International Journal of Primatology, Vol. 21, No. 5, 2000

Mitochondrial Sequences as Indicators of Generic Classification in Bush Babies

M. DelPero,[1] J. C. Masters,[2,4] D. Zuccon,[1] P. Cervella,[1] S. Crovella,[1] and G. Ardito[3]

Received April 22, 1999; accepted August 16, 1999

*Systematic relationships among the African bush babies are not well understood. Various generic designations are currently in use. Some authors refer all species to a single genus (*Galago*), while others recognize ≤4 genera. Phylogenetic reconstructions based on morphology, karyology, allozymes and vocal repertoires have generated inconsistent hypotheses of relationship. We analyzed partial sequences of three mitochondrial genes (270 bp from cytochrome* b, *387 bp from 12S rRNA, and 241 bp from 16S rRNA, total 898 bp) to resolve some uncertainties. We sampled taxa from each of three genera:* Galagoides alleni, G. demidoff *and* G. zanzibaricus; Galago senegalensis, G. gallarum *and* G. moholi; *and* Otolemur crassicaudatus *and* O. garnettii. *Outgroup taxa were Asian lorises:* Nycticebus coucang *and* Loris tardigradus. *We analyzed sequences separately and in combination, and modeled phylogenies using maximum parsimony, weighted parsimony, neighbor-joining and maximum-likelihood. We obtained some variation in phylogenetic inference depending on sequence and analytical method, but the results also gave strong phylogenetic signals. The lesser bush babies invariably formed a clade, showing evidence of very recent radiation. The greater bush babies also formed a clade, marked by somewhat greater interspecific genetic distances, which was allied with* Galagoides alleni *in most instances.* Galagoides demidoff *and* G. zanzibaricus *are not closely related, though both diverged early in the history of the group. A genus comprising*

[1]Dipartimento di Biologia Animale, Università di Torino, Via Accademia Albertina 17, 10123 Torino, Italy.
[2]Natal Museum, Private Bag 9070, Pietermaritzburg 3200, South Africa.
[3]Istituto di Antropologia, Università di Firenze, Via del Proconsolo 12, 50122 Firenze, Italy.
[4]To whom all correspondence should be addressed: Telephone: (27)-(33)-345-1404; Fax: (27)-(33)-345-0561; E-mail: jmasters@nmsa.org.za

0164-0291/00/1000-0889$19.50/0

Galagoides alleni, G. demidoff *and* G. zanzibaricus *is not supported by our data. The most likely alliance for* Galagoides alleni *is within the genus* Otolemur. *Of the three partial sequences employed in the study, 16S rRNA gave the most consistent results, while cytochrome* b *was least informative.*

KEY WORDS: galago; bush baby; mitochondrial genes; phylogeny; systematics.

INTRODUCTION

The Systematic Problem

The African bush babies, classified most consistently as a subfamily of the Loridae Gray, 1821 (= Lorisidae Gregory, 1915)—subfamily Galagonina Gray, 1825 (= Galaginae Mivart, 1864)—of the primate suborder Strepsirrhini, are among the most neglected groups of living primates. Their distribution in regions to which access is often difficult if not dangerous, and their shy, nocturnal habits have combined to obscure them from the notice of primatologists and systematists alike. For example, although Linnaeus included in his 10th edition a wide range of extant primate taxa, including cryptic animals like the slow loris, he was unaware of the existence of galagos.

Previous attempts at reconstructing galago phylogeny (de Boer, 1973; Olson, 1979; Eaglen, 1980; Schwartz and Tattersall, 1985; Zimmermann, 1990; Masters *et al.*, 1994) yielded conflicting hypotheses of relationships (Masters *et al.*, 1994). Taxonomy is inconsistently applied and appears to rest principally on two features: body size and the extent of prolongation of the premaxillae beyond the anterior margin of the lower jaw: essentially, the length of the rostrum. Thus in the most recent revision Nash *et al.* (1989, p. 59) advocated three genera to comprise 11 species of bush babies.

> *Galagoides*: dwarf galagos, and medium-sized galagos that show the same premaxillary elongation as found in their dwarf counterparts (including the species *alleni*, *demidoff*, *thomasi*, *zanzibaricus*)
>
> *Galago*: lesser galagos, with foreshortened muzzles (including *elegantulus*, *gallarum*, *matschiei*, *moholi*, *senegalensis*)
>
> *Otolemur*: greater galagos, with robust muzzles lacking premaxillary extension (including *crassicaudatus*, *garnettii*)

Taxonomic problems are exacerbated by questions of how many species are implied by the material in hand and how many still await discovery in the challenging African hinterland.

We obtained tissue samples from 8 species, representing all three genera: *Galagoides—G. alleni*, *G. demidoff* and *G. zanzibaricus;*

Galago—G. gallarum, *G. moholi* and *G. senegalensis*; and *Otolemur—O. crassicaudatus* and *O. garnettii*. We analyzed partial sequences from three mitochondrial genes, separately and in combination using several analytical techniques to investigate their potential to resolve uncertainties in galago systematics. The sequences were derived from two ribosomal genes, 16S and 12S rRNA, and a protein-coding gene, cytochrome *b* (cyt *b*). The genes and various sites within each gene are under different structural and functional constraints and thus will show very different patterns and rates of substitution (Mindell and Honeycutt, 1990; Hillis and Dixon, 1991; Meyer, 1994; Simon *et al.*, 1994). Previous studies have shown that sequences with relatively slow rates of change, such as the 12S rRNA gene, may be more appropriate for addressing deeper phylogenic questions, while genes with faster substitution rates, like cyt *b*, are suited to the resolution of relationships between more closely related species. Masters (1988, 1998) suggested that the extant radiation of bush babies is of a limited time depth, i.e., Plio-Pleistocene, with some very recent elements, e.g., the lesser bush baby radiation appears to have occurred within the last 1 Ma. Hence, we also sampled a highly variable portion of the 16S rRNA gene, which in some vertebrates has a mutation rate similar to the mitochondrial control region (Parker and Kornfield, 1996).

