Available online at www.sciencedirect.com
SCIENCE DIRECT®
Molecular Phylogenetics and Evolution 38 (2006) 155–170
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Molecular phylogenetic analysis of nycteribiid and streblid bat flies (Diptera: Brachycera, Calyptratae): Implications for host associations and phylogeographic origins

Katharina Dittmar [a,*], Megan L. Porter [b], Susan Murray [c], Michael F. Whiting [a]

[a] *Department of Integrative Biology, Brigham Young University, 401 WIDB, Provo, UT 84602, USA*
[b] *Department of Microbiology and Molecular Biology, Brigham Young University, Provo, UT, USA*
[c] *Department of Biology, Boston University, Boston, MA, USA*

Received 1 April 2005; revised 25 May 2005; accepted 6 June 2005
Available online 8 August 2005

Abstract

Bat flies are a small but diverse group of highly specialized ectoparasitic, obligatory bloodsucking Diptera. For the first time, the phylogenetic relationships of 26 species and five subfamilies were investigated using four genes (18S rDNA, 16S rDNA, COII, and cytB) under three optimality criteria (maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference). Tree topology tests of previous hypotheses were conducted under likelihood (Shimodaira–Hasegawa test). Major findings include the non-monophyly of the Streblidae and the recovery of an Old World- and a New World-Clade of bat flies. These data ambiguously resolve basal relationships between Hippoboscidae, Glossinidae, and bat flies. Recovered phylogenies resulted in either monophyly (Bayesian approach) or paraphyly (MP/ML topologies) of the bat flies, thus obscuring the potential number of possible associations with bats throughout the history of this group. Dispersal-vicariance analysis suggested the Neotropical region as the possible ancestral distribution area of the New World Streblidae and the Oriental region for the Old World bat flies. The genes examined show conflicting support across the nodes of the tree, particularly in the basal positions. Additionally, there is poor character support among all genes for the nodes associated with early hippoboscoid diversification. This results in extremely short basal branches, adding support to the idea of a rapid radiation among the four major groups of Hippoboscoidea.

Keywords: Nycteribiidae; Streblidae

1. Introduction

Bat flies are a group of highly specialized Diptera that are obligate ectoparasites on bats. Only the Old World genus *Ascodipteron* shows true female neosomy, and burrows into the skin of the host, thus being referred to as "endoparasitic" by some authors. Currently, 520 species are described (Húrka and Soós, 1986; Maa, 1989), which makes bat flies the most species-rich group among the calypterate Diptera associated with mammals. Two families are commonly recognized: Nycteribiidae and Streblidae (Marshall, 1981); however, in the Biosystematic Database of World Diptera streblid and nycteribiid bat flies are reported as part of the Hippoboscidae (http://www.sel.barc.usda.gov/Diptera/biosyst.htm). Bat flies are considered as part of the Hippoboscoidea, a calypterate superfamily encompassing several obligatory bloodsucking groups, including the medically important Glossinidae (tse-tse flies) and the Hippoboscidae, which feed on birds and mammals. Although both bat fly families show reduced compound eyes, no ocelli, a spider-like orientation of their legs, and adenotrophic vivipary

* Corresponding author. Fax: +1 801 422 0090.
E-mail address: katharinad@gmail.com (K. Dittmar).

1055-7903/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2005.06.008

(Meier et al., 1999), some of the characters that have led to the classification of two distinct bat fly families are the peculiar wing morphology with extensively membranous abdomen in the Streblidae, and the complete wing loss, the backwardly folded head, and dorsally inserted legs in the Nycteribiidae (McAlpine, 1989).

In addition to these morphological differences, the geographical and climatic distributions of both the families differ considerably. Streblidae are largely confined to subtropical and tropical climates and have a worldwide distribution, covering all biogeographic regions. However, Old- and New world faunae have no taxa in common (Kim and Adler, 1985). In addition to subtropical and tropical regions, Nycteribiidae occur in temperate climate, although their greatest diversity is in the Old World. Kim and Adler (1985) hypothesized that the center of origin for the Nycteribiidae might have been the Malaysian subregion (Oriental region) due to their high species diversity in this area.

Generally, bat flies are regarded as being not very host specific since most bat species exhibit high degrees of spatial overlap on geographic and habitat scales, and additionally often roost in dense colonies of conspecific individuals. Thus, there are ample opportunities for potential reassociations to hosts. However, it has been noted that bat fly assemblages may exhibit remarkable host specificity on all taxonomic scales (Patterson et al., 1999; Wenzel and Tipton, 1966). According to Marshall (1981) and Kim and Adler (1985), this is particularly obvious for the genus *Megastrebla* and the subfamily Cyclopodiinae, both exclusively associated to megachiropteran bats.

Bat flies are among the most specialized of all Diptera, and their highly specialized features have resulted in a confusing nomenclature. Earlier taxonomic work established two subfamilies of Streblidae: Streblinae and Nycteriboscinae [all New- and Old World taxa other than *Strebla* sensu Speiser] (Speiser, 1900). Later Jobling (1936) proposed the Trichobiinae (including *Nycterophilia*) and reduced the family Ascodipteridae to a subfamily level. Wenzel et al. (1966) proposed an additional subfamily, Nycterophiliinae, for the New World genus *Nycterophilia*. Most recently, five streblid subfamilies, Nycteriboscinae, Ascodipterinae, Trichobiinae, Streblinae, and Nycterophilinae, are recognized. Maa (1965) renamed the Nycteriboscinae to Brachytarsininae, as he regarded the type genus as a junior synonym. This is not recognized by all authors. Within Nycteribiidae, there are currently three subfamilies Nycteribiinae, Cyclopodiinae (Theodor, 1967), and Archinycteribiinae (Maa, 1971).

One main evolutionary trend within the hippoboscoid lineages is bloodsucking, which spurred the potential for convergent evolution of reduced and/or specialized features, and has consequently led to several contradicting classification schemes. Griffith (1972) challenged the common division of bat flies into two distinct families, and includes the Streblidae, Nycteribiidae, and Hippoboscidae sensu strictu as subfamilies within the Hippoboscidae sensu latu, based on several shared characters concerning the 6th tergum, the 6th and 7th abdominal spiracles, and the perigenital sclerite.

Although recent morphological and molecular studies (McAlpine, 1989; Nirmala et al., 2001;Yeates and Wiegmann, 1999) support the monophyly of the Hippoboscoidea, the phylogenetic relationships among the families, specifically between Nycteribiidae and Streblidae remain unclear. Previous morphological studies placed Streblidae and Nycteribiidae as sister groups supporting the hypothesis of a common origin, and a single lineage evolving with bats (Bequaert, 1954–1957; Hennig, 1969; McAlpine, 1989; Pollock, 1971; Schlein, 1970; Wenzel et al., 1966; Fig. 1A). Hippoboscidae is usually placed as a sister group to the Glossinidae (Fig. 1A), and this group is sister-clade to the bat flies. In the recent molecular analyses of Nirmala et al. (2001) based on 16S rDNA and 18S rDNA, however, Nycteribiidae and Streblidae never formed a monophyletic group suggesting independent origins of their association with bats (Fig. 1B). Also, Glossinidae and Hippoboscidae did not form sister groups. However, the limited number of bat fly species represented by DNA sequences in these analyses (three species) did not allow for a conclusive solution (Nirmala et al., 2001). Thus, no extensive formal analysis has been conducted yet to elucidate evolutionary relationships among the bat fly taxa.

The goals of this work are: (1) to explore the evolutionary relationships between Nycteribiidae and Streblidae, and among their subfamilies and genera, (2) to explore the hypothesis of a single versus multiple independent events of bat fly association to bats in general, and to Micro- and Megachiroptera in particular, and (3) to explore the patterns of contemporary bat fly distribution in light of their phylogeny by means of dispersal-variance analysis. To accomplish these goals, we use a molecular dataset composed of nuclear (18S rDNA) and mitochondrial (16S rDNA, COII, and cytB) genes. Additionally, we comment on evidence supporting a rapid radiation of the hippoboscoid groups.

2. Materials and methods

2.1. Taxon sampling

Insect specimens were collected in 96% ethanol and stored at −80 °C. Thirty in-group taxa were included in this analysis representing Hippoboscidae (three species), Glossinidae (one species), and bat flies (26 species). Our sampling distribution covers Old- and New World species of both Nycteribiidae and Streblidae. Exemplars of four out of five subfamilies of Streblidae and two out of

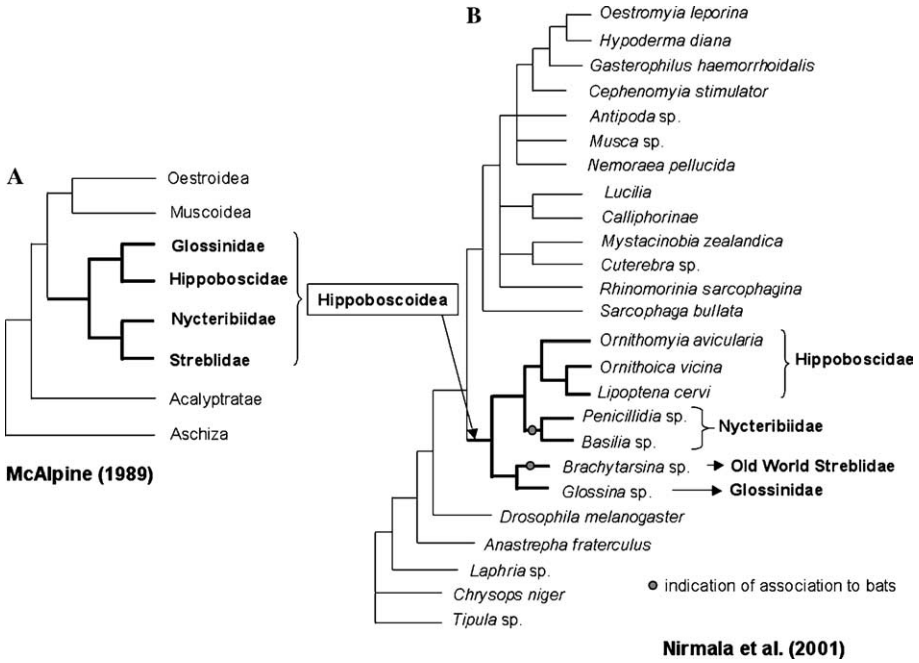

Fig. 1. (A) Morphology based phylogeny of Hippoboscoidea, according to McAlpine (1989). (B) Strict consensus of twelve trees obtained by MP analyses of the combined DNA matrix (16S rDNA + 18S rDNA) as presented by Nirmala et al. (2001).

