A NEWLY EMERGING INSECT PEST DAMAGING *Cinnamomum cassia* PLANTATIONS IN YEN BAI PROVINCE

Dao Ngoc Quang[1], Pham Quang Thu[1], Nguyen Quoc Thong[1],
Le Van Binh[1], Nguyen Van Thanh[1], Bernard Dell[1, 2]

ABSTRACT

Plantations of *Cinnamomum cassia* are important contributors to the livelihoods of communities living in the mountainous areas of northwest Vietnam. Recently, a *Cricula* species (Lepidoptera: Saturniidae) has emerged as a major threat to these plantations. Morphologically the species is similar to *Cricula variabilis* Naumann & Loffler in China, but preliminary analysis of the mitochondrial cytochrome oxidase 1 gene region indicates the need for detailed phylogenetic analysis to confirm the taxon in Vietnam. In a laboratory study (30±2°C, 85±5% RH and 12 h light: 12 h dark photoperiod), the life cycle took 69.6±5.2 days, females laid 200 to 310 eggs, eggs hatched after 8 to11 days, the larval period was 38 to 48 days, the pupal period was 9 to 15 days, and adults survived 4 to 8 days. There are four generations per year in the field. In ten plantations in Yen Bai province assessed in 2019, the damage incidence ranged from 47.2 to 71.5% and the damage index from 1.03 to 2.14. Cinnamon growers are concerned that the severity of defoliation will lead to a loss in bark yield and reduced income. Field studies are underway to develop an integrated pest management plan for this pest.

Keywords: *Cinnamomum cassia, Cricula variabilis, folivore, life cycle.*

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1. INTRODUCTION

Cinnamomum cassia (Lauraceae) is an evergreen tree, native to southern China and is widely cultivated in South and Southeast Asia [1] mainly for its bark. It has been cultivated commercially in Vietnam since the late 18th Century. The bark is removed for extracting essential oil when trees are 10-12 years old, but the best oil content occurs in older trees. The bark has high economic value due to its oil content [2], [1]; and is popular for seasoning food and processing into pharmaceutical products [2], [1], [3], [4]. In Vietnam, *C. cassia* has been planted in 4 ecological regions: the northwest, northeast, north central and south central. By the end of 2020, the area of *C. cassia* plantations in Yen Bai province in northwest Vietnam was about 78,000 ha, accounting for two-thirds of the total area of *C. cassia* plantations in Vietnam. Most of the *C. cassia* plantations in this area are owned by local people, with each household having about 5-10 hectares.

More than 70 insect pest species have been recorded damaging *C. cassia* [5] and the most common pests in Vietnam are species of *Aegeria, Biston, Sophophora* and *Phyllocnistis* genus [6], [7], and *Aetherastis grandisalba* [8], [9], [10], [7]. Recently, a species of *Cricula* (Lepidoptera: Saturniidae) has become a pest of *C. cassia* plantations in Vietnam. According to unpublished data of the Yen Bai's Department of Agriculture

[1] Forest Protection Research Centre, Vietnamese Academy of Forest Sciences
[2] Agriculture and Forest Sciences, Murdoch University, Murdoch, WA 6150, Australia
* Corresponding author: daongocquang@vafs.gov.vn

and Rural Development, this leaf eating pest was first observed causing damage to 262 hectares of *C. cassia* in Yen Bai province in 2016. Since then, local records reveal that damage has increased over time and expanded to Lao Cai and other provinces in northwest Vietnam. The pest in Vietnam has been tentatively assigned to *Cricula variabilis* which was first described in central China in 2010 [11]. In China, the larvae damage a range of trees within the Lauraceae and Fagaceae, including *Callicarpa kochiana, Castanopsis fissa, Cinnamomum burmanni, C. camphora, Machilus chinensis, Mucuna pruriens, Phoebe bournei* and *Schefflera octophylla* [12], [19].

To date, no formal studies have been undertaken on the biology of the *Cricula* damaging commercial stands of *C. cassia* in Vietnam. The purpose of this paper is to identify the taxon, describe the life cycle and morphological characteristics of *C. variabilis*, and to document the damage to *C. cassia* plantations. This information will help alert the forestry and biosecurity sectors to this new emerging pest.