MATERIALS AND METHODS

Details on the 8 bush baby and 2 outgroup species included in the analysis are in Table I. The outgroup taxa are Asian lorises—*Loris* and *Nycticebus*—and generally are classified within the same family or superfamily as bush babies.

Table I. Species, tissue sampled, and source of specimens included in the analyses

Species	Tissue sampled	Source
Galagoides alleni	Fibroblasts	Museum d'Histoire Naturelle, Brunoy (France)
Otolemur crassicaudatus	Blood	Institut für Biologie III, Tübingen (Germany)
Galagoides demidoff	Muscle	Duke University Primate Center (USA)
Galago gallarum	Blood	Somalia
Otolemur garnettii	Blood	Institut für Biologie III, Tübingen (Germany)
Galago moholi	Blood	University of the Witwatersrand, Johannesburg (S. Africa)
Galago senegalensis	Blood	Stuttgart Primate Facility (Germany)
Galagoides zanzibaricus	Muscle	Zambezia Province, Mozambique
Nycticebus coucang	Muscle	Duke University Primate Center (USA)
Loris tardigradus	Muscle	Duke University Primate Center (USA)

We extracted total genomic DNA following the standard proteinase K/SDS/phenol/chloroform method (Sambrook *et al.,* 1989) and amplified a 270 bp fragment from cyt *b*, a fragment of around 400 bp from 12S rRNA, and a fragment of about 240 bp from 16S rRNA by PCR using the following pairs of primers: cyt *b* L14841 5′-CAACATCTCAGCATGATGAAA-3′ and H15149 5′-CTCAGAATGATATTTGTCCTCA-3′ (Kocher *et al.*, 1989); 12S L1091 5′-CTGGGATTAGATACCCCACTAT-3′ and H1478 5′-GAGGGTGACGGGCGGTGTGT-3 (Kocher *et al.*, 1989); 16S VF 5′-TACATAACACGAGAAGACC-3′ and VR 5′-GTGATTGCGCTGTTATCC-3′ (Parker and Kornfield, 1996). For the rRNA fragments, we aligned sequences using the CLUSTAL W 1.7 program (Thompson *et al.*, 1994), with default pairwise and multiple alignment parameters and adjusted the resulting alignment visually.

We employed diverse tree-building methods to investigate the effect of different analytical approaches on tree topologies, viz., equally weighted parsimony and weighted parsimony using the branch and bound search option of PAUP 3.1 (Swofford, 1993), neighbor-joining, based on Kimura genetic distances; and maximum-likelihood, using the Felsenstein model (F84) implemented in the DNAML program of the PHYLIP 3.5c package (Felsenstein, 1993). We evaluated the effect of the transition:transversion bias in the data sets using different weighting schemes. Character state weighting allows greater weight to be given to rare changes, which are less likely to be homoplastic, and hence to be more reflective of phylogenetic history. We down-weighted transitions relative to transversions by a factor of 2 (ts:tv = 1:2), 5 (ts:tv = 1:5), or 10 (ts:tv = 1:10), as this range of ratios encompassed the observed substitution bias in the data. To obtain a heuristic estimate of confidence levels in particular nodes, we assessed the maximum parsimony results by bootstrapping (Felsenstein, 1985, 1988; Hillis and Bull, 1993), using the heuristic search option with a random addition sequence (10 random addition replications per bootstrap replication) for 1000 replications. We evaluated robustness of the neighbor joining tree with 500 bootstrap replicate data sets using the BOOTSEQ, DNADIST, NEIGHBOR and CONSENSUS programs from the PHYLIP package.

RESULTS

The sequences used are in Appendix A and in GenBank (accession numbers: 12S AF212942-AF212951; 16S AF212952-AF212961; Cyt *b* AF212962-AF212971).

16S rRNA

The best 16S alignment is 241 bp long, with 217 unambiguous sites. The outgroup taxa, *Nycticebus coucang* and *Loris tardigradus*, have sequences of 239 and 237 bp respectively, while the same sequences in bush babies range from 220 to 223 bp. Of the 217 unambiguous sites, 94 are variable; 49 of them are informative.

The equally-weighted data set produced three most parsimonious trees (tree length 141, CI = 0.77, RI = 0.72) that differ only in the relative positions of *Galagoides alleni* and *G. zanzibaricus.* Bootstrap support is strongest (≥70%) for the lesser bush baby clade, and the greater bush baby clade. When we applied a series of weighting schemes to the data (ts:tv = 2:1, 5:1, 10:1, plus transversions only) we consistently obtained a single most parsimonious tree (Fig. 1a), which is identical in topology to one of the three equally-weighted trees. Neighbor-joining and maximum-likelihood analyses generated the topology in Fig. 1b, which corresponds to another of the three equally weighted trees.

