Phylogenetic relationships of three “Nycticeiini” genera (Vespertilionidae, Chiroptera, Mammalia) as revealed by karyological analysis

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Abstract

GTG-banded karyotypes are presented for Scotoecus hirundo (2n = 30; FN = 50), Rhogeessa alleni (2n = 30; FN = 50), Scotophilus kuhlii (2n = 36; FN = 48) and Scotophilus leucogaster (2n = 36; FN = 50). These three genera belong to the family Vespertilionidae and have previously been placed into the tribe “Nycticeiini” (Tate 1942). Karyological analysis, however, points to a close relationship of Scotoecus hirundo to the tribes Pipistrellini and Vespertilionini (sensu Volleth and Heller 1994). Rhogeessa (Baeodon) alleni, a member of the karyologically diverse genus Rhogeessa, has two fusion chromosomes in common with the genus Plecotus. Together with morphological (Hill and Harrison 1987) and molecular-genetic results (Hoofer and Van Den Bussche 2003), chromosome analysis suggests a closer relationship of Plecotini and Rhogeessa. The two species examined of the genus Scotophilus show differences only in two small autosomal pairs and the Y chromosome. Chromosomal data did not reveal closer relationships of this genus to any other vespertilionid tribe.

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Key words: Vespertilionidae, Chromosomal evolution, Phylogenetic relationships

Introduction

The chiropteran family Vespertilionidae consists of about 407 species and 48 genera according to Simmons (2006). Tate (1942) placed 8 genera, amongst them Scotoecus, Scotophilus and Rhogeessa, into the tribe Nycticeiini according to a common morphological character, the missing second upper incisor. The same genera were included into Nycticeiini by Koopman (1984a, 1994) and Simmons (2006). However, Hill and Harrison (1987) classified the Nycticeiini genera according to bacular morphology into three different tribes. They assigned Rhogeessa, Nycticeius and Otonycteris to Plecotini. Scotoecus was placed, together with Scotorepens and Nycticeinops, into the Pipistrellini (sensu Hill and Harrison 1987). Together with Scotomanes, Scotophilus comprises the tribe Scotophilini of Hill and Harrison (1987). On the basis of a comprehensive study of mitochondrial DNA sequences, Hoofer and Van Den Bussche...
(2001, 2003) stated that “Nycticeiini, as traditionally recognized, does not represent a natural assemblage”. In agreement with Hill and Harrison (1987), they placed Sco
toceus into Pipistrellini and Scotophilus into Scotophilini. Rhogeessa and Bacodon, how-
ever, were assigned to Antrozoini based on mt DNA results (Hoofer and Van Den Bussche 2003).

In addition to similarities in the dental formula, Nycticeiini also show one striking karyological similarity: with the exception of Otonycteris, whose karyotype is similar to that of Plecotus (Zima et al. 1992), all Nycticeiini genera sensu Tate (1942) from which banded karyotypes have been published, miss at least two of the three large metacentric chromosomes (1/2, 3/4, 5/6) present in the basic karyotype (Volleth and Heller 1994). It is very likely, however, that the Robertsonian fission events leading to the acrocentric condition of arms 1–6 occurred independently several times in the family Vespertilionidae.

In this study, we present detailed karyotype descriptions of four species from three genera formerly placed with the Nycticeiini. The chromosomal character states are compared with published data to elucidate the phylo-
genetic relationships of the species studied. Chromosomal characters belong to the cate-
gory of rare genomic changes and provide an independent source of phylogenetic informa-
tion (Rokas and Holland 2000). Rare genomic changes are not prone to extensive convergent or parallel evolution as found in adaptive characters such as changes in the dental formula of vespertilionid bats.

**Material and methods**

**Chromosome preparations**

Metaphase spreads were obtained from fibroblast cultures (see Volleth 1987 for details) and stained by differential staining procedures as GTG, QFQ, CBG, RBG and AgNOR (see Volleth et al. 2001). Chromosomal arms were numbered using Bick-
ham’s scheme for American Myotis species (Bick-
ham 1979). Two chromosomal arm numbers separated by a solidus (/) indicate a bi-armed chromosome resulting from a Robertsonian trans-
location. For karyotype analysis the chromosomes were compared with the “basic karyotype” of Vespertilionidae (2n = 44, see Volleth and Heller 1994).

