Mycologia, 94(1), 2002, pp. 73–84.

Molecular and phenotypic description of *Coccidioides posadasii* sp. nov., previously recognized as the non-California population of *Coccidioides immitis*

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Abstract: *Coccidioides posadasii* sp. nov., formerly known as *non-California (non-CA) Coccidioides immitis,* is described. Phylogenetic analyses using single nucleotide polymorphisms, genes, and microsatellites show that *C. posadasii* represents a divergent, genetically recombining monophyletic clade. *Coccidioides posadasii* can be distinguished from *C. immitis* by numerous DNA polymorphisms, and we show how either of two microsatellite loci may be used as diagnostic markers for this species. Growth experiments show that *C. posadasii* has significantly slower growth rates on high-salt media when compared with *C. immitis,* suggesting that other phenotypic characters may exist.

Key Words: allele, Coccidioidomycosis, microsatellite, Onygenales, phylogeny, systematics

INTRODUCTION

Species can be defined as groups of organisms that share a common evolutionary history and, as a consequence, share exclusive characters. This is known as the evolutionary species concept (ESC; Simpson 1951, Simpson 1961, Wiley 1978) and is the most inclusive species concept to date (Mayden 1997). Molecular genetics (Reynolds and Taylor 1991) and cladistic analyses provide a method of diagnosing species under the ESC by describing shared exclusive characters (apomorphies) using an operational method known as phylogenetic species recognition (PSR). A subset of PSR uses genealogical concordance (GCPSR) to detect genetically isolated groups by comparing the gene trees from a number of different loci (Avise and Ball 1990). Different genes will have different genealogies within a species due to recombination, but between species the genealogies will be concordant due to the effects of genetic isolation and drift causing lineage sorting and coalescence. Detecting the common branches between these gene trees is the key to GCPSR.

GCPSR is finding increasing usage within both meiosporic and mitosporic fungal taxa (Taylor et al 2000), for instance the *Gibberella fujikuroi* complex (O'Donnell et al 1998), *Ajellomyces capsulatus* (Kwon-Chung) McGinnis and Katz (Kasuga et al 1999), *Aspergillus flavus* Link (Geiser et al 1998) and *Coccidioides immitis* Rixford and Gilchrist 1896 (Koufopanou et al 1997, 1998). Here, we use GCPSR to demarcate barriers to gene flow between individuals of the pathogenic fungus *Coccidioides immitis.* Our analysis and those of others clearly show the existence of two genetically isolated and deeply divergent clades within *C. immitis* and we use this as the basis for describing a new species, *Coccidioides posadasii.* Knowledge of genetically defined species enables workers to look closely for previously undetectable morphological and phenotypic differences. We use this approach to show that *C. immitis* has a tendency to grow faster than *C. posadasii* on high-salt media. This demonstrates that other, perhaps clinically important, characters may exist.

Coccidioides immitis is a dimorphic pathogenic fungus found in the southwestern United States, Mexico, Central and South America (Pappagianis 1988). In the saphrobic phase *C. immitis* is characteristically found inhabiting the arid, sandy soils of the Lower Sonoran Life Zone. Inhalation of arthroconidia causes a chronic pulmonary infection in humans and other vertebrates. In ca 0.5% of cases, secondary coccidioidomycosis occurs, a serious disseminated infection that is often fatal (Rippon 1988). Immunity generated from resolving the infection is specific and usually lifelong.

Coccidioidomycosis was originally described by Alejandro Posadas (and later confirmed by Robert Wernicke) from a soldier, Domingo Ezcurra, who acquired his infection in the Argentine pampas (Posadas 1892, Wernicke 1892). Posadas and Wernicke rec-

Accepted for publication June 4, 2001.
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ognized the presence of an organism, likened to a protozoon of the order Coccidia. Formal description of *C. immitis* was performed by Rixford and Gilchrist from a case observed in California (Rixford and Gilchrist 1896). However, the parasite was then still thought to be a protozoan. The correct taxonomic status of *C. immitis* as an ascomycete fungus was demonstrated by Ophüls and Moffit (1900) by culture on artificial media of the fungal mycelia using arthrospores isolated from laboratory infections of guinea pigs. The etiological relationship between *C. immitis* and coccidioidomycosis was also demonstrated by showing that arthroconidia cause infection in several types of laboratory animal. The lack of any known meiosporic state *in vitro* or *in vivo* hampered further classification until work by Sigler and Carmichael (1976) recognized the similarity between the asexual spores (arthroconidia) of *C. immitis* and those (aleurioconidia) found in the mitosporic genus *Malbranchea* Sacc., placing *C. immitis* in the order Onygenaceae. This relationship was confirmed by molecular phylogenetic methods (Bowman et al 1992, Pan et al 1994, Bowman et al 1996), and *Uncinocarpus reesii* Sigler and Orr was shown to be the sister group to *C. immitis*.

Research on the intraspecific relationships of *C. immitis* was first attempted by Zimmerman et al (1994), who compared RFLPs of total genomic DNA and showed that 15 clinical isolates formed two groups, referred to as Group I and Group II. Group I contained the isolate 'Silveira' that is extensively used in laboratory studies. Subsequent work by Burt et al (1997) using RFLPs of 10 DNA loci demonstrated the occurrence of highly significant differences in allele frequencies between clinical isolates from California, Arizona and Texas, the Californian population being the most divergent. This result was corroborated by Koufopanou et al (1997, 1998) who used genealogies of five nuclear genes to show that *C. immitis* consists of two non-interbreeding taxa, *CA* (centered in California) and *non-CA* (represented by clinical isolates from Arizona, Texas, Mexico, and Argentina). The 'Silveira' isolate was included in *non-CA C. immitis,* showing that Zimmerman's Group I and Koufopanou's *non-CA* were synonymous. Nucleotide sequence divergence between *CA* and *non-CA* showed that they had been reproductively isolated from one another for the past 11 million years, a result that was subsequently corroborated using a separate set of loci and *C. immitis* isolates (Fisher et al 2000b). That independent loci were randomly assorting with respect to one another within *CA* and *non-CA* showed that genetic recombination had occurred between individuals within the two groups, despite no teleomorph ever having been described for *C. immitis* (Burt et al 1996, Fisher et al 2000a). This observation suggests that the species described here are evolutionary species and could be recognized as biological species, as well as phylogenetic species, if a teleomorph were to be found.

Recently, the sampling of the *C. immitis* biogeographic distribution was extended to include previously unsampled populations from Southern California, Central and Southern Mexico, Venezuela, and Brazil, and analyzed using a suite of microsatellite markers (Fisher et al 1999, Fisher et al 2000b). Phylogenetic analyses showed that, despite the increased breadth and depth of sampling, the *C. immitis* phylogeny still contained two major clades (Fisher et al 2000c). Here, we use our dataset of microsatellite alleles to show that these clades correspond to the previous classifications of *CA* (Group II) and *non-CA* (Group I) *C. immitis*. Species rank is proposed for the two clades.

MATERIALS AND METHODS

One hundred and sixty-seven isolates identified by clinical laboratories as *Coccidioides immitis* were used in this study (APPENDIX). These isolates represent the entire known geographical distribution of the pathogen and are cryogenically preserved in the Roche Molecular Systems Culture Collection (RMSCC) for future reference (Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, California 94501, USA). Liquid cultures of each isolate were grown in a BL3 containment facility and total genomic DNA extracted from lyophilized mycelia according to the protocol described by Burt et al (Burt et al 1995). Polymerase chain reaction (PCR) amplification of nine microsatellite-containing loci (*GAC, 621, GA37, GA1, ACJ, KO3, KO7, KO1, KO9*) was performed for each isolate using the fluorescently labelled primers and conditions described previously (Fisher et al 1999). The multilocus genotype of each isolate was determined by electrophoresing the PCR products through a 6% denaturing polyacrylamide gel using an automated sequencer (Applied Biosystems), the alleles present at each locus being determined by reference against a TAMRA-labelled internal size standard. A standardized method for typing the alleles at each locus is available as a. pdf file at http://plantbio.berkeley.edu/~taylor/mf.html.

