptic species within this clade. On the other hand, genetic differences between *S. leucogaster* from Yemen and Ghana are half that between the Arabian population of *S.* aff. *dinganii* and its most closely related African population, although the geographical distance between Yemen and Ghana is five times larger than between Yemen and Ethiopia.

Morphological analysis of basic external measurements and detailed comparison of cranial dimensions corroborate the distinction of Yemeni *Scotophilus* into the two groups. While the affinity of group I (haplotype Yem1) specimens to the East African lineage of *S. dinganii* morpho-species was confirmed by available comparative morphological data, the affinity of group II (haplotype Yem2–5) specimens to *S. leucogaster* was based on

only the published GenBank sequence given a lack of comparative morphological information for Ghanian *S. leucogaster*. Nevertheless, the original voucher specimens (Carnegie Museum, Pittsburgh; CM113645 and CM113646) included in the study by Trujillo *et al.* (2009) were identified to species by D.A. Schlitter (Texas A&M University, College Station, TX), and their taxonomic affinity is thus based on traditional morphological grounds. Moreover, in contrast to *S. dinganii,* no evidence on paraphyly in *S. leucogaster* has yet been provided and no internal taxonomic structure has been suggested for this species in the northern hemisphere (Robbins *et al.* 1985; Simmons 2005). Finally, the existence of a genetically distinct topotypical form in Sudan, attributable to *S. leucogaster s.str.*, seems unlikely as the genetic divergence between the geographically distant populations of Yemen and Ghana reached 0.6% at the most. We therefore argue that assignation of the group II specimens to *S. leucogaster* is justified. Although distinguishable by the length of forearm, ear and tragus, and size and shape of skull, the validity of these distinctive morphological features in Yemeni *Scotophilus* has yet to be evaluated for mainland African populations of both species.

Marked differences between environmental conditions of the Yemeni localities further points to ecological segregation of the two species. Bats of group I occurred at four localities in mountainous habitats (elevation 1140–1615 m a.s.l.) in the region of Taizz, while bats of group II were recorded at three localities in lowland bare deserts (elevation 200–300 m a.s.l.) along the coastlines of the Red Sea and the Gulf of Aden. Besides the genetic similarity of the Yemeni group II to *S. leucogaster,* as suggested by comparison with the data published by Trujillo *et al.* (2009), the taxonomic affinity to this species is supported also by its lowland origin. *S. leucogaster* was described from the Sahel of western Sudan at a similar elevation to the Yemeni lowlands. On the other hand, the Yemeni group I clusters both in morphological and molecular genetic analyses with Ethiopian samples from mountainous areas. *S. dinganii s.str.* (type locality Durban, South Africa; Monadjem *et al.* 2010), whose distribution is likely limited to southern Africa, represents a phylogenetic lineage distinct from the East African lineage comprising Ethiopian and Kenyan populations of yellow-bellied house bats (Trujillo *et al.* 2009). Hence the name *S. dinganii* is not appropriate for this lineage. On the other hand, names of several other relevant forms described from this region are available: *Scotophilus nigrita colias* Thomas, 1904 from Kenya, and *Nycticejus flavigaster* von Heuglin, 1861 and *N. murino-flavus* von Heuglin, 1861 from Eritrea (Fig. 1). The two latter forms are of uncertain taxonomic position within the genus *Scotophilus* due to scarcity of information and inaccessibility of the type specimens, but based on the original description of colouration and size they can likely be allocated to *S. dinganii* morphospecies (Robbins *et al.* 1985). The *colias* form is currently recognized as the subspecies of *S. dinganii* from the northern hemisphere (Simmons 2005). As such, the name *S. colias* seems to be the only available name for East African *S.* aff. *dinganii* given current knowledge on the genus *Scotophilus* in Africa. Clearly, paraphyly exists in the Kenyan population of *S.* aff. *dinganii* (Fig. 2; see Trujillo *et al.* 2009 for comparison), and the lineage comprising specimens from Kakamega forest in the western part of Kenya and specimens from Ghana might be also attributed to the name *colias.* Nevertheless, morphologically the Yemeni and Ethiopian specimens resemble the type specimen of *S. nigrita colias* (Figs 4, 5), so we suggest the name *S. colias* as appropriate name for this lineage, which obviously represents a separate species within *Scotophilus.* Although the Yemeni bats are slightly smaller than the Ethiopian specimens, this size difference can be likely explained by clinal variation, as stated already by Harrison (1964). Our suggestion to use the name *S. colias* for designation of the East African yellow-bellied house bat should be regarded as plausible but tentative, based on limited morphological and molecular genetic data.

