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ORIGIN AND RAPID DIVERSIFICATION OF A TROPICAL MOSS

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Abstract.—Molecular sequences rarely evolve at a constant rate. Yet, even in instances where a clock can be assumed or approximated for a particular set of sequences, fossils or clear patterns of vicariance are rarely available to calibrate the clock. Thus, obtaining absolute timing for diversification of natural lineages can prove difficult. Unfortunately, without absolute time we cannot develop a complete understanding of important evolutionary processes, including adaptive radiations and key innovations. In the present study, the coding sequence of the nuclear gene, glyceraldehyde 3-phosphate dehydrogenase (*gpd*), extracted from the paleotropical moss, *Mitthyridium*, was found to exhibit clocklike behavior and used to reconstruct the history of 80 distinct molecular lineages that cover the full geographic range of *Mitthyridium*. Two separate clades endemic to two geographically distinct oceanic archipelagos were revealed by this phylogenetic analysis. This allowed the use of island age (as derived from potassium-argon dating) as a maximum age of origin of each monophyletic group, providing two independent time anchors for the clock found in *gpd*, the final piece needed to study absolute time. Based on results from both maximum age calibrations, which separately yielded highly consistent estimates, the ancestor of this moss group arose approximately 8 million years ago, and then diversified at the rapid rate of 0.56 ± 0.004 new lineages per million years. Such a rate is on par with the highest diversification rates reported in the literature including rapidly radiating insular groups like the Hawaiian silversword alliance, a classic example of an adaptive radiation. Using independent sources of data, it was found that neither the age nor diversification estimates were affected by the use of molecular lineages rather than species as the operational taxonomic units. Identifying the cause for this rapid diversification requires further testing, but it appears to be related to a general shift in reproductive strategy from sexual to asexual, which may be a key innovation for this young group.

Key words.—Adaptive radiation, island endemism, key innovation, *Mitthyridium*, molecular clock, mosses.

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Molecular clocks that tick away at a constant rate are rarely found in molecular sequences. Still, the instances where they have been found have proven instrumental to improving our understanding of processes behind organismal diversity (Hulbert 1993; Baldwin and Sanderson 1998). Despite the infrequency of finding clocklike evolution in molecular sequences, the benefits of understanding the timing of diversification have led researchers to use rate smoothing or other statistical approximations of a molecular clock rather than avoid addressing important questions like the age of eukaryotes (Doolittle et al. 1996; Feng et al. 1997), the origin of angiosperms (Sanderson and Doyle 2001; Davies et al. 2004), or the origin of HIV (Korber et al. 2000). Recently, the lack of clocklike behavior in molecular data is becoming less of a problem because statistical and theoretical progress now allows estimations of divergence times and rates in the absence of evolutionary rate constancy (Sanderson 1997, 2002; Thorne et al. 1998; Yoder and Yang 2000). These new methods make possible the addition of more molecular data and more taxa, which otherwise would cause a deviation from a strict clock, to the question of evolutionary origins. The additional data tend to shrink confidence intervals, making dates more useful to understanding evolutionary processes. The ability to date origins of lineages is critical to establishing absolute rates of diversification and to making quantitative comparisons among different clades that otherwise would be incomparable. Dating phylogenetic nodes and determining rates of lineage proliferation has allowed tests of viral transmission history (Korber et al. 2000), as well as estimations of population parameters (including demographics), effective population size, and gene flow (Thomson et al. 2000). Information on ages and rates of diversification of lineages also have allowed direct ties between diversification and ecology (Baldwin 1997; Baldwin and Sanderson 1998; Hulbert 1993; Sturmbauer et al. 2001; Verheyen et al. 2003) as well as ties between key innovations and their role in effecting rapid diversification (Zietara and Lumme 2002). Such information has allowed better characterization of evolutionary processes in numerous plant and animal groups including *Phylica* (Richardson et al. 2001a), *Inga* (Richardson et al. 2001a), ice plant (Klak et al. 2004), peacock irises (Goldblatt et al. 2002), and cichlid fish (Verheyen et al. 2003). As more knowledge about dating origins accumulates, our understanding of important processes responsible for organismal diversity, such as adaptive radiations and key innovations, should improve dramatically.

Regardless of whether a clock can be assumed or is approximated, the dates assigned to nodes, and consequently the absolute rates of diversification, can be erroneous if the calibrations used are either too ancient or too recent (Yoder and Yang 2000). This is especially true for tropical plant groups distributed primarily on islands, where fossils are virtually nonexistent. In such cases, potassium-argon dates may be the only source for calibrations, but these dates have been considered of minimal value for placing upper limits on ages of lineages in hot-spot archipelagos like Hawaii (Baldwin and Sanderson 1998). In general, an insular group under study might be as old as the oldest islands of the archipelago (including submarine islands), or even older if it arose on the mainland, potentially rendering the maximum-age calibration too ancient to be of use for dating recent origins. However, barring extinction, if it can be shown that a clade has formed from a most recent common ancestor as endemic to a datable

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volcanic island, the age of the island may serve as the maximum age of the divergence of the endemic clade from its sister. This island endemic method of calibration assumes that the phylogeny is complete and known without error, but rarely is an evolutionary question not laden by such assumptions.

Using islands to date the divergence of endemic lineages has been done before (Richardson et al. 2001b) and is explored here using *Mitthyridium* and a nuclear marker, glyceraldehyde 3-phosphate dehydrogenase (*gpd*). This gene has been used in angiosperm phylogeography (Olsen and Schaal 1999) and for phylogeny reconstruction in mosses (Wall 2002), where it was initially found to evolve rapidly and at a constant rate, suggesting its use for dating the origin of recently diverged clades and for estimating absolute rates of diversification. In the current paper, the clocklike behavior of this nuclear gene is tested further using sequences from a large number of specimens of *Mitthyridium*.

Mitthyridium is monophyletic and found in the paleotropics, from western Africa to Tahiti. It can be distinguished morphologically from its closest relatives in the larger clade, Calymperaceae, by its leaf margins with multiple rows of clear, dead, elongated cells, and also by its spreading style of growth and lateral sporophyte insertion (cladocarpy). *Mitthyridium* is dioecious, bearing male and female gametangia on separate plants, and has a range of reproductive strategies, from purely vegetative, to asexual and bearing leaf propagules (gemmae), to fully sexual and producing diploid sporophytes. *Mitthyridium* is a reportedly young clade in the process of diversification—an assessment based mostly on its narrow geographic distribution and the lack of clear morphological distinctions among putative members (Reese et al. 1986). Yet, a counter claim suggests that mosses in general lack evolutionary potential, are effectively living fossils, and are thus highly unlikely to have radiated rapidly (Crum 2001). Studies of the liverwort *Conocephalum* uncovered cryptic lineages (Odrzykoski and Szweykowski 1991; Ki et al. 2001); cryptic molecular lineages have also been detected in pleurocarpous mosses (Shaw et al. 2003). Whether *Mitthyridium* can also refute the claim that mosses are evolutionarily stagnant is questioned here.