2. MATERIALS AND METHODS

2.1. Insect rearing in the laboratory

Adults were obtained from pupae of *Cricula* collected from *C. cassia* plantations in Tran Yen district, Yen Bai province in 2019. The pupae were separated in Petri dishes (9 x 1.5 cm), and taken to the laboratory in Ha Noi and maintained at 30±2 (SE)°C, 85±5% RH and 12 h light: 12 h dark photoperiod. In order to obtain fertilized eggs, ten pairs of newly emerged adults were released into insect cages 0.6 x 0.6 x 1.0 m (length x width x height). Each cage contained fresh leaves of *C. cassia* trees for laying eggs. Cotton wool inserted into a container of 5% honey solution was placed in each cage to provide food for adults. After hatching, 100 newly emerged larvae were placed in insect cages 40 x 40 x 40 cm (length x width x height) with fresh leaves and kept in acclimatized chambers as for the pupae. The cages were inspected every day to replace the leaves and to record development and mortality of each instar.

2.2. Identification and description

Identification was based on the external morphology of 60 adult specimens (30 males and 30 females) and male genital characteristics, using the keys in Naumann and Löffler (2010) [11]. All specimens were reared in the laboratory. Fourteen male adult and twenty female adult specimens were used for description. A further 90 samples (30 eggs, 30 larvae and 30 pupae) were used to take measurements. The egg size and the length of the larvae were measured using a Leica M165C microscope (Leica Microsystems, Wetzlar, Germany). Images were captured using a Nikon DS-Fi2 camera (Nikon Cooperation, Ha Noi, Vietnam). Adult specimens examined were deposited in the insect collection of the Forest Protection Research Centre (FPRC) with numbers from M001 to M060.

Bodies of three males (M010-M012) were softened in boiled water for 1-2 hours. The adult genitalia were then detached from the abdomen with fine forceps. The genitalia were pretreated in a warm solution of 10% KOH for 10-20 minutes, washed in water several times, and then transferred into glycerol on microscope slides. Microscope images were obtained as described earlier and the structures were compared with the descriptions of Naumann and Löffler (2010) [11].

For molecular identification, the mitochondrial cytochrome oxidase 1 (CO1) gene region was sequenced using mtDNA extracted from legs of male and female adult specimens M001 (21°73’64″N 104°80’94″E) and M002 (21°73’64″N 104°80’94″E) respectively, using both forward and reversed primers COI-LEP-F (ATTCAACCAATCATAAAGATA TTGG) and COI-LEP-R (TAAACTTCTGGATGTCC AAAAAATCA) [14]. The detailed procedures have been described previously in Quang *et al.* (2021) [15]. The DNA sequences of M001 and M002 were edited by Geneious version 7.1.9 sofware (Biomatters Ltd.) and compared with the database on BOLDSYSTEMS via identification tool (https://v3.boldsystems.org/). The sequences of M001 and M002 were aligned and compared for the similarity with referenced sequences obtained from BOLDSYSTEMS via Geneious version 7.1.9 sofware.

2.3. Assessment of damage and severity

According to surveys undertaken by the Forest Protection Research Centre and Yen Bai's Department of Agriculture and Rural Development (unpublished data), *C. variabilis* in Vietnam has four generations per year and the second generation has the highest larval density and causes damage mainly in April and May. Therefore, six field surveys were carried out in April and May 2019 in 4 to 13-year-old pure plantations of *C. cassia* in Yen Bai province. Six plots (25 × 40 m) were randomly established in each plantation (Figure. 1). In total, 60 plots were established across four districts. In each sample plot, 30 trees were randomly selected for scoring damage index. The damage was ranked into five levels, where: 0 = trees without damage; 1 = trees with <25% leaves damaged; 2 = trees with 25 to <50% leaves damaged; 3 = trees with 50 to <75% leaves damaged; 4 = trees with more than 75% leaves damaged.

