A taxonomic study of the *Pandanus furcatus* and *P. tectorius* complexes (Pandanaceae) in Java

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ABSTRACT. Current taxonomic problems in *Pandanus* in Java include the interpretation of the *Pandanus furcatus* complex as well as the *P. tectorius* complex. A study of general morphological, stomatal and molecular characteristics (viz., the noncoding chloroplast intergenic spacer region *atp*B-*rbc*L) showed that *P. bantamensis* Koord., *P. pseudolais* Warb., and *P. scabrifolius* Martelli, previously considered synonyms of *P. furcatus*, and *P. tectorius* var. *littoralis* Martelli and *P. odoratissimus* L.f. are all distinct species.

Keywords. Anatomy, *atpB-rbcL*, Java, morphology, *Pandanus furcatus* complex, *Pandanus tectorius* complex

Introduction

The term species complex was used to describe a species aggregation sharing specific morphological and molecular features (Judd et al. 1999). Within such a complex, a complicated morphological overlap, without any discontinuities, has led to taxonomic difficulty (Pak and Kawano 1992). Although their taxonomic affinity may be difficult to determine, some form of taxonomic resolution is desirable.

According to Stone (1972), *Pandanus* Parkinson in Java contains many rather problematic species. He suggested that detailed studies were required to obtain a more refined taxonomic scheme. The main problem, as far as Javan plants are concerned, appears to be the status of taxa which are given as synonyms of *P. furcatus* Roxb. by Backer and Bakhuizen van den Brink f. (1968). These synonyms include *P. bantamensis* Koord., *P. oviger* Martelli, *P. pseudolais* Warb. and *P. scabrifolius* Martelli. Also, *P. odoratissimus* L.f was thought to be synonymous with *P. tectorius* var. *littoralis* Martelli. Stone (1972) made a short revision of Pandanaceae in Java based on herbarium specimens in the BO herbarium and living plants cultivated in the Hortus Bogoriensis, attempting to develop a more stable species concept for *Pandanus*

furcatus Roxb. In Stone's opinion, Backer's species concept for *Pandanus furcatus* was far too comprehensive and required readjustment because distinct species were lumped with *Pandanus furcatus*. Stone regarded *P. bantamensis*, *P. oviger*, *P. pseudolais* and *P. scabrifolius* as four different species, whereas the status of *P. tectorius* var. *littoralis* and *P. odoratissimus* was still more or less in question. The circumscription of these taxa are reviewed in light of new studies based on material from our recent fieldwork in Java. A practical difficulty here was that almost all morphological characters and/or character states used for evaluating a species complex are only slightly differentiated from one another and usually show considerable overlap. Therefore the identification of taxa within a species complex usually required a combination of several characters. The aim of the study was to provide a taxonomic resolution of the *P. furcatus* complex and *P. tectorius* complex, based on general and stomatal morphology, and a molecular approach utilising the noncoding chloroplast intergenic spacer region *atpB-rbcL*.

Materials and methods

Studies of herbarium specimens were conducted in the Herbarium Bogoriense (BO), Herbarium of the Royal Botanical Gardens, Kew (K) and National Herbarium of the Netherlands, Leiden (L). Observations of living plants and stomatal studies were undertaken at BO, while the molecular data was analysed in the Van der Klauw Laboratory, Leiden.

Data on morphology were collected from herbarium specimens and fresh field collections. The procedure for treating morphological variation followed that described by Rifai (1976) and Vogel (1987). Measurements were taken from spirit-preserved material, dried herbarium specimens and living material. Floral parts were measured from spirit-preserved material, and material rehydrated (by boiling) from dried specimens.

Leaf stomatal characteristics were investigated by first fixing the leaves (a small part of the middle to basal area) in FAA. Paradermal sections were taken from the upper and lower surfaces of leaves, then stained with safranin 1% in water and mounted in glycerine (Johansen 1940).