Methods

DNA Sequencing and Treatment of Operational Taxonomic Units

The single-copy nuclear gene *gpd* was sequenced from 112 samples of *Mitthyridium* from field and herbarium collections (GenBank accessions found at http://ucjeps.berkeley.edu/bryolab/students/dpwall/mono/introduction/table.shtml). This gene was shown previously (Wall 2002) to be valuable for phylogeny reconstruction and rich in informative characters including seven indels that yielded a high consistency index in the previous study (CI = 1.0) and in this one (CI = 0.67), suggesting small rates of homoplasy. Eighty of the 112 specimens samples were variable enough to be informative for phylogeny reconstruction; these encompass the complete geographic range known to be inhabited by *Mitthyridium*. Of these, 26 specimens were analyzed in detail in a previous study (Wall 2002); these included representatives from all previously characterized species of this poorly studied group, except *Mitthyridium megamorphum*, specimens of which did not yield usable DNA, and are shown in bold in Figure 1. This 26-lineage subset differs from the 80-lineage compartment in that all 26 lineages could be delimited by four separate and largely congruent lines of evidence: *gpd* (Wall 2002), morphology (Wall 2003), and two chloroplast sequences, *rps*4 and *trn*L (Wall 2002). However, not all of the 80 molecular lineages described here could be delimited by all lines of evidence (for further details see Wall 2003). The 26-lineage subset was used variously in the present study to test the existence of sampling biases in estimates of both age and diversification rate (described in more detail below).

In the present analysis, no attempt was made to assign Linnean ranks to clades in the reconstructed phylogeny. Instead, a rankless and purely phylogenetic approach to taxonomy, and consequently to estimates of the age and rate of diversification was taken. The vocabulary used here, clade (monophyletic and made up of lineages) and lineage (an ancestor-descendant sequence), transcend boundaries of traditional Linnean ranks and can be applied anywhere in a phylogenetic hierarchy. Each lineage was assigned a clade name, based on a phylogenetic taxonomy described in detail elsewhere (Wall 2003). A previous paper established the best outgroup candidate for *Mitthyridium*: *Syrrhopodon mahensis* (Wall 2002). This taxon was used as the only outgroup in all subsequent analyses. Inclusion of additional outgroups either singly or in combination did not affect the results reported (results not shown).

Two *gpd* primers that span a region of the gene composed of approximately 1000 bp were used. The region amplified spans four introns and three exons. The introns were found to be especially rich in indels and a useful source of variation for reconstructing relationships (Wall 2002). DNA extraction, amplification, and sequencing were done using the protocols described in Wall (2002). A single nexus file was assembled by manual alignment of *gpd* sequences. A character set was included to allow for easy removal of the introns, leaving 549 bp encoding 183 codons (Wall 2002). This allowed the division of *gpd* into three partitions: (1) full partition (includes introns and exons), (2) exon partition, and (3) intron partition.

Phylogenetic Methods

A series of likelihood analyses and likelihood ratio tests on progressively more complicated models was conducted to determine the model of sequence evolution that maximized the likelihood of the data for the 80 distinct *gpd* sequences. The most complicated model tested—HKY85 with rate heterogeneity among nucleotide positions approximated according to a Γ distribution—explained the data best and was used in all subsequent likelihood-based phylogenetic analyses. Phylogenetic analyses were run in PAUP* 4.0 (Swofford 2001) with the heuristic search setting. Starting trees were obtained via random stepwise addition and branch swapping was performed via tree bisection-reconnection (TBR). At least 100 replicate searches were conducted for all analyses. Clade support was assessed with the bootstrap; 10,000 replicates were run with ''fast'' stepwise addition. Shimodaira-

