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POPULATION STRUCTURE IN A SNAIL SPECIES FROM ISOLATED MALAYSIAN LIMESTONE HILLS, INFERRED FROM RIBOSOMAL DNA SEQUENCES

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ABSTRACT

We sequenced the first internal transcribed spacer (ITS-1) of the ribosomal DNA in nine populations of the vertiginid *Gyliotrachela hungerfordiana*, which lives on isolated (and threatened) limestone hills in the Malaysian peninsula. Current data suggest that the species is an obligate calcicole. The application of a tentative molecular clock suggests a Quaternary divergence for the *G. hungerfordiana* populations. A strong positive correlation between genetic and geographic distance was observed, which, combined with geological data, suggests that the hill populations may be interconnected by as yet unsampled populations.

Key words: internal transcribed spacer, ITS-1, Gastropoda, Pulmonata, Vertiginidae, *Gyliotrachela*, gene flow, Southeast Asia.

INTRODUCTION

Land snails have proverbially poor abilities for dispersal (e.g., Cowie, 1984; Schilthuizen & Lombaerts, 1994), which causes them to show evolutionary patterns at much smaller spatial scales than many other organisms of similar size. As a result, strong geographic structuring of populations is common in snails (e.g., in *Liguus;* Hillis et al., 1987). Another consequence is endemism, which is seen, for example, in the Mediterranean clausiliid genus *Albinaria*, of which almost 30 species are endemic to the island of Crete, with distribution areas of sometimes only one kilometer across (Gittenberger, 1991; Welter-Schultes, 1998).

An impressive situation of high endemism and geographic structuring of land snails in a strongly fragmented habitat exists in peninsular Malaysia. Here, limestone is exposed in the form of "tower karst" and other karstifications, limited to about three hundred hills, scattered over the peninsula. These hills are often very small, the largest with a diameter of a few kilometers, but most measuring only a few hundred meters across. In spite of their small size, the hills are a prominent feature of the landscape, because they usually stand isolated, are riddled with caves and are bounded by precipitous cliffs.

For more than a century, malacologists have been interested in the rich malacofauna that the hills support (de Morgan, 1885). High numbers of species are found, and the morphologies of some Diplommatinidae foreshadow the bizarre and extravagant forms found in this group in Borneo (Vermeulen, 1993, 1994; Gittenberger, 1995). But especially fascinating is the staggering degree of endemism in these calcicolous snails. Tweedie (1961) gave an overview of six taxa containing many obligate calcicoles (*Diplommatina, Opisthostoma*, Vertiginidae, *Discartemon, Oophana*, and *Sinoennea*). He listed the presence of 106 species on 28 hills or hill-clusters, of which 70 are endemic to only one locality. Some calcicolous species, however, are widespread and occur on almost all hills without a trace of morphological differentiation (e.g. *Gyliotrachela hungerfordiana* and some *Alycaeus* species).

Geologically, the hills form the exposed parts of a number of larger paleozoic limestone deposits, which are elsewhere overlain by non-calciferous alluvial deposits (Gale, 1986; Crowther, 1986). Some hills may thus have been connected in the past, while others have always been separate. Consequently, the hills form virtual "islands" for obligately calcicolous land snails, which they may reach by incidental dispersal. Alternatively, the pop-

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ulations on the hills may be relicts from a time when the hills were part of large continuous plateaus, which were subsequently fragmented.

In this paper, we examine a relatively widespread representative of the peninsular Malaysian hill malacofauna, using molecular and geological data, to answer the following questions: (1) what pattern of phylogeographic relationships exists among the populations of this widespread species, and (2) how has the population structure been shaped, that is, what are the relative influences of dispersal and habitat fragmentation over geological time?

By analyzing the variance in a noncoding nuclear DNA marker, we attempt to differentiate between various alternative population structures. In the case of ancient vicariance, we expect to find genetic distances that reflect the age of fragmentation of the limestone hills, while dispersal would result in genetic distances more or less related to geographic distance. Under the latter hypothesis (dispersal), indications of the type and frequency of dispersal may be gleaned from the degree of correlation between genetic and geographic distance; if dispersal is randomly oriented (i.e., corresponding to an island model of population structure; Wright, 1931), stochasticity would result in a poor fit, while dispersal occurring mainly among neighboring hills (i.e., corresponding to a stepping-stone model; Kimura, 1953) would be revealed by a strong correlation (Kimura & Weiss, 1964).

Sadly, there are other motives for working on this fauna. The hills of peninsular Malaysia are disappearing and becoming depauperate at an alarming rate. Forest clearing has destroyed the vegetation on some hills; and in the densely populated areas near Ipoh and Kuantan, many hills are being removed by quarrying. The true rate of species loss can only be guessed at, but the extinction of at least one endemic snail species, *Opisthostoma sciaphilum*, from Bukit Panching, has been documented (Schilthuizen et al., unpubl.).

MATERIAL AND METHODS

Selection of Taxa

We selected the widespread and morphologically uniform vertiginid *Gyliotrachela hungerfordiana* for study (Fig. 1). The related species *G. frequens* (Fig. 2) was selected to serve as an outgroup in the phylogenetic analysis.

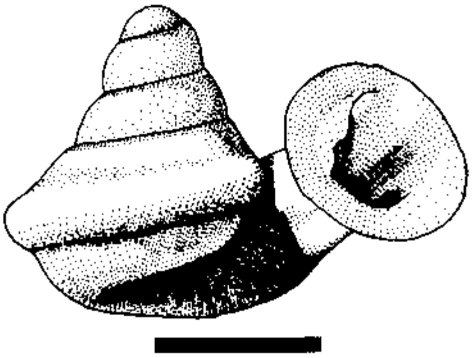

FIG. 1. *Gyliotrachela hungerfordiana* (von Möllendorff). Scale bar = 1 mm.

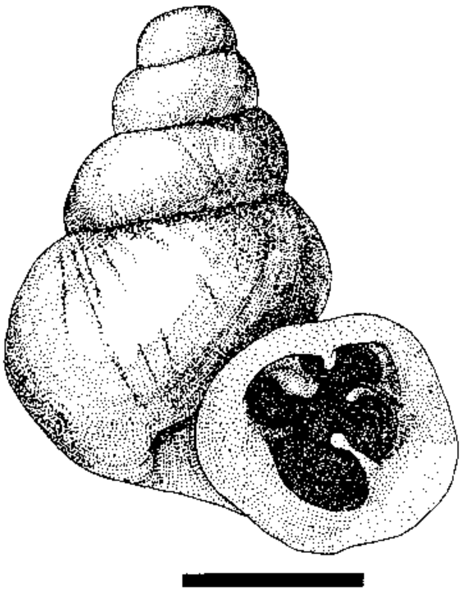

FIG. 2. *Gyliotrachela frequens* van Benthem Jutting. Scale bar = 1 mm.

