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The first record of the rare *Leucetta avocado* sponge from Sangihe Islands, Indonesia

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Abstract: Calcareous sponges represent the rarest class of marine sponges, constituting only 4% of the currently known sponge species globally. While abundant of demosponges have been reported from various locations in Indonesia, only 40 species of calcareous sponges have been identified in the country over the past 200 years. This suggests the rarity of these sponges and a significant knowledge gap regarding the Leucetta sponge in Indonesia. The objective of this study was to describe the biology of a calcareous sponge belonging to the genus *Leucetta*, which was collected from the Sangihe Islands in Indonesia. Morphological analysis was conducted using a modified acid digestion method, and the obtained data were compared to reported data for Leucetta sponges. The sponge exhibited triactine spicules, which are characteristic of Leucetta species. The number list of Indonesian *Leucetta* sponges was determined through a literature search. Previous research indicated that 40 identified calcareous sponges were reported in Indonesia between 1890 and 2023. Interestingly, *L. avocado* is known as a native sponge to Palau, although this species and closely related species have been reported elsewhere. Therefore, it is predicted that the current distribution of *L. avocado* in the Sangihe Islands and Bali could be attributed to a combination of the Indonesian Throughflow, sexual propagation, or stepping stone scenarios. This research provides new insights into the biology of *L. avocado* from Indonesia.

Keywords: calcareous; leucetta; triactine; sponge; sangihe islands

INTRODUCTION

Situated in the so-called Coral Triangle, the North Sulawesi region is recognized as part of a biodiversity hotspot for various marine organisms, including marine sponges. In the last four decades, hundreds of sponge species and their metabolites have been published from this region (Calcinai, Bastari, Bavestrello, et al., 2017; Calcinai, Bastari, Makapedua, et al., 2017; De Voogd & van Soest, 2002; Hanif et al., 2019). However, the majority of reported sponges from this area and worldwide belong to the Demospongiae class, which constitutes 85% of the known sponge species (Hooper & Van Soest, 2002). In contrast, there is a significant knowledge gap regarding calcareous sponges and their metabolites from Indonesia. For example, de Voogd and van Soest (2015) reported only 38 species of calcareous sponges from two major maritime expeditions (Siboga Expedition, March 1899 - February 1900, and Snellius Expedition, July 1929 - November 1950) and 7 years of sponge research in Indonesia (Van Soest & De Voogd, 2015). This fact stands in stark contrast to the discovery of Demospongiae sponges, where one week of research in Manado Bay, North Sulawesi, Indonesia, led to the discovery of 96 species (Calcinai, Bastari, Bavestrello, et al., 2017).

In a recent expedition in the Sangihe Islands, we were particularly attracted to a marine sponge collected from the islands. The specimen contained triactine spicules typical of calcareous sponges and exhibited morphological features resembling those of *Leucetta avocado* (De Laubenfels, 1954; Kelly et al., 2016). Curiously, despite intense sponge research in Indonesia, *L. avocado* has not been reported from Indonesian waters, except by Crews et al. (2003), who investigated the metabolites of Indo Pacific Leucetta sponges and found two *Leucetta* sponges from the Sangihe Islands (*L. avocado* and *L. primigenia*), as well as four from Papua New Guinea and Fiji (*L. avocado*, *L. primigenia*, *Leucetta* sp1, *Leucetta* sp2).

