

FIRST RECORDS OF TWO PARASITOIDS OF *Episparis tortuosalis* A SERIOUS PEST OF *Chukrasia tabularis* IN VIETNAM

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ABSTRACT

Chukrasia tabularis A. Juss is a valuable tree species that contributes to the economic significance of Vietnam where it is grown to produce furniture, doors, window frames, veneers, and decorative panels. However, *C. tabularis* plantations in the country are frequently attacked by the leaf defoliator *Episparis tortuosalis*. This study was designed to illustrate the morphological characteristics of *Ganaspis* wasps and *Megaselia* flies, which were found to be natural parasitoids of *E. tortuosalis* in *C. tabularis* plantations. Our field surveys revealed that the percentage of *E. tortuosalis* larvae and pupae parasitized by *Ganaspis* sp. was significantly lower than by *Megaselia* sp. and the parasitism rate of *Ganaspis* sp. varied significantly between Hoa Binh and Nghe An provinces meanwhile the parasitism rate of *Megaselia* sp. did not differ between these two study sites. These findings suggest that *Ganaspis* sp. and *Megaselia* sp. would be the potential biological control agents for effective and sustainable management of *E. tortuosalis* in the future.

Keywords: Parasitoid, *Ganaspis*, *Megaselia*, *Episparis tortuosalis*, identification, pest, morphology.

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1. INTRODUCTION

A wide array of insect pests have caused significant impacts on forest trees and forest ecosystems in different ways such as tree mortality, growth and vigor, defoliation and leaf loss, changes in species composition and structure, widespread of tree diseases, economic losses, and ecological consequences [1 - 4]. The severity of infestations varies depending on insect species. For example, bark and wood borers cause extensive damage to the vascular tissues and structural integrity of trees. This damage leads to tree mortality, especially when infestations occur in large numbers or during outbreaks [5]. These borers also selectively target specific tree species, leading to changes in the dominance and

distribution of tree species within a forest [6]. As a result, tree mortality disrupts forest ecosystems, affects biodiversity, finally loss of timber and forest resources [7]. In addition, defoliators cause a reduction in the photosynthetic capacity of trees, leading to reduced growth and energy reserves, stunted development, and decreased overall tree vigor [8]. Weakened trees are more susceptible to other stresses, such as drought, diseases, and secondary insect infestations [9].

Effective management of forest tree pests often involves a combination of multiple approaches, including monitoring, early detection, biological control, cultural practices, and targeted pesticide use when necessary [10, 11]. Among these methods, biological control using their natural enemies is a valuable tool in the integrated pest management (IPM) programs aiming to minimize their ecological and economic impacts while promoting sustainable forest management

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[12, 13]. Parasitoids are insects that lay their eggs on or inside another insect host, which the larvae then feed on and eventually kill. They are important natural enemies of many pest insects and are commonly used in biological control programs to manage pest populations [14]. Some wasps and flies are considered parasitoids because their larvae lead to the death of the host [15]. These parasitoid wasps and flies are used as the key component of biological control agents in forest ecosystems because of their contribution to preventing outbreaks, and reduction reliance on chemical pesticides, minimizing the negative impact on human health, non - target organisms, and the environment [16]. Interest in parasitoid - based biological control of insect pests has grown considerably in recent decades and has mostly focused on Bethilidae, Figitidae, Braconidae, Ichneumonidae, Phoridae, and Pteromalidae parasitoids [17, 18]. Many species of parasitic wasps and flies have been extensively studied and utilized for the biological control of forest insect pests such as *Hyssopus pallidus* in *Cydia pomonella* and *Anastatus orientalis* in *Lycorma delicatula* [19].

Episparis tortuosalis (Lepidoptera: Erebiidae: Pangraptini) is a main threat to some forest tree species in India, China and Southeast Asia such as *Chukrasia tabularis*, *Michelia champaca*, and *Azadirachta indica*. In Vietnam, one of the recent studies has reported widespread damage from *E. tortuosalis* in *C. tabularis* in plantations, home gardens and streetscapes [20]. The geographical range and the extent of damage caused by this pest have expanded rapidly across seven provinces in four ecological regions of the country between 2013 and 2020 [20]. The control measures of forest insect pests in Vietnam primarily rely on heavy use of chemical insecticides and the long-term frequent use of insecticides may lead to increased insecticide resistance, destruction of natural enemy populations and pest resurgence.