12S rRNA

The length discrepancies in the 16S sequences between lorises and galagos are not found in the 12S third domain sequences. The best alignment

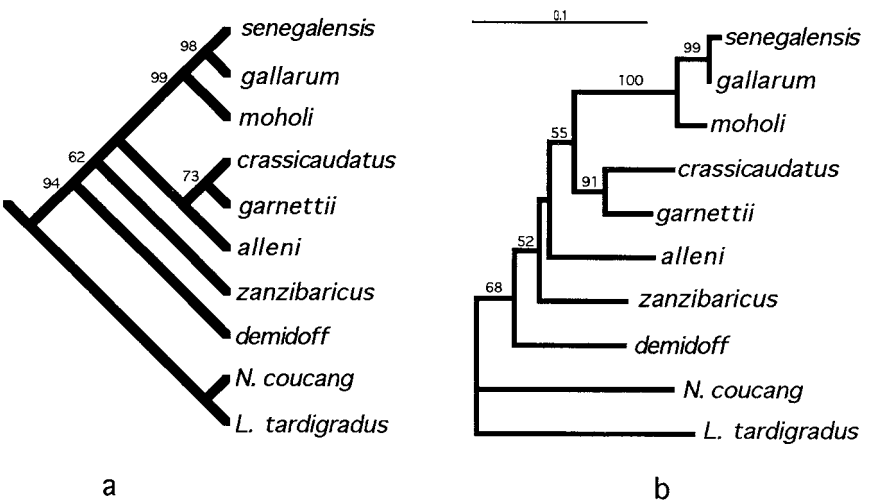


Fig. 1. Phylogenetic trees derived from the 16S rRNA analyses. (a) Most parsimonious tree obtained using different weighting schemes. (b) Neighbor-joining tree based on Kimura genetic distances. The same topology was also generated by maximum-likelihood analysis. Bootstrap values >50% are reported above internal branches.

is 387 bp long, and sequences range from 382 to 385 bp. We excluded 4 sites from the analysis because of alignment ambiguities. There are 92 variable positions, of which 54 are informative.

Parsimony analysis of the equally weighted data set yielded four most parsimonious trees (tree length 148, CI = 0.72, RI = 0.63), which are essentially unresolved, except for the lesser bush baby clade (bootstrap value = 100%). A weighting scheme of 2:1 resolved the earliest divergences on the tree (a branch of *Galagoides zanzibaricus* followed by a branch of *G. demidoff*), but left a polychotomy shared by *Galagoides alleni*, *Otolemur crassicaudatus*, *O. garnettii* and *Galago* spp. Weighting schemes of 5:1 and 10:1 resolved the tree (with bootstrap values <50%), apart from the relationships among the lesser bush baby species. This tree (Fig. 2a) is essentially the same as that derived from neighbor-joining. The maximum-likelihood tree has a similar topology, except that *Otolemur garnettii* and *O. crassicaudatus* do not form a clade (Fig. 2b).

Cytochrome *b*

Amplification of cyt *b* using the versatile primer pair of Kocher *et al.* (1989) was straightforward in all taxa but one: *Galagoides zanzibaricus*,

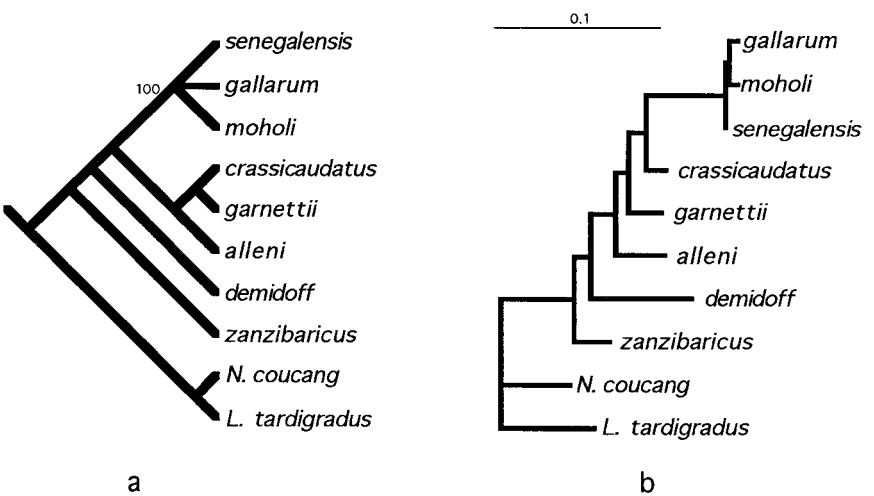


Fig. 2. Phylogenetic trees derived from the 12S rRNA analyses. (a) Consensus of the two most parsimonious trees obtained using weighted data (5:1 and 10:1). Bootstrap values >50% are reported above internal branches. Neighbor-joining yields the same topology. (b) Maximum-likelihood tree.

for which we repeatedly obtained a sequence containing a four-base insertion, i.e., a frameshift mutation, indicating that we were probably amplifying a nonfunctional nuclear pseudogene (Collura and Stewart, 1995). This problem was successfully bypassed using the primer L 14724 5′-CGAGATCTGAAAAACCATCGTTG-3′, which is directed toward the flanking tRNA (Collura and Stewart, 1995), together with the primer H15149.

