**Taxonomy, Biogeography and Pest Status of *Marmara* sp. in Northern Mexico and
California Citrus Production**

A Thesis

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TITLE: Taxonomy, Biogeography and Pest Status of *Marmara* sp.
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ABSTRACT

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A novel lepidopteran sex pheromone lure which was thought to be species-specific to the citrus pest *Marmara gulosa* (Lepidoptera: Gracillariidae) Guillén (McElfresh et al. 2009) has attracted male moths exhibiting two distinct morphologies. The morphological features examined were the sclerotized parts that make up the male genitalia; which include the valvular lobes, costal lobes, cucullar lobes, and the aedeagus. The sclerotized parts were methodically dissected and mounted on microscope slides. Digital images captured of the slide-mounted specimens proved to be inconsistent with the illustrations provided in the original *Marmara gulosa* species description (Guillén et al. 2001).

The morphologies of all specimens from both the San Joaquin and Coachella valley show remarkable consistency; regardless of host plant origin, collection method, and collection date. Two specimen groups (one from an orchard in Northern Mexico and the other from backyard citrus in Riverside, California) exhibited entirely different morphological features in which the three lobes mentioned above are fused together. This difference in morphology can only be noticed when examining the sclerotized parts of the male genitalia under a microscope; it is otherwise indistinguishable from *M. gulosa* via macroscopic features such as antennae shape and wing pattern. These two specimen groups with fused lobes are considered a new species belonging to the genus *Marmara* and currently remain undescribed.

A genetic study conducted at UC Riverside, which concentrated on the CO1 region of mitochondrial DNA, reported significant differences in genetics based on the geographic origins of specimens (Vickerman and Stouthammer 2006). Geographically, the genetic distribution described in the 2006 study does not coincide with the distribution of morphologies described in this paper. The species description of *Marmara gulosa* is in need of revision. In addition, further collection and research is required in order to describe and name the newly discovered *Marmara* species.

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1.0 Summary

Identification of a pest species that is causing damage to agriculture production is the first step in the development of an integrated pest management program (Flint and Gouveia 2001). A pest of citrus fruit in southern California, known as the citrus peelminer, was identified and described in 2001 as *Marmara gulosa* (Lepidoptera: Gracillariidae) by Guillén, et al. (2001). *Marmara gulosa* are microlepidopteran miners whose larvae create serpentine mines just under the epidermis of fruit rind and stems in citrus and other crops (Guillén, et al. 2001, Grafton-Cardwell et al. 2003). A visible mine scar results that render fruit, in particular citrus, unsuitable for fresh market sale (Atkins 1961, Reeves 1995). The current study provides evidence that revises the history and identification of *M. gulosa* as a pest of citrus in California and Mexico. Additionally, the current species concepts and methods of identification used to determine species in the genus *Marmara* are revised.

Figure 1. Peelminer damage on Valencia oranges grown in Mexico

The occurrence of citrus peelmining damage in the southwestern US was first recorded in 1917 on an orange (*Citrus sinensis*, Osbeck) collected in 1915 from Pasadena, CA (Vinal 1917). Since that time, outbreaks of peelmining have occurred sporadically in California and Arizona citrus orchards (Lockwood 1933, Woglum 1948, Atkins 1961, Atkins 1971, Reeves 1995, Gibson et al. 1997). All of these outbreaks were attributed to *Marmara salictella* (Lepidoptera: Gracillariidae) Clemens (Woglum 1948, Atkins 1961, Atkins 1971). *Marmara salictella* is a gracillariid miner known for mining young stems of willow species along the US eastern seaboard (Clemens 1863). Woglum (1948) referred to the pest in California as a “western form” of *M. salictella*. In the mid 1990s peelmining of citrus fruit in Coachella Valley of southern California became much more widespread, aggressive and persistent than evidenced in the past (Neff 2002, Grafton-Cardwell et al. 2003). This prompted studies by Guillén and coworkers on the biology, bionomics and ultimately the description of *M. gulosa* as a new species distinct from *M. salictella* (Guillén et al. 1999, 2001, 2003, 2004, 2007). The findings in these studies included the note that at least one, if not more, *Marmara* species are native to California and have only sporadically, been a pest in certain cropping systems (Atkins 1961, Atkins 1971, Reeves 1995, Gibson et al. 1997, Guillén et al. 2001).

Between 1999 and 2000, peelmining of citrus fruit became widespread and aggressive in the San Joaquin Valley (SJV) of California, similar to what was observed in Coachella Valley a few years earlier (Guillén 2001, Neff 2002, Grafton-Cardwell et al. 2003). The damage in the San Joaquin Valley was immediately associated with citrus peelminer, *M. gulosa*, from Coachella Valley. The identification of the San Joaquin Valley species causing peelmining damage was based on the following lines of evidence: 1) a tentative

identification provided by Guillén and coworkers based on similarity of mining, larval anatomical characteristics and adult features, 2) genetic analyses provided by Dr. Richard Stouthamer, Department of Entomology, UC Riverside, and 3) positive responses of males to *M. gulosa* female pheromones. All of these lines of evidence however, coincided with the report of accidental shipment of peelminer infested fruit into the San Joaquin Valley from southern California and Mexico following a severe freeze event in the winter of 1998/1999. Thus, the identification of the peelmining pest in the San Joaquin Valley was generally accepted by the research community as *M. gulosa*, but that its behavior in the San Joaquin Valley was due to invasion by an aggressive subpopulation of *M. gulosa* that heavily mined citrus and other crops. There were competing theories regarding how populations of *M. gulosa* achieved pest status in the San Joaquin Valley, including pesticide upset related to the use of Insect Growth Regulators for control of California red scale, *Aonidiella aurantii* Maskell or as the result of the freeze event itself. However, the freeze event and subsequent movement of fruit from southern California and northern Mexico to packing houses in Tulare Co. provided the most logical and easily accepted explanation for what appeared to be an invasion by a more aggressive biotype of *M. gulosa* into the San Joaquin Valley. In 1999, a field biologist working with the Tulare Co. Agricultural Commissioner's office observed citrus fruit with peelminer damage discarded outside packing houses. Peelminer damage appeared in orchards around the packinghouses as reported by county agriculture personnel (Haines 2002). Subsequently, infestations a greater distance from these initial sightings were noted and a wave of infestation moved northward, so that by summer 2001, peelminer infestation increased

dramatically with some orchards sustaining as much as 80% damage (Grafton-Cardwell 2003).

Further, a wider variety of plant species sustained peelmining damage than had previously been recorded in southern California (Haines 2002, Grafton-Cardwell 2003). The occurrence of feeding activity on new plant species and an increased radius of infestation from the initial site of discovery are evidence associated with an invasive species or biotype (Louda et al. 1997, Sakai et al. 2001, Grafton-Cardwell pers. com. 2003). Thus, it was hypothesized that an aggressive form of *M. gulosa* was unintentionally introduced from northern Mexico into the San Joaquin Valley during the 1998/1999 winter freeze event. This set the stage for biological control studies in Mexico, reported herein. Searching in the pest's native home provides the best-suited natural enemies (due to long-term co-evolution) for release and eventual establishment and control in the newly invaded area (Legner and Bellows 1999) was a primary precept of biological control used.