At present, no control measures have been formulated for *E. tortuosalis*. To develop an effective management measure for *E. tortuosalis*, information on its natural enemies is very

important. The objectives of the present paper were to identify external morphological characteristics of the parasitoid wasp and fly of *E. tortuosalis*, to evaluate the parasitism rates of these two parasitoid species in each collection province. This study is expected to lay insights into the potential application of natural enemies for the sustainable development of *C. tabularis* plantations in Vietnam.

2. RESEARCH METHODOLOGY

2.1. Collection sites and methods

During 2020 and 2021, one - hundred - last larval instars and one - hundred - pupae of *E. tortuosalis* were randomly collected from two *C. tabularis* plantations in Nghe An and Hoa Binh (50 larvae and 50 pupae from each province). The samples were then taken to the Forest Protection Research Centre (FPRC), Ha Noi where they were reared in the net cages (40 x 60 x 40 cm). The fresh leaves of *C. tabularis* were weekly provided to *E. tortuosalis* larvae as feeding material until pupation and adult moth emergence. When *E. tortuosalis* larvae pupae died, they were placed individually into a 50 mL glass bottle with the top covered by cotton mesh to allow air exchange. Newly emerging adults of parasitoids were collected directly and put into a 1.5 mL Eppendorf before pinning the specimens.

2.2. Identification of parasitoid specimens

Species identifications were undertaken by morphological comparison of specimens with previously published descriptions. Identification of parasitic wasps was based on descriptions by Kasuya *et al.* (2013) [21] and Gonzalez-Cabrera *et al.* (2020) [22]. Identification of parasitic flies was based on description by Disney *et al.* (2014) [23].

Morphological measurements of slide mounted specimens were taken to describe the species using a micrometer mounted in a compound microscope Leica M165C stereozoom microscope (Leica Microsystem, Wetzlar, 54 Germany) with a micrometric scale. The parasitoid wasp and fly images were captured by using a Nikon DS-63 Fi2 camera (Nikon Corporation, Ha Noi, Vietnam) mounted on a Leica M165C

microscope (Leica 64 Microsystems, Wetzlar, Germany). The images were further processed using Adobe Photoshop. Adult voucher specimens were deposited in the insect collection of the FPRC with numbers coded from FPRC111 to FPRC119 for the wasps and FPRC120 to FPRC130 for the flies.

2.3. Data analysis

The number of larvae and pupae dead by parasitoid wasps and flies were observed to calculate the percentage of parasitism (P%) using the following equation.

$$P\% = (n/N) \times 100$$

Where n is the number of parasitized *E. tortuosalis* larvae/pupae; and N is the total number of *E. tortuosalis* larvae and pupae collected.

Independent sample T-tests were performed to test for mean differences in parasitism rate of *Megaselia* sp. and *Ganaspis* sp. larvae and pupae between Hoa Binh and Nghe An provinces at $p < 0.05$ along with a 95% confidence interval. Data were analyzed using GenStat Release 12.1 software

package (VSN International Ltd., Hemel Hempstead, UK).

3. RESULTS

3.1. Morphological description of parasitoids

Two kinds of parasitoids were collected from larvae and pupae of *E. tortuosalis* in the laboratory of FPRC. Both of them are currently identified to generic level. Morphological descriptions of them are as below.

Ganaspis sp. (Hymenoptera: Figitidae: Eucoilinae): Body length 2.0 – 2.8 mm. Wing lengths 1.0 – 1.3 mm long. Body color black to dark brown. Leg color is light brown. Sculpture on vertex. Lateral surface of pronotum, mesosoma, and metasoma is entirely smooth. Head round in anterior view, more transverse in lateral view. Pubescence on head sparse glabrous. Sculpture along lateral margin of occiput absent. Gena short. Lateral margin of occiput round. Occiput smooth. Carina expanding from lateral margin of postocciput absent. Anterior ocellus far from posterior ocelli (Figure 1).