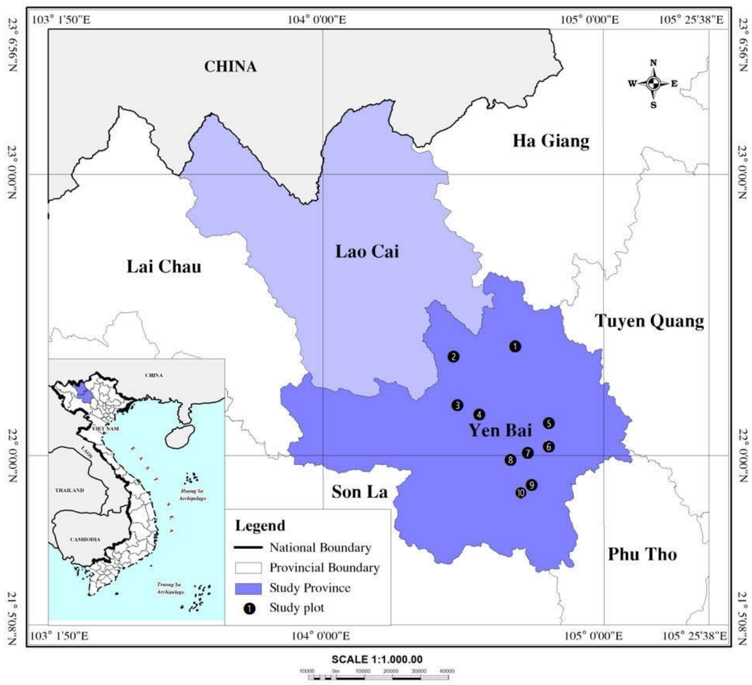

Figure. 1. Geographic location of *Cinnamomum cassia* plantations infested with *Cricula variabilis* in Yen Bai province, Vietnam where study sites were established

2.4. Data analysis

Following the results of damage classification, the damage incidence and damage index were calculated following Nguyen and Dao (2004) [16] and McMaugh (2005) [17], as follows:

$P\% = (n/N) \times 100$ (1)

Where: P% = damage incidence; n = the number of trees attacked by *C. variabilis*; N = total number of trees assessed;

$DI = (\Sigma n_i \times v_i)/N$ (2)

Where: DI = damage index; ni = the number of trees infested at damage index i; vi = the damage index at level i; N = total number of trees assessed. Based on the average damage index, the level of damage severity was ranked at 5 levels: DI = 0, no damage; $0 < DI \leq 1$, light damage; $1 < DI \leq 2$, moderate damage; $2 < DI \leq 3$, high damage; $3 < DI \leq 4$, very high damage.

3. RESULTS

3.1. Identification

Male and female adults (Figure 4) collected from Yen Bai province had almost the same morphology, colour and size as described for the type collection of *Cricula variabilis* [11] and in later publications [12], [13], [18]. Male genital structures (Figure 4) also matched the description of Naumann and Löffler (2010) [11]. Furthermore, the genitalia match those illustrated by Chen *et al.* (2019) [12].

The molecular work showed that the CO1 sequence of male M001 was 100% identical to that of female M002 and both sequences were 98.4% identical to the CO1 sequence of *Cricula variabllis* with the reference number SASNB201-09 on BOLDSYSTEM (Specimen Record | Public Data Portal | BOLDSYSTEMS).

Morphologically, the species of *Cricula* damaging *Cinnamomum cassia* plantations in Yen Bai province is similar to *Cricula variabilis* Naumann & Loffler in China. However, further detailed molecular analysis with a wider collection of Cricula in northern Vietnam is needed to confirm identity with the taxon in China.

