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Short Communication: The existence of *Homalomena rubescens* (Araceae) in Java, Indonesia based on morphological and molecular evidence

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Abstract. *Irsyam ASD, Hariri MR, Kamila NN, Kurniawan MFR, Suwandhi I, Irwanto RR. 2023. Short Communication: The existence of* Homalomena rubescens *(Araceae) in Java, Indonesia based on morphological and molecular evidence. Biodiversitas 24: 3821-3827.* *Homalomena* Schott is a member of Araceae family, with most species originating from Southeast Asia. Due to its stunning heart-shaped leaves and tolerance to shade, *H. rubescens* (Roxb.) Kunth is frequently used as an ornamental plant and is frequently found in tropical gardens. As an ornamental plant, it can escape cultivation. The existence of *H. rubescens* in Java has been enigmatic, and the likelihood of misidentification is high because it has morphological characteristics in common with *H. pendula* (Blume) Bakh.f. This research aims to confirm the presence of *H. rubescens* in Java, Indonesia based on examination of morphological characters and molecular analysis. An adventive population of *Homalomena rubescens* was found in the Babakan Siliwangi City Forest, Bandung City, West Java, as a result of a field study in 2022. Materials of the population were then collected and made into specimens in the Herbarium Bandungense for further morphological observations. Additionally, a molecular approach was used to confirm the identity. The collected specimen is unquestionably *H. rubescens* (Roxb.) Kunth based on morphological and molecular analysis. Therefore, in this paper, we formally announced *H. rubescens* as a newly naturalized species in Java, Indonesia.

Keywords: Araceae, DNA barcoding, *Homalomena*, Java, naturalized

INTRODUCTION

Homalomena Schott is a genus with 157 species distributed in tropical and subtropical Asia to Southwestern Pacific (Mayo et al. 1997; POWO 2023a), with the diversity center in Sumatra, Borneo, and New Guinea (Boyce and Wong 2008). Several new species of *Homalomena* have been recently described from Indonesia, i.e. *H. anthurioides* S.Y.Wong, P.C.Boyce & A.Hay (Wong et al. 2020), *H. pexa* S.Y.Wong, P.C.Boyce & A.Hay (Wong et al. 2020), and *H. puncticulosa* S.Y.Wong & P.C.Boyce (Wong and Boyce 2021). *Homalomena* is a strongly aromatic herb with no or only a very weak constriction between the lower and upper spathe, unisexual flowers, 3-4 stamens on each staminate flower, and 3-4 locular ovary on the pistillate flowers (Wong et al. 2013). Taxonomically, the genus, along with *Furtadoa* M. Hotta, is classified into the tribe Homalomeneae under the Araceae family (arums or aroids) (Mayo et al. 1997). The presence of pistillodes on male flowers distinguishes both genera, with *Homalomena* lacking sterile pistils (Mayo et al. 1997; Boyce and Wong 2008). *Homalomena* has a high potential for ornamental purposes due to its morphological features; some have already been cultivated by horticulturists (Boyce and Wong 2016; Yuzammi 2018; Wong and Boyce 2020; Wong et al. 2020). In Indonesia, *Homalomena cordata* Schott and *H. pendula* (Blume) Bakh.f. are most frequently used as ornamental plants (Munawaroh et al. 2017; Nisyawati and Mustaqim 2017; Asih and Kurniawan 2019; Asharo et al. 2022; Manurung et al. 2022; Rambey et al. 2022).

The information on the diversity of Araceae in Java has been considered completed by the publication of Flora of Java vol. III in 1968 with 27 genera occur on the island according to the book (Backer and Bakhuizen van den Brink 1968). In Java, the genus *Homalomena* is represented by four native species: *H. cordata* Schott, *H. humilis* (Jack) Hook.f., *H. pendula* (Blume) Bakh.f., and *H. rostrata* Griff. (Backer and Bakhuizen van den Brink 1968). The latter was mentioned under the name *H. sagittifolia* Jungh. ex Schott. in Flora of Java. The revised study of *Homalomena* in Java has since been discontinued. As a result, the most recent taxonomy information on *Homalomena* of Java is not yet available. It is challenging to accurately identify the *Homalomena* species in Java without the updated taxonomic reference.