Phylogenetic analyses were performed using the microsatellite genetic distances D_{AS} (Stephens et al 1992, Bowcock et al 1994) and $(\delta\mu)^2$ (Goldstein et al 1995). Here, D_{AS} = 1 − (the total number of shared alleles at all loci / n) where n is the number of loci compared. Pairwise distances calculated using the mean character distance option in PAUP* 4.0b1 (Swofford 1998) are identical to D_{AS} and were used here. The neighbor-joining algorithm in PAUP clustered these user-defined distances using the minimum evolution option, and support for each clade was estimated by 1000 neighbor-joining bootstrap replications of the dataset. Genetic distance between populations was assessed using the microsatellite distance $(\delta\mu)^2$. This measure more closely reflects the genetic distance that has accrued within loci by accounting for the size of alleles, as well as their frequen-

cies. $(\delta\mu)^2$ equals the square of the difference in mean allele size (x) between two populations A and B such that $(\delta\mu)^2 = (x_A - x_B)^2$. Confidence intervals for $(\delta\mu)^2$ were calculated by bootstrapping over loci using the program MICROSAT (Minch et al 1995). Alignments were deposited in Tree Base, and are available under study accession number S692.

We looked for differences in the phenotype of *CA* and *non-CA* by (i) comparing the growth rates of colonies on media with salt concentrations increasing to near-inhibitory levels and by (ii) comparing spore-size. Pilot experiments were performed where 8 isolates were chosen (four of the *CA* and four of the *non-CA* genotype) and grown on YEG agar (1% yeast extract, 1% glucose, 1.5% agar, Difco, BD Microbiology Systems, Sparks, Maryland 21152) containing the following concentrations of NaCl; 0.034 M (2%), 0.068 M (4%), 0.102 M (6%). Each isolate was initially grown on YEG plates and 3 mm diameter plugs removed from the colony margin. These were then placed on the test media, incubated in the dark at 30 C, and colonies were measured across their diameters after 15 d of growth. Subsequently, an expanded experiment was performed where 20 clinical isolates (10 each of the *CA* and *non-CA* genotypes) were grown in replicates of 4 on YEG agar containing either 0.034 M (2%) or 0.136 M (8%) NaCl, colony growth being measured after 4, 8, 10, and 15 d of incubation at 30 C.

The length of arthroconidia were measured for a selection of strains of the *CA* and *non-CA* genotypes. Cultures were grown on malt extract agar (4% malt extract, 1.5% agar, Difco, BD Microbiology Systems, Sparks, Maryland 21152), stained with lactophenol cotton blue (Hardy Diagnostics, Santa Maria, California 93455) and sealed with nail varnish. Lengths were then determined for a minimum of 20 arthroconidia from each culture.

RESULTS

All microsatellite loci were highly polymorphic, with between 8 and 26 alleles found at each locus (FIG. 1). Pairwise groupings using D_{AS} show that all isolates occur within one of two major clades, illustrated in FIG. 2A, B. Other than the support found for genetically identical isolates (shown as terminal branches joined by vertical lines), these are the only clades that are supported by bootstrap analysis. Mapping previously genotyped isolates from Koufopanou et al (1997) and Fisher et al (2000b) onto this tree shows that the 'upper' clade (comprising 106 samples) contains isolates that are exclusively of the *non-CA* genotype (FIG. 2A), and that the 'lower' clade (comprising 61 isolates) contains isolates that are exclusively of the *CA* genotype. This demonstrates that the original division of *C. immitis* into *CA* and *non-CA* (Koufopanou et al 1997) using GCPSR holds across the entire distribution of fungi represented in this study, and is the phylogenetic basis for our proposing species status for *CA* and *non-CA*. The distribution of alleles at each locus for *CA* (white) and *non-CA* (black) are shown in FIG. 1. These show that two loci, *GAC2* and *621,* are diagnostic for *CA* and *non-CA*. The other microsatellite-containing loci all show overlapping distributions in the sizes of alleles between *CA* and *non-CA,* however, in all cases the allele frequencies are dramatically different illustrating the deep genetic divergence between these two clades. The results of taking into account allele size as well as frequency are shown in FIG. 3. The tree inferred using $(\delta\mu)^2$ illustrates a tenfold increase in genetic distance between *CA* and *non-CA* genotypes when compared to the distance seen between populations within each clade, and has strong (99%) bootstrap support.

Non-CA C. immitis has a much wider biogeographical distribution when compared to *CA C. immitis*, being recovered from across the southwestern United States, southern California, northern, central and southern Mexico and South America. No isolates of *non-CA C. immitis* have been recovered from the San Joaquin Valley, California, except the isolate 'Silveira', commonly used in laboratory studies. *Non-CA* and *CA C. immitis* appear to be sympatric in southern California, and northern and southern Mexico; however low numbers of samples from central Mexico do not rule out the occurrence of *CA C. immitis* in this region.

We tested four different culture media in order to examine the colony growth rates of *CA* and *non-CA* isolates. In a pilot experiment, a trend was observed where *non-CA* isolates (filled circles) appeared to grow more slowly than *CA* isolates (open circles) as NaCl concentration in the medium was increased (FIG. 4). We investigated this effect further by increasing the scale of the experiment; 10 isolates from *CA* and *non-CA* were grown in quadruple replicates on media containing either 0.034 M (low salt) or 0.136 M (high-salt) NaCl. FIG. 5 shows the population mean and 95% confidence intervals for the growth rates of *CA* (open bars) and *non-CA* (filled bars) on the two media. While *CA* grew initially faster on low salt medium, by day 10 there was no significant difference between the two groups. On high-salt (inhibitory) medium, growth of *CA* and *non-CA* was restricted relative to the low-salt medium. However, on this medium significant inter-group variation was seen; growth of *CA* was significantly faster than *non-CA* for the duration of the experiment. The ranges of each group overlap, showing that this characteristic is not diagnostic between *CA* and *non-CA* (FIG. 5). Therefore, growth on high-salt plates appears to reflect phenotypic differences that have accrued between *CA* and *non-CA,* but these are not discrete and may not be used to distinguish between the two species.