Genomic DNA was extracted from leaf material dried in silica gel according to the protocol described by Doyle & Doyle (1987). Double-stranded DNA was directly amplified by PCR. Reaction volumes were 25 µl and contained 2.5 µl PCR buffer, 2.5 µl dNTPs, 1 µl each of the 5 mM primers, 0.3 µl *Taq Pol* and 12.7 µl ddH_2O. Approximately 5 µl genomic DNA was added to the PCR mixture. PCR was performed 3 min at 94°C for the activation of the polymerase, followed by 35 cycles of 49 sec at 94°C, 45 sec at 55°C, 2 min at 72°C, with a final extension period of 10 min at 72°C. The primers used in this study for *atpB-rbcL* intergenic spacers are (forward 5'-GAAGTAGTAGGATTGATTCTC- 3') and (reverse 5'-TACAGTTGTCCATGTACC AG-3'). The PCR product was checked on 1% agarose gel, and purified using a purification kit of Wizard SV Gel and PCR clean up system (PROMEGA) following the manufacturer's protocol prior to sequencing. The DNA

concentration was measured with the nanodrop. Cycle sequencing was performed by Macrogen Korea. The sequences were edited using sequencher 4.6 and MEGA 3.0 (Kumar et al. 2004).

Results

Morphological characters of the Pandanus furcatus complex

For this study, we found only three of the four species recognised within the *P. furcatus* complex, i.e., *P. bantamensis*, *P. pseudolais* and *P. scabrifolius*. Several characters studied are compared in Table 1. Prickles on prop-roots did not appear to display consistent differentiation among taxa, although *P. scabrifolius* did not have prickles on the prop-roots. Leaf dimensions were highly variable. *Pandanus pseudolais* tended to have longer leaves, whereas the leaves of *P. scabrifolius* had a shorter range of length measurements. *Pandanus bantamensis* had intermediate leaf length. The number of drupes per cephalium of *P. pseudolais* was higher than in the other two taxa. *Pandanus scabrifolius* had longer drupes compared with the other two taxa.

Four other characters studied were leaf base colour, peduncle shapes, fruit shapes and style shapes, that appeared to be useful for discriminating among species (Table 1). *Pandanus bantamensis* and *P. pseudolais* had a reddish brown leaf base, and *P. scabrifolius* a yellowish white leaf base. The peduncle was stout and quite straight in *P. scabrifolius*, and curved in the other two taxa. *Pandanus scabrifolius* also had a broadly ellipsoid cephalium, compared to the narrower cephalia in the other two species; it also had comparatively shorter style bifurcations (divisions a third the style length or less) than in the other two species (divisions about half the style length).

Table 1. The *Pandanus furcatus* complex: some morphological features of the vegetative parts, peduncle, fruiting cephalium, drupes and style in the three taxa studied.

Character	*P. bantamensis*	*P. pseudolais*	*P. scabrifolius*
Leaf length (cm)	216–441	299.4–574.5	204–372
Prickles on prop-root	present, prickles in rows	present, prickles in rows	smooth
Number of drupes	475–795	724–1053	473–483
Drupe length (cm)	2.7–4.5	2.6–4.5	4.8–5.2
Leaf base colour	Reddish brown	Reddish brown	Yellowish white
Peduncle shape	Slightly curved at the base	Strongly curved at the base	Straight
Cephalium shape	Narrow-ellipsoid	Narrow-ellipsoid	Broad-ellipsoid
Style bifurcation	Divisions about half the style length	Divisions about half the style length	Divisions a third of the style length or less

Morphological characters of the Pandanus tectorius complex

Leaf dimension, leaf shape, cephalium size and shape, and the number of phalanges per cephalium did not have any consistent differences between the taxa analysed (Table 2). However, *P. tectarius* var. *littoralis* had leaf apex prickles only on one surface (*P. odoratissimus* has prickles on both surfaces of the leaf apex); and carpel apices that were essentially fused, withour any deep grooves between carpels (*P. odoratissimus* had carpel apices that were free, leaving deep grooves between carpels) (Table 2).