A quarantine issue arose between the US and Mexico in late 2007 and early 2008 that changed our assumptions and prompted more careful taxonomic scrutiny of the collections of specimens of *M. gulosa* taken and stored from various locations in California and Mexico. In 2008 the Mexican authority SAGARPA rejected a shipment of stone fruits from Fresno Co. with what was identified as peelminer damage. Their actions were based on the lack of published records indicating that *Marmara* species occur in Mexico. Guillén et al. (2001) described *M. gulosa*'s known range as being north of Mexico, but did not collect or try to obtain specimens from Mexico to complete their study and verify the full extent of *M. gulosa*'s southern range. SAGARPA's decision to

halt fruit shipments was based on a lack of published evidence, not verified collecting data showing *Marmara* species did not occur in Mexico. Thus detailed taxonomic studies were initiated and are reported herein. The results show that citrus fruit in Mexico are attacked by a distinctly different species of *Marmara*. However, genetic analyses of specimens collected in Mexico show that both *M. gulosa* and an undescribed species of peelminer are present in Mexico. Thus some of the initial ideas linking peelmining activity in the San Joaquin Valley of California and the Hermosillo citrus growing region in Mexico must be re-considered. The existence of potentially different species with indistinguishable fruit damage patterns calls for reinterpretation of previous data regarding this pest species and its purported invasion into California. There are also new implications regarding importing and exporting fruit that serve as host plants for *M. gulosa* and other *Marmara* species across the US/MX border.

The research reported herein focuses on two research projects: 1) foreign exploration research evaluating the biology and natural enemies of peelminer populations in citrus production areas of northern Mexico, and 2) morphological analysis of male anatomy for identification of *Marmara* species associated with peelmining in both Mexico and California.

2.0 Literature Review

2.1 Citrus Cropping System: San Joaquin Valley

Citrus has been grown commercially in California since the mid 1800's (Sawyer 1996). Today citrus has become one of California's largest agricultural industries, with annual production values averaging around one billion dollars during the late 1990's and throughout the 2000's (USDA 2007). Citrus is extensively grown in the San Joaquin Valley. Orchard plantings form a "citrus belt" along the east side of the valley bordering the Sierra Nevada foothills, approximately spanning the distance between Bakersfield and Fresno. California's citrus industry relies heavily on fresh market sales. Growers receive the highest prices for fruit that can be sold in export markets. Most of California's pest management practices focus on producing unblemished fruit. Cosmetically damaged fruit can be used for juicing, but is worth less money. Common pests that cause cosmetic damage to fruit include thrips, scale insects, and katydids; these pests represent a relatively low number of insect species that directly attack the fruit relative to the total number of species in the system (UC ANR 2008). Peelmining, thus, poses a significant threat to citrus production because a single larva can create enough mining to reduce the value of the fruit (Guillén et al. 2001). The recurring threat that peelminer poses to California's citrus industry needs to be resolved through understanding of its biology, its biogeography and implementation of a control program that is compatible with all of the other control measures in use – i.e., integrated pest management.

2.2 Host Plant Species Associations of *Marmara gulosa*

Many *Marmara* species have a single or limited number of host plant species that are used for development (monophagous or oligophagous) and some feed only on specific structures or tissues of the host plant (De Gryse 1943, Guillén et al. 2001, DePrins 2008 pers. com, D. Davis 2008 pers. com). *Marmara gulosa* is considered to be one of the most highly polyphagous of the gracillariids, with rearing records from five families in four of six subclasses of dicotyledonous plants (Heywood 1993). The name *gulosa* was derived from the Latin word *gulosus*, meaning gluttonous or greedy, and refers to the wide host plant range observed in the species (Guillén et al. 2001). Host plant association records are dubious in the entomological literature because many older citations did not necessarily take into account that for a plant to serve as a true host, successful larval development must be verified. Gracillariids are minute insects and very rarely are adults observed in the wild; the most conspicuous evidence of host plant usage by gracillariids is the mining of the larvae; however, associating a particular adult with its previous larval mining is a painstaking process, thus, verification of an identity is something that requires more than a single observation of mining on a plant. If the identification of the insect species is unclear, that puts host association records in further doubt.

Peelmining activity attributed to *M. gulosa* was recorded in the San Joaquin Valley during 2000-2002 on 65 species of plants from 28 families (Neff 2002). Peelmining larvae were observed feeding on various host plant structures in San Joaquin Valley agricultural systems such as shoots and fruit of citrus (*Citrus* spp.) (Rutaceae),

leaves and stems of oleander (*Nerium oleander* L.) (Apocynaceae), stems and bolls of cotton (*Gossypium hirsutum* L.) (Malvaceae), shoots and fruit of various stonefruit (*Prunus* spp.) (Rosaceae), shoots and fruit of walnut (*Juglans* spp.) (Juglandaceae), olive fruit (*Olea europaea* L.) (Oleaceae), fruit of bell pepper (*Capsicum annuum* L.) (Solanaceae), fruit of squash (*Cucurbita* spp.) (Cucurbitaceae), shoots and fruit of avocado (*Persea* sp.) (Lauraceae), and shoots of willow (*Salix* sp.) (Salicaceae) (Grafton-Cardwell 2003). Citrus hosts susceptible to peelmining include varieties of grapefruit (*Citrus paradisi*, MacFayden), pummelos (*Citrus maxima*, Merr.), lemons (*Citrus limon*, Burmann), lime (*Citrus aurantifolia*, Tanaka), tangerine (*Citrus reticulata*, Blanco) and most smooth-skinned navel oranges (*Citrus sinensis*, Osbeck) varieties such as Atwood, Fukumoto, Thompson Improved and Lane Late.