Collecting

In July 1997, the first author visited 22 limestone hills in the West-Malaysian states of Pahang, Kelantan, Perak and Perlis. Living snails were discovered by eye using two strategies: (a) close inspection of limestone rock faces, either damp or dry, bare or covered in algae, mosses and lichens; and (b) sifting through damp and decaying leaf litter on limestone rocks or at the base of the limestone cliffs. All snails were put in 100% ethanol on the spot and kept at ambient temperatures until arrival in the laboratory for further processing. Identification of the material was carried out by the second author while the material remained in alcohol. *Gyliotrachela hungerfordiana* was collected from nine of the 22 localities (Fig. 3): loc. 5, State of Pahang: Gua Bama (ca. 10 km W of Kuala Lipis); loc. 8, State of Kelantan: Gua Musang, southern of the two hills that the road to Kuala Kerai passes between; loc. 9, State of Kelantan: rocks 59 km in the direction of Gua Musang, measured along the road from Kuala Krai; loc. 16, State of Perak: Bukit Tambun (ca. 6 km E of Ipoh); loc. 22, State of Perak: hill directly east of Sungai Siput Utara hospital; loc. 23, State of Perlis: hill ca. 1 km S of Kangar; loc. 24, State of Perlis: 9 km along the road from Kangar to Kaki Bukit; loc. 25, State of Perlis: Gua Kelam at Kaki Bukit; loc. 26, State of Perlis, Timah Tasoh (ca. 16 km NE of Kangar). All samples were taken between 27.vi.1997 and 17.vii.1997. *Gyliotrachela frequens* was taken only from locality 8. Voucher specimens have been deposited in the collection of the National Museum of Natural History "Naturalis", Leiden.

Molecular Techniques

DNA was isolated from pools of between one and five complete snails with their shells, using either a phenol/chloroform extraction as described previously (Schilthuizen et al., 1998a) or a sucrose-based protocol (van Moorsel & van Nes, unpublished), which can be briefly summarized as follows. Snails were ground in 200 μl of sucrose-buffer (0.1 M Tris; 0.02 M NaCl; 0.2 M sucrose; 0.05 M EDTA) and centrifuged. The pellet was incubated at 65°C for 60 min in 200 μl SDS-buffer (0.02 M Tris; 0.01 M EDTA, 1.25% SDS), 15 μl of cold KAc was added, and the mixture was incubated on ice for 60 min and centrifuged. The DNA was precipitated from the supernatant by the addition of two volumes of 100% ethanol and incubation at –20°C for 30 min. The DNA was dried and treated with 200 ng of RNase. Full details can be obtained from M.S. on request. Homogenization was always done with a sterile, disposable plastic pestle. The DNA was dissolved in 50 μl of Tris-EDTA buffer (phenol protocol) or 30 μl of ddH_2O (sucrose protocol) and stored at –20°C. The first internal transcribed spacer of the nuclear ribosomal DNA was amplified with the SuperTaq enzyme (HT Biotechnology, Cambridge, England) as described previously (Schilthuizen et al., 1995) and isolated using the "freeze-squeeze" technique (Tautz & Renz, 1983). Because PCR-amplification was at times too weak for direct sequencing, we resorted to cloning (PCR-based error is usually not a concern with this methodology; Schilthuizen et al., 1998b). After isolation, the fragments were ligated into Promega or Invitrogen T-tailed vectors, following the manufacturer's instructions. Colonies were screened for the presence of the correct insert by PCR. Plasmid DNA was isolated from the bacteria using QIAPrep spin columns (QIAGEN). One or two clones per sample were sequenced in both directions on an ABI automated sequencer.

Alignment

Before alignment, all chromatograms were checked and reading errors were corrected blindly where necessary (this never amounted to more than three corrections in a single sequence). Vector and primer sequences were removed. Sequences in the ingroup were sufficiently similar to allow manual alignment. Wherever alignment with the outgroup was ambiguous, missing data were introduced into the outgroup sequence.

Phylogenetic Analysis

Phylogenetic analyses of the data set were performed in PAUP3.1 (Swofford, 1993). Gaps were treated as missing data. Searches for the most parsimonious trees were carried out with the branch-and-bound option. Bootstrap replicates were carried out 100 times, using heuristic searches. In addition, Bremer (1988) support was determined. Kimura's 2-parameter genetic distances (Kimura, 1980) were calculated with the DNADIST program of the PHYLIP package (Felsenstein, 1995).

RESULTS

PCR-products ranged in length from 755 to 772 bp, including primers (52 bp), and the flanking regions of 18S (146 bp) and 5.8 S (87 bp). These lengths correspond well with other ITS-1 lengths reported in mollusks (Anderson

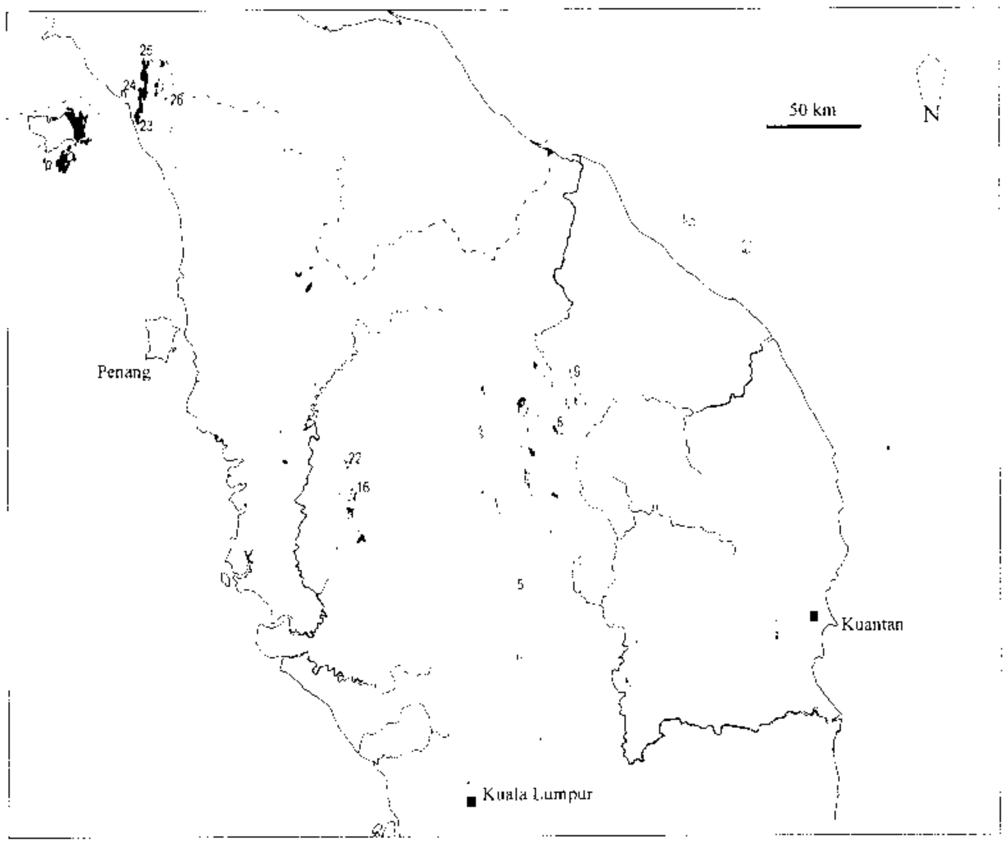

FIG. 3. A map of the northern part of Peninsular Malaysia, with the limestone hills drawn in black (modified after Gobbett, 1965). The numbers refer to localities where *Gyliotrachela hungerfordiana* and *G. frequens* were collected (see text for further details).

