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***Homona spargotis* Meyrick, 1910: First report on a *Eucalyptus grandis* W. Hill × *Eucalyptus pellita* F. Muell. (Myrtaceae) hybrid in Sumatra, Indonesia (Lepidoptera: Tortricidae)**

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Abstract

All potential pests (e.g., defoliating lepidopterans) of *Eucalyptus* L'Hér. (Myrtaceae) are important to be recorded because of multiple species from this plant genus are ubiquitously planted in forest plantations across the tropics, including Sumatra, Indonesia. The objective of this study was to record, for the first time, a species closest to the avocado leafroller, *Homona spargotis* Meyrick, 1910 (Lepidoptera: Tortricidae) on a *Eucalyptus grandis* W. Hill × *Eucalyptus pellita* F. Muell. hybrid in Sumatra with the insect species identification performed via molecular analysis. The used molecular protocol was able to identify a similar, yet distinct insect species to *H. spargotis* through analysis of its larvae, which defines its geographical distribution to Sumatra and identified a host species (i.e., *E. grandis* × *E. pellita*) for this insect.

KEY WORDS: Lepidoptera, Tortricidae, defoliation, *Ericiana spargotis*, Myrtales, Sumatra, Indonesia.

***Homona spargotis* Meyrick, 1910: Primer registro sobre un híbrido de *Eucalyptus grandis* W. Hill × *Eucalyptus pellita* F. Muell. (Myrtaceae) en Sumatra, Indonesia (Lepidoptera: Tortricidae)**

Resumen

Todas las plagas potenciales (por ejemplo, lepidópteros defoliadores) del *Eucalyptus* L'Hér. (Myrtaceae) son importantes para ser registradas porque múltiples especies de este género de plantas son ubíquistas en plantaciones en bosques al otro lado de los trópicos, incluyendo Sumatra, Indonesia. El objetivo de este estudio fue registrar, por primera vez, una especie próxima del aguacate, *Homona spargotis* Meyrick, 1910 (Lepidoptera: Tortricidae) sobre un híbrido del *Eucalyptus grandis* W. Hill × *Eucalyptus pellita* F. Muell. en Sumatra, la identificación de la especie del insecto funcionó vía el análisis molecular. El protocolo molecular usado podría identificar una especie de insecto similar, sin embargo, una especie de insecto similar a *H. spargotis* a través del análisis de sus larvas, que definiría su distribución geográfica en Sumatra e identificaría una especie de anfitrión (por ejemplo *E. grandis* × *E. pellita*) para este insecto.

PALABRAS CLAVE: Lepidoptera, Tortricidae, defoliación, *Ericiana spargotis*, mirtácea, Sumatra, Indonesia.

Introduction

Eucalyptus L'Hér. (Myrtales: Myrtaceae) is largely planted in Sumatra, Indonesia for production of paper, pulp and viscose goods (PRASETYO *et al.*, 2019; INAIL *et al.*, 2019). These goods are used to supply the local market as well as being imported to countries such as China, India, Malaysia,

Singapore, and South Korea (SZULECKA *et al.*, 2016). *Eucalyptus* plantations in Sumatra are predominantly grown with *Eucalyptus pellita* F. Muell. and the hybrids of *Eucalyptus grandis* W. Hill. × *E. pellita* (BOPHELA *et al.*, 2019; TACHI *et al.*, 2020; TAVARES *et al.*, 2020). Several lepidopteran species have been recorded to infest *Eucalyptus* in Sumatra, including *Ophiusa disjunctus* (Walker, 1858) (Erebidae) (RAIMON *et al.*, 2020), *Polyphagozerra coffeae* (Nietner, 1861) (Cossidae) (TAVARES *et al.*, 2020; TACHI *et al.*, 2020), *Strepsicrates* sp. (Tortricidae) (KKADAN *et al.*, 2020a, 2020b), and *Tetracona amathealis* (Walker, 1859) (Crambidae) (MELIA *et al.*, 2021).