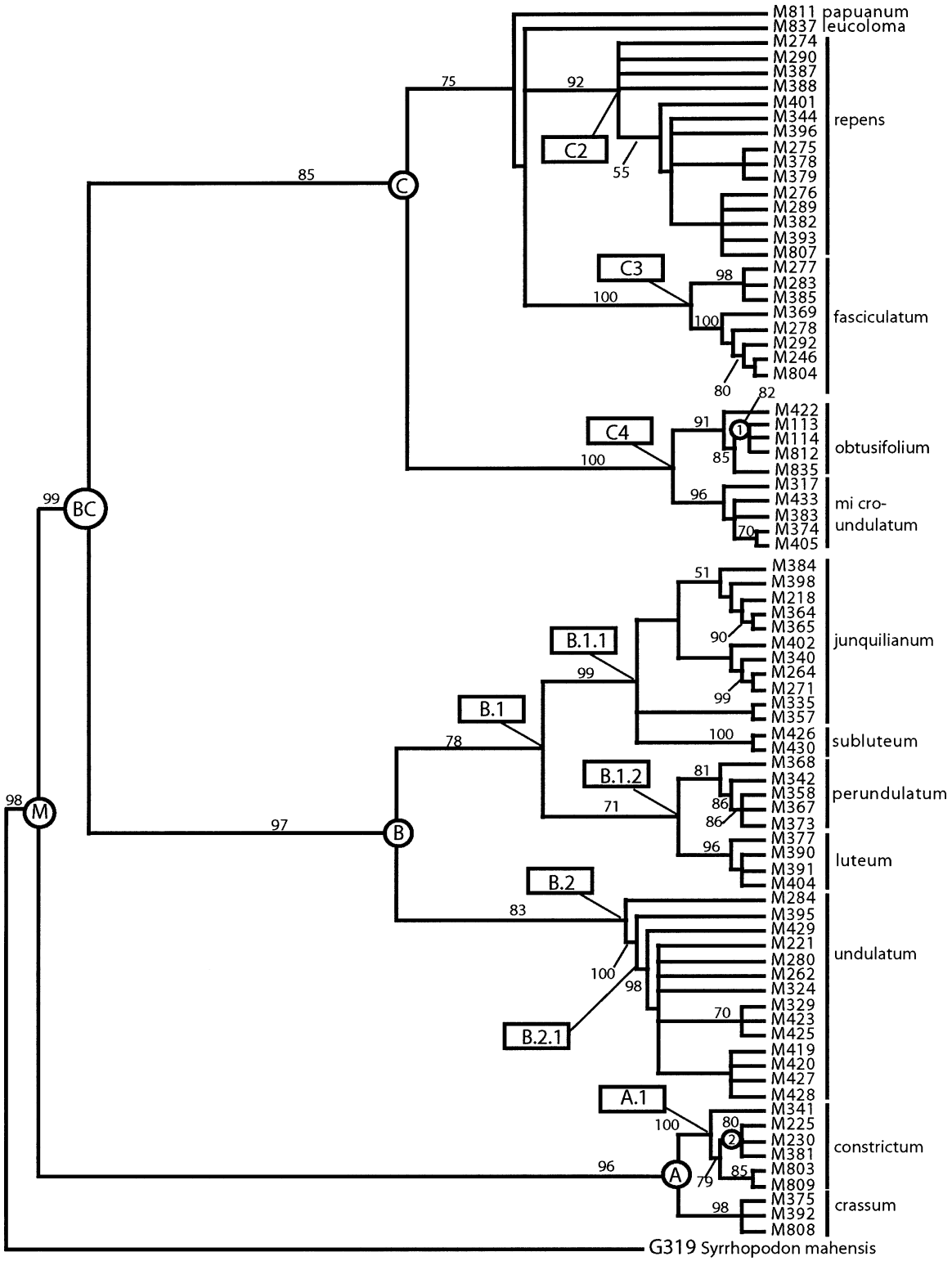

FIG. 1. Maximum likelihood phylogeny of *Mitthyridium* using the HKY85 + Γ model of sequence evolution. The names assigned to lineages are based on a more detailed phylogenetic taxonomy reported elsewhere (Wall 2003). The nodes are labeled with circled or boxed letters for discussion in the body of the manuscript. M, ancestral node of all *Mitthyridium* lineages. The circled, numbered nodes correspond to the (1) Mo'orea calibration (1.8 mya) and (2) Samoan calibration (2.9 mya). The 26 taxa in bold represent the 26-lineage subset analyzed in the present manuscript and in a previous study (Wall 2002).

Hasegawa (SH) tests (Shimodaira and Hasegawa 1999) were used to examine the congruence of the exon and intron partitions of *gpd*; this test may be biased against rejecting the null hypothesis of congruence (Goldman et al. 2000). Three tests were conducted. In the first two, the likelihoods of the exon and intron data were maximized, respectively, given each of three trees: exon derived, intron derived, and the full *gpd* derived. In the third, the likelihood of the full *gpd* data partition was maximized given each of the trees separately. The SH tests were performed using the bootstrap with full optimization and 1000 replicates. In each case the trees were found to be statistically indistinguishable ($P = 0.497$, $P = 0.233$, and $P = 0.396$), though the tree with the consistently highest likelihood score was derived from the full *gpd* data partition. This tree was used in subsequent tests of evolutionary rate constancy.

Tests for a Molecular Clock

A previous study (Wall 2002) could not reject a molecular clock hypothesis for exonic *gpd* sequences in 26 lineages of *Mitthyridium*. In extension, a likelihood ratio test was used to test the molecular clock hypothesis (Felsenstein 1988; Huelsenbeck and Rannala 1997; Muse 2000) among the complete set of 80 *gpd* lineages. The null hypothesis (L_0) is that the molecular clock holds and the likelihood is maximized under the constraint of equal rates across lineages. The alternative hypothesis (L_1) relaxes the clock constraint by assigning a different rate to each lineage and maximizing the likelihood. The likelihood ratio test statistic (the negative of twice the difference between the log likelihood values obtained under the null and alternative hypotheses) is approximately χ^2 distributed with degrees of freedom equal to the difference in the number of parameters (Huelsenbeck and Rannala 1997).

The tree in Figure 1 was used to test the clocklike behavior of *gpd*. This tree was derived from all *gpd* data, but, as described above, was found to be statistically indistinguishable from the trees derived from the exons and introns alone. Degrees of freedom for the test of rate constancy across lineages are equal to the difference between the number of parameters in the rate-constant and rate-variable models. In the rate-constant model for the 80 taxa examined there were 54 internal node ages and one rate parameter (55 parameters), and in the rate-variable model there was one parameter for each branch length on the unrooted topology (158 parameters), leaving 103 degrees of freedom. Tests of evolutionary rate constancy were conducted with and without the introns of the *gpd* data partition.

Divergence Time Estimation

After testing the data for conformity to a molecular clock, maximum likelihood estimates of divergence times were obtained using the program r8s (Sanderson 2003). Standard errors of the estimated divergence times were obtained using the procedure described in Baldwin and Sanderson (1998). The outgroup, *Syrrhopodon mahensis*, was used to determine the appropriate rooting of the ingroup tree, but removed prior to divergence time calculations. One hundred bootstrap matrices were generated by SEQBOOT in PHYLIP 3.6 (Felsenstein 2001), which were imported into PAUP* to obtain branch lengths for the tree depicted in Figure 1 (minus the outgroup) assuming the HKY85+ Γ model of sequence evolution and a molecular clock. The collective tree file was then processed using r8s to obtain nodal age estimates and confidence intervals. To account for effects of phylogenetic uncertainty on age estimates, the procedure above was repeated using trees generated from 25 bootstrap matrices of the *gpd* exon data and the Mo'orea calibration described below.

Time Calibration

Fossils of *Mitthyridium* are unknown. Thus, an alternative calibration point was needed. The areas of highest diversity of *Mitthyridium*—Malaysia, Australia, and Borneo—have very complex geotectonic histories (Kroenke 1996), making the use of geochronology from these areas unreliable. However, the eastern geographic range of *Mitthyridium* includes the Pacific hotspots of Samoa and the Society Islands. These island chains have less complicated histories and reliable potassium-argon dates. Instances of island endemism in lineages of *Mitthyridium* allowed the use of ages from Mo'orea and the high islands of Western Samoa for independent calibrations of the rate-constant *gpd* tree and independent determination of the age of the most recent common ancestor of *Mitthyridium*. Both of these ''island-endemic'' calibrations assume that the lineages on the islands used for dating arose on those islands, and that they are monophyletic and truly endemic. Inasmuch as the data show, these assumptions are valid.