three subfamilies of Nycteribiidae are part of this study. The subfamilies Nycterophiliinae (Streblidae) and Archinycteribiinae (Nycteribiidae) were not included due to difficulty in acquiring specimens. In some cases, multiple exemplars of the same species are present, representing different geographic or host origins. Nycteribiidae are represented by 8 species and Streblidae by 18 species. In one case, no species identification could be made, since characters did not match any of the known keys, indicating a new species (*Ascodipteron* n. sp.). Unless otherwise stated, family, subfamily, and genus names are used in the sense of McAlpine (1989), Maa (1965), and Wenzel and Tipton (1966). A representative out-group sampling among the Diptera exemplars include members of the remaining calypterate superfamilies Oestroidea (*Sarcophaga bullata*, Sarcophagidae; *Belvosia* sp., Tachinidae; *Cuterebra* sp., Oestridae) and Muscoidea (*Musca domestica*, Muscidae), the Acalyptratae (*Drosophila melanogaster*, Drosophilidae), the Orthorrhapha (*Chrysops niger*, Tabanidae), and the nematoceran family Simuliidae (*Simulium damnosum*). Details of all exemplars are provided in Table 1.

2.2. Nucleotide sampling and laboratory procedures

DNA was extracted from whole specimens using the Qiagen DNeasy (Valencia, CA, USA) protocol for animal tissue. After extraction, the specimens were permanently mounted in Berlese solution, and cataloged as specimen vouchers. Specimens and DNA vouchers are deposited in the Insect Genomics Collection (IGC), Monte L. Bean Museum, Brigham Young University (voucher numbers are provided in Table 1). One nuclear and three mitochondrial genes were targeted: nuclear—18S ribosomal DNA (18S rDNA, ~1900 bp); mitochondrial—16S ribosomal DNA (16S rDNA, ~700 bp), cytochrome oxidase II (COII, 700 bp), and cytochrome B (cytB, 400 bp). The primers, specific PCR amplification, sequencing, and cleaning protocols are given in Whiting (2002), Dittmar de la Cruz and Whiting (2003), and Bybee et al. (2004).

2.3. Sequences and alignment

The editing, contig assembly, proofreading, and manual alignment of consensus sequences were performed in Sequencher 4.2 (GeneCodes, 2003). NCBI's BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/) was used to confirm the source of the sequences as dipteran. Complete nucleotide sequences are available in GenBank under the accession numbers listed in Table 1. Alignments of 16S rDNA, COII, and cytB contained no ambiguities, and were done manually. Preliminary multiple 18S alignments were determined with ClustalX (Thompson et al., 1997), using dynamic programming under default settings for gap opening (10) and gap extension (0.10). Manual correction was undertaken using the 18S rDNA *Drosophila* secondary structural model predicted by the Gutell Lab at the University of Texas at Austin (http://www.rna.icmb.utexas/edu) as a template. All Nycteribiidae in this analysis have two unique insertions in their 18S rDNA (Nirmala et al., 2001), covering the

Table 1
In-group and out-group samples with Gen Bank Accession Numbers and Insect Genomics Collection Voucher Number (IGColl. No., Whiting Lab)

Species	IGColl. No.	Gen Bank Accession Numbers				Host	Locality
		18S	16S	CO2	cytB		
INGROUP							
Streblidae							
Ascodipteron n. sp.	DI 137	DQ133083	DQ133048	DQ133119	DQ133154	*Hipposiderus bicolor*	Tiger Cave, Penang, Malaysia
Ascodipteron phyllorhinae Adensamer, 1896	DI 136	DQ133077	DQ133042	DQ133113	DQ133149	*Hipposiderus bicolor*	Tiger Cave, Penang, Malaysia
Brachyotheca lobulata[1] Speiser, 1900	DI 142	DQ133060	DQ133045.1	DQ133096	DQ133132	*Hipposiderus bicolor*	Gua Samat, Kula Krau, Pahang, Malaysia
Brachyotheca lobulata[2]	DI 143	DQ133062	DQ133045.2	DQ133098	DQ133134	*Hipposiderus bicolor*	Gua Batu Belah, Krau Wildlife Reserve, Malaysia
Brachyotheca lobulata[3]	DI 144	DQ133054	DQ133045.3	DQ133090	DQ133126	*Hipposiderus sp.*	Gua Hiyau, Malaysia
Brachyotheca lobulata[4]	DI 150	DQ133056	DQ133045	DQ133092	DQ133128	*Hipposiderus sp.*	Dark Cave, Kuala Lumpur, Malaysia
Megastrebla (Aoroura) nigriceps[1] Jobling, 1934	DI 138	DQ133085	DQ133049	DQ133121	DQ133155	Eonycteris s. *spelaea*	Bat Cave, Pahang, Malaysia
Megastrebla (Aoroura) nigriceps[2]	DI 139	DQ133066	DQ133032	DQ133102	DQ133138	*Eonycteris s. spelaea*	Dark Cave, Batu Caves, Kuala Lumpur, Malaysia
Megastrebla p. parvior Maa, 1962	DI 135	DQ133055	DQ133024	DQ133091	DQ133127	*Eonycteris s. spelaea*	Dark Cave, Batu Caves, Kuala Lumpur, Malaysia
Raymondia huberi Frauenfeld, 1855	DI 17	DQ133072	X	DQ133108	DQ133144	unknown	Ethiopia
Strebla guajiro Garcia & Casal, 1965	DI 132	DQ133080	X	DQ133116	DQ133151	*Glossophaga* sp.	Grutas Xpukil, Yucatan, Mexico
Strebla mirabilis Waterhouse, 1879	DI 133	DQ133082	DQ133047	DQ133118	DQ133153	unknown	Venezuela
Trichobius caecus Edwards, 1918	DI 156	DQ133063	DQ133029	DQ133099	DQ133135	*Pteronotus* sp.	Volcan de los Murcielagos, Yucatan, Mexico
Trichobius corynorhini Cockerell, 1910	DI 134	DQ133079	DQ133044	DQ133115	DQ133150	*Corynorhinus townsendi*	Upper Pictograph Cave, Nevada, USA
Trichobius diaemi Wenzel, 1976	DI 157	DQ133061	DQ133028	DQ133097	DQ133133	*Desmodus* sp.	Volcan de los Murcielagos, Yucatan, Mexico
Trichobius dugesii Townsend, 1891	DI 159	DQ133069	DQ133035	DQ133105	DQ133141	unknown	Cenote de Xkhalkumin, Yucatan, Mexico
Trichobius hirsutulus Bequaert, 1953	DI 158	DQ133053	DQ133023	DQ133089	DQ133125	*Myotis sp.*	Las Grutas, Yucatan, Mexico
Trichobius intermedius Peterson and Húrka, 1974	DI 153	DQ133070	DQ133036	DQ133106	DQ133142	fruit bat	Grutas Xpukil, Yucatan, Mexico
Trichobius longipes Rudow, 1871	DI 155	DQ133088	DQ133052	DQ133124	DQ133158	*Phyllostomus* sp.	Las Grutas, Yucatan, Mexico
Trichobius major[1] Coquillett, 1899	DI 148	DQ133084	X	DQ133120	X	*Eptesicus fuscus*	Upper Pictograph Cave, Nevada, USA
Trichobius major[2]	DI 149	DQ133081	DQ133046	DQ133117	DQ133152	*Myotis* sp.	Upper Pictograph Cave, Nevada, USA
Trichobius parasiticus Gervais, 1844	DI 154	DQ133087	DQ133051	DQ133123	DQ133157	unknown	Grutas Xpukil, Yucatan, Mexico
Trichobius yunkeri[1] Wenzel, 1966	DI 151	DQ133065	DQ133031	DQ133101	DQ133137	unknown	Cenote Hoctun, Yucatan, Mexico
Trichobius yunkeri[2]	DI 152	DQ133086	DQ133050	DQ133122	DQ133156	unknown	Cenote Hoctun, Yucatan, Mexico
Nycteribiidae							
Basilia (Tripselia) coronata inivisa Theodor 1967	DI 141	DQ133071	DQ133037	DQ133107	DQ133143	*Myotis ater*	Gua Ikan, Dabong, Kelantan, Malaysia
Basilia corynorhini Ferris, 1916	DI 131	DQ133057	DQ133025	X	DQ133129	*Corynorhinus townsendi*	Upper Pictograph Cave, Nevada, USA
Basilia forcipata Ferris, 1924	DI 147	DQ133064	DQ133030	DQ133100	DQ133136	*Myotis volans*	Upper Pictograph Cave, Nevada, USA
Dipseliopoda biannulata Oldrich, 1953	DI 18	DQ133073	DQ133038	DQ133109	DQ133145	unknown	African Continent
Eucampsipoda inermis Theodor, 1955	DI 146	DQ133076	DQ133041	DQ133112	DQ133148	Eonycteris *spelaea*	Dark Cave, Kuala Lumpur, Malaysia
Eucampsipoda penthetoris Theodor, 1955	DI 145	DQ133068	DQ133034	DQ133104	DQ133140	*Eonycteris s. spelaea*	Bat Cave, Ipong, Malaysia
Penicillidia sp.		AF322435	AF322426	X	X		
Phthiridium fraterna Theodor, 1967	DI 140	DQ133058	DQ133026	DQ133094	DQ133130	*Hipposiderus bicolor*	Gua Samat, Kula Krau, Pahang, Malaysia
Hippoboscidae							
Lipoptena cervi	DI 181	DQ133078	DQ133043	DQ133114	X	deer	Leipzig, Germany
Ornithoica vicina		AF073888	X	X	X		
Ornithomyia avicularia		AF322421	X	X	X		
Glossinidae							
Glossina sp.		AF322431	AF072373	X	X		

OUTGROUPS							
Sarcophagidae							
Sarcophaga bullata	DI124	DQ133067	DQ133033	DQ133103	DQ133139		Rock Canyon, Provo, Utah, USA
Tachinidae							
Belvosia sp.	DI68	DQ133059	DQ133027	DQ133095	DQ133131		Liberty County, Florida, USA
Oestridae							
Cuterebra sp.	DI161	DQ133075	DQ133040	DQ133111	DQ133147		Leeds campground, Washington County, Utah. USA
Muscidae							
Musca domestica	DI180	DQ133074	DQ133039	DQ133110	DQ133146		1400N, 1160W, Provo, Utah, USA
Drosophilidae							
Drosophila melanogaster		M21017	AJ400907	AJ400907	AJ400907		
Simulidae							
Simulium damnosum		U36205	AF081904	DQ133093	X		
Tabanidae							
Chrysops niger		AF073889	X	X	X		

Superscript numbers behind binomial names indicate different specimens of the same species, from different hosts and/or habitats.

stem–loops E19-1 (V4 expansion region), and the stem–loops 34, 35, and the V6 expansion region of 18S rDNA—as defined by Hancock et al. (1988). Both insertions were removed from the analyses due to high ambiguity of the alignment.