The lack of updated Flora of Java is exacerbated by the numerous discoveries of new plant alien species in Java. Many newly discovered alien species, including aroids, have been reported to occur on the island in recent years

(Mustaqim and Nisyawati 2016; Al Anshori et al. 2020; Hariri et al. 2020; Effendi and Mustaqim 2021; Mustaqim and Setiawan 2021; Peniwidiyanti et al. 2021; Al Anshori et al. 2022; Irsyam et al. 2022; Irsyam et al. 2023). Furthermore, the commerce in ornamental plants has grown significantly, allowing ornamental species to be easily introduced to new geographic areas. As a result of the introduction, two newly naturalized aroids have been found in Java, namely *Syngonium wendlandii* Schott and *Alocaisa cucullata* (Lour.) G. Don (Mustaqim and Nisyawati 2016; Irsyam et al. 2023). *Homalomena* is also a member of a genus that is commonly sold as an exotic ornamental plant. Therefore, it is possible that unrecorded *Homalomena* can still be discovered in Java.

In 2022, we found a spontaneous population of *H. rubescens* (Roxb.) Kunth during a botanical exploration in West Java. However, misidentification might occur due to restricted and difficult access to sources. Other *Homalomena* species, such as *H. pendula* and *H. cordata*, are frequently misidentified as *H. rubescens* by ornamental plant traders and enthusiasts. Therefore, it is important to provide the most recent information on *Homalomena* in Java. This study aimed to provide updated data on *Homalomena* in Java as part of the preparation of Alien Flora of Java and confirm the identity of *H. rubescens* in Java. In this paper, we scientifically report the occurrence of *H. rubescens* in Java based on morphological and molecular analyses. Morphological description, updated key to the genus *Homalomena* of Java, photographs, and a brief discussion are provided here.

MATERIALS AND METHODS

Study area

The study was conducted in several districts and cities in West Java, Indonesia, namely Bandung District, West Bandung District, Bandung City, Bogor City, Bogor District, Cianjur District and Sumedang District, from October to November 2022. The study was carried out using the exploration method according to Rugayah et al. (2004).

Procedures

Morphological observation and herbarium processing

Plant materials were collected from the field according to Croat (1985). The materials were collected on its complete organs consisting of rhizome, leaves, inflorescences, and infructescences. The materials were processed into dried and wet specimens at the Herbarium Bandungense (FIPIA), School of Life Sciences and Technology, Institut Teknologi Bandung, Jatinangor, Sumedang District. The specimens were identified using some taxonomic references: Mayo et al. (1997), Schott (1858), Hooker (1894), Clay and Hubbard (1987), and Oonsalung et al. (2008). The observation of herbarium specimen of *Homalomena* was carried out in Herbarium Bogoriense (BO) and herbarium Bandungense (FIPIA). The digital specimens of *Homalomena* spp. were also observed through https://bioportal.naturalis.nl/.

