# Genetic Diversity of *Trichomonas vaginalis* Clinical Isolates Determined by *Eco*RI Restriction Fragment Length Polymorphism of Heat-Shock Protein 70 Genes

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## Abstract

Restriction fragment length polymorphism (RFLP) analysis using a multilocus heat-inducible cytoplasmic heat-shock protein 70 (Hsp70) hybridization probe with *Eco*RI-digested genomic DNA was used in molecular typing of 129 *Trichomonas vaginalis* isolates. Results indicate that *Trichomonas* organisms exhibit considerable polymorphism in their Hsp70 RFLP patterns. Analysis of seven American Type Culture Collection reference strains and 122 clinical isolates, including 84 isolates from Jackson, Mississippi, 18 isolates from Atlanta, Georgia, and 20 isolates from throughout the United States, showed 105 distinct Hsp70 RFLP pattern subtypes for *Trichomonas*. Phylogenetic analysis of the Hsp70 RFLP data showed that the *T. vaginalis* isolates were organized into two clonal lineages. These results illustrate the substantial genomic diversity present in *T. vaginalis* and indicate that a large number of genetically distinct *Trichomonas* isolates may be responsible for human trichomoniasis in the United States.

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## Introduction

*Trichomonas vaginalis* is a flagellated protozoan parasite that infects the urogenital tract of humans, causing the most common non-viral, sexually transmitted disease in the world. The annual global incidence of trichomoniasis is greater than 170 million cases, including 7.4 million in the United States.[1,2] The prevalence of trichomoniasis among reproductive-age women in the United States, 3.1% overall, shows a pronounced racial disparity, 1.3% in non-Hispanic white women versus 13.3% in non-Hispanic black women, and can exceed 50% in sexually transmitted disease clinic patients and high-risk adolescents.[3,4] Trichomoniasis is associated with vaginitis, cervecitis, dysuria, and urethritis, and is a risk factor for herpes simplex virus type 2 infection, human immunodeficiency virus transmission and acquisition, and possibly pelvic inflammatory disease and cervical neoplasia.[5–10] *Trichomonas* infection in pregnant women increases the incidence of preterm delivery, premature rupture of membranes, low birth weight infants, and can be transmitted to neonates during passage through the birth canal.[11–13] In men, trichomoniasis is a cause of chronic prostatitis and non-gonococcal urethritis.[14] Despite serious health consequences, efforts to detect and eliminate *Trichomonas* are confounded by large numbers of asymptomatic infections and the presence of drug-resistant strains.[15] In addition, the relationships between *Trichomonas* strains and the variable clinical manifestations of trichomoniasis are not well understood, in part because of the lack of reliable and widely accepted techniques to type and track individual isolates.

Multiple approaches to typing *Trichomonas* isolates have been described; antigenic characterization, isozyme analysis, repetitive sequence hybridization, ribosomal gene and intergenic region sequence polymorphisms, pulsed-field gel electrophoresis, random amplified polymorphic DNA analysis, and restriction fragment length polymorphism (RFLP).[16–26] These studies produced differing results, even when using similar techniques, in attempting to demonstrate concordance between parasite genotypes and phenotypic expressions during infection, such as virulence and metronidazole resistance. The *T. vaginalis* genome composition provides a potential explanation for this difficulty in correlating genotype with phenotype.[27] The *Trichomonas* genome is highly repetitive with 65% of the genome consisting of repeated sequences, including 59 repeat families of transposable elements representing 25% of the genome. The movement of these transposable elements through the genome can generate considerable genetic diversity. The population structure of *Trichomonas* has been proposed to be clonal in nature because there is little evidence of sexual recombination and segregation. [28] A consequence of clonality in *Trichomonas* is that in the presence of active mechanisms to generate diversity, genetic differences can become fixed in the population and lead to a large and diverse population structure.

In this study, we assess the level of genetic diversity in *T. vaginalis* isolates using restriction fragment length polymorphism of cytoplasmic heat-shock protein 70 (Hsp70) genes.[25] The cHsp70s in *Trichomonas* are a large multilocus gene family present in at least eight nearly identical copies (96–97% sequence identity).[27] The use of a multilocus gene family for *Trichomonas* genome analysis provides a tool with considerable discrimination power to analyze genetic diversity in *T. vaginalis* isolates.