2.2.1 Host Plant Range Expansion. Peelminer populations in the San Joaquin Valley expressed behaviors that can be interpreted as those of an invasive species/population (Grafton-Cardwell pers. com. 2003). When herbivorous insects are moved away from their natural habitats, they have the potential to exploit new niches in the new environment (Huffaker et al. 1984, Louda 1997). An insect population that is able to do this is more likely to become a pest (Sakai 2001). Typically, a phytophagous insect in its native habitat has predators, pathogens, and climatic pressures that keep populations under control. In normal population dynamics, the number of phytophagous insects will naturally rise, creating more resources for its associated predators, pathogens, and parasites that begin to exert mortality that lowers the phytophagous populations; this is known as imperfect density dependent regulation (Huffaker et al. 1984). Due to this natural regulation of populations, phytophagous insects in their native habitats should

rarely exhibit abnormally large and sustained population increases. When the insect is either moved or the habitat is significantly altered to provide more favorable weather conditions, less pressure from natural enemies, and/or fewer limitations on resources, the insect may find conditions to be more suitable for growth. A species may adapt to the new habitat, expressing increased reproductive potential and eventual pest status (Lee 2002). Host plants available in the pest's new habitat may be more desirable than the plants provided by its native habitat. A phytophagous insect's host plant range can expand when introduced to a new area (Louda 1997). Adaptation of species can occur in response to environmental shifts in host plant species (Lee 2002). This has been demonstrated with the North American soapberry bug, *Jadera haemotoloma*, and its morphological and lifecycle adaptations to the introduced host plant *Koelreuteria elegans* (Sapindaceae) (Carroll et al. 2001). Ovipositional mistakes are one method by which a lepidopteran insect can expand its host range (Chew & Robbins 1984, Singer 1984). Other microlepidoptera such as those in the family Tortricidae are known to frequently shift host plants (Powell 1980, Thompson & Pellmyr 1991). Most of the other members of the genus *Marmara* are host plant specific (Gullin et al. 2001). During the summers of 2001 and 2002 peelminer was observed on many more crops than normal such as bell peppers, table grapes, avocado, and various stone fruit (Grafton-Cardwell 2003). Field observations of population dynamics and host plant range expansion are not enough however, to determine if peelminer is native or invasive in the San Joaquin Valley, when one considers the possibility of a native species having the ability to adapt to altered environments.

2.2.2 Host Plants and Voltinism. The many alternate host plants provide opportunities for the pest to overwinter or escape host plant-specific natural enemies. Environmental conditions permitting, the peelminer can potentially complete seven consecutive generations per year in the San Joaquin Valley (Grafton-Cardwell 2001, O’Neil 2007). However, citrus orchards alone cannot provide a suitable host environment for all seven generations. Some of the most notable alternate hosts in the San Joaquin Valley are oleander, cotton, willow, and walnut. These plants figure into the seasonal cycle of peelminer, presumptively providing resource “bridges” from one host plant species to another as the season progresses. Peelmining activity takes place on newly expanding tissues and it appears that it is the type of tissue and its developmental stage that is important for the growth and development of *Marmara* species and not necessarily the host plant species *per se*.

Figure 2. Appearance of peelminer activity on SJV oleander.

Figure 3. Peelminer activity during the previous year caused the scarring seen in this SJV walnut orchard.

2.3 *Marmara gulosa* Biology in the San Joaquin Valley

According to Guillén et al. (2001), a female peelminer moth oviposits eggs on the epidermis of a host plant. A larva bores through the egg and directly into the epidermis of the host plant. Upon entering the plant from the egg, it begins feeding under the uppermost layers of the epidermis creating its characteristic serpentine mine. Peelminer larvae have four to five feeding instars; with each successive instar the larvae increase in size. The final subcutaneous instar is a nonfeeding stage which retains the cuticle of the former feeding instar. This nonfeeding instar is referred to as a “pinkie” due to its noticeable pink coloration. A final spinning instar results when the larva simultaneously sheds both the final sapfeeding and nonfeeding cuticles. Finally, the spinning instar cuts out of its mine, leaving behind a characteristic half-circle exit hole (Fig. 4).

Figure 4. Semicircular cut in the citrus rind epidermis from which the spinning stage larva emerges to seek a sheltered place for pupation. Note the cast cuticle with darkened head capsule left behind in the mine.

After emerging from the mine, the spinning larva seeks a sheltered place either on or off of its natal plant to spin a cocoon and pupate. Adults emerge and presumably begin mating and oviposition behaviors. Adult female moths release a sex pheromone to attract males for mating.

Experiments showed peelminer in Tulare Co. completes a generation in 580 F (320 C) degree-days (Grafton-Cardwell et al. 2001, O'Neal 2007). Peelminer was found to have a lower developmental threshold of 55°F (12.9°C) and an upper development threshold of 91.4°F (33°C) (O'Neal 2007). Sustained temperatures at and above the upper development threshold are rarely seen in SJV orchards, but are common in Coachella Valley and Hermosillo citrus growing areas. The ideal temperature range for peelminer development is between 77° and 84° F (25° and 29°C).

2.4 Management of *Marmara gulosa*

Whether the peelmining problem in the San Joaquin Valley is from a native or invasive population, the grower is faced with a significant management issue that requires data-based recommendations while long term solutions are developed.

2.4.1 Development of Pheromone Lures for Monitoring. Sex pheromones have been identified for three different species within the subfamily Gracillariidae (Witzgall et al. 2007). The compounds that have been discovered to attract male *M. gulosa* range from common lepidopteran pheromone components to more complex and unique molecular structures (McElfresh et al. 2009). An experimental pheromone lure was synthesized by researchers at the University of California, Riverside for *M. gulosa* (McElfresh et al. 2009). The compound 8*E*,10*E*-14:Formate with ~1% of 8*E*,10*E*-14:Acetate were found to be the most effective components for attracting male *M. gulosa* moths according to the field studies conducted in San Joaquin Valley, Coachella Valley, and Sonora MX (McElfresh et al. 2009).

2.4.2 Chemical Options. The citrus peelminer poses a challenge for chemical pest control because the larvae are protected from spray applications when they are underneath the cuticle of the rind. Pesticide trials on citrus peelminer so far have had from zero to very marginal success (Grafton-Cardwell 2001, 2003, 2004, 2005, 2006, 2007). Only one insecticide, Diflubenzuron (Micromite, Dimilin) was shown to be effective during *M. gulosa*'s brief and vulnerable egg stage, but its use requires appropriate timing to target the egg stage in the field (Grafton-Cardwell, 2001, 2006).

The degree day studies mentioned above currently aid application timing (Grafton-Cardwell 2007, O'Neil 2007).

2.4.3 Biological control options. Biological control approaches differ significantly if a pest is native or invasive (Nechols 1999). Native species become pests presumably for reasons other than the absence of an effective natural enemy (Legner & Bellows 1999). Thus, the importation of natural enemies from abroad is usually not a consideration as it is for invasive species. When developing a biological control plan for native species, consideration must be given to alternate agro-ecosystems, urban landscapes, and natural ecosystems; each must be surveyed to determine the complete geographic and host plant range of the pest (Nechols 1999) and any native natural enemies present. If a suitable natural enemy is found it may be “augmented” or “conserved.” Augmentation biological control is an approach that enhances the numbers of natural enemy species already present, but does not function to reduce pest densities to acceptable levels (Ridgeway and Vinson 1977). Conservation biological control works to manipulate or enhance the cropping system environment to create conditions which are more favorable for natural enemies (Barbosa 1998).

Since *M. gulosa* pest populations were assumed to be invasive to the San Joaquin Valley, the classical approach to biological control was implemented with exploration trips to northern Mexico seeking natural enemies.

