

Passiflora chiapasensis
Photo by Kristen Porter-Utley

Evolution of the Small-Flowered Passionflowers

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Introduction

Passionflowers (family Passifloraceae) are tropical vines that are widely known for their striking, complex flowers and delicious fruits. They possess some of the most variable reproductive and vegetative structures of all plants. For this project, we have chosen to study a subgroup (~250 species) of passionflowers (genus *Passiflora*, subgenus *Decaloba*) that possess small flowers (Fig. 3). These plants are incredibly interesting for a variety of reasons; they have beautiful flowers, a wide variety of interesting pollinators such as bees, bats, butterflies and hummingbirds, have floral structures that are adapted for these different types of pollinators (Fig. 1), and have interesting coevolutionary relationships with Heliconid butterflies.

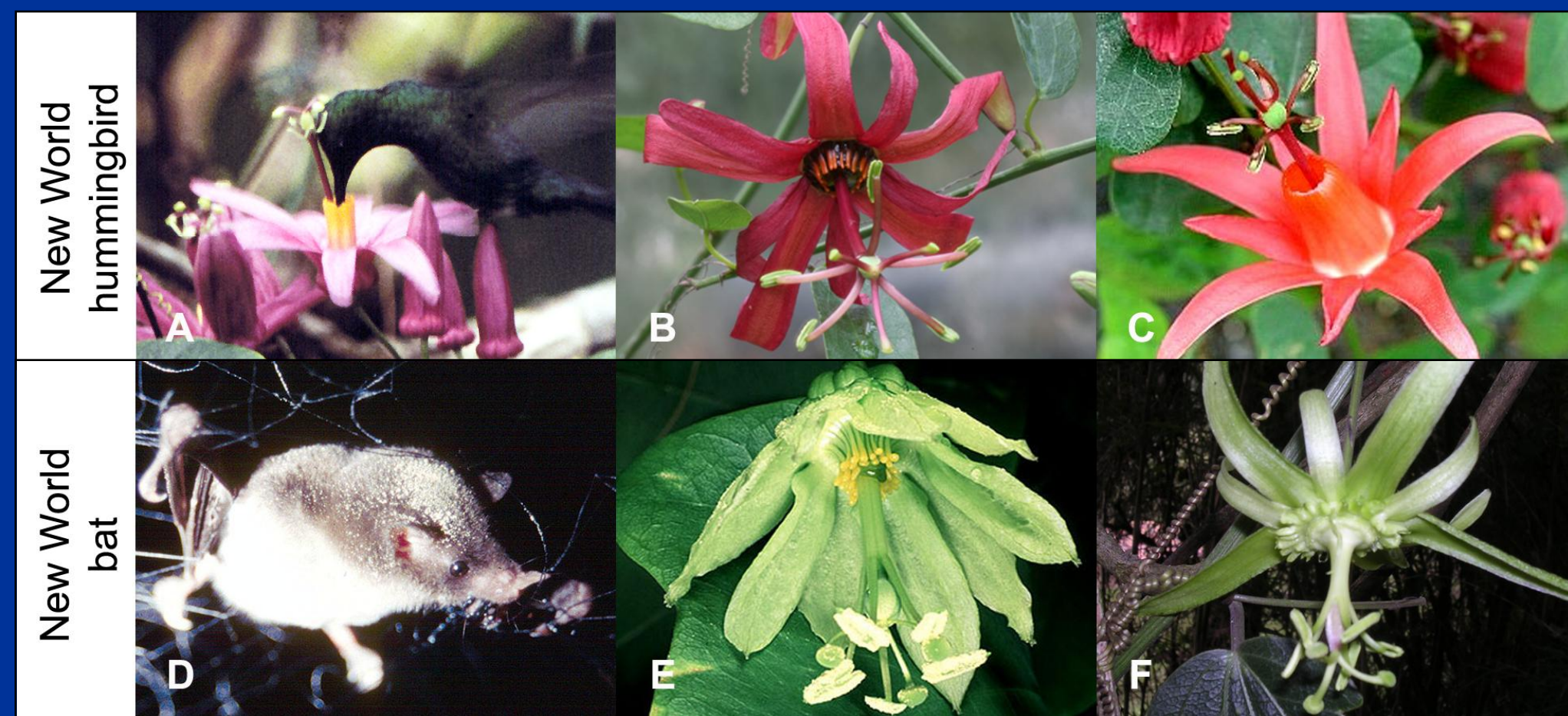


Figure 1: Vertebrate pollination syndromes in subgenus *Decaloba*. A. Hummingbird pollinating *P. tulae* in Puerto Rico, photo E. Kay. B. *P. perfoliata* from Jamaica. C. *P. murucuja* from the West Indies. D. Bat captured in a mist net pollinating *P. penduliflora*, photo E. Kay. E. *P. penduliflora* from Cuba. F. *P. viridescens* from Ecuador and Peru.

