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New localities of *Margaritaria indica* (Phyllanthaceae) in Singapore, with notes on fruiting and propagation

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Abstract. This paper documents the discovery of the second and third *Margaritaria indica* (Dalzell) Airy Shaw individuals in Singapore since the first record of this species in 2012. The second individual is on Kusu Island, although the sex remains uncertain as no flowering or fruiting has been observed to date. The third individual is in Bukit Brown Cemetery on the main Singapore island, and is confirmed to be female from the fruits produced. Seeds collected from this individual germinated in extremely low numbers, possibly indicating the presence of at least one mature male tree in the vicinity. Propagation trials for this species by stem cutting and seed are discussed.

Key words. *Margaritaria indica*, Phyllanthaceae, Bukit Brown, Kusu Island

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INTRODUCTION

The genus *Margaritaria* L.f. (Phyllanthaceae, previously Euphorbiaceae) consists of 14 species with a pantropical distribution excluding the Pacific Islands (Webster, 1979; Barker, 2001). The genus was first published in 1782 by Linnaeus with *Margaritaria nobilis* as the type species (Linnaeus, 1782). *Margaritaria* is one of the basal genera of Phyllanthaceae with centres of diversity in Madagascar and South America, suggesting a Gondwanan origin. It primarily consists of dioecious plants with canopy-reaching habit, flowers with distinct nectaries and tardily dehiscent fruits (Kawakita & Kato, 2017). In Singapore, the genus is represented by *Margaritaria indica*, which is of conservation interest because the species is listed locally as Critically Endangered (CR) with designation Category D (Low et al, 2014).

Distribution of *Margaritaria indica*. India, Sri Lanka, Myanmar, Thailand, northern Vietnam, southern China, Taiwan, across Malesia to Australia (Barker, 2001). In Malesia, it has been recorded from Sumatra, Peninsular Malaysia, Singapore, Java, Borneo, the Philippines, the Lesser Sunda Islands, Moluccas and the Aru Islands (Barker, 2001). The distribution of this species is described as "rare and very scattered in primary forests, riverine forests and peat swamps" (Barker, 2001) with spotty distributions (Webster, 1979). It is also found in secondary forests (Low et al., 2014).

***Margaritaria indica* in Singapore.** *Margaritaria indica* has two forms, namely *Margaritaria indica* f. *indica* and *Margaritaria indica* f. *vestita* (Barker, 2001). The Singapore individuals belong to *Margaritaria indica* f. *indica* as the leaves are glabrous. Prior to this study, only one individual was known from Singapore, located at University Town (UTown), National University of Singapore, in 2012 (Low et al., 2014).

MATERIALS, METHODS & RESULTS

Discovery of new *Margaritaria indica* individuals. In 2018, the second *Margaritaria* individual was found on Kusu Island. A voucher specimen was first collected in 2018 on a suspicion that the tree was *Margaritaria indica* (Ali Ibrahim, pers. comm.; Table 1). The tree was re-visited in 2020 for the collection of more material. The height and girth were estimated to be 18 m and 1.8 m respectively. This individual was growing in full sun at the base of the hillock within 20 m from the shoreline, with a dense undergrowth consisting of mostly exotic species (Fig. 1). The trunk was completely covered with *Epipremnum aureum* (Linden ex André) G.S.Bunting, which obscured the bark features. There were many epicormic shoots ('watershoots') from the basal part of the trunk that grew to over 3 m high, and it was from these that the second confirmatory voucher in 2020 was collected.

The third *Margaritaria indica* individual was discovered at Bukit Brown Cemetery Blk 4 during routine tree inspection work, just off a dirt trail, in close proximity to a medium-sized *Syzygium polyanthum* (Wight) Walp. The tree was 9 m

tall and its girth was 0.6 m with single stem (Figs. 2–5). Two vouchers were collected, one on 6 February 2020 and another on 24 February 2020, the second with more developed fruits, and both were deposited in the Singapore Botanic Gardens' Herbarium (SING) (Table 1). The collections from all three individuals conform to the descriptions of the species in Barker (2001) and Low et al. (2014). Based on the collection from the individual at Bukit Brown Cemetery, the seeds were 3.5–4 × 3–4 mm with a thin layer of flesh and a linear embryo about 1.6 mm long.

Fig. 1. The individual on Kusu Island. (Photograph by: Reuben C. J. Lim).