The eastern Society Islands, Mo'orea and Tahiti, bear an endemic group of lineages belonging to the group *M. obtusifolium* that are monophyletic to the exclusion of a lineage found on the western island of Raiatea, and lineages on more western Pacific islands such as Fiji (see Figs. 1 and 3). The older island, Mo'orea, dates back to no more than 1.8 million years (Duncan and McDougall 1976; Diraison et al. 1991; Binard et al. 1993; Duncan et al. 1994). Barring extinction from older islands in the Society Island archipelago, this date was assumed to represent the maximum age of origin of the eastern lineages of *obtusifolium* (those found on Mo'orea and Tahiti) and was used for calibrating the molecular clock (Fig. 1; circled number 1). It is hereafter referred to as the Mo'orea calibration.

The second calibration used was the oldest age of the three major Samoan islands, Upolu, Savai'i, and Tutuila, where an endemic clade of *M. constrictum* was found. All three members of the Samoan *constrictum* clade were found to posses an indel character that was not found in any other *constrictum* lineage (Wall 2002). This character provided strong confirmation of both the monophyly and endemism of the clade. Indeed, the lack of this indel character in a Samoan lineage of the sister *constrictum* clade that is more geographically widespread, found on Vanuatu and Raiatea, lent strong support to the hypothesis of the clade's endemism. With these data, the assumption was made that the Samoan clade (marked by a circled number 2 in Fig. 1) is endemic to and no older than the islands it inhabits—the high islands of Samoa. These islands date back to no more than 2.9 million years before the present (Duncan 1985; Natland and Turner

1985; Clourad and Bonneville 2000); this age was considered the maximum age of origin of that clade and was used as a second calibration of the molecular clock, hereafter called the Samoa calibration.

Tests of the Accuracy of the Age Estimations

A check on the accuracy of the nodal ages and diversification rates estimated by the two calibrations using the full set of 80 *gpd* lineages was made by comparison with estimates from the 26-lineage subset mentioned above (also, bold taxa in Fig. 1). Exonic sequences of *gpd* from this 26-lineage subset previously were found to evolve at a constant rate (Wall 2002) and could thus be used to recalculate the age of origin of *Mitthyridium*. For this calculation only the Mo'orea calibration was used.

A second, independent estimator of the most recent common ancestor of *Mitthyridium* was possible by use of chloroplast DNA (combined *rps*4 and *trn*L amplicons) described in Wall (2002) from the lineages in the 26-lineage subset (additional lineages introduced zero-length branches and increased the likelihood of spurious age estimates). The molecular clock hypothesis was easily rejected in both cpDNA gene datasets. Thus, the nonparametric rate smoothing (NPRS) algorithm originally described by Sanderson (1997) was implemented in r8s (Sanderson 2003) to obtain age estimates. This method smoothes local variation in evolutionary rate by autocorrelation. A maximum likelihood tree based on the combined cpDNA and methods described above were used to obtain standard error on the estimates. For this test, only the Mo'orea calibration was used.

Diversification Rate Estimates

The measure λ defined separately by Kendall (1949) and Moran (1951) was used to estimate diversification rates after determining the age of the group *Mitthyridium*, $\lambda = N - 2/s$, where N is the number of extant taxa and s is the summed durations of the branches of the phylogeny (Nee 2001). This measure assumes a Yule pure-birth process of diversification, which is adequate as long as the value of λ does not change within the phylogeny, although changes in λ may be a common phenomenon (Mooers and Heard 1997; Nee 2001). This measure is moderately free from biases imposed by methods of taxonomic ranking because it is scaled by duration. The variance in λ was obtained using Moran's estimator, $\text{var}(\lambda) = \lambda^2/(N - 2)$, The value of λ was calculated at various locations in the phylogeny (Fig. 1) to investigate changes in its value. To account for uncertainty in the divergence time estimates, λ was calculated for each of the 100 bootstrapped character datasets. Finally, to account for possible sampling biases caused by the use of lineages rather than species as the operational taxonomic unit in this study, λ was calculated for *gpd* using the ultrametric phylogenies from both the 80- and 26-lineage subsets. The lineages in the smaller subset are strongly supported by molecular and morphological evidence (Wall 2002, 2003) and could easily be circumscribed into species.

Lineage-through-Time Plots

Lineage-through-time plots allow the researcher to differentiate between populations that have maintained a constant size and those that have grown exponentially. The numbers of lineages through time from Figure 1 were first log-transformed and then transformed using an ''epidemic'' transformation formula (Slatkin and Hudson 1991; Nee et al. 1995). The epidemic transformation allows for confirmation that lineages have appeared at an exponential rate and also demonstrates whether any acceleration or deceleration in exponential growth occurred (Nee et al. 1995). The two sets of transformed number of lineages were plotted against time to observe trends in the pattern of diversification in *Mitthyridium*.

Comparative Tests

Geological locality was broken into a 19-state character ordered from west to east, starting with Gabon and proceeding eastward longitudinally to Tahiti. MacClade 4.0 (Maddison and Maddison 2000) and ACCTRAN character optimization over 1000 dichotomous versions of Figure 1, produced by resolving polytomies randomly, was used to determine the pattern of dispersal in *Mitthyridium*.

The pattern of reproductive and geographic differences was assessed in the 112 specimens to infer the evolution of reproductive strategies in *Mitthyridium*. This procedure included counting numbers of males, females, gemmae (asexual propagules that have low-dispersal potential), and sporophytes. These data were coded into two-state (presence/absence) characters to conduct concentrated-changes tests (Maddison 1990) under a set of hypotheses designed to illuminate the trends in reproductive strategy and the association of those trends with geography, specifically, continent (mainland source) versus island. Geography was coded into a two-state continent/island character. The tree in Figure 1 was used after random resolution of the polytomies.

Results and Discussion

Test for Rate Constancy and Age Estimation

Eighty of the 112 *gpd* sequences were variable enough to be informative and of use to reconstruct the maximum likelihood phylogeny shown in Figure 1 (the additional 32 specimens were either nearly or entirely identical to other sequences in the set of 80). Not surprisingly, when the introns and exons of *gpd* were combined, a hypothesis of evolutionary rate constancy among the lineages was easily rejected. However, when examining the exonic sequences alone, a null model enforcing a molecular clock could not be rejected ($-\ln L_0 = 1486.60961$, $-\ln L_1 = 1431.84541$; $-2 \log\Lambda = 110$; $P = 0.1$; df = 103), offering an excellent opportunity to estimate absolute dates of origin and rates of diversification.

