Identification of the Egg Parasitoids of Auchenorrhyncha (Hemiptera) of Economic Importance in Taiwan: Collaborative Research between Taiwan Agricultural Research Institute and University of California at Riverside Scientists

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ABSTRACT

An overview of the three collaborative projects between Taiwan Agricultural Research Institute (TARI), Taiwan, ROC, and University of California at Riverside, USA, scientists is given, as follows: 1) collecting (H.-T. Shih), rearing (S.-H. Huang), and identification (S. V. Triapitsyn) of the egg parasitoids (Hymenoptera: Mymaridae and Trichogrammatidae) of rice leafhoppers and planthoppers (Hemiptera: Cicadellidae and Delphacidae) of economic importance in west central Taiwan; 2) rearing (H.-T. Shih) and identification (S. V. Triapitsyn) of the egg parasitoids (Mymaridae and Trichogrammatidae) of the leafhopper *Kolla paulula* (Walker) (Cicadellidae), vector of the phytopathogenic bacterium *Xylella fastidiosa*; and 3) a preliminary study of the biodiversity and taxonomy of Mymaridae in Taiwan (S. V. Triapitsyn) based largely on the specimens in the insect collection of TARI.

Keywords: rice pests, Cicadellidae, Delphacidae, *Kolla paulula*, vector, Mymaridae, Trichogrammatidae, egg parasitoid, biological control, biodiversity, taxonomy, Taiwan

INTRODUCTION

Since 2013, scientists from the Taiwan Agricultural Research Institute (TARI), Wufeng, Taichung, Taiwan, ROC (H.-T. Shih) and the Entomology Research Museum, University of

California, Riverside, California, USA (S. V. Triapitsyn) have collaborated on the three research projects which are important for applied entomology in Taiwan, particularly for biological control of some key agricultural pests belonging to Auchenorrhyncha (Hemiptera). Several years later, S.-H. Huang (Chiayi Agricultural Experiment Station, TARI) joined the effort on one of these projects, rearing egg parasitoids of rice leafhoppers and planthoppers in Chiayi and Yunlin counties of Taiwan.

As outlined by Triapitsyn [15], the most common egg parasitoids of Auchenorrhyncha belong to two families of Hymenoptera, Mymaridae and Trichogrammatidae of the superfamily Chalcidoidea. Worldwide, they are largely responsible for the natural control of leafhopper (Cicadellidae), planthopper (Delphacidae), and treehopper (Membracidae) species, including some economically important pests. Therefore, knowledge of their taxonomy (for correct identification) and biology is very important for biological control, ecological, and biodiversity studies. Triapitsyn [15] also provided an overview of the history and current status of the taxonomy and biology of these two groups of egg parasitoids in Taiwan.

COLLABORATIVE PROJECTS

1. Collecting, rearing, and identification of egg parasitoids of rice leafhoppers and planthoppers of economic importance in west central Taiwan

Project rationale. Recently, Triapitsyn [20] gave a critical analysis of the history of prior identifications of the egg parasitoids (Mymaridae only) of rice leafhoppers and planthoppers in Taiwan. Chu and Hirashima [3] summarized the earlier Taiwanese literature on the natural enemies of rice leafhoppers and planthoppers, while Hirashima [5] provided information on their survey in Taiwan. Lin [6] and Chen [1] also reported on the egg parasitoids of rice leafhoppers and planthoppers in Taiwan, while Chen and Yu [2] studied *Anagrus* Haliday egg parasitoids of brown rice planthopper, *Nilaparvata lugens* (Stål) (Delphacidae). Unfortunately, almost no voucher specimens from these earlier studies in Taiwan could be found in the museum collections either there (including that of TARI) or in Japan [20], so it was impossible, with a very few exceptions [20], to verify their identity with confidence; it seems likely that most of them were misidentified at species level [20]. Therefore, we decided to conduct a new survey of egg parasitoids of rice leafhoppers and planthoppers in west central Taiwan (Taichung as well as Chiayi and Yunlin counties) with the aim of identifying them properly. The latter, however, has proven to be not a small task by itself as a lot of confusion exists in Asia (in the Oriental

region and southern part of the eastern Palaearctic region) in regard of their identification. For instance, without providing much of critical analysis, Gurr *et al.* [4] summarized many published records of parasitoids of Asian planthopper pests of rice; among the mymarid egg parasitoids they included both valid species and, separately, their several current synonyms, as well as some obvious misidentifications, species of unknown identity such as *Gonatocerus longicrus* (Kieffer), and a genus which is not known to have species that parasitize eggs of either leafhoppers or planthoppers (*Anaphes* spp.).