Of the 270 bp analyzed, 101 were variable, 69 of which were informative. An equally weighted analysis yielded five maximally parsimonious trees (tree length 193, CI = 0.64, RI = 0.51), a strict consensus of which is unresolved, except for the lesser bush babies (bootstrap values ≥99%). A 50% majority rule consensus tree produced a *Galagoides zanzibaricus-Galago* clade, but the remaining taxa formed a star-burst clade. A 2:1 weighting scheme did not improve resolution (Fig. 3a). Weighting schemes of 5:1 and 10:1 gave identical results, as follows: *Galagoides demidoff* was the most basal divergence, followed by *G. alleni. Otolemur* spp. formed the sister taxon to a clade of *Galagoides zanzibaricus-Galago* (Fig. 3b). Omitting third positions all but drowned the phylogenetic signal. Neighbor-joining also generated a clade of *Galagoides zanzibaricus-Galago*, but it was sister to a clade of *Galagoides alleni-Otolemur*, with *Galagoides demidoff* as the most basal divergence (Fig. 3c). Maximum-likelihood gave a pectinate tree with little internal structure (Fig. 3d). Thus, our cyt *b* results varied dramatically depending on the mode of analysis. The only strong bootstrap support was for the lesser bush baby clade.

A Combined 16S-12s-cyt *b* Data Set

The equally weighted data set produced two most parsimonious trees (tree length 490, CI = 0.69, RI = 0.55), one of which has the same topology as Fig. 2a except that the relationships among the lesser bush babies are resolved (bootstrap values 97–100%). The same tree was obtained when we weighted the data 2:1, and used maximum-likelihood (Fig. 4a). In the second tree, *Galagoides zanzibaricus* and *G. demidoff* formed a clade that was sister to *Galago* spp., but the relationship disappeared when character weighting was applied. Weighting schemes of 5:1 and 10:1 and a transversions-only analysis yielded the same tree as in Fig. 4a, except that the branching order of *Galagoides demidoff* and *G. zanzibaricus* was reversed. Bootstrap values from these weighted analyses provided 65% support for the clade of *Otolemur crassicaudatus-O. garnettii*, in addition to 100% support for the clade of *Galago* spp. Neighbor-joining generated an identical topology (Fig. 4b) with strong bootstrap support for the clades of *Galago* (100%), *Otolemur* (87%), and *Galagoides alleni-Otolemur* (73%).

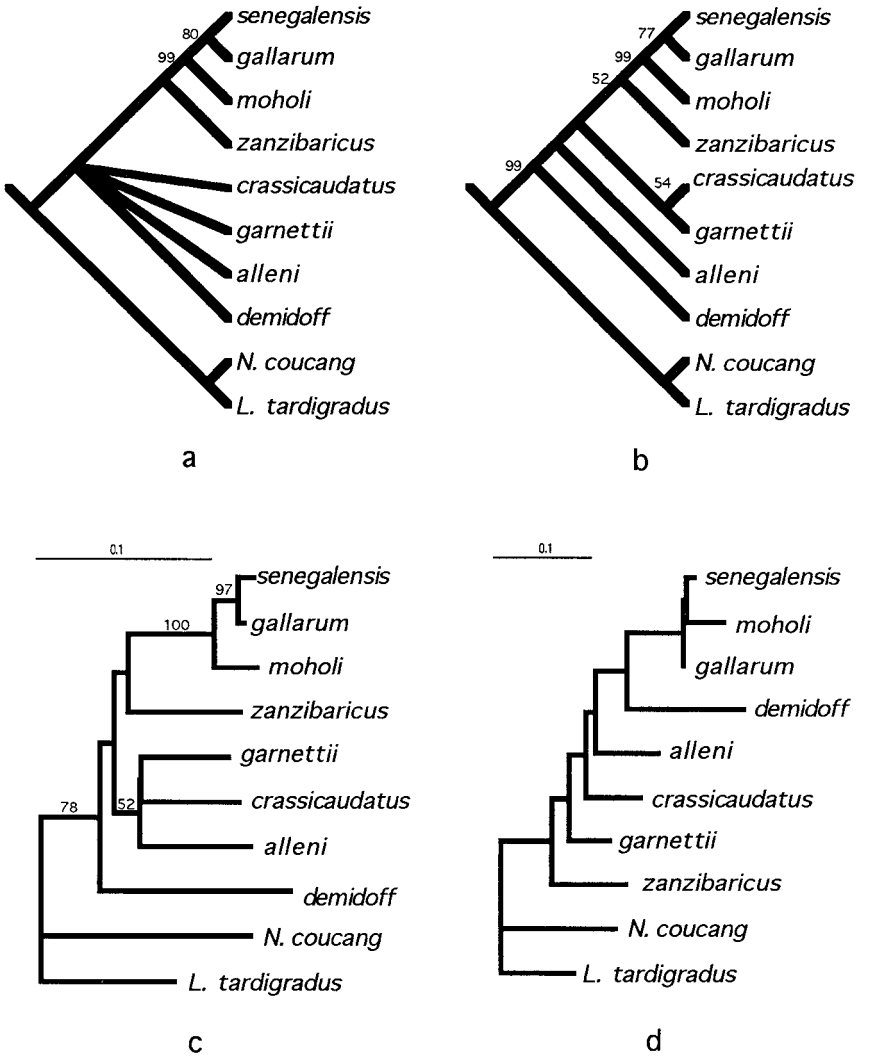


Fig. 3. Phylogenetic inferences from cytochrome *b* analyses. (a) Consensus of the two most parsimonious trees obtained using a 2:1 weighting scheme. (b) Most parsimonious tree resulting from a 10:1 weighting scheme. Bootstrap values >50% are reported above internal branches. (c) Neighbor-joining tree produced using Kimura genetic distances. (d) Maximum-likelihood tree.