**Specimens examined**

*Rhogeessa alleni*, 1 m, Zapotitlan, Puebla, Mexico, SMF 77908; *Scotophilus leucogaster*, 1 m, Ouagadougou, Burkina Faso, SMF 77907; *Scotophilus kuhlii*, 1 m, Ulu Gombak Field Studies Centre, Selangor, Malaysia, SMF 69317 (SMF = catalogue number of the Senckenberg Museum Frankfurt/ Main); *Scotoecus hirundo*, 1 f, Comoé National Park, Ivory Coast, field-no. 2948, currently housed at the University of Ulm.

For the *Scotophilus* specimens, species identification follows Koch-Weser (1984) and Heller and Volleth (1989).

**Results**

*Scotoecus hirundo* (de Winton, 1899)

The female studied shows a karyotype with a diploid chromosome number of 30 and an autosomal fundamental number (FN) of 50. There are 11 large to small meta- to submetacentric autosomal pairs. The X chromosome is a medium-sized subtel
centric element. The three large metacentric chromosomes, composed of arms 1 and 2, 3 and 4, 5 and 6, which are present in the basic karyotype of Vespertilionidae (see Volleth and Heller 1994) and in the majority of vespertilionid species, are not present in *Scotoecus hirundo*. Instead, the following Robertsonian fusion products have been found: 1/12, 2/7, 3/13, 4/8, 5/14, 6/11, 9/10, 15/20, 18/19, 21/22. The small metacentric chromosome 16/17 of the basic karyotype is altered in *Scotoecus*, probably due to a small pericentric inversion, bringing the proximal part of arm 16 to the long arm. Further, the three small acrocentric chromosomes have been identified as arms 23, 24 and 25 (Fig. 1). Some of the chromosomal arms of the vespertilionid karyotype have been found to exist in two different states (Volleth and Heller 1994). In *Scotoecus*, arms 1 and 2 probably show state II, arms 11 and 23 clearly state I, arms 12 and 15 clearly state II. Due to the rearranged condition of the X
chromosome, the state could not be determined for the X. According to these characters, *Scotoecus* is closely related to, but not a member of the Pipistrellini (sensu Volleth and Heller 1994). In addition to the centromeric dots, CBG-banding revealed terminally located heterochromatic bands on both homologues of arm 1 and 3, and of one homologue of arm 5. Further, the distal part of the short arm of chromosome 16/17 and a small intercalary segment of the proximal part of arm 2 were CBG-positive. Due to the addition of heterochromatic material in arm 2, this arm is clearly longer than those of other vespertilionid species (Fig. 2). The Nucleolus Organizing Regions (NORs) are located in the secondary constriction (SC) of chromosomal arm 15. An unknown rearrangement led to the subtelocentric X chromosome of *Scotoecus* with three small heterochromatic bands, one of each situated proximal, central and distal on the long arm. Together with additional material (SMF 91994–97) from the same locality, this specimen represents the first record of *S. hirundo* for Ivory Coast.

*Scotophilus kuhlii* Leach, 1821

The karyotype of the male studied consists of 7 meta- to submetacentric and 10 acrocentric autosomal pairs. The diploid chromosome number is $2n = 36$ and the fundamental number is $FN = 48$. Due to centric fission events, the three large metacentric autosomal pairs of the basic vespertilionid karyotype are present here as acrocentric elements. Robertsonian fusions resulted in bi-armed

![Fig. 1. GTG-banded karyotype of a *Scotoecus hirundo* female. Scale bar=5 μm.](image)
autosomes 8/14, 10/20, 12/23 and 15/21. In addition to centric fusion of arms 9 and 13, a small pericentric inversion was found in the resulting metacentric chromosome, which transferred a small GTG-negative, early replicating segment from arm 9 to 13. Chromosomal arms 7, 11 and 24 are present as acrocentric elements. The composition of three chromosomes, provisionally called A, B and C, could not be determined. Chromosomal arms 1, 2 and 7 are present in state II, arms 11, 15, and 23 are present in state I (Fig. 3). The X chromosome is submetacentric with the banding pattern of state I. The acrocentric Y chromosome with a size equal to arm 21 consists of CBG-positive heterochromatin except for a small region close to the centromere. The centromeres of the acrocentric chromosomes are very clearly stained after CBG-banding whereas the metacentric chromosomes show only weak staining in the centromeric regions. The SC on the smallest autosome, 24, was found to bear the only NOR in this species.