Fig. 2. Base of the individual at Bukit Brown Cemetery, showing the characteristic scaly bark that leaves a reddish bole after it falls off. (Photograph by: Ying Wei Jong).

Fig. 3. Stem of the individual at Bukit Brown Cemetery (left) in close proximity to a *Syzygium polyanthum* (right). (Photograph by: Ying Wei Jong).

Fig. 4. Crown of the individual at Bukit Brown Cemetery festooned with climbers. (Photograph by: Ying Wei Jong).

Fig. 5. Fruits on the individual at Bukit Brown Cemetery. (Photograph by: Ying Wei Jong).

Table 1. Vouchers of *Margaritaria indica* in SING. The second and fourth vouchers were collected from the same individual on Kusu Island, while the third and fifth vouchers were collected from the same individual at Bukit Brown.

S/No.	Date collected	Collectors	Collector's no.	Barcode number	Collection locality
1.	29 May 2012	P. Leong, Y. W. Low, M. Tay & R. Singh	SING 2012–252	SING0166292	University Town, National University of Singapore
2.	11 April 2018	I. Ali & S. K. Ganesan	SING 2018–385	SING0256617	Kusu Island
3.	6 February 2020	Y. W. Jong & A. R. Rushan	SING 2020–219	SING0291112	Bukit Brown Cemetery
4.	11 February 2020	H. K. Lua, R. C. J. Lim, X. Y. Ng, Y. S. Yeoh & et al.	SING 2020–296	SING0291114	Kusu Island
5.	24 February 2020	Y. W. Jong, A. R. Rushan, X. Y. Ng & R. C. J. Lim	SING 2020–220	SING0291113	Bukit Brown Cemetery

Fruit collection and seed germination. On 6 February 2020, fruits were encountered for the individual in Bukit Brown Cemetery; the fruits were about 0.9–1 cm across, with seeds covered in a green aril, and the endosperm when dissected was green as well. On 24 February 2020, another collection was made, and while the fruits were still green, they were slightly larger at over 1 cm in diameter. Several ripe, brown fruits were seen on the tree as well, but these were lost in the tall grass during the cutting of the branches for collection, or were beyond reach. Collected seeds were processed in the Singapore Botanic Gardens Seed Bank. Ten seeds were randomly chosen for cut testing. Only three out of 10 seeds were found to have fully formed endosperms and healthy embryos. Seeds with healthy white embryos were most likely to be viable (Figs. 6–7). The rest had empty interior cavities or half-formed endosperm without any embryos.

The rest of the seeds were also separated using the water floatation method, where healthy or viable seeds tend to sink while empty seeds float. Sinkers were further randomly assigned into two batches that were planted in separate germination media of either coarse sand or a mixture of sand and cocopeat. Floaters were all planted in a mixture of coarse sand and cocopeat. The results of the germination trials are presented in Table 2.