## Materials and Methods

### Trichomonas parasites

*Trichomonas vaginalis* isolates were obtained from the American Type Culture Collection (ATTC, Manassas, VA), the Centers for Disease Control and Prevention (CDC, Atlanta, GA), and urban teaching hospitals in Jackson, Mississippi, and Atlanta, Georgia. Reference strains were obtained from ATCC or provided by the CDC (ATCC and CDC isolates). Twenty isolates were obtained from cultures submitted to the CDC for metronidazole susceptibility testing

(MSA isolates) after patients did not respond to a minimum of two courses of metronidazole treatment. Eighteen isolates were cultured from women at a teaching hospital in Atlanta, Georgia (GMH isolates).[19] Eighty four isolates came from sexually transmitted disease clinic or emergency department patients at the University of Mississippi Medical Center (UMC), Jackson, Mississippi. This set included isolates from males presenting with urethritis (B isolates, n = 6), women participating in a trial of oral versus intravaginal metronidazole treatment[29] (C isolates, n = 9), a study of single versus multiple doses of metronidazole treatment (D isolates, n = 9), women with vaginitis and their sexual partners when available (P, S, and T isolates, n = 8), and random culture of urine specimens, regardless of patient symptoms, submitted to the UMC emergency department (E isolates, n = 52). Informed consent was obtained when required by the UMC Institutional Review Board for Human Investigation. *Trichomonas* trophozoites were cultured from urethral/penile discharge and posterior vaginal fornix or urine sediment in trypticase-yeast maltose medium and harvested as previously reported.[19,25]

### Nucleic acid isolation

Genomic DNA was isolated from log phase ($10^6$ parasites/mL) cultures using a DNA extraction kit (CDC, GMH, and MSA isolates) or a modified DEPC-Triton X-100 method (ATCC, B, C, D, E, P, S, and T isolates).[19,25] Purity and concentration of DNA were determined by optical density measurements at 260 nm and 280 nm.

### Hsp70 RFLP analysis

Two-microgram aliquots of *T. vaginalis* genomic DNA were digested with *Eco*RI, resolved by electrophoresis for at 1,305–1,400 volts/hour in 24 cm × 7 mm, 0.9% agarose gels, transferred to nylon membranes, and hybridized with a radiolabelled 1.8-kb *T. vaginalis* cytoplasmic Hsp70 cDNA fragment (GenBank U93873, nt 31–1832).[30] Blots were washed to a final stringency of 0.2× SSC (30 mM NaCl, 3 mM sodium citrate), 0.5% sodium dodecyl sulfate at 45–55°C and exposed for autoradiography at −70°C.

### Phylogenetic analysis

The phylogenetic relatedness of the *T. vaginalis* isolates was assessed using FreeTree, a software program designed for analysis of binary character data (presence or absence of character state) generated in DNA fingerprinting methods.[31] The genetic distances between isolates were calculated using the Nei-Li coefficient of similarity and a phylogenetic tree constructed from the distance matrix using the neighbor-joining method.[32,33] Statistical support for topological elements (nodes) within the phylogram was estimated by operational taxonomic unit–based jackknifing with 10,000 re-sampled datasets.[31]

## Results

A total of 129 *Trichomonas* clinical isolates obtained from 127 individual patients were typed by Hsp70 RFLP of *Eco*RI-digested genomic DNA. These isolates are listed in Table 1 with the year of initial culture, sex of patient, and geographic origin included when known. Eighty four isolates were obtained in Jackson, Mississippi, 18 in Atlanta, Georgia, and 25 from diverse locations across the United States; the geographic origin of 2 strains was not available. Although most of the *Trichomonas* isolates were obtained from symptomatic women, the 52 E series isolates (E1–E98, Table 1) were cultured at random and may include asymptomatic patients. The E series study showed an infection rate of 8% (100 of 1,248 samples collected) in UMC emergency room patients, 9.4% in females, and 0.9% in males.

Figure 1 shows a representative Southern blot with Hsp70 RFLP patterns for 12 *Trichomonas* isolates. A common set of *Trichomonas* isolates was included in each analysis

