

RESEARCH PAPER

## A new report of moss-camouflaging mantis Nanomantinae in Bali, Indonesia

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### ABSTRACT

In 2019 we found moss praying mantis around the forest in the Bedugul Area of Bali, Indonesia. This species belongs to the family Mantidae, subfamily Nanomantinae, and predicted as member of *Calofulcinia* genus based on diagnostics of morphological characteristics, behavior, and habitat. Information about the morphological characteristics and data from the molecular sequencing database of the genus *Calofulcinia* has been very limited. In this research, the barcoding DNA of mitochondrial Cytochrome Oxidase I (mtCOI) of the species is noted using paired primers LCO1490 and HCO2198. Based on the sequence analysis (identity matrix and phylogeny analysis), the moss mantis from Bali has low similarity and genetic relationship with other Mantodea species from GenBank database. The data indicate the moss mantis from Bali has not been reported previously. In addition, the distribution of moss mantis species in Bedugul, Bali, Indonesia has just been described and recorded.

**Key words:** Bedugul area, *Calofulcinia*, DNA barcoding, insect predator, mantidae, mantodea

### INTRODUCTION

Mantodea (praying mantises) is a group of predatory insects with 15 families and more than 2400 species distributed worldwide (Ehrmann, 2002). One of the unique species is moss mantis including *Calofulcinia* sp., *Arria* sp., and *Majangella* sp. (Giglio-Tos, 1915; La Greca, 1966; Brannoch & Svenson, 2016; Unnahachote et al., 2021; Svenson & Vollmer, 2014). Its habitat is found in moss plants grown in terrestrial areas and trees. Based on its habitat, this species is known as the moss mantis. *Calofulcinia* sp. was reported under the tribe of Fulciniini, subfamily Nanomantinae and family of Mantidae (Giglio-Tos, 1915; La Greca, 1966; Brannoch & Svenson, 2016). In addition, the moss mantis species found in Thailand belongs to genus *Arria*, Haaniidae family (Unnahachote et al., 2021). On the other hand moss mantis genus *Majangella* was first reported by Giglio-Tos in 1915, belong to family of Mantidae subfamily Majanginae. The distribution of *Majangella* is across parts of Southeast Asia including Malaysia and Borneo, Indonesia (Svenson & Vollmer, 2014). The

identification of *Calofulcinia*, *Arria* and *Majangella* generally relies on morphological characteristics, however this method needs special taxonomic ability. For clearer identification, the morphological identification is better combined with molecular identification using DNA barcoding. DNA barcoding has been gradually verified as an effective tool for identifying species in a wide range of taxonomic groups. DNA barcoding using *Mitochondrial Cytochrome Oxidase Subunit I* (mtCOI) has been utilized for identification of many insects (Jiang et al., 2014). In addition, mitochondrial DNA has also been used to describe genetic variation (Loftus et al., 1994). DNA barcoding using mtCOI of the moss mantis species from Indonesia has been limited information. Therefore, the sequence of the mtCOI gene of the moss mantis from Bali is a useful approach for the DNA database.

Additionally, the distribution of the moss mantis *Calofulcinia* sp. in Indonesia has not been previously reported. According to Williams (2002), the moss mantis *Calofulcinia* sp. is distributed in Australia and Papua New Guinea. This new location data will expand the known distribution of the moss mantis worldwide. In its habitat, the mantis plays a very important role as a predator. One of the unique behaviours of the praying mantis is to sit-and-wait for its prey from feeding activity (Carle et al., 2018). Future areas of research should focus further on these behavioural aspects of the moss mantis while morphological and molecular identification has been completely studied.

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## MATERIALS AND METHODS

**Research Site.** The sampling areas of moss mantis were around the forest in Bedugul area, Bali, Indonesia at an altitude around 1000–1500 m above sea level. The location is in the mountainous area with three lakes surrounded by forest (Figure 3).