We measured the size of arthroconidia for four

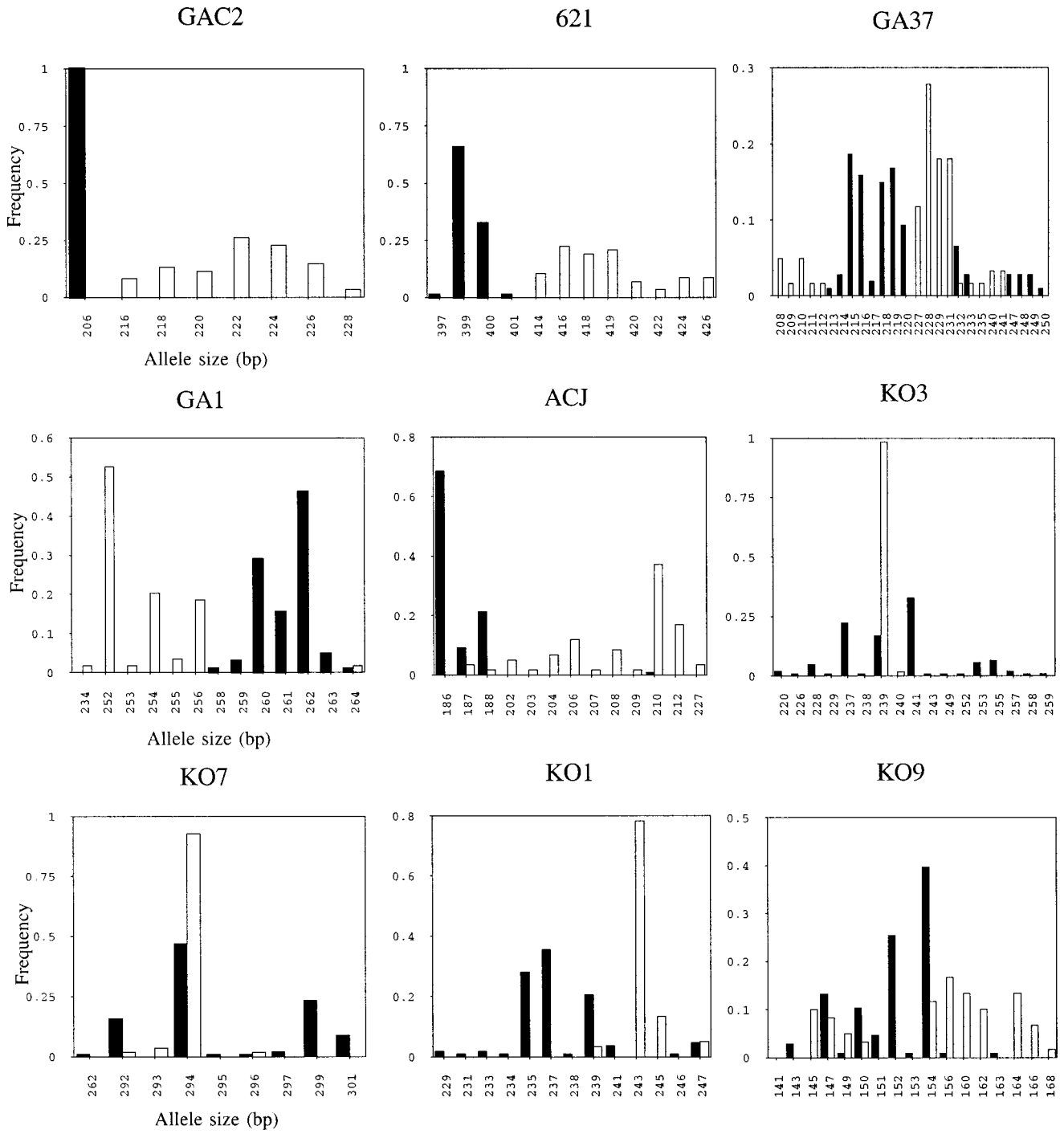

FIG. 1. Allele frequency distributions showing the sizes of PCR products at nine microsatellite loci for *C. immitis* (white bars; $n = 61$) and *C. posadasii* (black bars $n = 106$).

cultures of *CA* (RMSCC 2006, 2007, 2009, 2010) and three of *non-CA* (1037, 1038, 1039). Means of the two groups were as follows: *CA*; 5.96 μm (SD = 0.93) and *non-CA*; 5.88 μm (SD = 0.85). Use of a two tailed *t*-test showed that the means of these distributions were not significantly different from each other ($t = 1.977$, d.o.f. = 138, $P = 0.602$). From this we concluded that the length of arthroconidia was not a diagnostic character for *CA* and *non-CA*.

TAXONOMY

Based on these and other published data, a new species is proposed for *non-CA C. immitis*. We have chosen to recognize and name this evolutionary species

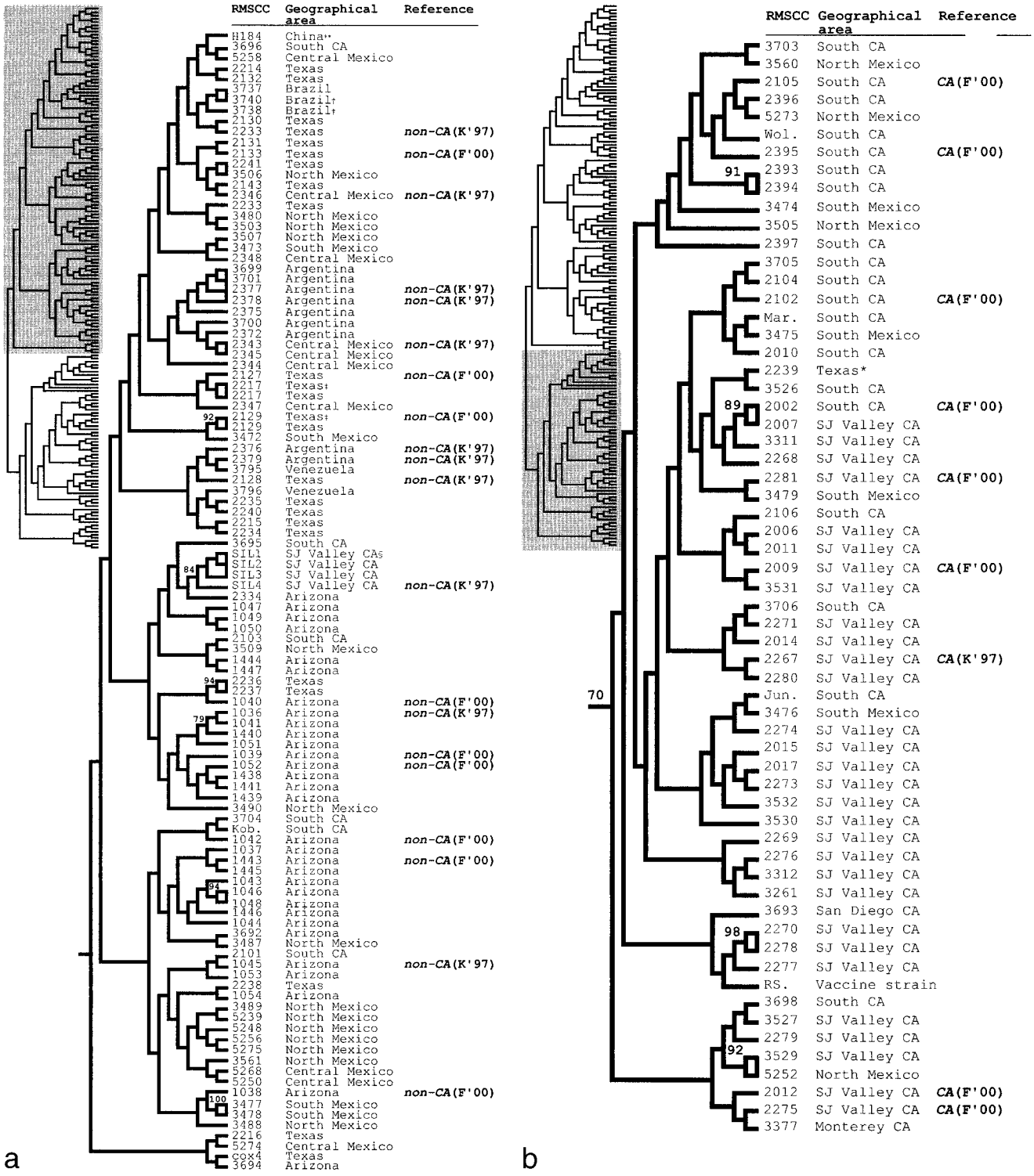

FIG. 2. Cladogram inferred using D_{AS} and neighbor-joining showing A. the *C. immitis* (*CA*) clade and B. the *C. posadasii* (*non-CA*) clade. Bootstrap values >50% are shown as numbers above branches, terminal branches joined by vertical lines denote isolates with identical genotypes. The tree is mid-point rooted. * 2239 was isolated in Texas, but the patient became infected in California (Burt et al 1997). ** 2375 was isolated from a Chinese patient with a history of travel in the USA. † non-clinical isolates from armadillo burrows, Brazil. ‡ 'blind' duplicated isolates used as genotype controls. § 1932, 1933, and 2030, respectively are isolates of 'Silveira' from R. Cox. SILV is an isolate of 'Silveira' from D. Pappagianis and shows length mutations at 2 loci compared with 1932, 1933, and 2030. RMSCC 2233A/B were two separate genotypes recovered from a single clinical sample. Z'94, K'97, B'97 and F'00 are references to the original papers that describe genotypes of the isolate (see Appendix).

