

IDENTIFICATION, DISTRIBUTION AND ECOLOGY OF MALARIA MOSQUITOES IN THE EASTERN SLOPES OF THE ANDES MOUNTAINS, BOLIVIA

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INTRODUCTION

Malaria continues to be the most important vectored infectious disease of humans, killing 1-3 million people worldwide each year. Though over a dozen species of anopheline mosquitoes have been implicated in malaria transmission in South America, little is known about the ecology and distribution of most species. A lack of reliable and efficient means of identifying malaria mosquitoes has been a major obstacle for studies on their biology, ecology, and behavior, and responsible for our fragmentary understanding of the epidemiology of malaria in this region of the world. This study examined entomological aspects of the epidemiology of malaria in a Neotropical valley system along the eastern slopes of the Andes Mountains in Bolivia, the Chapare/Carrasco Valleys. This study had three main goals: 1) the development of quick and reliable molecular diagnostics for identifying malaria mosquito species, 2) the determination of species numbers and distributions in an ecologically complex valley system in Bolivia with endemic malaria, and 3) the relationship between aquatic breeding sites and other physical parameters in relation to mosquito species distributions.

METHODS & RESULTS

1. Development of Molecular Diagnostics

By using species specific sequences from a rDNA spacer (the internal transcribed spacer 2, Fig. 1) multiplex PCR were developed for identifying all life stages of nine species of malaria mosquitoes.