3.2. Morphology

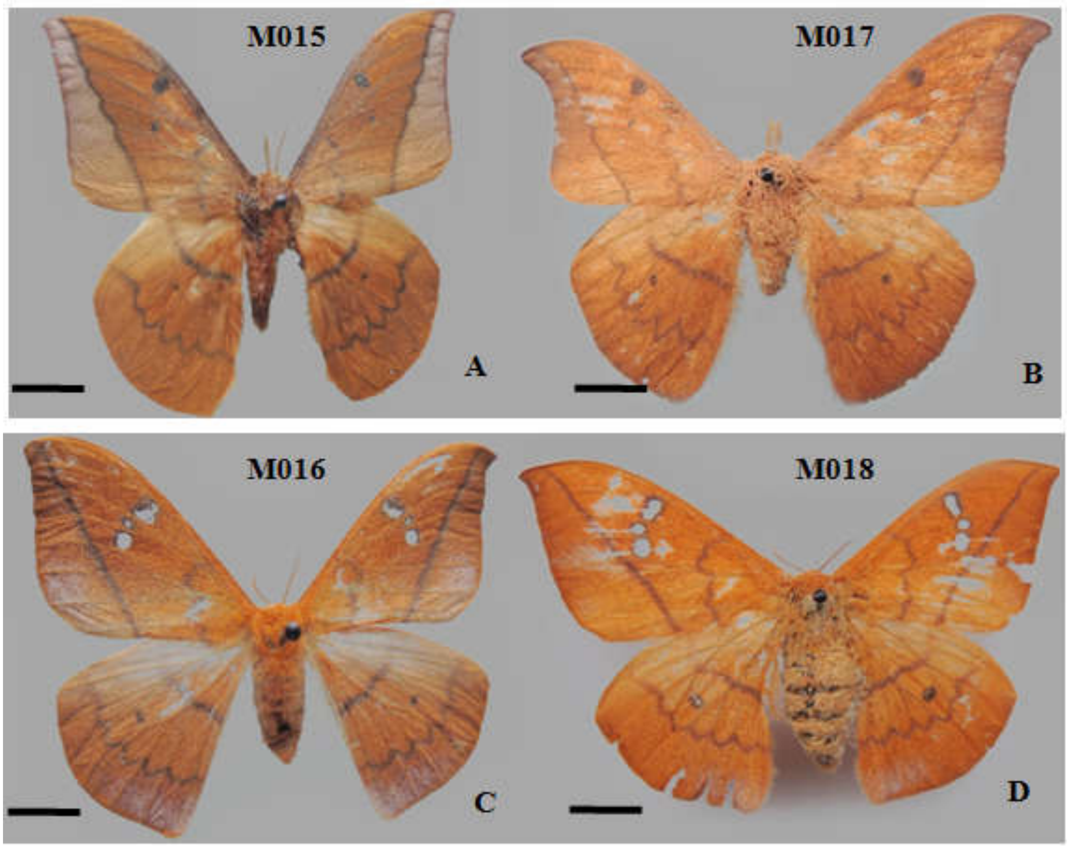

Figure 2. Morphological characteristics of *Cricula variabilis* adults collected from the field in May 2019 (A, C) and July 2019 (B, D). Upper male, lower female, scale bars = 0.5 cm

Figure. 3. *Cricula variabilis* male genitalia. (A) dorsal view with aedeagus removed, (B) aedeagus in lateral view. Scale bars = 0.5 mm