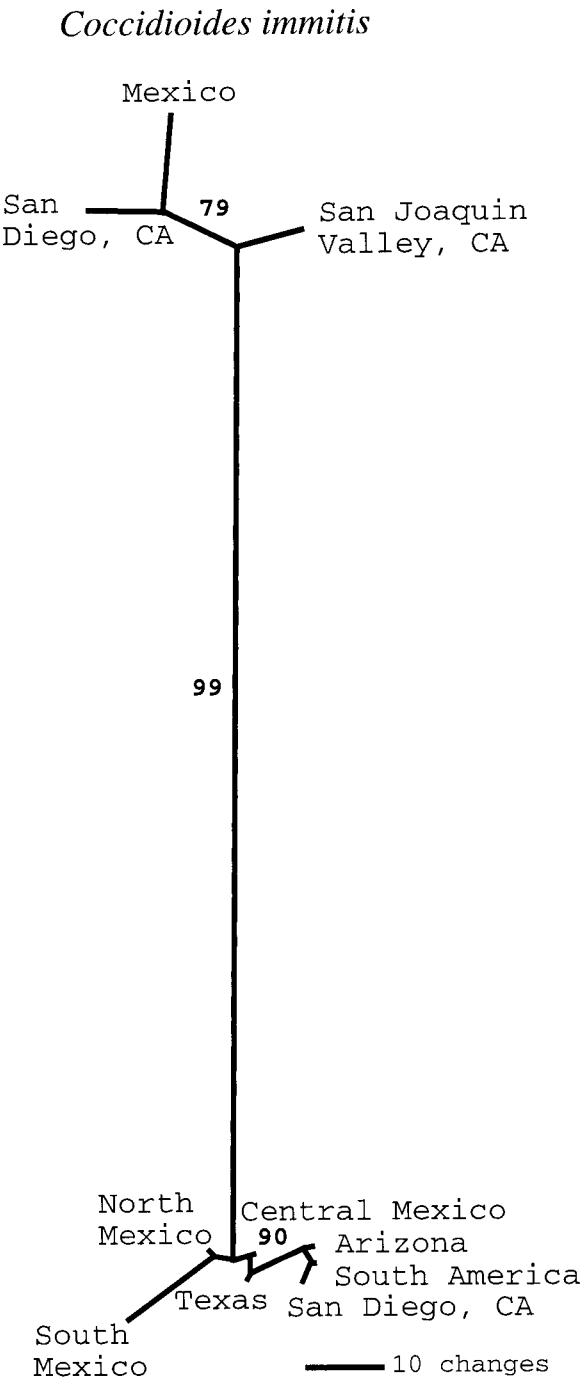

FIG. 3. Unrooted phylogram inferred using $(\delta\mu)^2$ and neighbor-joining showing the relationships of phylogeographic populations within *C. immitis* and *C. posadasii*. Bootstrap values >50% are shown.

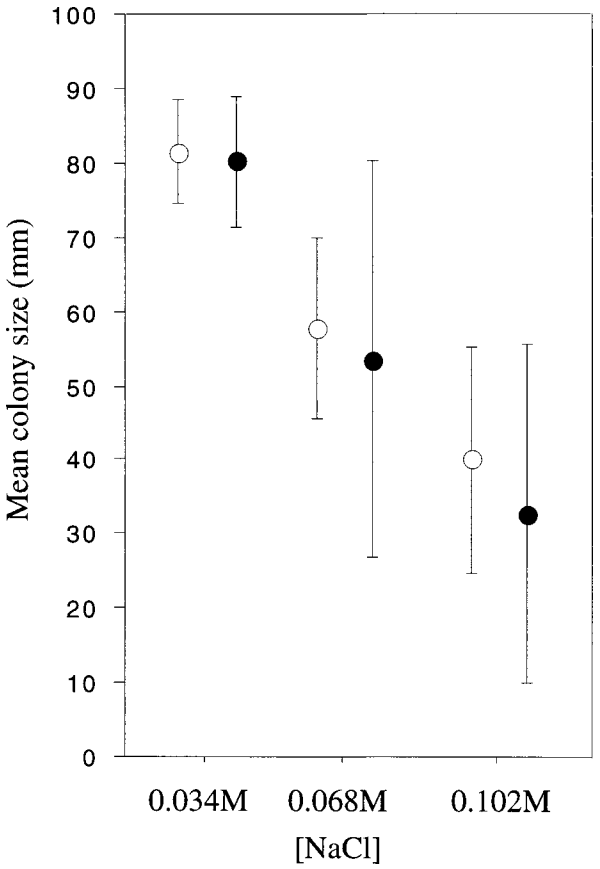

FIG. 4. Summary of mean colony size ± 95% confidence intervals after 15 d growth on YEG media containing increasing concentrations of NaCl. White circles = *C. immitis* (*CA*; *n* = 4) and black circles = *C. posadasii* (*non-CA*; *n* = 4).

because the original description of *C. immitis* was from a patient in San Francisco who most likely contracted his infection within California (Rixford and Gilchrist 1896). Based on the geographical distributions of *CA* and *non-CA*, this infection was most likely to have been caused by *CA C. immitis*, which should therefore retain the name.

Coccidioides posadasii Fisher, Koenig, White et Taylor, sp. nov.

= *Coccidioides immitis*, non-California population sensu Koufopanou et al, Proc. Natl. Acad. Sci. *USA* 94 5478–5482 (1997).

Morphologia idem ac Coccidioides immitis, distinguibilis characteribus sequentibus nucleotiditis fixationibus mutuis inter Coccidioidem immitem et Coccidioidem posadasii: positiones synthase chitinis 192 (A), 288 (T); positiones dioxygenase 872 (C), 1005 (C), 1020 (G), 1179 (C), 1272 (T); positiones orotidine decarboxylase 473 (A), 506 (C), 606 (C), 647 (A); positiones serine 477 (G), 517 (C), 632 (C), 744 (G), 887 (C); positio chitinase 910 (T) (Koufopanou et al 1997, Koufopanou et al 1998); positio z 134 (T) (Burt et al 1997). Loci sequentes microsatellitum distribu-

