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LEAFLET-CUTTING PROPAGATION OF *ATHYRIUM ACCEDENS* (BLUME) MILDE (WOODSIACEAE)

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ABSTRACT. — *Athyrium accedens* (Blume) Milde (Woodsiaceae) is a recently recorded species of fern in Singapore. It is considered to be nationally critically endangered as it has been only reported near and along small streams in Dairy Farm Nature Park. Ex situ conservation of the species may be a viable option if there is a suitable method to propagate it. A method of vegetative propagation by leaflet-cutting using sections of the rachis each with an attached pinna is tested. We report on the success of producing independent plantlets complete with roots within 12 weeks ready for planting out. Interestingly, it was also discovered by accident that the spores of the species germinated well on sphagnum moss and the resulting gametophytes started to produce recognisable sporophytes within 18 weeks. Thus, both clonal propagation by leaflet-cuttings and non-aseptic spore germination are viable ways of producing propagation material for the species.

KEY WORDS. — *Athyrium accedens*, Woodsiaceae, Singapore, conservation status, leaflet-cutting, spores

INTRODUCTION

This paper seeks to document the sightings and propagation of a recently recorded fern species, *Athyrium accedens* (Blume) Milde in Singapore (Lai, 2010). *Athyrium accedens* is a member of the fern family Woodsiaceae, which consists of 15 genera and approximately 700 species worldwide (Smith et al., 2006, 2008). Most of the species are terrestrial and have subcosmopolitan distribution. About 85% of the family members belong to two major genera, *Diplazium* and *Athyrium*. In Singapore, Chong et al. (2010) reported 10 *Diplazium* species.

Athyrium accedens is a relatively large fern, up to 1.8 m tall. The green stipes are covered with prominent protuberances. Usually for younger stipes, brownish toothed scales are found at the end of the tips of these protuberances. The fern possesses pinnate fronds that are about 180 by 50 cm. Each mature frond has up to 15 pairs of shallowly lobed pinnae, which are gradually reduced in size towards the base of stipes. The pinnae, 9–29 by 3.5–7 cm, are oblong and gradually taper to an acute apex. Adjacent groups of veinlets often unite to form series of areoles. Sori are distinctly J-shaped and arranged along the veinlets. A vegetative bud is sometimes present at the base of the pinnae. These buds develop into plantlets over time and may serve as a means of vegetative propagation.

PAST AND PRESENT RECORDS

Lai (2010) first sighted the fern in 2010 at Diary Farm, Bukit Timah Nature Reserve. Three specimens were deposited at the Herbarium, Singapore Botanic Gardens (SING; Table 1). We observed that the species is represented by not more than 30 mature plants, in three clusters within a radius of 150 m. Two of these are located along a stream on loose sandy soil exposed by erosion, and one is found on dry land under shade within 50 m of the stream. This generally agrees with the observation of Holttum (1966) that it is a species found along shady stream banks. Given its rarity, we would like to suggest a conservation status of Critically Endangered for *Athyrium accedens*, a new record for Singapore.

Table 1. Previous Singapore collections of *Athyrium accedens* (Blume) Milde deposited in the Herbarium, Singapore Botanic Gardens (SING).

S/No.	Bar Code No.	Collector	Collector's No.	Date	Locality
1.	0153627	J. Lai	LJ850	26 Aug.2010	Dairy Farm, BTNR
2.	0153628	J. Lai	LJ850	26 Aug.2010	Dairy Farm, BTNR
3.	0153629	J. Lai	LJ850	26 Aug.2010	Dairy Farm, BTNR

MATERIAL AND METHODS

Leaflet-cutting. — Leaf material for the leaflet-cutting propagation experiment was collected from a group of plants along Wallace Trail of Dairy Farm Nature Park on 11 Jul.2012 by CKY, SYT, and CYK. Three plants of about 1.5 m tall were selected and a whole leaf was collected from each plant. Two partial leaves were also collected from two of the above-mentioned plants.