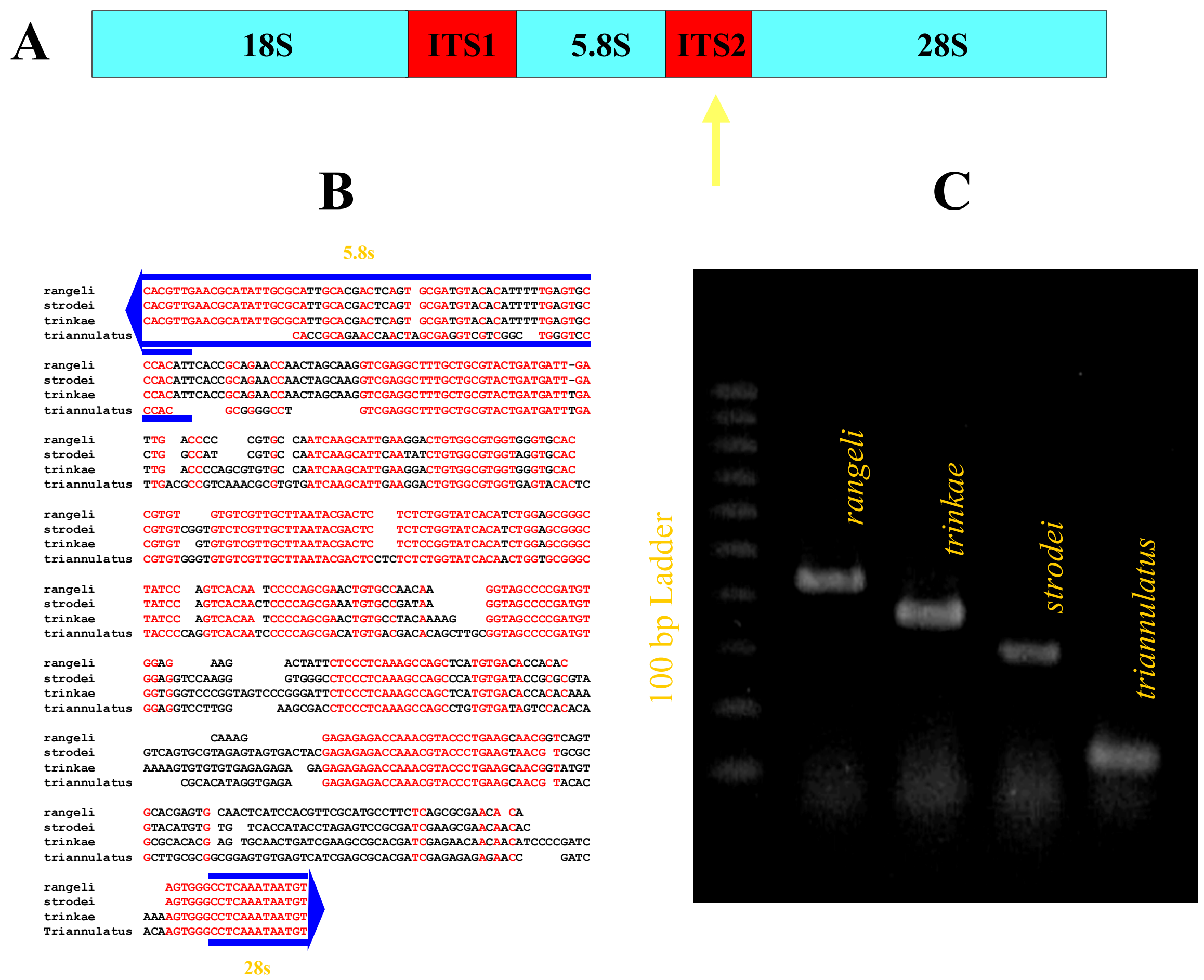


Fig. 1. A) rDNA unit showing ITS2 region sequenced for alignment; B) example alignment of the ITS2 of four species of malaria mosquitoes showing variable regions (black letters); C) gel showing species specific banding pattern for 4 species.

• Species Distributions and Number

Up to 50 mosquito larvae were sampled and identified (using PCR) from 56 aquatic breeding sites along the length of the Chapare/Carrasco valleys (Fig. 2A). The distributions of most species is non-random across all 56 sites (e.g., Fig. 2B). Some species such as *An. trinkae*, for example, are found only at higher elevations on the eastern slopes of the valley (Fig. 2B). Though 13 species have been identified (Fig. 3), two species, *An. trinkae* and *An. rangeli*, comprise over 54% of all mosquitoes collected.