To begin to resolve the evolutionary relationships of the species in subgenus *Decaloba* we extracted DNA from 86 of the 250 species in the subgenus and amplified the nuclear ribosomal internal transcribed spacer (ITS) gene for 41 taxa. The ITS gene has been important in the reconstruction of phylogenetic relationships in *Passiflora* (Muschner et al., 2003; Hansen, 2004; Hearn, 2004; Krosnick and Freudenstein, 2005; Krosnick et al., 2006), providing sufficient signal to resolve relationships at the subgenus to species level (Fig. 3).

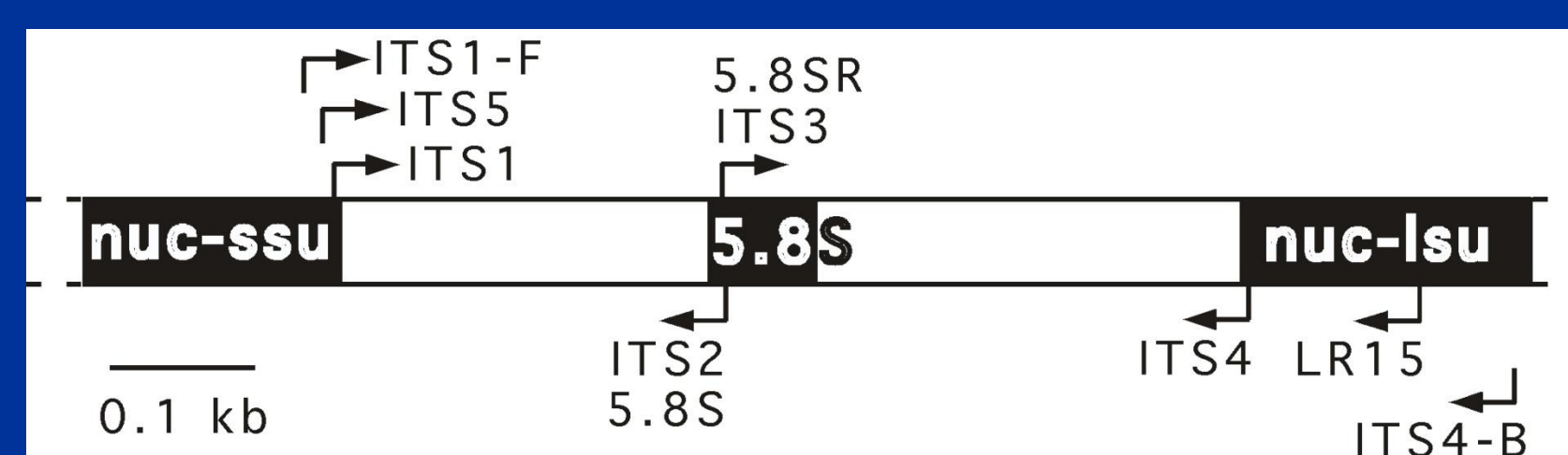


Figure 3: Map of ITS gene and the primers (ITS4 and ITS5) used to amplify it (photo from Binder and Hibbett, 2003).

Abstract

Throughout the course of the academic year our research has been focused on the systematics of a large group of passionflowers (*Passiflora* subgenus *Decaloba*). Protocols have been designed and modified for the amplification and sequencing of the nuclear ribosomal internal transcribed spacer (ITS) for the species in the subgenus. The DNA sequences for this gene will allow for the verification, or possibly call for the reinterpretation, of the currently proposed hypothesis of evolutionary relationships in subgenus *Decaloba*. Little is known about the evolution of the species and it is likely that, if left unstudied, many of these species will be destroyed before we know anything about their biology or the interactions that these plants have with other organisms in nature. At the end of this project insight into the evolutionary relationships of this relatively understudied group of plants will be presented.

