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A new endemic clove tree pest of *Cryptophasa* Lewin, from Sangihe Island, Indonesia (Lepidoptera: Xyloryctidae)

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Abstract

A novel endemic pest of clove tree, *Cryptophasa warouwi* **sp. nov.**, has been discovered on Sangihe Island. This new species can be distinguished from its closest relative species, *C. watungi* Sutrisno & Suwito, 2015 which is found in North Sulawesi, by its dark brown straw-coloured wings in both males and females. The most distinctive diagnostic characters of this new species are observed in its genitalia structure: a bent-downward uncus with a strongly sclerotized finger-shaped apex, a bent phallus gradually widened towards coecum, and a double, membranous corpus bursae branching off at mid-ductus corpus bursae of female genitalia. Additionally, DNA barcodes revealed this new species to be embedded among Australian *Cryptophasa* species despite having fasciculated male antennae that have been considered diagnostic of the genus *Paralecta*. This suggests that the male antennae may not be a reliable character for separating *Cryptophasa* from *Paralecta*. A more comprehensive study including all *Cryptophasa* and *Paralecta* will be required to elucidate the definition of each genus. Images depicting both adults and genitalia are provided for this newly recognized species.

Key words: clove, description, genitalia, Syzygium, tunnels

Introduction

The genus *Cryptophasa* Lewin, 1805 was established based on *Cryptophasa irrorata* Lewin, 1805 by subsequent designation. The adults are characterized by the smooth and generally glossy wing, pectinated antenna in males terminated by a thread at extremity, filiform antenna in females, upcurved. terminating in a point, and a short or not discernible haustellum (Lewin 1805; Duponchel 1844). Currently, the type species *C. irrorata* has been redescribed with emphasis on the morphology of the male genitalia (McMillan 2019).

This genus holds the distinction of being the largest within the family Xyloryctidae, with over 95 described species worldwide. It is predominantly distributed across Australia and New Guinea with about 55 species in Australia and 42 species in Indonesia (36 species in Papua, 3 in Moluccas Islands, 2 in Sulawesi, and 1 in Java). Moreover, it is highly likely that numerous undescribed species await discovery, especially given the genus diversity in New Guinea (Snellen 1878; Meyrick 1917, 1925, 1928, 1930, 1938; Diakonoff 1948, 1954, 1966; Common 1990; Holloway *et al.* 2001; McMillan 2013; Sutrisno *et al.* 2015).

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The larvae of *Cryptophasa* are known as branch and stem borers, creating tunnels and sealing the opening with silk webbing and excrement. The larvae are notably active at night, cutting off leaves, which they bring into the tunnel for food (Common 1990). The recently described *C. watungi* Sutrisno & Suwito, 2015, has been reported to cause substantial damage to clove trees (*Syzygium aromaticum* L.) in North Sulawesi since 2013. This species exhibits a rapid expansion in range and poses a significant threat to clove trees in the four districts of North Sulawesi Province. Signs of damage on clove trees have been reported from Sangihe Island, potentially attributed to *C. watungi* larvae (Sutrisno *et al.* 2015; Rante & Watung 2016). However, the larvae of this species have been found not only in clove trees but also in watery rose apple trees (*Syzigium aqueum* (Burm.f.) Alston), raising the possibility of a different species. Consequently, a comprehensive study of adults, considering external and genital characters, became imperative, as genitalia traits are species-specific within the genus *Cryptophasa* (Sutrisno *et al.* 2015; McMillan 2019).

This paper presents the description of a new species of *Cryptophasa*, so far found only on Sangihe Island. We also conduct a genetic distance analysis to delineate the new species from other closely related species within *Cryptophasa* and from the related genus *Paralecta*. The findings are discussed briefly, emphasizing their implication. We anticipate that this discovery will advance our understanding of *Cryptophasa* diversity within the Wallacea Region and shed light on its pest status. Such information holds significance in developing strategic pest control plans, conducting pest risk analysis, compiling quarantine pest lists, and more.

Materials and methods

The present study is based on materials collected during field surveys conducted in the clove tree plantations on Sangihe Island, Indonesia, spanning the years 2016–2023. Adult specimens were reared from the pupae in the Laboratory of Entomology, Faculty of Agriculture, Sam Ratulangi University, Manado, North Sulawesi. All materials examined in this study are deposited in the Museum Zoologicum Bogoriense (MZB), Research Center for Biosystematics and Evolution, National Research and Innovation Agency, Cibinong, Indonesia.