*Scotophilus leucogaster* (Cretzschmar, 1826)

This species shows a karyotype with $2n = 36$ and $FN = 50$. It differs only slightly from that of *S. kuhlii*. The following differences were found: chromosome C is a small submetacentric chromosome whereas it is acrocentric in *S. kuhlii* (Fig. 3). Chromosomal arm 24 is here the short arm of a submetacentric chromosome, the long arm of which consists completely of heterochromatic material (Fig. 4). The NOR in the SC of arm 24 is separated by a small heterochromatic segment from the centromere. The Y chromosome is a very small submetacentric chromosome with a completely heterochromatic short arm.

*Rhogeessa (Baeodon) alleni* Thomas, 1892

This species possesses a karyotype with $2n = 30$ and $FN = 50$. There are 11 large to small metacentric and three small subtelocentric autosomal pairs (Fig. 5). Out of the three large metacentric chromosomes of the basic karyotype, only chromosome 3/4 is present in *Rhogeessa alleni*. Arm 1 is fused with arm 5, arm 2 with arm 6. In addition, the following Robertsonian fusion products have been found: 7/8, 9/12, 10/14, 11/15, 13/18 and 19/21. The subtelocentric chromosomes are composed of arms 22, 23 and 24 in the long arms and a small heterochromatic short arm each. The composition of two small submetacentric chromosomes,
provisionally called A and B, could not be determined. Chromosomal arms 11, 15, and 23 are present in state I, arms 1, 7, and 12 in state II. Arm 2 is enlarged by addition of a small GTG-positive segment surrounded by two small CBG-positive heterochromatic bands (see Fig. 6). One possible explanation is the insertion of arm 25 at this position. However, in several non-related species, CBG-positive material has been found in the proximal segment of arm 2 (see Scotoecus hirundo for example). The X chromosome shows a GTG-banding pattern similar to state I. The small Y chromosome is submetacentric, late-replicating with a completely heterochromatic short arm. The NORs are situated in the SCs of the long arms of chromosomes 23 and 24. CBG-banding revealed clearly stained centromeric dots in addition to tiny heterochromatic short arms of chromosomes 22 to 24 and a heterochromatic band in the distal segment on the short arm of chromosome B.

Discussion

Comparison with published chromosomal data

Genus Scotoecus

Nagorsen et al. (1976) presented conventionally stained chromosomes of Scotoecus hindei (Thomas, 1901), a taxon that is currently considered as a synonym of S. hirundo (Robbins 1980; Koopman 1994; but see Hill 1974; Taylor and van der Merwe 1998; Cotterill 2001). The published karyotype with $2n = 30$ and FN = 50 seems to be very similar to the one presented here.

Genus Scotophilus

Several authors have published conventionally stained karyotypes of this genus. Many of them have already been cited by Zima and Horáček (1985). However, assignment to species differs due to conflicting taxonomic treatments and problems of nomenclature (e.g. Harrison and Brownlow 1978; Robbins
1978; Koopman 1984b; Robbins et al. 1985). As a result, different karyotypes were assigned to the same species name by different authors (e.g. S. temmincki with $2n = 36$, $FN = 52$ in Pathak and Sharma 1969 and with $2n = 36$, $FN = 48$ in Harada and Kobayashi 1980; Naidu 1985). At first glance, published data on Scotophilus species lead to the impression that a remarkable number of different karyotypes, all with a $2n = 36$, is present in this genus. A closer examination, however, reveals that the chromosomal variation in Scotophilus seems to be restricted to the two smallest autosomal pairs (pair “C” and 24 in Fig. 3). Scotophilus kuhlii shows both pairs in an acrocentric condition, resulting in an $FN$ of 48 (Harada et al. 1982; Sreepada and Gururaj 1994; Rickart et al. 1999; Lin et al. 2002). Scotophilus temmincki (Horsfield, 1824), which has been synonymised with S. kuhlii (Hill and Thonglongya 1972), was also reported with $2n = 36$ and $FN = 48$ (Harada and Kobayashi 1980; Naidu 1985). The two smallest pairs of the Scotophilus leucogaster karyotype are bi-armed. However, one arm of the SC-bearing chromosome consists of heterochromatic material and is thus not counted for the $FN$ ($FN = 50$). The karyotype of the Asian S. heathii (Horsfield, 1831), resembles very much the S. leucogaster karyotype, showing a bi-armed chromosome with the SC in the short arm (Sharma et al. 1974; Bhatnagar and Srivastava 1974) and a long arm of polymorphic size (Sreepada and Gururaj 1994), thus being probably heterochromatic as in S. leucogaster. The confirmation of the heterochromatic nature of this arm would
reduce the published FN from 52 to 50. Further remarkable is the fact that populations of *S. heathii* with a subtelocentric X chromosome have been reported (Sreepada and Gururaj 1994).