**Extraction of Total DNA.** Extraction of total DNA was conducted on a single insect based on a modified method performed using CTAB (Doyle and Doyle, 1987). A single preserved insect (femur) was frozen in liquid nitrogen and its tissue was finely ground using mortar and pestle and then transferred to a micro tube (2.1 mL). Five hundred microliters of buffer extracting solution (EDTA-20 mM, Tris-HCl, pH 8-100 mM, NaCl-1.4 M, CTAB-2%, and Mercaptoethanol-0.2%) were added to the micro tube followed by incubation at 65 °C for 60 min with occasional mixing by gently inverting the tube. After incubation, equal volumes of phenol:chloroform:isoamyl alcohol (25: 24: 1) were added and the tube was inverted several times followed by centrifugation at 12,000 rpm for 15 min. The upper phase was transferred to a new micro tube followed by adding sodium acetate (1/10× volume) and cold isopropanol (2/3× volume), then the mixture was incubated overnight at -20 °C to precipitate DNA. After the incubation, the tube was centrifuged at 12,000 rpm for 10 min and the supernatant was removed. The pellet containing total DNA was washed with 70% ethanol and centrifuged at 8000 rpm for 5 min. Pellet DNA was then air-dried and resuspended in a TE buffer (Promega, USA) solution (1x) pH 8.0 and stored at -80 °C for further use.

**Polymerase Chain Reaction (PCR).** DNA amplification uses primer set LCO1490 (5'-GGTCAACA AATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAATCA-3') targeting ± 710 bp of mtCO1 fragment (Folmer et al., 1994). The PCR mix consisted of 12.5 µL GoTaq Green 2× (Promega, USA), 1 µL 10 µM forward primer, 1 µL 10 µM reverse primer, 9.5 µL nuclease-free water (Promega, USA) and 1 µL of total DNA to final reaction volume of 25 µL. The DNA was amplified in GeneAmp PCR system 9700 (Thermo Fisher, USA) for 5 min at 94 °C for pre-heating, followed by 30 cycles of denaturation (60s at 94 °C), annealing (35s at 52 °C), and extension (90s at 72 °C), with final extension of 7 min at 72 °C. Amplicons was then visualized on 1% agarose gel using electrophoresis in TBE 0.5× buffer (GRiSP, Portugal).

### Nucleotides Sequence and Phylogenetic Analysis.

PCR products then sequenced at 1st BASE Laboratories (Malaysia). Sequence was assembled using CLC sequence viewer 7.5, then aligned with sequence isolates from GenBank using Bioedit 7.2.5 (Alzohairy, 2011). to analyse the sequence homologies. Phylogenetic analysis of the moss mantis was conducted by comparing the sample sequences and moss mantis sequences and an outgroup comparison from GenBank. A phylogenetic tree of the moss mantis was constructed using the program ClustalX (Thompson et al., 1997), Bio Edit 7.2.5 (Genious) (Tamura et al., 2013), and MEGA 6.06 (Tamura et al., 2013). Phylogenetic tree was constructed using Mega 6.06 (Tamura et al., 2013) in Neighbor Joining algorithm with 1000 bootstraps replicates.

## RESULTS AND DISCUSSION

The species was found on moss in a terrestrial area including rocks, walls of buildings, and the bark of trees around the forest in the Bedugul area. Surprisingly, a high number of mantises were found on moss that grows on the bark of trees and walls of buildings. Bedugul is a tourist area with many hotels around the forest. This phenomenon is the reason why an abundant number of moss mantises were found on the walls of buildings or trees around the hotels. The female adults lay their eggs in a cluster around the moss (Figure 1B). The adults' size is approximately 40 mm in length. The female and male species are identifiable by their wings. The males have complete wings, but the females are absent (Figure 1 C and D). A deep morphological characteristic for clear identification of the species is needed. Based on the comparison of habitat diagnostics, behaviour, and general morphological characteristics, the species is named the moss mantis, but a scientific name does not designated yet. Habitus: When compared to genus *Majangella*, the moss mantis from Bali is smaller (length from head to tip of the abdomen ranges 25–30 mm) and similar size with *Calofulcinia australis*, (La Greca, 1966). However, *Majangella* is medium sized mantises (length from head to tip of the abdomen ranges 34–37 mm) (Svenson & Vollmer, 2014). On the other hand, the moss mantis from Bali has different antenna characteristic with male nymph of *Arria muscoamicta* sp. nov. (Unnahachote et al., 2021). Based on general morphological characteristics and their habitat, the moss mantis from Bali is very similar to the genus *Calofulcinia* (Figure 1). Brannoch & Svenson (2016), reported the subfamily Nanomantinae includes *Sceptuchus* (Hebard, 1920) and *Sinomantis* (Beier, 1933), while *Nanomantis* (Saussure, 1899), *Fulcinia* (Stal C, 1877) *Tylomantis* (Westwood, 1889), *Fulciniella*