2.4. Phylogenetic analysis

Phylogenetic analyses were performed on the computational cluster of the College of Biology and Agriculture at Brigham Young University (http://babeast.b yu.edu). The topologies were reconstructed using equally weighted maximum parsimony (MP) and maximum likelihood (ML) as implemented in PAUP* 4.0b10 (Swofford, 2002), as well as Bayesian methods coupled with Markov Chain Monte Carlo inference (BMCMC, MrBayes 3.04b, Ronquist and Huelsenbeck, 2003).

A continuous discussion in phylogenetics is if different datasets should be analyzed as combined data or as individual evidence. An important argument against combining data has been the idea of different underlying phylogenetic histories (Bull et al., 1993; De Queiroz et al., 1995). However, phylogenetic analyses of combined datasets have also been shown to reveal hidden support for relationships in conflict among analyses of individual markers (Gatesy et al., 1999). In these analyses, we used the combined dataset but addressed potential incongruence and conflict between genes by using the methodology proposed by Wiens (1998). For this method, separate MP analyses were conducted on the three mitochondrial genes combined (because all genes in the mitochondrial genome are linked and should therefore share the same phylogenetic history) and the 18S gene to detect potential areas of strongly supported incongruence, as indicated by conflicting nodes with bootstrap proportions (BP) $\geqslant 70\%$.

The best fit likelihood models of nucleotide substitution for the total combined dataset and each individual gene were determined using likelihood ratio tests as implemented in ModelTest 3.5 (Posada and Crandall, 1998).

The MP/ML analyses were run using 5000/100 random addition (RA) replicates and tree bisection–reconnection branch swapping. Non-parametric bootstrap values (10,000/100 bootstrap replicates, 100/1 heuristic random addition replicates) were calculated using PAUP* 4.0b10 (Swofford, 2002) to assess confidence in the resulting relationships (Felsenstein, 1985). Partitioned Bremer support values (Baker and DeSalle, 1997) for individual nodes on the strict consensus MP topology from the combined dataset were calculated using TREEROT v2b (Sorenson, 1999) in conjunction with PAUP* 4.0b10 (Swofford, 2002).

Recently, "mixed model" analyses have been incorporated into Bayesian statistical frameworks, allowing for

the accommodation of heterogeneity across sites by partitioning data so that different models of evolution can be assigned to the respective partitions. A priori information of sequence evolution obtained by ModelTest was incorporated into the BMCMC analyses as four linked gene partitions. Bayesian phylogeny estimation was achieved using random starting trees, run for 3×10^6 generations, with a sample frequency of 1000, and 10 chains (nine heated, temperature = 0.2). Analyses were repeated three times to check for likelihood and parameter mixing and congruence. Likelihood scores of sample points were plotted against generation time to determine stationarity levels. Sample points before stationarity were discarded as "burn-in." Repeated analyses were compared for convergence on the same posterior probability distributions (Huelsenbeck et al., 2002). The maximum a posteriori tree (MAP tree) is presented showing to percentage converted posterior probabilities (p*P*%) (Huelsenbeck et al., 2002).

2.5. Hypothesis testing

The topologies from our analyses were compared to hypotheses from previous analyses (McAlpine, 1989 and Nirmala et al., 2001) as well as additional arrangements using the Shimodaira and Hasegawa test (1999; SH). For the SH test, we used two approaches. In the first, alternative topologies were constructed in MacClade (Maddison and Maddison, 2000) by rearranging relevant branches on the ML tree to represent the hypotheses to be tested, and to account for the different taxon sampling in other analyses (e.g., Nirmala et al., 2001). In the second approach, we constrained the search to the clades of interest and the resulting best scored hypotheses were then compared to the single ML tree. The SH test was performed with 10,000 replicates, resampling the partial likelihoods for each site (RELL model) using PAUP*. Hypotheses and results of the first analysis are displayed in Table 2.

2.6. Internal branch tests

Internal branch tests (IBTs) were conducted to determine whether the lengths of the internal branches in the topologies were significantly different from zero (Nei and Kumar, 2000). Branches with lengths not significantly different from zero can be regarded as a polytomy, indicating poor character support. The IBT was performed in MEGA3 version (Kumar et al., 2004) using each dataset separately and the combined dataset to build distance based trees under neighbor-joining (NJ) and minimum evolution (ME). The trees were produced using the most complex model of nucleotide substitution available in MEGA2 (Tamura–Nei parameters), both with gamma distribution (Felsenstein, 2004; Swofford et al., 1996).

2.7. Character mapping—association with bats

To further assess host association, two characters [I. association with bats: (0) no association with bats, (1) association with bats; II. specific host association: (0) with Microchiroptera, (1) with Megachiroptera] were mapped most parsimoniously under ACCTRAN and DELTRAN optimization as implemented in McClade 4.0 (Maddison and Maddison, 2000).

2.8. Dispersal-vicariance analysis

We analyzed current distributions (excluding regions of known recent introduction) and fossil records (Rasnitsyn and Quicke, 2002) to assign each terminal to the six major biogeographic regions of the world as defined by Wallace (1876)—Neotropic, Nearctic, Ethiopian, Palaearctic, Oriental, and Australian regions. This was done by genus, rather than by species, taking into account that our taxon sampling for this analysis is not reflective of the number of described species. Only in the case of paraphyly were terminals considered as individual species (*Trichobius* spp., *Basilia* spp.), and distributions assigned accordingly. We chose to limit the number of regions to this scale, because substantial geographical overlap exists between some of the genera in question. Thus, at this point, increasing the geographical resolution (i.e., using subregions) would decrease the resolution of our analysis.

Dispersal-vicariance analysis (DIVA v1.1; Ronquist, 1996, 1997) was used to infer the optimal ancestral

Table 2
Shimodaira and Hashegawa (1999) test results for comparisons of alternative hypotheses

Hypothesis	Possible arrangements	$\Delta \ln L$	*P*
Monophyletic Streblidae	1. (((N,S)H)G)	27.9	0.0158
	2. ((N,H) (S,G)) (Nirmala et al., 2001)	38.6	0.019
Monophyletic batflies with monophyletic families	1. ((N,S) (H,G)) (McAlpine, 1989)	49.5	0.0186
Monophyletic batflies	1. (((N,OWS) NWS) (H,G)) MAP topology	5.138	0.2437
Monophyletic Hippoboscidae + Glossinidae	1. (((N,OWS) (H,G)) NWS) Maximum parsimony topology	24.8	0.0221
	2. ((N,OWS) ((NWS (H,G)))	25.7	0.0157

Δ = difference–ln *L*. *P* = significance. N, Nycteribiidae; S, Streblidae; H, Hippoboscidae; G, Glossinidae; NWS, New World Streblidae; OWS, Old World Streblidae.

distributions using the bat fly phylogenies from our analysis. Also, possible dispersal events in the history of the bat fly taxa were identified. Multiple runs were conducted to ensure consistency of the results. We were particularly interested in the possible ancestral distribution on a broad scale of the recovered bat fly clades. Therefore, all phylogenetic hypotheses were subjected to the analysis, since independently of the basal relationships, the same major clades were always recovered. Clades composed of multiples of the same species (e.g., *Trichobius yunkeri*) were collapsed to one terminal. Dispersal-vicariance analysis does not require hierarchical area relationships, and does not assume that dispersal events are necessarily associated with speciation, or that ancestors were restricted geographically to a single unit area.

DIVA v1.1 works with two main assumptions: (1) Speciation is mostly the result of vicariance. If the ancestral species is restricted to a single area, it will consequently speciate allopatrically, with the descendants co-occurring in the same area. If an ancestor is associated with multiple unit areas, it speciates allopatrically resulting in the distribution of descendants in two mutually exclusive areas. The cost of these scenarios is always zero. (2) The addition (dispersal) or the loss (extinction) of an area has a cost of one per area. The algorithm optimizes a biogeographical reconstruction minimizing dispersal cost by excluding an area in the ancestral distribution that is not occupied by any descendant, and by including at least one unit area from each of the two descendant species (Ronquist, 1996). One problem of this type of analyses is that the root node is inherently the least reliable in the optimization, usually manifesting itself in being widely distributed (Ronquist, 1996). Therefore, we used the Muscoidea and Oestroidea as a more distant outgroup.

3. Results

3.1. Sequences and alignment

The aligned nucleotide sequences resulted in an 18S data partition of 1859 bp, a 16S partition of 512 bp, a CoII partition of 663 bp, and a cytB partition of 373 bp. The total dataset (~3.5 kb) contained 1007 parsimony informative sites, which in the protein coding genes are largely confined to the third codon position. In the nycteribiid taxa, the stem–loops E19-1 (V4 expansion region), and the stem–loops 34, 35, and V6 expansion region of 18S rDNA show a significant length extension in comparison to the mean length of those regions observed in Hippoboscidae, Glossinidae, and Streblidae ($P < 0.001$, Student's t test, http://nimitz.mcs.kent.edu/~blewis/stat/tTest.html). Generally, within the bat flies, the length of stem–loop E19 varies between 40 and 211 bp, whereas the variable region covering stem–loops 34, 35, and V6 is between 189 and 309 bp long. The expansion trend in the Nycteribiidae always affects both, region E19-1 (V4 expansion region) and the conjunct of stem–loops 34, 35, and V6 expansion regions, confirming Hancock et al.'s (1988) hypothesis about the "coevolution" of expansion segments within a species.