Pinned specimens were examined under a stereomicroscope illuminated with an incandescent light source. Genitalia dissections were prepared by the standard method of boiling in 10% potassium hydroxide solution for approximately 10–11 minutes. Genitalia dissections were carried out under a stereomicroscope (Robinson 1976; Sutrisno *et al.* 2015).

Terms used for morphological and genital characters in this paper follow Holloway *et al.* (2001) and McMillan (2019).

For comparative purposes, we examined the following specimens of *C. watungi* Sutrisno & Suwito. 1 \Diamond . Indonesia, North Sulawesi, Bolaang Mongondow Selatan, Pinolosian, Lungkap. N 00° 27' 25.3" E 124° 11' 02. 6". Alt. 552 m. Emerge on 05.X.2014. leg. J.F. Watung, Genitalia slide MZB. Lepi. 172 (MZB); 1 \Diamond Indonesia, North Sulawesi, Bolaang Mongondow Selatan, Pinolosian, Lungkap. N 00° 27' 25.3" E 124° 11' 02. 6". Alt. 552 m. Emerged on 25.IX.2014. leg. J.F. Watung; 1 \Diamond ; Indonesia, North Sulawesi, Bolaang Mongondow Selatan, Pinolosian, Lungkap. N 00° 27' 25.3" E 124° 11' 02. 6". Alt. 552 m. Emerged on 16.X.2014. leg. J.F. Watung, Genitalia slide MZB. Lepi. 173 (MZB).

We also extracted DNA from the following larvae of *C. watungi*: 1 ex. (Genbank # OR800731). Indonesia, North Sulawesi, Minahasa, Kakas, Touliang. 29.vi.2023. leg. J.F. Watung. hostplant clove; and *C. warouwi* **sp. nov**.: 1 ex. (Genbank # OR800730). Indonesia, Sangihe, Tabukan Selatan, Bukide; N 03° 30′ 44.3″ E 125° 34′ 38. 4″. Alt. 540 m.12.vii.2020. leg. J.F. Watung. hostplant clove; 1 ex. (Genbank # OR800732). Indonesia, Sangihe, Tabukan Selatan, Bukide; N 03° 30′ 44.3″ E 125° 37′ 08. 9″. Alt. 82 m. 15.vii.2023. leg. J.F. Watung. Hostplant watery rose guava.

In order to compare *Cryptophasa* species within the same genus and from the closely related *Paralecta*, a distance method analysis based on *COI* gene marker was performed. To generate sequence data for this study, DNA extraction was carried out from the thorax of each individual using the Dneasy tissue kit (Qiagen, Hilden, Germany) following standard protocol. PCR was conducted by the following primers: LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.* 1994). PCR profile consisted of an initial denaturation step at 94 °C for 10 min, followed by 35 cycles at 92 °C for 30 sec, 47 °C for 30 s, and 72 °C for 1.5 min, and a final extension step of 10 min at 72 °C. Sequencing was performed by Macrogen inc. (Korea).

We also included 15 sequences of *Cryptophasa*, 3 of *Paralecta*, and 1 of *Tonica* from Genbank in the distance analysis. In total 21 sequences for the ingroup and a single sequence for the outgroup *Tonica* were included in the analysis. The names, accession numbers and references are provided in Table 1. Sequence alignment was accomplished using MUSCLE (Edgar 2004) implemented in Geneious Prime 2023 (www.geneious.com). A distance analysis was performed by using Neighbor-Joining tree with Kimura Two-parameter model as implemented in MEGA 11 (Saitou & Nei 1987; Tamura *et al.* 2021).

The resulting NJ tree is presented in Fig. 6 and the pairwise distance table is available upon request.

