tarsal (Mt-I) retained from a formerly five-toed foot. These primitive characteristics are found in the oldest known artiodactyls like early Eocene *Diacodexis* (*42*, *44*), but also in later anthracotheriid artiodactyls such as late Eocene-Oligocene *Bothriodon* from Europe ("*Hyopotamus*") (*45*), and *Elomeryx* and possibly *Heptacodon* from North America (*46*, *47*). If hippopotamids are derived from anthracotheres (*48*), then it appears plausible that hippopotami may be the closest living relatives of whales.

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33. Order Cetacea, Suborder Archaeoceti, Family Protocetidae. *Artiocetus clavis*, new genus and species. **Etymology**: *artios*, entire or even-numbered, and *ketos*, Gr., whale; *clavis*, L., key or clavicle; reflecting possession of shared characteristics of Artiodactyla and Cetacea, and alluding to both the key intermediacy of this taxon and retention of a rudimentary clavicle in the shoulder girdle. **Holotype**: GSP-UM 3458 (Geological Survey of Pakistan–University of Michigan collection, Quetta): virtually complete skull with much of the axial skeleton; parts of the shoulder girdle and forelimb including a rudimentary clavicle, scapula, distal radius and ulna; and parts of pelvic girdle and hind limb including an ilium, distal femur with a patella, and complete astragalus and cuboid. Eruption of all permanent teeth and fusion of most epiphyses shows that the specimen was fully mature. **Type locality**: Kunvit, Kohlu District, eastern Balochistan Province, Pakistan (30°05′44′N latitude, 69°47′20″E longitude). **Diagnosis**: Medium-sized protocetid archaeocete (estimated weight, 420 kg) with a skull distinctive in having anteriorly positioned nares, a relatively narrow frontal shield, and a relatively broad cranial base (*29*). Femoral shaft is roughly cylindrical. Astragalus differs from that in contemporary *Rodhocetus balochistanensis* in being smaller and relatively lower, with smaller ectal and fibular facets. **Description**: See supplementary material (*29*).
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35. Order Cetacea, Suborder Archaeoceti, Family Protocetidae. Genus *Rodhocetus* Gingerich et al. (*8*). *Rodhocetus balochistanensis*, new species. **Etymology**: *balochistanensis*, referring to provenence of type specimen. **Holotype**: GSP-UM 3485: braincase of skull with much of axial skeleton; parts of forelimb including distal humerus, radius and ulna, virtually complete carpus and manus; and parts of pelvic girdle and hind limb including acetabular rim of pelvis, femur, patellae, tibia, virtually complete tarsus and pes. Fusion of most epiphyses shows that the specimen was fully mature. **Type locality**: Kunvit, Kohlu District, eastern Balochistan Province, Pakistan (30°05′20″N latitude, 69°47′39″E longitude). **Diagnosis**: Smaller *Rodhocetus* [estimated body weight, 450 kg compared with 590 kg in *R. kasrani*; see (*49*)]. Anterior thoracic vertebrae average 13% smaller in linear dimensions (meaning *R. kasrani* is 16% larger), whereas the femur is slightly longer. Femoral shaft has a more distinct third trochanter but is otherwise similarly diamond-shaped in cross-section. Astragalus differs from that in contemporary *Artiocetus clavis* in being larger and relatively higher, with larger ectal and fibular facets. **Description**: See supplementary material (*29*).
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Rapid Diversification of a Species-Rich Genus of Neotropical Rain Forest Trees

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Species richness in the tropics has been attributed to the gradual accumulation of species over a long geological period in stable equatorial climates or, conversely, to speciation in response to late Tertiary geological events and unstable Pleistocene climates. DNA sequence data are consistent with recent diversification in *Inga*, a species-rich neotropical tree genus. We estimate that speciation was concentrated in the past 10 million years, with many species arising as recently as 2 million years ago. This coincides with the more recent major uplifts of the Andes, the bridging of the Isthmus of Panama, and Quaternary glacial cycles. *Inga* may be representative of other species-rich neotropical genera with rapid growth and reproduction, which contribute substantially to species numbers in the world's most diverse flora.