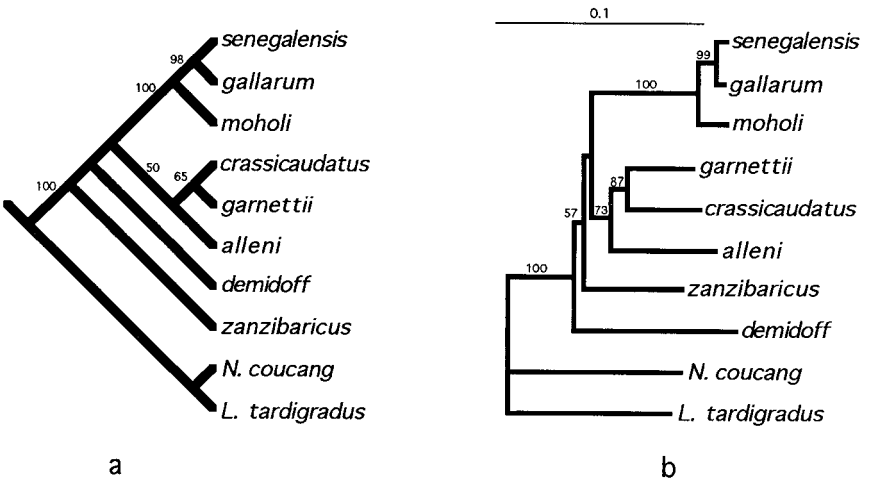


Fig. 4. Phylogenetic trees derived from the combined 16S-12S-cyt *b* data set. (a) Tree topology generated by equally weighted parsimony, 2:1 weighted parsimony and maximum-likelihood analyses. (b) Neighbor-joining tree using Kimura genetic distances; an identical topology was recovered for the most parsimonious tree resulting from 5:1, 10:1 and TV only weighted analyses. Bootstrap values >50% are reported above internal branches.

DISCUSSION

Phylogenetic Reconstruction

Our results showed some variability in phylogenetic inference, depending on the sequence and analytical method employed, but repeatedly indicate some phylogenetic relationships, which we discuss in the light of previous scenarios based on karyology (de Boer, 1973), morphology (Olson, 1979; Eaglen, 1980; Wesselman, 1984; Schwartz and Tattersall, 1985), vocal repertoires (Zimmermann, 1990), allozymes (Masters *et al.,* 1994; Masters, 1998), and highly repeated (hr) DNA sequences (Crovella *et al.,* 1994).

The Lesser Bush Baby Radiation Is Very Recent

The clade of lesser bush babies was supported throughout, indicating a high signal:noise ratio. Genetic distances between species range from <1% to 4.5% across species and genes. These results accord with a recent radiation for the group, as suggested by comparative karyological findings (de Boer, 1973), allozymes (Masters, 1998) and hrDNAs (Crovella *et al.,*

1994) which failed to distinguish *Galago moholi* and *G. senegalensis.* The majority of our analyses show *Galago senegalensis* and *G. gallarum* as sister taxa, with *G. moholi* most divergent. Olson (1979) obtained the same result with morphological data. However, Zimmermann's (1990) analysis of vocalizations failed to recover such a clade: in her reconstruction, *Galago senegalensis* is sister to a clade of *G. alleni-G. matschiei*, and *G. moholi* is sister to it.

The Greater Bush Babies Comprise a Clade, with Greater Intraclade Genetic Distances than Those Observed in Lesser Bush Babies

The greater bush baby clade is consistently supported, though genetic distances between the species range from 10.6% (cyt *b*) to 4% (12S). Karyological evidence supports this grouping, i.e, they are the only two species with 2n = 62 (de Boer, 1973), though their karyotypes differ by 7 pericentric inversions (Masters *et al.*, 1987). Morphological (Olson, 1979) and vocalization data (Zimmermann, 1990) also group the two species as a clade, which Nash *et al.* (1989) distinguished as genus *Otolemur.*

The Position of Galagoides alleni

Our analyses placed *Galagoides alleni* fairly consistently as the sister taxon to *Otolemur* (Figs. 1a, 2a, 3c, 4a, 4b), though bootstrap support for this node was generally low. The relationship was indicated previously by molar morphology: Wesselman (1984) described a 3.03 Ma fossil taxon, *Galago howelli,* from maxillary, mandibular and dental fragments that showed striking similarities to *Galagoides alleni,* on one hand, and *Otolemur* on the other. Schwartz and Tattersall (1985) proposed a clade of *Galagoides alleni-Otolemur* on the basis of dental synapomorphies in extant taxa. Allozymes (Masters *et al.*, 1994) and hr DNA sequences (Crovella *et al.*, 1994) grouped *Galagoides alleni* with the *Otolemur* spp. to the exclusion of *Galago* spp. However, other data indicate very different associations. Based on similarities in chromosomal structure and number (2n = 40 in *Galagoides alleni,* 2n = 38 in *Galago senegalensis* and *G. moholi*) de Boer (1973) argued for grouping *Galagoides alleni* with *Galago*, while Zimmermann's (1990) analysis of vocal repertoires also placed *G. alleni* among *Galago* spp., as sister to *Galago matschiei.* Morphological analyses made *Galagoides alleni* the sister to the dwarf galagos (Eaglen, 1980), or the dwarf galagos plus *G. zanzibaricus* (Olson, 1979). Based on Olson's analysis, Nash *et al.* (1989) defined the composition of genus *Galagoides.*