According to the environmental separation between the specimens analysed, we hypothesize that Yemeni records of house bats from Mashgab, Najd An Nashamah, Suq ad Dabab near Taizz and from Wadi Maytam near Ibb, as well as published records from Ibb and Al Hadr (Al-Jumaily 2004; Benda *et al.* 2011), also represent *S. colias* (Fig. 1). Correspondingly, lowland specimens can be assigned to *S. leucogaster.* These include the revised Yemeni records from Wadi Zabid and Wadi Tuban, and published records from southern Yemeni lowlands of the Aden area (Harrison 1964; Wranik *et al.* 1991) and from western Yemeni lowlands in the Al Hudaydah region (Al-Safadi 1991; Harrison & Bates 1991). The Saudi Arabian specimens from the Reydah escarpment (Harrison & Bates 1991; Gaucher 1993) could be also perhaps assigned to

this form, since they were collected at a rather lowland elevation around 500 m a.s.l.

Ventral pelage colouration in the NMP Yemeni *Scotophilus* was yellow in 19 specimens, while in seven bats it was greyish white. This difference was consistent with the respective haplotypes of groups I and II within *Scotophilus* phylogeny, which corroborates the hypothesis that venter colour distinguishes the *S. dinganii* morphospecies and *S. leucogaster.* However, there was one exception, the group II specimen from Wadi Tuban in the Aden area, which had yellowish tint in ventral pelage. Information on previous records from lowland localities by Harrison (1964), Harrison & Bates (1991) and Gaucher (1993) also state yellow colour present in ventral pelage of captured house bats, which according to our hypothesis could belong to *S. leucogaster.* Thus, we conclude that differences in ventral colour, while useful, cannot be used to diagnose the two species present in the Arabian Peninsula with 100% accuracy, and that *S. leucogaster* exhibits as much individual variability in pelage colour as other African house bat species.

## ACKNOWLEDGEMENTS

We thank Masaa Al-Jumaily and Abdul Karim Nasher (Sana'a University, Yemen) for their assistance during field excursions to Yemen, and Louise Tomsett (BMNH, London, U.K.) for access to the type specimen of *S. nigrita colias.* We also thank Victor van Cakenberghe (University of Antwerp, Antwerpen, Belgium) for comments and discussion on the taxonomy of *Scotophilus,* and Woody Cotterill (University of Cape Town, Cape Town, South Africa) and an anonymous reviewer for improvement of language and clarity of the manuscript. Sue McLaren (Carnegie Museum, Pittsburgh, PA, U.S.A.) kindly provided information on the reference specimens of *S. leucogaster.* The study was supported by grants of the Czech Science Foundation (nos. 206/09/P624, 206/09/0888) and the Ministry of Culture of the Czech Republic (no. MK00002327201).

## REFERENCES

AL-JUMAILY, M.M. 1998. Review of the mammals of the Republic of Yemen. *Fauna of Arabia* **17**: 477–502.

AL-JUMAILY, M.M. 2004. Recent records of bats from Yemen including *Plecotus cf. austriacus* (Fischer, 1829) (Mammalia: Vespertilionidae) new to the country. *Myotis* **41–42**: 57–68.

AL-SAFADI, M.M. 1991. Chiropteran fauna of Yemen Arab Republic. *Mammalia* **55**: 269–274.

BAKER, R.J. & BRADLEY, R.D. 2006. Speciation in mammals and the genetic species concept. *Journal of Mammalogy* **87**: 643–662.

BENDA, P., AL-JUMAILY, M.M., REITER, A. & NASHER, A.K. 2011. Noteworthy records of bats from Yemen with description of a new species from Socotra. *Hystrix, N.S.* **22**: 23–56.

GAUCHER, P. 1993. First record of *Scotophilus leucogaster* (Cretzschmar, 1826) (Mammalia: Chiroptera: Vespertilionidae) in Saudi Arabia. *Mammalia* **57:** 146–147.

GOODMAN, S.M., JENKINS, R.K.B. & RATRIMOMANARIVO, F.H. 2005. A review of the genus *Scotophilus* (Chiroptera: Vespertilionidae) on Madagascar, with the description of a new species. *Zoosystema* **27**: 867–882.