& Adlard, 1994; Schilthuizen et al., 1995; Armbruster et al., unpubl.).

We obtained sixteen sequences from *G. hungerfordiana* and one sequence for the outgroup, *G. frequens* (Appendix, Table 1). They have been deposited in GenBank under accession numbers AF118000-AF118016. Only small genetic distances were found among the *G. hunderfordiana* sequences, the largest being 0.048 between sequence *a* from locality 5 and sequence *b* from locality 23. A comparison between pairwise genetic distances and pairwise geographic distances between sequences revealed a strongly significant ($p < 0.005$) positive correlation (Fig. 4, Appendix, Table 2). The phylogenetic analysis produced 18 most parsimonious trees (length = 89 steps, RI = 0.95), which showed two alternative topologies for three monophyletic groups of sequences, and otherwise only minor differences in topology within each of these three monophyletic groups (Figs. 5, 6). The fact that duplicate sequences from a single locality always formed monophyletic groups might justify the small sample sizes. Geographic structuring is apparent in the trees also, as these show monophyly for the sequences derived from populations in Perlis, Pahang + Kelantan, and Perak.

DISCUSSION

Unfortunately, it is difficult to estimate reliably from the molecular data the time since divergence. Unlike the situation for mitochon-

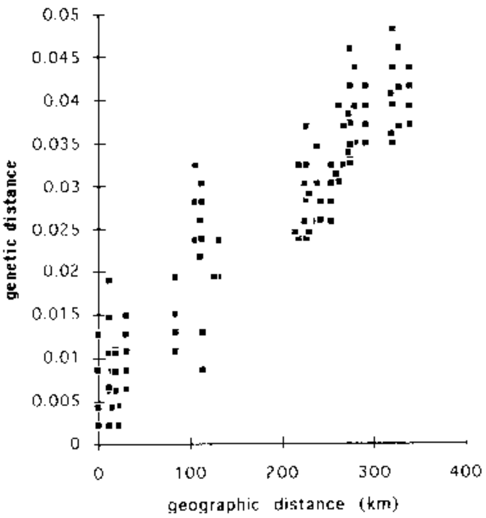

FIG. 4. The relationship between geographic distance and Kimura's 2-parameter distance for the sequences of *Gyliotrachela hungerfordiana*.

drial DNA, corroborated molecular clocks for the ITS regions are hardly available yet, and where they are, they differ by orders of magnitude among taxonomic groups. In the angiosperm families Cucurbitaceae and Winteraceae, substitution rates of 3.62 × 10^{-3} and 3.4 × 10^{-4} per site per million years (MY) were calculated, respectively (Jobst et al., 1998; Suh et al., 1993), while in Chlorophyta, a rate of 0.8 – 2.0 × 10^{2} was estimated (Bakker et al., 1995). In animals, rates of substitution in ITS appear to be somewhat higher. Schlötterer et al. (1994) give a figure of 1.2 × 10^{2} for *Drosophila*. and preliminary data for clausiliid land snails from Greek islands indicate a similar rate (van Moorsel, unpublished data).

Here, we will adopt a substitution rate of 1 × 10^{-2} per site per MY as a very rough molecular clock. Applying this rate to the average genetic distance between sequences on either side of the node basal to all *G. hungerfordiana* sequences in the trees, we obtained an estimated divergence time of 1.8 MYA for the populations of *G. hungerfordiana*. It should be stressed that, given the lack of agreement in the few calibrated molecular clocks available, not too much confidence should be placed on this date. However, it may be safe to assume a Late Tertiary or Quaternary origin for *G. hungerfordiana*.

Given the low degree of genetic divergence among the *G. hungerfordiana* populations, it seems unlikely that vicariance has played an important role; hills which have been studied geologically are thought to be older than Late Tertiary/Quaternary (Gale, 1986). However, in view of the uncertainty about the calibration of the ITS-1 molecular clock, this reasoning may be little meaningful. More importantly, geological data indicate that most of the hills from which the species was sampled have never been part of one continuous plateau (Paton, 1961). It is for this reason not likely that vicariance events have been important in its distribution pattern. Rather, the limestone hills on which it lives now must have been colonized after dispersal.

Several mechanisms for passive dispersal in small snails have been suggested, including wind and water mediated dispersal. In reference to *Gyliotrachela* and similar snails, Tweedie (1961) has suggested that flooding may be important in producing dispersal among hills that are situated close together. However, the drainage patterns in the peninsula preclude any long-range dispersal by this mechanism. Stagnant water may also provide means of dispersal, and geological data (Gale, 1986; Crowther, 1986) indicate that lacustrine conditions have prevailed around several limestone hills in the past. But here, too, dispersal would be across very small distances. Another possibility is wind-dispersal. Kirchner et al. (1997) demonstrate how *Truncatellina,* a vertiginid very similar in size to *G. hungerfordiana*, could be blown over distances of several kilometers during storms.

Some additional characteristics of dispersal may be gleaned from Figure 4, which suggests a linear relationship between geographic and genetic distance. If dispersal from one hill to another were infrequent and undirected (i.e., a population structure corresponding to Wright's [1931] island model, where all possible pairs of subpopulations are equally likely to exchange migrants), such a clear relation would not be expected. The fact that genetic distance is reliably predicted ($r^2 = 0.77$) by geographic distance, suggests that a structured network of dispersal connects the hills. This corresponds to a stepping-stone model (Kimura, 1953). Under such a model, genetic similarities drop steeply with increasing numbers of intervening populations (Kimura & Weiss, 1964). The fact that we observe a strong relationship with geographic distance, suggests that the hill popu-