The Positions of Galagoides Zanzibaricus and G. Demidoff

The two most consistent topologies generated by our combined 12S-16S-cyt *b* data set (Fig. 4) differed only with respect to the divergence order of these two taxa. Both show large genetic distances from the other taxa, with values for *Galagoides demidoff* slightly higher (*G. zanzibaricus:* cyt *b* 13.3–15.9%, 12S 7.5–8.6%; *G. demidoff:* cyt *b* 17.0–19.76%, 12S 10.0–11.9%; 16S values were intermediate), and the distances between *G. demidoff* and *G. zanzibaricus* are of a similar order (cyt *b* 19.9%, 12S 8.9%). In fact, the ingroup taxon with the greatest cyt *b* and 12S distances from *Galagoides zanzibaricus* is *G. demidoff,* which is consistent with the interpretation that occasional groupings of the two taxa, e.g., in the combined 12S/16S/cyt *b* data set, equally weighted analyses only, are products of long-branch attraction (Felsenstein, 1978). Our data do not provide sufficient information to allow a choice between the phylogenies in Figs. 4a and 4b. The only conclusion we draw with confidence is that both taxa diverged early in the radiation of the group.

Galagoides zanzibaricus was traditionally placed in *Galago* and, apart from its original description (Matschie, 1893), most early taxonomists described it as a subspecies of *Galago senegalensis* (e.g., Schwarz, 1931; Hill, 1953). On the basis of chromosomal similarities (2n = 36), de Boer (1973) upheld this classification, though he pointed out structural differences between their karyotypes. Our data refute a close relationship between *Galagoides zanzibaricus* and *Galago* spp. Zimmermann's (1990) vocalization analysis placed *Galagoides zanzibaricus* as sister to *Otolemur,* which our data do not support.

Data from various sources attest to the early divergence of *Galagoides demidoff*: de Boer's (1973) karyological comparisons, Zimmermann's (1990) vocal analysis, and the fossil record. One of the oldest fossils that can be assigned tentatively to a living taxon, is a >3-Ma lower molar of *Galagoides demidoff* (Wesselman, 1984). Schwartz and Tattersall's (1985) craniodental analysis allied *Galagoides demidoff* with *Galago* spp. to the exclusion of a clade of *Galagoides alleni-Otolemur,* which is not supported by our data.

Taxonomic Implications

Our data imply the following:

1. A close phylogenetic relationship of *Galagoides alleni, G. demidoff* and *G. zanzibaricus* is not indicated by 12S rRNA, 16S rRNA or cyt *b* sequences.

2. The most likely generic allocation for *Galagoides alleni* is *Otolemur,* along with *O. crassicaudatus* and *O. garnettii.*
3. A close relationship between *Galagoides zanzibaricus* and *Galago* spp. is not supported, and the species does not belong in genus *Galago.*
4. A close relationship between *Galagoides zanzibaricus* and *G. demidoff* is not supported; associating them together in a single genus may be creating a wastebasket taxon of plesiomorphic species.

Comparing the Performances of cyt *b,* 12S and 16S rRNA

Comparing the resolving power of the ribosomal sequences, 16S gave greater resolution than 12S, both at deeper and shallower levels of divergence. The highest bootstrap values and the highest CIs were derived from 16S sequence data; the lowest values from cyt *b;* 16S and cyt *b* showed equivalent levels of sequence divergence among taxa, but 16S gave much more consistent resolution. The only strongly supported cyt *b* node is that of the lesser bush babies, a very recent radiation. The inconsistency of our cyt *b* results was unexpected, since the same gene has been used effectively in phylogenetic studies of the Malagasy lemurs (Yoder, 1994; DelPero, 1995, Yoder *et al.,* 1996), the sister group of bush babies and lorises. A 12S analysis of lemur phylogeny (DelPero *et al.,* in press) was less effective than cyt *b* at resolving closely related species, but showed more resolving power at the level of genera within families, or closely related families. Like cyt *b,* it did not resolve more distantly related families (>12% divergence in 12S sequences) with much consistency. Similar limitations were experienced by Ortí and Meyer (1997) in their 12S analysis of relationships among characiform fishes. McNiff and Allard (1998) found complete 12S sequences of doubtful usefulness in resolving the relationships among the Archonta, refuting the suggestion of Springer and Douzery (1996) that 12S is reliable to a time depth of 100 Mya.

Each gene clearly has a particular window of phylogenetic applicability, and it appears that the use of data combined from several genes with different windows—even if each gene is represented by a relatively short sequence—has the potential to reveal relationships at a variety of phylogenetic levels.