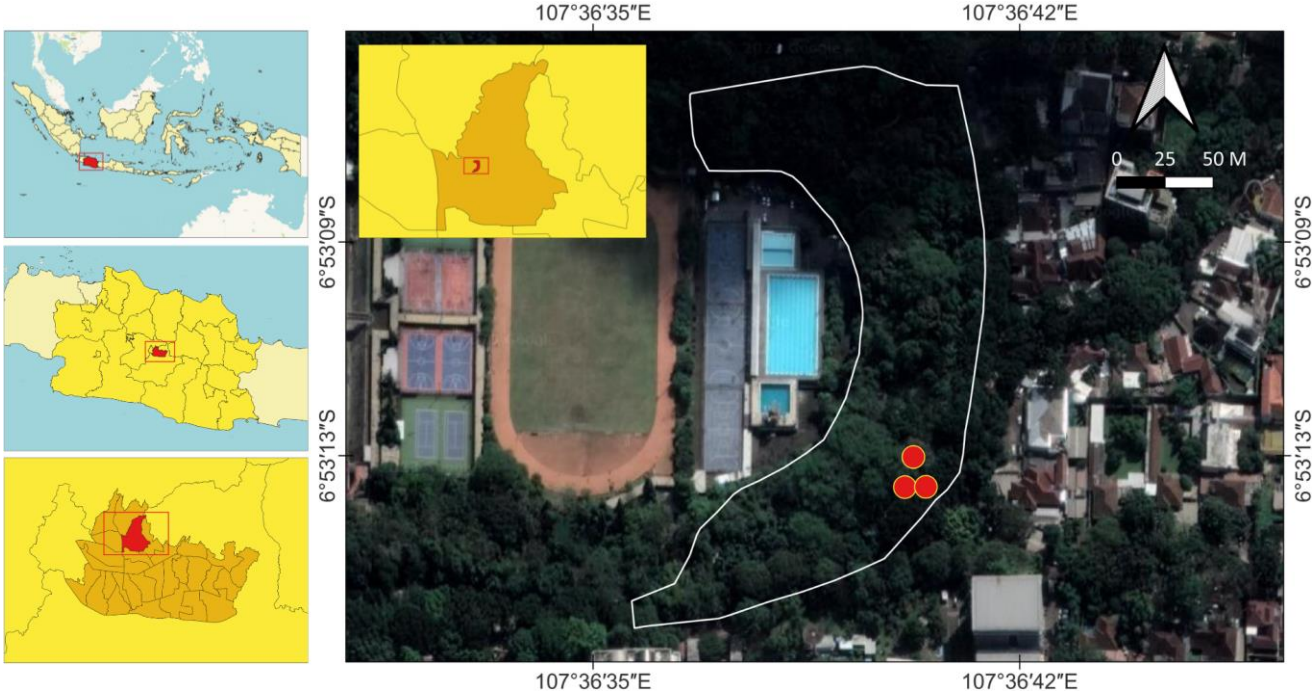

Figure 1. The locality map of the spontaneous population of *Homalomena rubescens* in Babakan Siliwangi City Forest, Bandung City, West Java, Indonesia

Molecular identification

A healthy and fresh leaf was selected and put inside a zip-lock bag filled with 70% ethanol. Following the manufacturer's recommended protocol, the GeneJET Plant Genomic DNA Purification Kit (Thermo Scientific™, K0791) was used to extract the genomic DNA from the preserved leaf. The region was amplified using the universal ITS primer that Sun et al. (1994) previously described. For the PCR reaction, a final volume of 50 µL of a mixture containing 10 ng of the DNA template, 5 µM forward and reverse primers, 25 µL of MyTaq™ HS Red Mix (Bioline, USA), and 11 µL nuclease-free water was used. The following PCR settings were used: 1 cycle at 95°C for 3 min, 35 cycles at 95°C for 30 sec, 58°C for 45 sec, and 72°C for 45 sec, and 1 cycle at 72°C for 5 min following Aprilianingsih et al. (2022). Using a GelDoc UV trans-illuminator from BioRad, the PCR products were separated on a 1% florosafe stained-agarose gel. The PCR product was sent to 1st Base through the service of PT Genetika Science Indonesia for the sequencing procedure.

Data analysis

Morphological data obtained from vegetative and generative organs were analyzed descriptively. Meanwhile the sequence data was analyzed using the ClustalW method. The forward and reverse ITS sequences were combined into a contig sequence that was then submitted to NCBI BLAST to determine the homology level (https://blast.ncbi.nlm.nih.gov/) (Hung and Weng 2016). In order to determine the significance of nucleotide similarities for supporting the morphological identification, two sequences that emerged from the BLAST result were used for further analysis using MultAlin (Pervez et al. 2014).

RESULTS AND DISCUSSION

Homalomena rubescens (Roxb.) Kunth, Enum. Pl. 3: 57 (1841). Figures 2 and 4.

Species description: Herb, terrestrial, erect, up to 1 m tall, rhizomatous. *Rhizomes* long, slender, reddish brown, internodes 6-20 mm. *Leaves* alternate; sheath reddish; petiole stout, green with reddish tinged, up to 75.5 cm long, aromatic when bruised; lamina cordate, 14.5-35.5×10-26.5 cm, base cordate, basal lobes outwardly directed, margin entire, apex acuminate, nerves rather straight, near the apex arcuate, adaxial surface dark green, abaxial surface glaucous. Juvenile leaves deltoid to elliptic, base truncate to subcordate or rounded. *Inflorescences* shorter than petioles, 1-5 in a sympodium. *Peduncle* slender, up to 17.5 cm long, terete, thicker below spathe, green with reddish tinged, peduncle first erect, after anthesis pendulous. Spathe 7-8 cm long, 2 cm in diam., non-constricted, thick, with a terminal stout mucro to 4-9 mm long, green with reddish tinged outside and whitish inside, convolute and closed after anthesis. Spadix 7-7.2 cm long; stipe c. 5 mm long green; female zone 1.8-2 cm long, yellowish green; male zone 3.7-5 cm long, ellipsoid, cream-colored, apex blunt, fertile to the apex. *Flowers* unisexual, perigone absent. *Pistillate flowers* densely arranged; gynoecium ovoid, c. 2 mm tall; staminode 1 per flower, minute, ca. 1 mm long, white; ovary 1-1.5 mm in diam., yellowish green, with 3 parietal placentae, ovules numerous; stigma discoid, 0.5 mm in diam., greenish. *Staminate flowers* consisting of usually 3-4 free stamens, often somewhat irregularly arranged; stamen truncate, subrectangular in view from above, ca. 1 mm wide.