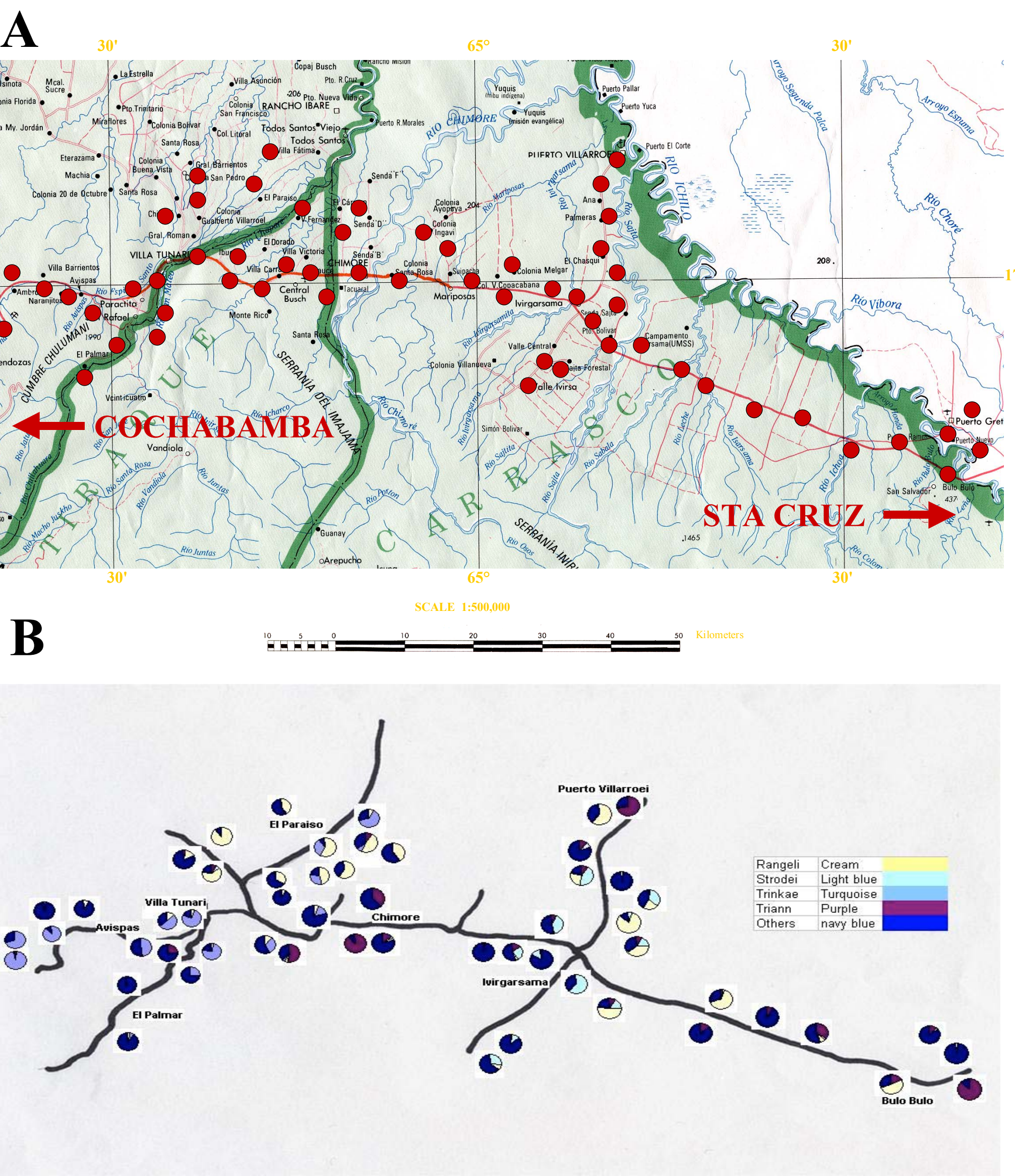


Fig. 2. A) Map of the Chapare/Carrasco valleys, Bolivia showing 56 breeding sites where larvae were collected; B) example of the distribution of four of the nine species identifiable by PCR in this study.