Table 2. The *Pandanus tectorius* complex: morphological characteristics of *P. tectorius* var. *littoralis* and *P. odoratissimus*.

Characters	*P. tectorius* var. *littoralis*	*P. odoratissimus*
Leaf length (cm)	112–199	98–126
Leaf shape	ligulate or sword-shaped	ligulate or sword-shaped
Cephalium size (cm)	26 × 25	22 × 25
Cephalium shape	broad-ellipsoid	broad-ellipsoid
Number of phalanges	79–83	73–83
Prickles on leaf apex	only on one side of leaf, the other side smooth	on both sides of leaf
Carpel apices	fused, without deep grooves between carpels	not fused, with deep grooves between carpels

Stomatal characters

Five classes of stomatal features (Tomlinson 1965, Kam 1971) are known in *Pandanus*, depending on the number of papillae that develops on the subsidiary and neighbouring cells. In the *Pandanus furcatus* complex, *P. bantamensis*, *P. pseudolais* and *P. scabrifolius* had stomatal Class 2 (papillae only occurring on lateral subsidiary cells), Class 1 (papillae absent: unspecialized stomata) and Class 3 (papillae in both terminal and lateral subsidiary cells) of Tomlinson (1965), respectively. Within the *Pandanus tectorius* complex, *P. tectorius* var. *littoralis* had stomatal Class 4 (papillae occurring in both lateral subsidiary and neighbouring epidermal cells) and *P. odoratissimus* had stomatal Class 2 (papillae only occurring on lateral subsidiary cells).

Molecular characteristics

Although the small number of taxa investigated would not be expected to yield any meaningful phylogenetic analysis, the *atpB-rbcL* intergenic spacer region provided potential markers for distinguishing the different species in both of the complexes.

There were 29 polymorphic sites in this region for the three species of the *Pandanus furcatus* complex investigated (Table 3), of which 19 sites were different between *P. bantamensis* and *P. pseudolais*, 24 sites were different between *P. pseudolais* and *P. scabrifolius*, and 21 sites were different between *P. bantamensis* and *P. scabrifolius*. Likewise, there were 71 polymorphic sites in this region for the two species of the *Pandanus tectorius* complex investigated (Table 4).

Table 3. The *Pandanus furcatus* complex: polymorphic sites in the *atpB-rbcL* intergenic spacer region.

Species	Nucleotide at polymorphic site at indicated position																		
	4	6	16	17	18	121	123	149	150	166	167	168	249	271	272	273	292	293	296
P. bantamensis	A	G	A	A	A	C	C	G	A	A	G	T	T	A	A	T	T	T	A
P. pseudolais	T	T	A	C	C	A	T	A	A	A	A	T	A	G	G	A	A	T	A
P. scabrifolius	T	G	C	C	T	T	C	A	G	G	T	G	T	G	A	G	T	A	T

Species	Nucleotide at polymorphic site at indicated position									
	297	446	447	448	520	521	522	550	551	552
P. bantamensis	G	T	A	G	A	C	C	T	C	A
P. pseudolais	T	G	G	G	A	T	A	T	T	A
P. scabrifolius	A	T	A	A	T	A	C	A	T	C

Discussion

Pandanus furcatus complex

The species recognised within the *Pandanus furcatus* complex shared some morphological similarities, but could be distinguished by leaf base colour, peduncle shape, fruit shape, style shape, and stomatal characteristics. A number of sites in the *atpB-rbcL* intergenic spacer region were also polymorphic for these species. We conclude that *Pandanus bantamensis*, *Pandanus pseudolais* and *Pandanus scabrifolius* are three distinct species. Our study thus corroborates the conclusions of Stone (1972).

Table 4. The *Pandanus tectorius* complex: polymorphic sites in the *atpB-rbcL* intergenic spacer region.