2.5 Taxonomic Characters for *Marmara* Identification

The two illustrations below (figures 5 and 6) from Guillén et al., (2001) show the various features of male genitalia used to describe and separate species of *Marmara*. The terminology and descriptions serve as the basis for the additional studies reported herein and are commonly used to build species concepts in Lepidoptera (Klots 1970).

**Figure 5. Original illustrations of *Marmara gulosa* male reproductive structures (Guillén et al. 2001).
CoL – costal lobe, CuL – cucullar lobe, VL – valvular lobe. Aedeagus is pictured on the right.**

Figure 6. Original illustrations of *Marmara salictella* male reproductive structures (Guillén et al. 2001).

A male's genitalia are located at the terminus of the abdomen and are made up of a series of paired sclerites that are used to couple with a female's external genitalia. The aedeagus serves as the intromittent organ that passes male gametes to a bursa or other storage structure within a female's body.

The illustrations (figures 5 and 6) show the male sclerites in an open position to facilitate descriptions and measurements; they do not appear in this orientation in life. The sclerites are positioned into a more compact cylindrical shape. The orientation of the sclerites and the shape of the aedeagus have been hypothesized as a type of “lock and key” approach to avoid hybridization during the mating process (Dufour 1844, Eberhard 1985); an idea with wide general acceptance. However, the application of such a hypothesis to explain subtle variation between species or considerable variation among specimens within a species has come under scrutiny in recent times (e.g. Gilligan and Wenzel 2008).

A significant consideration in describing *M. gulosa* in 2001 was to show that the populations in the western US represented a different species from that in the eastern US, originally described as *M. salictella*. That *M. gulosa* was becoming a significant economic pest in citrus production also added motivation to having a formal description as some of the older literature described peelmining on citrus as being caused by the “western form of *M. salictella*.” (Woglum 1948).

2.6 Biogeography and Identification of *Marmara* species

The differences between behaviors noted in Mexico and California prompted interest regarding peelminer’s biogeography. Peelminer, although present, was never a significant pest or concern to citrus growers in Mexico. Action was not taken to properly identify and compare peelminer specimens from California and Mexico until October 2007, when the Mexican authority, SAGARPA, rejected a shipment of peelminer scarred fruit from California with the claim that *M. gulosa* was not a documented species in Mexico. A morphological comparison of adult male genitalia was prompted using both laboratory reared and pheromone trapped moths. The sclerotized structures that make up the male genitalia are the characteristics currently used to distinguish among closely related gracillariid species.

The hypothesis was that *M. gulosa* occurred in Mexico and analysis of the morphology was expected to validate that assumption. However, two distinct species were found among the specimens observed; one identified as close to *M. gulosa* and another undescribed species (D. Davis, pers. com., September 2008). The findings

showed that more than one “citrus peelminer” species occurs within the range spanning between northern Mexico and the San Joaquin Valley. The specimens examined that were identified as close to *M. gulosa* did not exactly match the illustrations of *Marmara gulosa* as presented in Guillén et al. (2001) and cannot unequivocally be identified as such. Thus, a redescription of *M. gulosa*, with additional specimens, is needed along with a revision of the genus for all of North America. A full revision notwithstanding, this study improves our ability to anatomically identify the species of peelminer that cause damage in a variety of host plants in Mexico and California and fit well with the data from molecular analyses used to identify species.

3.0 Materials and Methods

3.1 Mexico foreign exploration

A biological control program was developed in 2002 to locate *M. gulosa* populations in northern Mexico and study their biology and natural enemies for potential importation of promising natural enemies species into the San Joaquin Valley (Grafton-Cardwell et al. 2006). Additionally, parasitoid wasps attacking *M. gulosa* were also collected from southern California (Guillén et al. 2007). Efforts in establishing a parasitoid biological control agent in the San Joaquin Valley concentrated on *Cirrospilus coachellae* Gates (Grafton-Cardwell et al. 2006, Guillén et al. 2007); a species shown to regulate peelminer populations in Coachella Valley orchards (Guillén et al. 2007). Early attempts at establishing *C. coachellae* in the San Joaquin Valley were not successful (Grafton-Cardwell 2006, D. Headrick, unpublished data). The possibility of more efficient natural enemies present elsewhere in the citrus peelminer's range warranted further investigation.

3.1.1 Pre-release sampling. Personnel from the California department of Food and Agriculture (CDFA), UC Riverside, and Cal Poly, San Luis Obispo participated in a pre-release survey of *M. gulosa* in the San Joaquin Valley in 2000-2001 to determine the diversity of natural enemies, mainly parasitic Hymenoptera, attacking this pest (Godfrey et al. 1999, unpublished data). Mined plant material was examined for signs of parasitoids. Plant material was excised and held in growth chambers (28 °C, 12:12 l:d, 90% humidity) to allow for development and adult emergence. Adult specimens were sent to UC Riverside (Dr. John Heraty) for identification. *Pnigalio*, *Closterocerus*, *Baryscapus*, *Nechrysocharoides*, and *Hemiptarsenus* (Hymenoptera: Eulophidae) were

the common genera found attacking *M. gulosa* larvae in mines in the San Joaquin Valley, but in low numbers (Gates 2002). In the Coachella Valley of southern California, *Cirrospilus coachellae* Gates was shown to be part of a complex of eulophid parasitoids, that has provided demonstrable population regulation of *M. gulosa* in commercial citrus settings (Guillén et al. 2001). *Cirrospilus coachellae* was not detected in the San Joaquin Valley and so it was chosen for an introduction program for *M. gulosa* that began in 2001. The results of that project are ongoing and to be reported in a follow up paper (DHH, E. E. Grafton-Cardwell, K Godfrey, unpublished data).

3.1.2 Establishment of a foreign field research program. There is extensive citrus production throughout Sonora including the Hermosillo area, as well as in Valle de Yaqui. Initial contact was made with university researchers working on a related pest species, citrus leafminer, *Phyllocnistus citrella* Stainton, in northern Mexico (Dr. Alejandro González, University of Monterrey, Nuevo Leon, MX) and representatives of Junta Local de Sanidad Vegetal de Hermosillo, René Martínez de Castro Navarrete, President, and Carmelo Silva Sánchez, Gerente General. They in turn assigned Leobardo “Fito” Lopez Madrid, an agricultural engineer, to aide in the location of collecting sites for *M. gulosa* populations.