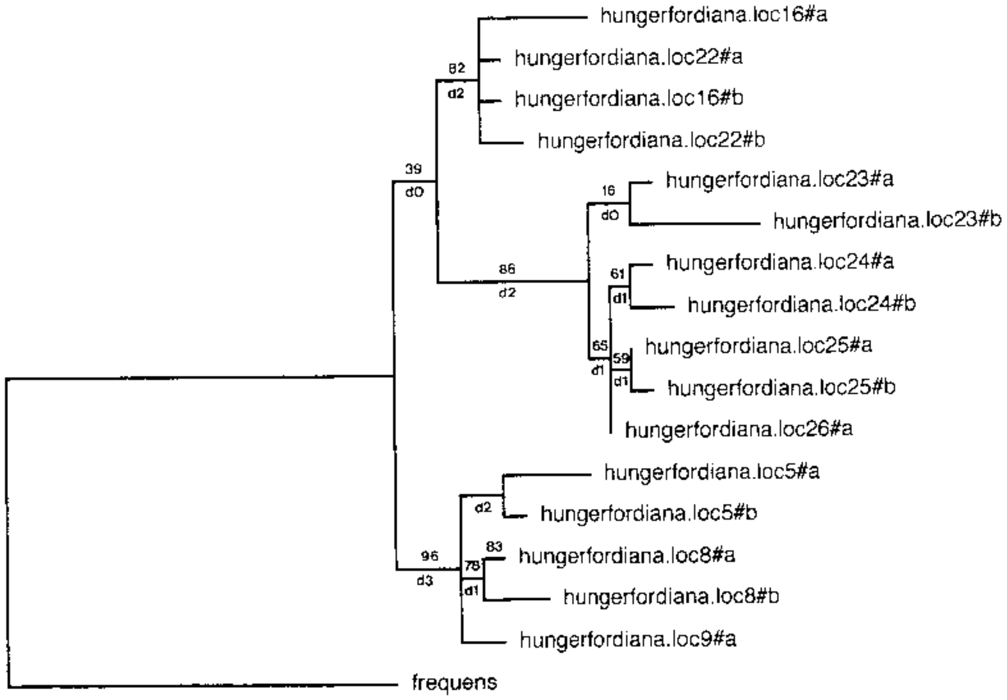

FIG. 5. A representative most parsimonious tree of the *Gyliotrachela* sequences. Bootstrap percentages and decay indices have been indicated on the branches.

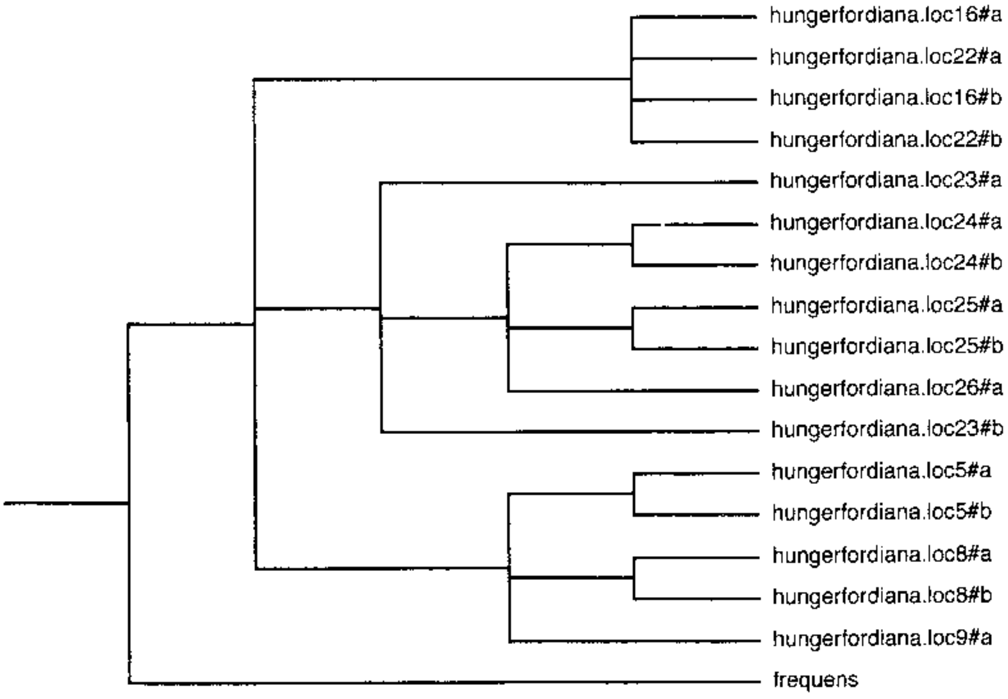

FIG. 6. Strict consensus over all 18 most parsimonious trees.

lations cannot represent directly adjacent populations in a two-dimensional stepping-stone lattice. Rather, to obtain this result, it is necessary to postulate unsampled populations in between. Unfortunately, the population genetics of ribosomal DNA are as yet far from clear (Hillis et al., 1991; Rich et al., 1997), which makes a quantitative analysis of dispersal parameters and spatial details of the population structure impossible. Therefore, it is not possible to tell whether the hills that separate our sample sites (e.g., the six or more hills between sites 8 and 9) will suffice as additional stepping stones. This might be tested, for instance, by exhaustively sampling the hills in a given subregion.

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APPENDIX

TABLE 1. Aligned sequences for *Gyliotrachela hungerfordiana* and *G. frequens*. The 5′ end of the 18S region is at position 146, the 3′ end of the 5.8S region is at position 694.

	10	20	30	40	50
hungerfordiana.loc5#a	AGCGGTTCAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
hungerfordiana.loc5#b	AGCGGTTCAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
hungerfordiana.loc8#a	AGCGGTTCAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
hungerfordiana.loc8#b	AGCGGTTCAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
hungerfordiana.loc9#a	AGCGGTTCAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
hungerfordiana.loc16#a	AGCGGTTCAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
hungerfordiana.loc16#b	AGCGGTTCAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
hungerfordiana.loc22#a	AGCGGTTCAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
hungerfordiana.loc22#b	AGCGGTTCAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
hungerfordiana.loc23#a	AGCGGTTCAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
hungerfordiana.loc23#b	AGCGGTTCAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
hungerfordiana.loc24#a	AGCGGCTCAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
hungerfordiana.loc24#b	AGCGGTTCAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
hungerfordiana.loc25#a	AGCGGTTCAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
hungerfordiana.loc25#b	AGCGGTTCAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
hungerfordiana.loc26#a	AGCGGTTCAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC
frequens	AGCGGTTCAG	TGAGGGCCTC	GGATTGGTCT	CGGTCTGGTG	CGCAAGTGCC

	60	70	80	90	100
hungerfordiana.loc5#a	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
hungerfordiana.loc5#b	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
hungerfordiana.loc8#a	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
hungerfordiana.loc8#b	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
hungerfordiana.loc9#a	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
hungerfordiana.loc16#a	GGCGCCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
hungerfordiana.loc16#b	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
hungerfordiana.loc22#a	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
hungerfordiana.loc22#b	GGCATCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
hungerfordiana.loc23#a	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
hungerfordiana.loc23#b	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
hungerfordiana.loc24#a	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
hungerfordiana.loc24#b	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
hungerfordiana.loc25#a	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
hungerfordiana.loc25#b	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
hungerfordiana.loc26#a	GGCACCGCTG	GCCGAGAAGA	AGCTCGAACT	CGATCGCTTG	GAGAAAGTAA
frequens	GGCACCGCTG	GCCGAGAAGA	AGCCCGAACT	CGATCGCTTG	GAGAAAGTAA

	110	120	130	140	150
hungerfordiana.loc5#a	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
hungerfordiana.loc5#b	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
hungerfordiana.loc8#a	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
hungerfordiana.loc8#b	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
hungerfordiana.loc9#a	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTAACGG
hungerfordiana.loc16#a	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTATCGG
hungerfordiana.loc16#b	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTATCGG
hungerfordiana.loc22#a	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTATCGG
hungerfordiana.loc22#b	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTATCGG
hungerfordiana.loc23#a	AAGTCGTAAC	AGGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTATCGG
hungerfordiana.loc23#b	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTATCGG
hungerfordiana.loc24#a	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTATCGG
hungerfordiana.loc24#b	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTATCGG
hungerfordiana.loc25#a	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTATCGG
hungerfordiana.loc25#b	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTATCGG
hungerfordiana.loc26#a	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTATCGG
frequens	AAGTCGTAAC	AAGGTTTCCG	TAGGTGAACC	TGCGGAAGGA	TCATTATCGG

TABLE 1. Continued.