Specimen collection and preparation. During 2015 and 2016, egg parasitoids of selected species of rice pests belonging to the Auchenorrhyncha were reared in Chiayi and Yunlin counties by S.-H. Huang by exposing their sentinel eggs in rice plants in pesticide-free rice fields. For obtaining sentinel eggs, 30-day-old rice plants were used; each pot contained 3 to 5 tillers. Five gravid female adults each of the green rice leafhopper *Nephotettix cincticeps* (Uhler) (Cicadellidae) or the planthoppers (Delphacidae) *Laodelphax striatella* (Fallén) (small brown planthopper), *Nilaparvata lugens* (Stål) (brown planthopper), and *Sogatella furcifera* (Horváth) (whitebacked planthopper) were released into caged pots with rice plants for oviposition. The host leafhoppers or planthoppers were removed from the cages after 24 hours, and the pots with their sentinel eggs were taken to the rice fields and exposed there for parasitization for 48 hours. Then the rice plants with both unparasitized (Fig. 1) and parasitized (Fig. 2) eggs were taken back to the laboratory. The tillers with host eggs were transferred to vials lined with a moistened filter paper to avoid desiccation. The parasitized host eggs turned reddish (Fig. 2). The emerging parasitoids were collected daily, labeled, and placed in 95% ethanol for storage at -20°C until shipped to H.-T. Shih and/or S. V. Triapitsyn for identification and record keeping.

In Taichung, egg parasitoids of rice leafhoppers and planthoppers were collected using a Malaise trap (Fig. 11) and yellow pan traps in an experimental organic rice field at TARI, Wufeng, during S. V. Triapitsyn's visit in October 2016. Thereafter several Malaise traps were installed in rice fields in Taichung by H.-T. Shih and M.-R. Tzeng, who collected and processed the samples. The obtained data will be used for both parasitoid identification and for obtaining information on their species composition, abundance, and population dynamics. These will be reported elsewhere in a forthcoming joint publication to be submitted to the Journal of Taiwan Agricultural Research.

Most of the collected or reared specimens were identified directly in ethanol. Selected specimens were dried from ethanol using a critical point drier, point-mounted, and labeled; these were then sorted to morphospecies, and some of the specimens were slide-mounted by Vladimir V. Berezovskiy. Both ethanol-preserved, pointed- and slide-mounted voucher specimens were deposited in the insect collection of TARI and the Entomology Research Museum, University of California, Riverside, California, USA.

Specimen identification. The most common and abundant parasitoid reared from sentinel eggs of all four aforementioned host species (one leafhopper and three planthoppers) was *Anagrus nilaparvatae* Pang and Wang (Mymaridae) (Figs 3, 4), both in Chiayi and Yunlin counties of Taiwan. *Anagrus nilaparvatae* was originally described from Wushan (Fuoshan), Guangdong, China, as an egg parasitoid of brown planthopper *Nilaparvata lugens* [(7)]. Since then it was reported, in numerous publications partially summarized by Gurr *et al.* [(4)], from many countries in the Oriental and eastern Palaearctic regions as the main egg parasitoid of several economically important leafhoppers and planthoppers in rice field agroecosystems. As noted by Triapitsyn and Berezovskiy [(22)] and Triapitsyn [(15)], *A. nilaparvatae* is morphologically practically indistinguishable from the common, widespread, polyphagous Palaearctic species *A. incarnatosimilis* Soyka (as *A. incarnatus* Haliday). Its proper taxonomic identity was recently figured out as part of this project [(23)] using both morphometric analysis and genetic studies.

The second most common egg parasitoid of rice leafhoppers in Chiayi and Yunlin counties of Taiwan was *Mymar taprobanicum* Ward (Mymaridae), a rather common and almost cosmopolitan species. Illustrated here is a female of *M. taprobanicum* (Fig. 5) reared from eggs of green rice leafhopper *Nephotettix cincticeps*. Also reared in west central Taiwan were a few specimens of *Gonatocerus aegyptiacus* Soyka (Mymaridae) (Fig. 6) from eggs of *Nephotettix cincticeps* and *Oligosita* sp. (Trichogrammatidae) (Fig. 7) from eggs of small brown planthopper *Laodelphax striatella*.