Distribution: This species is distributed in Sikkim, Bhutan, Bangladesh, and Myanmar. In Malesia, the species previously reported were from Singapore, Sumatra, and Borneo (Chong et al. 2009; Maretni et al. 2017; Isnaini et al. 2019). In this study, the spontaneous population of *H. rubescens* was discovered in Babakan Siliwangi City Forest, Bandung City, West Java (Figure 1).

Habitat: In Java, the plant grows on roadsides, abandoned lands, and ditches at 200-778 masl.

Specimens examined: Java: West Java, Bandung City, Babakan Siliwangi City Forest, 20.X.2022, *ASD Irsyam, I Suwandhi, NN Kamila & MR Hariri s.n.* (FIPIA); Bandung City, Babakan Siliwangi City Forest, 05.XI.2022, *ASD Irsyam & RR Irwanto s.n.* (FIPIA).

Vernacular names: *Cariang* (Sundanese).

Updated key to the genus *Homalomena* in Java (modified from Backer and Bakhuizen van den Brink (1968))

1.a. Low, creeping-ascending herbs; leaves distributed over the thin stem, lamina elliptic-narrowly lanceolate, with acute or rounded-cuneate base …… ……. *Homalomena humilis*

1.b. Robust, mostly erect herbs; leaves crowded near the apex of stout stem, with cordate, sagittate, or hastate base……..2

2.a. Spathe when still inrolled constricted, consisting of two very unequal portions…….*Homalomena rostrata*

2.b. Spathe when still inrolled ellipsoid, not constricted ……..……..... 3

3.a. Leaves with slightly outwardly directed basal lobes, nerves rather straight, near the apex arcuating into leaf-margin, petiole stout ..…… 4

3.b. Leaves with inwardly directed basal lobes, all nerves arcuating into leaf-margin, petiole slender to rather stout……...*Homalomena cordata*

4.a. Petiole and spathe are reddish-tinged …….…… ……..……..….... *Homalomena rubescens*

4.b. Petiole and spathe are completely green or red …… ……. *Homalomena pendula*

Homalomena rubescens is an exotic species, with its native range extending from South Asia (Sikkim) to Myanmar (POWO 2023b). It is cultivated as an ornamental both within and outside its native range (Clay and Hubbard 1987; Oonsalung 2008; Chong et al. 2009). The occurrence of the species in Java was enigmatic. *Homalomena rubescens* was included in Miquel's Flora van Nederlandsch Indie in 1855 (Miquel 1855). He based his book on specimens from the Leiden Herbarium. However, no specimens from Java were obtained by older botanists, as demonstrated by firsthand observation in Herbarium Bogoriense and

Herbarium Bandungense, as well as digital specimen observation. Specimen number L.0741113 was collected by Heinrich Gustav Adolf Engler from an unknown place in January 1883, and specimen L.3719178 was collected from Kaling Tana Siaad by an anonymous collector. The other specimen of *H. rubescens*, WAG.1666007, has also been examined through the Naturalis Bioportal. Jan W. Moll collected this specimen from an unknown place on December 19th, 1903. The specimen observed by Miquel might be misidentified as *H. rubescens*. According to Furtado (1939), juveniles of the allied species *H. pendula* (Blume) Bakh.f., *H. rubescens*, and *H. lindenii* (Rodigas) Ridl. have small deltoid leaves, and their herbarium specimens are frequently confused. Thus, in this study, we confirm the occurrence of *H. rubescens* in Java, Indonesia. In this study, we explored several localities in West Java and the naturalized *H. rubescens* has only been discovered in Bandung City. However, there is a possibility that the species might be found elsewhere on Java, outside of our study locations.

