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First report of *Tetracona (Agrotera) amathealis* (Walker, 1859) on a *Eucalyptus grandis* W. Hill × *Eucalyptus pellita* F. Muell. (Myrtaceae) hybrid in Indonesia (Lepidoptera: Crambidae, Spilomelinae)

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Abstract

Tetracona amathealis (Walker, 1859) (Lepidoptera: Crambidae) is previously known from Australia and New Guinea, where it feeds on the native *Eucalyptus tereticornis* Sm. (Myrtaceae). The objective of this study was the report, for the first time, *T. amathealis* in Indonesia as well as to record new host plants for this insect. Were collected manually from a *Eucalyptus grandis* W. Hill. × *Eucalyptus pellita* F. Muell. hybrid commercial plantation in Riau, Sumatra, Indonesia and a sample sent for identification via molecular analysis. Our findings revealed that the molecular approach used (through sequencing of its mt-COI gene) successfully allowed the identification of the species as *T. amathealis*. This insect is recorded for the first time in Indonesia on a new host, a *E. grandis* × *E. pellita* hybrid.
KEY WORDS: Lepidoptera, Crambidae, Spilomelinae, *Tetracona amathealis*, first record, Indonesia.

Primer registro de *Tetracona (Agrotera) amathealis* (Walker, 1859) sobre un híbrido de *Eucalyptus grandis* W. Hill × *Eucalyptus pellita* F. Muell. (Myrtaceae) en Indonesia (Lepidoptera: Crambidae: Spilomelinae)

Resumen

Tetracona amathealis (Walker, 1859) (Lepidoptera: Crambidae) previamente es conocida de Australia y New Guinea, donde se alimenta del oriundo *Eucalyptus tereticornis* Sm. (Myrtaceae). El objetivo de este estudio fue registrar, por primera vez, *T. amathealis* en Indonesia así como registrar una nueva planta nutricia para el insecto. Manualmente, fueron recogidos de un híbrido de *Eucalyptus grandis* W. Hill. × *Eucalyptus pellita* F. Muell. Plantación comercial en Riau, Sumatra, Indonesia y una simple muestra enviada para identificación vía análisis molecular. Nuestras conclusiones revelaron que el enfoque molecular usado (a través del ordenación en serie de su gen mitocondrial mt-COI) permitió la identificación de la especie con éxito como *T. amathealis*. Este insecto se registra, por primera vez, en Indonesia sobre una nueva planta nutricia un híbrido de *E. grandis* × *E. pellita*.
PALABRAS CLAVE: Lepidoptera, Crambidae, Spilomelinae, *Tetracona amathealis*, primer registro, Indonesia.

Introduction

Eucalyptus L'Hér. (Myrtales: Myrtaceae) is a plant genus with most species native to Australia (PRASETYO *et al*., 2017; INAIL *et al*., 2019). It is largely planted in Riau, Sumatra, Indonesia, where is utilized to produce paper, pulp and viscose materials (TAVARES *et al*., 2020). Several lepidopteran species infest *Eucalyptus* in Riau, including *Ophiusa disjungens* (Walker, 1858) (Erebidae)

(LAKSAMANA *et al*., 2020), *Polyphagozerra coffeae* (Cossidae) (TAVARES *et al*., 2020; TACHI *et al*., 2020) and *Strepsicrates* sp. (Tortricidae) (KKADAN *et al*., 2020a).

Edward Meyrick described the genus *Tetracona* in 1884 with *Aediodes amathealis* Walker, 1859 as type species (MEYRICK, 1884). The genus was for a long period treated as a synonym of *Agrotera* Schrank, 1802, but a recent taxonomic revision of *Agrotera* resulted in the re-instatement of *Tetracona* (CHEN *et al*., 2017). The genus is placed in the tribe Agroterini based on the structure of the uncus, featuring a broad base and simple chaetae on the uncus head, a well-developed medial process of the gnathos, the rectangular, elongate valvae with parallel costal and ventral margins, and the notably elongate saccus (at least in *Tetracona amathealis* (Walker, 1859)) (MALLY *et al*., 2019). The genus with its three species (*T. amathealis*, *Tetracona multispina* Jie & Li, 2020 and *Tetracona pictalis* Warren, 1896) is known from Australia, China and New Guinea (HERBISON-EVANS *et al*., 2013; JIE *et al*., 2020).