The *Homona spargotis* Meyrick, 1910 (Lepidoptera: Tortricidae), of the *coffearia* group, was known previously as *Ericiana spargotis* and native to the Northern Territory and Queensland, Australia and currently apparently distributed in the Australasian realm, where is considered a potential pest on a variety of tropical crops (HERBISON-EVANS & CROSSLEY, 2004; HULCR *et al.*, 2007). The larvae feed primarily on avocado, *Persea americana* Mill. (Laurales: Lauraceae) and sporadically on coffee, *Coffea arabica* L. (Gentianales: Rubiaceae), custard-apple, *Annona reticulata* L. (Magnoliales: Annonaceae), lychee, *Litchi chinensis* Sonn. (Sapindales: Sapindaceae), macadamia, *Macadamia* spp. (Proteales: Proteaceae), narra, *Pterocarpus indicus* Willd. (Fabales: Fabaceae), pariparoba, *Piper umbellatum* L. (Piperalis: Piperaceae), star fruit, *Averrhoa bilimbi* L. (Oxalidales: Oxalidaceae), and tea plant, *Camellia sinensis* (L.) Kuntze (Ericales: Theaceae) (HERBISON-EVANS & CROSSLEY, 2004; HULCR *et al.*, 2007). *H. spargotis* has not been recorded in Australia from any native host, except *Macadamia* spp. and *P. indicus* suggesting its potential as polyphagous on rainforest plants (WHITTLE *et al.*, 1987).

Homona spargotis larvae form a shelter made of rolled leaves held with silk where it lives and feeds. The adult moths are a patchy brown (HERBISON-EVANS & CROSSLEY, 2004), inactive during daytime and found resting in leaf litter and in the canopy (WHITTLE *et al.*, 1987). The wingspan is about 14.0-19.5 mm for males and 20.0-28.0 mm for females (MEYRICK, 1910; WHITTLE *et al.*, 1987). The eggs are yellow and flattened. They are laid on the upper section of the host plant crown in overlapping masses of several hundred (HERBISON-EVANS & CROSSLEY, 2004). The moths have an unusual resting posture, with the hindwings protruding from under the forewings, and the protruded parts folded over (ZBOROWSKI & EDWARDS, 2007).

Not only to identify species, DNA barcoding may also be used to establish species boundary, as recently shown to distinguish multiple species within the *Homona* genus (HULCR *et al.*, 2007) and Hesperiiidae (HEBERT *et al.*, 2004). Genetic distances calculated from the highly conserved mitochondrial cytochrome c oxidase subunit I (mt-COI) gene were shown to form bimodal distribution, clearly separating genetic distance between insects belonging to the same species vs. across species, i.e., intraspecific vs. interspecific genetic distance. Insects within the same species were shown to share similar DNA sequences, corresponding to unique haplotypes associated with morphologically and ecologically distinct caterpillars (HULCR *et al.*, 2007), hence they have a much smaller pairwise genetic distances.

The objectives of the current study were to report the finding of *Homona spargotis* in Sumatra, Indonesia, confirmed by molecular analysis, and to add a new plant host for this insect.

Material and methods

COLLECTION OF *HOMONA SPARGOTIS*

Homona spargotis larvae were collected manually from trees of an *E. grandis* × *E. pellita* hybrid in a commercial stand during the first semester of 2020 in Pangkalan Kerinci area (0° 20' N × 101° 51' E, 10 m altitude) in Riau, Sumatra, Indonesia. Larvae were placed in one-liter plastic containers and taken to the Entomology Laboratory of the Asia Pacific Resources International Holdings Ltd. (APRIL) of the PT. Riau Andalan Pulp and Paper (RAPP), where they were kept in a room at 26 ± 2° C, 75 ± 15% RH and 14:10 (L:D) h photoperiod. Caterpillars received daily fresh shoots of *E. grandis* × *E. pellita* as a food.

MOLECULAR IDENTIFICATION OF *HOMONA SPARGOTIS*

One caterpillar was used for species name confirmation through molecular analysis. DNA extraction and PCR of the mt-COI gene were carried out as previously described (MELIA *et al.*, 2021). The PCR product was then sent to a service provider for Sanger Sequencing. Trimming and consensus sequence building were carried out using the sangeranalyseR package (CHAO *et al.*, 2020) with default settings. We used the consensus sequence to find the most similar sequence in NCBI GenBank using blastn (ALTSCHUL *et al.*, 1990). The top hit, sorted by the highest score, is kept. The consensus sequence, top hits from the blast search as well as several sequences from the *Homona* sp. Meyrick were aligned using Clustal Omega (SIEVERS *et al.*, 2011), and conserved sequences were identified using Gblocks (TALAVERA & CASTRESANA, 2007). The output was used to build a phylogenetic tree using the maximum likelihood approach (FELSENSTEIN *et al.*, 1981) with a bootstrap value of 1,000 and GTR as the DNA substitution model (TAVARÉ, 1986), which were implemented in the phangorn R package (SCHLIEP *et al.*, 2011).