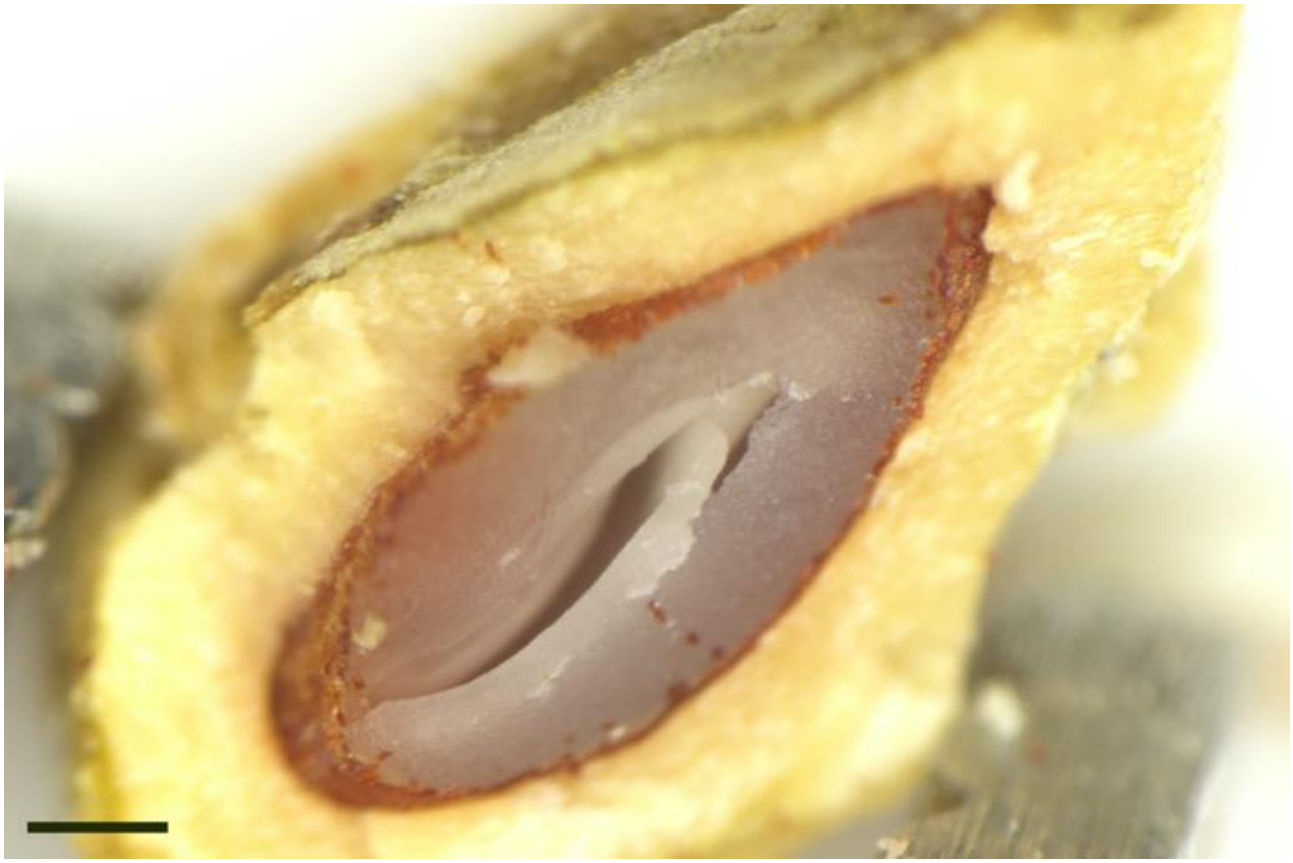

Fig. 6. Viable seed with endosperm and linear embryo filling the interior cavity. Scale bar = 0.5 mm. (Photograph by: Xin Yi Ng).