Species	Nucleotide at polymorphic site at indicated position															
	31	32	33	61	62	63	85	86	87	115	117	119	120	199	200	201
P. tectorius var. *littoralis*	A	G	T	T	C	T	T	A	A	G	T	T	C	C	T	C
P. odoratissimus	G	T	A	G	A	C	G	T	T	A	A	A	G	T	G	A

Species	Nucleotide at polymorphic site at indicated position															
	204	229	231	232	233	241	242	260	269	334	335	336	380	405	438	441
P. tectorius var. *littoralis*	T	A	G	G	A	A	T	T	T	C	A	T	A	G	C	C
P. odoratissimus	A	G	T	T	C	T	A	A	A	T	T	G	T	A	A	C

Species	Nucleotide at polymorphic site at indicated position															
	464	465	476	477	507	520	521	571	573	578	579	586	589	590	591	608
P. tectorius var. *littoralis*	A	G	A	A	G	T	C	A	A	A	C	G	A	A	T	T
P. odoratissimus	G	A	T	T	C	A	A	T	T	G	G	C	C	C	A	G

Species	Nucleotide at polymorphic site at indicated position															
	609	613	614	623	739	740	748	770	771	774	775	789	809	810	856	857
P. tectorius var. *littoralis*	G	A	C	T	C	A	T	C	A	A	C	T	G	T	A	G
P. odoratissimus	A	T	T	C	A	G	G	A	C	G	A	G	A	A	T	A

Species	Nucleotide at polymorphic site at indicated position						
	858	895	896	897	901	902	903
P. tectorius var. *littoralis*	A	A	A	A	G	G	A
P. odoratissimus	C	G	T	C	T	C	C

Key to three species of the *Pandanus furcatus* complex

1a. Leaf base reddish brown; cephalium narrowly ellipsoid (the length almost 3 times the width); style bifurcations about half the style length 2
1b. Leaf base yellowish white; cephalium broadly ellipsoid (the length at most twice the width); style bifurcations a third of the style length or less ..... *P. scabrifolius*

2a. Peduncle slightly curved at the base; cephalium with 475–795 drupes; stomata type "Class 2", with papillae on lateral subsidiary cells ..... *P. bantamensis*
2b. Peduncle strongly curved at the base; cephalium with 724–1053 drupes; stomata type "Class 1", without papillae ..... *P. pseudolais*

Pandanus tectorius complex

Although *Pandanus tectorius* var. *littoralis* and *P. odoratissimus* are very closely related (Stone 1994). Stone (1967) had proposed delimiting *P. odoratissimus* by just two characters: fleshy shoulders on phalanges and large white spines on the leaves. In our study, *P. odoratissimus* was found to have large white spines on the leaves as described, but the same could also be found in *P. tectorius* var. *littoralis*. *Pandanus odoratissimus* also does not have fleshy shoulders on the phalanges. Our observations tally with those of Stone (1979) who stated that *P. odoratissimus* has large white spines, but does not have fleshy shoulders. In this study, we have found several contrasting morphological characters that could be useful for distinguishing these two species. *Pandanus tectorius* var. *littoralis* has a leaf apex that is prickly on only one side of the leaf and fused carpel apices without any significant grooves in between; whereas *P. odoratissimus* has a leaf apex that is prickly on both sides of the leaf and free carpel tips separated by deep grooves in between. As many as 71 sites in the *atpB-rbcL* intergenic spacer region were also found to be polymorphic between these two taxa. We therefore accept these two taxa as specifically distinct.