In the Malaise trap installed in the rice field at TARI, also collected were the rice green leafhopper *Nephotettix nigropictus* (Stål) and the zig-zag leafhopper *Maiestas dorsalis* (Motschulsky) (Cicadellidae) (identifications by S. V. Triapitsyn). The known egg parasitoid *Pseudoligosita nephotetticum* (Mani) (Trichogrammatidae) (Fig. 8) was collected there in yellow pan traps but not in the Malaise trap standing next to them, whereas *Anagrus nilaparvatae* was collected in the same location in both types of traps.

2. Rearing and identification of egg parasitoids of the leafhopper *Kolla paulula* (Walker), vector of *Xylella fastidiosa*

In Taiwan, the cicadelline (Cicadellidae: Cicadellinae: Cicadellini) leafhopper *Kolla paulula* (Walker) is a known vector of the phytopathogenic bacterium *Xylella fastidiosa* [8], the causative agent of Pierce's disease of grapes and similar diseases of other affected plants, and thus of particular economic importance. Two species of parasitoids were reared by W.-F. Hung and H.-T. Shih from its eggs in plant tissue of spreading dayflower, *Commelina diffusa* (Commelinaceae), and then identified by S. V. Triapitsyn as *Pseudoligosita nephotetticum* (Mani) (Trichogrammatidae) (Fig. 9) and one male of *Cosmocomoidea* sp. (Mymaridae) (Fig. 10) [as *Gonatocerus* (*Cosmocomoidea*) sp.] [24]. *Pseudoligosita nephotetticum* was redescribed and illustrated; also provided was a summary of the known records of egg parasitoids (Mymaridae and Trichogrammatidae) of other leafhoppers from the tribe Cicadellini in the entire world [24].

3. A preliminary study of the diversity and taxonomy of Mymaridae in Taiwan

General information. Biogeographically, the fairyfly (Mymaridae) fauna of Taiwan is primarily Oriental, although at high altitudes it fits more that of the Palaearctic ecozone. While generic identifications of most Mymaridae in the region are generally relatively easily available, species identifications are still a major problem. Expensive and time-consuming special preservation and mounting techniques, such as critical point drying from ethanol and making microscopic slides in Canada balsam, are usually required to be able to identify most mymarids to species based on morphology. Overall, prior to this study, taxonomic reports on the diversity of Mymaridae in Taiwan were scarce and either included some random identifications of the Taiwanese specimens as parts of broader generic revisions or those of specimens of interest to biological control projects, mainly of egg parasitoids of rice pests.

Keys and taxonomic revisions of importance. Keys and taxonomic revisions that include Taiwanese specimens of certain fairyfly genera are unfortunately few, but some can be helpful to recognize some species: Taguchi [9, 10, 11, 12, 13] for a number of genera, Triapitsyn [16] for *Nepolynema* Triapitsyn, Triapitsyn [17] for *Himopolynema* Taguchi (a revision of the Taiwanese species with descriptions of several new taxa), Triapitsyn [18] for *Arescon* Walker, Triapitsyn

[19] for *Alaptus* Westwood, Triapitsyn *et al.* [21] for *Zeyanus* Huber, Triapitsyn and Berezovskiy [22] for *Anagrus* Haliday, etc.

Recent achievements. Triapitsyn [20] just published an annotated checklist of Mymaridae of Taiwan, which includes records of 28 genera and 60 named species. Of these, 13 genera and 33 species were newly recorded from Taiwan, including 5 new species described. Information on their distribution and available host records was also provided, along with illustrations of some species and keys to several genera. In addition, a number of the undescribed species were identified in the course of this study; these await thorough taxonomic revisions of the genera to which they belong. The previous published records of mymarid egg parasitoids of rice leafhoppers and planthoppers in Taiwan were critically analyzed.

This checklist was based largely on the collection of Mymaridae in TARI, almost all mounted on individual microscopic slides, captured mostly by sweeping during the 1950s-1970s by Kwei-Shui Lin (1921-2002), a taxonomist and prolific collector of parasitic Hymenoptera, who worked at TARI in Taipei City before moving to Taichung. He collected almost 3,000 specimens of fairyflies throughout Taiwan (the bulk from Taipei City and its environs). These were examined and identified by S. V. Triapitsyn mostly during visits to TARI in August 2013 and October 2016.