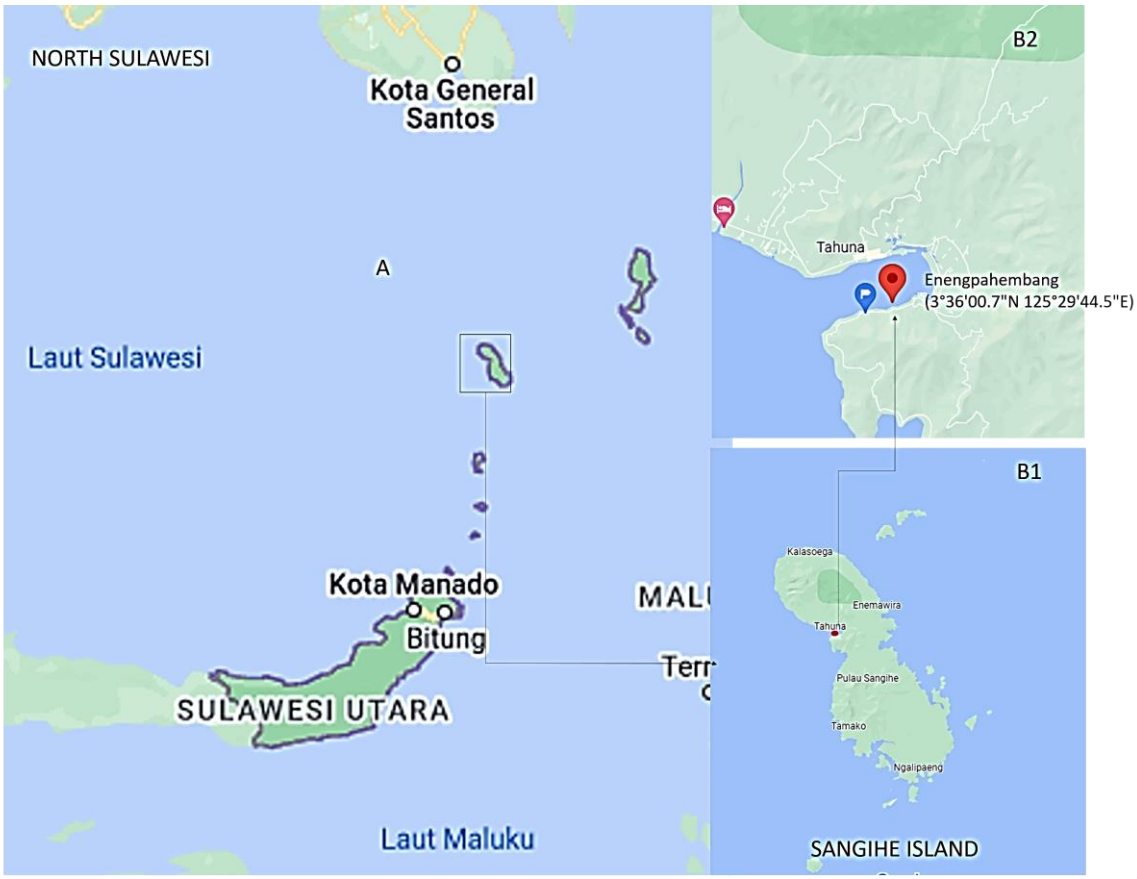

Figure 1. Map of the study area. A-North Sulawesi. B2-Studied islands (Google Maps)

In this report, we present the biology of three calcareous sponges collected from the Sangihe Islands. The native status of this sponge to Palau makes its discovery in the Sangihe Islands, as well as in Bali, intriguing. The fact that the Sangihe Islands serve as one of the gates for the Indonesian Throughflow (ITF) from the Pacific to the Indian Ocean (Pang et al., 2022) may explain the presence of this species in the Sangihe Islands and Bali.

MATERIALS AND METHODS

Location and Sample Collection

The specimen (EP_7) was collected during our survey in Enepahembang Sangihe Islands in May 2020 at the geographical position (3º47'16.7" N and 126º45'06.6" E) (Figure 1). The specimen was collected by SCUBA diving collected between a depth of 6 m and 7 m in Enepahembang coral reef. Underwater photo-graph of the specimen was taken *in situ* and the specimen was preserved in alcohol 95% soon after it exposed to air (GoPro 4). Morphological characteris-tic of the specimens (growth form, surface characte-ristics, substrate attachment) were recorded under-water.

Morphological analysis

A portion of the specimen, weighing approxi-mately 50 grams (wet weight), was cut from the host sponge using a sharp knife while SCUBA diving. The cut specimens were placed in a plastic bag, cleaned, and then immersed in a 300 mL bottle containing methanol. They were transported to the laboratory and stored at approximately 18 °C until further use.

Spicules from the sponge were purified using a modified acid digestion method (Hooper, 2002). In brief, a small fragment weighing 1 mg (dry weight) from each specimen was heated in an oven at 108 °C for 1 hour to completely remove any remaining water. The dried fragment was then treated with 1.0 mL of commercial bleach (Bayclin), left at room temperature for 3 hours, washed three times with water, and finally rinsed with 70% alcohol. The purified spicules were observed under a light Motic BA210 digital biological microscope, and images of 15 spicules for each specimen were taken using a Samsung Type A12 mobile phone camera. The captured images were then transferred to a laptop for measurement using our previously reported method (Rieuwpassa et al., 2023).