Exploration research trips were conducted in Nov 2002, Sep 2003, Nov 2004, July 2005 and late Nov/Dec 2006 to determine the presence of *M. gulosa* by examination of potential host plant material, focusing on citrus, to document its life history in the field; collect and identify natural enemies, and import any potential biological control

candidate species to the quarantine facility, Department of Entomology, UC Riverside. Field sites in MX were located and accessed with the aid of local officials with the Sanidad de Vegetal de Hermosillo. A total of 15 field sites were visited SW of Hermosillo along Calle 20 Sur to Bahia Kino and 5 sites in Valle de Yaqui, west of Ciudad Obregón near Bacúm in 2004 and 2006. Orchards were examined for an equivalent of 8 hours per field site (four people surveying for 2 h) unless otherwise stated. All field collected and reared specimens used for identification were placed into 95% ethanol for morphological and molecular identification. Life stages on host plants were documented by examining infested plant material (mine age on fruit in MX was estimated by comparing observations of mining during growth and development studies in the San Joaquin Valley, CA). Parasitism was determined by the presence of a parasitoid pupal case or exit hole. Natural enemies observed in the pupal stage in the field were excised from plants and placed in rearing containers consisting of interlocking-top, 950 cc (=1 qt) plastic bags into which plant material and paper toweling were placed.

In 2004, a pheromone lure for *M. gulosa* became available from Dr. J. Millar UC Riverside. Delta traps [brand trademark] with sticky inserts, plus rubber septa impregnated with 1 mg of the “h4” version of the pheromone, were deployed to aid in locating *M. gulosa* populations in the field in MX.

All preserved specimens were returned to UC Riverside (*M. gulosa* to R. Stouthamer and Hymenoptera to J. Heraty) for identification. Stouthamer & Vickerman (2006) compared mitochondrial and ribosomal DNA sequences of *M. gulosa* populations on different hosts from different locations in CA and MX.

3.2 Male Genitalia Morphology Analysis

Seven different specimen groups of *Marmara* were used to isolate and compare the anatomical structures that make up the male genitalic capsule. The isolated male genitalia were compared to published illustrations of *M. salictella* and *M. gulosa* (Guillén et al. 2001). The seven groups consisted of trapped adult males collected in 2005 in Hermosillo, Mexico on citrus, reared adults from 2008 Tulare Co. on *Salix sp.*, trapped adult males from 2005 Tulare Co. from citrus orchards, trapped adult males from 2008 Tulare Co. on *Nerium oleander*, trapped adult males from 2008 Tulare Co. on walnut, *Juglans regia*, trapped adults from 2008 Riverside Co. on citrus, and reared adults from 2001 Riverside Co. (Coachella Valley) on grapefruit, *C. paradisi*.

Table 1. The 5 groups used for dissection.

Separate dissection groups:				
Specimen group name	Collection date	Collection method	Specimen group origin	Host plant
“-“	2005	pheromone trap	Hermosillo, Mexico	<i>Citrus sinensis</i>
“a”	2008	lab reared	Tulare Co.	<i>Salix sp.</i>
“b”	2005	pheromone trap	Tulare Co.	<i>Citrus sinensis</i>
“c”	2008	pheromone trap	Tulare Co.	<i>Nerium oleander</i>
“d”	2008	pheromone trap	Tulare Co.	<i>Juglans regia</i>
“e”	2008	pheromone trap	Riverside Co.	<i>Citrus sinensis</i>
“f”	2001	lab reared	Riverside Co.	<i>Citrus paradisi</i>

In order to isolate the sclerotized genitalia, adult male abdomens were separated from the body and placed into 15mL 10% KOH solution. The 10% KOH solution was brought to boil for 20 seconds and then allowed to cool. The abdomens became transparent with the sclerotized reproductive parts clearly visible within. The KOH-

treated abdomens were washed for 5 minutes in a 60% EtOH solution and then placed in 80% EtOH for storage and later dissection. Once in the 80% EtOH, the aedeagus was separated from the valva. One ml syringes fitted with 29-guage hypodermic needles were the primary tools used for manipulating the sclerotized parts and microdissection.

The aedeagus and valva were washed in 90% and then 100% EtOH, each for 5 minutes, before mounting on glass microscope slides. The slides and cover slips were cleaned with an optical lens wipe and 100% EtOH prior to specimen preparation and slide mounting. After cleaning, a single drop of Hoyer's solution was placed on the slide as the mounting medium. The parts were lowered into the Hoyer's solution and set to the desired orientation before placing the cover slip on top. Slide mounting orientation mimicked that illustrated in Guillén et al. (2001). The aedeagus and associated valvular lobes were mounted apart, but adjacent to each other on a slide. Each aedeagus was positioned with a lateral side upward and the valvular lobes were mounted with the ventral side upward.

Specimens taken from pheromone lure sticky traps were placed in Goo-Gone®, brand degreaser to remove the trap adhesive. Further dissecting was carried out in the dish of Goo-Gone. After the valva and aedeagus were separated from the abdomen, the parts were placed directly in 100% EtOH and washed for 10 minutes. The parts were then washed for another 10 minutes in a second dish of 100% EtOH. After the second wash, the parts were mounted on microscope slides using Hoyer's solution as a mounting medium and the same orientation as reared specimens. None of the trapped specimens were macerated with KOH. The maceration process makes the sclerotized parts flexible

and easier to manipulate, and thus, the specimens that did not undergo KOH maceration were very brittle and required extra care to avoid damage.

A Leica® MZ16 dissecting microscope was used with firewire interface to a PC running Leica Application Suite (LAS) ® software on the Windows XP Pro® operating system. Maximum magnification was 184 diameters of magnification. Digital images were captured using this setup.

4.0 Results

4.1 Mexico Foreign Exploration and Field Studies 2002-2006

The Hermosillo Growing Region

Hermosillo is the capital city for the state of Sonora. The city is situated 172 miles south of the Nogales, AZ US border crossing point. The climate is Sonoran desert; hot and dry most of the year with a short rainy season during August and September. Average annual precipitation is 42.2 cm (16.61 in). Temperatures are lowest during December ranging from 2.8° - 30.5°C (37° - 85°F). The warmest month is July with temperatures ranging from 45° - 22.2°C (113° - 72°F).

The citrus varieties grown in this region include Washington Navel, Valencia, and several different varieties of grapefruit, tangerine, lemon, and lime. There were some notable differences in farming practices, based on both the foreign exploration team's observations and input from local agricultural engineers. Unlike the dense grid of farms that make up the San Joaquin Valley, citrus orchards are isolated amidst the expanse of Sonoran desert. Water for the orchards is supplied by local wells. Water management practices included drip, microsprinkler, and furrow irrigation. Weed management efforts appeared to be minimal, relying mainly on mechanical tillage. Some orchards visited had dense weed coverage in between rows. Insecticide use is infrequent due to lack of resources such as water, equipment, and funds. Some orchards were certified organic for international export. In most orchards, trees showed some degree of water stress. The removal of suckers (1st year vegetative shoots) several times a year is a common practice to aid in water conservation.

Figure 7. Map showing the locations of Hermosillo and Ciudad Obregón.

Foreign exploration team members:

USA: Dr. David Headrick – Project Manager. Professor, Horticulture & Crop Science dept., Cal Poly, San Luis Obispo, Bert Quesada, Ag IPM Consultants Inc., Jim Stewart – Pest Management Associates (PMA) Inc., and Ag IPM Consultants Inc., Paul Semet, MS candidate, Cal Poly, San Luis Obispo, Sage Finch, Master's of Agriculture Science from Cal Poly, San Luis Obispo.