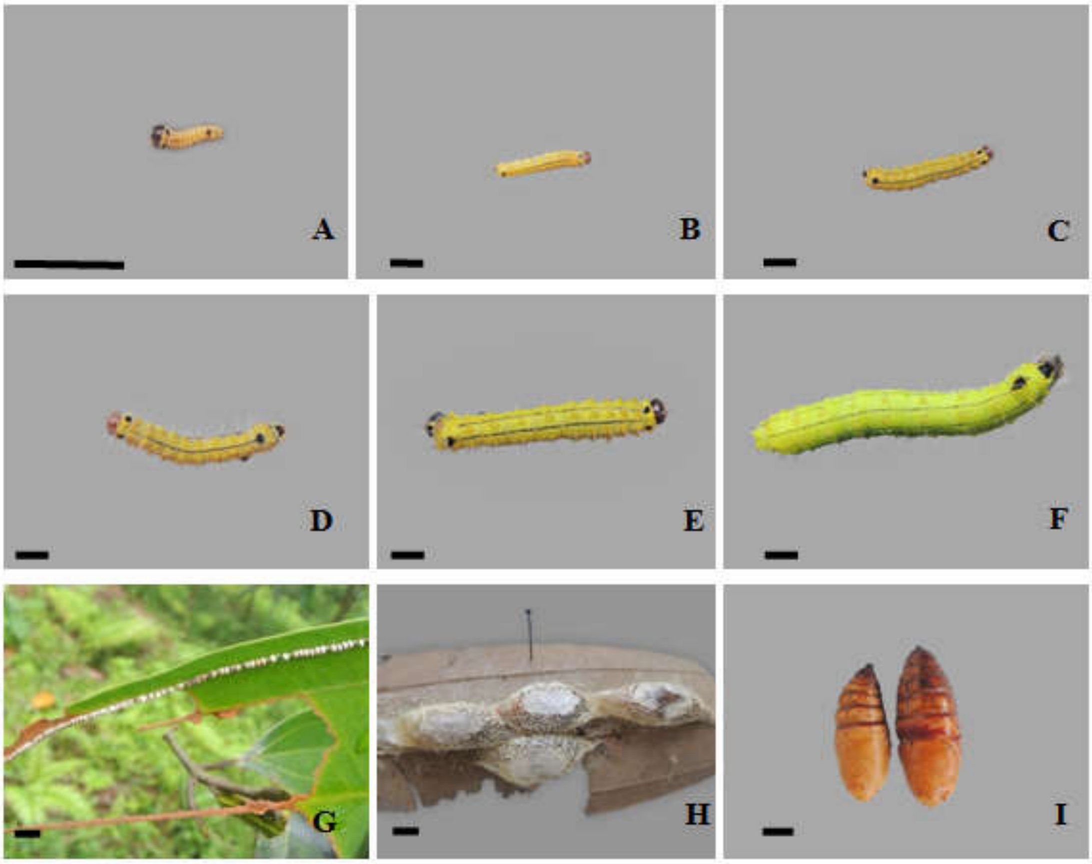

Figure 4. *Cricula variabilis* developmental stages. (A-F) larvae grown in the laboratory: (A) 1st instar, (B) 2nd instar, (C) 3rd instar, (D) 4th instar, (E) 5th instar, (F) 6th instar. (G) Egg and (H) cocoon on *C. cassia* leaf collected from plantation; (I) pupae male (left) and female (right). Scale bars = 0.5 cm

Adults (Figure 2): Body light to dark brown, mostly covered by brown scales, the fore- and hind-wings postmedian are darkened somewhat by grey scales. Wings triangular with acute forewing apex. Postmedian line with slight outward apical curvature. *Male* (Figure. 2A, C): Forewing 28.5-32 mm (mean 30.7±5.7 mm) in length, with up to four fenestrae. Antennae feather-shaped, dark brown, 4.0-8.0 mm in length (mean 5.9±1.1 mm). *Female* (Figure. 2B, D): larger than males. Forewing 31.5-43.0 mm (mean 35.2±7.0 mm) in length, with three fenestrae in the forewing and a single fenestrum in the hindwing. Antennae filiform, dark brown, 7.0-11.0 mm (mean 8.0±1.1 mm) in length. Male genitalia (Figure. 3): Uncus short with two lateral, almost triangular acute processes, gnathos trapezoid, juxta with two short lateral triangular processes, valva pubescent, phallus with four acute and slender dorsal spines.

Eggs (Figure 4G): oval, ivory white then becoming grey white, 1.5±0.13 mm in length, 1.2±0.2 mm in width. The eggs are laid in one or two parallel rows along the leaf.

Larvae (Figure 4A-F): newly emerged larvae brownish grey, becoming yellowish green then pale green by the sixth instar. The body is covered with numerous white hairs. Mean body length: 1st instar (Figure. 4A) 3.4±1.0 mm; 2nd instar (Figure 4B) 14.2±1.5 mm; 3rd instar (Figure. 4C) 20.5±2.4 mm; 4th instar (Figure. 4D) 29.3±3.1 mm; 5th instar (Figure. 4E) 39.8±3.4 mm; 6th instar (Figure. 4F) 50.2±3.8 mm.

Pupae (Figure 4H, I): light brown, enclosed by yellow cocoons, female 24.2±1.5 mm in length and 12.3±0.9 mm in width, male 19.6±1.2 mm in length and 9.0±0.9 mm in width.

3.3. Life cycle

In the laboratory study, the life cycle took 69.6 days ± 5.2 days, females laid 200-310 eggs during an oviparous period, eggs hatched after 8-11 days, and the larval and pupal periods were 38-48 and 9-15 days, respectively. Adults survived 4-8 days. Newly hatched larvae fed on eggshells before moving away to search for young leaves and start feeding from the edge of the leaf. In the field, larvae often fed in the morning and late afternoon, and moved down to seek shelter to avoid intense sunlight (Figure 5C). Pupae are usually located on the lower leaf surface, branches, or stems. Females mainly emerge during the day and males at night.