The neotropical flora comprises about 90,000 plant species—37% of the world's total and more than the floras of tropical Africa (35,000 spp.) and Asia (40,000 spp.) combined (*1*). Most of these species are found in rain forests, which have higher plant species diversity than any other habitat on the planet. How this diversity arose is unexplained (*2*, *3*). Early theories (the "museum model") suggested that a stable tropical climate allowed species to accumulate over time, with low rates of extinction in the absence of major environmental perturbations

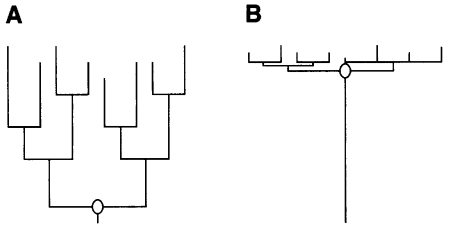

Fig. 1. (**A**) Gradual accumulation of diversity from the most recent common ancestor (MRCA, indicated by the circle) of the extant species, resulting in a well-resolved phylogeny. (**B**) Rapid and recent burst of diversification from the MRCA of the extant species, resulting in a poorly resolved phylogeny.

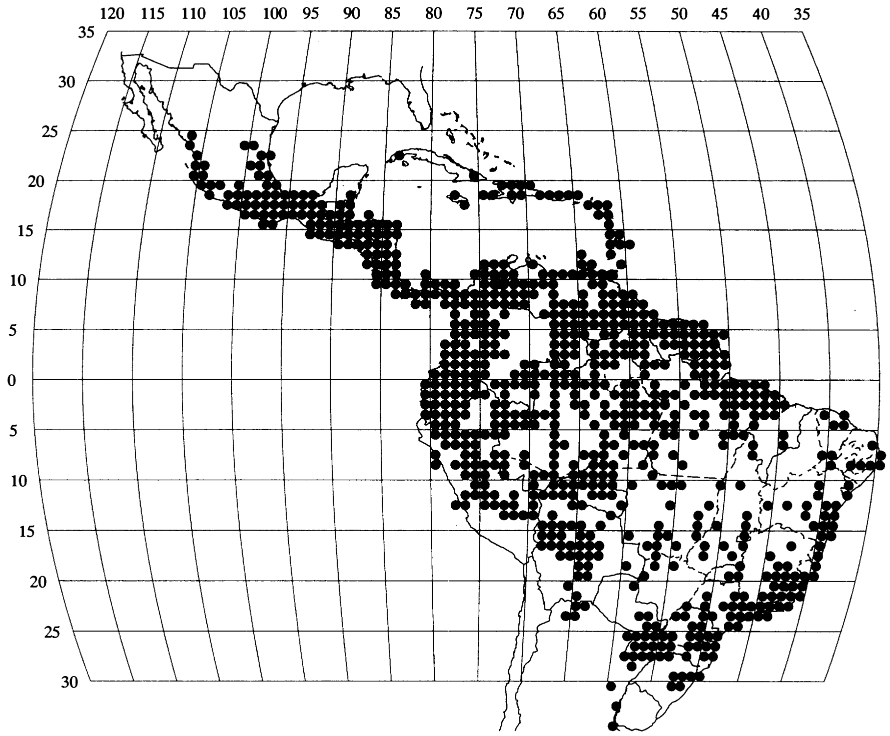

Fig. 2. Total distribution of all species of *Inga*.

(*4*). However, more recent discoveries suggest that neotropical climates were unstable over the past 2 million years (My) during the Pleistocene [summarized in (*5*)]. Cyclical glacial events led to periods of cooler and/or drier climate in which rain forest species may have withdrawn to small refugial pockets. This has led to suggestions that the present species diversity of the rain forests could be more recent, resulting from speciation through allopatric differentiation of populations in separate refugia [e.g., (*6*)]. Other recent geological phenomena that have been suggested as driving recent neotropical speciation are the uplift of the northwestern Andes from $\sim$5 million years ago (Ma) and the bridging of the Isthmus of Panama $\sim$3.5 Ma (*7*).

The Tertiary fossil record for the neotropics shows a diversity of angiosperm families similar to that of today's floras (*8*), but the record is not complete enough to assess species richness. Another potential way of distinguishing between the museum model and recent speciation is to use molecular phylogenies. If the present diversity has arisen by way of the museum model, there should be numerous nucleotide substitutions among species, and the tree shape should be consistent with a graded origin of species from their most recent common ancestor (MRCA) (Fig. 1A). In contrast, if the diversity has arisen recently, there should be few nucleotide substitutions differentiating species, and the tree should have short branch lengths between the MRCA node where diversification began and the branch tips (Fig. 1B).