The interpretation of the published chromosomal data for the African species *S. dinganii* (A. Smith, 1833) and *S. viridis* (Peters, 1852) is complicated by the fact that populations from South Africa have been found with an acrocentric X chromosome (Schlitter et al. 1980). Specimens of both species from Namibia and/or Somalia, however, have been published with a metacentric X chromosome by Ruedas et al. (1990). In the third study dealing with *S. dinganii* (specimen originally published as *S. nigrita* (Schreber, 1774) by Peterson and Nagorsen 1975, but subsequently assigned to *S. dinganii* by Robbins 1978, see also Robbins and Baker 1978), the shape of the X-chromosome remained uncertain as only one female was studied (Peterson and Nagorsen 1975). Taking into account only the karyotype with a metacentric X chromosome, the karyotype of *S. dinganii* and *S. viridis* would be composed of 8 metacentric and 9 acrocentric autosomal pairs, with the SC located on the smallest metacentric chromosome and an acrocentric homologue to “C”. The karyotype of the African *S. nux* differs from that of *S. dinganii* and *S. viridis* only in the larger SC-bearing chromosome (Ruedas et al. 1990), which resembles very much that of *S. leucogaster*. Banded karyotypes have only been published in two papers (Ruedas et al. 1990; Sreepada and Gururaj 1994). Unfortunately, the quality of the published figures is not sufficient for a detailed comparison.

Genus *Rhogeessa*

The karyological variation within the genus *Rhogeessa* has been discussed in detail by Baker et al. (1985). The results of the banding analysis (Bickham and Baker 1977; Baker et al. 1985) revealed a large number of...
Robertsonian translocation events. Therefore it is not surprising that the species studied here, *Rhogeessa alleni*, has only two fusion chromosomes in common with *R. parvula* (2n = 44) and the *R. tumida* cytotypes (2n = 30, 32, 34, 52). These are chromosomes 19/21 and B.

**Phylogenetic considerations**

*Scotoecus*

Formerly, the classification of the different vespertilionid tribes was based on the dental formula of the respective genera (e.g. Tate 1942). According to this character, all members of the genera treated here would belong to the same tribe, Nycticeiini. Hill and Harrison (1987), however, introduced the shape of the baculum (os penis) as an additional character. The genus *Scotoecus* was placed by Hill and Harrison (1987) in their tribe Pipistrellini. Previously, we showed that chromosomal characters are better suited features for classification into certain tribes, e.g. Pipistrellini and Vespertilionini (Volleth and Heller 1994). Members of both tribes show state II of chromosome 15 and the X chromosome as synapomorphic features. *Scotoecus hirundo* was found to have state II of chromosome 15 and a rearranged X chromosome. However, *S. hirundo* does neither show state II of chromosome 11 (autapomorphic feature of Vespertilionini) nor state II of chromosome 23 (autapomorphic feature of Pipistrellini), see Table 1. Therefore, it is closely related to the members of both tribes, if the unlikely event of an independent paracentric inversion leading from state I to state II of chromosome 15 is excluded. The shape of the baculum as well as the results of the mt DNA analysis (Hoofer and Van Den Bussche 2003), however, imply a closer phylogenetic relationship of *Scotoecus* to the Pipistrellini than to the Vespertilionini. Within the genus *Scotoecus*, the recognition of different taxa as distinct species or as subspecies of *S. hirundo* is problematical (Hill 1974; Taylor and van der Merwe 1998; Cotterill 2001) and warrants further morphometric and molecular investigations.

**Table 1.** Distribution of karyological features: chromosomal arms occurring in two states (I, II).

<table>
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<tr>
<th>Taxon</th>
<th>11</th>
<th>15</th>
<th>23</th>
<th>X</th>
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<td>Ancestor¹</td>
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<td>I</td>
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<td>Pipistrellini²</td>
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<td>II</td>
<td>II</td>
<td>II</td>
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<td>Vespertilionini²</td>
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<td><em>Scotoecus</em></td>
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<td><em>Scotophilus</em></td>
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<tr>
<td><em>Rhogeessa</em></td>
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¹ Presumed ancestor of the subfamily Vespertilioninæ.
² According to Volleth and Heller (1994).
³ Determination of state not possible due to rearranged X chromosome.