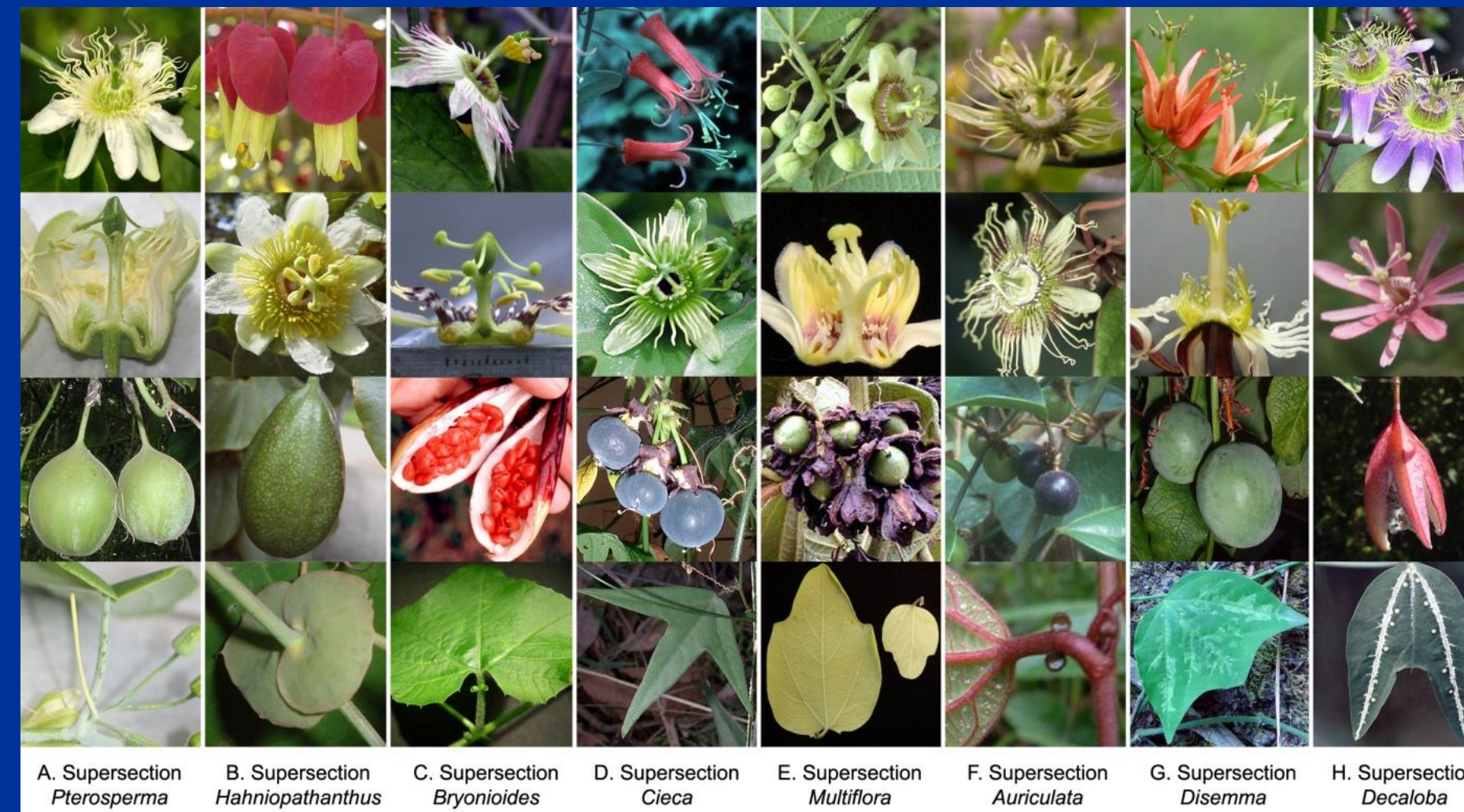


Figure 3: Floral and vegetative diversity among the eight subgroups (supersections) of subgenus *Decaloba* (image from Jorgensen et al., 2006).

Methods



Figure 4: Four Students extracting DNA from leaf material. In photo Nicole Siddall, Scott Massed, Jacob Neal, Erika Brooks. Photo by Kristen Porter-Utley.