The leaves were cut up into rachis sections (also referred to as "leaflet-cuttings"). Only completely free rachis sections, each with a pinna, were used in the experiment, thus the terminal pinnae were discarded. The rachis was trimmed so that each section has only 1–2 cm of the rachis protruding from the basiscopic side and less than 1 cm protruding from the acroscopic side. Each pinna was cut back to a length of about 3 cm to reduce transpiration. The presence of developing adventitious bud on each section and the stage of development of the bud were noted. The stage of development were recorded as "bud", i.e., globular structure without clear leaf or root (Fig. 1d); "plantlet", i.e., with leaf (or leaves) discernible only (Fig. 1e); or "independent plantlet", i.e., with leaf (or leaves) and root(s) discernible (Fig. 1f). Stages prior to the independent plantlet stage are collectively termed "incipient plantlet" stages.

The sections were randomly assigned to be planted (basioscopic side down) in wet sphagnum moss or a soilless potting medium mixture consisting of equal parts Plantaflor Humus (Verkaufs-GmbH, Germany), vermiculite, and coconut husk cubes (Fig. 2a). Humidity was maintained by placing the cuttings in a glass tank covered with a clear plastic sheet at the top. The sections were placed under a 12-hour daily lighting regime using Super Sun T5 (AquaZonic) fluorescent light tube providing 32.33 $\mu molm^{-2}$ of illumination (averaged from three measurements taken with LI-250A light meter [LI-COR Biosciences, USA]).

The cuttings were examined once every two weeks and their development was recorded, following the terminology detailed earlier for "the stage of development of the bud". This continued until a cutting died or when an "independent plantlet" developed and detached itself from the cutting and the cutting dies. Should the cutting still be alive in the second scenario, it would be observed till the end of the experiment or till it died, whichever came to pass earlier.

Statistical analysis. — The mean duration of the survival of leaflet-cuttings that failed to produce plantlets, and the mean duration to the production of independent plantlets were calculated for the "treatments" using different media and presence or absence of adventitious buds (incipient plantlets). Fisher's exact test was done to detect any departure from random the presence of incipient plantlet or choice of media and successful production of independent plantlets by leaflet-cuttings.

RESULTS AND DISCUSSION

One hundred leaflet-cuttings were made, out of which 10 produced independent plantlets. Of the cuttings that produced plantlets, nine had incipient plantlets and one did not. Of the 90 cuttings that did not successfully produce an independent plantlet, only four had incipient plantlets. Leaflet-cuttings that failed to produce plantlets survived for 5.22 ± 3.64 weeks (mean ± standard deviation), while cuttings that produced plantlets survived for 8.60 ± 3.53 weeks. Mean duration till the production of independent plantlets was 5.00 ± 1.70 weeks. From Fisher's exact test, it was clear that the successful production of independent plantlets was non-randomly related to the presence of incipient plantlets (one-tailed $p = 0.000$), i.e., the presence of incipient plantlets in leaflet-cuttings would more often than not "predict" the successful formation of incipient plantlets.

Of the cuttings, 49 were placed in soilless potting medium mixture and 51 in sphagnum moss. Six successfully produced independent plantlets in the former and four in the latter. Fisher's exact test showed that there was no significance difference between the two media (one-tailed $p = 0.345$). However, interestingly, leaflet-cuttings that had failed to produce plantlets survived for 4.38 ± 3.22 weeks in sphagnum and 6.14 ± 3.89 weeks in soilless potting medium mixture. The duration till the production of independent plantlets was 4.50 ± 1.00 weeks in sphagnum and 5.33 ± 2.07 weeks in soilless potting medium mixture. Therefore, while there may not be any difference in the media in successfully in producing plantlets, soilless potting medium mixture appears to keep the cuttings alive for longer, but the plantlets take longer to reach independence.