The age of the most recent common ancestor of *Mitthyridium* and all internal nodes was estimated using the Mo'orea and Samoa maximum age calibrations independently. For the first, the mean age for the most recent common ancestor of *Mitthyridium* was estimated to be 7.95 ± 2.02 million years ago (mya). Figure 2 shows a chronogram, in which branches are scaled to time, based on that calibration. When using the

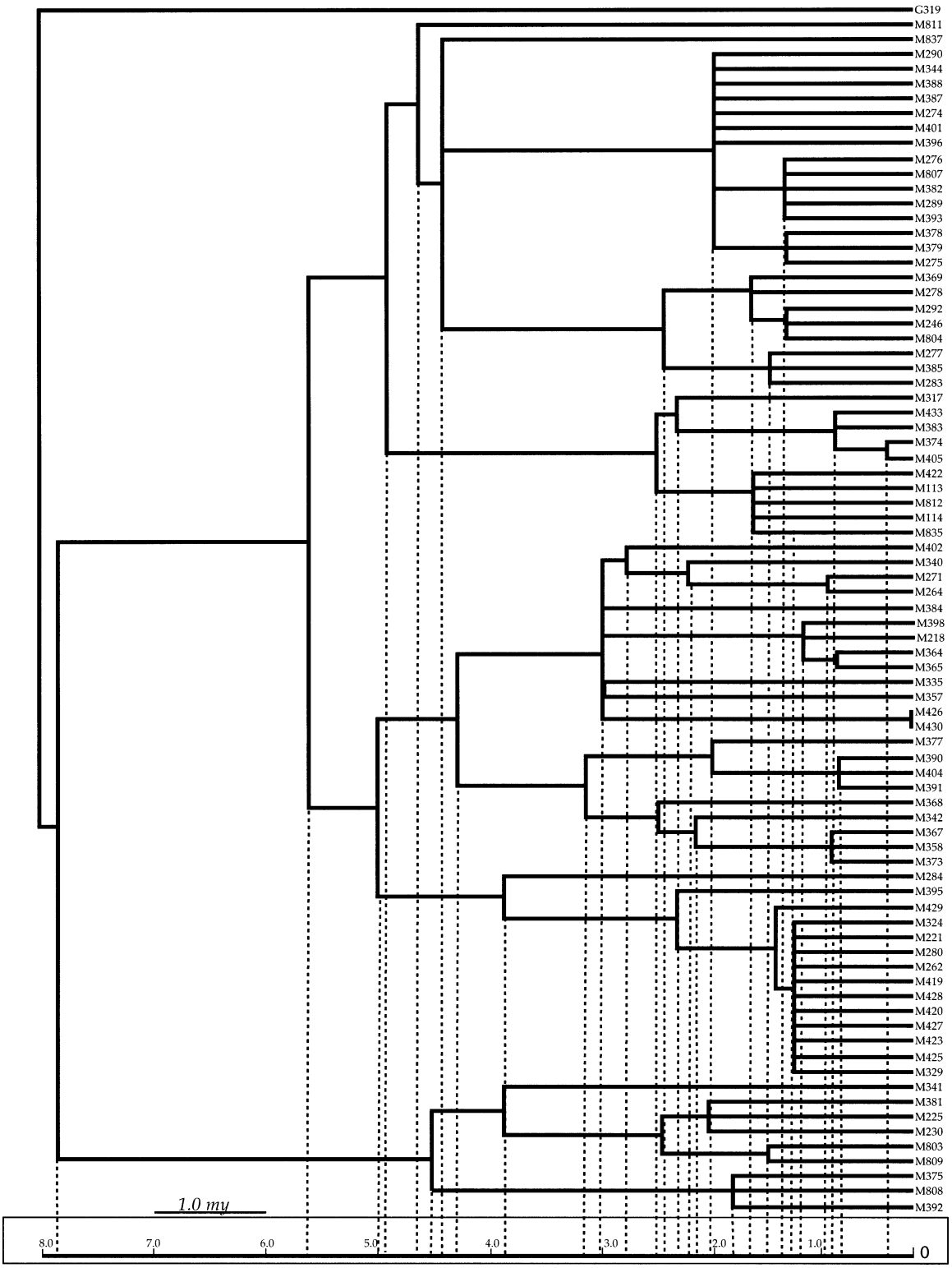

FIG. 2. Chronogram of 80 lineages in the group *Mitthyridium* plus one outgroup. The branches, based on the nuclear gene glyceraldehyde 3-phosphate dehydrogenase, are scaled to time constrained by the calibration date of 1.8 million years (my), corresponding to the potassium-argon dates reported for the island of Mo'orea (Duncan and McDougall 1976; Diraison et al. 1991; Binard et al. 1993; Duncan et al. 1994). Branches of less than 10,000 years were collapsed.

Samoan calibration, the mean age estimated for the most recent common ancestor of *Mitthyridium* was highly similar and found to be 8.13 ± 1.3 mya. The average age estimated for most recent common ancestor of *Mitthyridium* across the 25 bootstrap phylogenies was 8.3 ± 1.9 mya, suggesting that the estimates are robust to phylogenetic uncertainty in the *gpd* dataset.

Interestingly, the two age anchors, Mo'orea and Samoa, proved to be mutually consistent. That is, the Samoa calibration estimated the age of the Mo'orea node to be 1.66 ± 0.44 mya, very near the actual constraint age of 1.8 mya, and the independent Mo'orea calibration estimated the Samoan node to be 2.81 ± 0.82 mya, again very near the actual constraint age of 2.9 mya. Although this is not conclusive evidence for the accuracy of the two independent island calibrations, it is consistent with such a hypothesis.

Recalculation of the age of *Mitthyridium*'s origin using the 26-lineage subset produced consistent results. First, with the *gpd* exon sequences, an estimate of 7.35 ± 1.5 mya was obtained. Second, using nonparametric rate smoothing and cpDNA from Wall (2002), an age estimate of 6.43 ± 1.04 mya was obtained for the origin of *Mitthyridium*.

Choosing the most conservative estimate from the results above, the *Mitthyridium* clade was born approximately 8 million years before the present time. The area of origin was most likely Peninsular Malaysia (Fig. 3), which is the most parsimonious locality when optimizing geography on the phylogeny. The lineages emanating from nodes BC, A, B, and C, did not appear until about 5 mya (Figs. 2, 3). Shortly after, as illustrated by the dotted lines in Figure 2, two major bursts of diversification occurred. The first, between about 5 and 4 mya, was responsible for establishing all of the major lineages of *Mitthyridium* on continental areas of Malaysia, Borneo, the Philippines, and probably Australia. The second, beginning about 3 mya and continuing to the present day, formed the remaining lineages. Table 1 lists the age estimates for key nodes in the early diversification of *Mitthyridium*.