3.2. Phylogenetic analysis

ModelTest determined the GTR + I + Γ model as best fit nucleotide substitution for the combined dataset, while the separate partitions converged on the following models: TrN + I + Γ (18S rDNA), GTR + I + Γ (16S rDNA), GTR + I + Γ (COII), and TrN + I + Γ (cytB). The heuristic search of the combined dataset resulted in four most parsimonious trees (treelength: 4395), the strict consensus of which is shown in Fig. 2. The topologies resulting from the BMCMC MAP (pP: 0.072) and ML analyses (ln$L$: 25423.84) are depicted in Figs. 5 and 6, respectively. Similar to previous analyses, all topologies support the monophyly of the superfamily Hippoboscoidea with high support values (BP ⩾ 90; pP% = 100). All trees show strong support for the monophyly of the Nycteribiidae (BP = 100; pP% = 100), while Streblidae as a family, and the Old World Streblidae as a taxonomic group are paraphyletic on all topologies. Within the bat flies, two major clades are recovered on all topologies: the New World Clade (NWC), being composed of exclusively New World Streblidae and an Old World Clade (OWC) encompassing Old World Streblidae and Nycteribiidae (including the secondary New World *Basilia* spp.).

Our data do not robustly resolve the placement of Hippoboscidae and Glossinidae—each have contradictory placements under different optimality criteria. In the MP topology, a monophyletic Hippoboscidae + Glossinidae are placed sister to the OWC (Fig. 2). Thus, they are nested within the bat fly taxa suggesting bat fly paraphyly. These relationships, however, receive no significant nodal support values (BP < 50). In the BMCMC analysis, they are recovered as the most basal Hippoboscoidea lineage, which would render the bat flies monophyletic (Fig. 3). Yet again, only moderate nodal support is recovered for this placement (pP% = 84), and the positioning of the Glossinidae (as represented by *Glossina* sp.) as sister to the Hippoboscidae is basically not supported (pP% = 52). In the ML tree, Hippoboscidae and Glossinidae are not recovered as monophyletic sister-clades, but Hippoboscidae are placed as sister group to the OWC, whereas Glossinidae are sister to the NWC (Fig. 4). Again, none of these relationships receives strong support values (BP < 50).

Within Nycteribiidae, a clear, strongly supported division of the subfamilies, Cyclopodiinae and Nycteribiinae, can be seen (Figs. 2–4). All topologies show the genus *Basilia* as paraphyletic, but its species relationships differ among the topologies. Although in the MP

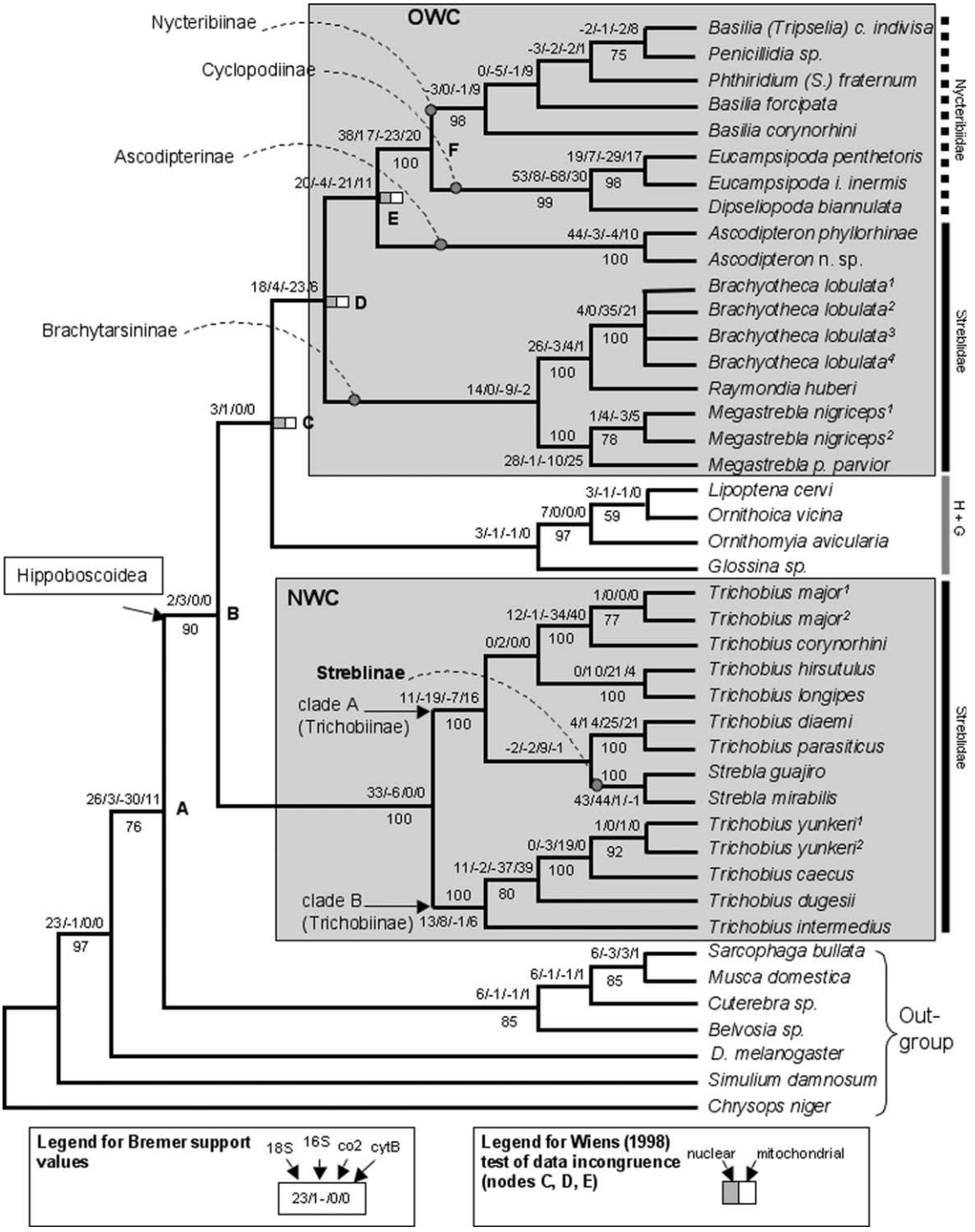

Fig. 2. Strict consensus topology of four most parsimonious trees (treelength: 4395, CI: 0.505; RI: 0.757) for the combined dataset of bat flies. Numbers above branches describe partitioned Bremer support values. Values below branches denote bootstrap support (BP). Grey rectangles on node C, D, and E describe a BP $\geqslant$70% for the separate 18S rDNA analysis in the incongruence test according to Wiens (1998). White rectangles indicate BP $\leqslant$70%, and topological conflict recovered by the mitochondrial partition. OWC, Old World Clade; NWC, New World Clade; H + G, Hippoboscidae + Glossinidae; S, Stylidia.

tree the Old World species *Basilia* (*Tripselia*) *coronata indivisa* is nested with *Penicillidia* sp., in the MAP and ML topologies *Basila* (*T.*) *c. indivisa* groups with *Phtiridium fraterna* instead. In addition althoygh the MP tree shows the New World taxa *B. forcipata* and *B. corynorhini* basal to the rest of Nycteribiinae (BP < 50, Bremer = −6), the MAP (p*P*% = 100) and the ML (BP = 83) trees recover a sister group relationship,

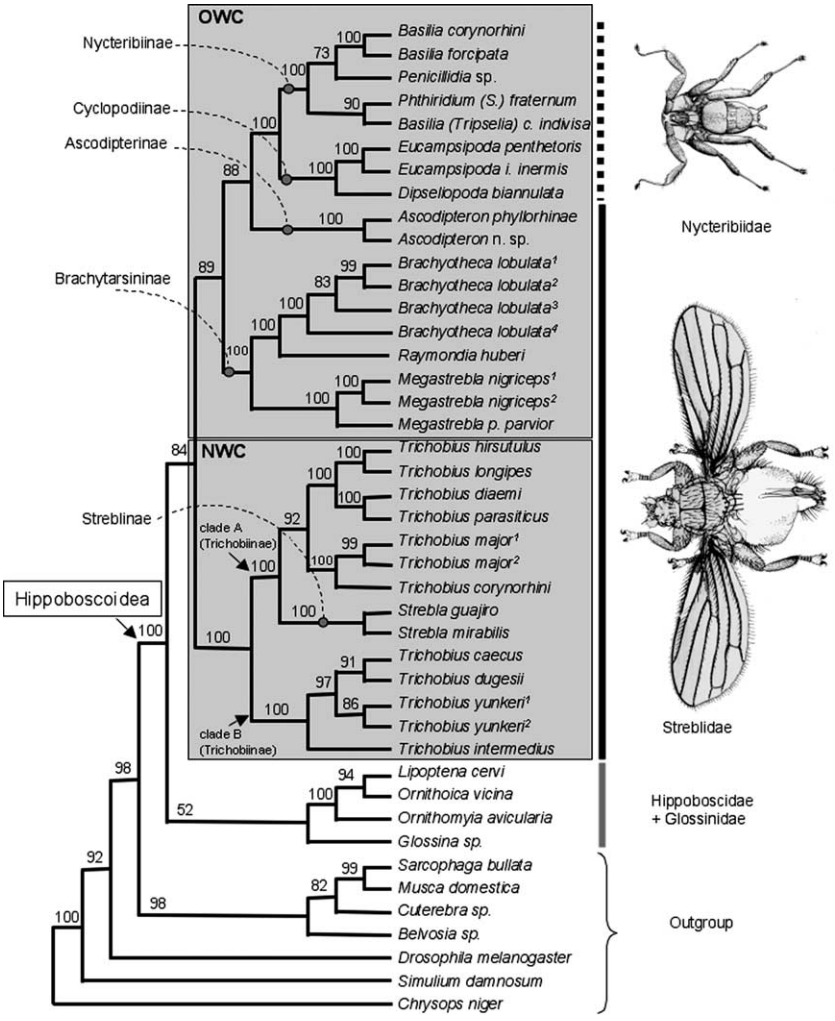

Fig. 3. Maximum a posteriori (MAP) tree of bat fly relationships as recovered by Bayesian analysis (pP = 0.072). Numbers above the branches denote posterior probabilities to percentage converted. OWC, Old World Clade; NWC, New World Clade; S, Stylidia. Pictures of nycteribiid and streblid bat fly adapted after Theodor (1967), and Jobling (1936), respectively.

placing them in the most derived position on the tree (Figs. 3 and 4).