Morphologically, *H. rubescens* can be easily distinguished from other *Homalomena* species with red petioles, for example, *H. pendula*. The color of the petiole and spathes, as well as the size of the petiole, are the primary defining characteristics of both species. *Homalomena rubescens* has a green with a reddish-tinged petiole, but *H. pendula* has a broad variety of petiole colors ranging from red to completely green as mentioned by Furtado (1939). The color difference between the petiole and the spathe is similar. The petiole of *H. rubescens* is thicker than that of *H. pendula*, which is thin and slender. Both species have outwardly directed basal lobes of lamina and *H. cordata* has inwardly directed basal lobes.

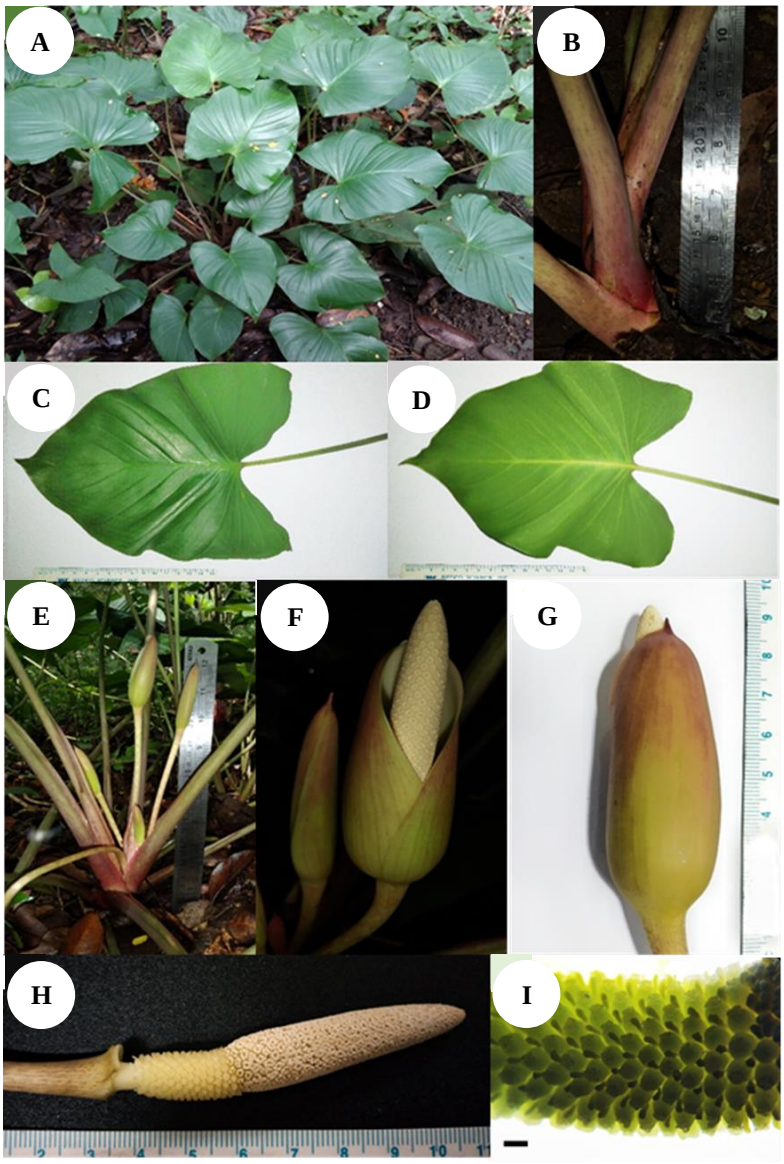

Figure 2. *Homalomena rubescens* (Roxb.) Kunth. A. Habit; B. Sheaths base; C. Adaxial leaf surface; D. Abaxial leaf surface; E. Inflorescences; F. Front view of spathe; G. Back view of spathe; H. Spadix without spathe, showing stipe, female zone, and male zone; I. Pistillate flowers. Bar scale: 1 mm