Tian *et al.* [14] reported a surprisingly low generic diversity index for the Mymaridae of Taiwan which they suggested, along with Tibet's, to be significantly lower than that for Fujian, China. However, Triapitsyn [20] demonstrated that to be a misleading conclusion because the mymarid fauna of Taiwan was at that time too poorly known. One would expect, due to the large size of the island, diversity of its habitats, and relative proximity to Fujian, a greater fairyfly diversity than reported, along with some possible endemism (particularly in the mountains). Thus, the mymarid fauna of Taiwan could likely be almost as diverse as that of Fujian.

Looking ahead. Clearly, what is now known about biodiversity and taxonomy of Mymaridae in Taiwan, as summarized recently by Triapitsyn [20], is not enough, primarily because of the lack of systematic collecting efforts of micro-Hymenoptera throughout Taiwan Island and in the surrounding smaller islands using both various modern trapping methods and rearings from known hosts. Likewise, there hasn't been any significant effort to sort, dry, mount, label, and identify any fairyflies from the numerous existing bulk alcohol samples, primarily those from Malaise traps, in any major Taiwanese collections of insects. Thus, more

species are expected to be discovered there once such efforts take place sometimes in the future, provided interest, taxonomic expertise, and appropriate funding become available.

As noted by Triapitsyn [20], at least 5 more genera of Mymaridae are likely to occur in Taiwan, besides those included in that checklist. Indeed, after it was accepted for publication and it was too late to add any taxa to it, one more genus, *Dorya* Noyes and Valentine, was found in Taiwan (John T. Huber, personal communication, material in the Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada). Thus, once all or almost all the genera are found and properly recorded from there, the next step would be to compile a key to both sexes of the genera occurring in Taiwan and to identify (and describe, if necessary) species in each genus. The latter, however, may require revising at least the Oriental species of some genera.

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We thank Mr. Vladimir V. Berezovskiy (UCRC) for mounting specimens, Ms. Mei-Rong Tzeng (TARI) for processing Malaise trap samples, Dr. John T. Huber (Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada) for sharing valuable information, and Dr. Chi-Feng Lee for access to the mymarid collection of TARI.

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Fig. 1. Unparasitized eggs of brown planthopper *Nilaparvata lugens* (Taiwan).

Fig. 2. Eggs of brown planthopper, *Nilaparvata lugens*, parasitized by *Anagrus nilaparvatae* (Taiwan).

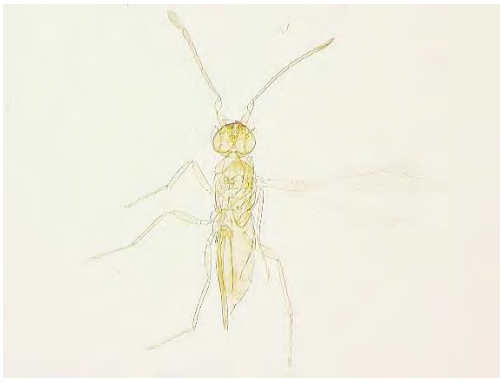

Fig. 3. Female of *Anagrus nilaparvatae*, egg parasitoid of brown planthopper *Nilaparvata lugens* (Lucao, Taiwan).

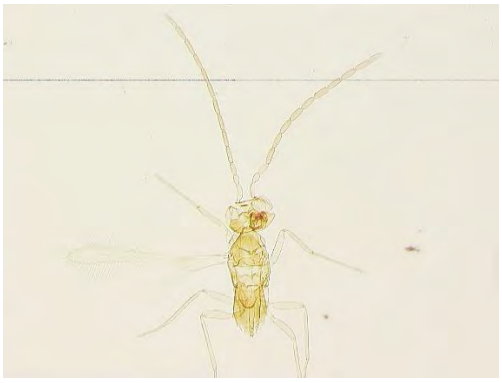

Fig. 4. Male of *Anagrus nilaparvatae*, egg parasitoid of brown planthopper *Nilaparvata lugens* (Lucao, Taiwan).