Diversification Rates

Using approximately 8.1 mya as the actual time of origin of the most recent common ancestor to all extant lineages of *Mitthyridium*, the mean rate of diversification (λ) estimated for *Mitthyridium* across the 100 bootstrapped matrices is 0.56 ± 0.003 new lineages per million years, which is in accord with other reports of extremely rapid diversification rates such as in both the Hawaiian silversword alliance (Baldwin and Sanderson 1998) and Neogene horses (Hulbert 1993).

To determine whether this rate of diversification could have resulted from sampling biases brought on by the use of molecular lineages rather than species as the operational taxonomic unit, the rate was recalculated using *gpd* exonic sequences from the 26-lineage subset and was found to be highly similar at 0.52 ± 0.01. Given the similarity of the two rates calculated, it is unlikely that the rate of 0.56 is an artifact of the use of lineages rather than the traditional rank of species. Thus, further analyses below are restricted to the larger, 80-lineage compartment.

Other similar rises in diversity have been reported in angiosperms (Wojciechowski et al. 1999; Richardson et al. 2001a,b), but given that mosses have traditionally been considered ''living fossils'' with limited or no evolutionary potential (Crum 2001), it is surprising to find such a rapid rate of diversification in a moss. It is possible that, like liverworts (Odrzykoski and Szweykowski 1991), much of the diversity in mosses lies at the molecular rather than the morphological level. To date, only one other study has revealed rapid evolution in a moss group (Shaw et al. 2003). Further similar studies will likely find that other rapid rises of diversity happened repeatedly throughout moss evolution.

In general, the rapid rate of diversification in *Mitthyridium* is constant except for two notable shifts. Node A, which contains the lineages of *constrictum* and *crassum*, shows a significant decrease in diversification rate (Fig. 3). Clade B2, containing the lineages of *undulatum* that diversified across the Fijian archipelago, has a sharply increased λ of 0.71 ± 0.006, one lineage per million years (Fig. 3). The changes in λ may indicate that the data violate the assumptions of the Yule pure-birth process, though extinction is not likely to be a major influencing factor in such a young group. Furthermore, the estimates of λ are likely to be underestimates because they are based on maximum island ages. However, an alternative look at the pattern of diversification was conducted through the use of lineage-through-time plots.

Lineages Through Time

The logarithmic transformation of the number of lineages through time shows a dramatic flattening-off, which suggests that *Mitthyridium* underwent rapid exponential growth in lineage number over the time period covered by the coalescent events in its phylogenetic history (about 8 mya) followed by a decreased rate of lineage appearance near the present time (Fig. 4). The epidemic transformation was applied to the lineage-through-time data to confirm that the lineages have appeared at an exponential rate and also to determine whether any acceleration or deceleration in exponential growth occurred (Nee et al. 1995). The line of this epidemic plot trended upward twice, indicating two phases of growth accelerated above the exponential rate (Fig. 4). These upward trends are broken by a short deceleration in the lineage growth rate. The first phase of rapid growth was between about 5 and 4 mya and corresponds to the appearance of the major lineages in *Mitthyridium* (Fig. 2). The second was between about 3 and 0.7 million years (Fig. 4) and corresponds roughly to the establishment of *Mitthyridium* lineages on increasingly remote oceanic islands as illustrated by the dotted lines in Figure 2 closer to the right side of the phylogeny.

Pattern of Dispersal

The general pattern of dispersal in *Mitthyridium* is predominantly west to east ($P < 0.01$; Wilcoxon signed-ranks test) and appears to fit a stepping-stone pattern. For example, optimization of locality to the phylogeny (Fig. 3) demonstrated four shifts from Peninsular Malaysia to Borneo, two shifts from Borneo to Australia, and two shifts from Samoa to Mo'orea. Furthermore, optimization of locality indicates that the deep, most recent common ancestors inhabited Peninsular Malaysia and subsequently dispersed eastward. For example, the lineages composing clade A, whose ancestor