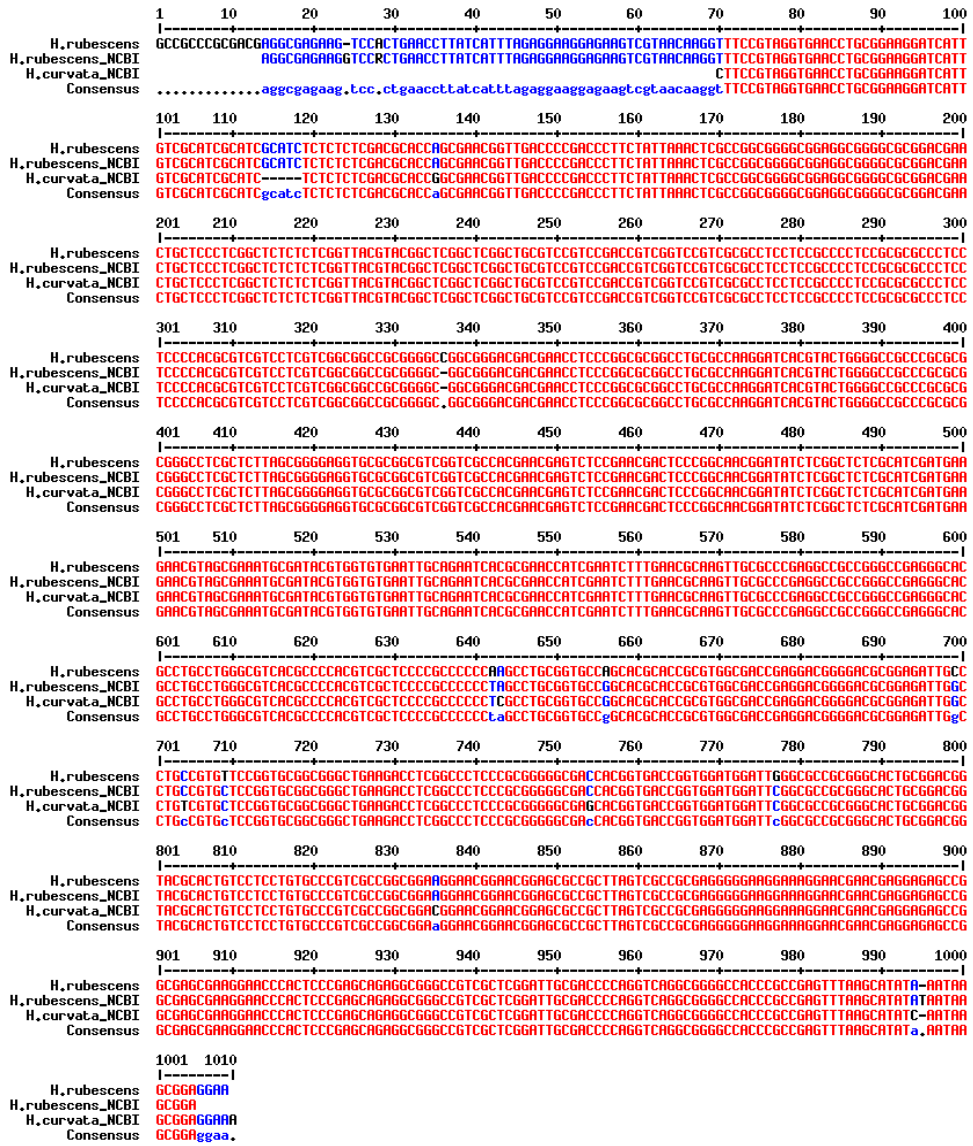

Figure 3. The nucleotide comparison of *Homalomena rubescens* from NCBI and Babakan Siliwangi Bandung were nearly identical, as revealed by the multiple alignment sequences. Note that red indicates conserved nucleotide, blue indicates varied nucleotide, and black indicates singlet variation (singleton)

The sequence of *H. rubescens* from Babakan Siliwangi is nearly 99.09% identical to *H. rubescens* KM580744.1 (NCBI database), according to a molecular analysis using the ITS sequence. This analysis also found that 98% of the queries were covered by this sequence. *Homalomena curvata* JX076777.1, which had a high identity similarity of 98.19% and a 93% query cover, was the other homologous organism. Among the 100 hits from the BLAST result, *H. pendula* identity matched 94.65%, with 44% of the query only including fragments of the 5.8S ribosomal RNA gene, the entire sequence of internal transcribed spacer 2, and the fragments of the large subunit ribosomal RNA gene (Figure 3). The specimen collected from the Babakan Siliwangi City Forest, Bandung City, is undoubtedly *H. rubescens* based on morphological analysis, as shown by this result. Given that *Homalomena* is one of the most diverse and taxonomically challenging aroid genera in the Asian tropics, the absence of a trustworthy taxonomy poses significant difficulties for field workers. However, species verification using ITS empowered the researcher. There is an urgent need for a thorough study to clarify the taxonomy and phylogeny of the genus (Li and Boyce 2010).

