Journal of Sustainability Science and Management
Volume 15 *Number* 7, October 2020: 49-55

eISSN: 2672-7226

Vanilla montana Ridl.: A NEW LOCALITY RECORD IN PENINSULAR MALAYSIA AND ITS AMENDED DESCRIPTION

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Submitted final draft: 25 April 2020 *Accepted: 11 May 2020* http://doi.org/10.46754/jssm.2020.10.006

Abstract: Among the seven *Vanilla* species native to Peninsular Malaysia, *Vanilla montana* was the first species to be described. But due to its rarity, it took more than 100 years for the species to be rediscovered in two other localities. This paper describes the first record of *V. montana* in Negeri Sembilan with preliminary notes on its floral development and some highlights on the ecological influences. We also proposed a conservation status for the species. The data obtained will serve as an important botanical profile of the species, and it will add to our knowledge gaps on the distribution of this distinctive orchid in Malaysia.

Keywords: Biodiversity, florivory, endangered *Vanilla*, Orchidaceae, Negeri Sembilan.

Introduction

In Peninsular Malaysia, the genus *Vanilla* Plum. ex. Mill. has been a recent topic of interest as the native species had been extensively investigated via the classical and advance botanical approaches to respond to the crop improvement calls for their commercialised sister species, *Vanilla planifolia* Jacks ex Andrews (Ong, 2018; Raffi & Go, 2019). Only seven species are known to be native to the peninsula (Raffi *et al.*, 2017a; 2017b). *Vanilla montana* Ridl., once collected from Gunung Korbu in Perak, which is located on the Titiwangsa Range in Peninsular Malaysia, was the first species of *Vanilla* described by Henry Nicholas Ridley in 1915. However, its occurrence and distribution are difficult to locate as the taxonomic identification of the species is challenging due to its rarity, scarcity of flowering materials and the fact that its vegetative characteristics highly resemble other *Vanilla* species. Since its last sighting, this long-lost species was then claimed to be 'missing' for more than a century, and was unaccounted for during a series of orchid biodiversity surveys of the montane areas in the peninsula (Go *et al.*, 2015a). Surprisingly, it was rediscovered in another undisclosed location by Ong (2017). A third locality for *V. montana* was recorded during two different field excursions at a summit region on the tail-end of the Titiwangsa Range. Initially, the species identification was hampered since the entire locality was represented by a single capsule, but the taxonomic work was validated when some of the vines produced flowers three months later. A total of three short inflorescences with seven flowers were retrieved. The discovery was important as it allowed in-situ observations of the flowering stages, and additional data on the geographical range and threat risks could be gathered. This paper aims to document the new *V. montana* locality, present the preliminary notes on its floral development with some highlights on the ecological influences, update the geographical distribution pattern and provide a provisional conservation status for this species. The results of this study would serve as a fundamental source of information on the botanical profile of the species for its conservation management.

Materials and Methods

Samples Collection and Preservation

Convenience samplings were conducted at one of the mountains in Negeri Sembilan on January 2019 and from March to April 2019. Fruiting samples were collected, preserved using the standard herbarium technique of Bridson and Foreman (1992), and deposited in the herbarium of Universiti Sains Malaysia (USMP). The flower specimens were dissected and preserved in spirit.

Morphological Assessment and Description

The morphological characteristics of the flowers and pods were diagnosed and compared with the descriptions by Ridley in his 1915's protologue and with revisions provided by Ong (2017; 2018). The taxonomy of *V. montana* was then updated and the latest species synopsis was prepared, following Soto-Arenas and Cribb (2010).

Notes on the Flowering Stage

Data on temperature, precipitation, humidity and other general climate information when the flowering events took place were downloaded from AccuWeather (www.accuweather.com) and METMalaysia (www.met.gov.my). In-situ observations of the anthesis stage were made on seven (n = 7) available flower specimens. The anthesis sequences and sightings of possible pollinators were documented.

Provisional Conservation Status Assessment

The general distribution pattern of the species was suggested according to Rabinowitz (1981). The provisional conservation status of *V. montana* was assessed via the extent of occurrence (EOO) and area of occupancy (AOO) estimations using Geospatial Conservation Assessment Tool, GeoCAT (geocat.kew.og), and the extinction risk was determined based on Criterion B and D by IUCN (2012).

Results

Taxonomic Update of V. montana

The taxonomy of *V. montana* was updated with the description of the two undescribed reproductive structures, the rostellum and stigmata. Additional information on the morphological characteristics that were not previously reported were described and illustrated.

Vanilla montana Ridl. *J. Fed. Malay States Mus.*, *6*: 58 (1915); Ong, *Orchid Rev*. *125* (138): 146 (2017); Ong, *Malesian Orchid J*. *21*: 101 (2018).