Old World Streblidae are paraphyletic, with Ascodipterinae supported as monophyletic, and sister to Nycteribiidae (Figs. 2–4). Brachytarsininae are monophyletic, forming a sister group to Nycteribiidae + Asc odipterinae. The genus *Megastrebla* was recovered as monophyletic with high support in all analyses (BP = 100; pP% = 100), with the genera *Raymondia* and *Brachyotheca* as sister groups, also with high support [BP (MP&ML) = 100%, Bremer = 28, pP% = 100]. New World Streblidae also form a monophyletic group. Trichobiinae are paraphyletic because of the inclusion of Streblinae (Figs. 2–4), and there is a clear, strongly supported division into two distinct clades (BP = 100; pP% = 100)—Trichobiinae clade A and clade B (Figs. 2–4). Streblinae are recovered as a monophyletic clade, nested within Trichobiinae, although contradictory relationships are found with respect to their grouping with the Trichobiinae clade A.

Most of the higher level nodes within Hippoboscoidea receive only moderate to low bootstrap support (MP + ML) and pP values are low. The partitioned Bremer support values (MP-tree, Fig. 2) indicate a high degree of disagreement on certain nodes among the partitions, particularly between 18S rDNA and CoII. Specifically, although there is strong support from the 18S rDNA partition on most basal nodes (Fig. 2; nodes A, D, E, and F), at the same nodes CoII delivers contradicting negative values, resulting in low overall Bremer support values for those nodes (Fig. 2). Additionally, on nodes B and C that split the three major clades (OWC, NWC, and Hippoboscidae + Glossinidae), it becomes apparent that none of the partitions contribute significant character support (Fig. 2).

The separate analyses of the mitochondrial and nuclear datasets to address potential incongruence (Wiens, 1998) resulted in ten and six most parsimonious trees, respectively (data not shown). Generally, both

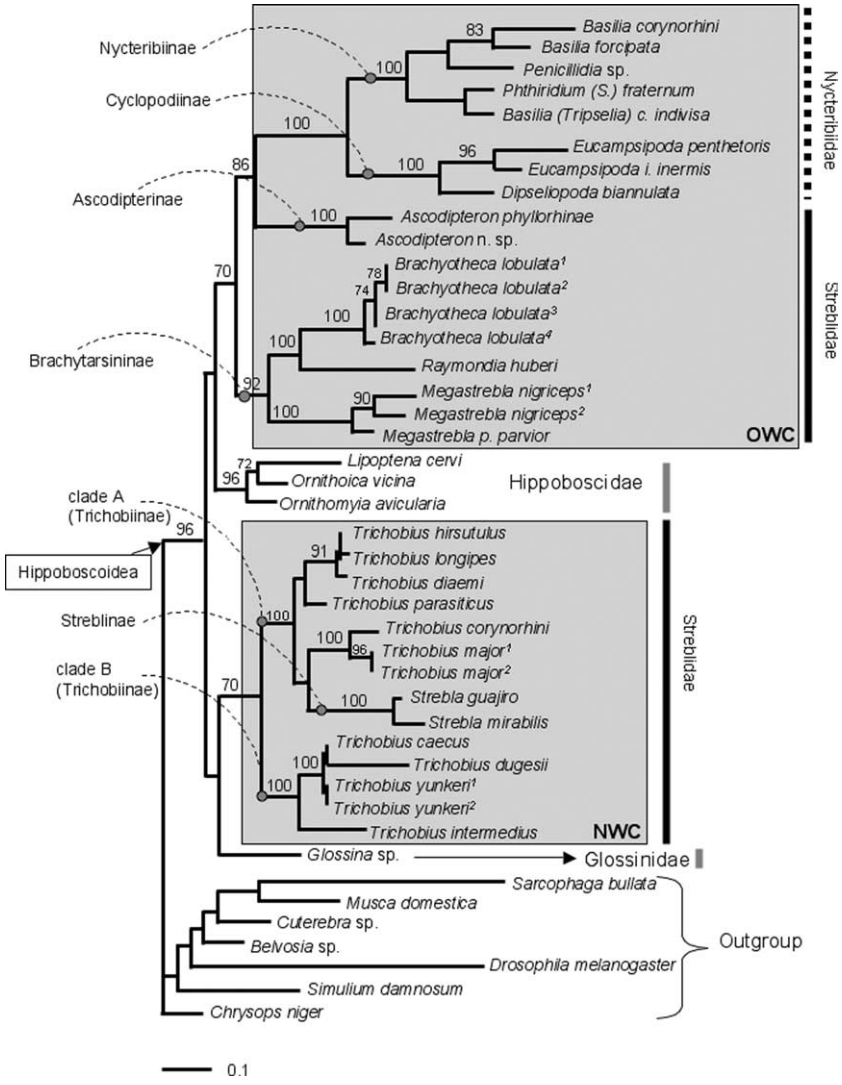

Fig. 4. Maximum Likelihood phylogram of bat fly relationships. The scale bar indicates a branch length of 0.1 substitutions per site. Numbers above the branches indicate bootstrap support values. OWC, Old World Clade; NWC, New World Clade; S, Stylidia.

consensus topologies show differing relations between families and subfamilies and congruent relationships at the generic and specific levels. However, most of the conflicting nodes receive no significant bootstrap support values, whereas all congruent nodes receive equally high bootstrap support values in either dataset. On three nodes (C–E; Fig. 2), moderate conflict was detected, since the nuclear partition received a high bootstrap support (BP $\geqslant$ 70%), whereas the mitochondrial partition supports a different relationship with low bootstrap support (BP $\leqslant$ 70%).

3.3. Hypothesis testing

According to both SH-tests only one hypothesis ("Monophyletic bat flies," equal to the MAP-topology; Table 2) was not significantly different from the ML tree (the best scored hypothesis; $P = 0.2427$). Thus, the potential monophyly of the bat fly clades cannot be significantly rejected as a worse topology over the recovered paraphyly in the ML tree. All other hypotheses proved to be significantly different (worse) than the ML topologies. The hypotheses supported by the MP topology (monophyletic clade of Nycteribiidae + Old World Streblidae and Hippoboscidae + Glossinidae versus New World Streblidae) were significantly rejected in the SH-test ($P = 0.0157$). Also, a monophyletic bat fly clade composed of monophyletic Streblidae and Nycteribiidae, reflecting McAlpine's (1989) hypothesis, was significantly rejected ($P = 0.0186$). Additionally, a monophyletic Streblidae, with branches arranged after Nirmala et al.'s (2001) hypothesis, would be significantly worse than the recovered paraphyly in all analyses ($P = 0.0158$).

3.4. Internal branch tests

In all datasets, none of the basal branches leading to the Old World bat fly clade, the New World bat fly clade, the Hippoboscidae, or the Glossinidae were significantly different from zero, showing length confidence probabilities much lower than 95% (Kumar et al., 2004; Nei and Kumar, 2000). These results confirm extremely short branches on the nodes splitting the four major hippoboscoid lineages.

3.5. Character mapping—association with bats

Depending on the topology, the mapping of the association of bat flies resulted in either a single (Fig. 5A) or two independent host shifts to bats (Figs. 5B and C). Additionally, its biological practicality left aside, in the case of the MP tree an initial association to bats coupled with a secondary host shift to mammals and birds (Hippoboscidae + Glossinidae) represents an equally parsimonious solution under ACCTRAN optimization.

Independent of the tree topology, two host shifts to Megachiroptera were recovered (Fig. 5), involving the genus *Megastrebla*, and the nycteribiid subfamily Cyclopodiinae. Moreover, our phylogenies support that the ancestral association of both the Old World and the New World bat flies were Microchiropteran bats.

3.6. Dispersal-vicariance analysis

The number of inferred dispersal events and the optimizations of ancestral distribution regions was consistent in all runs, and did not differ among the two major bat fly clades independent of the topology used (OWC = 25 dispersal events; NWC = 2 dispersal events). Therefore, we show the results on only one tree (MAP tree, Fig. 6). As predicted, the inferred ancestral distribution of the basal nodes (splitting Hippoboscidae, Glossinidae, NWC, and OWC) was widespread, always including three biogeographic regions (Nearctic, Neotropic, and Oriental).