A completely randomised two-way ANOVA with Type II sums of squares was done to detect any difference in the above two factors (medium choice and presence of incipient plantlets) on the duration of survival of cuttings that failed to produce plantlets, and on the duration till production of independent plantlets. The type of substrate used was found to significantly increase the survival of cuttings that failed to produce plantlets ($p = 0.037$), but it did not influence the time needed for producing independent plantlets. The presence of incipient plantlets had no significant effect. Therefore, soilless potting medium mixture did keep the cuttings alive for longer, though the longer survival had no detectable effect in promoting formation of independent plantlets.

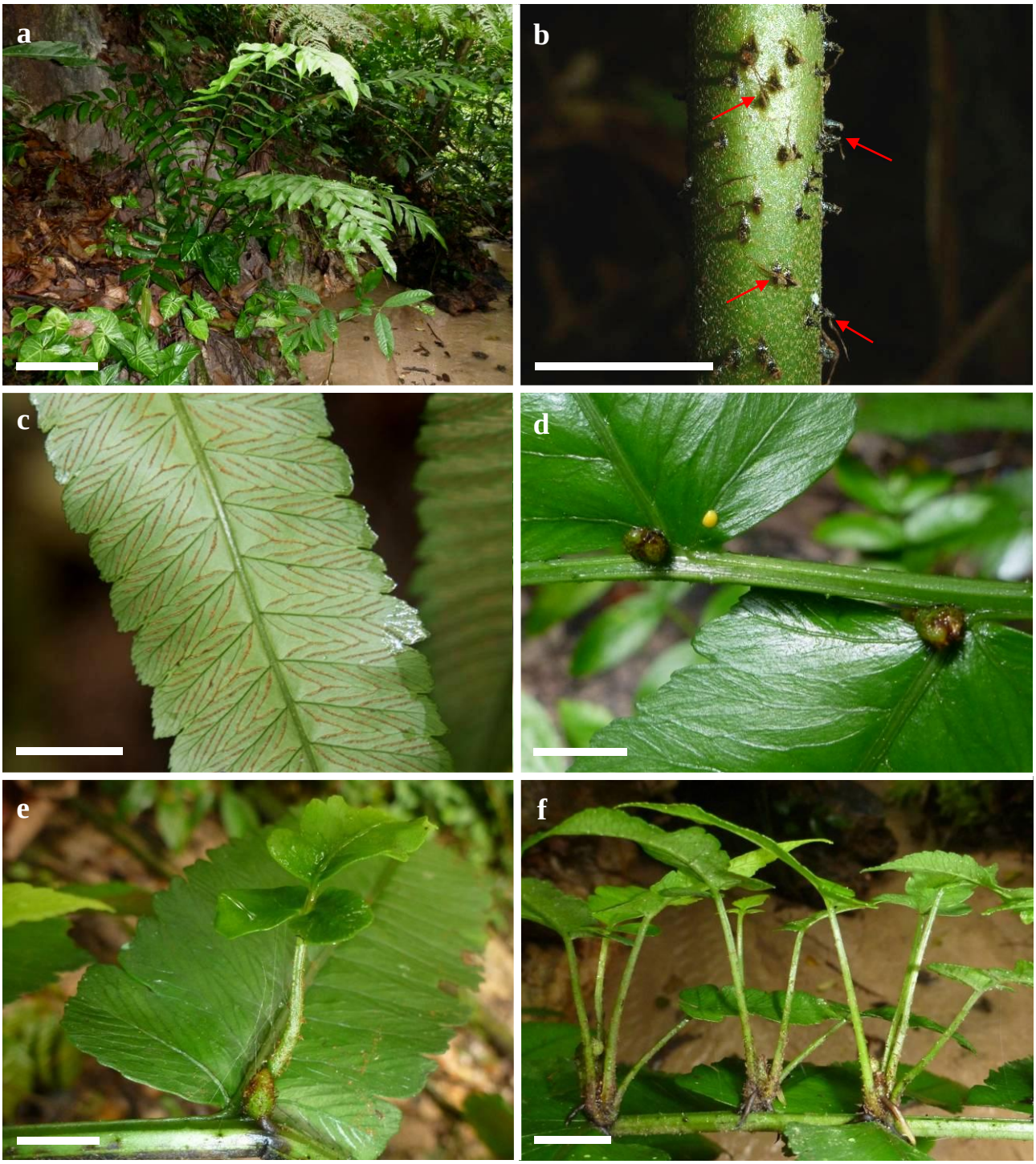

Fig. 1. *Athyrium accedens*: a, habit; b, close up of a young stipe with protuberances bearing minute scales (arrowed) at the end of the tips; c, close up of lower surface of a fertile pinna. The sori are arranged along the veinlets. d, adventitious buds at the base of pinnae; e, plantlet with single young frond growing out from a bud; f, plantlets, with prominent fronds and roots, that will soon detach from the parent plant as new independent individuals. Scale bars = 50 cm (a), 1 cm (b, d, e), and 2 cm (c, f). (Photographs by: Yeo Chow Khoon [a, b, d–f] and Koh Choon Yen [c]).

We conclude that the leaflet-cutting propagation method has a reasonable success rate at producing clonal plantlets within about six weeks if leaf-cuttings with incipient plantlets were used. Thus, resources for plant propagation could be used most economically by not choosing to take cuttings without incipient plantlets. This practice would also ensure that the stock plant providing the source of cuttings would be harmed less by having leaves without incipient plantlets left largely intact.We also noticed that each leaflet-cutting did not produce multiple plantlets as we had hoped for, and that keeping the leaflet-cutting alive for a prolonged duration did not yield more plantlets.

As we have not compared the yield of plantlets by our leaflet-cutting propagation method to a healthy intact leaf on the plant, we cannot ascertain if our method would produce more plantlets than an intact leaf over its whole natural

Fig. 2. Experimental set-up: a, rachis sections on sphagnum moss; b, gametophyte at about 10 weeks old; c, young sporophyte at 18 weeks. Scale bars = 5 cm (a) and 2 cm (b, c). (Photographs by: Yeo Chow Khoon).