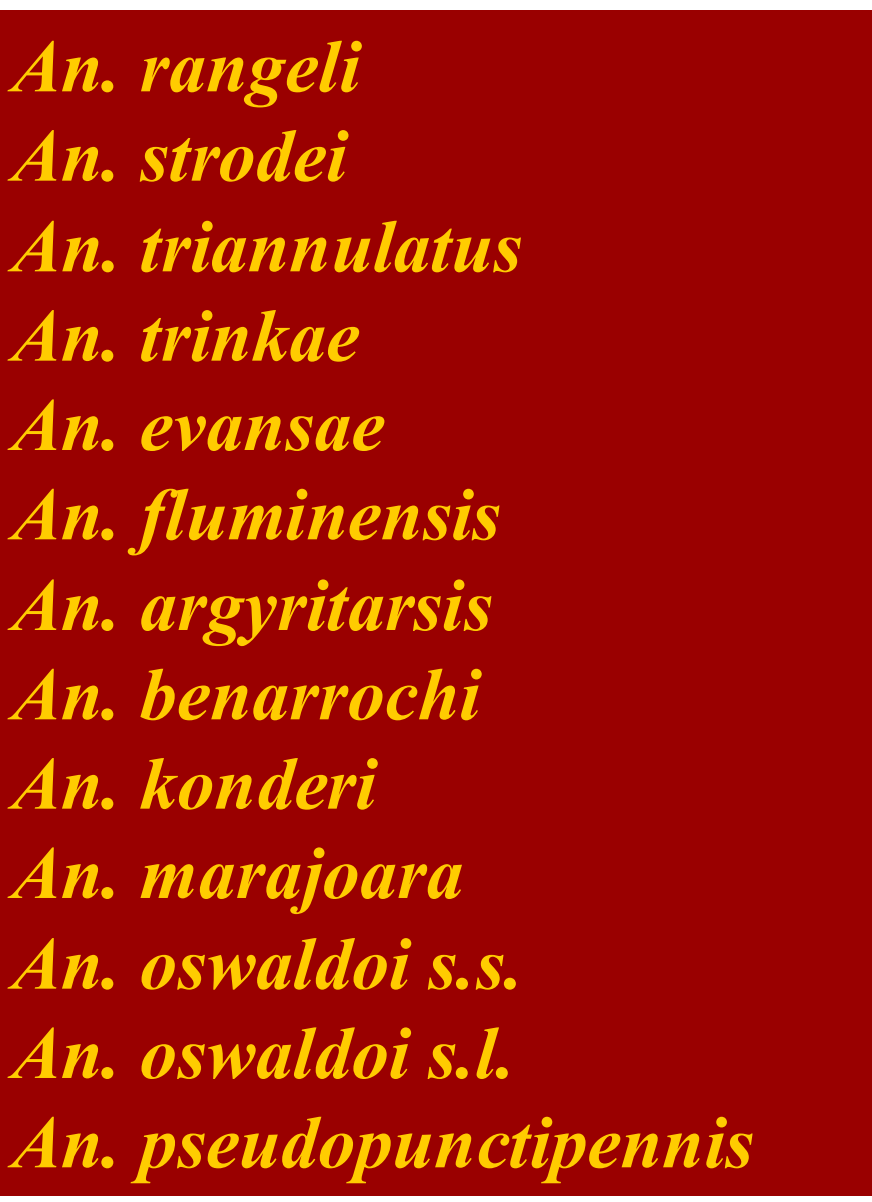


Fig. 3. All species identified in the Chapare/Carrasco valleys to date.

3. Aquatic Parameters & Species Distributions

Principal component analysis and Spearman's Correlations indicated significant associations between the presence/absence of particular species and particular parameters of aquatic breeding sites (Fig. 4A, 4B). ANOVA indicated significant associations of certain species with elevation above sea level.