Lip with a single whitish translucent ridge-like callus in front of tuft at central midlobe. **Rostellum** 3.2 × 3.5 mm, blade concave, ovate, tapering towards apex and wider at the base, apex and base truncated, whitish-yellow. **Stigmata** 1.0 mm × 3.5 – 4.0, transversely oblong to rectangular, concave, apex irregularly incised, whitish yellow (Figure 1).

Distribution and habitat. New record for Negeri Sembilan. Flowering period from March

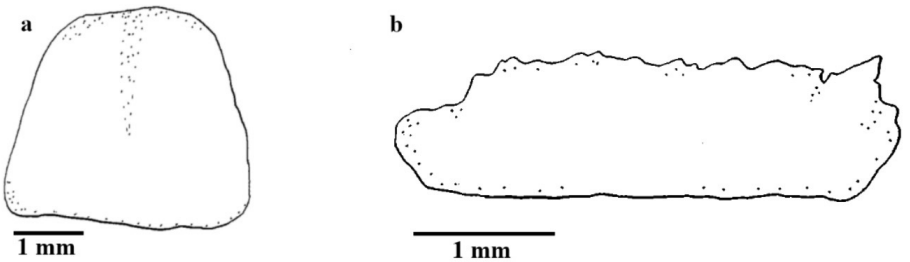

Figure 1: The reproductive structures of *V. montana*, drawn from the spirit collection, FAR001. a) The rostellum and b) stigmata.

to April 2019. Elevation of 895 – 900 m above sea level (a.s.l). Ground rooted climbing herb, later dangling from tall tree branches near slopes exposed to direct sunlight in hill dipterocarp forest.

Additional specimens examined. Peninsular Malaysia, Negeri Sembilan, 18 January 2019, Farah Alia Nordin FAN. TB. 024. Flowers in spirit, 1 April 2019, Akmal Raffi FAR 001.

Synopsis of *V. montana.* It is recognised as the only leafy species localised in hill dipterocarp to lower montane forests with a relatively short inflorescence of c. 1.4 – 1.6 cm in length. It bears two to four successive flowers per raceme, opening one at a time. Bracts ovate with an acute apex and truncate base, c. 0.6 cm wide at base x 0.7 cm long. Flowers large, c. 5.0 x 4.6 cm in dimension, open widely. Sepals and petals greenish-yellow, petals relatively wider than sepals; dorsal sepals c. 5.2 x 1.4 cm, lateral sepals c. 5.0 x 1.7 cm, lateral petals c. 5.1 x 1.8 cm. Lip trilobed, obtrullate when spread out, c. 4.2 x 3.3 cm, lip white with dark red to purple veins with two types of trichome arrangement on the midlobe; trichomes at the apex arranged in five rows, each row with up to seven single hairs, purple with white tip or base or all white; central trichomes arranged in tuft, white, gated with single whitish translucent ridge-like; sidelobes c. 1.5 x 2.3 cm when spread out, encapsulating the column, margin wavy. Columns adnate to the lip base for about 3.0 cm, white with dark red to purple marks on the abaxial of the column towards the anther-cap, beneath anther and stigmata. Anther tetragonal, whitish- yellow. Stigmata short (0.1 cm) in length, transversely oblong to rectangular, apex irregularly incised, whitish yellow. Pods cylindrical, angular, c. 8.0 (–10) cm in length.

In-situ Observations of the Flowering Stage Of V. montana

Fluctuations in monthly precipitation and humidity were determined as the main flowering promoters for the matured vines of *V. montana* in Negeri Sembilan. This was observed by the drastic reduction in the amount of monthly precipitation in January and February 2019 (from 154 mm to 85 mm per month) followed by significantly higher precipitation in the subsequent month of March at 242 mm per month. The minimum annual relative humidity in Negeri Sembilan recorded in February each year also served as a synergist to the development of the flower buds. Temperature fluctuations showed the least effect on the flowering evocation process as the average daily temperature was 30 ºC; the highest and lowest temperatures recorded were at 34 ºC and 25 ºC, respectively, for three consecutive months. High light intensity was also speculated as another promoting factor as the individuals of *V. montana* populating the forest fringes under semi-shaded tree canopy tended to produce inflorescence on the vines that were highly exposed to the sunlight.