	160	170	180	190	200
hungerfordiana.loc5#a	TATAAT----	-----CATCA	GGCTGCAGCG	GGGCGCGCAG	CGGCTTATGA
hungerfordiana.loc5#b	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCGCAG	CGGCTTATGA
hungerfordiana.loc8#a	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCGCAG	CGGCTTATGA
hungerfordiana.loc8#b	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCGCAG	CGGCTTATGA
hungerfordiana.loc9#a	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCGCAG	CGGCTTATGA
hungerfordiana.loc16#a	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCGCAG	CAGCTTATGA
hungerfordiana.loc16#b	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCGCAG	CGGCTTATGA
hungerfordiana.loc22#a	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCGCAG	CGGCTTATGA
hungerfordiana.loc22#b	TATAAT----	-----CATCA	GGCATCAGCG	GGGCGCGCAG	CGGCTTATGA
hungerfordiana.loc23#a	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCACGCAG	CGGCTTGTGA
hungerfordiana.loc23#b	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCGCAG	CGGCTTGTGA
hungerfordiana.loc24#a	TACAAT----	-----CATCA	GGCAGCAGCG	GGGCGCGCAG	CGGCTTGTGA
hungerfordiana.loc24#b	TACAAT----	-----CATCA	GGCAGCAGCG	GGGCGCGCAG	CGGCTTGTGA
hungerfordiana.loc25#a	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCGCAG	CGGCTTGTGA
hungerfordiana.loc25#b	TATAAT----	-----CATCA	GGCAGCAGCG	GGGCGCGCAG	CGGCTTGTGA
hungerfordiana.loc26#a	TATAAT----	-----CATCA	GGCAGC----	----------	-GGCTTGTGA
frequens	TATTATTACA	AAATACGTCA	GGC-------	----------	------ATGA

	210	220	230	240	250
hungerfordiana.loc5#a	TGAAATTA--	---TGCTGAT	TGAACGTCTG	TC--------	----------
hungerfordiana.loc5#b	TGAAATTA--	---TGCTGAT	TGAACGTCTG	TC--------	----------
hungerfordiana.loc8#a	TGAAATTA--	---TGCTGAT	TGAACGTCTG	TC--------	----------
hungerfordiana.loc8#b	TAAAATTA--	---TGCTGGT	TGAACGTCTG	TC--------	----------
hungerfordiana.loc9#a	TGAAATTA--	---TGCTGAT	TGAACGTCTG	TC--------	----------
hungerfordiana.loc16#a	TGAAAATA--	---TGCTGAT	TGAACGTCTG	TC--------	----------
hungerfordiana.loc16#b	TGAAAATA--	---TGCTGAT	TGAACGTCTG	TC--------	----------
hungerfordiana.loc22#a	TGAAAATA--	---TGCTGAT	TGAACGTCTG	TC--------	----------
hungerfordiana.loc22#b	TGAAAATA--	---TGCTGAT	TGAACGTCTG	TC--------	----------
hungerfordiana.loc23#a	TGAAAATA--	---TGCTGAT	TGAACGCCTG	TC--------	----------
hungerfordiana.loc23#b	TGAAAATA--	---TGCTGAT	TGAACGCCTG	TC--------	----------
hungerfordiana.loc24#a	TGAAAATA--	---TGCTGAT	TGAACGCCTG	TC--------	----------
hungerfordiana.loc24#b	TGAAAATA--	---TACTGAT	TGAACGCCTG	TC--------	----------
hungerfordiana.loc25#a	TGAAAATA--	---TGCTGAT	TGAACGCCTG	TC--------	----------
hungerfordiana.loc25#b	TGAAAATA--	---TGCTGAT	TGAACGCCTG	TC--------	----------
hungerfordiana.loc26#a	TGAAAATA--	---TGCTGAT	TGAACGCCTG	TC--------	----------
frequens	TG--TATAGA	TAATGCTGAT	GGAACGTGTC	TCGTCTCGTC	TCGTCTCGTC

	260	270	280	290	300
hungerfordiana.loc5#a	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCCC	CGGTCGGTTG
hungerfordiana.loc5#b	---TCCCGTT	GCCAATCGGG	GACCGCAAGA	AGCGCCGCCC	CGGTCGGTTG
hungerfordiana.loc8#a	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCCC	CGGTCGGTCG
hungerfordiana.loc8#b	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCCC	CGGTCGGTCG
hungerfordiana.loc9#a	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCCC	CGGTCGGTTG
hungerfordiana.loc16#a	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCCC	CGGTCGGTTG
hungerfordiana.loc16#b	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCCC	CGGTCGGTTG
hungerfordiana.loc22#a	---TCCCGTT	GCCGATCGGG	GACCGCGAGA	AGCGCCGCCC	CGGTCGGTTG
hungerfordiana.loc22#b	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCCC	CGGTCGGTTG
hungerfordiana.loc23#a	---TCCCGTT	GCCGATCGGC	GACCGCAAGA	AGCGCCGCCC	CGGTCGGTTG
hungerfordiana.loc23#b	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCCC	CGGTCGGTTG
hungerfordiana.loc24#a	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCCC	CGGTCGGTTG
hungerfordiana.loc24#b	---TCCCGTT	GCCGATCGGG	GACCGcAAGA	AGCGCCGCCC	CGGTCGGTTG
hungerfordiana.loc25#a	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCCC	CGGTCGGTTG
hungerfordiana.loc25#b	---TCCCGTT	GCCGATCGGG	GACCGCAAGG	AGCGCCGCCC	CGGTCGGTTG
hungerfordiana.loc26#a	---TCCCGTT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCCC	CGGTCGGTTG
frequens	TCGTCTCATT	GCCGATCGGG	GACCGCAAGA	AGCGCCGCCC	CGGTCGGTTG

TABLE 1. Continued.