No.	Species	Accession No.	References
1	Cryptophasa albacosta	KF406205.1	Hebert et al., 2013
2	C. alphitodes	KF405353.1	Hebert et al., 2013
3	C. byssinopsis	KF405269.1	Hebert et al., 2013
4	C. epadelpha	KF406242.1	Hebert et al., 2013
5	C. irrorata	KF405816.1	Hebert et al., 2013
6	C. molaris	KF406147.1	Hebert et al., 2013
7	C. nephrosema	KF400906.1	Hebert et al., 2013
8	C. opalina	KF405730.1	Hebert et al., 2013
9	C. phycidoides	KF405419.1	Hebert et al., 2013
10	C. rubescens	KF405463.1	Hebert et al., 2013
11	C. rubra	KF406185.1	Hebert et al., 2013
12	C. russata	KF406113.1	Hebert et al., 2013
13	C. sceliphrodes	KF405897.1	Hebert et al., 2013
14	C.tetrazona	KF406034.1	Hebert et al., 2013
15	C. xylomima	KF406080.1	Hebert et al., 2013
16	C. warouwi	OR800730 & OR800732	unpublished
17	C. watungi	OR800731	unpublished
18	Paralecta spp.	KF403223.1, KF399744.1 & KF395782.1	Hebert <i>et al.</i> , 2013
19	Tonica effractella	KF393432.1	Hebert <i>et al.</i> , 2013

TABLE 1. Sequences included in the molecular study.

Checklist of Cryptophasa species in Indonesia and their distributions

- C. aethoptera Meyrick, 1938 (Papua)
- C. aggesta Meyrick, 1925 (Papua)
- C. amphicroca Meyrick, 1925 (Papua)
- C. antalba Diakonoff, 1966 (Papua)
- C. argophanta Meyrick, 1917(Moluccas)
- C. arithmologa Meyrick, 1938 (Papua)
- C. chionocra Diakonoff, 1954 (Papua)
- C. chionosema Meyrick, 1938 (Papua)
- C. chionotarsa Meyrick, 1925 (Papua)
- C. chlorotis Diakonoff, 1954 (Papua)
- C. choliki Sutrisno & Suwito, 2015 (Sulawesi)
- C. crocochorda Meyrick, 1925 (Papua)
- C. crossosticta Meyrick, 1938 (Papua)
- C. curialis Meyrick, 1925 (Papua)
- C. ensigera Meyrick, 1925 (Papua)

C. geron Diakonoff, 1954 (Papua) C. hades Diakonoff, 1954 (Papua) C. hormocrossa Meyrick, 1925 (Papua) C. iorhypara Diakonoff, 1954 (Papua) C. kwerbaensis Sutrisno & Suwito, 2015 (Papua) C. luciflua Merick, 1938 (Papua) C. malevolens Meyrick 1928 (Papua) C. megaloma Meyrick, 1910 (Papua) C. merocentra Meyrick, 1925 (Papua) C. mesotoma Meyrick, 1925 (Papua) C. neocrates Myerick, 1925 (Papua) C. nesograpta Meyrick, 1925 (Papua) C. niphadobela Diakonoff, 1954 (Papua) C. obscura Diakonoff, 1954 (Papua) C. oecodoma Myerick, 1930 (Papua) C. phaeochtha Meyrick, 1925 (Papua) C. psammochtha Meyrick, 1925 (Papua) C. psathyra Diakonoff, 1948 (Papua) C. pseudogramma Meyrick, 1930 (Papua) C. psiloderma Diakonoff, 1948 (Papua) C. ranunculus Diakonof, 1954 (Papua) C. sceliphrodes Meyrick, 1925 (Papua) C. sepiogramma Meyrick, 1938 (Papua) C. transversella Snellen, 1878 (Sunda Islands, Sulawesi, and Papua) C. vacuefacta Meyrick, 1925 (Papua) C. watungi Sutrisno & Suwito, 2015 (North Sulawesi) C. warouwi Sutrisno & Watung, sp. nov. (Sangihe Island)

Description of the new species

Cryptophasa warouwi Sutrisno & Watung, sp. nov.

Figs 1C–D, 2C–D, 3B

Diagnosis. The male of *C. warouwi* **sp. nov.** is easily distinguished from the closest species, *C. watungi*, by the forewing dark brown streak along the entire costa, which is gradually paler towards CuP, being light brown from CuP towards dorsum, the dark brown spots on discal cell of forewing, predominantly dark brown and become paler from the cubito-anal (CuA₁ and CuA₂) area towards dorsum, and white on the discal cell of hindwing. The female has the forewing with a white ochreous ground color tinged with brown from costa to dorsum, more pronouncedly so toward the margin, with a dark brown spot at the discal cell, a margin with a prominent basal line of alternating white and dark brown dashes (Fig. 1C–D). A bent-down uncus (black arrow) with a strongly sclerotized, finger-shaped apex (black arrow) a slightly sclerotised, medially bent phallus (black arrow), and a double corpus bursae without signum black arrow) are the best diagnostic for the male and female genitalia of this species (Fig. 2C–D, 3B).