The possible ancestral distribution area inferred for the OWC was always the Oriental region (Fig. 6). With respect to the New World Nycteribiidae (*Basilia* spp.), which occupy derived positions in the MAP and the ML topologies, the ancestral region was also inferred to be the Oriental region. In the MP tree, the position of the New World *Basilia* spp. is basal to the Old World Nycteribiinae of our taxon sampling. This would suggest the Neotropical + Oriental region as the ancestral area for New World *Basilia*; however, this scenario would additionally require a back-dispersal from the Neotropics to the Oriental region for *Basilia* (Old World), *Penicillidia*, and *Phthiridium.* This is a highly unlikely scenario, and since branch support for this particular phylogenetic

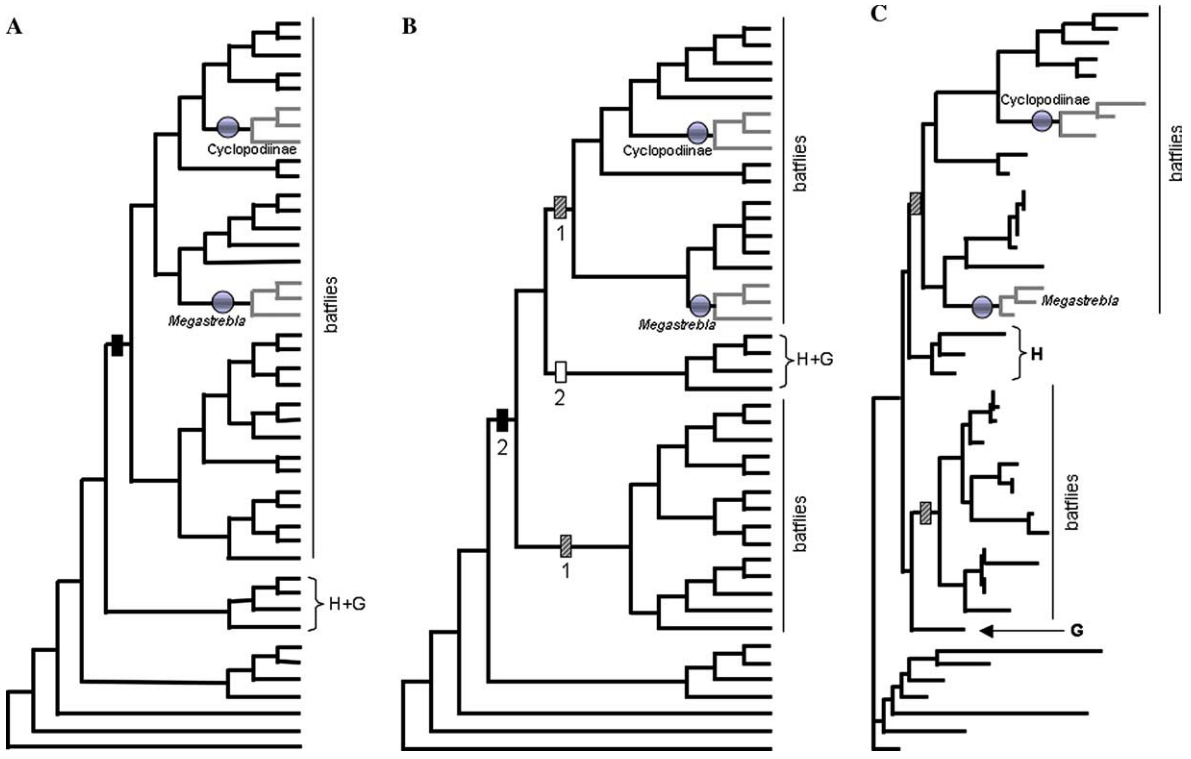

Fig. 5. Character mapping of bat fly association to bats. Black rectangles indicate the host shift to bats, white rectangles a secondary host shift to a host other than bats (loss). Shaded rectangles indicate independent shifts to bats. Numbers represent the results of ACCTRAN (1) and DELTRAN (2) optimizations. Grey circles represent events of host shifts to Megachiroptera (from Microchiroptera). (A) MAP tree; (B) MP (strict) tree; (C) ML phylogram. H, Hippoboscidae; G, Glossinidae.

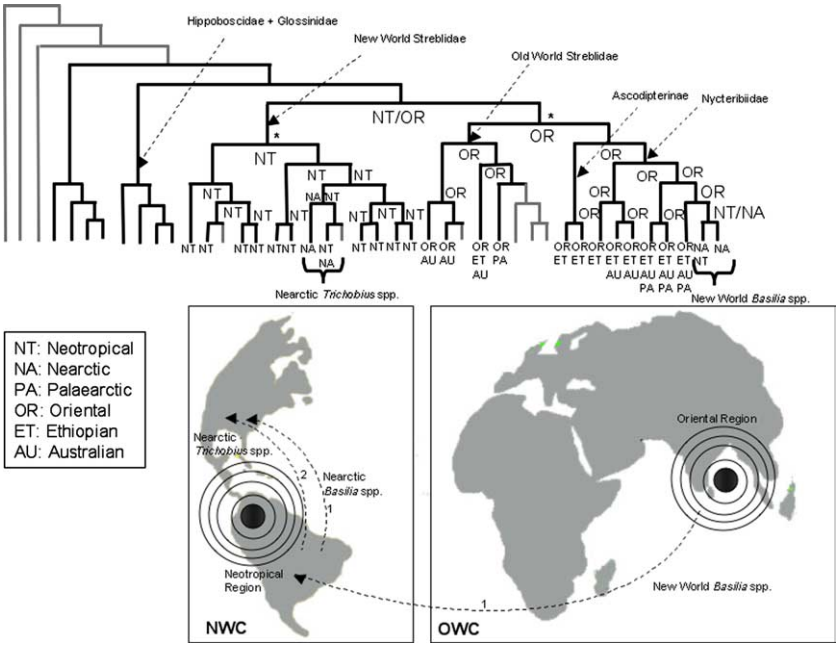

Fig. 6. Results of the dispersal-vicariance (DV) analysis of bat flies using the MAP topology (BMCMC). Grey branches indicate lineages not included in the DV analysis. Stars indicate the most basal nodes of interest; inferences beyond that point were not considered in the interpretation of this analysis. The concentric circles indicate potential ancestral distribution areas for the respective clade. OWC, Old World Clade; NWC, New World Clade. Arrows represent two specific dispersals: (1) the dispersal of *Trichobius* spp. to the Nearctic; and (2) the dispersal of *Basilia* spp. from the Old World to the Neotropics and the Nearctic.

relationship is low we do not present it as a viable option.

The ancestral distribution of the New World bat fly clade is always recovered as being the Neotropics (Fig. 6), where the highest species diversity of the New World bat flies occurs. Only two dispersal events from the Neotropic to the Nearctic were inferred, involving the ancestors of the *Trichobius major*/*T. corynorhini* clade.

4. Discussion

4.1. Bat fly phylogeny

Overall, although the different reconstruction methods vary in topological relationships of the in-group families to each other, all main bat fly clades were recovered consistently. The most striking result of the present analysis is the clear division of the bat flies into an Old World and a New World clade, which is present under all applied optimality criteria. This places the New World Streblidae apart from all Old World taxa (Nycteribiidae + Old World Streblidae), and renders Streblidae paraphyletic, contradicting current taxonomy. All alternative hypotheses implying the monophyly of the Streblidae (e.g., McAlpine, 1989; Nirmala et al., 2001) were rejected as significantly worse in the tree topology tests (Table 2), giving additional support to our findings. Nevertheless, regarding Nycteribiidae, our results agree with previous hypotheses in recovering them as a monophyletic family (Nirmala et al., 2001), which is congruent with the current taxonomy.

Our analyses further support the previous taxonomic hypothesis of the division of the Nycteribiidae into two subfamilies (Cyclopodiinae and Nycteribiinae) by recovering both of these clades as monophyletic sister groups. The apparent paraphyly of the genus *Basilia* (Nycteribiidae), however, challenges the idea of *Basilia* being a continuous entity, as supported by Theodor (1967) and Maa (1971). Previous morphological studies by Scott (1917) had instated a genus separate from *Basilia*, named *Tripselia* (which would include the species *B.* (*T.*) *coronata indivisa* of our analysis). This was based mainly on their lack of eyes. It seems from our analysis that this division was justified. *Basilia* is globally widespread and contains species that are so diverse in structure that no satisfactory scheme for subgeneric classification exists (Maa, 1971). Our results are equivocal in terms of the sequence of speciation within *Basilia*.

In both topologies, the streblid genus *Ascodipteron* is shown as the basal clade to all Nycteribiidae, albeit with low support. Although, due to similar morphology with the rest of the Streblidae, the placement of the Ascodipterinae as sister group to the Nycteribiidae seems to be counterintuitive, this placement has been recovered in all of our trees. Ascodipterinae is the only group where female species are able to shed their wings, halteres, and

legs after finding a host, henceforth leading a sedentary life on the host. Nycteribiidae, in turn, which appear to be more derived, are the first group being entirely wingless, and unlike Streblidae that can leave their hosts, they are mostly confined to a particular host. In a taxonomic sense, this might justify the re-elevation of the Ascodipterinae to a family status.

The third major lineage in the Old World clade comprises what is currently defined as the subfamily Brachytarsininae. According to Maa (1971), several morphological characters, such as the large, prominent eyes, the weak, but distinct sixth longitudinal vein, and the occasional traces of segmentation in the male abdominal convexium, suggest the "primitiveness" of the genus *Megastrebla* in respect to *Brachytarsina* (not represented in this dataset) and all Streblidae in general. Our analyses, however, do not place *Megastrebla* in a basal position to all other Streblidae.

Despite the presence of morphological synapomorphies (Jobling, 1938) uniting the Trichobiinae, the results of our analyses suggest that this subfamily is paraphyletic. Streblinae are placed nested within subclade A of Trichobiinae (Figs. 2–4). This is surprising, taking into account that Trichobiinae include the most generalized species of the New World bat flies and Streblinae have an extremely different morphology. Characteristically, Streblinae are dorso-ventrally flattened, and seem to represent specializations toward a more polyctenoid body form. Jobling (1936) characterized this subfamily on the basis of the shape and the width of the head, the shape and position of the palpi, and the shape and relative size of the mesonotum. Wenzel and Tipton (1966), however, note a high variability among those characters within the subfamily, and hypothesized about the potential multiple independent evolution of these characters. Our topologies support a clear division of the Trichobiinae into two distinct clades. While subclade A (Figs. 2 and 3) contains nearctic and neotropical species, subclade B is entirely composed of neotropical species. Perhaps, not surprisingly, given similar geographical distributions, *Trichobius major* and *T. corynorhini* are recovered as sister species. Also, Wenzel (1976) defined a *T. parasiticus* complex, including among others *T. diaemi*. Our analysis corroborates this hypothesis by placing both as sister species. However, he included the *T. parasiticus* complex within the *T. dugesii* group, a hypothesis that is contradicted by our analyses as they appear as a paraphyletic assemblage, nested within different clades.

4.2. Basal relationships, support, and conflict in the data

Like all previous analyses, our phylogenies confirm the monophyly of the Hippoboscoidea, though the placement of the families are not well resolved with our data. This lack of resolution may be due to two reasons.