	310	320	330	340	350
hungerfordiana.loc5#a	ACCGCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana.loc5#b	ACCGCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana.loc8#a	ACCGCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana.loc8#b	ACCGCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana.loc9#a	ACCGCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana.loc16#a	ACCGCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana.loc16#b	ACCGCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana.loc22#a	ACCGCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana.loc22#b	ACCGCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana.loc23#a	ACCGCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana.loc23#b	ACCGCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana.loc24#a	ACCGCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana.loc24#b	ACCGCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana.loc25#a	ACCGCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana.loc25#b	ACCGCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
hungerfordiana.loc26#a	ACCGCTCCCC	TGTTTCGGGG	TACCTAGTCT	TATCTCGCAC	TCAATACGGC
frequens	ACCGCTCCCC	TGTTTCGGGG	TACCTAGTCT	CGTCTCGCAC	TCAATACGGC
	360	370	380	390	400
hungerfordiana.loc5#a	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGTCTAAGGA
hungerfordiana.loc5#b	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGTCTAAGGA
hungerfordiana.loc8#a	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGTCTAAGGA
hungerfordiana.loc8#b	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGTCTAAGGA
hungerfordiana.loc9#a	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGTCTAAGGA
hungerfordiana.loc16#a	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGTCTAAGGA
hungerfordiana.loc16#b	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGTCTAAGGA
hungerfordiana.loc22#a	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGTCTAAGGA
hungerfordiana.loc22#b	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGTCTAAGGA
hungerfordiana.loc23#a	CCACGGTGAC	GGCAAGAGCT	CTCAGCTCGC	CGGGTCGTCA	GGTCTAAGGA
hungerfordiana.loc23#b	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGTCTAAGGA
hungerfordiana.loc24#a	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGTCTAAGGA
hungerfordiana.loc24#b	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGTCTAAGGA
hungerfordiana.loc25#a	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGTCTAAGGA
hungerfordiana.loc25#b	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGTCTAAGGA
hungerfordiana.loc26#a	CCACGGTGAC	GGCAAGAGCT	TTCAGCTCGC	CGGGTCGTCA	GGTCTAAGGA
frequens	CCACGGTGAC	GGCATGAGCT	CTCAGCTCGC	CGGGTCGTCA	GGTCTAAGGA
	410	420	430	440	450
hungerfordiana.loc5#a	GCGCTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	GTTG-TGAGG
hungerfordiana.loc5#b	GCGCTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	GTTG-TGAGG
hungerfordiana.loc8#a	GCGCTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	GTTG-TGTGG
hungerfordiana.loc8#b	GCGCTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	GTTG-TGTGG
hungerfordiana.loc9#a	GCGCTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	GTTG-TGTGG
hungerfordiana.loc16#a	GCGCTGCTCC	GACTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-CGTGG
hungerfordiana.loc16#b	GCGCTGCTCC	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-CGTGG
hungerfordiana.loc22#a	GCGCTGCTCC	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-CGTGG
hungerfordiana.loc22#b	GCGCTGCTCC	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-CGTGG
hungerfordiana.loc23#a	GCGCTGCTCT	GATTGCCCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-TGTGG
hungerfordiana.loc23#b	GCGCTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-TGTGG
hungerfordiana.loc24#a	GCGCTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-TGTGG
hungerfordiana.loc24#b	GCGCTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-TGTGG
hungerfordiana.loc25#a	GCGCTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-TGTGG
hungerfordiana.loc25#b	GCGCTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-TGTGG
hungerfordiana.loc26#a	GCGCTGCTCT	GATTGCTCTG	TGAGCGGCGC	CGCCCCGGTG	ATTG-TGTGG
frequens	GCGCCGCTCT	GACTGCTCTA	TGAGCGGCGC	CGCCCCGGTA	GTTGGTGTGG

TABLE 1. Continued.

	460	470	480	490	500
hungerfordiana.loc5#a	-ATAATGGAG	G---------	---GTACCTG	TGCGCTCGAC	CCGCT-CTGC
hungerfordiana.loc5#b	-ATAATGGAG	G---------	---GTACCTG	TGCGCTCGAC	CCGCT-CTGC
hungerfordiana.loc8#a	-ATAATGGAG	G---------	---GTACCTG	TGCGCTCGAC	CCGCT-CTGC
hungerfordiana.loc8#b	-ATAATGGAG	G---------	---GTACCTG	TGCGCTCGAC	CCGCT-CTGC
hungerfordiana.loc9#a	-ATAATGGAC	G---------	---GTACCTG	TGCGCTCGAC	CCGCT-CTGC
hungerfordiana.loc16#a	GATAATGGAG	G---------	---GTACCTG	TGCGCTCGAC	CCGCT-CTGC
hungerfordiana.loc16#b	GATAATGGAG	G---------	---GTACCTG	TGCGCTCGAC	CCGCT-CTGC
hungerfordiana.loc22#a	GATAATGGAG	G---------	---GTACCTG	TGCGCTCGAC	CCGCT-CTGC
hungerfordiana.loc22#b	GATAATGGAG	G---------	---GTACCTG	TGCGCTCGAC	CCGCT-CTGC
hungerfordiana.loc23#a	-ATAACGGAG	G---------	---GTACCTG	TGCGCTCGAC	CCGCTGCTGC
hungerfordiana.loc23#b	-ATAACGGAG	G---------	---GTACCTG	TGCGCTCGAC	CCGCTGCTGC
hungerfordiana.loc24#a	-ATAACGGAG	G---------	---GTACCTG	TGCGCTCGAC	CCGCTGCTGC
hungerfordiana.loc24#b	-ATGACGGAG	G---------	---GTACCTG	TGCGCTCGAC	CCGCTGCTGC
hungerfordiana.loc25#a	-ATAACGGAG	G---------	---GTACCTG	TGCGCTCGAC	CCGCTGCTGC
hungerfordiana.loc25#b	-ATAACGGAG	G---------	---GTACCTG	TGCGCTCGAC	CCGCTGCTGC
hungerfordiana.loc26#a	-ATAACGGAG	G---------	---GTACCTG	TGCGCTCGAC	CCGCTGCTGC
frequens	-ATCAAGGAG	GCAAGGCCGG	AGGGTACCTG	TGCGCTCGAC	CG-CT-CTGC

	510	520	530	540	550
hungerfordiana.loc5#a	TCGGCGGATC	CGGGTGCGAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc5#b	TCGGCGGATC	CGGGTGCGAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc8#a	TCGGCGGATC	CGGGTGCGAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc8#b	TCGGCGGATC	CGGGTGCGAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc9#a	TCGGCGGATC	CGGGTGCGAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc16#a	TCGGCGGGTC	CGGGTGCGAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc16#b	TCGGCGGGTC	CGGGTGCGAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc22#a	TCGGCGGGTC	CGGGTGCGAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc22#b	TCGGCGGGTC	CGGGTGCGAT	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc23#a	TCGGCGGGTC	CGGGCGCGAG	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc23#b	TCGGCGGGTC	CGGGTGCGAG	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc24#a	TCGGCGGGTT	CGGGTGCGAG	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc24#b	TCGGCGGGTT	CGGGTGCGAG	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc25#a	TCGGCGGGTT	CGGGTGCGAG	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc25#b	TCGGCGGGTT	CGGGTGCGAG	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
hungerfordiana.loc26#a	TCGGCGGGTT	CGGGTGCGAG	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?
frequens	TCGGCGGCTC	TGGGTGCGAC	AGCTCCTGCG	GTGCAAACGC	AGGCCGCGA?