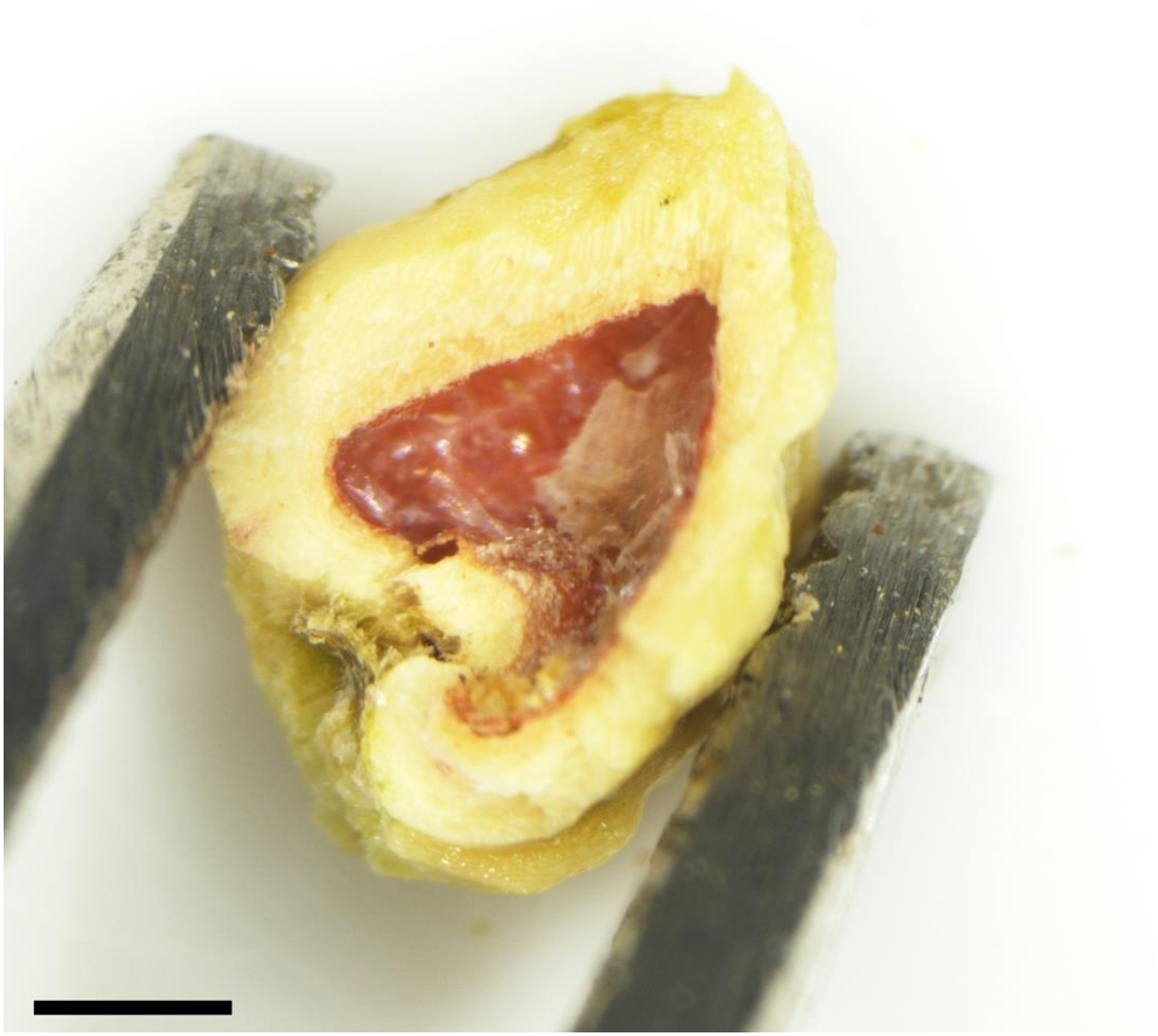

Fig. 7. Non-viable seed with empty interior cavity and absence of embryo. Scale bar = 2.5 mm. (Photograph by: Xin Yi Ng).

Table 2. Germination trial results for the two fruit collections from the Bukit Brown Cemetery individual.

Collection date	Seed type	Media	Number sown	Results (percentage sown)	Germination period
6 February 2020	Immature seeds	Coarse sand and cocopeat 1:1 mix	34	0 germinated (0%)	150 days
24 February 2020	Floating seeds	Coarse sand and cocopeat 1:1 mix	58	0 germinated (0%)	180 days
6 February 2020	Sinking seeds	Coarse sand and cocopeat 1:1 mix	217	2 germinated (0.9%)	47–150 days
6 February 2020	Sinking seeds	Pure coarse sand	217	10 germinated (4.6%)	47–150 days

The germination rate of *Margaritaria indica* was observed to be higher in pure coarse sand at 68 days after sowing, with two seedlings having germinated, compared with coarse sand and cocopeat in which only one seedling germinated. At 110 days after sowing, eight more seedlings had germinated in pure coarse sand, while only two had germinated in the coarse sand and cocopeat mix (Fig. 8). More seeds were hence observed to have germinated in pure coarse sand.

Fig. 8. Transplanting of *Margaritaria indica* seedlings. a, newly germinated seedling; b, young seedling with reddish stem (red arrow); c, several seedlings grew quickly after being transplanted into a seedling mix for 28 days (red arrows). (Photographs by: Reuben C. J. Lim).

Stem cuttings. Prior to this discovery, propagation of *Margaritaria indica* depended on vegetative propagation through stem cuttings. A vegetative propagation trial was similarly carried out on material from the Bukit Brown Cemetery individual. Cuttings of about 10 cm in length were made from the terminal end of a leafy twig until fully woody parts were reached, all leaves were removed except for the last fully expanded leaf which was half cut away to reduce water loss, and then the proximal end was dipped into rooting hormone and planted into the media (Table 3; Fig. 9a). The cuttings were then assigned into one control group (coarse sand) and one treatment group (coarse sand + cocopeat), with each group consisting of 29 cuttings. It was found that the number of surviving cuttings planted in a mixture of coarse sand and cocopeat was significantly higher than that of those planted in coarse sand ($p < 0.001$, Fisher's exact test; Table 3). It was also observed that cuttings that were non-woody or semi-woody had a higher rate of rooting compared to woody, older stems.