to facilitate identification of individual bands and comparison of different Southern blot results. In each isolate tested, 7–11 distinct DNA bands, ranging in size from 1.8 kb to 22.5 kb, were detected. Examples of isolates producing 7 bands (D1, B137, B157, and D10), 8 bands (B114), 9 bands (B110, P1 and T1F), and 10 bands (C3) are shown in Figure 1. More than 50% of *T. vaginalis* isolates contained 9 bands (n = 68), 35 isolates had 8 hybridizing DNA bands, and isolates with 7 (n = 16), 10 (n = 7), or 11 (n = 3) hybridizing bands were less frequent. Thirty-one DNA fragments were identified; Hsp70 RFLP fragments ranging in size from 1.8 kb to 22.5 kb are shown in Figure 1. The observed frequency for each band in the 129 *T. vaginalis* isolates is shown in Table 2. Four bands, 2.7 kb, 3.7 kb, 5.6 kb, and 5.8 kb (Figure 1), were present in nearly all isolates tested but many bands were represented in fewer isolates; 10 bands were each present in less than 9 isolates. The Hsp70 RFLP patterns were reproducible because 72 isolates were run multiple times, including reference isolates run 7–15 times each, and in every instance an identical Hsp70 RFLP pattern was produced. The Hsp70 RFLP patterns from individual *T. vaginalis* isolates were also stable over time, generating identical patterns from DNA samples obtained several years apart.

The Hsp70 RFLP patterns indicate a high degree of diversity among *Trichomonas* clinical isolates. A total of 105 different Hsp70 RFLP patterns were observed; 85 isolates produced unique patterns and 20 additional Hsp70 RFLP patterns were shared among 44 isolates. Table 3 shows the isolates that comprise these 20 shared patterns; most are paired identical isolates but two groupings contain four isolates each (MSA826, D4, T1F, T1M and D1, D2, D5, D10). In Figure 1, seven Hsp70 RFLP patterns are seen, including matching patterns for D4–T1M–T1F, D1–D10, and B137–B157. Multiple identical Hsp70 RFLP patterns were obtained from isolates widely separated in time of collection and in geographic origin: GMH1 (Atlanta, GA, 1997) and E8 (Jackson, MS, 2000); MSA812 (Birmingham, AL, 1996) and E19 (Jackson, MS, 2001); MSA819 (Brooklyn, NY, 1998) and GMH17 (Atlanta, GA, 1999); MSA826 (Grover Beach, CA, 1996) and three strains from Jackson, MS, D4 (1994) and T1M and T1F (2000); MSA830 (Great Bend, KS, 1997) and E40 (Jackson, MS, 2001); MSA836 (Greenville, SC, 1997) and GMH10 (Atlanta, GA, 1996); and MSA844 (Rochester, NY, 1998), which is identical to E35 (Jackson, MS, 2001). These results illustrate the widespread dispersion of individual isolates as well as their temporal stability over at least six years. One pair of sexual partners in the study (T1F and T1F, Figure 1) had the same Hsp70 RFLP pattern but another partner pair (S1M and S1F) had different patterns. Also of interest, the *Trichomonas* isolate cultured from patient D4 prior to metronidazole treatment differed from isolate D4F cultured after treatment, indicating that re-infection rather than treatment failure had occurred.

The phylogenetic relatedness of the 129 *Trichomonas* isolates was determined by analysis of their Hsp70 RFLP patterns using the program FreeTree.[31] A phylogram built from the Nei-Li distance matrix using the neighbor-joining method for tree construction is shown in Figure 2. This phylogenetic analysis shows two major groups of *T. vaginalis* strains: group I with 78 isolates consisting of three subgroups (A, B, and C) of 37, 33, and 8 *T. vaginalis* isolates, respectively, and group II with 51 isolates consisting of two subgroups (D and E) containing 28 and 23 isolates, respectively. Each of these groups and subgroups is characterized by deeply diverging branches containing individual *T. vaginalis* isolates. The operational taxonomic unit–based jackknifing values calculated for each node indicate that there is excellent statistical support for the placement of *T. vaginalis* isolates within the phylogram, especially at the most distal nodes. The long terminal branches and the high bootstrap support values at the distal nodes are suggestive of a clonal radiation of *T. vaginalis* with little genetic flow between the different strains. Isolates possessing 7–10 hybridizing bands were distributed throughout the phylogram with representative members present in subgroups A–E, but the three isolates that contained 11 hybridizing bands (E17, E83, E95) were all located in subgroup E. The *T. vaginalis* isolates are also distributed throughout the figure without apparent regard to their geographical origin or sex of the patient. Although additional phenotypic information such as

metronidazole sensitivity and presence of *Trichomonas* virus was available for some isolates analyzed, these data were not sufficient to permit any determination of concordance between these traits and the phylogenetic relationships shown here.