RESULTS

Checklist of Species

During this study, a list of 41 species of calcareous sponges was compiled based on previous records (Van Soest & De Voogd, 2015; Balansa 2003, Crews et al., 2003, Dalisay et al., 2009) and the present study, encompassing 11 families and 21 genera (Table 1). Previous studies have documented the presence of 39 species from 21 genera and 11 families of calcareous sponges identified from sponge collections obtained from various important research expeditions in Indonesia to date (Van Soest & De Voogd, 2015). Among these, two species of the genus Leucetta (*L. primigenta* and *L. avocado*) were reported from the Sangihe Islands (Crews et al., 2003), indicating the rarity of known calcareous sponges in Indonesia.

The list includes two new records of calcareous sponges from Indonesia, namely *L. avocado* and *Leucetta* sp., discovered in the last three years in the Sangihe Islands, North Sulawesi. While this represents the second report of L. *avocado* from the Sangihe Islands and the third from Indonesia, following a 2011 report on L. *avocado* from Bali (Daniele, 2011), an additional specimen described in detail in the following section brings the total number of known calcareous sponge species in Indonesia to 40 (Table 1).

Systematic

Phylum: Porifera, Class Calcarea, Subclass: Calcaronea, Family: Leucettidae, Genus *Leucetta*.

Material Examined

The specimen (EP_7) was collected at a depth of 7 m from Enepahembang East Tahuna, Sangihe Islands (3°36'00.7"N 125°29'44.5"E). Colour. Dark green alive (EP_7, olive green in alcohol 95%).

Morphology

The specimen had a tubular growth form, consisting of various tubules reaching 12-15 cm long with the basal of the three individual tubes connected and coalesced. This specimen contained thin, regular tubes, a rough reticulated surface, smooth and sharp rims as well conspicuous oscula 4-5 cm long, located very close (2-3 mm) to the next individual tube 2-3 mm apart. The interior colour of each tube (green) was darker than the interior ones (pale green) and the texture of this specimen was compressible and easily torn. The specimen contained two sizes of triactine or three branching spicules with the smaller spicules 34.68-35.00-39.21 x 4.06-4.84-7.65 µm and the larger ones 68.90-94.84-118.43 x 1.87-2.30-3.75 µm respectively. The exterior and interior colour of EP_7 (green olive) was much darker compared to those reported by Kelly et al. (2016) and Daniele (2011).

According to de Laubenfels, who first described this species in 1954, L. *avocado* can exist as a singular tube or as two fused individual tubes with deep furrows at the bottom of each tube. The color of this species ranges from light green to olive green, with the exterior color being darker than the interior. It exhibits two sizes of tricaine spicules. In addition, EP_7 showed two sizes of triactine spicules that matched those of specimens from Palau, although the small triactine spicules of EP_7 was smaller than those of *L. avocado* from Palau, measuring 300x20 µm, while the larger triactine spicules measured 400x35 µm (Figure 2A and 2B, Table 2). Given that these characteristics correspond to those reported for *L. avocado* (Daniele, 2011; De Laubenfels, 1954; Kelly et al., 2016), EP_7 was identified as *L. avocado*. This species was reported as a common sponge in the reefs of Palau, typically found at depths between 5 and 10 m (De Laubenfels, 1954). However, it was later discovered at deeper depths (5-30 m) in the same area (Kelly et al., 2016). In addition to Palau, *L. avocado* has also been reported from Indonesia (Sangihe Islands), Fiji, and Papua New Guinea (Crews et al., 2003), and more recently from Bali, Indonesia (Daniele, 2011).

DISCUSSION

Past studies on sponges have documented numerous species, particularly from Eastern Indonesia, including species that are common to Indonesia and the Indo Pacific, as well as new species to science (Calcinai, Bastari, Bavestrello, et al., 2017; Calcinai, Bastari, Makapedua, et al., 2017). However, most of these studies have focused on sponges of the class Demospongiae, which is the major class of marine sponges comprising 85% of the known marine sponge species (Hooper & Van Soest, 2002). Despite nearly 200 years since the Siboga Expedition, there have been very few reports on Indonesian calcareous sponges. The main report on Indonesian calcareous sponges comes from a 2015 report (Van Soest & De Voogd, 2015). Even in the reports on *L. avocado* and *L. primigenia* from Sangihe (Crews et al., 2003) and *L. avocado* from Bali (Daniele, 2011), there is no detailed information provided, except for picture and their chemistry.