Species boundary analyses were performed using mt-COI sequences from 65 insects belonging to the *Homona* genus submitted to NCBI GenBank, which were all available sequences from *H. spargotis* (13 sequences) and *Homona aestivana* (Walker, 1866) (12 sequences) as well as a maximum of 20 sequences each from *Homona mermerodes* Meyrick, 1910 and *Homona trachyptera* Diakonoff, 1941. These four species were selected following previously published data examining species determination amongst generalist moths (HULCR *et al.*, 2007). Sequences were then aligned to find conserved blocks using the same approach described previously. The resulting aligned sequences were used to calculate genetic distance as implemented in the phangorn R package with default settings. A total of 2080 genetic distances was calculated between all possible pairwise combinations within our dataset, which were then plotted as a histogram.

Results

REPORT OF *H. SPARGOTIS* IN SUMATRA

This is the first report of *H. spargotis* in Sumatra (Fig. 1).

REPORT OF *H. SPARGOTIS* ON *E. GRANDIS* \times *E. PELLITA*

The *H. spargotis* larvae were collected while feeding on an *E. grandis* \times *E. pellita* hybrid, which represents a new host plant species for this insect.

MOLECULAR ANALYSIS

Species identification through sequencing of the mt-COI gene has become an accepted approach given the difficulty of identification through insect body morphology. Here, we report a leafroller whose closest species is *H. spargotis* based on molecular analysis (Table 1). This similarity is stable as our sample are clustered together with *H. spargotis* in 67% of the 1,000 bootstraps (Fig. 2). Despite the sequence resemblance to *H. spargotis*,

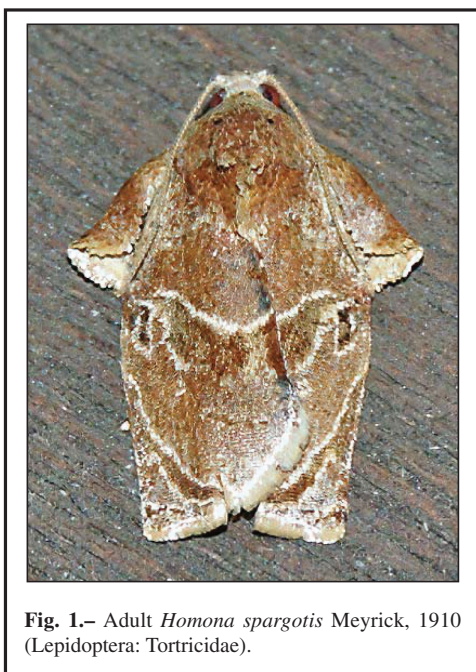


Fig. 1.— Adult *Homona spargotis* Meyrick, 1910 (Lepidoptera: Tortricidae).

our sample is clustered less tightly with the rest of *H. spargotis*, as compared to other intra-species clustering within the *Homona* genus (Fig. 2).

Origin			Score	E value	Identity	Accession	
Pangkalan	Kerinci,	Riau,	856	0.0	94%	GU688782.1	<i>Homona spargotis</i>
Sumatra, Indonesia							