GOODMAN, S.M., RATRIMOMANARIVO, F.H. & RANDRIANANDRIANINA, F.H. 2006. A new species of *Scotophilus* (Chiroptera: Vespertilionidae) from western Madagascar. *Acta Chiropterologica* **8**: 21–37.

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

HARRISON, D.L. 1964. *The Mammals of Arabia. Volume I. Introduction, Insectivora, Chiroptera, Primates.* Ernest Benn Ltd, London.

HARRISON, D.L. & BATES, P.J.J. 1991. *The Mammals of Arabia,* 2nd edn. Harrison Zoological Museum, Sevenoaks, U.K.

HASEGAWA M., KISHINO, H. & YANO, T. 1985: Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22**: 160–174.

HAYMAN, R.W. & HILL, J.E. 1971. Part 2. Order Chiroptera. In: *The Mammals of Africa: an Identification Manual,* (eds) J. Meester & H.W. Setzer, pp. 1–73. Smithsonian Institution Press, Washington, D.C.

HUANG, X. 1992. A contig assembly program based on sensitive detection of fragment overlaps. *Genomics* **14**: 18–25.

JACOBS, D.S., EICK, G.N., SCHOEMAN, M.C. & MATHEE, C.A. 2006. Cryptic species in an insectivorous bat, *Scotophilus dinganii. Journal of Mammalogy* **87**: 161–170.

KIMURA, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**: 111–120.

KOOPMAN, K.F. 1975. Bats of the Sudan. *Bulletin of the American Museum of Natural History* **154**: 353–444.

MONADJEM, A., TAYLOR, P.J., COTTERILL, F.D.P. & SCHOEMAN, M.C. 2010. *Bats of Southern and Central Africa. A Biogeographic and Taxonomic synthesis.* Wits University Press, Johannesburg.

NYLANDER, M.A.A. 2004. MrModeltest v2.2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University. Online at: http://www.ebc.uu.se/ systzoo/staff/nylander.html.

ROBBINS, C.B. 1978. Taxonomic identification and history of *Scotophilus nigrita* (Schreber) (Chiroptera: Vespertilionidae). *Journal of Mammalogy* **59**: 212–213.

ROBBINS, C.B., DE VREE, F. & VAN CAKENBERGHE, V. 1985. A systematic revision of the African bat genus *Scotophilus* (Vespertilionidae). *Annales de Museé Royal de l'Afrique Centrale, Sciences Zoologiques* **246**: 51–84.

RONQUIST, J. & HUELSENBECK, J.J. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

ROZAS, J., SANCHEZ-DELBARRIO, J.C., MESSEGUER, X. & ROZAS, R. 2003. DNASP, DNA polymorphism analysis by the coalescent and other methods. *Bioinformatics* **19**: 2496–2497.

SAKAI, T., KIKKAWA, Y., TSUCHIYA, K., HARADA, M., KANOE, M., YOSHIYUKI, M. & YONEKAWA, H. 2003. Molecular phylogeny of Japanese Rhinolophidae based on variations in the complete sequence of the mitochondrial cytochrome *b* gene. *Genes and Genetic Systems* **78**: 179–189.

SIMMONS, N. 2005. Order Chiroptera, In: *Mammal Species of the World: a Taxonomic and Geographic Reference,* (eds) D.E. Wilson & D.M. Reeder, 3rd edn, pp. 312–529. Johns Hopkins University Press, Baltimore, Maryland.

TAYLOR, P.J. 2000. *Bats of Southern Africa.* University of Natal Press, Pietermaritzburg, South Africa.

THOMAS, O. 1904. On new bats from British Africa and Cameroons. *Annals and Magazine of Natural History* (7)**13**: 207.

TRUJILLO, R.G., PATTON, J.C., SCHLITTER, D.A. & BICKHAM, J.W. 2009. Molecular phylogenetics of the bat genus *Scotophilus* (Chiroptera: Vespertilionidae): perspectives from paternally and maternally inherited genomes. *Journal of Mammalogy* **90**: 548–560.

WRANIK, W., FEILER, A. & OBADI, N. 1991. Notes on some bats from the southern part of Yemen (Mammalia, Chiroptera). *Zoologische Abhandlungen des Staatlichen Museums für Tierkunde, Dresden* **46**: 207–213.

YANG, Z. 1996. Among-site variation and its impact on phylogenetic analysis. *Trends in Ecology and Evolution* **11**: 367–372.

Responsible Editor: G.N. Bronner