## Discussion

The genetic diversity among *T. vaginalis* isolates is considerable with 105 unique Hsp70 RFLP patterns identified in the 129 isolates analyzed. The Hsp70 RFLP patterns are reproducible, temporally stable over six years, and geographically disjunct. The detection of genetically identical isolates from diverse geographic locations is consistent with the theory of clonal propagation proposed for *T. vaginalis*, suggesting a common clonal descent.[28] Significant genetic variability in *T. vaginalis* can exist even in a localized environment; the 84 isolates from Jackson, Mississippi and 18 isolates from Atlanta, Georgia produced 69 and 17 different Hsp70 RFLP patterns respectively. Much of the observed variability can be attributed to differences in hybridizing bands $\geq 26.5$ kb, each present in 37% or less of isolates, and bands present in 80–100% of isolates are $\geq 25.8$ kb. The large, diverse set of repeated, mobile genetic elements in the *T. vaginalis* genome provides a mechanism for generating size diversity.[27] Similar repetitive families are present in other human parasites with clonal population structures such as *Leishmania*, *Plasmodium*, *Giardia*, and *Trypanosoma*.[34] The ability of repeat elements to increase in copy number and transpose throughout the genome would naturally have an increased impact on larger DNA fragments. However, the preservation of 2.7 kb, 3.7 kb, 5.5 kb, and 5.8 kb Hsp70 RFLP fragments in nearly all *T. vaginalis* isolates indicates that transposon-mediated size changes are constrained in the sequences surrounding some *T. vaginalis* Hsp70 genes. The variable number of Hsp70 hybridizing bands detected in individual isolates, ranging from 7 to 11, predominantly reflects differences in Hsp70 copy number. The *Trichomonas* genome is highly repetitive with many multi-copy genes, and the existence of gene copy number variation between different *T. vaginalis* isolates has been demonstrated. [20,27] It has also been proposed that *Trichomonas* has undergone a recent and substantial increase in genome size caused by duplication.[27] However, six isolate pairs differ by the conversion of a larger band to two smaller bands of corresponding size, indicating that *Eco*RI restriction site polymorphism within Hsp70 genes may account for some of this heterogeneity.

Diversity among *Trichomonas* isolates has been observed in data from prior studies characterizing antigenic, enzymatic, or genetic markers.[16–26] However, those analyses either used a limited number of strains and were not informative of the larger *T. vaginalis* population structure, or were based on random amplified polymorphic DNA analysis, which can be compromised by problems with reproducibility caused by sensitivity of the PCR and contaminating DNA from mycoplasma infection.[35] The Hsp70 RFLP technique used here, coupled with the large number of isolates examined, offers a reliable and reproducible approach for demonstrating genetic diversity in *Trichomonas*. Although there are potential biases inherent in sample selection for this study, E series isolates cultured from randomly selected urine samples could represent *T. vaginalis* strains with increased survivability in urine and random selection could also produce multiple isolates from the same patient, it is difficult to envision how these biases could significantly impact our findings. In the United States, *T. vaginalis* isolates are diverse and are comprised of two major clonal lineages. Whether this population structure proves to be representative of *T. vaginalis* isolates across the globe is an interesting objective for future studies.

Trichomoniasis is characterized by significant heterogeneity in its clinical manifestations such as pathogenicity, metronidazole sensitivity, sequelae to infection, and susceptibility to acquisition of other infectious agents. Whether this clinical variability is caused by host factors or to differences in the phenotypic expression of individual *T. vaginalis* isolates is not clear. However, the genetic diversity among *Trichomonas* isolates demonstrated here suggests that

an association between *Trichomonas* genotypes and highly variable clinical pathology may exist. Understanding this relationship will be a critical factor in devising strategies to control this disease and its clinical sequelae. Similar phenotypic variation in the course of human infection has been noted for other pathogens, which exhibit substantial genomic variation, including *Leishmania* species, *Trypanosoma cruzi*, *Toxoplasma gondii*, and *Plasmodium falciparum*.[36–39]

Genetic diversity in *Trichomonas* is an attribute that will need to be carefully considered in future investigations of trichomoniasis. The recent advent of proteomic and genomic techniques based on the *T. vaginalis* sequence presents an opportunity to elucidate the genetic factors controlling clinical manifestations, association with other disease entities, and drug resistance. The selection of *Trichomonas* isolates used to characterize the basis for its biologic variability will need to focus on those that are genetically similar to maximize the discovery of relevant genetic elements and avoid differences rooted in genetic diversity. In addition, efforts to design a vaccine for trichomoniasis will benefit from an understanding of the genetic diversity present in *Trichomonas* by focusing efforts on antigenic determinants which are broadly representative of the *T. vaginalis* population.