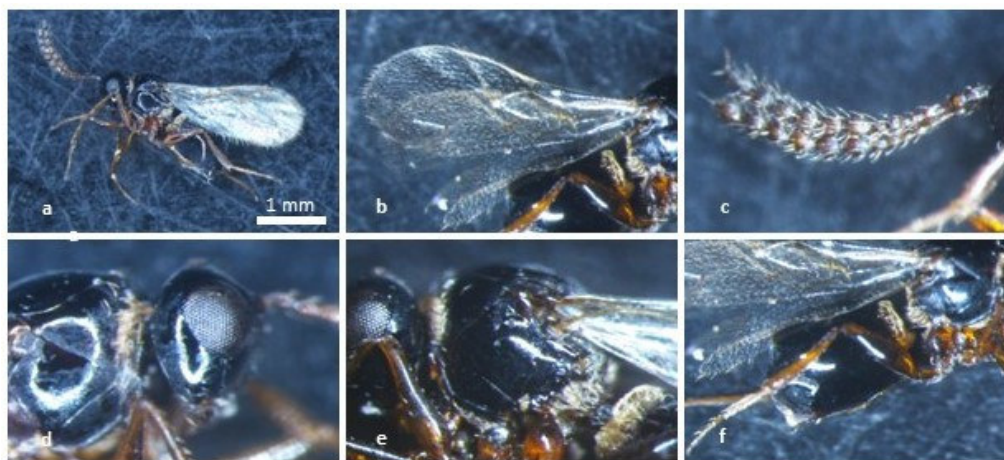


Figure 1. External morphological characteristics of *Megaselia* sp. (a) Lateral view; (b) Forewing and Hindwing; (c) Antenna; (d) Head; (e) Thorax; (f) Abdomen

Megaselia sp. (Diptera: Phoridae): Body length 2.3 - 3.3 mm. Wing lengths 1.1 - 1.6 mm long. Body color black to dark yellow except for brown on the head. Leg color is dark yellow. Costal cilia 0.01 - 0.03 mm long. The outer portion of two axillary bristles 0.04 mm long. Frons dense. Palps dark yellow. Mesopleuron bare. Labrum short. Thorax with two notopleural bristles.

Scutellum with an anterior pair of hairs and a posterior pair of bristles. Hypopygium with a pale anal tube. Cercus and proctiger hairs are shorter than epandrium hairs. Lobes of hypandrium vestigial. Dorsal hair palisade of mid tibia extends about 0.6 times its length. Hairs below basal half of hind femur are longer than those of anteroventral row of outer half (Figure 2).



Figure 2. External morphological characteristics of *Megaselia* sp. (a) Newly emerged adult; (b) Lateral view; (c) Dorsal view; (d) Ventral view; (e) Head; (f) Eye; (g) mouth; (h) Thorax; (i) Ventral view of genital terminalia

3.2. Parasitism rates of parasitoids

In general, the percentage of larval and pupal *E. tortuosalis* parasitized by *Ganaspis* sp. (5.3 - 7.0% and 6.1 - 8.9%, respectively) was significantly lower than by *Megaselia* sp. (11.7 - 12.1% and 16.8 - 17.5%, respectively) (Table 1).

There was a significant difference ($p < 0.001$) in the percentage of *E. tortuosalis* larvae and pupae parasitized by *Ganaspis* sp. wasps between Hoa Binh and Nghe An. In contrast, this result was not observed in *Megaselia* sp. flies ($P = 0.448 - 0.521$) (Table 1).

Table 1. Parasitism rates of *E. tortuosalis* larvae and pupae caused by *Ganaspis* sp. and *Megaselia* sp. in Hoa Binh and Nghe An provinces

Parasitoid species	Province	Mortality rate (%)	
		Larvae	Pupae
<i>Ganaspis</i> sp.	Hoa Binh	5.3	6.1
	Nghe An	7.0	8.9
<i>P value</i>		<0.001	<0.001
<i>Megaselia</i> sp.	Hoa Binh	11.7	16.8
	Nghe An	12.1	17.5
<i>P value</i>		0.448	0.521