Mexico: Leobardo “Fito” Lopez Madrid, Agricultural Engineer, Sanidad Vegetal de Hermosillo (Board of Plant Health of Hermosillo); Fito was a key member for locating, collecting, and trapping peelminer populations in Mexico. He also served as the main contact for the research team in Mexico and conducted peelminer monitoring between visits.

Figure 8. 2004 research team. Left to right: Bert Quesada, Leobardo Lopez Madrid, Dr. David Headrick. Photo taken by Paul Semet.

4.1.1 Foreign Exploration Narrative

November, 2002 - In November 2002 an initial fact finding trip was made to Hermosillo, Son. MX with the objective of locating *M. gulosa* in citrus production in the region and documenting any natural enemy activity. Several citrus orchards were visited and their usefulness for future studies was rated and recorded. November was chosen for

the first trip as lifecycle information from Coachella Valley suggested that would be an active time for *M. gulosa* (Guillén et al. 2001, Gates et al. 2002). Although differences eventually became apparent, it was originally hypothesized that the desert climate in Coachella Valley would be similar to that in Hermosillo.

Observations were made during the trip of a few live *M. gulosa* larvae on Rio Red variety grapefruit was made. The survey resulted in 16 larvae dissected out of mines and preserved in 95% EtOH solution for genetic analyses. Evidence of parasitism was also noted. Old mining activity was noted on willows near citrus areas, but no live larvae were found.

September, 2003 - The 2003 travel was planned for September two months earlier in the season, based on the age of the mines observed in November the previous year. The typical rainy season for the region is during the months of August and September.

November, 2004 - The objective for this trip was to test the new “h4” pheromone lures by trapping *M. gulosa* preceding their movement into overwintering plant species. A pheromone trap which was set early in the week caught one microlepidoptera, which was not a peelminer but a suspected leafminer, (*Phyllocnistis citrella* Stainton, Lepidoptera: Gracillariidae). Personnel in Mexico were trained to use the traps for monitoring population fluctuations throughout the year. Alternate host plants *Salix* spp. and *Nerium oleander* were also examined for presence of overwintering larvae. Oleander is a reliable overwintering host plant for collecting *M. gulosa* in the San Joaquin Valley (Neff 2001, Grafton-Cardwell et al. 2008). In Hermosillo, the closest stand of oleander bordering a citrus orchard was in Ciudad de Obregon, a farming region about 160 miles

to the Southeast of Hermosillo. An active population of peelminer larvae was found in the oleander stand adjacent to Valencia and Navel oranges. This collection site was known as “Block 421”. Peelminer larvae were field dissected from oleander stems and placed into vials with 95% EtOH. A total of 70 preserved peelminers were obtained for genetic studies.

Figure 9. Location of peelminer population found in oleander.

Figure 10. View of “Block 421” oleander stand and adjacent citrus orchard.

Figure 11. Collecting peelminer larvae from “Block 421” oleander stand.

July 18th-22nd, 2005 - The next visit in 2005 needed to coincide with a period of active mining. Personnel in Mexico used pheromone trapping in May 2005 to track populations of peelminer in citrus orchards. The trip was initiated in July 2005 after flights of male peelminer moths were detected in the traps. By confirming peelminer activity before visitation, time spent collecting was more likely to yield live peelminer pupae and parasitoids.

In the first orchard visited, “Mazocoba” (limes and grapefruit), both healthy and parasitized peelminer larvae were found. The levels of peelminer infestation were very low compared to those observed in Tulare County orchards. There was a noticeable abundance of recently deceased mines and parasitism, suggesting that the peelminer populations had already peaked for the year and was now on the decline. By observing the age, number of exit holes, and prematurely dead larvae associated with the mining evidence on citrus fruits, it was determined that no more than three generations occur during the growing season.

November 25th-29th 2006 - Studying the citrus leafminer (CLM), a close relative of citrus peelminer, was an objective due to its recent arrival in the major citrus growing regions of CA. The time of year chosen, very late November, was intended to coincide with nursery and new planting activity associated with CLM damage. A secondary objective of the 2006 visit was to continue locating overwintering peelminer and their parasites.

Additional objectives for peelminer were to survey overwintering activities in Yaqui Valley citrus, willow, and oleander. Any evidence of parasitism in overwintering peelminer populations would be collected in 95% EtOH for additional identification studies.

The “Block 421” oleander stand located in Ciudad de Obregon, which had served as a successful collection site in 2004, did not yield any live peelminer during this visit. Evidence of two previously parasitized peelminer larvae was found in the oleander stand.

A current generation of peelminer was observed attacking citrus sucker growth. The larvae found on the suckers were mostly first instars. Pheromone traps had male peelminer moths during the visit. L. Lopez reported trapping 196 males during the following week of December 5th, 2006. One of the parasitoids collected during this trip was later identified as *C. coachellae* (Heraty, UC Riverside, 2006).

4.1.2 Life History of *Marmara gulosa* in Sonora The results are also reported in the seasonal sequence of *M. gulosa* activity by month (July, Sep, Nov), in addition to the chronological narrative above. Stouthamer & Vickerman (2006) reported the insect specimens collected in Sonora, MX in 2002 from larval mines on citrus were *M. gulosa* by using the mitochondrial DNA CO1 region and that they were similar to the San Joaquin Valley specimens, but different from Coachella Valley populations.

July. Traps were deployed in three orchards (grapefruit, Valencia, & Key lime) at the same Campo (Mazocoba, Calle 20 Sur, Bahia Kino) beginning in late May (Table 2).

Examination of fruit of Key lime bore evidence of a generation of *M. gulosa* that began in late June corresponding to the trapping data.

Table 2. Trap data from Hermosillo citrus orchards by date and variety, recorded by personnel in MX.

2005 Week of:	# of males	Citrus type
28 May	0	grapefruit/orange
4 June	0	grapefruit/orange
11 June	1	grapefruit/orange
18 June	3	grapefruit/orange
25 June	0	grapefruit/orange
2 July	1	grapefruit/orange
2 July	75	Key lime
9 July	28	Key lime

Four people examined fruit for 3 hours and determined that the majority of fruit had mines created by mature *M. gulosa* larvae, most of which were emerged. A second generation of *M. gulosa* larvae was actively mining the same fruit in mid July, but at lower densities (not quantified). There also appeared to be heat-related mortality of the second generation of larvae on fruit; which was consistent with observations in CA.

