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Detection and phylogenetic analysis of *Coccidioides posadasii* in Arizona soil samples

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ABSTRACT

Reports of coccidioidomycosis are on the rise in the southwestern US. However, the ecology of the pathogen, *Coccidioides*, remains obscure and there is limited knowledge of the environmental antecedents of disease outbreaks. Detection of the fungus in the environment remains a critical challenge to modeling the source of disease. Using BALB/c mice as a biosensor, 8.9 % of soils analyzed from the Tucson area (Pima County, Arizona) were found to contain the pathogen. The genotypes of 66 *Coccidioides* strains, recovered from 11 soils, were determined with diagnostic microsatellite loci. Comparison of these genotypes to clinical isolates revealed all were *Coccidioides posadasii* and they grouped with Arizona isolates. Among sites where multiple strains were recovered, two indicated a clonal population, while others yielded a diversity of genotypes. A secondary goal of this research was to assess applicability of PCR, with its potential for high-throughput screening, as a method for identifying *Coccidioides*-containing soils.

Introduction

Coccidioides immitis and *Coccidioides posadasii* are dimorphic fungi that change from a saprobic mycelial phase in soil into a parasitic spherule phase when arthroconidia are inhaled by a mammalian host (Cole & Sun 1985). Coccidioidomycosis, a disease that can be severe in humans and other animals, can result from this exposure. Approximately 60 % of human infections are asymptomatic. Thirty percent result in a range of symptoms that can persist for months but are ultimately self-limiting. Fewer than 10 % of patients develop complications that require medical intervention. However, these cases may require lifelong therapies, or result in death. *C. immitis* is proposed to be endemic to the semiarid desert areas of the central valley and southern deserts of California, and possibly into Baja California, whereas *C. posadasii* is proposed to be endemic to southern Arizona, New Mexico, western Texas, northern Mexico and parts of Central and South America (Fisher *et al.* 2002).

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Based on human infection and positive skin test rates in the US, the highest exposure to *Coccidioides* is in the southern portion of the central valley of California (Kern and Tulare counties) and the Sonoran desert areas of southern and central Arizona (Pima, Pinal and Maricopa counties) (Fig 1; Ajello 1971; Kirkland & Fierer 1996; Pappagianis 1988). There has been a steady increase in reported cases of coccidioidomycosis with 1551 cases reported to the Arizona Department of Health Services in 1998, 5535 reported in 2006 and over 10000 reported in 2009 (Komatsu *et al.* 2003; Sunenshine *et al.* 2007; Hector *et al.* 2011). Despite this increase, and the associated interest due to the economic and human costs of coccidioidomycosis, little is known about the environmental source of the inoculum. On a regional scale, the major predictors of disease are climate, soil disturbance, and dust/wind events (Pappagianis 1994; Pappagianis *et al.* 1994; Comrie 2005; Comrie & Glueck 2007). However, at finer scales, the ecology of the fungus remains obscure and environmental antecedents of the disease are largely unstudied (Cox & Magee 2004). Reports have suggested it may be associated with alkaline soil that has a high salt content, rodent burrows and Amerindian middens at archeological sites; however, little is known about the specific niche of *Coccidioides* in the soil or how environmental factors impact risk of human infection (Galgiani 1999).

Previous studies of the ecology of *Coccidioides*, conducted primarily between the 1930s and the mid 1970s, demonstrated that it was difficult to isolate the fungus from soil. Overall reported recovery rates from soil are low, from 0 % to 15 % (Elconin *et al.* 1957; Maddy 1958; Swatek *et al.* 1967; Swatek &

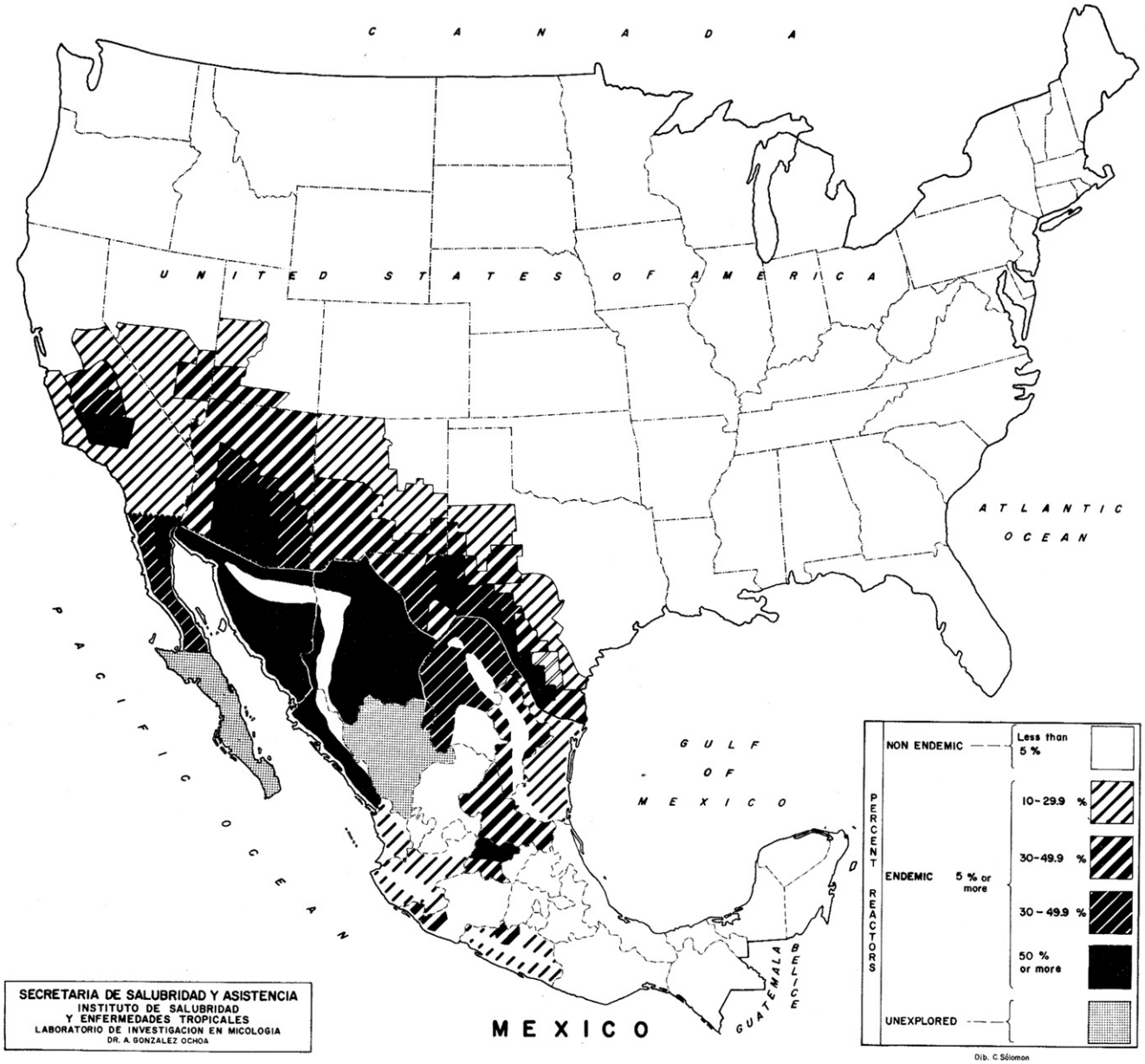

Fig 1 – Map of endemic area (from Ochoa 1967, reprinted with permission from UA Press). Darker areas have higher incidence of coccidioidomycosis and are therefore thought to be highly endemic.