We carried out a molecular phylogenetic investigation of *Inga* (Leguminosae-Mimosoideae), a genus of about 300 tree species growing in neotropical rain forests (Fig. 2). A recent taxonomic monograph (*9*) demonstrated that *Inga* species are clearly distinct by unique combinations of continuously varying characters. Forest inventories demonstrate that species-rich tree genera such as *Inga*, *Ocotea* (Lauraceae, 300 to 500 spp.), and *Eschweilera* (Lecythidaceae, 100 spp.) are often the most important component of the woody element in these forests, both in terms of biomass and species numbers [e.g., (*10*)]. Such genera are thus appropriate to test hypotheses of plant diversification patterns in neotropical rain forests.

Thirty-two species from throughout the geographic range and representing 7 of the 14 sections of *Inga* were sequenced for the internal transcribed spacers (ITS, including the 5.8*S* gene) of nuclear ribosomal DNA (*11*). Thirty-one species from throughout the geographic range and representing 10 of the 14 sections were sequenced for the plastid locus *trnL-F*. In total, we have sampled 45 *Inga* species (*12*). Parsimony analyses of the resulting sequence matrices (*12*) resulted in the phylogenetic trees shown in Fig. 3 (*13*). For both *trnL-F* and ITS, *Inga* forms a monophyletic group with respect to the outgroup taxa (other mimosoid legumes; the unique morphological feature of a fleshy seed coat also supports *Inga*'s monophyly). Key features of the phylogenetic trees from both genes are the small number of substitutions among *Inga* species, and the unresolved phylogenies, both of which are consistent with the recent speciation hypothesis.

We used a molecular clock approach to formalize estimates of divergence times among species. Table 1 is a summary of substitution rate estimates of ITS and *trnL-F* for a range of plant groups. On average, *Inga* species have 14 ITS substitutions from the MRCA *Inga* node to the tip of each branch. Ten out of the 11 ITS rate estimates in Table 1 fall between 1.72×10^{-9} and 7.83×10^{-9} substitutions per site per year (s/s/y). Using these rates, estimates of the time since the onset of diversification in *Inga* are between 13.4 and 2.0 Ma with a mean estimate of 5.9 Ma. The *trnL-F* rates indicate that diversification of *Inga* species began between 4.3 million and 300 thousand years ago, with a mean of 1.8 Ma. The rates calibrated from Winteraceae ITS are an order of magnitude different from the remaining estimates, indicating that *Inga* diversification began between 20.3 and 113.8 Ma. The Winteraceae estimate is unlikely to be accurate for *Inga*: (i) Winteraceae have a long generation time, which would result in a slower rate of accumulation of neutral mutations (*14*), whereas *Inga* species have an exceptionally fast generation time for trees. Forty-seven percent of *Inga* species (106) for which there are data are fast-growing and prefer open, disturbed sites. The remaining species that inhabit primary rain forest are mostly small- or medium-sized trees, and even canopy species such as *I. edulis* that reach 30 m are able to produce seed within 2 or 3 years of germination (*15*). (ii) The Winteraceae rate estimate itself may be inaccurate because it is based on an uncertainly dated geological event (the split of New Zealand and Australia) and data from an incomplete fossil record.

In the absence of a fossil record for *Inga*, we have obtained an independent estimate of substitution rates based on geological dating of the bridging of the Isthmus of Panama [$\sim$3.5 Ma