Active parasitism of *M. gulosa* was evident. In 3 hours of searching the 4 team members found 51 larvae with exit holes made by emerged parasitoids and 7 live peelminer larvae. About half of the second generation larvae were prematurely dead

within the mine. A total of 7 live peelminer larvae, 23 parasitoids, and 22 parasitized peelminer larvae were collected. Whole fruit with parasitized larvae were removed from the field and individually dissected and placed into rearing bags for subsequent emergence. At the time, there was no *M. gulosa* colony to support the importation of natural enemies into the quarantine facility at UCR and all emerged parasitoid adults (n = 8) were placed into 95% ethanol for later identification. The only parasitoid species successfully reared and identified from *M. gulosa* populations from northern Sonora was *Cirrospilus coachellae* (John Heraty, UC Riverside, 2006). An additional eulophid species may be present and attacking *M. gulosa* based on the shape of the meconium. None of the latter species were successfully reared for identification.

September. Nine orchards were visited. Peelminer activity was evident on fruit and vegetative growth of citrus, however, all of the mining was old and no living larvae were present. Population densities overall were very low (4 infested fruit per hour of searching for a total of 18 hours), but spotty in distribution, as observed in CA. For example, two sites had mining on fruit that was easy to locate; with five people searching, 20 infested fruit were found in 30 minutes (= 8 infested fruit per hour of searching). There was no evidence of parasitism on *M. gulosa*, except for one emerged, solitary parasitoid pupa in a mine on a grapefruit.

November. Traps with *M. gulosa* pheromone lures were placed into citrus orchards. Trap catches in Key lime increased from 1 to nearly 200 in fewer than two

weeks time indicating an adult flight marking the start of a new generation (Table 3).

Fruit and vegetative shoots were examined for mining activity.

Table 3. Trap catches by date, number of males per trap and citrus variety from Hermosillo, Sonora, MX.

Date	# males	Citrus type
26 Nov 2006	1	Key Lime
26 Nov 2006	1	grapefruit
28 Nov 2006	28	Key lime
28 Nov 2006	1	grapefruit
5 Dec 2006	196	Key lime

Mining on citrus fruit. All mining on fruit (Grapefruit, Valencia's, navels, and Key lime) was old and no living larvae were observed on fruit in Nov. Vegetative growth: Live *M. gulosa* larvae were found on the vegetative growth of the grapefruit variety referred to as "Rio Red" and two unknown varieties of Valencia's and Key lime. Mining was most common by first instars in Nov. A total of 16 live larvae were dissected and saved in 2002.

Mining on other host plant species. *Salix* sp. was examined at two sites and mining activity on the current season's growth was observed, but no living *M. gulosa* were found. Active mining was observed in Nov 2004 on *N. oleander* at two sites, one in Hermosillo, the other in Valle de Yaqui; approximately 70 larvae were dissected and placed into 95% ethanol. No mining was observed at either of these same sites in Nov

2006. Additionally, there was no mining observed in the citrus orchard adjacent these sites in either year. New shoot growth on *N. oleander* in 2004 had extensive, active mining, but no parasitism. In 2006, two oleander stems, each with one miner, had been parasitized as evidenced by exit holes and meconium deposits. Upon inspection one appeared to be *C. coachellae* and the other, a different species of parasitoid. Stouthamer & Vickerman (2006) noted, using the techniques described above, that the larvae dissected from mines on *N. oleander* did not genetically resemble *M. gulosa*, but may be a related species.

Additional trapping, April 2006. Traps with *M. gulosa* pheromone lure were taken to a property in La Purisima, Baja California Sur, MX. Four traps were placed in an orchard with Washington navels and bordered by *N. oleander*. Traps were left for one week during which time less than six adult male *M. gulosa* were trapped.

4.2 Male Genitalia Morphology Analysis

Below are micrographs that provide detailed information on the size and shape of the major sclerites used in the description and identification of *Marmara* species based on Guillén et al. (2001).

Figure 12. Valvular lobe descriptive information.

Figure 13. Cucullar lobe descriptive information.

Figure 14. Costal lobe descriptive information.

Figure 15. Aedeagus descriptive information.

The aedeagus, costal lobes, and cucullar lobes were isolated and identified in each specimen and then compared with the illustrations of *Marmara gulosa* and *Marmara salictella* in Guillén et al. (2001). The valvular lobes of the San Joaquin Valley and Coachella Valley specimens most closely resembled valvular lobes of the *Marmara gulosa* illustration. This particular anatomical feature was determined by Guillén et al.,

(2001) to be the most important diagnostic feature distinguishing *M. gulosa* from *M. salictella*.

Thus, all specimens derived from San Joaquin Valley and Coachella Valley during this study were determined to be *Marmara gulosa* following consultation with Dr. Don Davis (Smithsonian Institution, Department of Entomology) and Dr. Marc Epstein (California Department of Food and Agriculture, Plant Pest Diagnostics Branch). Additionally, an undescribed species of *Marmara* was also identified from material collected in Hermosillo MX and Riverside, CA.

It was stated in the article that other anatomical features of the male genitalia have too much variability in structure among specimens and therefore cannot be relied upon for diagnosis (Guillén et al. 2001, D. Davis 2008 personal communication). However, the specimens used in the current study showed a high degree of consistency in genitalic structure from a variety of locations, years collected and host plant species (see illustrations below, figures 16-20) and more closely resembled that illustrated for *M. salictella*. But because these lobes have been deemed unsuitable for trustworthy diagnosis, the single distinguishing feature of the valvular lobe is used. Additional examination of these anatomical features may be necessary to fully understand the utility of male genitalic anatomy in species descriptions.

Slide mounting technique should be considered when making observations of the anatomy in question. Manipulating the minute sclerites of male genitalia onto glass microscope slides was challenging and technique does affect the final appearance of slide-mounted specimens. A costal lobe slightly rotated with hairs facing downward from the viewer can make the hairs appear to extend from the apex of the lobe, when they

actually terminate subapically. Valvular lobes can be easily folded or ripped in the slide mounting process.

The specimens taken from the 2005 Mexico trap were found to be a new undescribed species of *Marmara* with dramatically different genitalia. The two trapped male specimens from Riverside in the summer of 2008 resembled the undescribed species trapped in Mexico. The anatomical distinctions of this species include no separate valvular, costal, and cucullar lobes. Instead these lobes appear fused together into a single structure. Only four stout hairs followed by a few fine hairs are present on the costal lobe. The aedeagus has a larger dorsal knob followed by a longer downward pointing tube when compared to the aedeagus of *M. salictella*. Although Mexico and Riverside specimens have a matching knob and tube at the tip of the aedeagus, the aedeagus differs slightly in shape. The condition of specimens from the 2005 Mexico trap also must be taken into consideration. They were held in trap adhesive covered by plastic wrap for 3 years prior to dissection. Many of the specimens were dry and damaged. While features that distinguish it from *M. salictella* are unmistakably present, more specimens need to be examined and compared before a formal description of the new species can be made. Additionally, no females of this undescribed species have been examined to date.

Figure 16. 2001, reared on grapefruit from Coachella Valley.

Figure 17. 2008, reared on Salix from Tulare Co.

Figure 18. 2005, pheromone trap in SJV citrus orchard.