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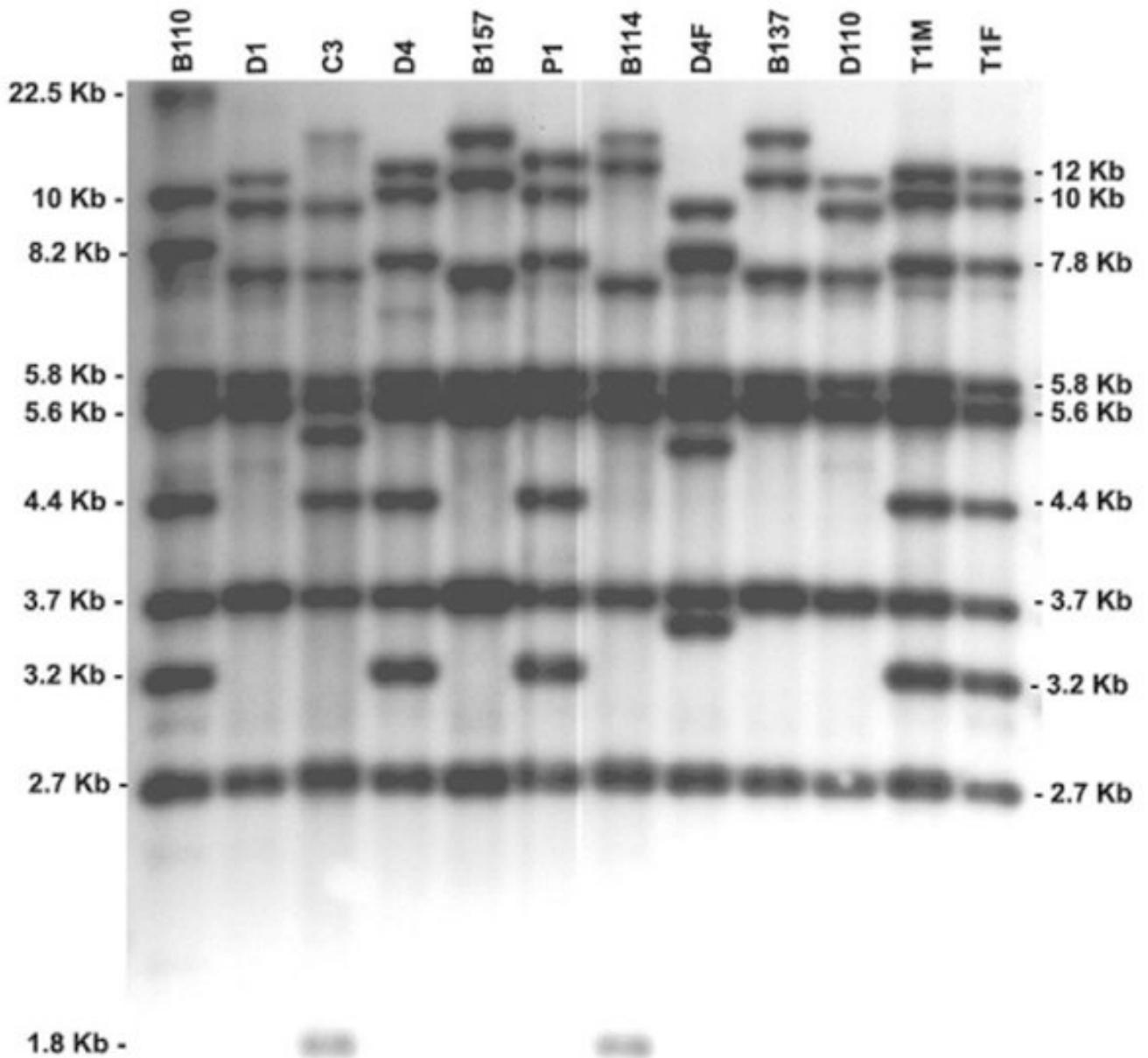
**Figure 1.**
Restriction fragment length polymorphism of *Trichomonas vaginalis* isolates analyzed on Southern blots containing 1–2 μg *Eco*RI-digested genomic DNA hybridized with a *T. vaginalis* cytoplasmic heat-shock protein 70 (HSP70) cDNA probe. Selected Hsp70 RFLP band sizes in kilobases, calculated from the migration of molecular weight markers (not shown), are indicated in the left and right margins. *Trichomonas* isolates, labeled above each lane are described in Table 1.
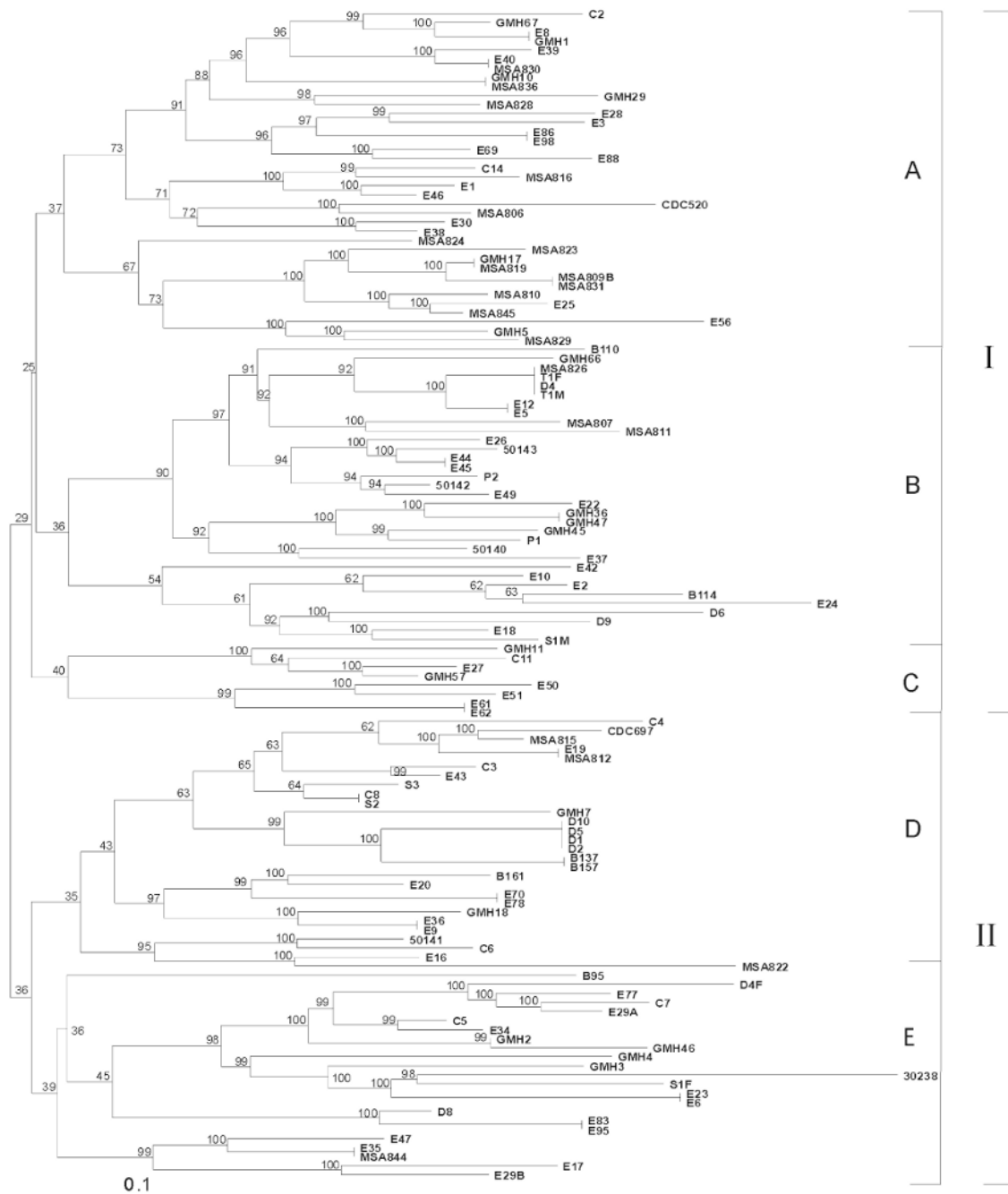
**Figure 2.**
Phylogenetic tree of 129 *Trichomonas vaginalis* isolates constructed on the basis of the presence or absence of 31 individual heat-shock protein 70 (Hsp70) RFLP bands using the phylogeny inference package FreeTree.[33] Numbers at the upper left of each node show the operational taxonomic unit jackknifing percent values based on 10,000 re-sampled datasets, which estimate statistical support for the existence of those branches. The sum of the horizontal branch lengths between individual *Trichomonas* isolates reflects the relative genetic distance separating the isolates.