	560	570	580	590	600
hungerfordiana.loc5#a	GCTTAAAGA?	GTCGGCC-GT	A-----TGCT	CGAGCA?ACC	CGCCCGCTCC
hungerfordiana.loc5#b	GCTTAAAGA?	GTCGGCC-GT	A-----TGCT	CGAGCA?ACC	CGCCCGCTCC
hungerfordiana.loc8#a	GCTTAAAGA?	GTCGGCC-AT	A-TATATGCT	CG?GCA?ACC	CGCCCGCTCC
hungerfordiana.loc8#b	GCTTAAAGA?	GTCGGCC-AT	A-TATATGCT	CG?GCA?ACC	CGCCCGCTCC
hungerfordiana.loc9#a	GCTTAAAGA?	GTCGGCCCAT	A-----TGCT	CGAGCG?ACC	CGCCCGCTCC
hungerfordiana.loc16#a	GCTTAAAGA?	GTCGGCC-AT	G-----TGCT	CGAGCA?ACC	CGCCCGCTCC
hungerfordiana.loc16#b	GCTTGAAGA?	GTCGGCC-AT	G-----TGCT	CGAGCA?ACC	CGCCCGCTCC
hungerfordiana.loc22#a	GCTTAAAGA?	GTCGGCC-AT	G-----TGCT	CGAGCA?ACC	CGCCCGCTCC
hungerfordiana.loc22#b	GCTTAAAGA?	GTCGGCC-AT	G-----TGCT	CGAGCA?ACC	CGCCCGCTCC
hungerfordiana.loc23#a	GCTTAAAGA?	GTCGGCC-AC	G-----TGCT	CGAGCA?ACC	CGCCCGCTCC
hungerfordiana.loc23#b	GCTTAAAGA?	GTCGGCC-AT	G-----TGCT	CGAGCA?A-C	CGCCCGCTCC
hungerfordiana.loc24#a	GCTTAAAGA?	GTCGGCC-AT	G-----TGCT	CGAGCA?ACC	CGCCCGCTCC
hungerfordiana.loc24#b	GCTTAAAGA?	GTCGGCC-AT	G-----TGCT	CGAGCA?ACC	CGCCCGCTCC
hungerfordiana.loc25#a	GCTTAAAGA?	GTCGGCC-AA	G-----TGCT	CGAGCA?ACC	CGCCCGCTCC
hungerfordiana.loc25#b	GCTTAAAGA?	GTCGGCC-AA	G-----TGCT	CGAGCA?ACC	CGCCCGCTCC
hungerfordiana.loc26#a	GCTTAAAGA?	GTCGGCC-AT	G-----TGCT	CGAGCA?ACC	CGCCCGCTCC
frequens	GCTTAAAGA?	GTCGGCC-AT	GCTCGCGGCT	-GACCC-GCC	CGCCC--T--

TABLE 1. Continued.

	610	620	630	640	650
hungerfordiana.loc5#a	GTCTTCCT--	TATTT-AATT	TGTTACGCTT	GGTGGCTCGT	CTGTCTTATT
hungerfordiana.loc5#b	GTCTTCCT--	TATTT-AATT	TGTTACGCTT	GGTGGCTCGT	CTGTCTTATT
hungerfordiana.loc8#a	GTCTTCCT--	TATTT-AATT	TGTTACGCTT	GGTGGCTCGT	CTGTCTTATT
hungerfordiana.loc8#b	GTCTTCCT--	TATTT-AATT	TGTTACGCTT	GGTGGCTCGT	CTGTCTTATT
hungerfordiana.loc9#a	GTCTTCCT--	TATTT-AATT	TGTTACGCTT	GGTGGCTCGT	CTGTCTTATT
hungerfordiana.loc16#a	GTCTTCCT--	TATTT-AATT	TGTTACGCTT	GGTGGCTTGT	CTGTCTTATT
hungerfordiana.loc16#b	GTCTTCCT--	TATTT-AATT	TGTTACGCTT	GGTGGCTCGT	CTGTCTTATT
hungerfordiana.loc22#a	GTCTTCCT--	TATTT-AATT	TGTTACGCTT	GGTGGCTCGT	CTGTCTTATT
hungerfordiana.loc22#b	GTCTTCCT--	TATTT-AATT	TGTTACGCTT	GGTGGCTCGT	CTGTCTTATT
hungerfordiana.loc23#a	GCCTTCCT--	TATTT-AATT	TGTTACGCTT	GCTGGCTCGT	CTGTCCTATT
hungerfordiana.loc23#b	GCCTTCCT--	CATTT-AATT	TGTTACGCTT	GCTGGCTCGT	CTGTCCTATT
hungerfordiana.loc24#a	GCCTTCCT--	TATTT-AATT	TGTTACGCTT	GCTGGCTCGT	CTGTCCTATT
hungerfordiana.loc24#b	GCCTTCCT--	TATTT-AATT	TGTTACGCTT	GCTGGCTCGT	CTGTCCTATT
hungerfordiana.loc25#a	GCCTTCCT--	TATTT-AATT	TGTTACGCTT	GCTGGCTCGT	CTGTCCTATT
hungerfordiana.loc25#b	GCCTTCCT--	TATTT-AATT	TGTTACGCTT	GCTGGCTCGT	CTGTCCTATT
hungerfordiana.loc26#a	GCCTTCCT--	TATTT-AATT	TGTTACGCTT	GCTGGCTCGT	CTGTCCTATT
frequens	GTC-TCCTCT	CATTTTATTT	TGTTACGCT-	----------	-TGTCCGAT-

	660	670	680	690	700
hungerfordiana.loc5#a	TGTCAGTTAC	CGAAAAA---	---------C	AAGATTGCTT	GTCGTACAAC
hungerfordiana.loc5#b	TGTCGGTTAT	CGAAAAA---	---------C	AAGATTGCTT	GTCGTACAAC
hungerfordiana.loc8#a	TGTCCGTTAT	CGAAAAA---	---------C	AAGATTGCTT	GTCGTACAAC
hungerfordiana.loc8#b	TGTCGGTTAT	CGAAAAG---	---------C	AAGATTGCTT	GTCGTACAAC
hungerfordiana.loc9#a	TGTCGGTTAT	CGAAAAA---	---------C	AAGATTGCTT	GTCGTACGAC
hungerfordiana.loc16#a	TGTCGGTTAT	CGAAAAA---	---------C	AAGATTGCTT	GTCGTACAAC
hungerfordiana.loc16#b	TGTCGGTTAT	CGAAAAA---	---------C	AAGATTGCTT	GTCGTACAAC
hungerfordiana.loc22#a	TGTCGGTTAT	CGAAAAA---	---------C	AAGATTGCTT	GTCGTACAAC
hungerfordiana.loc22#b	TGTCGGTTAT	CGAAAAA---	---------C	AAGATTGCTT	GTCGTACAAC
hungerfordiana.loc23#a	TGTCGGTTAT	CGAAAAA--A	AAAACAAA-C	AAGATTGCTT	GTCGTACAAC
hungerfordiana.loc23#b	TGTCGGTTAT	C?AAAAAAAA	AAAAC?AAAC	AA?ATTGCTT	GTCGT?CAAC
hungerfordiana.loc24#a	TGTCGGTTAT	CGAAAAAAAA	AAAA-----C	AAGATTGCTT	GTCGTACAAC
hungerfordiana.loc24#b	TGTCGGTTAT	CGAAAAAAAA	AAA------C	AAGATTGCTT	GTCGTACAAC
hungerfordiana.loc25#a	TGTCGGTTAT	CGAAAAAAAA	A--------C	AAGATTGCTT	GTCGTACAAC
hungerfordiana.loc25#b	TGTCGGTTAT	CGAAAAAAAA	A--------C	AAGATTGCTT	GTCGTACAAC
hungerfordiana.loc26#a	TGTCGGTTAT	CGAAAAA---	-AAACAAAAC	AAGATTGCTT	GTCGTACAAC
frequens	----GGTTAT	CGAAAAA-CC	AAAACAAAA-	-----TGCTT	GTCGTACAAC