Key distinguishing *Pandanus tectorius* and *P. odoratissimus*

1a. Leaf apex prickly only one one side; stomata with papillae on neighbouring epidermal and subsidiary cells; carpel apices fused and without any significant grooves in between ..... *P. tectorius* var. *littoralis*
1b. Leaf apex prickly on both sides; stomata with papillae on lateral subsidiary cells only; carpel apices free and separated by deep grooves in between .... *P. odoratissimus*

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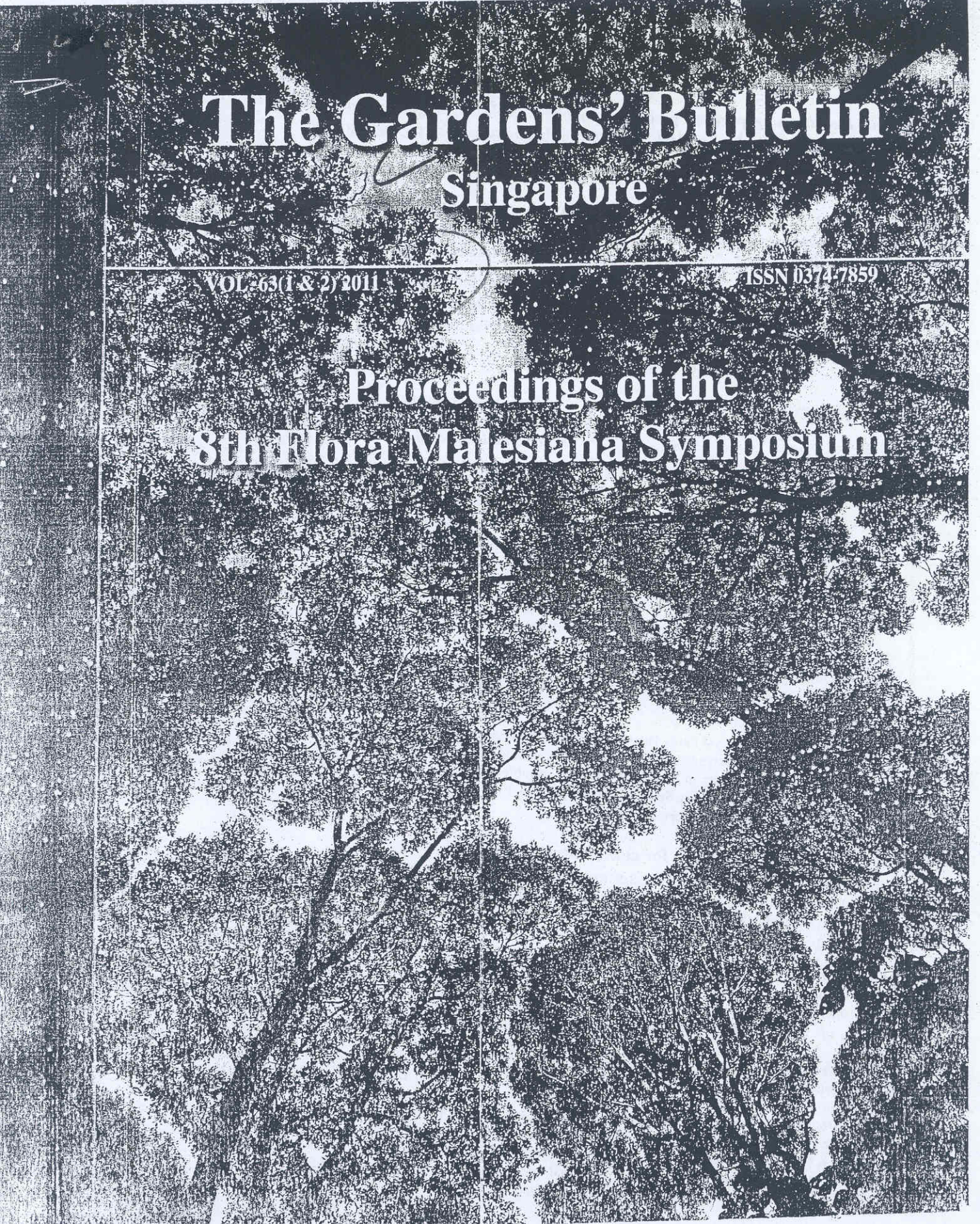

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