**Table 1**

*Trichomonas vaginalis* isolates analyzed by heat-shock protein 70 restriction fragment length polymorphism*

| Isolate | Year | Sex of patient | Location |
|---|---|---|---|
| ATCC30238 | 1963 | F | Baltimore, MD |
| ATCC50140 | 1983 | F | MA |
| ATCC50141 | 1983 | F | CT |
| ATCC50142 | 1983 | F | NY |
| ATCC50143 | 1980 | F | Columbus, OH |
| CDC520 | | F | |
| CDC697 | | F | |
| B95 | 1993 | M | Jackson, MS |
| B110 | 1993 | M | Jackson, MS |
| B114 | 1993 | M | Jackson, MS |
| B137 | 1993 | M | Jackson, MS |
| B157 | 1993 | M | Jackson, MS |
| B161 | 1993 | M | Jackson, MS |
| C2 | 1993 | F | Jackson, MS |
| C3 | 1993 | F | Jackson, MS |
| C4 | 1993 | F | Jackson, MS |
| C5 | 1993 | F | Jackson, MS |
| C6 | 1993 | F | Jackson, MS |
| C7 | 1993 | F | Jackson, MS |
| C8 | 1993 | F | Jackson, MS |
| C11 | 1993 | F | Jackson, MS |
| C14 | 1993 | F | Jackson, MS |
| D1 | 1994 | F | Jackson, MS |
| D2 | 1994 | F | Jackson, MS |
| D4 | 1994 | F | Jackson, MS |
| D4F | 1994 | F | Jackson, MS |
| D5 | 1994 | F | Jackson, MS |
| D6 | 1994 | F | Jackson, MS |
| D8 | 1994 | F | Jackson, MS |
| D9 | 1994 | F | Jackson, MS |
| D10 | 1994 | F | Jackson, MS |
| E1 | 2000 | F | Jackson, MS |
| E2 | 2000 | F | Jackson, MS |
| E3 | 2000 | F | Jackson, MS |
| E5 | 2000 | | Jackson, MS |
| E6 | 2000 | | Jackson, MS |
| E8 | 2000 | | Jackson, MS |
| E9 | 2001 | F | Jackson, MS |
| E10 | 2001 | F | Jackson, MS |
| E12 | 2001 | | Jackson, MS |
| E16 | 2001 | F | Jackson, MS |
| E17 | 2001 | F | Jackson, MS |
| E18 | 2001 | F | Jackson, MS |
| E19 | 2001 | F | Jackson, MS |
| E20 | 2001 | | Jackson, MS |
| E22 | 2001 | M | Jackson, MS |
| E23 | 2001 | F | Jackson, MS |
| E24 | 2001 | M | Jackson, MS |
| E25 | 2001 | | Jackson, MS |
| E26 | 2001 | | Jackson, MS |
| E27 | 2001 | F | Jackson, MS |
| E28 | 2001 | | Jackson, MS |
| E29A | 2001 | | Jackson, MS |
| E45 | 2001 | | Jackson, MS |
| E46 | 2001 | | Jackson, MS |
| E47 | 2001 | | Jackson, MS |
| E49 | 2001 | F | Jackson, MS |
| E50 | 2001 | F | Jackson, MS |
| E51 | 2001 | F | Jackson, MS |
| E56 | 2001 | F | Jackson, MS |
| E61 | 2001 | F | Jackson, MS |
| E62 | 2001 | F | Jackson, MS |
| E69 | 2001 | F | Jackson, MS |
| E70 | 2001 | F | Jackson, MS |
| E77 | 2001 | F | Jackson, MS |
| E78 | 2001 | M | Jackson, MS |
| E83 | 2001 | F | Jackson, MS |
| E86 | 2001 | F | Jackson, MS |
| E88 | 2001 | F | Jackson, MS |
| E95 | 2001 | F | Jackson, MS |
| E98 | 2001 | F | Jackson, MS |
| P1 | 1995 | F | Jackson, MS |
| P2 | 1995 | F | Jackson, MS |
| S1M | 1998 | M | Jackson, MS |
| S1F | 1998 | F | Jackson, MS |
| S2 | 1998 | F | Jackson, MS |
| S3 | 1998 | F | Jackson, MS |
| T1M | 2000 | M | Jackson, MS |
| T1F | 2000 | F | Jackson, MS |
| GMH1 | 1997 | F | Atlanta, GA |
| GMH2 | 1997 | F | Atlanta, GA |
| GMH3 | 1997 | F | Atlanta, GA |
| GMH4 | 1997 | F | Atlanta, GA |
| GMH5 | 1997 | F | Atlanta, GA |
| GMH7 | 1997 | F | Atlanta, GA |
| GMH10 | 1997 | F | Atlanta, GA |
| GMH11 | 1997 | F | Atlanta, GA |
| GMH17 | 1997 | F | Atlanta, GA |
| GMH18 | 1997 | F | Atlanta, GA |
| GMH29 | 1997 | F | Atlanta, GA |
| GMH36 | 1997 | F | Atlanta, GA |
| GMH45 | 1997 | F | Atlanta, GA |
| GMH46 | 1997 | F | Atlanta, GA |
| GMH47 | 1997 | F | Atlanta, GA |
| GMH57 | 1997 | F | Atlanta, GA |
| GMH66 | 1997 | F | Atlanta, GA |
| GMH67 | 1997 | F | Atlanta, GA |
| MSA806 | 1995 | F | Jackson, MS |
| MSA807 | 1995 | F | Crestview, FL |
| MSA809B | 1996 | F | Brooklyn, NY |
| MSA810 | 1996 | F | Gilbert, AZ |
| MSA811 | 1996 | F | Benton Harbor, MI |
| MSA812 | 1996 | F | Birmingham, AL |
| MSA815 | 1996 | | Hartford, CT |
| MSA816 | 1996 | F | Rochester, NY |
| MSA819 | 1996 | F | Brooklyn, NY |