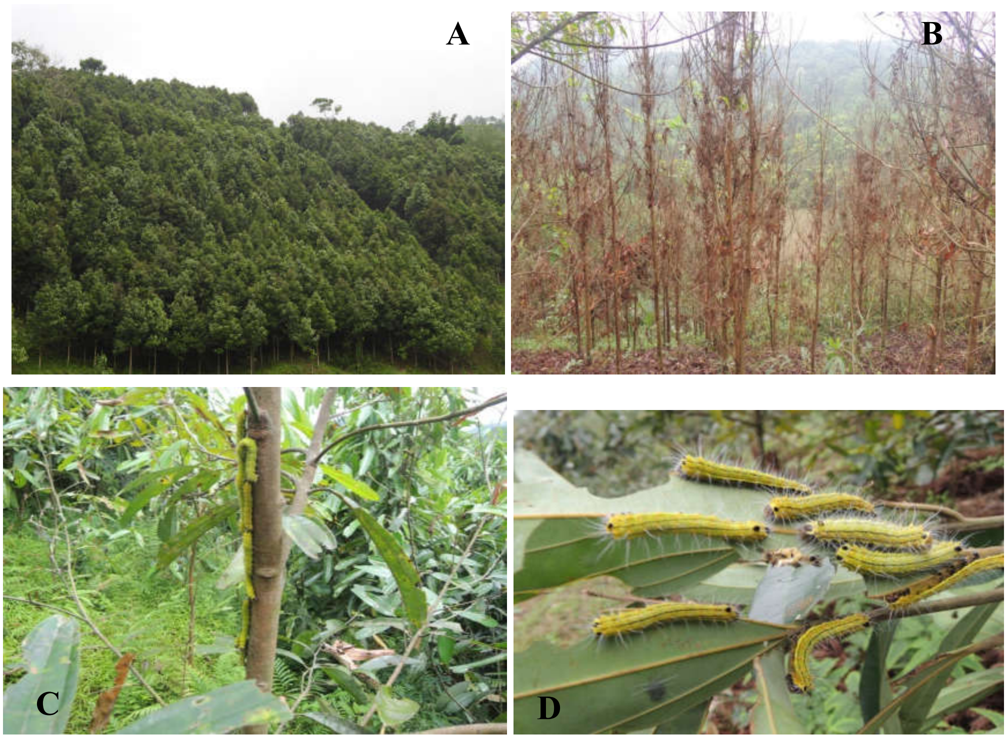

Figure. 5. (A) Healthy *Cinnamomum cassia* plantation, (B) Damaged plantation with most of its foliage consumed by caterpillars, (C) Larva of *Cricula variabilis* moving down the bole to avoid sunlight, (D) fifth instar larvae feeding on leaves

3.4. Damage to *Cinnamomum cassia plantations*

Infestation levels (P%) and damage index (DI) of *C. variabilis* in all ten plantations in Yen Bai province in 2019 was ranged from 47.2 to 71.5% and 1.03 to 2.14, respectively. The most serious damage was in 8-year-old plantations in Tran Yen district where the infestation level (P%) was 71.5±6.5% and average damage index was 2.14±0.21 (Table 1).

Table 1. Plantation details and extent of damage from *Cricula variabilis* in 2019

Location (district)*	Age (year)	Area (ha)	Coordinates	Density (tree/ha)	Elevation (m)	Damage incidence (P%)	Damage index (DI)
Luc Yen[1]	13	6	22°06’66″ N 104°76’96″ E	1,650	328	47.2±5.10	1.03±0.10
Van Yen[2]	7	6	21°85’16″ N 104.61’76″ E	3,300	443	66.1±6.2	2.03±0.19
Van Yen[3]	4	7	21°85’14″ N 104°60’77″ E	6,600	254	53.2±4.6	1.52±0.13
Van Yen[4]	13	5	21°84’25″ N 104.60’68″ E	1,650	330	51.1±5.0	1.41±0.11
Van Yen[5]	8	5	21°84’30″ N 104°61’93″ E	3,300	379	59.5±5.2	1.44±0.13
Tran Yen[6]	7	8	21°82’12″ N 104°78’64″ E	3,300	230	66.2±6.4	1.45±0.16
Tran Yen[7]	8	8	21°73’64″ N 104°80’94″ E	3,300	165	71.5±6.5	2.14±0.21
Tran Yen[8]	12	4	21°80’87″ N 104°80’85″ E	1,650	262	54.4±5.4	1.43±0.13
Tran Yen[9]	5	5	21°733’9″ N 104°80’72″ E	6,600	182	55.3±4.8	1.51±0.14
Van Chan[10]	7	6	21°44’61″ N 104°79’88″ E	3,300	427	51.6±4.2	1.24±0.12