The development of inflorescence until the anthesis of the first flower was noted to be approximately one month. The peak time frame for the anthesis was recorded between 0700 hours to 1230 hours, starting with the opening of the lateral sepals, followed by the dorsal sepals, petals and lips (Figure 2). There is not much information on the pollinator of *V. montana,* but unidentified ants were noticed to linger at the flower's compartment during anthesis. As for now, until further in-situ investigations on the pod development are carried out, the flowers were hypothesised to be chasmogamous based on the ovulated superior ovaries that remained intact on the inflorescence after the flowers wilted. Furthermore, exceptional phenomenon of florivory was documented, in which half of the available flower units were destroyed (Figure 3). The chew marks on the consumed parts was consistent with marks left by a small mammal, which was assumed to be a squirrel. The criteria for the florivory events were described as follows; a) occurring each day during anthesis, b) the earliest was documented before 0900 hours up to midday, c) the lips were targeted as the main consumed structure and d) the growth of the superior ovaries was impeded, wilting a few days later.

Figure 2: The timeline of the peak anthesis sequences of *V. montana*. a) The lateral sepals opening at 1000 hours, b) the lip opening at 1120 hours, c) the dorsal sepals and petals openings at 1130 hours, and d) the complete anthesis at 1230 hours

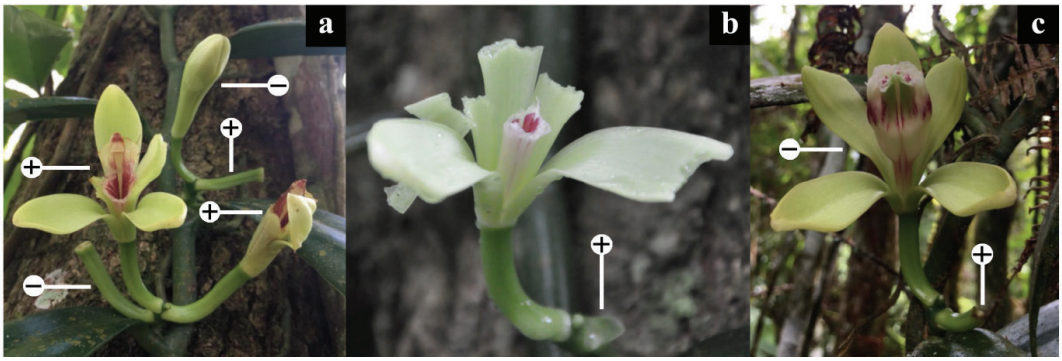

Figure 3: Florivory evidences on *V. montana* flowers during anthesis. a) The affected (shown by "+") and survived (shown by "-") flower, b) the severed flower unit with multiple chew marks and wilted superior ovary due to florivory (shown by "+") and c) the survived, fully bloomed flower (shown by "-") and complete flower consumption up to the superior ovary (shown by "+")

New Locality and Current Extinction Risks

So far, wild populations were known to be strictly localised to the Titiwangsa Range in Peninsular Malaysia, and the newly recorded populations in Negeri Sembilan were noticed to be at the lowest elevation ever documented. The main population comprising three hosts with less than 10 matured individuals was clustered in a small area of 3 m^2, while another population represented by a matured vine was scattered approximately 50 metres along the same trail. There were most likely, in total, three juvenile vines spotted growing on the forest floor and no signs of seedling germination in the entire area. The successful pollination rate was estimated to be very low based on the limited capsule production. Furthermore, the populations were regarded as vulnerable to human interferences and landslides as they were adjacent to paved roadsides and located at steep slopes. In general, the species distribution can be determined as constantly sparse and geographically restricted in several habitats. Further investigation on the distribution of the species using EOO and AOO as parameters had resulted in a significantly narrow geographic range of 669.318 km^2 and 12.000 km^2, respectively. Thus, the conservation status of *V. montana* was provisionally assessed as endangered (EN) based on Criterion B and D (B1ab+2ab (iii); D1).

Discussion

Reproductive Structures and Stages of V. montana

In this study, the lips were observed to vary in colour, in which some were dark red, instead of purple. Similar occurrences were reported in *Vanilla borneensis* Rolfe, ranging from reddish to pinkish lips, and *Vanilla kinabaluensis* Carr with whitish to yellowish tepals (Cribb, 2014). The variation is speculated to be associated with

the responses of plant pigments, flavonoids, in which their modification is affected by the age of the plant, the surrounding environment, as well as sugar and organic acid availability as some of the influencing factors (Miller *et al*., 2011). Thus, the identification of a *Vanilla* species using colour has its limitations, and morphological characteristics are considered to be more significant. The microstructure documentation of both rostellum and stigmata was beneficial in the species discrimination, as well as to comprehend the species pollination strategy. Changes in the rostellum overall size and stigmata appearance could determine the species' capacity to undergo self-fertilisation via rostellum dehydaration, or "stigmata leak" (Lubinsky *et al.*, 2006). The first description of the *V. montana* stigmata showed generic characteristics of having a transversely oblong to rectangular shape, with small lobes on the apex as the other Peninsular Malaysian species, suggesting the genus to possess plesiomorphic morphology from its progenitor, except for the size. There was a distinct reduction in the stigmata, with a larger width, approximately 50 %, in both measurements recorded in other localities (Raffi *et al*., 2017a; 2017b). The angular feature of the pod was a continuous trait derived from the superior ovary since all the available ovaries were observed to have a straight lateral line, forming a slightly obcordate surface, which would contribute to this unique character.