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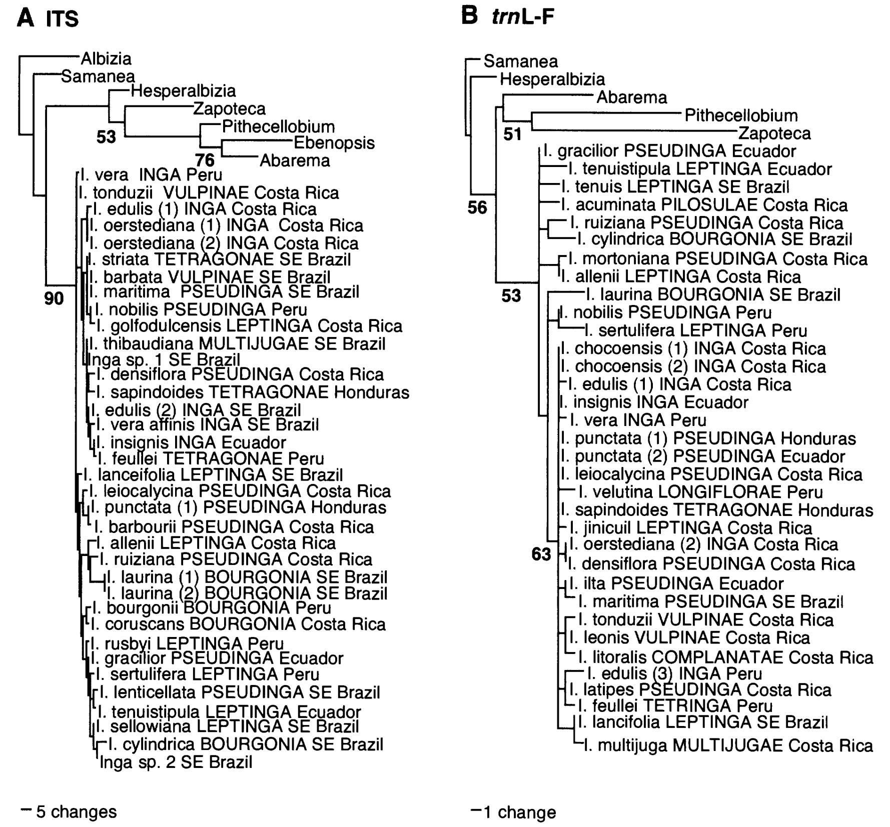

Fig. 3. (**A**) One of the most parsimonious trees from the ITS analysis. The ITS data matrix had 260 variable characters and 104 potentially informative characters out of a total of 609 characters, i.e., 17% of characters were variable in two or more taxa. Heuristic search produced over 5000 trees of 430 steps with consistency index (CI) = 0.74 (0.55 excluding autapomorphies) and retention index (RI) = 0.67. Bootstrap percentages are indicated below branches. (**B**) One of the most parsimonious trees from the *trnL-F* analysis. The *trnL-F* data matrix had 102 variable characters and 16 potentially informative characters out of a total of 1009 characters, i.e., 1.6% of characters were variable in two or more taxa. Heuristic search produced 4065 trees of 111 steps with CI = 0.94 (0.71 excluding autapomorphies) and RI = 0.86. Bootstrap percentages are indicated below branches.

(*16*)]. This may have been the earliest time that *Inga* species, which are dispersed by primates, migrated between South and Central America; before this date, the continents were separated by an oceanic barrier. If we assume that migration first occurred 3.5 Ma, we can estimate substitution rates by comparing numbers of nucleotide substitutions between related pairs of Central and South American species. For the ITS data, the endemic Central American species that is the most divergent from its nearest South American relative is *I. allenii*, with five substitutions from its MRCA. Translating this sequence divergence to a substitution rate based on 3.5-My divergence gives 2.34×10^{-9} s/s/y. For the *trnL-F* data, *I. mortoniana* is the most divergent endemic Central American species, with three substitutions from its MRCA (this gives a rate of 1.3×10^{-9} changes per site per year). If we apply these calibrations to the mean number of substitutions from the MRCA of all extant *Inga* species inferred from our phylogenetic trees, we obtain estimates for the onset of diversification of 9.8 Ma for ITS and 1.6 Ma for *trnL-F*. Both data sets were demonstrated to exhibit rate heterogeneity among lineages, so nonparametric rate smoothing (NPRS) trees were produced (*17–19*). Dates calibrated on the basis of these trees and sequence divergence between taxa on either side of the Isthmus of Panama gave dates for the onset of diversification of *Inga* of 3.5 (*trnL-F*) and 5.9 Ma (ITS).