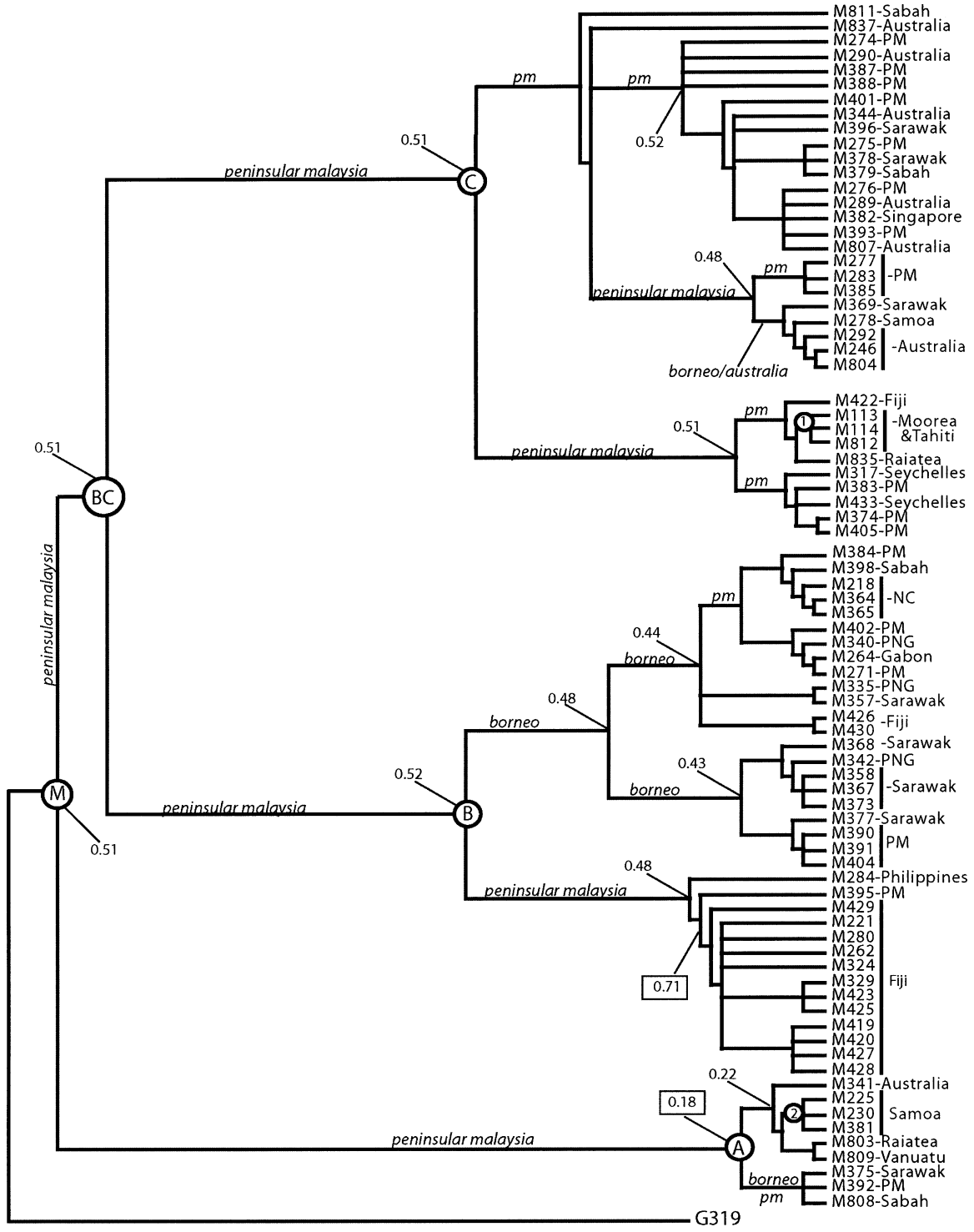

FIG. 3. Rates of diversification, λ, and the geological locations occupied by the lineages in *Mitthyridium*. The two boxed values of λ indicate the highest and lowest diversification rates, respectively. PM, Peninsular Malaysia; PNG, Papua New Guinea; NC, New Caledonia. Nodes are labeled as in Figure 1.

originated in Malaysia or Borneo, dispersed and established first about 3.9 mya, probably in Australia and/or Vanuatu (equivocal) and finally reached and spread through Samoa between about 2.5 and 1.9 mya (Figs. 2, 3). Similar patterns are found elsewhere, as for example in clade B2. The ancestral population of this group was established in Peninsular Malaysia and the Philippines about 4 mya. The establishment of a population in the Fijian islands did not occur until almost 3 million years later, at which time *undulatum* radiated rapidly in Fiji. Also, in clade C4, a series of dispersal events

TABLE 1. Summary of divergence times for key nodes in the diversification of *Mitthyridium*. Node labels are pictured in Figure 1. M is the most recent common ancestor of all *Mitthyridium* lineages. Nodes are listed in order of age from oldest to youngest. Age-Mo indicates age estimates and standard errors were calculated using the Mo'orea calibrator, Age-Sa are estimates based on the Samoa calibrator. The calibrators were used to scale ultrametric phylogenetic trees made from 100 replicate *gpd*-exon datasets.

Node	Age-Mo	Age-Sa
M	7.95 ± 2.02	8.11 ± 1.31
BC	5.91 ± 1.49	6.04 ± 1.00
A	4.0 ± 1.12	4.02 ± 0.47
B	5.15 ± 1.39	5.24 ± 0.87
C	5.27 ± 1.33	5.39 ± 0.93
Samoa	2.81 ± 0.82	—
Mo'orea	—	1.66 ± 0.44

from Peninsular Malaysia was followed by the establishment of *obtusifolium* populations on Fiji, Raiatea, and the eastern Society Islands of Mo'orea and Tahiti. A slightly different event occurred in the western-distributed sister group *microundulatum*, because Peninsular Malaysia appears to have been a source for two independent establishment events on the Seychelles.

The deceleration breaking up these two phases of rapid diversification occurred between about 4.3 and 3.2 mya, as discussed above (Figs. 4) and as depicted in Figure 2 (note the space between sets of dotted lines). The causes of this deceleration are unknown, but may be related to the difficulty in reaching more remote islands and establishing viable populations there.

Conclusions

Mitthyridium originated approximately 8 million years ago on Peninsular Malaysia. From there, the group diversified rapidly in both easterly and westerly directions to islands in the Indian and Pacific Oceans. This rate of diversification was rapid, at 0.56 ± 0.004 and on par with other key examples of rapid diversification in nature (Hulbert 1993; Baldwin and Sanderson 1998; Klak et al. 2004). This rate of diversification remained constant except for one deceleration between approximately 4.3 and 3.2 million years before the present time, plausible causes of which are discussed below. The measure used to estimate diversification (λ) proved particularly robust to differences in taxonomic units, almost certainly because the estimation of age is based on branch length and time, rather than number of extant taxa alone as in other measures (Slowinski and Guyer 1989). Indeed, a final conclusion one can make from these results is that the age and diversification estimates reported do not appear to be an artifact of the use of molecular lineages rather than species. A matrix of 26 taxa that can easily be distinguished from one another via multiple independent lines of data, and could be clearly circumscribed as different species, yielded estimates of the age of origin of *Mitthyridium* (6.43 ± 1.04 mya) and a measure of diversification (0.52 ± 0.01) that are highly consistent with those from the larger 80-lineage compartment.

However, open questions remain, the most prominent of which is why did *Mitthyridium* diversify so rapidly? There are at least two ways to investigate causes of diversification.