(Giglio-Tos, 1915), *Fulciniola* (Giglio-Tos, 1915), and *Pilomantis* (Giglio-Tos, 1915) belong to *Nanomantinae*, including *Calofulcinia* (Giglio-Tos, 1915).

The mtCOI DNA sequence of the moss mantis from Bali, Indonesia was compared with other Mantodea

from GenBank and was found to have low similarity with other mtCOI DNA sequences of Mantodea species worldwide. The data shows similarity around 79–82% (Table 1). The phylogenetic tree shows the moss mantis in only one branch, and it is different from other

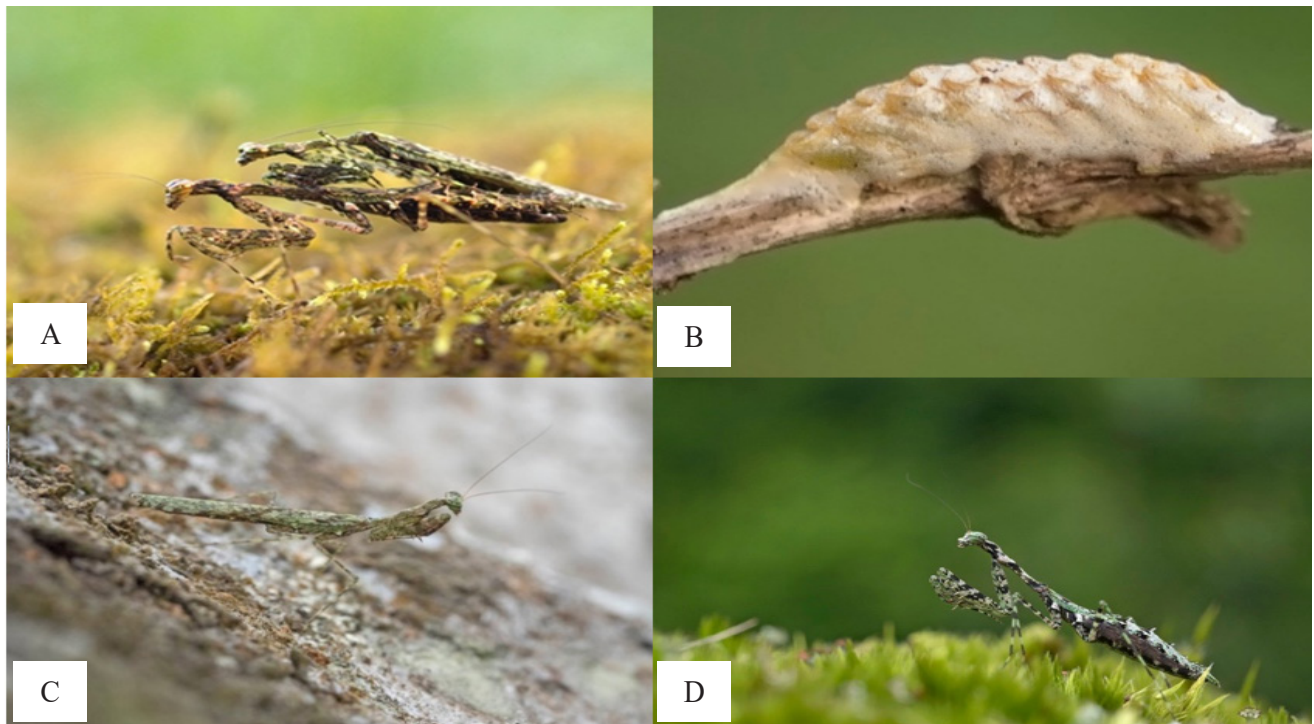


Figure 1. Brief morphology and behaviour of moss mantis from Bali, Indonesia. A. Mating of imago; B. Egg of moss mantis; C. Male of moss mantis imago; D. Female of moss mantis imago.