As mentioned earlier, calcareous sponges represent a rare class of sponges, with only 40 known species in Indonesia to this day (Van Soest & De

Table 1. Checklist of calcareous sponges from Indonesia

No	Family	Genus	Species	References
1	Family Clathrinidae	Genus *Clathrina*	*Clathrina purpurea sp. nov.*	
2			*Clathrina chrysea*	Van Soest and De Voogd (2015)
3			*Clathrina heronensis*	
4			*Clathrina beckingae sp. nov.*	
5			*Clathrina aff. luteoculcitella*	
6			*Clathrina sororcula sp.nov*	
7			*Clathrina stipitata (Dendy, 1891) comb. nov*	
8		Genus *Arthuria klautau*	*Arthuria tenuipilosa (Dendy, 1905)*	
9			*Arthuria tubuloreticulosa sp. nov*	
10		Genus *Ernstia klautau*	*Ernstia indonesiae sp. nov.*	
11			*Ernstia chrysops sp. nov*	
12			*Ernstia klautauae sp. nov.*	
13			*Ernstia naturalis sp. nov*	
14	Family Levinellidae	Genus *Burtonulla*	*Burtonulla sibogae*	
15	Family Leucaltidae	Genus *Ascandra*	*Ascandra kakaban sp.nov.*	
16			*Ascandra crewsi sp. nov*	
17		Genus *Leucaltis*	*Leucaltis nodusgordii (*Poléjaeff, 1883) *comb. nov*	
18	Family Leucascidae	Genus *Ascaltis* Haeckel, 1872	*Ascaltis angusta sp.nov*	
19		Genus *Leucascus*	*Leucascus flavus* Cavalcanti,	
20		Genus *Ascoleucetta*	*Ascoleucetta sagittata*	
21	Family Leucettidae De Laubenfels 1936	Genus *Leucetta* Haeckel, 1872	*Leucetta chagosensis* Dendy, 1913	
22	Family Leucettidae		*Leucetta microharphis* Haeckel, 1872	(Dalisay et al., 2009; Daniele, 2011), this article
24	De Laubenfels 1936		*Leucetta avocado* De Laubenfels 1954	
25			*Leucetta sp.*	Balansa (2003)
26			*Leucetta primigenta*	Crews et al. (2003)
27	Family Leucettidae De Laubenfels 1936	Genus *Pericharax* Poléjaeff, 1883	*Pericharax orientalis sp. nov*	Van Soest and De Voogd (2015)
28	Family Lelapiellidae Vacelet, 1977	Genus *Lelapiella* Vacelet, 1977	*Lelapiella sphaerulifera* Vacelet, 1977	
29	Family Sycettidae Dendy, 1893a	Genus *Sycetta* Haeckel, 1872	*Genus Sycetta* Haeckel, 1872	
30		Genus Sycon Risso, 1827	*Sycon spec.*	
31	Family Grantiidae Dendy, 1893b	Genus *Leucandra* Haeckel,1872	*Leucandra irregularis (*Burton, 1930) *comb. nov*	
32	Family Jenkinidae Borojevic, Boury-Esnault & Vacelet, 2000	Genus *Anamixilla Poléjaeff, 1883*	*Anamixilla torresi* Poléjaeff, 1883	
33			*Anamixilla singaporensis sp. nov.*	
34		Genus *Uteopsis Dendy & Row, 191*	*Uteopsis argentea (*Poléjaeff, 1883)	

(Continue)

35	Family Heteropiidae Dendy, 1893a	Genus *Sycettusa* Haeckel, 1872	*Sycettusa sibogae (*Burton, 1930)	Van Soest and De Voogd (2015)
36		Genus *Grantessa* Lendenfeld, 1885	*Grantessa borojevici sp. nov.*	
37			*Grantessa tenhoveni sp. nov.*	
38		Genus *Heteropia* Carter, 1886	*Heteropia minor* Burton, 1930	
39		Genus *Vosmaeropsis* Dendy, 1893a	*Vosmaeropsis grisea* Tanita, 1939	
40	FamilyAmphoriscidae Dendy, 1893a	Genus *Amphoriscus* Haeckel, 1870	*Amphoriscus semoni* Breitfuss, 1896	
41		Genus *Leucilla* Haeckel, 1872	*Leucilla australiensis* (Carter, 1886)	