In wild individuals of *Margaritaria indica*, the main stem is orthotropic with plagiotropic side branches. While the survival and rooting rates of stem cuttings are high, stem cuttings from plagiotropic branches continue to exhibit such growth forms without ever forming an erect orthotropic form as wild individuals grown from seed (Fig. 9b). Insufficient numbers of orthotropic cuttings have been collected thus far as these are generally much taller and thicker than side branches. No stem cuttings of orthotropic origin have been successfully rooted. More trials are needed for stem cuttings of orthotropic origin to determine if these would have the same form as seed-grown individuals. Another drawback of vegetative propagation, such as through stem cuttings, is that all individuals produced through this method are clones of the parent with limited genetic diversity, and since the current stem cuttings originate from a female individual, it will not be possible to obtain a male individual from the cuttings for natural pollination and production of viable seeds.

Table 3. Propagation trial results for stem cuttings collected from the individual at Bukit Brown Cemetery.

Media	Number planted	Results	Rooting period
Coarse sand and cocopeat 1:1 mix	29	22 rooted (75.9%)	30 days
Pure coarse sand	29	5 rooted (17.2%)	30 days

Fig. 9. Vegetative propagation of *Margaritaria indica.* a. rooted cuttings from the Bukit Brown Cemetery individual in coarse sand and cocopeat with new leaves on day 25 after planting; b, rooted cutting from the NUS individual after one year, exhibiting continued plagiotropic growth. (Photographs by: Reuben C. J. Lim).

Reproductive ecology of *Margaritaria indica*. The small and inconspicuous flowers of *Margaritaria indica* suggest biotic vector-driven pollination, and the blue-purple sarcotesta suggest avian seed dispersal (Barker, 2001). Compared to seed dispersal, pollination is more likely the bottleneck of reproduction for dioecious species like *Margaritaria indica.* With few individuals spread across large distances, effective cross-pollination among different *Margaritaria indica* individuals could prove challenging because different sexes are spatially separated and require specific pollinator movement direction from male to female flowers for successful pollination (Vamosi et al., 2006). Notes on the specimen cited in Barker (2001), Podzorski SMHI2036, mentioned that the flowers were visited by bees, while in Japan, the flowers are frequently visited by flies (Kawakita & Kato, 2017). Since the genus *Margaritaria* has a highly relictual distribution (Kawakita & Kato, 2017), *Margaritaria indica* might not currently have obligate pollinators across its whole distribution area but instead be pollinated by various unspecialised insect pollinators in different regions, contributing to its spotty distribution. Particularly for Singapore, the low density of individuals spread across major barriers such as urban areas and stretches of sea could pose difficulties for effective cross-pollination.

The inability to form viable seeds could be one of the reasons for the low germination rate of the seeds as well as the low ability of the species to disperse from the site. Pollination, the transfer of pollen grains from an anther to a stigma, is necessary for fruit set, but fertilisation, which is when the nucleus of a pollen grain fuses with the nucleus of an ovule to produce a zygote, is a subsequent step from pollination and may not occur (Srivastava, 2002). This could explain the high numbers of fruits observed on the tree, but only an exceptionally low number of viable seeds collected. In this case it is possible that pollen from another male *Margaritaria indica* individual was transferred successfully to this female individual, but the pollen was of low quality or not viable to effect successful fertilisation for most seeds. Some searches had been conducted at Bukit Brown cemetery for trees over 0.4 m in girth in the vicinity of the female individual, but no male tree was found within a 50-metre radius from the female individual. Further studies are required for better understanding of how to increase the viable seed production of this species. No record of apomixis has been described for this genus.

CONCLUSIONS

Margaritaria indica is a dioecious species, hence the discovery of an individual with viable seeds implies the presence of at least one mature male tree in the vicinity of Bukit Brown cemetery and/or the neighbouring MacRitchie Reservoir area of the Central Catchment Nature Reserve. Prior to this discovery, the conservation effort for this nationally critically endangered species largely depended on vegetative propagation as part of the Species Recovery Programme of the National Parks Board Singapore (Lim et al., 2019). Therefore, the discovery of viable seeds and new individuals also paves the way for propagation through sexual reproduction, through which new individuals of both sexes may be grown from seeds and planted around known individuals to facilitate the production of more viable seeds in the future. We recommend regular monitoring of these two newly discovered individuals and seed collections from the Bukit Brown Cemetery individual to grow the next generation and safeguard the future of this species in Singapore.

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