*Scotophilus*

On dental and bacular reasons, the Afro-Oriental genus *Scotophilus* was placed together with the Oriental genus *Scotomanes* into a separate tribe, Scotophilini, by Hill and Harrison (1987). This has been criticised by Frost and Timm (1992) because *Scotophilus* and *Eptesicus* show a rather similar shape of the baculum. However, chromosomal characters clearly exclude *Scotophilus* from Eptescini (sensu Volleth and Heller 1994) if the presence of the NOR on chromosomal arm 15 is used as a distinctive feature. Both genera have in common the presence of arms 1–6 as acrocentric elements instead of the three metacentrics of the basic karyotype. However, besides these two genera, fission of all three metacentrics (1/2, 3/4, and 5/6) must have occurred independently at least three times in the evolution of vespertilionid bats. Therefore, this character is clearly not suited for elucidating phylogenetic relationships. Molecular genetic studies indicate that *Scotophilus* is more closely related to the Plecotini than to the other vespertilionid tribes (Kawai et al. 2002; Hoofer and Van Den Bussche 2003), but is also the most divergent genus within Vespertilioninæ based on mt DNA data (Hoofer and Van Den Bussche 2003: 28). Provisionally, we follow Hill and Harrison (1987) and Hoofer and Van Den Bussche (2003) in assigning *Scotophilus* to its own tribe, Scotophilini. Concerning the relationships within the genus *Scotophilus*, the results of the karyologi-
cal and mt DNA analysis (Hoofer and Van Den Bussche 2003) are in excellent agreement. With both methods, the Asian S. heathii is more closely related to the African Scotophilus species than to the Asian S. kuhlii.

**Rhogeessa**

Due to the fact that one of the three metacentrics of the basic karyotype, i.e. 3/4, is present in *R. alleni* but not in the other species, *R. alleni* is thought to branch off early from the common *Rhogeessa* stem. Based on a multivariate analysis of morphological characters, this opinion was also given by LaVal (1973), justifying the classification of *R. alleni* as a separate subgenus, Baeodon. The basal position of *R. alleni* was also revealed in the molecular phylogeny of Hoofer and Van Den Bussche (2003), who treated this taxon as a full genus, i.e. Baeodon alleni, following Corbet and Hill (1991). On baculare grounds, the genus Rhogeessa has been allied to the plecotine bats together with Nycticeius humeralis (Rafinesque, 1818) and Otonycteris by Hill and Harrison (1987). Molecular genetic analyses (Hoofer and Van Den Bussche 2001, 2003) suggest closer phylogenetic relationships between traditional Plecotini, Rhogeessa, Lasiurus, Scotophilus and other New World vespertilionine genera.

If further DNA studies would support these relationships, the fact that *Rhogeessa alleni* shares two fusion chromosomes (9/12, 13/18) with the Nearctic genus Corynorhinus and the Palaearctic plecotines could be interpreted as a common feature and not as a mere homoplasy. The Palaearctic plecotines (Plecotus, Otonycteris, Barbastella) differ from Corynorhinus in only one autosome, i.e. a derived state of chromosome 16/17 (Zima et al. 1992). On the other hand, Corynorhinus has only one apomorph chromosomal feature in common with the Nearctic Plecotini Euderma and Idionycteris (i.e. fusion chromosome 11/14; Stock 1983). If we assume that Rhogeessa is closely related to traditional Plecotini, the karyotype of Corynorhinus would be the most ancestral one within Plecotini. A New World origin of this tribe seems then to be very likely.

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**Zusammenfassung**

Phylogenetische Beziehungen von drei “Nycticeiini” Gattungen (Vespertilionidae, Chiroptera, Mammalia) aufgrund karyologischer Analysen

Für Scotoecus hirundo (2n = 30; FN = 50), Rhogeessa alleni (2n = 30; FN = 50), Scotophilus kuhlii (2n = 36; FN = 48) und Scotophilus leucogaster (2n = 36; FN = 50) werden GTG-gelanderte Karyotypen vorgestellt. Diese drei Gattungen gehören zur Familie Vespertilionidae und wurden früher dem Tribus “Nycticeiini” zugeordnet. Die zytogenetischen Befunde sprechen jedoch für eine nahe Verwandtschaft von Scotoecus hirundo zu den Tribus Pipistrellini und Vespertilionini. Rhogeessa (Baeodon) alleni, eine Art der karyologisch vielfältigen Gattung Rhogeessa, besitzt zwei Fusionschromosomen, die auch bei der Gattung Plecotus gefunden wurden.

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