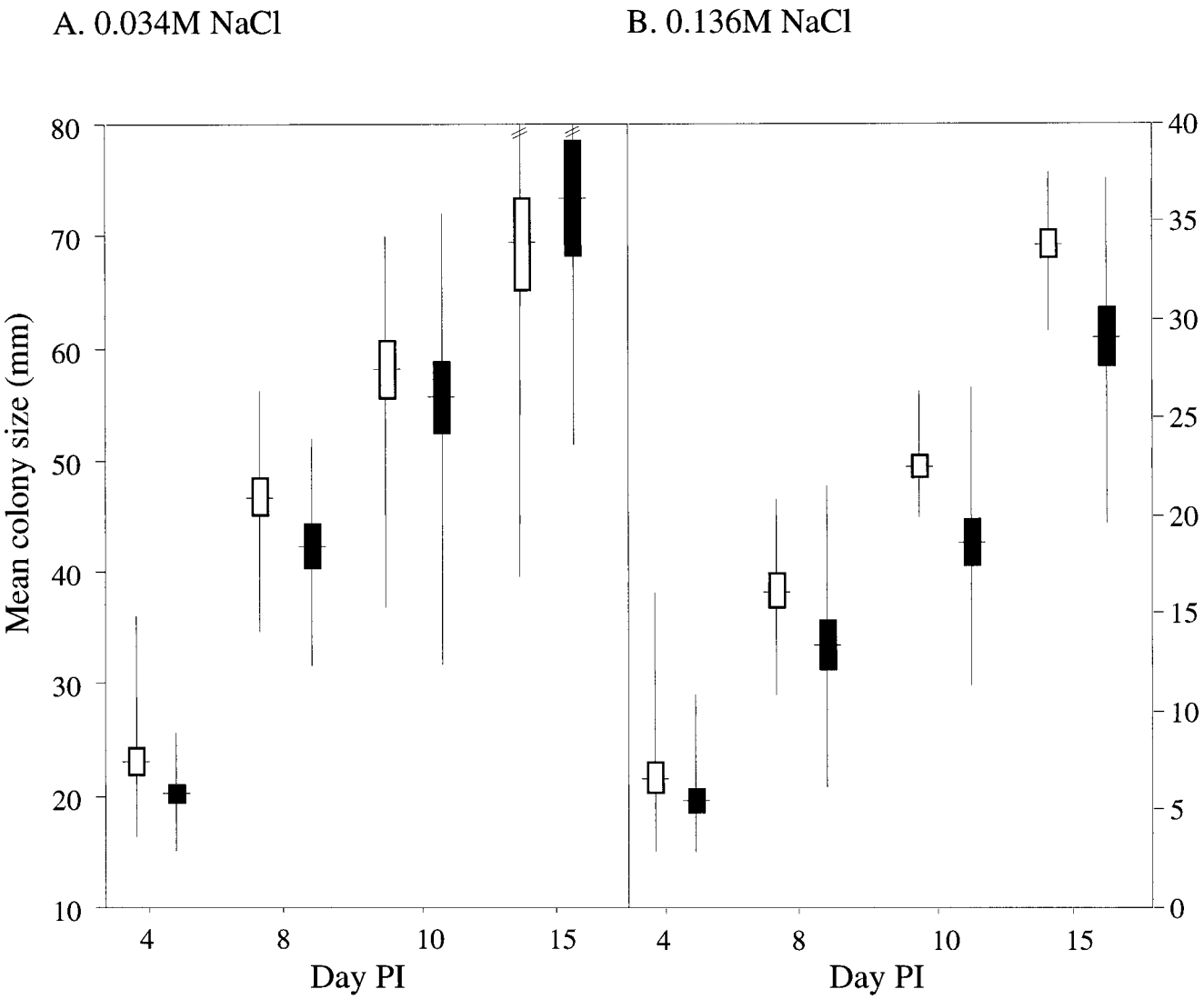

FIG. 5. Summary of mean colony size ± 95% confidence intervals (rectangles) and total ranges (lines) on A. low (0.034 M) and B. high (0.136 M) salt-containing YEG media. White bars = *C. immitis* (*CA*; $n = 10$) and black bars = *C. posadasii* (*non-CA*; $n = 10$). Day PI signifies day post inoculation.

tionibus propriis allelones: GAC2, PCR amplitudo operis primeribus GAC2.1 et GAC2.2 = 206 bp; 621, PCR amplitudo operis primeribus 621U et 621L = 397–401 bp.

Coccidioides posadasii is morphologically indistinguishable from *Coccidioides immitis. C. posadasii* is diagnosed by the following nucleotide characters (given as the gene, the nucleotide position in the gene, and, parenthetically, the nucleotide fixed in *C. posadasii*) showing reciprocal fixation between *C. immitis* and *C. posadasii*: Chitin synthase positions 192 (A), 288 (T); Dioxygenase positions 872 (C), 1005 (C), 1020 (G), 1179 (C), 1272 (T); Orotidine decarboxylase positions 473 (A), 506 (C), 606 (C), 647 (A); Serine proteinase positions 477 (G), 517 (C), 632 (C), 744 (G), 887 (C); Chitinase position 910 (T) (Koufopanou et al 1997, Koufopanou et al 1998). *z* position 134 (T) (Burt et al 1997). The following microsatellite loci have exclusive allele distributions: *GAC2,* PCR product size using primers GAC2.1 and GAC2.2 = 206bp; *621,* PCR product size using primers 621U and 621L = 397–401bp. Distribution: southwestern United States, Central and South America.

HOLOTYPE: Pappagianis isolate 'Silveira' (RMSCC Silveira, appendix) isolated in the San Joaquin Valley, California 1951. Widely used laboratory isolate, maintained by the American Type Culture Collection (ATCC) #28868. This isolate is currently on regulatory hold at the ATCC due to *C. immitis* being a controlled pathogen. A killed sample of RMSCC Silveira has been lodged in the Jepson Herbarium, University of California at Berkeley, Berkeley, California 94720, USA. Frozen samples of RMSCC Silveira are stored at Roche Molecular Systems, Ala-

meda, California and the Centers for Disease Control and Prevention, Atlanta, Georgia.

Etymology. posadasii; after Alejandro Posadas who described the first case of coccidioidomycosis, which was from Argentina (Posadas 1892)

DISCUSSION

This study describes the division of *Coccidioides immitis* into two species, *C. immitis* and *C. posadasii*, using GCPSR. We have chosen to give species status to *C. posadasii* for the following reasons: (i) Genetic isolation appears to be absolute as no incongruencies have been detected between *C. immitis* and *C. posadasii* in the genealogies of the 12 nuclear loci studied (five described by Koufopanou et al 1997 and seven described by Fisher et al 2000b), thus fulfilling the criteria of GCPSR, (ii) the two species are deeply divergent, estimations of the time of genetic isolation ranging from 11 (Koufopanou et al 1998)— 12.8 (Fisher et al 2000b) million years ago, (iii) *C. immitis* and *C. posadasii* have been shown, separately, to genetically recombine in nature (Burt et al 1996, Fisher et al 2000a) and (iv) we have sampled the entire worldwide distribution of this pathogen, therefore we feel that the possibility is small of discovering further cryptic species of *Coccidioides* that would change the taxonomic relationships described here.

Coccidioides immitis appears to have the smaller biogeographic distribution of the two species, centered on the San Joaquin Valley, California. In this region, *C. immitis* appears to be the only species, however two clinical isolates of *C. posadasii*, Silveira and K-727, were recovered from this area. Silveira was isolated from a patient in Bakersfield in 1951 and K-727 from a patient in 1992; however, it is not known whether either patient had a history of travel to other parts of the southwest where these infections may have been acquired. Such movements of patients suffering from coccidioidomycosis have been previously detected using molecular markers (Burt et al 1997). *Coccidioides immitis* and *C. posadasii* appear to be sympatric in southern California and Mexico. Here, significant intraspecific differences in allele frequencies between populations show that these are *bona fida* populations, and are not solely due to the immigration of patients with infections that were gained from other areas (Fisher et al 2000c).

In this paper, we have used phylogenetic analyses of microsatellite loci to identify isolates as *C. immitis* or *C. posadasii*. This approach was used because acquiring multiple gene genealogies for all the isolates described in this paper would be very difficult due to the amount of sequencing involved. Instead, the microsatellites provided a means of acquiring multilocus genotypes for many isolates with far less effort and expense. However, this technique must be used with caution as concerns exist when using microsatellites for phylogenetic analyses due to their mode and rate of mutation. Constraints exist on the sizes of microsatellites, causing a limit on the genetic distance that can accrue between genetically isolated taxa (Garza et al 1995, Lehmann et al 1996). Further, the high mutation rates seen at microsatellite loci cause the re-appearance of alleles that were previously lost from populations, resulting in mutational convergence (homoplasy) of alleles that are identical by size but not by descent. Such homoplasy can be directly observed from the allele distributions illustrated in FIG. 1, where *C. immitis* and *C. posadasii* share identical alleles at all loci except *GAC2* and *621*. A cursory analysis of the data might lead to the conclusion that genetic isolation between *C. immitis* and *C. posadasii* was not absolute, due to the occurrence of alleles that are shared between the two taxa. We have addressed these concerns in a previous study by comparing the phylogeny inferred using the microsatellites against the phylogeny inferred using genes, and there showed that (i) they were identical, and that (ii) microsatellite alleles shared between the two taxa were a result of mutational convergence and not interbreeding (Fisher et al 2000b). This demonstrated that the *Coccidioides* microsatellite loci were correctly diagnosing taxonomic units that had been identified using the GCPSR, and were therefore suitable for extending the GCPSR to include the isolates used in this study. However, it should be recognized that, without performing this initial study, using microsatellite phylogenies alone to describe a species may not be valid.