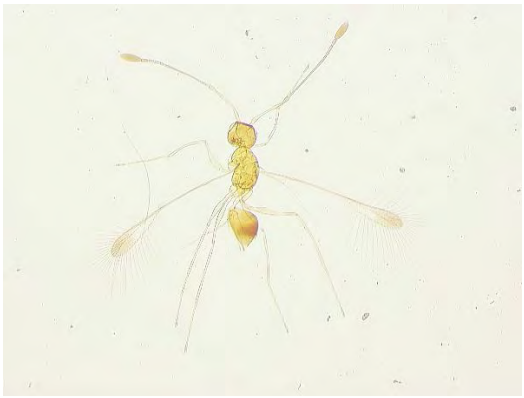

Fig. 5. Female of *Mymar taprobanicum*, egg parasitoid of green rice leafhopper *Nephotettix cincticeps* (Gukeng, Taiwan).

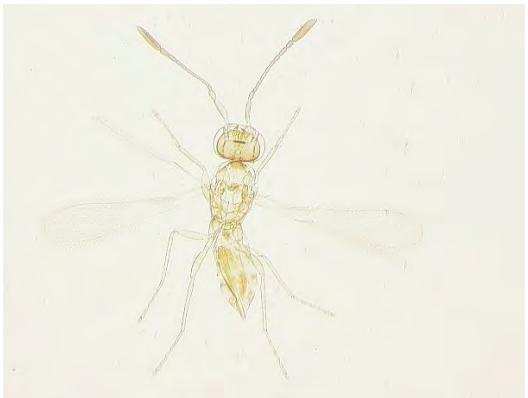

Fig. 6. Female of *Gonatocerus aegyptiacus*, egg parasitoid of green rice leafhopper *Nephotettix cincticeps* (Gukeng, Taiwan).

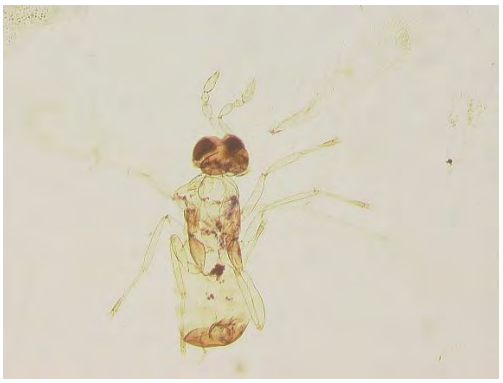

Fig. 7. Male of *Oligosita* sp., egg parasitoid of small brown planthopper *Laodelphax striatella* (Gukeng, Taiwan).

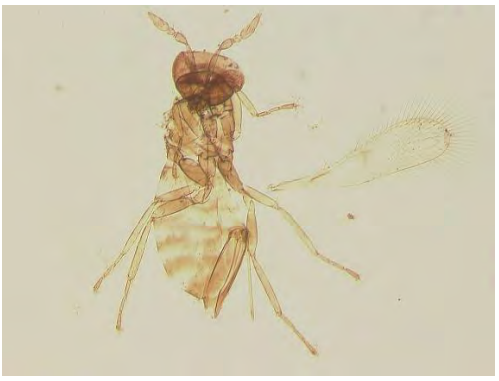

Fig. 8. Female of *Pseudoligosita nephotetticum* (rice field, TARI, Wufeng, Taichung, Taiwan).

Fig. 9. Female of *Pseudoligosita nephotetticum*, egg parasitoid of *Kolla paulula* (Wufeng, Taichung, Taiwan).

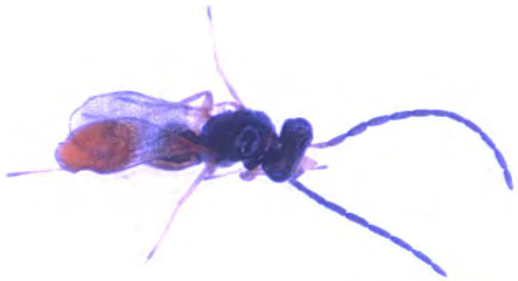

Fig. 10. Male of *Cosmocomoidea* sp., egg parasitoid of *Kolla paulula* (Wufeng, Taichung, Taiwan).

Fig. 11. A Malaise trap in the experimental organic rice field (TARI, Wufeng, Taichung, Taiwan).