Total genomic DNA was extracted from fresh and herbarium leaf material and polymerase chain reactions (PCR) were run to amplify the ITS gene (Fig. 4). Total genomic DNA was extracted from silica dried leaves using the CTAB method of Doyle and Doyle (1987). Amplification of ITS was performed using primers designed by White (1990) and the method of the *Passiflora* Research Network. The ITS region was sequenced directly from the cleaned cycle sequencing product in the DNA Sequencing Lab at Rancho Santa Ana Botanic Garden, California.

Results

DNA was effectively extracted from both live and herbarium (dating as far back as the 1980s) plant tissue. Amplification of the ITS gene was successful through PCR (figure 5). We were able to effectively amplify 41 taxa.

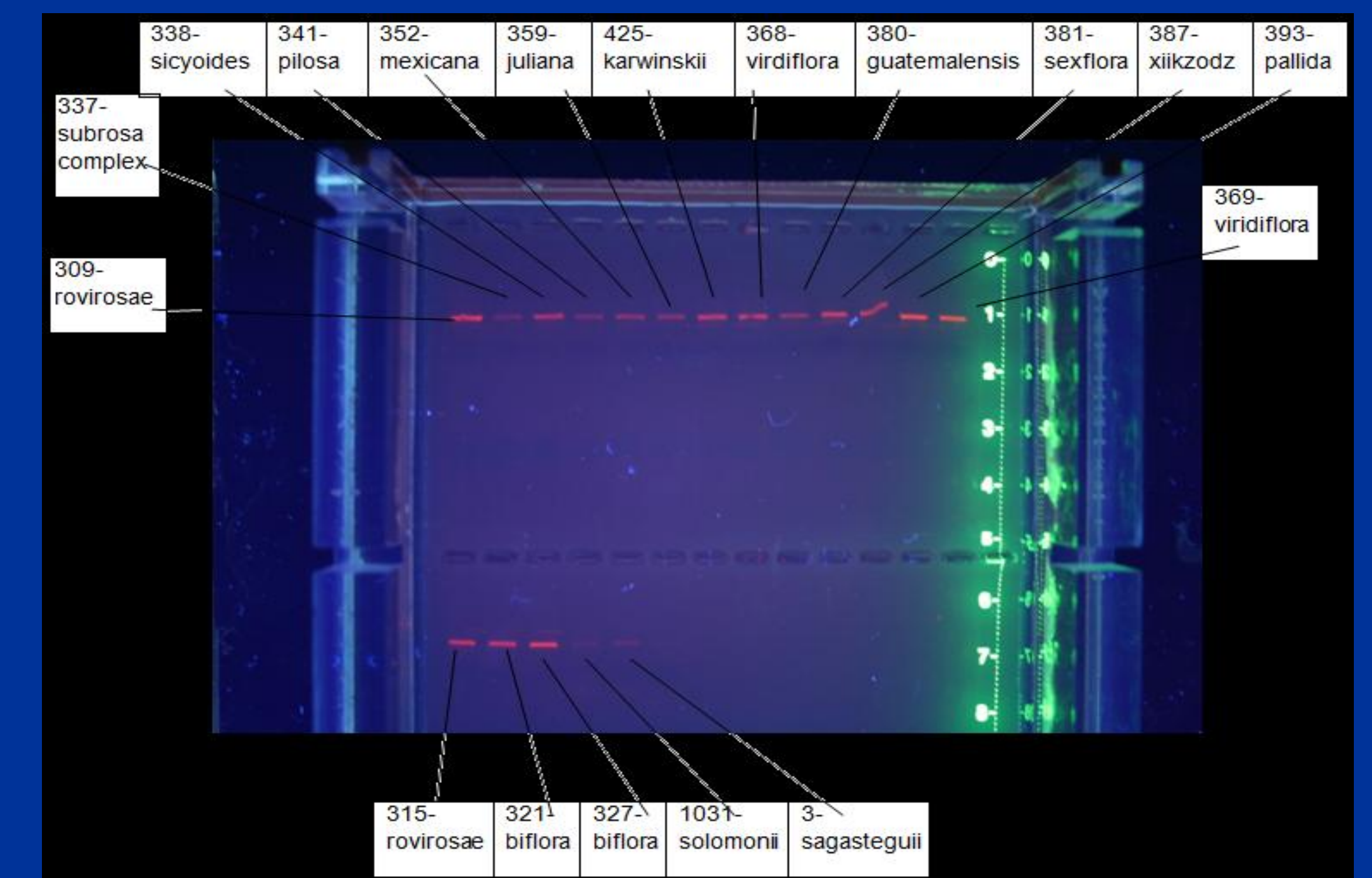


Figure 5: Photograph of PCR products on an agarose gel. The pink bands represent the amplified ITS gene for 16 species in subgenus *Decaloba*.