Previous studies revealed that Araceae had higher naturalization than predicted (Pemberton and Liu 2009; Mustaqim and Nisyawati 2016; Witt 2017; Mustaqim 2019; Iamonico 2021; Wohlwend et al. 2021; Irsyam et al. 2023). In this study, an adventive population of *H. rubescens* has been discovered in Babakan Siliwangi City Forest, Bandung City (Figure 1). We suspect that the population is a result of naturalization. *Homalomena rubescens* is found in an abandoned area under the bridge, roadsides, and ditch at elevation ±778 masl. The species is relatively common in Babakan Siliwangi and may have already naturalized outside our study area.

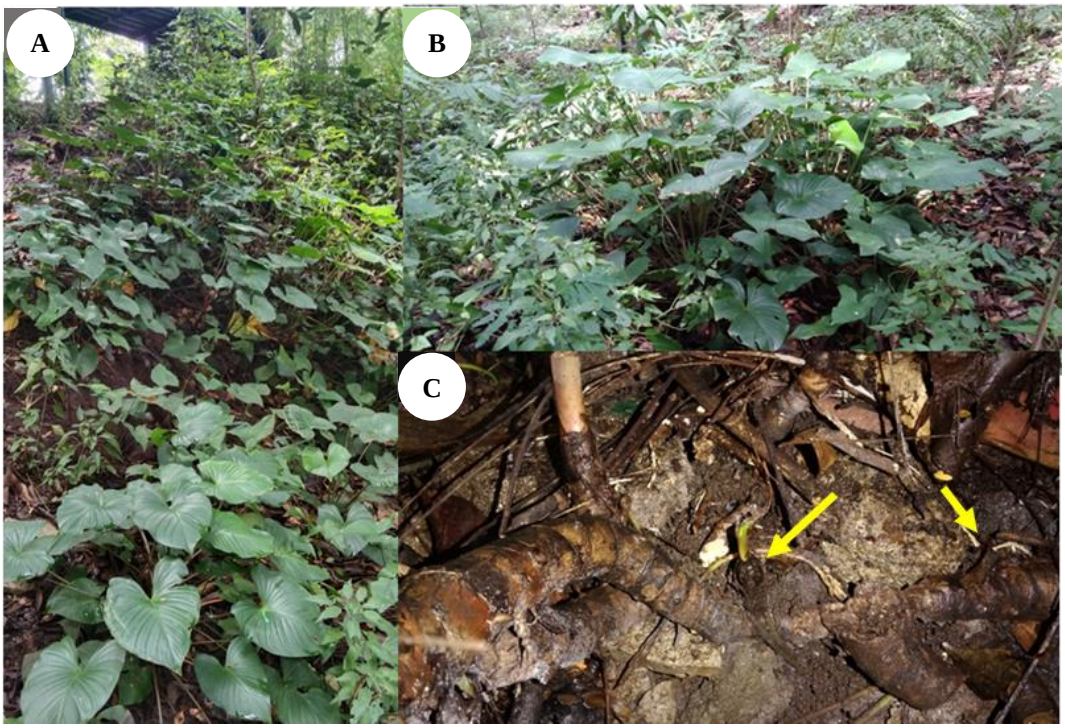

Figure 4. The naturalized population observed in Babakan Siliwangi City Forest, Bandung, C. Rhizome with new shoots (arrows)

Homalomena rubescens was brought to Java for decorative use, and the Sundanese ethnic group in West Java refers to it as *cariang*. It has been planted in gardens due to its red-colored petioles and spathes (Oonsalung 2008). However, the history of its introduction to the island is not known.

Naturalized plants can sustain self-replacing populations in the wild without any human intervention. They spread their population in the new distributional areas using seeds, tillers, tubers, bulbs, fragments, and other self-replicating structures (Pyšek et al. 2004). The observed *H. rubescens* can produce flowers and mature infructescences. However, in addition to generative reproduction, it can also reproduce vegetatively through stolons and stem fragments. The primary dispersal mechanism is most likely vegetative fragments in discarded soil or waste from human activity. The pieces of rhizome can also develop into new, self-contained individuals. Furthermore, the rhizomes colonize nearby patches, eventually producing new shoots (Figure 4). The other mechanism is that seeds are dropped near their parent plant or spread by water. The occurrence of *H. rubescens* in Java Island requires more attention. In the future, it might be distributed more widely on the island.

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