Figure 19. 2008, pheromone trap in SJV oleander.

Figure 20. 2008, pheromone trap in SJV walnut orchard.

Figure 21. 2005, pheromone trap from citrus orchard in Mexico.

Figure 22. 2008, pheromone trap in Riverside backyard orchard.

Figure 23. Side-by-side comparison: Left – 2001 specimen lab-reared from Riverside Co. *Citrus paradisi*; Right – 2008 specimen lab-reared from Tulare Co. *Salix* sp.

Figure 24. Aedeagus comparison: A. 2008 Tulare Co. *Salix* sp. (lab reared); B. 2008 Tulare Co. *Nerium oleander* (pheromone trap); C. 2001 Riverside Co. *Citrus paradisi* (lab reared); D. 2005 Tulare Co. *Citrus sinensis* (pheromone trap).

Figure 25. Specimens with fused structures of valva. Left – 2005 Hermosillo, Mexico (pheromone trap); Right – 2008 Riverside Co. (pheromone trap).

Figure 26. Top – 2005 Hermosillo, Mexico (pheromone trap); Bottom - 2008 Riverside Co. (pheromone trap).

Table 4. Morphological feature resemblance to original illustrations by specimen group.

	Valvular lobe	Costal lobe	Cucullar lobe	Aedeagus
2008 Willow SJV	G	S	S	S
2008 Oleander SJV	G	S	S	S
2008 Walnut SJV	G	S	S	S
2005 Citrus SJV	G	S	S	S
2001 Citrus Coachella	G	S	S	S
2008 Citrus Riverside	U	U	U	U
2005 Citrus Mexico	U	U	U	U

G – gulosa, S – salictella, U – undescribed species

5.0 Discussion

Center of Endemism, *M. gulosa* Population Growth & Movement

Previous studies related to *Marmara* species distribution show more species diversity north rather than south within the United States (Guillén et al. 2001). One study on the variety of Hymenopteran species which parasitize mining insects within California show that there is more parasitoid species variety in the San Joaquin Valley area than in the deserts around Riverside County (Gates et al. 2002). The majority of Hymenopteran parasitoids found attacking *Marmara* sp. are within the family Eulophidae. The parasitoids found attacking mining insects are host generalists and niche specialists (Askew and Shaw 1974). There are no parasitoids specific to *M. gulosa* (Gates 2002). A total of six different parasitoid species have been identified in Tulare Co. and four in Coachella Valley (Grafton-Cardwell, Godfrey, & Headrick 2008). Only one species of parasitoid, *C. coachellae*, was identified from Sonora (Heraty, UC Riverside, 2006). The presence of a second eulophid species is suspected based on the appearance of meconium (Headrick, unpublished data). The three different regions, Sonora Mexico, Coachella Valley, and Tulare County form a North-South transect. Increased species diversity along a transect line can indicate an area of evolutionary longevity (Rosenzweig 1995). This distribution is contrary to the usual tendency towards increased species diversity with a decrease in latitude (Brown and Lomolino 1998). The difference in diversity is reasonable when considering the transect's climate becomes more dry and desert-like towards the south. Observing species diversity of both the *Marmara* genus and their associated parasitoids along this transect suggests that Mexico may not be the center of

endemism for *M. gulosa*. Instead, the evidence collected suggests that Sonora Mexico is a southern boundary of its natural range.

The data presented from the genetic studies at UC Riverside showed that the Coachella population of *Marmara* was genetically different from the populations in SJV, Sonora, and Arizona; with the exception of the one population collected from “Block 421” in Ciudad de Obregon (Vikerman and Stouthammer 2006). Pheromone trap and micro dissection data showed that populations from SJV and Coachella were morphologically different from the specimens collected from Riverside and Mexico. The geographical distribution of *Marmara* biotypes (and possibly species) does not match when comparing the results from the genetic and the morphological analyses. Failure to develop internally transcribed spacer (ITS) microsatellite markers from ribosomal DNA was noted in the genetic study, instead differences in CO1 regions were used as markers for subdivision (Vikerman and Stouthammer 2006). These differences observed in CO1 regions do not correspond with the differences observed in morphology. The genetic study concluded with the recommendation, “It would be very useful to determine if the Coachella Valley population and the other populations of what we consider the Citrus Peelminer are indeed the same species or not by conducting cross breeding experiments” (Vikerman and Stouthammer 2006). This suggestion also holds true for the populations classified by morphology, in order to test the “lock and key” hypothesis of male genitalia in Lepidoptera (Dufour 1844, Eberhard 1985, Gilligan & Wenzel 2008).

Differences Between *Marmara* Activity in California and Mexico

Peelmining damage occurs in citrus orchards in Northern Mexico, but never exhibits the same pattern of migration from crop to crop within a season and population increase as seen in the San Joaquin Valley. A few hypotheses of its benign activity in Mexico were formed. One is that the orchards and farms in Mexico are far more isolated than in the San Joaquin Valley, with harsh desert in between. This would make it more difficult for adult moths to find and exploit a new host plant. The highest temperatures in Northern Mexico are during July and beyond the pest's developmental threshold (O'Neil 2007). The development of peelminer can be stunted due to the high temperatures (O'Neil 2007). Water management practices in Sonora, Mexico are very different from those in the San Joaquin Valley. Generally, the San Joaquin Valley orchards are watered more frequently. There have not been any studies regarding the effects of water management on citrus fruit susceptibility to peelminer. Although these behavioral differences in can now be attributed to the presence of an entirely different species in Mexico, future studies of environmental and physiological factors which stunt peelminer population growth should be helpful in IPM control efforts.

Invasive Species

The study has implications on the quarantine protocols and procedures that sometimes allow states and nations to make claims based on incomplete data. That no species description with Mexico included in the native range was published allows for artificial barriers to be used in marketing and quarantine. Species descriptions should include as much of the actual range as possible without the use of artificial human

borders defining or delimiting biological borders. As a result, this incident helped spur the re-evaluation of citrus peelminer via morphological comparison. Closer inspection of specimens from the 2005 Mexican pheromone trap stored at Cal Poly San Luis Obispo was originally expected to prove that *M. gulosa* did indeed exist in Mexico. However, the morphological analysis done in 2008 proved with certainty that the specimens from the Mexican pheromone trap are not *M. gulosa*, but an undescribed species of *Marmara*. While SAGARPA's claim that *M. gulosa* was not present in Mexico held true, there is and always has been "citrus peelminer" activity present there. The context in which SAGARPA used the term "*Marmara gulosa*" was identical to the way the term was used in California prior to the morphology analysis: referring to any and all peelminer found in citrus and common alternate host plants.

It is now evident that there is more than one species of microlepidopteran "peelminer" that can attack citrus fruit ranging from the San Joaquin Valley south through northern Mexico. Additionally, the pheromone lure currently available attracts two distinctly different morphologies of *Marmara*.

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