*Note: * The numbers refer to the locations given in Fig. 1, P% is damage incidence, DI is mean damage index. Values are mean (n = 6) ± SE*

4. DISCUSSION

This is the first report of a *Cricula* species defoliating *Cinnanomum* cassia plantations in Vietnam. Adults have morphological characteristics similar to *Cricula variabilis* Naumann & Loffler collected in central China [11], [12], [13], [18]. The CO1 sequence of the two samples (M001, M002) in this study had 98.4% identity to the CO1 sequence of *Cricula variabilis* (SASNB201-09) on BOLDSYSTEM. The latter specimen was collected by V. Siniaev in Guangxi province (China) in 2008, and deposited in the Research Collection of Stefan Naumann (http://www.boldsystems.org/index.php/Public_RecordView?processid=SASNB201-09). A molecular study, with a number of gene regions across a large number of specimens from different geographical areas, is required to determine the extent to which the taxon in Vietnam is distinct from the type collection.

The *C. variabilis* samples in this study were collected at low (165-465 m) elevations in Yen Bai province, from where it is likely to be spreading to nearby provinces. In China, the larvae of C. variabilis damaged host trees at altitudes from 680 to 840 m above sea level in Guangdong province [12]. There, Phoebe bournei (Lauraceae) is the main host tree, and when all leaves of this host are eaten the larvae move to other tree species such as *Callicarpa kochiana*, *Castanopsis fissa*, *Mucuna pruriens* and *Schefflera octophyyla* [12]. In south China, Zhang *et al.* (2020) [13] reported that members of the Lauraceae and Fagaceae were damaged, including *Cinnamomum burmanni, C. camphora,* and *Castanopsis fissa*. Also, Jin-kun *et al.* (2020) [19] reported that *C. variabilis* damages Machilus chinensis in China.

Studies on the genus *Cricula* have been carried out for many years in Vietnam [11], [20] but this is the first to report of *C. variabilis* in Vietnam. In Vietnam there are 11 species of *Cricula* [11], [20]. Three species (*C. trifenestrata*, *C. jordani* and *C. vietnama*) have been recorded in Tam Dao for a long time [21]. Eight species were added to the record by Nässig *et al.* (2010) [22]. These authors collected *C. falcata* and *C. hoffinanni* in Lam Dong province in the Central Highlands; *C. frederkingi, C. griseorubrescens* and *C. sponai* in Thua Thien - Hue province in Central Vietnam; *C. fansipanensis* and *C. schintlmeisteri* in Lao Cai province in the highlands of Northwest Vietnam; and *C. hoabinhnguyeni* in Bac Kan and Cao Bang provinces in North Vietnam. Most specimens were collected during the cold season from October to December, some even during snow fall. According to surveys conducted by the Forest Protection Research Centre, *C. variabilis* was first seen in a *C. cassia* plantation in Tran Yen district, Yen Bai province in 2016. It is not known whether *C. variabilis* is an alien invasive species or whether there has been opportunistic population buildup of previously undetected natural populations. It is worth noting that the forest protection surveys undertaken by the Forest Protection Research Centre have tended to focus on major pests and small outbreaks could be missed. It appears that the pest is spreading quickly and thus has the potential to threaten *C. cassia* plantations more broadly in Northwest Vietnam. Therefore, there is a need for further studies to determine the geographical extent of *C. variabilis* in Vietnam, to define damage thresholds and to devise integrated management solutions. These are necessary to effectively manage this pest, reduce or prevent its spread, and to safeguard the incomes of rural households.

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