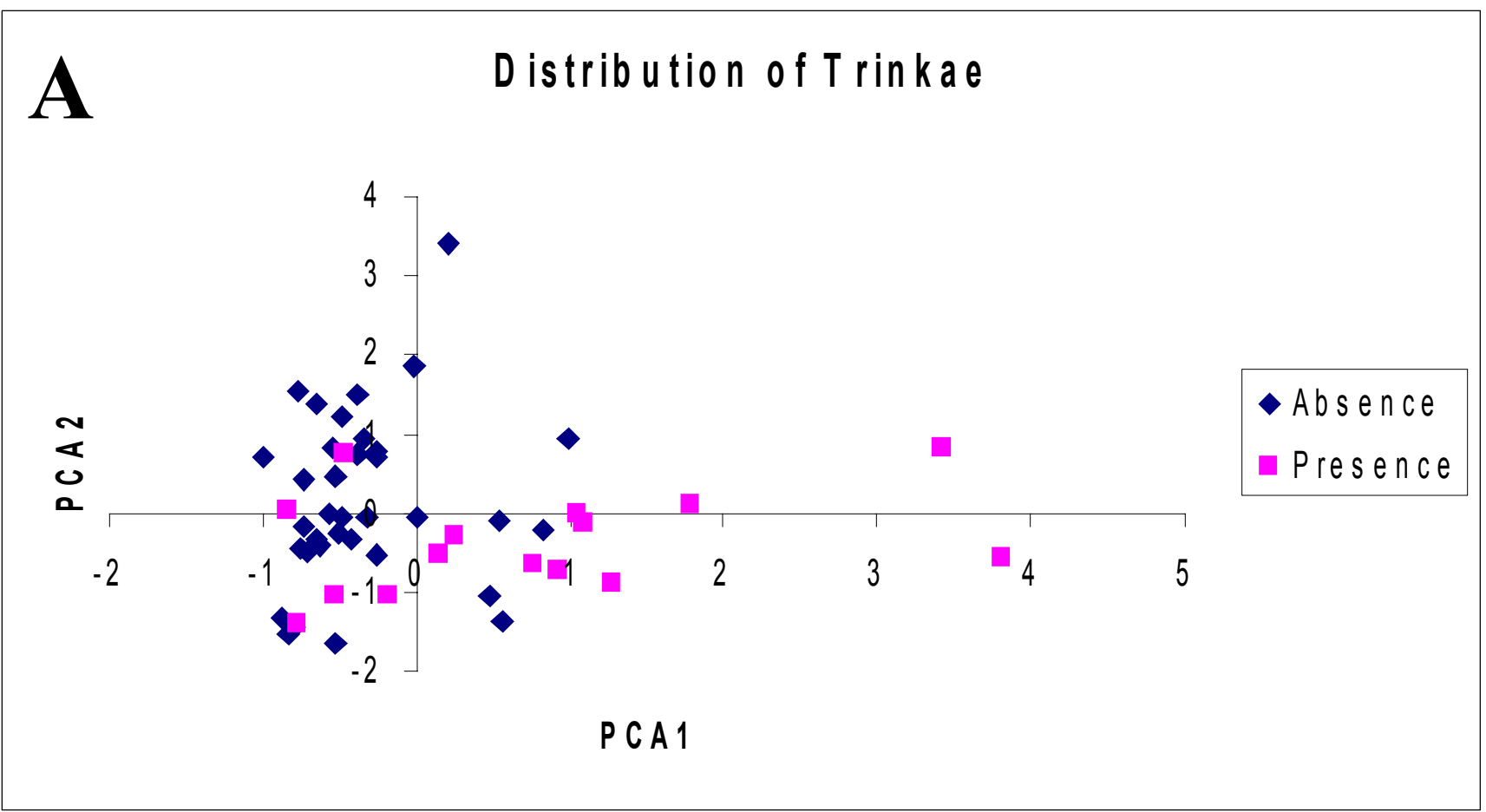


Fig. 4. A) An example of Principal Component Analysis for the presence/absence of *An. trinkae* at 56 aquatic sampling sites in relation to 14 aquatic parameters; B) Spearman's Correlation Probability values for four species of anopheline mosquitoes with respect to 14 water quality parameters.

WATER QUALITY PARAMETERS	triannulatus	strodei	rangeli	trinkae
DO	.029	.958	.689	.785
Turbidity	.195	.934	.332	.014
NO ₃	.755	.237	.542	.782
COD	.860	.001	.480	.647
NH ₄ -N	.418	.586	.328	.893
pH	.059	.028	.180	.001
TDS	.066	.021	.866	<.001
Conductivity	.085	.021	.873	<.001
PO ₄	.408	.745	.498	.379
Total solids	.247	.037	.955	<.001
Dissolved solids	.076	.043	.923	<.001
Suspended solids	.002	.468	.824	.965
Volatile solids	.338	.077	.398	.009
Fixed solids	.256	.045	.858	<.001

CONCLUSIONS

- Multiplex PCR of nine species produce species specific amplicons useful for identifying any life stage. The development of multiplex PCR that can diagnose nine vectors of human *Plasmodium* in the Neotropics should make it more feasible to initiate studies on anopheline mosquito ecology and the dynamics of transmission of malaria, particularly since the primers can be used with a minute amount of sample material for species identification.
- Some species, such as *An. trinkae*, *An. strodei*, and *An. marajoara* exhibit narrow distribution ranges, whereas others species such as *An. triannulatus* and *An. rangeli* are broadly distributed. At least 13 species have been identified in the Chapare/Carrasco valleys, but two species, *An. trinkae* and *An. rangeli* comprise over 54% of all specimens collected. Distribution patterns of some species were significantly associated with certain water quality parameters (e.g., dissolved solids, pH, conductivity, etc.), elevation, aquatic categories (river, pond, ditch, etc.) and their associated flora (algae, grasses, etc.).

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