| Isolate | Year | Sex of patient | Location | Isolate | Year | Sex of patient | Location |
|---|---|---|---|---|---|---|---|
| E29B | 2001 | | Jackson, MS | MSA822 | 1996 | F | Clearwater, FL |
| E30 | 2001 | | Jackson, MS | MSA823 | 1996 | F | Austin, TX |
| E34 | 2001 | M | Jackson, MS | MSA824 | 1996 | F | Philadelphia, PA |
| E35 | 2001 | | Jackson, MS | MSA826 | 1996 | F | Grover Beach, CA |
| E36 | 2001 | | Jackson, MS | MSA828 | 1997 | F | Minneapolis, MN |
| E37 | 2001 | M | Jackson, MS | MSA829 | 1997 | F | Columbus, OH |
| E38 | 2001 | F | Jackson, MS | MSA830 | 1997 | F | Great Bend, KS |
| E39 | 2001 | | Jackson, MS | MSA831 | 1997 | F | Brooklyn, NY |
| E40 | 2001 | | Jackson, MS | MSA836 | 1997 | F | Greenville, SC |
| E42 | 2001 | | Jackson, MS | MSA844 | 1998 | F | Rochester, NY |
| E43 | 2001 | | Jackson, MS | MSA844 | 1998 | F | Rochester, NY |
| E44 | 2001 | | Jackson, MS | MSA845 | 1998 | F | Nacogloches, TX |

* ATCC = American Type Culture Collection. A blank space indicates that no information is available.

**Table 2**

Number of *Trichomonas* isolates possessing individual heat-shock protein 70 hybridizing bands

| Band size (kb) | No. isolates | Band size (kb) | No. isolates | Band size (kb) | No. isolates |
|---|---|---|---|---|---|
| 1.8 | 26 | 5.6 | 6 | 9.6 | 37 |
| 2.0 | 1 | 5.8 | 127 | 10.0 | 24 |
| 2.4 | 3 | 6.5 | 1 | 11.5 | 27 |
| 2.7 | 128 | 7.0 | 34 | 12.0 | 30 |
| 3.2 | 34 | 7.3 | 18 | 12.5 | 16 |
| 3.4 | 2 | 7.5 | 48 | 13.3 | 19 |
| 3.5 | 26 | 7.8 | 36 | 15.0 | 39 |
| 3.7 | 129 | 8.0 | 13 | 16.5 | 5 |
| 4.4 | 103 | 8.2 | 24 | 18.0 | 4 |
| 5.2 | 9 | 9.0 | 8 | 22.5 | 2 |
| 5.5 | 128 | | | | |

**Table 3**

*Trichomonas* isolates sharing identical heat-shock protein 70 restriction fragment length polymorphism patterns

| | |
|---|---|
| GMH1, E8 | B137, B157 |
| GMH36, GMH47 | D1, D2, D5, D10 |
| MSA809B, MSA831 | E5, E12 |
| MSA812, E19 | E6, E23 |
| MSA819, GMH17 | E9, E36 |
| MSA826, D4, T1F, T1M | E44, E45 |
| MSA830, E40 | E61, E62 |
| MSA836, GMH10 | E70, E78 |
| MSA844, E35 | E83, E95 |
| S2, C8 | E86, E98 |