In the flowering evocation process, drastic climate changes were noted to be responsible for inducing the flowering of matured vines, which were similar to the proposed environmental cues of *V. planifolia* Andrews by Hernández and Lubinsky (2010). Higher light intensity was also deduced as one of the contributors in initiating and sustaining the plant reproductive stages. Puthur (2005) reported that the increment of light intensity on the shaded area in a *Vanilla planifolia* plantation in India had resulted in a high production of flowers per raceme and pods per plant. The anthesis peak time frame was noted to be congruent with *Vanilla norashikiniana* Go et Raffi (Go & Raffi, 2017), suggesting that the optimum viability of both pollen and stigmata to ensure successful pollination most likely occurs before noon. Although the notes on the pollination mechanism by ants were not reported in the *Vanilla*, observations on *Spathoglottis* and *Chenorchis* proved ants as promising pollinators that were attracted to the extra-cellular nectarines, eventually promoting pollen transfers between flowers on the same inflorescence (Liu *et al*., 2008; Nordin, 2018). This study presented a new insight on the in-situ florivory phenomenon on *V. montana*, which could be classified under the direct trophic effect that resulted in a negative impact on the flower's viability and capsule production (McCall & Irwin, 2006). The foraging behaviour by the unidentified small mammal was speculated to be driven by the extra cellular sugar-contained liquid offerings of the flowers, and these were in accordance to the attributes reported in both taxa groups (Weber *et al*., 2015; Popic *et al.*, 2016). Florivory in the genus *Vanilla* within its natural habitat was considered very rare and the only possible florivory documentation from Southeast Asia was observed in the *Atractomorpha* visitation on *Vanilla aphylla* Blume (Tan *et al.*, 2017). Malo *et al*. (2001) reported that florivory incidences in Orchidaceae were higher if the populations were small and prone to human disruption, which is relevant to the *V. montana* population in Negeri Sembilan.

Conservation Status Assessment

Vanilla montana is provisionally assessed as endangered due to its biogeographical distribution and relatively small number of natural populations. The species may be facing the possibility of extinction, similar to *Vanilla cribbiana* Soto Arenas, *Vanilla hartii* Rolfe, *Vanilla inodora* Schiede, *Vanilla insignis* Ames, *Vanilla odorata* C.Presl, *Vanilla phaeantha* Rchb.f., *Vanilla planifolia* Andrews, *Vanilla pompona* Schiede, and *Vanilla somai* Hayata (IUCN, 2020). The endemic montane flora of Peninsular Malaysia, including orchids that occupy a restricted geographical range, were regarded to be highly vulnerable to extinction, especially from habitat disruption (Kiew,

1992). Prolonged negligence in implementing fundamental conservation measures would cause *V. montana* to experience the same fate as other rare orchids, such as *Corybas*, whose extinction risk to the whole member of this peninsula was leveled up to critically endangered (Go *et al*., 2015b). Bory *et al.* (2008) suggested that the best approach to conserve the wild populations is via in-situ conservation and this is in agreement with previous ex-situ observations of collected *Vanilla* cuttings, which were usually subjected to vigorous leave abscission and lengthy consequential dormancy. Therefore, three field conservation approaches are proposed as pragmatic measures to protect the natural populations, which are periodical survey, reintroduction of affected population in the same locality with a similar elevation using cuttings from the initial individuals, as well as performing hand-pollination across flowers from different vines (if available) to ensure the success of pod development and to broaden the species' genetic diversity. These actions are expected to significantly maintain and increase the number of individuals in the known localities.

Conclusion

The botanical profile of *V. montana* of Peninsular Malaysia is updated with some insights on the rarely documented events during its flowering stage. The present distribution pattern suggests that the species should be categorised as endangered, and immediate conservation measure must be implemented to prevent the drastic depletion of this endemic species.

Acknowledgements

The authors are grateful to Ong Poh Teck from the Forest Research Institute Malaysia (FRIM) and the Negeri Sembilan Forestry Department for their assistance during the study. This study was financially supported by the Geran Insentif Pusat Pengajian Sains Kajihayat, Universiti Sains Malaysia. We thank Mohd Tajuddin Abdullah (UMT), Micheal Charles Rajaram and anonymous reviewers for their comments to improve the initial draft manuscript. The first author was supported by the Centre for Research and Innovation (CRIM) postdoctoral grant to the Institute of Biodiversity and Sustainable Development (BIO-D TROPIKA), Universiti Malaysia Terengganu (UMT).

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