The calibration methods for *Inga* are crude. First, it is possible that *Inga* species dispersed across the Panama Isthmus before it closed because 10 species (8 widespread species and 2 island endemics) are found on islands in the Caribbean. However, this represents only 3% of *Inga* species diversity. Such over-water dispersal events must therefore be rare, a fact reinforced by a maximum seed viability of only 1 to 2 weeks, which reduces to a few days if seeds are removed from the pod (*20*). Second, the application to *Inga* of rates drawn from unrelated taxa may be inappropriate, because different lineages with different life-history attributes may have different substitution rates. However, there is a marked consistency in the rates from different groups in Table 1. Any of the *trnL-F* estimates and all of the ITS estimates with the exception of Winteraceae suggest that the diversification of *Inga* occurred within the last 14 My. Most estimates place diversification in *Inga* within the last 10 My, which is reassuringly consistent with the geological calibration based on the bridging of the Isthmus of Panama. Each of the species included in this study is a representative of a larger group of closely related species, and it is unlikely that our sampling has failed to include more divergent, ancient lineages.

Pairwise comparisons from the ITS data indicate that 30% of the species sampled diverged from their closest relative within the last 2 My, implying a Pleistocene origin. Further support for such recent speciation comes from the two accessions of the widespread *I. edulis* that were collected from different parts of its range. Polymorphism in *trnL-F* and ITS sequences within this species are of a similar magnitude to that detected between species. In contrast, in other species where multiple accessions (e.g., *I. chocoensis*, *I. oerstediana*, and *I. laurina*) were collected from closely spaced localities, they have identical sequences. The variability within *I. edulis* mimics patterns of variation between species and is consistent with a lack of time since speciation for the accumulation of interspecific differences that are greater than intraspecific polymorphism.

Extensive introgressive hybridization could cause both homogenization of ribosomal DNA (rDNA) repeats and chloroplast capture [e.g., (*21*)], leading to the low ITS and *trnL-F* diversity found here. However, Koptur (*22*) demonstrated that seven sympatric species of *Inga* are not cross-compatible, and morphological intermediates between species are rare, which indicates that the extensive hybridization required by this scenario is highly improbable. It is also unlikely that concerted evolution of rDNA repeats and introgression of the chloroplast genome would be correlated across all species of such a large genus across such a wide geographic range.

Neotropical rain forests are characterized by species-rich genera such as *Inga*, whose species often grow sympatrically, and contribute disproportionately to the high diversity of Amazonia and other areas. For example, *Inga* contributes 19 species to a total of 307 that grow in 1 ha of rain forest in Cuyabeno, Ecuador (*23*), which is perhaps the most species-rich area of rain forest in the world (*24*). Patterns of morphological variation in *Inga*, where species differ in combinations of continuously varying characters, are common in species-rich tropical genera (*25*). Gentry (*26*) interpreted similar morphological patterns in species-rich neotropical shrub and herb genera such as *Piper* (Piperaceae) as consistent with explosive speciation. We have demonstrated this kind of rapid diversification in *Inga*, which may be more representative of these shrub and herb genera than it is of other species-rich tree genera because of its rapid generation time. Gentry speculated that nearly half the neotropical flora

Table 1. Estimated timing of divergence from the MRCA of all *Inga* species based on the average number of substitutions from the onset of diversification of *Inga* according to molecular clocks calibrated from a range of other taxa. The calibrated rates for *Saxifraga* and *Aichryson* were estimated by us from data in the original publications. Entries in bold are from the present study. NA, not applicable; cp, chloroplast.