Francis Walker described *T. amathealis* in 1859 (synonyms *Pyralis ornatalis* Walker, 1866) based on material collected at Moreton Bay in Queensland, Australia (WALKER, 1859). It is found in Australia (Queensland, northern New South Wales and Western region) and New Guinea (CHEN *et al*., 2017). The wingspan is about 20 mm. The basal half of the forewings is golden and the marginal half grey. An orange submarginal band divides these two areas. The hindwings are grey with a gold inner margin (HERBISON-EVANS *et al*., 2013). The *T. amathealis* leafrollers are sometimes considered a *Eucalyptus tereticornis* Sm. pest (HERBISON-EVANS *et al*., 2013), an economically important plant native to eastern Australia and southern New Guinea (BOOTH, 2019).

Molecular method is an important way to confirm identification of lepidopteran species such as Crambidae (BARRERA *et al*., 2017) and Noctuidae (SULISTYONO *et al*., 2020). Insects recovered from traps such as light and sticky traps often have their body parts damaged leading to a difficult species determination through morphology analysis (MORINIÈRE *et al*., 2016; FUJIWARA *et al*., 2017). The collection of insect immatures without a defined rearing protocol is also a challenge for species identification via morphology analysis (ANTONINI *et al*., 2009; SHIN *et al*., 2015), since most insects are identified based on their adult body morphology examination (TAHIR *et al*., 2018).

The objective of this study was to report the findings of *T. amathealis* in Indonesia, confirmed by molecular technique, as well as its attack on a *Eucalyptus grandis* W. Hill. × *Eucalyptus pellita* F. Muell. hybrid. We provided also a key for *Tetracona* species identification in Indonesia.

Material and methods

Collection of *T. amathealis*

Tetracona amathealis leafrollers were collected manually from trees of an *E. grandis* × *E. pellita* hybrid in a commercial stand in the first semester of 2020 in Teso East area (0º 30' N × 101º 26' E, 33 m altitude) in Riau. Leafrollers 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 ± 2ºC, 75 ± 15% RH and 14:10 h (L:D) photoperiod. Leafrollers received daily fresh *E. grandis* × *E. pellita* shoots as a food.

Molecular identification of *T. Amathealis*

One leafroller individual was sent to the Molecular Biology and Genomics Laboratory of APRIL in Pangkalan Kerinci, Riau for species name confirmation. DNA extraction and PCR of the mitochondrial cytochrome c oxidase subunit I (mt-COI) gene were carried out as described by SULISTYONO *et al*. (2020). The PCR product was then sent 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 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 *Strepsicrates* Meyrick, 1888 (Lepidoptera: Tortricidae, Eucosmini) were aligned using Clustal Omega (SIEVERS *et al*., 2011), and the output was used to build a phylogenetic tree. The maximum likelihood approach (FELSENSTEIN *et al*., 1981) was used to build a dendrogram, with a bootstrap value of 1,000 and GTR (LE *et al*., 2008) as the DNA substitution model, both of which were implemented in the phangorn R package (SCHLIEP *et al*., 2011).

Results

Report of *T. amathealis* in Indonesia

This is the first report of *T. amathealis* (Fig. 1) as well as of a member in the genus *Tetracona* in Indonesia. We used molecular method to confirm the identity of this insect species, through sequencing of its mt-COI gene.

Report of *T. amathealis* on *E. grandis* × *E. pellita*

The *T. amathealis* were collected while feeding on a *E. grandis* × *E. pellita* hybrid, which represents two new host plants for this insect.

Fig. 1.– Adult *Tetracona amathealis* (Walker, 1859).