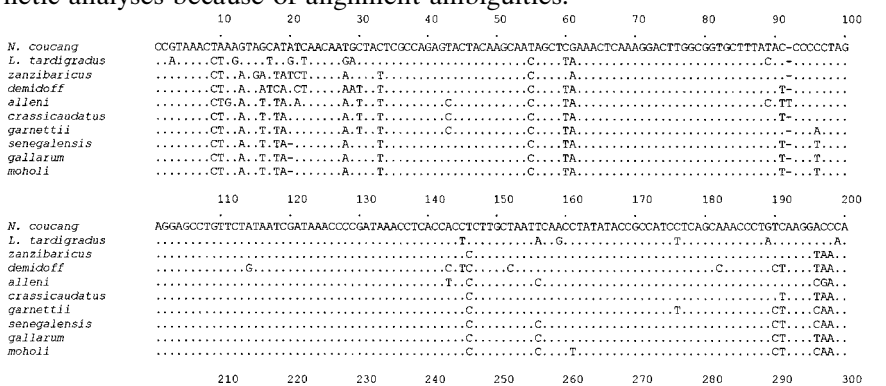
ACKNOWLEDGMENTS

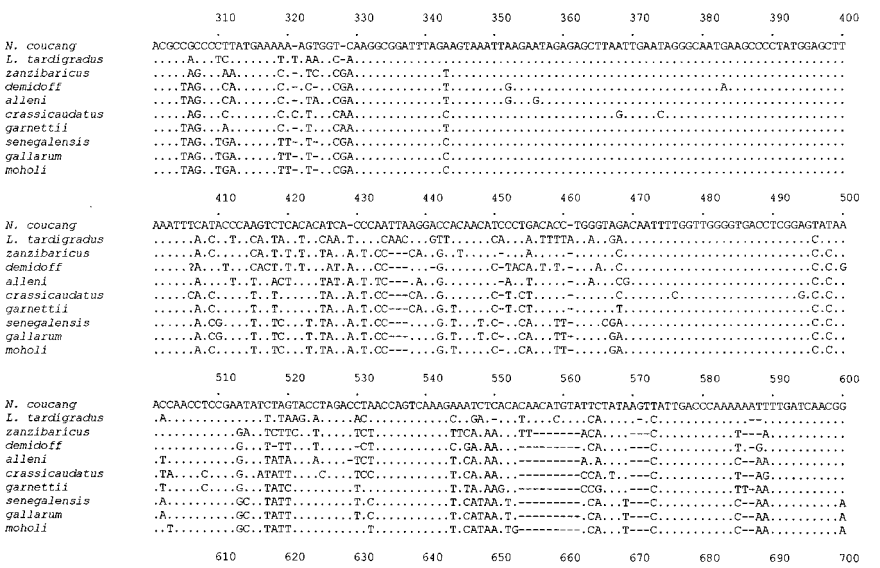
Several people assisted us in the collection of samples: Drs. M. J. Whiting and Chris Chimimba, Transvaal Museum, Pretoria; Prof. Dr. H.

Erkert and PD Dr. E. Müller, Universität Tübingen; Dr. David Haring, Duke University Primate Center; Dr. Steven Maeder, University of the Witwatersrand; Dr. David Reim, State University of New York at Stony Brook; Prof. Y. Rumpler, Université Louis Pasteur, Strasbourg; the U.S. Army; and Prof. Dr. Elke Zimmermann, Universität Stuttgart–Hohenheim. We thank also Dr. Jacopo Moggi-Cecchi for playing the role of the winged messenger. Two anonymous reviewers provided very helpful advice for improving the manuscript. JCM thanks Profs. Jack Stern, Brigitte Demes and Fred Grine, and Marianne Crisci for their various forms of support of the *G. gallarum* specimen and her during the tenure of both at Stony Brook. Funding for the project was provided by the FRD Science Liaison Centre (South Africa), CNR (Italy), MURST (Italy), and the Università di Torino (courtesy of Dr. Paola Bigatti), and is gratefully acknowledged.

APPENDIX A

Alignment of nucleotide sequences. Positions 1-387 correspond to the 12S rRNA sequence; 388-628 to 16S rRNA; and 629-898 to cytochrome *b*. Dots indicate nucleotide identity to the sequence of *Nycticebus coucang* in the top line. Sites 321-324, 554-572 and 585-589 were excluded from the phylogenetic analyses because of alignment ambiguities.





REFERENCES

Collura, R. V., and Stewart, C.-B. (1995). Insertions and duplications of mtDNA in the nuclear genomes of Old World monkeys and hominoids. *Nature* 378: 485–489.

Crovella, S., Masters, J. C., and Rumpler, Y. (1994). Highly repeated DNA sequences as phylogenetic markers among the Galaginae. *Am. J. Primatol.* 32: 177–185.

De Boer, L. E. M. (1973). Cytotaxonomy of the Lorisoidea (Primates: Prosimii). 1. Chromosome studies and karyological relationships in the *Galagidae. Genetica* 44: 155–193.

DelPero, M., Crovella, S., Cervella, P., Ardito, G., and Rumpler, Y. (1995). Phylogenetic relationships among Malagasy lemurs as revealed by mitochondrial DNA sequence analysis. *Primates* 36: 431–440.