Table 1.– Origin, score, E value, identity, and accession

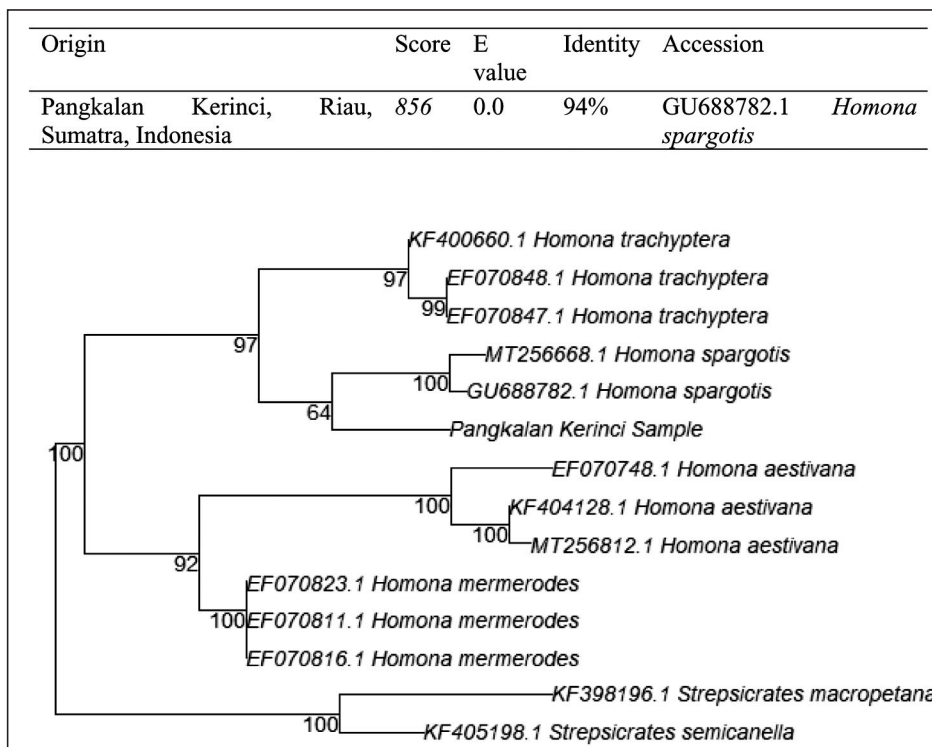


Fig. 2.– Phylogenetic tree on *Homona spargotis* Meyrick, 1910 (Lepidoptera: Tortricidae).

We calculated and plotted the distribution of all possible pairwise genetic distance in four *Homona* species included in our dendrogram (Figs. 2-3). Interestingly, we confirm the existence of bimodal distribution, separating intra species with inter species genetic distances (Fig. 3). Based on our data, any pairwise genetic distance that exceeds 0.045 are likely to come from samples of different species. The closest genetic distance of our sample to any *H. spargotis* is 0.053 (to *H. spargotis* with NCBI accession EF070841.1), which falls within the range of inter species genetic distances. Thus, we concludes our sample is *Homona spargotis*.