	710	720	730	740	750
hungerfordiana.loc5#a	TTTGAGCGGT	GGATCACTCG	GCTCGTGCGT	CGATGAAGAG	CGCAACCAGC
hungerfordiana.loc5#b	TTTGAGCGGT	GGATCACTCG	GCTCGTGCGT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc8#a	TTTGAGCGGT	GGATCACTCG	GCTCGTGCGT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc8#b	TTTGAGCGGT	GGATCACTCC	GCTCGTGCGT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc9#a	TTTGAGCGGT	GGATCACTCG	GCTCGTGCGT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc16#a	TTTGAGCGGT	GGATCACTCG	GCTCGTGCGT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc16#b	TTTGAGCGGT	GGATCACTCG	GCTCGTGCGT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc22#a	TTTGAGCGGT	GGATCACTCG	GCTCGTGCGT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc22#b	TTTGAGCGGT	GGATCACTCG	GCTCGTGCGT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc23#a	TTTGAGCGGT	GGATCACTCG	GCTCGTGCGT	CGATGAAGAG	CGCGGCCGGC
hungerfordiana.loc23#b	TTT???????	??????????	??????????	??????????	??????????
hungerfordiana.loc24#a	TTTGAGCGGT	GGATCACTCG	GCTCGTGCGT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc24#b	TTTGAGCGGT	GGATCACTCG	GCTCGTGCGT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc25#a	TTTGAGCGGT	GGATCACTCG	GCTCGTGCGT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc25#b	TTTGAGCGGT	GGATCACTCG	GCTCGTGCGT	CGATGAAGAG	CGCAGCCAGC
hungerfordiana.loc26#a	TTTGAGCGGT	GGATCACTCG	GCTCGTGCGT	CGATGAAGAG	CGCAGCCAGC
frequens	TTTGAGCGGT	GGATCACTCG	GCTCGTGCGT	CGATGAAGAG	CGCAGCCAGC

TABLE 1. Continued.

	760 770 780 790 800
hungerfordiana.loc5#a	TGCGTGAATT AATGTGAATT GCAGAACACA
hungerfordiana.loc5#b	TGCGTGAATT AATGTGAATT GCAGAACACA
hungerfordiana.loc8#a	TGCGTGAATT AATGTGAATT GCAGAACACA
hungerfordiana.loc8#b	TGCGTGAATT AATGTGAATT GCAGAACACA
hungerfordiana.loc9#a	TGCGTGAATT AATGTGAATT GCAGAACACA
hungerfordiana.loc16#a	TACGTGAATT AATGTGAATT GCAGAACACA
hungerfordiana.loc16#b	TGCGTGAATT AATGTGAATT GCAGAACACA
hungerfordiana.loc22#a	TGCGTGAATT AATGTGAATT GCAGAACACA
hungerfordiana.loc22#b	TGCGTGAATT AATGTGAATT GCAGAACACA
hungerfordiana.loc23#a	TGCGTGAATT AATGTGAATT GCAGAACACA
hungerfordiana.loc23#b	?????????? ?????????? ??????????
hungerfordiana.loc24#a	TGCGTGAATT AATGTGAATT GCAGAACACA
hungerfordiana.loc24#b	TGCGTGAATT AATGTGAATT GCAGAACACA
hungerfordiana.loc25#a	TGCGTGAATT AATGTGAATT GCAGAACACA
hungerfordiana.loc25#b	TGCGTGAATT AATGTGAATT GCAGAACACA
hungerfordiana.loc26#a	TGCGTGAATT AATGTGAATT GCAGAACACA
frequens	TGCGTGAATT AATGTGAATT GCAGAACACA

TABLE 2. Pairwise Kimura's 2-parameter distances among the *G. hungerfordiana* sequences.

hungerfordiana.loc5#a	0.0000															
hungerfordiana.loc5#b	0.0085	0.0000														
hungerfordiana.loc8#a	0.0129	0.0107	0.0000													
hungerfordiana.loc8#b	0.0193	0.0150	0.0084	0.0000												
hungerfordiana.loc9#a	0.0128	0.0085	0.0064	0.0107	0.0000											
hungerfordiana.loc16#a	0.0324	0.0280	0.0259	0.0301	0.0236	0.0000										
hungerfordiana.loc16#b	0.0280	0.0236	0.0215	0.0258	0.0193	0.0085	0.0000									
hungerfordiana.loc22#a	0.0280	0.0236	0.0215	0.0258	0.0193	0.0085	0.0042	0.0000								
hungerfordiana.loc22#b	0.0281	0.0237	0.0216	0.0259	0.0193	0.0085	0.0042	0.0042	0.0000							
hungerfordiana.loc23#a	0.0394	0.0349	0.0328	0.0372	0.0304	0.0282	0.0238	0.0238	0.0238	0.0000						
hungerfordiana.loc23#b	0.0481	0.0436	0.0414	0.0458	0.0391	0.0368	0.0324	0.0324	0.0324	0.0126	0.0000					
hungerfordiana.loc24#a	0.0414	0.0365	0.0349	0.0392	0.0324	0.0302	0.0258	0.0258	0.0258	0.0063	0.0147	0.0000				
hungerfordiana.loc24#b	0.0459	0.0413	0.0392	0.0436	0.0369	0.0345	0.0302	0.0302	0.0302	0.0105	0.0190	0.0042	0.0000			
hungerfordiana.loc25#a	0.0415	0.0370	0.0349	0.0392	0.0325	0.0302	0.0258	0.0259	0.0259	0.0064	0.0127	0.0042	0.0084	0.0000		
hungerfordiana.loc25#b	0.0437	0.0392	0.0370	0.0414	0.0347	0.0324	0.0280	0.0280	0.0280	0.0085	0.0148	0.0063	0.0105	0.0021	0.0000	
hungerfordiana.loc26#a	0.0405	0.0359	0.0337	0.0382	0.0313	0.0289	0.0244	0.0244	0.0244	0.0043	0.0108	0.0022	0.0065	0.0022	0.0044	0.0000