DelPero, M., Masters, J. C., Cervella, P., Crovella, S., Ardito, G., and Rumpler, Y. (in press). Phylogenetic relationships among the Malagasy lemuriforms (Primates: Strepsirrhini) as indicated by sequence data from the 12S rRNA mitochondrial gene. *Zool. J. Linn. Soc.*

Eaglen, R. H. (1980). *The systematics of living Strepsirhini, with special reference to the Lemuridae.* Ph.D. thesis, Duke University, Durham, North Carolina.

Felsenstein, J. (1978). Cases in which parsimony or compatibility methods will be positively misleading. *Syst. Zool.* 27: 401–410.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783–791.

Felsenstein, J. (1988). Phylogenies from molecular sequences: Inference and reliability. *Annu. Rev. Genet.* 22: 521–565.

Felsenstein, J. (1993). *PHYLIP* (*Phylogeny Inference Package*), *Version 3.5c.* Department of Genetics, University of Washington, Seattle.

Hill, W. C. O. (1953). *Primates. Comparative Anatomy and Taxonomy, Vol. I. Strepsirhini.* Edinburgh University Press, Edinburgh.

Hillis, D. M., and Bull, J. J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst. Biol.* 42: 182–192.

Hillis, D. M., and Dixon, M. T. (1991). Ribosomal RNA: Molecular evolution and phylogenetic inference. *Q. Rev. Biol.* 66: 411–453.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X., and Wilson, A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA* 86: 6196–6200.

Masters, J. C. (1988). Speciation in the greater galagos (Prosimii: Galaginae): Review and synthesis. *Biol. J. Linn. Soc.* 34: 149–174.

Masters, J. C. (1998). Speciation in the lesser galagos. *Folia Primatol.* 69 (suppl. 1): 357–370.

Masters, J. C., Rayner, R. J., Ludewick, H., Zimmermann, E., Molez-Verrière, N., Vincent, F. and Nash, L. T. (1994). Phylogenetic relationships among the Galaginae as indicated by erythrocytic allozymes. *Primates* 35: 177–190.

Masters, J. C., Stanyon, R., and Romagno, D. (1987). Standardized karyotypes for the greater galagos, *Galago crassicaudatus* E. Geoffroy, 1812 and *G. garnettii* (Ogilby, 1838) (Primates: Prosimii). *Genetica* 75: 123–129.

Matschie, P. (1893). Über anscheinend neue Africanische Säugethiere. *Sber. Ges. Naturf. Freunde Berl.* 4: 107–114.

McNiff, B. E., and Allard, M. W. (1998). A test of Archonta monophyly and the phylogenetic utility of the mitochondrial gene 12S rRNA. *Am. J. Phys. Anthropol.* 107: 225–241.

Meyer, A. (1994). Shortcomings of the cytochrome *b* gene as a molecular marker. *Trends Ecol. Evol.* 9: 278–280.

Mindell, D. P., and Honeycutt, R. L. (1990). Ribosomal RNA in vertebrates: Evolution and phylogenetic implications. *Annu. Rev. Ecol. Syst.* 21: 541–566.

Nash, L. T., Bearder, S. K., and Olson, T. R. (1989). Synopsis of *Galago* species characters. *Int. J. Primatol.* 10: 57–80.

Olson, T. R. (1979). *Studies on aspects of the morphology of the genus Otolemur.* Ph.D. thesis, University of London, London.

Ortí, G., and Meyer, A. (1997). The radiation of characiform fishes and the limits of resolution of mitochondrial ribosomal sequences. *Syst. Biol.* 46: 75–100.

Parker, A., and Kornfield, I. (1996). An improved amplification and sequencing strategy for phylogenetic studies using the mitochondrial large subunit rRNA gene. *Genome* 39: 793–797.

Sambrook, J. E., Fritsch, E. F., and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Press, Cold Spring Harbor, New York.

Schwartz, J. H., and Tattersall, I. (1985). Evolutionary relationships of living lemurs and lorises (Mammalia, Primates) and their potential affinities with European Eocene Adapidae. *Anthropol. Pap. Am. Mus. Nat. Hist.* 60: 1–100.

Schwarz, E. (1931). On the African long-tailed lemurs or galagos. *Ann. Mag. Nat. Hist.* (ser. 10) 7: 41–66.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., and Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am.* 87: 651–701.

Springer, M. S., and Douzery, E. (1996). Secondary structure and patterns of evolution among mammalian mitochondrial 12S rRNA molecules. *J. Mol. Evol.* 43: 357–373.

Swofford, D. L. (1993). *Phylogenetic analysis using parsimony* (*PAUP*). Illinois Natural History Survey, Urbana, IL.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22: 4673–4680.

Wesselman, H. B. (1984). The Omo micromammals: Systematics and paleoecology of early man sites from Ethiopia. *Contrib. Vertebr. Evol.* 7: 1–219.

Yoder, A. D. (1994). Relative position of the Cheirogaleidae in strepsirhine phylogeny: A comparison of morphological and molecular methods and results. *Am. J. Phys. Anthropol.* 94: 25–46.

Yoder, A. D., Cartmill, M., Ruvolo, M., Smith, K., and Vilgalys, R. (1996). Ancient single origin for Malagasy primates. *Proc. Natl. Acad. Sci. USA* 93: 5122–5126.

Zimmermann, E. (1990). Differentiation of vocalizations in bushbabies (Galaginae, Prosimiae, Primates) and the significance for assessing phylogenetic relationships. *Z. Zool. Syst. Evolut.-forsch.* 28: 217–239.