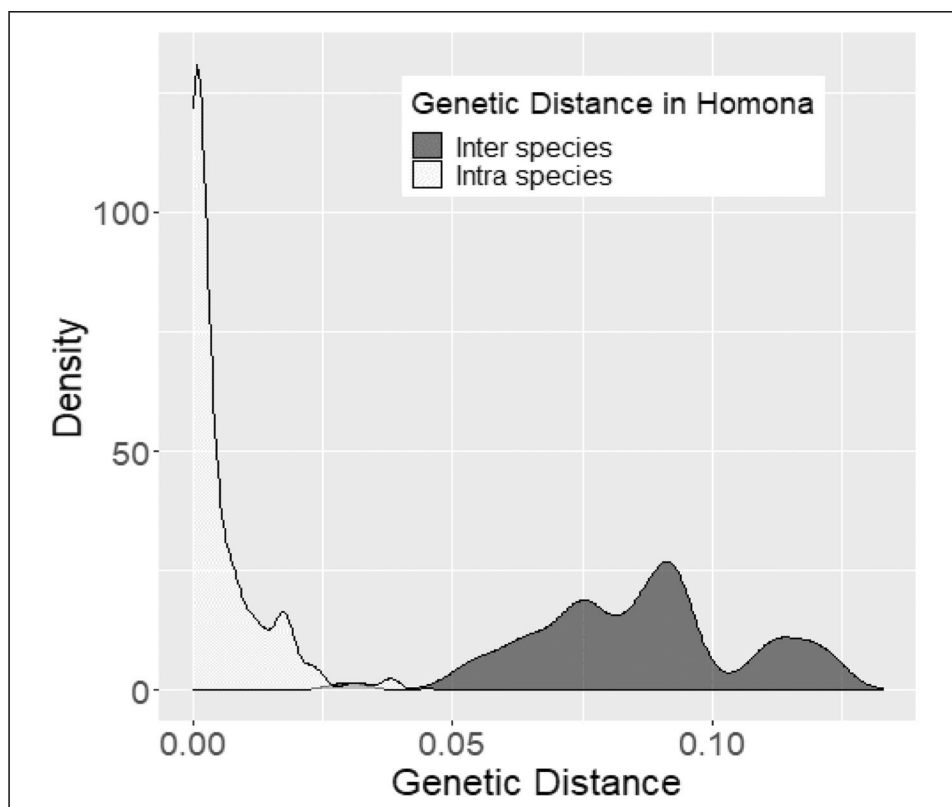


Fig. 3.— Distribution of pairwise genetic distances amongst 65 *Homona* Walker (Lepidoptera: Tortricidae) samples.

Discussion

REPORT OF *H. SPARGOTIS* IN SUMATRA

Homona spargotis has been previously reported in the Australasian realm (HERBISON-EVANS & CROSSLEY, 2004) without however confirmation of the countries with distribution of this insect. Figures of the male aedeagus in DIAKONOFF (1939, 1948) of specimens collected from Java, Indonesia agree with those of *H. spargotis* in WHITTLE *et al.* (1987). Other specimens collected beyond Australia to New Guinea (WHITTLE *et al.*, 1987) and in Sri Lanka (KOCHANESKY *et al.*, 1978) are also confirmed as *H. spargotis*. Specimens collected in Sulawesi are similar to those of *H. spargotis* described in WHITTLE *et al.* (1987). The combination of a semicircular costal fold on the fore wing together with dark grey hind wings readily distinguishes the male of *H. spargotis* from any other species of *Homona* Walker, 1863 known from Australia (WHITTLE *et al.*, 1987).

REPORT OF *H. SPARGOTIS* ON *E. GRANDIS* \times *E. PELLITA*

Homona spargotis, the closest described species from *H. spargotis* collected in the present study

while feeding on a *E. grandis* × *E. pellita* clone, has been previously recorded as a predominant pest of *P. americana* and a sporadic pest of *C. arabica*, *A. reticulata*, *L. chinensis*, *Macadamia* spp., *P. indicus*, *P. umbellatum*, *A. bilimbi*, and *C. sinensis*. On *P. americana*, the infestation of *H. spargotis* appears to be determined by the presence of growth flushes. Numbers increase quickly at flushing and the moth virtually disappears when no new shoots are present (WHITTLE *et al.*, 1987).

Homona spargotis, in the current study, was seen along with the tea mosquito bug, *Helopeltis theivora* Waterhouse, 1886 (Hemiptera: Miridae), a sapsucker and other mirid species on the commercial stands of *E. grandis* × *E. pellita* clone in Sumatra (KKADAN *et al.*, 2020c). Potential monitoring and control measures of *H. spargotis* include the use of light traps (HULCR *et al.*, 2007) and sex pheromone traps (for monitoring) (WHITTLE *et al.*, 1987). *Homona spargotis* is recorded to have two flying activity periods on *P. americana* in Australia, one from March to June and another from August to December however its moths were collected using light traps throughout the summer months (WHITTLE *et al.*, 1987).

MOLECULAR ANALYSIS

We assessed the possibility of our sample belonging to another species that is similar to *H. spargotis* following an approach previously used to determine species boundaries in six tortricid moths (HULCR *et al.*, 2007) and HesperIIDae (HEBERT *et al.*, 2004). Both studies showed that genetic distances within species are much closer, as compared to between species, which can be exploited to draw species boundaries.

The confirmation of the existence of bimodal distribution, separating intra species with inter species genetic distances has been previously reported (HULCR *et al.*, 2007; HEBERT *et al.*, 2004).

Conclusions

The used molecular protocol was able to identify the insect species as *H. spargotis* through analysis of its larvae, which extends the geographical distribution to Sumatra and add a new host plant family (i.e., Myrtaceae) and genus (i.e., *Eucalyptus*) and a host species (i.e., *E. grandis* and *E. pellita*) for this insect.

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