Recognition of the two species has enabled us to show that *C. posadasii* grows more slowly on media containing high salt concentrations. The range in growth rates overlaps between the two species, showing that, although the difference in growth rates is significant, this phenotype is not diagnostic. The presence of differences in the amino acid composition of proteins between the two species (Koufopanou et al 1997, Peng et al 1999) suggests that other phenotypic differences may exist. This difference may extend to variation in the antigenicity of key proteins or pathogenicity factors. Recognizing the existence of *C. posadasii* is instrumental in allowing researchers and physicians to investigate these possibilities.

ACKNOWLEDGMENTS

We thank Jim Trappe for providing the Latin diagnosis, and Jamie Platt for advice. The National Institutes of Health and the California Healthcare Foundation contributed to funding this study.

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APPENDIX. Isolates of *Coccidioides* used in this study

Species	RMSCC	Source	Geographic origin	References	Locus								
					GAC2	621.2	GA37	GA1	ACJ	KO3	KO7	KO1	KO9
C. posadasii	H184	Z. Quin (DNA)	China		206	?	22	262	186	237	294	235	154
C. posadasii	3699	R. Negroni	Catamarca, Argentina		206	400	215	262	186	241	294	239	154
C. posadasii	3700	R. Negroni	Jujuy, Argentina		206	400	218	260	186	241	294	239	154
C. posadasii	3701	R. Negroni	Catamarca, Argentina		206	400	215	262	186	241	294	239	154
C. posadasii	3737	B. Wanke	Piaui, Brazil		206	400	215	261	186	241	294	235	154
C. posadasii	3738	B. Wanke	Piaui, Brazil (from armadillo burrow)		206	400	215	262	186	241	294	235	154
C. posadasii	3740	B. Wanke	Piaui, Brazil (from armadillo burrow)		206	400	215	261	186	241	294	235	154
C. posadasii	2372	R. Negroni	Argentina		206	399	218	261	186	241	294	239	154
C. posadasii	2375	R. Negroni	Argentina		206	399	215	262	186	241	294	239	154
C. posadasii	2376	R. Negroni	Argentina	Koufopanou et al ('97)	206	399	215	262	186	241	294	237	152
C. posadasii	2377	R. Negroni	Argentina	Koufopanou et al ('97)	206	400	215	262	186	241	294	239	154
C. posadasii	2378	R. Negroni	Argentina	Koufopanou et al ('97)	206	400	215	262	186	241	294	239	154
C. posadasii	2379	R. Negroni	Argentina	Koufopanou et al ('97)	206	400	215	262	186	241	294	237	152
C. posadasii	3795	G. San-Blas	Venezuela		206	400	220	262	186	241	294	237	152
C. posadasii	3796	G. San-Blas	Venezuela		206	400	220	262	186	239	299	235	149
C. posadasii	3695	Naval Hospital	Barstow, CA		206	400	214	260	186	239	301	239	147
C. posadasii	3696	Naval Hospital	San Diego, CA		206	400	215	262	186	237	294	235	154
C. posadasii	3704	UCSD Medical Center	San Diego, CA		206	401	214	261	188	239	301	237	150
C. posadasii	2101	T. Kirkland	San Diego, CA		206	399	218	263	188	252	294	235	143
C. posadasii	2103	T. Kirkland	San Diego, CA		206	399	219	261	186	239	294	239	152
C. posadasii	Kober	T. Kirkland	San Diego, CA		206	399	214	261	188	241	297	247	150
C. posadasii	2214	R. Cox	San Antonio, TX	Burt et al ('97)	206	400	216	262	186	241	294	235	154
C. posadasii	2215	R. Cox	San Antonio, TX	Burt et al ('97)	206	400	215	259	186	241	262	235	147
C. posadasii	2216	R. Cox	San Antonio, TX	Burt et al ('97)	206	400	220	263	186	239	294	237	147
C. posadasii	2217	R. Cox	San Antonio, TX	Burt et al ('97)	206	400	232	262	186	237	292	239	154
C. posadasii	2130	R. Cox	San Antonio, TX		206	400	233	262	186	241	294	235	154
C. posadasii	2131	R. Cox	San Antonio, TX		206	400	216	262	186	241	292	235	154
C. posadasii	2132	R. Cox	San Antonio, TX		206	400	216	262	186	237	294	235	154
C. posadasii	2133	R. Cox	San Antonio, TX	Fisher et al ('00)	206	400	219	262	186	241	292	235	154
C. posadasii	2233A	M. Rinaldi	San Antonio, TX	Burt et al ('97), Koufopanou et al ('97)	206	400	232	262	186	241	294	235	154
C. posadasii	2233B	M. Rinaldi	San Antonio, TX		206	399	232	262	186	241	294	235	154
C. posadasii	2234	M. Rinaldi	San Antonio, TX	Burt et al ('97)	206	399	215	262	186	241	292	235	147
C. posadasii	2235	M. Rinaldi	San Antonio, TX	Burt et al ('97)	206	400	247	262	186	237	292	235	152
C. posadasii	2236	M. Rinaldi	San Antonio, TX	Burt et al ('97)	206	400	219	260	186	220	299	237	150
C. posadasii	2237	M. Rinaldi	San Antonio, TX	Burt et al ('97)	206	?	219	?	186	220	299	237	150
C. posadasii	2238	M. Rinaldi	San Antonio, TX	Burt et al ('97)	206	400	219	?	188	241	294	237	152
C. posadasii	2240	M. Rinaldi	San Antonio, TX	Burt et al ('97)	206	400	216	262	186	228	292	247	147
C. posadasii	2241	M. Rinaldi	San Antonio, TX	Burt et al ('97)	206	399	216	262	186	241	292	235	154
C. posadasii	2127	R. Cox	San Antonio, TX	Burt et al ('97), Fisher et al ('00)	206	399	232	262	186	237	292	239	154
C. posadasii	2128	R. Cox	San Antonio, TX	Burt et al ('97), Koufopanou et al ('97)	206	399	247	262	186	241	294	235	152
C. posadasii	2129	R. Cox	San Antonio, TX	Burt et al ('97), Fisher et al ('00)	206	399	219	262	186	237	294	237	154
C. posadasii	2133	R. Cox	San Antonio, TX	Burt et al ('97)	206	399	219	262	186	241	292	235	154
C. posadasii	cox4	R. Cox	San Antonio, TX		206	400	215	?	188	239	299	237	151
C. posadasii	Silveira1	R. Cox	San Joaquin Valley, CA		206	400	216	260	186	253	301	237	151
C. posadasii	Silveira2	R. Cox	San Joaquin Valley, CA		206	400	216	260	186	253	301	237	151
C. posadasii	Silveira3	R. Cox	San Joaquin Valley, CA		206	400	216	260	186	253	301	237	151
C. posadasii	Silveira4	D. Pappagianis	San Joaquin Valley, CA	Koufopanou et al ('97), Zimmerman et al ('94)	206	400	216	260	186	253	301	239	152
C. posadasii	1036	J. Galgiani	Tucson, AZ	Burt et al ('97), Koufopanou et al ('97)	206	399	220	260	186	239	299	237	147
C. posadasii	1037	J. Galgiani	Tucson, AZ	Burt et al ('97)	206	399	215	260	188	237	301	237	152
C. posadasii	1038	J. Galgiani	Tucson, AZ	Burt et al ('97), Fisher et al ('00)	206	399	220	261	186	249	294	235	150
C. posadasii	1039	J. Galgiani	Tucson, AZ	Burt et al ('97), Fisher et al ('00)	206	399	218	261	186	237	299	237	152
C. posadasii	1040	J. Galgiani	Tucson, AZ	Burt et al ('97), Fisher et al ('00)	206	399	215	260	186	255	292	237	150
C. posadasii	1041	J. Galgiani	Tucson, AZ		206	399	220	260	186	239	299	237	147
C. posadasii	1042	J. Galgiani	Tucson, AZ	Burt et al ('97), Fisher et al ('00)	206	399	215	262	188	239	297	233	143
C. posadasii	1043	J. Galgiani	Tucson, AZ	Burt et al ('97)	206	399	215	260	188	255	299	237	147