Reason 1: Partition Bremer support values indicate a general scarcity of characters in all partitions on nodes B and C (Fig. 2), involved in resolving the relationships between Nycteribiidae, Old World Streblidae, Hippoboscidae, and Glossinidae. Combined with the IBT analysis, which corroborates our hypothesis of significantly shorter branches at these nodes, this pattern could have resulted from an episode of rapid lineage diversification (sensu Schluter, 2000), leaving little opportunity for character fixation in the early stages of hippoboscoid evolution. Not surprisingly, as obligate vertebrate parasites, fossil records of bat flies are virtually non-existent. The Hippoboscidae, however, are known from the early Miocene in Germany (16.4–23.8 Mya) (Statz, 1940) and the late Miocene of Italy (5.3–11.2 Mya) (Bradley and Landini, 1984). Unfortunately, due to preservation issues, fossil findings do not necessarily coincide with the actual appearance of the group. This might explain why Glossinidae, which are apparently closely related to the Hippoboscidae and the bat flies, date back to the much earlier Eocene (Rasnitsyn and Quicke, 2002). Interestingly, it is hypothesized that bats diverged from primitive eutherians in the late Cretaceous, and all of the 18 living families of bats were already present by the late Eocene (ca. 33.7 Mya) (Teeling et al., 2003; Simmons, 2005). Taking the well-supported monophyly of the Hippoboscoidea into account, combined with Manter's rule (Manter, 1966) of parasites evolving slower than their hosts, one could hypothesize that the radiation of the bat flies could have been initiated sometime at the end of the late Eocene, at the height of bat diversification (Simmons, 2005).

Reason 2: On nodes A, D, E, and F, there seems to be particularly strong conflict between the 18S rDNA and the CoII partitions, with the conflicting characters canceling phylogenetic signal in either gene, resulting in a low overall Bremer support value and inconsistency in reconstruction across the methods investigated. This trend is supported by low bootstrap support values, low p*P* values, and the detection of moderately incongruent gene histories (Wiens, 1998) on nodes C, D, and E (Fig. 2). Biological processes, such as the action of natural selection or genetic drift, may cause the history of the genes to differ, and ultimately obscure the history of the taxa. Although our dataset is more extensive than any previous compilation, it still represents only a small number of concatenated genes, thus having a significantly higher probability of supporting conflicting topologies (Rokas et al., 2003). Therefore, the addition of other, more informative genes might contribute to a better resolution of basal ingroup relationships.

Likelihood topology tests suggest that the monophyly of the bat flies as recovered in the MAP tree is not significantly worse than the paraphyly recovered in the ML tree. They do, however, significantly reject the supposed

monophyly of Hippoboscidae + Glossinidae and of the Streblidae, regardless of the topology of the rest of the tree. All this information taken together makes it clear that although there is some confidence in the non-monophyly of the Streblidae and of the supposed Hippoboscidae + Glossinidae clade, no firm statements regarding the basal relationships of the four main ingroup lineages to each other can be made, consequently impeding us from hypothesizing about the mono- or paraphyly of bat flies as a whole.

4.3. Evolution of host association

The parsimony character mapping on the branches, leading to hypotheses of single or multiple independent events of host associations, depends entirely on the mono- or paraphyly of the clade of interest. Since the basal relationships in our trees remain unresolved (see previous section), we cannot significantly reject Nirmala et al.'s (2001) hypothesis of two independent events of bat association, but can also not convincingly argue for just a single event.

Standard evolutionary theory has it that the parallel, independent evolution of association to the same host group among closely related parasite groups, as supported by our ML and MP trees, is regarded as being highly unlikely. This is partly based on the assumption that the adaptations required for a parasitic lifestyle are mostly dramatic in nature (e.g., mouthparts, life-cycles), requiring energy that is unlikely to be invested twice within one lineage (e.g., bat flies). Under the scenario of a rapid radiation, however, an evolutionary opportunity stimulating rapid bursts of speciation and phenotypic evolution is provided (Schluter, 2000). These speciation bursts usually subside later, as the supply of new niches is exhausted, and species richness generally declines afterwards due to extinction events. Therefore, the possibility of the simultaneous survival of two independent ancestral lineages stemming from a rapid speciation event, which were geographically separated at an early stage of their evolution (as indicated in our topologies) and both specialized to the ecological niche of bats, cannot be ruled out with certainty. A scenario of an initial association to bats, with a secondary switch from bats to mammals and birds, as proposed by the ACCTRAN optimization on our MP tree is intriguing, but highly unlikely under both morphological and ecological considerations.

On all of our topologies, the evolution of two independent associations with Megachiroptera, once within Old World streblid (*Megastrebla* spp.), and again nycteribiid bat flies (Cyclopodiinae), are supported (Fig. 5). This is assuming that Megachiroptera are a monophyletic group of hosts. Currently, Megachiroptera are composed of one family, the Pteropodidae, which are regarded as being monophyletic (Bastian et al., 2001; Simmons, 2005). However, recent phylogenetic studies on bats have shown that two families of Microchiropteran bats are closely related to the Megachiropteran bats than to the Microchiroptera (Teeling et al., 2002, 2005). In the future, this might change the taxonomic grouping of the Megachiroptera; and more conservatively, one would have to interpret the character mapping as two independent associations within the family Pteropodidae rather than the Megachiroptera. However, these results corroborate previous observations by Theodor (1967), Hutson and Olroyd (1980), and Maa and Marshall (1981) based on host records. Our phylogenies also suggests that Megachiroptera might have been colonized as hosts after the Microchiroptera, since microchiropteran association is recovered on all nodes prior.

4.4. Phylogeography of bat flies

The recovery of two major clades (OWC and NWC) on the topology support a scenario where the ancestral bat flies split into two geographically distinct groups at an early stage of bat fly speciation, and subsequent diversification within these groups occurred only afterwards. This is consistent with Wenzel and Tipton (1966) suggesting an ancient origin of the New World and Old World bat fly taxa, based on the observation that Old- and New World taxa have no streblid fauna in common, even in the cosmopolitan bat families Emballonuridae, Molossidae, and Vespertillionidae. Additionally, the general outcome of our dispersal-vicariance analysis suggests two distinct ancestral distribution areas for the New World and Old World bat fly taxa, being the Neotropical and the Oriental regions, respectively. Kim and Adler (1985) have hypothesized that the center of origin of the Nycteribiidae might have been the Malaysian subregion. While the resolution produced by our taxon sampling does not allow us to pinpoint this specific region, their hypothesis is corroborated by our analysis in the context of the Malaysian subregion being part of the Oriental region.

Our analyses strongly suggest that current distributions within the Old World clade are the result of multiple (25) independent widespread dispersal events following speciation, covering a wide array of biogeographical regions (e.g., Palaearctic, Australian, and Ethiopian regions), whereas the New World clade seems to have stayed more restricted within its ancestral region of the Neotropics (two dispersals). However, the number of dispersal events is likely to increase with the inclusion of more taxa in further analyses.

For the New World members of the genus *Basilia*, our results indicate the dispersal of the genus *Basilia* (Nycteribiidae) to the New World as occurring secondary to the New World–Old World split of the main clades, separating them from the ancestors of the

Basilia/*Penicillidia* group (Fig. 6). Assuming that the New World *Basilia* are monophyletic, it also suggests that the dispersal route of the ancestors of the New World *Basilia* was directed mainly to the Neotropics, which is confirmed by the fact that their highest species diversity occurs in this region. The colonization of the Nearctic by the New World *Basilia* can be considered to have occurred through the Neotropics, rather than directly from the Oriental region. Assuming that the Neotropical area represents the ancestral distribution area of the New World Streblidae, the optimal solution indicates a dispersal of the *Trichobius major*/*T. corynorhini* clade to the Nearctic, with the ancestor of both species being distributed throughout the Neotropic and Nearctic (Fig. 6).

5. Concluding remarks

The present study was intended to provide a first molecular phylogeny of the ectoparasitic bat flies. The main conclusions of our analyses are:

1. Nycteribiidae are monophyletic.
2. Streblidae are paraphyletic.
3. No conclusive statement regarding the mono- or paraphyly of the bat flies can be made at this point of the analysis.
4. Two major clades, the New World clade (being entirely composed of Streblidae) and the Old World clade (uniting the Old World Streblidae and the Old- and New World Nycteribiidae) are supported under all optimality criteria.
5. The basal relationships of the four hippoboscoid families cannot be unambiguously resolved, due to conflict in the data, and a potential rapid radiation during early hippoboscoid diversification.
6. Currently, there is as much evidence for two independent evolutionary events of bat fly association with bats, as there is for a single event. However, regardless of the topologies, two independent events of association to megachiropteran bats are supported.
7. Dispersal-vicariance analysis supports the Oriental region as the ancestral distribution area of the Old World clades and the Neotropical region for all New World taxa, except the New World members of the Nycteribiidae, which are a result of secondary dispersal from the Old World.

The conflicting results between gene trees and alpha taxonomy indicate that further work will be necessary before a taxonomic revision can be formalized. Also, the subfamilial relationships of the bat flies remain poorly understood. Undoubtedly, not only more taxa, but also more informative genes and an updated morphological matrix have to be included in further analyses to reach a more comprehensive reconstruction of bat fly phylogeny.

Acknowledgments

We thank Dr. B. Peterson for assistance identifying some specimens for this study, Liz Price, John Jasper, Krupa Patel, Hashim Bin Man, and Gavin Svenson for collecting assistance. This study was funded by the Karl-Enigk-Foundation for Experimental Parasitology (Hannover, Germany), NSF DEB 9983195 and NSF DEB 0120718.

References

Baker, R.H., DeSalle, R., 1997. Multiple sources of character information and the phylogeny of Hawaian drosophilids. Systematic Biology 46, 674–698.

Bastian, S.T., Tanaka, K., Anunciado, R.V.P., Natural, N.G., Sumalde, A.C., Namikawa, T., 2001. Phylogenetic relationships among megachiropteran species from the two major islands of the Philippines, deduced from DNA sequences of the cytochrome *b* gene. Candian Journal of Zoology 79, 1671–1677.