lifespan. However, we are confident that our method has the distinct advantage of keeping the leaflet-cuttings in a controlled and safe environment for us to collect all the plantlets produced, while plantlets produced by a plant growing in the open in nature or under cultivation may be easily dislodged and lost to a horticulturist interested in propagating the fern. Furthermore, the dislodged plantlets may not find suitable sites for their establishment, while our method will certainly allow all plantlets a good chance of surviving and reaching maturity.

We also noted that sporophytes start developing from the gametophytes as early as eight weeks after spores had been inadvertently scattered on the sphagnum medium in the same tank that the leaflet-cutting experiment was performed in (Fig. 2b), while few gametophytes were seen developing on the soilless potting medium. The sporophytes were recognisable of our study species at about 18 weeks after the “sowing” of the spores on sphagnum (Fig. 2c). This shows that the conditions for producing plantlets from the leaflet-cuttings on sphagnum are also suitable for the spore

germination of this species. Thus, the fern could easily be brought into cultivation without the application of costly sterile methods such as micropropagation.

CONCLUSIONS

In conclusion, while *Athyrium accedens* is known to be a species with specific habitat preferences, we believe that it is probably restricted to these habitats owing to the fastidious requirement of the gametophytes and limited dispersal ability of the adventitious plantlets. The adventitious plantlets may also have low establishment rate after dispersal, which seems to be supported by the small numbers of mature plants seen despite the numerous plantlets found on the wild individuals. The existence of one cluster of plants on dry land is indicative that the species can accept a broader range of environmental conditions than what was previously suspected. This is corroborated by our observations of the plants we grew from plantlets doing well under light shade (<10%) with daily watering in our nursery. Therefore, the production of propagation material for planting in suitable locations without depleting the wild stock is clearly a viable way of conserving the rare species with ornamental potential. We have shown that this can be done both clonally and sexually.

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