Description. *Male* (Fig. 1C): Forewing length 14 mm. Head grey. Labial palpus grey, slender, about 2 times vertical diameter of eye, pointed last segment directed slightly forward. Antenna fasciculate-ciliated, extended to about half of length of forewing, dorsal surface covered with longitudinal rows of black scales along entire length. Thorax white, tegula dark grey. Legs black, with hind tibia externally covered with greyish-white scales. Forewing oblong, costa slightly arched at base, then nearly straight, apex obtuse, dorsum slightly oblique, rounded, entire costa black, discal cell (black arrow) with two dark spots, from discal cell towards dorsum white, terminal cilia black. Hindwing black fuscous, except at 2/3 basal costa white, from cubito-anal (CuA₁ and CuA₂) area towards dorsum white fuscous with brown tinge, terminal cilia grey with orange-tinged basal line at margin, basal line paler towards tornus. Abdomen slender with first segment white, second segment toward seventh segment brown becoming gradually darker brown, distal segment black mixed with white.



FIGURES 1A–D. A. *Crytophasa watungi* \Diamond , B. *C. watungi* 3f, C. *C. warouwi* **sp. nov.**, \Diamond , D. *C. warouwi* \heartsuit . ds= discal spot, blt= basal line of termen.



FIGURES 2A–D. A. Valva of *Cryptophasa watungi*, **B.** Phallus of *C. watungi*, **C.** Valva of *C. warouwi* **sp. nov.**, **D**. Phallus of *C. warouwi* **sp. nov.** uc= uncus, tg= tegumen, vlv= valva, fb= fibula, jt= juxta, scl= sacculus, sc= saccus, vc= vinculum.



FIGURES 3A–B. A. Female genitalia of *Cryptophasa watungi*, B. Female genitalia of *C. warouwi* **sp. nov.** db= ductus bursae, cb= corpus bursae, pa= posterior apophysis, lp= lamella postvaginalis, ol= ovipositor lobe.

Male genitalia (Figs 2C–D): Uncus divided dorsally into two lobes, divided into two apical processes, bent downward, strongly sclerotized at finger-shaped apex (black arrow). Gnathos laterally fused with two sclerotized arms to base of uncus. Tegumen broad, gently arched, margins slightly sclerotized; articulated with vinculum. Vinculum U-shaped, with weakly developed saccus. Juxta weakly sclerotized, paddle-shaped. Valva tapered with scattered short setae. Costa of valva slightly arched inwardly; apex blunt. Lower margin of supravalva gently curved, slightly sinuate towards base. Basis of valva elongate-rectangular. Sacculus elongate, triangular towards base, with scattered setae; subapical cleft (black arrow). Phallus slightly sclerotised, slightly bent at middle (black arrow).

Female (Fig. 1D): Forewing length 24 mm. Head white. Labial palpus grey, slender, directed upward. Antenna fasciculate-ciliated (cilia less dense than those of male), dorsal surface covered with longitudinal rows of black scales along entire length. Thorax entirely white. Legs black, with last tibia partly covered with grey scales. Forewing white ochreous tinged with brown, denser towards margin; discal cell with a black reniform spot; margin with indistinct black dots; terminal cilia grey with yellow-orange basal line. Hindwing fuscous; dorsum with indistinct black dots; cilia yellow-orange. Abdomen dark fuscous, except for first segment greyish-white.

Female genitalia (Figs 3B): Ovipositor lobes tubular with scattered setae (black arrow), anterior apophyses equal in length to posterior apophyses. Ductus bursae membranous, forked at middle (black arrow). Corpus bursae a pair elongate, membranous sacs without signa (black arrow).