APPENDIX. Continued

Species	RMSCC	Source	Geographic origin	References	Locus								
					GAC2	621.2	GA37	GA1	ACJ	KO3	KO7	KO1	KO9
C. posadasii	1044	J. Galgiani	Tucson, AZ	Burt et al ('97)	206	399	216	260	188	253	299	237	154
C. posadasii	1045	J. Galgiani	Tucson, AZ	Burt et al ('97), Koufopanou et al ('97)	206	399	218	260	188	237	294	238	153
C. posadasii	1046	J. Galgiani	Tucson, AZ	Burt et al ('97)	206	399	218	260	188	237	299	247	147
C. posadasii	1047	J. Galgiani	Tucson, AZ	Burt et al ('97)	206	399	216	260	186	239	295	231	152
C. posadasii	1048	J. Galgiani	Tucson, AZ		206	399	218	260	188	237	299	247	147
C. posadasii	1049	J. Galgiani	Tucson, AZ	Burt et al ('97)	206	399	216	260	186	239	292	241	152
C. posadasii	1050	J. Galgiani	Tucson, AZ	Burt et al ('97)	206	399	249	260	186	228	292	241	152
C. posadasii	1051	J. Galgiani	Tucson, AZ	Burt et al ('97)	206	399	220	261	186	239	299	237	152
C. posadasii	1052	J. Galgiani	Tucson, AZ	Burt et al ('97), Fisher et al ('00)	206	399	218	259	188	239	299	237	152
C. posadasii	1053	J. Galgiani	Tucson, AZ	Burt et al ('97)	206	399	219	259	188	237	?	235	150
C. posadasii	1054	J. Galgiani	Tucson, AZ	Burt et al ('97)	206	399	219	261	188	237	294	237	152
C. posadasii	1438	J. Galgiani	Tucson, AZ	Burt et al ('97)	206	399	218	260	188	237	299	237	152
C. posadasii	1439	J. Galgiani	Tucson, AZ	Burt et al ('97)	206	399	218	260	186	239	299	237	152
C. posadasii	1440	J. Galgiani	Tucson, AZ		206	399	220	260	186	239	299	237	147
C. posadasii	1441	J. Galgiani	Tucson, AZ	Burt et al ('97)	206	399	218	260	188	255	299	237	152
C. posadasii	1443	J. Galgiani	Tucson, AZ	Fisher et al ('00)	206	399	216	263	188	239	292	237	152
C. posadasii	1444	J. Galgiani	Tucson, AZ		206	399	217	263	186	237	299	239	152
C. posadasii	1445	J. Galgiani	Tucson, AZ	Burt et al ('97)	206	399	216	263	188	237	299	237	152
C. posadasii	1446	J. Galgiani	Tucson, AZ	Burt et al ('97)	206	397	217	260	188	255	299	247	152
C. posadasii	1447	J. Galgiani	Tucson, AZ	Burt et al ('97)	206	399	219	260	186	243	299	239	152
C. posadasii	2334	J. Galgiani	Tucson, AZ		206	399	216	260	186	253	301	237	150
C. posadasii	3692	Naval Hospital	Yuma, AZ		206	399	213	262	188	?	299	237	147
C. posadasii	3694	Naval Hospital	Arizona, AZ		206	400	215	260	210	237	299	239	151
C. posadasii	3480	I. Gutierrez	Sonora, North Mexico		206	399	233	262	186	241	294	239	154
C. posadasii	3487	I. Gutierrez	Sonora, North Mexico		206	399	250	?	188	257	296	237	147
C. posadasii	3488	I. Gutierrez	Sonora, North Mexico		206	399	248	261	188	226	294	246	147
C. posadasii	3489	I. Gutierrez	Sonora, North Mexico		206	399	?	262	187	238	294	?	?
C. posadasii	3561	I. Gutierrez	Chihuahaua, North Mexico		206	399	232	260	187	241	294	235	152
C. posadasii	5239	I. Gutierrez	Chihuahaua, North Mexico		206	399	219	262	187	237	?	235	152
C. posadasii	5248	I. Gutierrez	Chihuahaua, North Mexico		206	399	219	262	187	239	294	237	150
C. posadasii	5256	I. Gutierrez	Chihuahaua, North Mexico		206	?	219	261	187	258	?	237	150
C. posadasii	5275	I. Gutierrez	Chihuahaua, North Mexico		206	399	219	261	187	257	294	237	150
C. posadasii	3490	I. Gutierrez	Coahuila, North Mexico		206	399	220	258	186	237	301	237	143
C. posadasii	3503	I. Gutierrez	Coahuila, North Mexico		206	399	233	262	186	241	294	235	154
C. posadasii	3506	I. Gutierrez	Coahuila, North Mexico		206	399	216	262	186	241	?	235	?
C. posadasii	3507	I. Gutierrez	Coahuila, North Mexico		206	399	218	262	186	255	294	234	154
C. posadasii	3509	I. Gutierrez	Coahuila, North Mexico		206	399	248	261	186	239	294	239	152
C. posadasii	2343	R. Diaz	Nuevo Leon, Central Mexico	Koufopanou et al ('97)	206	399	218	262	186	241	294	239	154
C. posadasii	2344	R. Diaz	Nuevo Leon, Central Meixco		206	399	219	262	186	241	294	239	156
C. posadasii	2345	R. Diaz	Nuevo Leon, Central Mexico		206	399	218	262	186	241	294	239	154
C. posadasii	2346	R. Diaz	Nuevo Leon, Central Mexico	Koufopanou et al ('97)	206	399	248	262	186	241	292	235	154
C. posadasii	2347	R. Diaz	Nuevo Leon, Central Mexico		206	399	218	262	186	237	292	239	154
C. posadasii	2348	R. Diaz	Nuevo Leon, Central Mexico		206	399	247	262	186	255	294	235	154
C. posadasii	5258	I. Gutierrez	San Luis Potosi, Central Mexico		206	400	215	262	187	237	294	235	154
C. posadasii	5268	I. Gutierrez	Durango, Central Mexico		206	399	216	260	187	241	294	233	154
C. posadasii	5250	I. Gutierrez	Tamaulipas, Central Mexico		206	399	219	260	187	229	?	241	154
C. posadasii	5274	I. Gutierrez	Aguascalientes, Central Mexico		206	400	220	264	187	259	?	237	163
C. posadasii	3472	I. Gutierrez	Michoacan, South Mexico		206	399	219	262	186	228	299	241	154
C. posadasii	3473	I. Gutierrez	Michoacan, South Mexico		206	399	218	262	186	255	294	235	154
C. posadasii	3477	I. Gutierrez	Michoacan, South Mexico		206	399	249	261	186	228	294	229	154
C. posadasii	3478	I. Gutierrez	Michoacan, South Mexico		206	399	249	261	186	228	294	229	154
C. immitis	3693	Naval Hospital	San Diego, CA		218	419	231	253	188	239	294	243	145
C. immitis	3698	Naval Hospital	Barstow, CA		224	416	233	252	212	239	294	247	?
C. immitis	3703	UCSD Medical Center	San Diego, CA		226	419	210	256	212	239	294	243	164
C. immitis	3705	UCSD Medical Center	San Diego, CA		218	416	229	252	212	239	294	243	147
C. immitis	3706	UCSD Medical Center	San Diego, CA		222	418	229	252	212	239	294	243	160