Discussion

Amplification of the ITS gene was successful. The final step, DNA sequencing, will be completed by the end of the spring 2009 semester. We did successfully sequence two taxa (Figs. 6 and 7). For one of the taxa (*P. rovirosae*), we received clean sequence data (Fig. 6). For the other (*P. juliana*) we received a mixed signal (Fig. 7). A possible reason for this is because more than one copy of the ITS gene is located in the cells of the species, due to hybridization. Cloning the PCR products may yield better results.

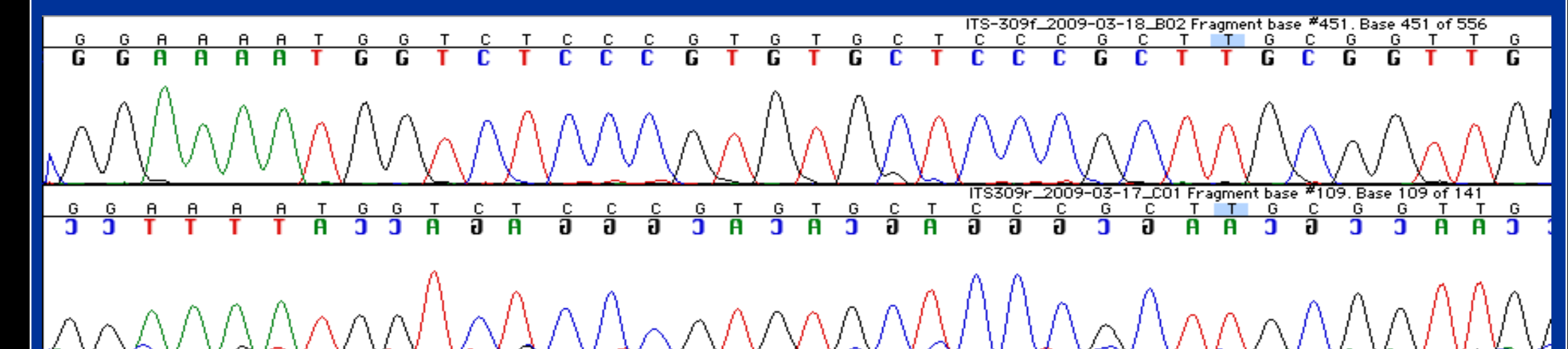


Figure 6: Cycle sequence of *Passiflora rovirosae*, worked

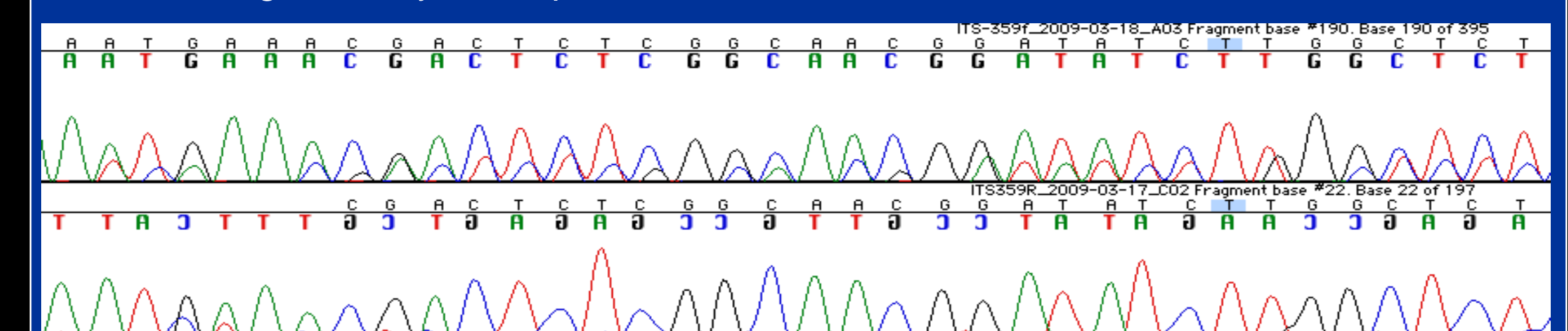


Figure 7: Cycle sequence of *Passiflora juliana*, did not work