Holotype: ♂. Indonesia, Sangihe, Tabukan Selatan, Bukide; N 03° 30' 44.3" E 125° 34' 38. 4". Alt. 540 m. Emerged on 19.X.2020. leg. J.F. Watung, Genitalia slide MZB. Lepi. 689AB (MZB)

Paratype: 1 ♂, 1 ♀. Indonesia, Sangihe, Tabukan Selatan, Bukide; N 03° 30′ 44.3″ E 125° 37′ 08. 9″. Alt. 82 m. Emerged on 22.vii.2023. leg. J.F. Watung, Genitalia slide MZB. Lepi. 689 (MZB); 1♂, 1♀; Indonesia, Sangihe, Tabukan Selatan, Bukide; N 03° 33′ 52.95″ E 125° 32′ 24. 25″. Alt. 252 m. Emerged on 20.vii.2023. leg. J.F. Watung, Genitalia slide MZB. Lepi. 690 (MZB)

Etymology: The species name is dedicated to Dr. Ir. Jootje Warouw, a senior entomologist and retired professor in the Faculty of Agriculture, Sam Ratulangi University who conducted research on pest control in Sangihe and Talaud Islands.

Distribution: Sangihe Island only (Fig. 4).

Remark: The adults used in the description were obtained from rearing pupae.

Note: *Cryptophasa warouwi* **sp. nov.** is closely related to *C. watungi* (Fig. 6) by average 6.1% p-distance of its cox1 sequence. Meanwhile the average distance with *Paralecta* sp. is 10.7% p-distance of its cox1 sequence. The NJ tree also shows that *C. watungi* and *C. warouwi* is embedded among other *Cryptophasa* species, not in *Paralecta* (Fig. 6).



FIGURE 4. Distribution of Cryptophasa warouwi sp. nov. (blue dots) on Sangihe Island, Indonesia.

Discussion

The genus *Cryptophasa* showcases notable diversity, often characterized by pronounced size differences and instances of color dimorphism between males and females of the same species. However, the external characters initially employed to define the genus by Lewin (1805) and Meyrick (1890) have been contradicted by recent studies (Common 1990; Sutrisno *et al.* 2015; McMillan 2019). A distinguishing character shared by several species within this genus is the deep division of the uncus dorsally into two lobes, further subdivided into two apical processes, and strongly sclerotized at apex. The trait is notably observed in *C. irrorata* Lewin, 1805, the type species of this genus (Lewin, 1805; McMillan 2013; Sutrisno *et al.* 2015; McMillan 2019).

The shape of antennae appears to be an unreliable diagnostic character for defining *Cryptophasa*. Our results reveal that *C. watungi* and *C. warouwi* **sp. nov.** from Indonesia possess fasciculate antennae in males, a feature traditionally associated with *Paralecta*. However, our analysis based on DNA barcode marker (cox1) placed these two species within *Cryptophasa* species from Australia, distinct from *Paralecta*. *Paralecta antistola* Meyrick, 1930 (the type species of *Paralecta*) and *C. warouwi* **sp. nov.** is distinct in the wing pattern, the wing pattern of these two species is highly distinctive in both males and females (Plate 6: 9 & 10) (Robinson *et al.* 1996). A comprehensive study including all *Cryptophasa* and *Paralecta* will be necessary to clarify the definition of each genera.

Interestingly, our recent surveys reveal that the larvae of this new species are not exclusive to the clove trees but also infest watery rose apple and guava trees in the same sampling location on Sangihe Island. It is also noteworthy that not a single male specimen was collected in the field during our survey, suggesting that males may not be attracted to light.



FIGURES 5A-D. A. Signs of damages by Cryptophasa warouwi on clove trees, B. Severe infestations, C. Larva; D. Pupa.

The larvae causing damage to clove plants were initially identified in 2016, primarily affecting three plantations across two villages in North Tabukan District and one village in Tamako District of Sangihe Island. At that time, the level of infestation was relatively low, and it was regarded as a potential pest (Rante & Watung 2016). However, during field surveys conducted in July and September 2023, infestations were observed in several additional villages, including four villages in North Tabukan District, two in Northeast Tabukan, three in Central Tabukan, two in Manganitu, and two in Tamako. The degree of infestation varied, ranging from low levels in Bowo'mbuase Village (Manganitu) to severe infestations in Bukide Village (North Tabukan) and Basauh Village (Southeast Tabukan Selatan). Severe infestations resulted in extensive branch and twig damage, leading to a notable reduction in the density of clove tree leaf crowns (Figs. 5A–B). Given the current widespread and severe nature of infestations, it is now appropriate to categorize this pest as an important threat with the potential for significant damage in the field.



FIGURE 6. A Neighbor Joining tree (rooted) based on Kimura Two-parameter model.

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