Table 1. Sequence identity matrix of mtCOI DNA sequence of *Calofulcinia* sp. from Bali comparison with Mantodea species in GenBank.

Isolate	1*	2	3	4	5	6	7	8	9	10	11
1.*	ID	82%	81%	80%	81%	81%	80%	80%	80%	79%	81%
2.	82%	ID	84%	82%	83%	81%	83%	84%	85%	81%	82%
3.	81%	84%	ID	83%	84%	81%	85%	84%	87%	84%	83%
4.	80%	82%	83%	ID	83%	79%	82%	79%	85%	81%	81%
5.	81%	83%	84%	83%	ID	82%	86%	77%	85%	84%	84%
6.	81%	81%	81%	79%	82%	ID	83%	79%	80%	81%	82%
7.	80%	83%	85%	82%	86%	83%	ID	83%	87%	88%	86%
8.	80%	84%	84%	79%	77%	79%	83%	ID	78%	80%	79%
9.	80%	85%	87%	85%	85%	80%	87%	78%	ID	84%	90%
10.	79%	81%	84%	81%	84%	81%	88%	80%	84%	ID	86%
11.	81%	82%	83%	81%	84%	82%	86%	79%	90%	86%	ID

1. *Calofulcinia* sp. (Bali) (Acc. No. LC492501), 2. *Caliris* sp. (Acc. No. KY689126), 3. *Amantis nawai* (Acc. No. NC037203), 4. *Leptomantella albella* (Acc. No. FJ802889), 5. *Odontomantis* sp. (Acc. No. KY689121), 6. *Eomantis yunnanensis* (Acc. No. NC037208), 7. *Sphodromantis lineola* (Acc. No. NC037204), 8. *Theopompa* sp. (Acc. No. KU201314), 10. *Statilia apicalis* (Acc. No. KR011175), 11. *Mantis reliogosa* (Acc. No. MN356097). \*Investigated *Nanomantinae* in this study

Mantodea species in GenBank, except with *Eomantis yunnanensis* (same family Nanomantidae) (Figure 2). Both sequence analyses (identity matrix and phylogeny) show the mtCOI DNA was recorded for the first time.

The moss mantis in Bali was found in moss growing on rocks, walls of buildings, and tree bark around the forest in Bedugul area (Figure 3). The Bedugul area is a high land area with altitude 1000–1500

m above sea level. The average annual relative humidity is more than 80%. The average annual temperature is 18.7–25 °C and the rainfall averages 2315–2392 mm annually (SCA Bali, 2017). In addition, the information on the distribution of *Calofulcinia* is very limited, and the location in Bedugul, Bali, Indonesia has never been previously reported.

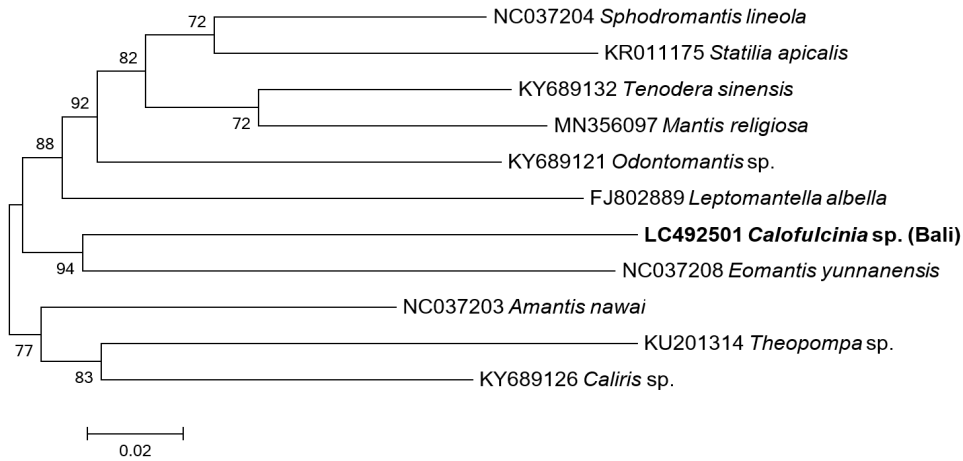


Figure 2. Phylogenetic analysis of moss mantis from Bali based on alignment of partial nucleotide sequences using Mega 6.06. in Neighbor Joining algorithm with 1000 bootstraps replicates.