Bequaert, J.C., 1954–1957. The Hippoboscidae or louse-flies (Diptera) of mammals and birds. Part II. Taxonomy, evolution and revision of American genera and species. Entomologica Americana 34–36, 1–611.

Bradley, F., Landini, W., 1984. I fossili del "tripoli" messiano di Gabbro (Livorno). Palaeontographica Italiana 73, 5–33.

Bull, J.J., Huelsenbeck, J.P., Cunningham, D., Swofford, D.L., Waddell, P.J., 1993. Partitioning and combining characters in phylogenetic analysis. Systematic Biology 42, 384–497.

Bybee, S., Taylor, S., Nelson, C.R., Whiting, M.F., 2004. A phylogeny of robberflies (Asilidae) at the subfamilial level: molecular evidence. Molecular Phylogenetics and Evolution 30, 789–797.

De Queiroz, A., Donoghue, M.J., Kim, J., 1995. Separate versus combined analysis of phylogenetic evidence. Annual Review of Ecology and Systematics 26, 657–681.

Dittmar de la Cruz, K., Whiting, M.F., 2003. Genetic and phylogeographic structure of populations of *Pulex simulans* (Siphonaptera) in Peru inferred from two genes (CytB and CoII). Parasitology Research 91, 55–59.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Felsenstein, J., 2004. Infering Phylogenies. Sinauer Associates Inc., Sunderland.

Gatesy, J., O'Grady, P., Baker, R.H., 1999. Corroboration among data sets in simultaneous analysis: hidden support for phylogenetic relationships among higher level artiodactyl taxa. Cladistics 15, 271–314.

Griffith, G.C.D., 1972. The phylogenetic classification of Diptera, Cyclorrhapha, with special reference to the structure of the male postabdomen. The Hague.

Hancock, J.M., Tautz, D., Dover, G.A., 1988. Evolution of the secondary structures and compensatory mutations of the ribosomal RNAs of *Drosophila melanogaster*. Molecular Biology and Evolution 5, 393–414.

Hennig, W., 1969. Ueber die Stammesgeschichte der Insekten. Verlag Waldemar Kramer, Frankfurt (Main).

Huelsenbeck, J.P., Larget, B., Miller, R.E., Ronquist, F., 2002. Potential applications and pitfalls of Bayesian inference phylogeny. Systematic Biology 51, 673–688.

Húrka, K., Soós, A., 1986. Family Nycteribiidae. Family Streblidae. In: Soós, A., Papp, L. (Eds.), Catalogue of Palaearctic Diptera

Scatophagidae–Hypodermatidae, vol. 11. Elsevier, Amsterdam, Netherlands, pp. 226–236.
Hutson, A.M., Olroyd, H., 1980. Family Streblidae. In: Crosskey, R.W. (Ed.), Catalogue of the Diptera of the Afrotropical Region. British Museum of Natural History, London, UK, pp. 772–774.
Jobling, B., 1936. A revision of the subfamilies Streblidae and the genera of the subfamily Streblinae (Diptera, Calypteratae) including a redescription of *Metelasmus pseudopterus* Coquillett and a description of two new species from Africa. Parasitology 28, 355–380.
Jobling, B., 1938. A revision of the species of the genus *Trichobius* (Diptera, Acalypterae, Streblidae). Parasitology 28, 358–387.
Kim, K.C., Adler, P., 1985. Patterns of insect parasitism in mammals. In: Kim, K.C. (Ed.), Coevolution of Parasitic Arthropods and Mammals. John Wiley and Sons, New York, pp. 157–196.
Kumar, S., Tamura, K., Nei, M., 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment briefings in Bioinformatics 5, 150–163.
Maa, T.C., 1965. An interim world list of bat flies (Diptera: Nycteribiidae and Streblidae). Journal of Medical Entomology 1, 377–386.
Maa, T.C., 1971. Studies in bat flies (Diptera: Streblidae, Nycteribiidae). Pacific Insects Monograph 28. Bernice P. Bishop Museum, Entomology Department, Honolulu, Hawaii. pp. 1–248.
Maa, T.C., 1989. Nycteribiidae, Streblidae. In: Evenhuis, N.L. (Ed.), Catalog of the Diptera of the Australasian and Oceanian Region. Bishop Museum Press, Honolulu, Hawai, pp. 790–795.
Maa, T.C., Marshall, A.G., 1981. Diptera: Pupiparia of the New Hebrides: taxonomy, zoogeography, host association and ecology. Quarternary Journal of the Taiwan Museum 34, 213–232.
Maddison, D., Maddison, W., 2000. MacClade 4: Analysis of Phylogeny and Character Evolution. Sinauer Associates, Sunderland, Massachussetts.
Manter, H.W., 1966. Parasites of fishes as biological indicators of recent and ancient conditions. In: McCauley, J.E. (Ed.), Host-Parasite Relationships. Oregon State University Press, Oregon, pp. 59–71.
Marshall, A.G., 1981. Ectoparasitic Insects. Academic Press, New York.
McAlpine, J.F., 1989. Phylogeny and Classification of the Muscomorpha. In: McAlpine, J.F. (Ed.), Manual of Nearctic Diptera. Research Branch, Agriculture Canada, pp. 1397–1518.
Meier, R., Kotrba, M., Ferrar, P., 1999. Ovoviviparity and viviparity in the Diptera. Biological Reviews 74, 199–258.
Nei, M., Kumar, S., 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York.
Nirmala, X., Hypsa, V., Zurovec, M., 2001. Molecular phylogeny of Calyptratae (Diptera: Brachycera): the evolution of 18S and 16S ribosomal rDNAs in higher dipterans and their use in phylogenetic inference. Insect Molecular Biology 10, 475–485.
Patterson, B.D., Ballard, J.O.W., Wenzel, R., 1999. Distributional evidence for cospeciation between neotropical bats and their bat fly ectoparasites. Studies on Neotropical Fauna and Environment 33, 76–84.
Pollock, J.N., 1971. Origin of tse-tse flies: a new theory. Journal of Entomology 40, 101–109.
Posada, D., Crandall, K.A., 1998. ModelTest: testing the model of DNA substitution. Bioinformatics 14, 817–818.
Rasnitsyn, A.P., Quicke, D.L.J., 2002. History of Insects. Kluwer Academic Publishers, Dordrecht.
Rokas, A., Williams, B.L., King, N., Carroll, S.B., 2003. Genome-scale approaches to resolving incongruence in molecular phylogenies. Nature 425, 798–804.
Ronquist, F., 1996. DIVA, Version 1.1. Computer Program and manual available by Anonymous FTP from Upsala University ftp.uu.se or ftp.systbot.uu.se.
Ronquist, F., 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. Systematic Biology 46, 195–203.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Schlein, Y., 1970. A comparative study of the thoracic skeleton and musculature of Pupiparia and the Glossinidae (Diptera). Parasitology 60, 327–373.
Schluter, D., 2000. The Ecology of Adaptive Radiation. Oxford University Press, New York.
Scott, H., 1917. Notes on Nycteribiidae with descriptions of two new genera. Parasitology 9, 593–610.
Shimodaira, H., Hashegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Molecular Biology and Evolution 16, 1114–1116.
Simmons, N.B., 2005. An Eocene big bang for bats. Science 307, 527–528.
Sorenson, M.D., 1999. TreeRot, Version 2. Boston University, Boston, Massachussetts.
Speiser, P.G.E., 1900. Ueber die Strebliden, Fledermausparasiten aus der Gruppe der pupiparen Dipteren. Archive der Naturgesellschaft 66, 31–70.
Statz, G., 1940. Neue Dipteren (Brachycera et Cyclorhapha) aus dem Oberoligozaen von Rott. Palaeontographica A 91, 120–174.
Swofford, D.L., Olsen, G., Waddell, P.J., Hillis, D.M., 1996. Phylogenetic inference. In: Hillis, D.M., Moritz, C., Mable, B.K. (Eds.), Molecular Systematics. Sinauer Associates Inc, Sunderland, pp. 407–514.
Swofford, D.L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony (* and Other Methods), Version 4.0b8. Sinauer Associates, Sunderland, Massachussetts.
Teeling, E.C., Madsen, O., Van Den Bussche, R.A., de Jong, W., Stanhope, M.J., Springer, M.S., 2002. Microbat paraphyly and the convergent evolution of a key innovation in Old World rhinolophoid microbats. Proceedings of the National Academy of Sciences 99, 1431–1436.
Teeling, E.C., Madsen, O., Murphy, W.J., Springer, M.S., O'Brien, S.J., 2003. Nuclear gene sequences confirm an ancient link between New Zealand's short-tailed bat and South American noctilionoid bats. Molecular Phylogenetics and Evolution 28, 308–319.
Teeling, E.C., Springer, M.S., Madsen, O., Bates, P., O'Brien, S.J., Murphy, W.J., 2005. A molecular phylogeny for bats illuminates biogeography and the fossil record. Science 307, 580–584.
Theodor, O., 1967. An Illustrated Catalogue of the Rothschild Collection of Nycteribiidae (Diptera) in the British Museum of Natural History. British Museum of Natural History, London.
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24, 4876–4882.
Wallace, A.R., 1876. The Geographical Distribution of Animals. Macmillan Press, London.
Wenzel, R., 1976. The streblid bat flies of Venezuela. Brigham Young University Science Bulletin 20, 1–177.
Wenzel, R., Tipton, V.J., 1966. Some relationships between mammal hosts and their ectoparasites. In: Wenzel, R., Tipton, V.J. (Eds.), Ectoparasites of Panama. Field Museum of Natural History, Chicago, pp. 677–723.
Wenzel, R., Tipton, V.J., Kiewlicz, A., 1966. The streblid bat flies of Panama. In: Wenzel, R., Tipton, V.J. (Eds.), Ectoparasites of Panama. Field Museum of Chicago, Chicago, pp. 405–667.
Whiting, M.F., 2002. Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. Zoologica Scripta 31, 3–17.
Wiens, J., 1998. Combining Datasets with different phylogenetic histories. Systematic Biology 47, 568–581.
Yeates, D.K., Wiegmann, B., 1999. Congruence and controversy: towards a higher-level phylogeny of Diptera. Annual Review of Entomology 44, 397–428.