Table 2. The type and size of EP_7 and *Leucetta avocado* from Palau

Specimen	Types of spicules	Size (mm)	Reference
EP_7	Large and small triactines	Large 138.43-152.5-154.5 Small 34.68-35.00-39.21	This study
Palau	Large and small triactines	Large 400x35 Small 300x30	De Laubenfels (1954)

Voogd, 2015), which stands in stark contrast to the diversity of their Demospongiae counterparts (Calcinai, Bastari, Bavestrello, et al., 2017). Therefore, the present finding of *L. avocado* in Sangihe contradicts the notion that the diversity of calcareous sponges equals that of demosponges, and the lack of study and taxonomic complexity is a major obstacle to the current lack of knowledge on calcareous sponges (Van Soest & De Voogd, 2015). Instead, it supports the idea that calcareous sponges are an uncommon class of sponges in Indonesia (Breitfuss, 1897). This is also evident in North Sulawesi, where despite 50 years of marine bioprospecting on Indonesian sponges, only a few calcareous sponges, including *L. chagosensis*, *Leucetta* sp., *L. microraphis* from Manado Bay, and *L. primigenia* and *L. avocado* from Sangihe Islands, have been reported (Crews et al., 2003; Dalisay et al., 2009; Hanif et al., 2019). Moreover, while we have collected over 100 Demospongiae sponges from various locations in the Nusa Utara region in the last five years (Riyanti, Balansa, et al., 2020; Riyanti, Marner, et al., 2020), we have so far discovered only one calcareous sponge described in the present study from this region.

Although the presence of *L. avocado* has been previously reported from Indonesia, particularly from the Sangihe Islands (Crews et al., 2003), and later from Bali (Daniele, 2011), the authors did not provide detailed descriptions of the sponge's morphological characteristics, probably due to the publication's focus on chemistry and biodiversity, respectively. This may explain why this species was not included in the list of known calcareous sponges from Indonesia (Van Soest & De Voogd, 2015). Therefore, this study represents a record of *L. avocado* from Indonesia. It expands on the previous reports by Crews and Danielle, emphasizing the triactine spicules and other morphological characteristics of the calcareous sponges and resembling those reported by De Laubenfels, who first discovered this species in Palau in 1954 (De Laubenfels, 1954).

Curiously, *L. avocado* has been frequently reported mainly from Palau (De Laubenfels, 1954; Kelly et al., 2016) and the Pacific, and is considered a native sponge to Palau. Therefore, the discovery of L. *avocado* along with *L. primigenia* from Sangihe (Crews et al., 2003), Bali, and the present study in Sangihe raises the question of how this sponge reached Indonesia. Given the position of the Sangihe Islands and the Indonesia Throughflow (ITF) in the northern part of Indonesia, it is assumed that the presence of L. avocado in Sangihe and Bali may be due to a combination of oceanographical and biological factors.

It has been estimated that more than 15 million cubic meters per second of seawater flows from the Pacific to the Indian Ocean through Indonesia, known as the Indonesia Throughflow (ITF), with Talaud and Sangihe Islands acting as "the entrance" and Bali as "the exit gates" of the ITF (Pang et al.,