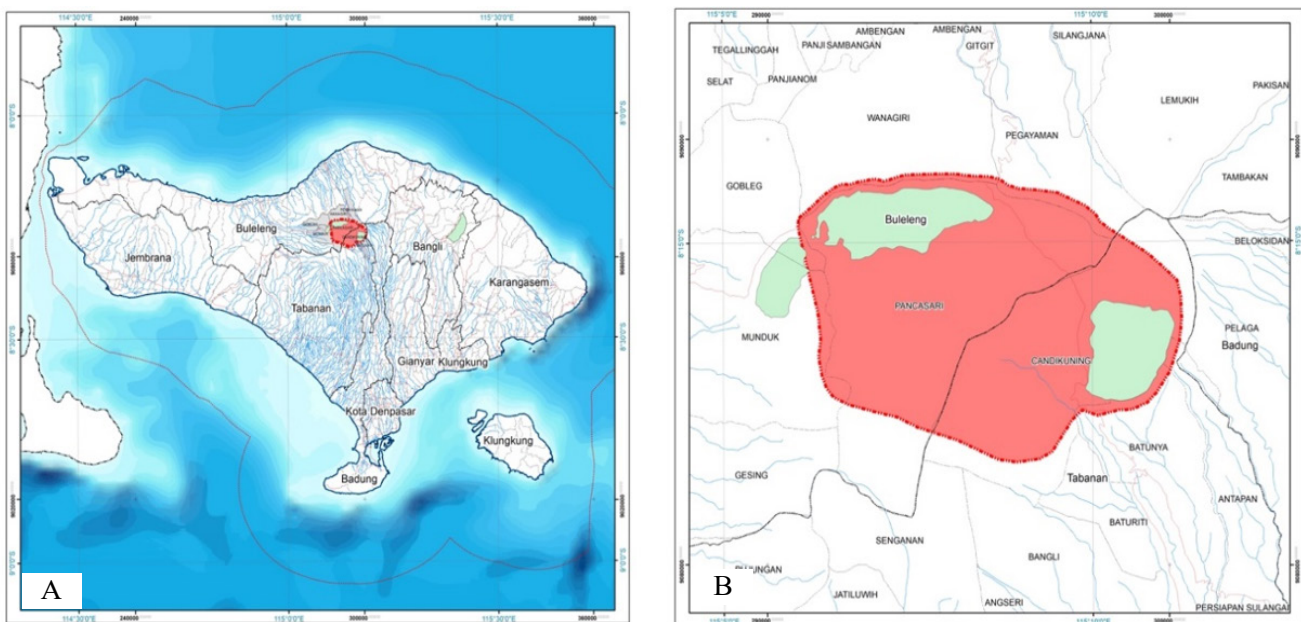


Figure 3. The distribution of moss mantis in Bali, Indonesia. A. Bali Island; B. Bedugul Area (red colour mark).

### CONCLUSIONS

The moss mantis from Bali, Indonesia is predicted to belong to the genus *Calofulcinia*, subfamily Nanomantinae and the family of Mantidae. The mtCOI DNA sequence of the moss mantis from Bedugul, Bali, Indonesia is the first recorded in GenBank.

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## AUTHORS' CONTRIBUTIONS

IPS, GNASW, and DGWS contributed to the article equally. IPS and GNASW conceived the research and collected the samples from the field. IPS carried out the morphological identification, DGWS performed the molecular analysis. IPS and DGWS prepared the manuscript and final editing by PWS and IMA. All authors read and approved the final manuscript

## COMPETING INTEREST

Authors declare that there is no competing interest regarding the publication of manuscripts.

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