4. DISCUSSION

This study is the first that reports two parasitoids of *E. tortuosalis* in *C. tabularis* plantations in Vietnam. *Megaselia* is the largest genus in the family Phoridae (cuttle flies), a member of the order Diptera [24]. There are currently more than 1,400 species of the approximately 4,300 species in this genus have been described so far, and large number of species are found in the tropical and subtropical countries of the world [25, 26]. *Megaselia* genus has high species diversity and richness, and life histories are tremendously diverse despite only a small proportion being known [26]. The genus *Megaselia* exhibits remarkable ecological diversity. Different species within this genus can be found in a wide range of habitats, including forests, grasslands, caves, and human-made structures such as houses, barns and compost heaps. Some species are associated with decaying organic matter, while others are found in association with insects, fungi, or vertebrate carrion. The genus *Ganaspis* is a group of parasitic wasps belonging to the family Figitidae (gall wasps or fig wasps) within the order Hymenoptera [27]. This genus contains numerous species, and taxonomists continue to study and describe new species within this group. Different species within this genus exhibit varying levels of host specificity. Many *Ganaspis* species target specific insect hosts, often associated with galls or plant tissues. Taxonomists continue to study the diversity, biology, and behavior of these *Megaselia* and *Ganaspis* species to better understand the relationships among different species and their interactions with hosts and the environment because this information is crucial for developing effective pest management strategies and conservation efforts.

Researchers and forest owners explore the potential of using parasitoid wasps and flies as biocontrol agents to reduce the reliance on chemical pesticides and promote sustainable pest management practices. Some species of *Ganaspis* wasps and *Megaselia* flies have been reported to parasitize different insects such as *G. brasiliensis*

in some frugivorous *Drosophila* species [28 - 30], *Ganaspis* sp. in *Atherigona varia* [31], *M. opacicornis* in *Chrysomela lapponica* [32], *M. scalaris* in *Spodoptera frugiperda*, *Mischocyttarus cassununga*, *Macrotermes gilvus* and honeybee [33 - 35], *M. rufipes* in *Apis mellifera* [36]. Thus, *Ganaspis* sp. and *Megaselia* sp. based biological control agents in this study will hold a great promise as a valuable tool in the development of natural enemies for sustainable management of *E. tortuosalis* in Vietnam. Interestingly, our results found that the percentage that *E. tortuosalis* larvae and pupae parasitized by *Megaselia* sp. flies were significantly higher than those by *Ganaspis* sp. wasps. This finding implies that *Megaselia* sp. might be more important than *Ganaspis* sp. in controlling and killing *E. tortuosalis*.

The expansion programs of *C. tabularis* plantation areas have been strongly promoted and supported by the Ministry of Agriculture and Rural Development in recent years. Therefore, *C. tabularis* is among the most important forest tree species in the country. Increase in defoliation from *E. tortuosalis* presents many challenges for plantation growers [20] since when *C. tabularis* trees are lose its leaves, they can suffer from reduced photosynthesis, which might lead to decreased growth, weakened immune systems, and reduced fruit or seed production. The application of parasitoid wasps and flies in the preventive control of insect pests is limited by several practical issues, particularly rearing and releasing issues [37]. Some species of *Ganaspis* wasps and *Megaselia* flies are commonly used as laboratory model organisms in scientific research because they are relatively easy to rear in the laboratory and their short generation time and large brood sizes make them useful for studying various biological phenomena, such as development, behavior, and genetics. Though parasitoid wasps and flies do not feed directly on artificial diets, a proper artificial diet for mass - rearing their hosts would contribute to increasing the individual populations for mass releases in the field. Indeed, methods for rearing and releasing *Ganaspis* sp. and *Megaselia* sp. have been studied

[38 - 40]. Therefore, further studies are required to focus on biological features, mass rearing, and releasing techniques of *Ganaspis* sp. and *Megaselia* sp. under *C. tabularis* plantation conditions in Vietnam.

5. CONCLUSION

This study provides the first reported data regarding the external morphological identification of two parasitoid species of *E. tortuosalis* including *Megaselia* sp. and *Ganaspis* sp. Although these two parasitoid wasps and flies do not seem to sufficiently reduce pest populations in fields, but this study is the basis for developing them into potential biological control agents. Additional studies are required to clarify unknown aspects of two parasitoids such as taxonomy, biology, and rearing techniques as well as releasing methods of *Megaselia* and *Ganaspis* species and implement biological control as a successful and important component of integrated pest management.

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