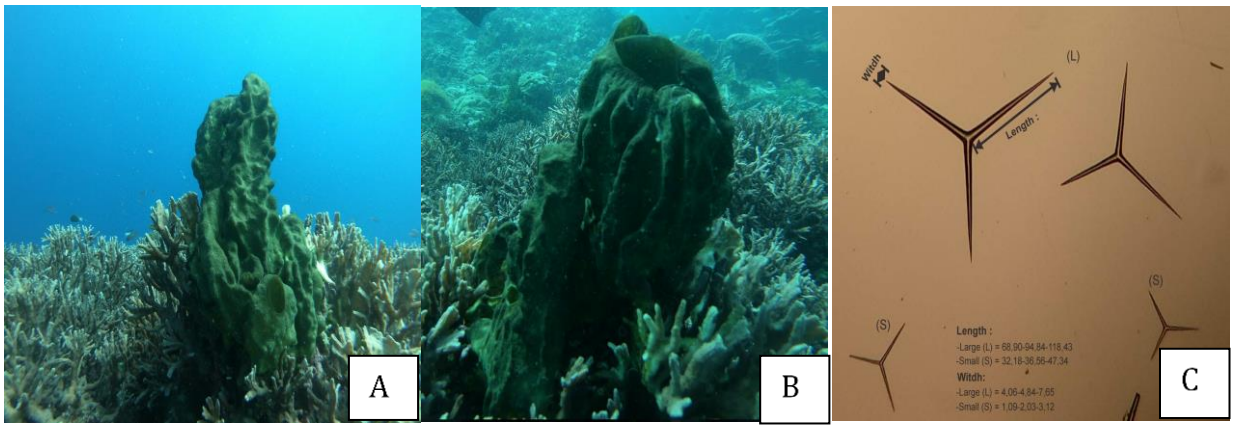

Figure 2. *In situ* photographs of the long volcano-shaped *Leucetta avocado* from Enepahembang Sangihe Islands (D and E) (Photos: Hertjumes Aatjin). Two sizes of triactine typed spicules of EP_7 from Sangihe Islands (C).

2022). The vast amount of water in the ITF is predicted to carry eggs and larvae of marine organisms from the Pacific to the Indian Ocean through Indonesia, contributing to the high marine biodiversity in Indonesia (L. C. Huffard et al., 2012). Therefore, it is predicted that the ITF may have carried the larvae of *L. avocado* from Palau to Talaud and later to Sangihe Islands, possibly through a sexual propagation scenario involving sponge fragments (Maldonado & Uriz, 1999). While sponges are known to have limited dispersal capabilities and show philopatry (Griffiths et al., 2021), fragments detached from a host due to foraging by fish or invertebrates or wave actions can remain in the water for up to 28 days and be dispersed several kilometres away from their original location (Maldonado & Uriz, 1999). If the fragments are brooding adults, they could also facilitate sexual propagation and allow the recruitment of the species in a new location far from the original location (Maldonado & Uriz, 1999). In this case, the Sangihe Islands and Palau are the new and original locations of *L. avocado*, respectively, separated by nearly 1000 km. In fact, van Soest and colleagues even discovered the rare calcareous sponge *Paragrantia waguensi* in Okinawa, about 1500 km away from its original location in Mie Prefecture (Van Soest et al., 2015).

Furthermore, we assume that *L. avocado* may have reached Bali through the same scenario, with the sponge fragments presumably originating from Sangihe Islands, considering the distance. Alternatively, the embryos of *L. avocado* from Sangihe could have reached Bali through a stepping-stone mode (Padua et al., 2016). This assumption is supported by the fact that the areas along the IFT corridor (North Sulawesi, Makassar Strait to Lombok Strait) are well-known for their coral reefs (L. C. Huffard et al., 2012) and have been major targets for marine sponge bioprospecting in the last 50 years (Hanif et al., 2019). Thus, the stepping-stone process involving the fragmentation of *L. avocado* followed by the fusion between *L. avocado* and similar species from the genus *Leucetta* may have facilitated the dispersal of *L. avocado* to Bali from Palau.

However, the above scenarios still need to be investigated. Firstly, it is unknown whether L. *avocado* has the same life cycle or philopatric behaviour in nature as other members of the calcareous sponges. Secondly, information about the genetic diversity, dispersal patterns, and distribution of Indonesian *L. avocado* is also lacking, which is crucial in understanding the scenario of embryo dispersal from Palau to Sangihe and other parts of Indonesia. Moreover, the factors influencing the different sizes of *L. avocado* in Sangihe have yet to be studied. Therefore, future studies should focus on the biodiversity, genetic diversity, life cycle, and philopatric nature of the Leucetta genus in the Nusa Utara region.

CONCLUSIONS

The present study represents the record of the rare calcareous sponge *Leucetta avocado*, adding the list to the known calcareous sponges from 39 to 41 species and an intriguing speculation of its dispersal from Palau to Indonesia, giving a new insight into the biology of *L. avocado* as the first record of this species from Indonesia.

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