Rate source	Habit/estimated minimum generation time	Genomic region	Calibrated rate (s/s/y)	Average divergence time (My)
Aichryson, Crassulaceae (*29*)	Annual or perennial herbs/1–2 years	ITS2	5.69×10^{-9}	2.0
Astragalus, Leguminosae (*30*)	Annual or perennial herbs/1–2 years	ITS	3.5×10^{-9}	6.6
Dendroseris, Compositae (*31*)	Shrubs/ ~4 years	ITS	3.94×10^{-9} to 6.06×10^{-9}	5.8 to 3.8
Gossypium, Malvaceae (*32*)	Perennial shrubs/~1–3 years	ITS1	5.0×10^{-9} to 9.0×10^{-9}	7.3 to 4.0
Gossypium, Malvacae	Perennial shrubs/~1–3 years	ITS2	2.5×10^{-9} to 4.5×10^{-9}	4.6 to 2.6
***Inga*, Leguminosae**	**Trees and shrubs/2–3 years**	**ITS**	$\mathbf{2.34 \times 10^{-9}}$	**9.8**
Lupinus, Leguminosae (*33*)	Annual or perennial herbs, occasionally shrubby/1–2 years	ITS1/ITS2	$3.6 \times 10^{-9}/3.3 \times 10^{-9}$	10.1/3.5
Phylica, Rhamnaceae (*34*)	Trees and shrubs/~2–3 years	ITS	2.44×10^{-9}	9.4
Silverswords, Compositae (*35*)	Woody perennials/~2–3 years	ITS	3.00×10^{-9}	7.9
Saxifraga, Saxifragaceae (*36*)	Annual or perennial herbs/1–2 years	ITS	1.72×10^{-9}	13.4
Robinsonia, Compositae (*37*)	Woody pachycaul/~2–3 years	ITS	7.83×10^{-9}	2.8
Winteraceae (*38*)	Trees and shrubs/~6–10 years	ITS1	3.2×10^{-10} to 5.2×10^{-10}	113.8 to 70.1
Winteraceae	Trees and shrubs/~6–10 years	ITS2	3.6×10^{-10} to 5.7×10^{-10}	32.1 to 20.3
Aichryson, Crassulaceae	Annual or perennial/1–2 years	*trnL-F* intergenic spacer	8.24×10^{-9}	0.3
***Inga*, Leguminosae**	**Trees and shrubs/2–3 years**	***trnL-F***	$\mathbf{1.30 \times 10^{-9}}$	**1.6**
Phylica, Rhamnaceae,	Trees and shrubs/~2–3 years	*trnL-F*	4.87×10^{-10}	4.3
Wolfe *et al.*, 1987 (*39*)	NA	cp upper limit	3.00×10^{-9}	0.7
Zurawski *et al.*, 1984 (*40*)	NA	cp generally	1.00×10^{-9}	2.1

might be accounted for by explosive speciation. Our data provide strong evidence that in the case of *Inga*, the neotropics are an active laboratory of speciation and suggest that a substantial proportion of species diversity in Amazonia may have arisen recently. This diversification may have been promoted by the later phases of Andean orogeny (the largest center of diversity for *Inga* is in the Andean foothills of western Amazonia), the bridging of the Panama Isthmus, and Quaternary climatic fluctuations.

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11. Multiple divergent copies of ITS were amplified from some individuals. ITS copies that were identified as pseudogenes, on the basis of their unstable secondary structure, high substitution rates, occurrence of insertions or deletions (in the 5.8S coding regions), and high number of deamination-type substitutions, were excluded from our analyses.
12. Supplementary Web material is available on *Science* Online at www.sciencemag.org/cgi/content/full/293/5538/2242/DC1.
13. Data were analyzed with the parsimony algorithm of the software package PAUP* for Macintosh (*27*). Searches were conducted on the separate ITS and *trnL-F* data sets. Tree searches were conducted under the equal and unordered weights criterion (Fitch parsimony) with 1000 random sequence additions and TBR (tree bisection-reconnection) swapping, but permitting only five trees to be held at each step, which was implemented to reduce time spent searching on suboptimal trees. All shortest trees collected in the 1000 replicates were then used as starting trees for another round of heuristic search, and all these trees were swapped on to completion or swapped on until 5000 trees were produced, at which point we limited the number of trees and swapped on the 5000 trees collected. As a guide to the support offered to various monophyletic groups by the data, bootstrap values were calculated with 1000 replicates.
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17. The ITS and *trnL-F* data sets were tested for rate heterogeneity. Branch lengths were fitted to one of the trees derived from each of the parsimony analyses with a three-parameter maximum likelihood (ML) model with transition-transversion ratio and a gamma distribution of rate variation among sites. The hypothesis of rate constancy was evaluated with a likelihood ratio test (*28*) that is twice the difference in log likelihood of branch lengths between a rate-constrained tree (forcing the molecular clock in PAUP*) and a tree that has no constraints on branch lengths. These log likelihoods were significantly different for both data sets (ITS: 3020.5 versus 2943.6; $P < 0.005$; *trnL-F*: 2114.0 versus 2078.4; $P < 0.01$) so the constrained hypothesis was rejected as significantly worse than the unconstrained hypothesis. Because of this rate heterogeneity, ultrametric trees were produced from the unconstrained ML branch lengths by using the NPRS method in TreeEdit version 1.0 alpha 4-61.
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