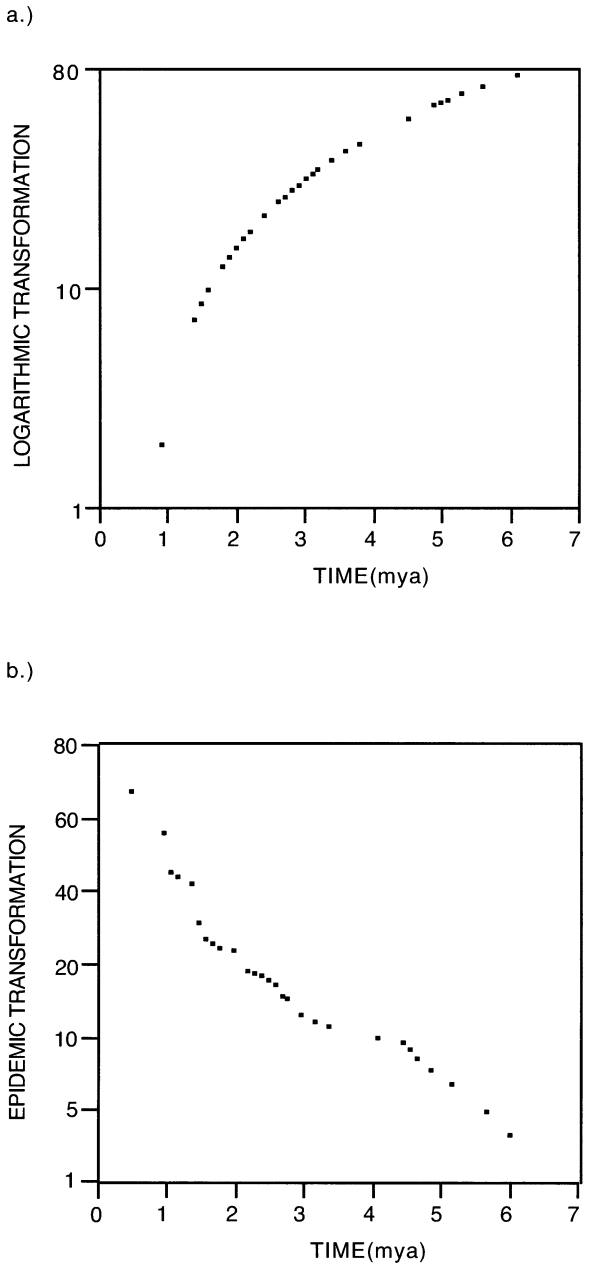

FIG. 4. Lineages-through-time plots of the group *Mitthyridium*. (a) Logarithmic transformation. (b) Epidemic transformation based on the formula in Nee et al. (1995). mya, million years ago.

One is to search for common denominators in the ecology or climate that may drive the formation of diversity, and another is to look at characteristics of the organism, such as the reproductive life-history strategies, for evidence of a key innovation that could be responsible for the rise in diversity.

Unfortunately, the geotectonic history of the localities occupied by *Mitthyridium* offers minimal insight into ecological or geological influences on dispersal, establishment, and diversification (De Boer and Duffels 1996). The higher islands of the Samoan archipelago and the Society Islands (including Bora Bora) were not present until after about 3 mya, which likely represents the earliest conceivable time of establish-

ment of *Mitthyridium* lineages on these islands. The islands west of the Samoa archipelago have more complex geological histories. Around the time of the origin of *Mitthyridium* (6–9 mya) the other half of the Fiji platform (Vanua Levu) was forming and Fiji was in a general period of flux that included collisions with New Caledonia (Kroenke 1996). This environmental instability may help to explain in part the later appearance of new lineages and the late onset of the rapid radiation shown in clade B2 (Fig. 2). Otherwise the microecological setting for *Mitthyridium* lineages varies little. Substrate specificity is nearly identical across the lineages and the elevational preferences are homoplasious (data not shown).

On the other hand, *Mitthyridium* is diverse in reproductive behavior, ranging from fully sexual to asexual to simply vegetative, lacking any sign of reproduction beyond regular gametophytic growth. *Mitthyridium* bears male and female gametangia on separate plants. Sporophytes and spores, the most probable units of long-distance dispersal, are rarely produced (Reese et al. 1986; Wall 2003). This biology leads to the prediction that dispersal to oceanic islands must be rare, which may help to explain the relatively late appearance of new lineages on islands and the one-million year lag period between the two phases of ''epidemic'' growth. This general variability in reproductive behavior also predicts that differences should exist between clades that have different percentages of sexual or asexual reproduction.

Support for the second prediction is offered by the marked differences in λ reconstructed for clade A and clade B2. Clade B2 is more restricted geographically than clade A and has a conspicuously lower percentage of gemmae production than clade A (53% vs. 90%, though the sample sizes are small). These were the only clades whose λ differed from the mean rate of diversification in *Mitthyridium*.

Could an ability to shift reproductive modes be a key innovation responsible not only for the survival, but also the diversity of this young group? *Mitthyridium*, like other mosses, is able to shift reproductive strategies under different environmental circumstances (Stark et al. 1999, 2000). Such a shift, if fixed in a population, likely could be a powerful creative force in evolution.

A preliminary step in investigating the relationship between establishment and reproductive strategy in *Mitthyridium* was taken through comparative tests (Table 2). Several conclusions may be drawn, although tentatively, from the results of these concentrated-changes tests. Most notable are that males reach islands more frequently than females, and, perhaps related, gemmae rather than sporophytes are significantly associated with dispersal to islands. Given that the joint presence of males and females in a single moss clump is required for sexual reproduction (i.e., the production of sporophytes), males and females would have to disperse together and remain in very close proximity for sexual reproduction to occur. Since the likelihood of any propagule reaching an island is small, the chance of both a male and female reaching an island and landing in sufficiently close proximity is certainly smaller. These results taken together suggest that island-dispersed members of *Mitthyridium* may indeed be shifting their reproductive mode from sexual to asexual (perhaps permanently). It is tantalizing to suggest that this reproductive plasticity was the key innovation that facilitated rapid diversification in *Mitthyridium*; however, more research, such as tests that link shifts in reproduction directly with changes in diversification rates, is required before that conclusion may be made.

TABLE 2. Patterns of correlated character evolution. Ten tests were conducted to help characterize the pattern of sexual expression in *Mitthyridium* and its relationship with geographical distribution, here restricted to mainland (0) versus island (1). The independent and dependent characters are listed along with their distinguished characters. Each row asks whether the ''gains'' in the dependent character are more concentrated than expected by chance on distinguished branches of the independent character. Asterisks indicate significance.

Independent	Dependent	Distinguished character	*P*
Locality	females	island-0 | present-1	0.060
Gemmae	females	present-1 | present-1	0.198
Males	females	present-1 | present-1	0.355
Males	sporophytes[1]	present-1 | present-1	0.001*
Gemmae	males	present-1 | present-1	0.245
Locality	gemmae	island-0 | present-1	0.014*
Locality	sporophytes	island-0 | present-1	0.124
Locality	males	island-0 | present-1	0.045*
Sporophytes	gemmae	present-1 | present-1	0.200
Sporophytes	females[1]	present-1 | present-1	0.030*

[1] The independent and dependent variables are interchangeable.

Further studies that characterize the phylogenetic signal left by different evolutionary processes (Hey 1992; Kirkpatrick and Slatkin 1993; Sanderson and Donoghue 1994, 1996; Mooers 1995; Heard 1996; Mooers and Heard 1997) should go a long way toward improving our understanding of adaptive radiations and the role of key innovations. Such research coupled with easier mechanisms to study absolute time and rates of diversification (Sanderson 1997, 2002; Paradis 1998; Thorne et al. 1998; Korber et al. 2000; Yoder and Yang 2000) should make studies of the causes behind shifting diversification rates more commonplace. Although this is among the first studies showing that even mosses have evolved recently and rapidly, it likely will not be the last. With more research, especially on moss clades that are narrowly distributed, we may find that an elevated rate of diversification is more common in mosses than previously thought possible.

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