Discussion

Report of *T. amathealis* in Indonesia

Tetracona amathealis, reported in the current study in Indonesia, has been previously reported in Australia (i.e., Queensland, northern New South Wales and Western region) and New Guinea. Other reported *Tetracona* species in Asia includes *T. multispina* in China, Jiangxi Province (i.e., Huangzihao, Fuliang; Tongboshan; Wuyuan, Shangbao; Dabali, Xunwu; Doushui, Shangyou; Shangyou Arboretum) (JIE *et al*., 2020) and *T. pictalis* in Australia (i.e., Queensland) (NUSS *et al*., 2003).

The mt-COI gene utilized to identify *T. amathealis* is often used in taxonomic studies due to its lower divergence within species and larger divergence across species. The most similar sequence to our sample is of that *T. amathealis* (Table 1), which was deposited at the Australian National Insect Collection. Phylogenetic analysis confirms that our sample cluster together with *T. amathealis* in all 1,000 bootstraps, indicating the similarity to *T. amathealis* is stable (Fig. 2).

Table 1.– Origin, score, E value, identity, and accession of *Tetracona (Agrotera) amathealis*.

Origin	Score	E value	Identity	Accession	
Teso East area, Riau, Sumatra, Indonesia	623	0.0	95%	HQ952617.1;	*Agrotera amathealis*

Report of *T. amathealis* on *E. grandis* × *E. pellita*

Tetracona amathealis, collected in the present study while feeding on a *E. grandis* × *E. pellita* hybrid, has been previously recorded to feed on *E. tereticornis* (HERBISON-EVANS *et al*., 2013). This insect 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* hybrid in Riau (KKADAN *et al*., 2020b).

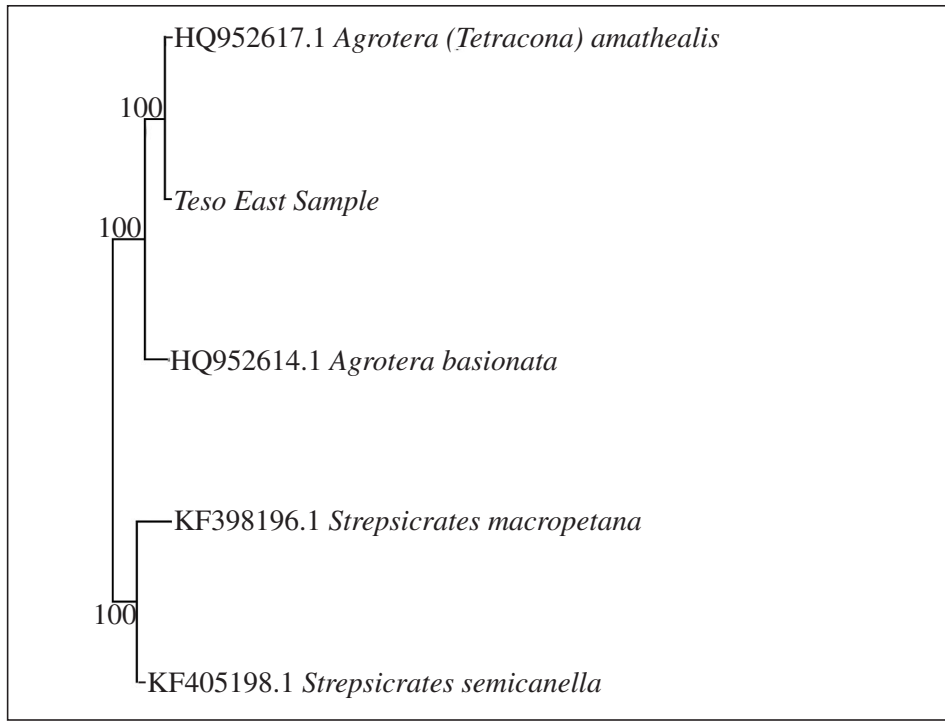

Fig. 2.– Dendrogram of the Teso East area sample of *Tetracona (Agrotera) amathealis* using the maximum likelihood approach with 1,000 bootstraps.

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