APPENDIX. Continued

Species	RMSCC	Source	Geographic origin	References	Locus								
					GAC2	621.2	GA37	GA1	ACJ	KO3	KO7	KO1	KO9
C. immitis	2102	T. Kirkland	San Diego, CA	Fisher et al ('00)	224	419	229	252	212	239	294	243	147
C. immitis	2104	T. Kirkland	San Diego, CA		226	416	229	252	208	239	?	243	147
C. immitis	2105	T. Kirkland	San Diego, CA	Fisher et al ('00)	226	419	231	256	210	239	294	243	145
C. immitis	2106	T. Kirkland	San Diego, CA		228	419	227	252	?	239	294	243	156
C. immitis	Mart	T. Kirkland	San Diego, CA		228	?	229	252	210	239	?	243	147
C. immitis	Wolf	T. Kirkland	San Diego, CA		222	419	231	256	210	239	294	243	164
C. immitis	Junior	T. Kirkland	San Diego, CA		216	416	212	252	202	239	294	243	145
C. immitis	2393	A. Catanzaro	San Diego, CA		216	419	228	256	210	239	294	243	162
C. immitis	2394	A. Catanzaro	San Diego, CA		216	419	228	256	210	239	294	243	162
C. immitis	2395	A. Catanzaro	San Diego, CA	Fisher et al ('00)	220	419	231	254	210	239	294	243	164
C. immitis	2396	A. Catanzaro	San Diego, CA		226	419	231	256	210	239	294	243	164
C. immitis	2397	A. Catanzaro	San Diego, CA		226	?	231	256	210	239	294	239	160
C. immitis	2239	M. Rinaldi	San Antonio, TX		224	426	210	234	210	239	294	243	156
C. immitis	2002	R. Talbot	San Joaquin Valley, CA	Burt et al ('97), Fisher et al ('00)	224	416	228	252	204	239	294	243	160
C. immitis	2006	R. Talbot	San Joaquin Valley, CA	Burt et al ('97)	220	418	227	252	204	?	?	243	156
C. immitis	2007	R. Talbot	San Joaquin Valley, CA		224	?	228	252	204	239	294	243	160
C. immitis	2009	R. Talbot	San Joaquin Valley, CA	Burt et al ('97), Fisher et al ('00)	218	414	227	252	210	239	?	245	156
C. immitis	2010	R. Talbot	San Joaquin Valley, CA	Burt et al ('97)	224	416	231	252	210	239	293	243	154
C. immitis	2011	R. Talbot	San Joaquin Valley, CA	Burt et al ('97)	224	418	227	252	208	239	294	243	156
C. immitis	2012	R. Talbot	San Joaquin Valley, CA	Burt et al ('97), Fisher et al ('00)	222	420	228	264	208	239	294	245	156
C. immitis	2014	R. Talbot	San Joaquin Valley, CA	Burt et al ('97)	222	426	229	?	206	239	294	?	149
C. immitis	2015	R. Talbot	San Joaquin Valley, CA	Burt et al ('97)	220	418	227	254	208	239	294	243	160
C. immitis	2017	R. Talbot	San Joaquin Valley, CA	Burt et al ('97)	222	424	228	254	210	239	294	243	160
C. immitis	2267	R. Talbot	San Joaquin Valley, CA	Burt et al ('97), Koufopanou et al ('97)	220	426	228	252	206	239	296	243	150
C. immitis	2268	R. Talbot	San Joaquin Valley, CA	Burt et al ('97)	224	418	240	252	212	239	294	243	166
C. immitis	2269	R. Talbot	San Joaquin Valley, CA	Burt et al ('97)	220	418	228	252	210	239	294	239	154
C. immitis	2270	R. Talbot	San Joaquin Valley, CA		218	414	228	254	202	239	294	243	162
C. immitis	2271	R. Talbot	San Joaquin Valley, CA	Burt et al ('97)	222	418	229	252	206	239	294	243	145
C. immitis	2273	R. Talbot	San Joaquin Valley, CA	Burt et al ('97)	220	424	208	254	210	239	294	243	160
C. immitis	2274	R. Talbot	San Joaquin Valley, CA	Burt et al ('97)	222	416	235	254	204	239	294	243	149
C. immitis	2275	R. Talbot	San Joaquin Valley, CA	Burt et al ('97), Fisher et al ('00)	222	422	228	255	227	239	294	243	162
C. immitis	2276	R. Talbot	San Joaquin Valley, CA	Burt et al ('97)	222	418	228	252	210	239	294	247	168
C. immitis	2277	R. Talbot	San Joaquin Valley, CA	Burt et al ('97)	218	414	208	254	210	239	294	243	156
C. immitis	2278	R. Talbot	San Joaquin Valley, CA	Burt et al ('97)	218	414	228	254	202	239	294	243	162
C. immitis	2279	R. Talbot	San Joaquin Valley, CA	Burt et al ('97)	224	416	227	254	206	239	294	245	160
C. immitis	2280	R. Talbot	San Joaquin Valley, CA	Burt et al ('97)	222	420	228	252	206	239	294	243	147
C. immitis	2281	R. Talbot	San Joaquin Valley, CA	Burt et al ('97), Fisher et al ('00)	222	424	240	252	210	239	294	243	149
C. immitis	3526	R. Talbot	San Joaquin Valley, CA		224	426	211	252	212	239	294	243	156
C. immitis	3527	R. Talbot	San Joaquin Valley, CA		224	416	229	252	207	239	294	245	164
C. immitis	3529	R. Talbot	San Joaquin Valley, CA		222	416	241	255	212	239	294	245	166
C. immitis	3530	R. Talbot	San Joaquin Valley, CA		220	416	208	252	187	239	294	243	154
C. immitis	3531	R. Talbot	San Joaquin Valley, CA		218	418	229	252	187	239	294	245	156
C. immitis	3532	R. Talbot	San Joaquin Valley, CA		216	418	209	254	203	239	294	243	154
C. immitis	3311	R. Talbot	San Joaquin Valley, CA		224	424	210	252	206	239	294	243	162
C. immitis	3312	R. Talbot	San Joaquin Valley, CA		222	418	231	252	206	?	294	247	154
C. immitis	3261	R. Talbot	San Joaquin Valley, CA		222	414	231	252	210	239	294	245	154
C. immitis	3377	D. Pappagianis	Monterey, CA		224	422	228	254	208	239	292	243	166
C. immitis	5252	I. Gutierrez	Baja, North Mexico		222	416	241	?	212	240	?	245	145
C. immitis	5273	I. Gutierrez	Baja, North Mexico		226	419	231	256	?	239	294	243	164
C. immitis	3560	I. Gutierrez	Chihuahaua, North Mexico		226	419	229	256	209	239	?	243	164
C. immitis	3505	I. Gutierrez	Coahuila, North Mexico		226	420	228	256	212	239	294	243	154
C. immitis	3474	I. Gutierrez	Michoacan, South Mexico		222	420	232	256	210	239	294	243	166
C. immitis	3475	I. Gutierrez	Michoacan, South Mexico		224	426	229	252	210	239	293	243	164
C. immitis	3476	I. Gutierrez	Michoacan, South Mexico		216	416	228	254	210	239	294	243	145
C. immitis	3479	I. Gutierrez	Guerrero, South Mexico		226	424	228	252	210	239	294	243	156
C. immitis	RS	T. Kirkland	Vaccine strain-origin unknown		218	414	230	252	214	239	294	243	150