

A PHYLOGENETIC PLACEMENT OF DICKSONIOID FERN *Calochlaena javanica* (DICKSONIACEAE)

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Bayu Adjie & Wenni Setyo Lestari. 2014. Penempatan Secara Filogenetika Tumbuhan Paku *Calochlaena javanica* (Dicksoniaceae). *Floribunda* 5(1): 17–20. — *Calochlaena* adalah marga baru yang dipisahkan dari *Culcita* pada tahun 1988, beranggotakan lima jenis. Pohon filogenetika telah dibuat berdasarkan sekuens DNA kloroplas pada gen *rbcL*, dan *C. javanica* tergabung dalam marganya dan juga anggota suku *Dicksoniaceae* lainnya. Deskripsi, distribusi dan morfologi sporanya juga disajikan dalam makalah ini.

Kata kunci: *Calochlaena javanica*, *Dicksoniaceae*.

Bayu Adjie & Wenni Setyo Lestari. 2014. A Phylogenetic Placement of Dicksonioid Fern *Calochlaena javanica* (Dicksoniaceae). *Floribunda* 5(1): 17–20. — The genus *Calochlaena* was separated from *Culcita* in 1988, and consisting of about five species. Based on cpDNA sequences of *rbcL* gene, the phylogenetic tree was made and *C. javanica* is nested within the genus and the rest of dicksonioid ferns. The description, distribution and spore morphology are presented.

Keywords: *Calochlaena javanica*, *Dicksoniaceae*.

The genus *Culcita* C. Presl recognized by Maxon (1922) to have eight species were divided into two subgenera *Culcita* and *Calochlaena*. Significant differences morphologically as any two genera of dicksonioid ferns, it is likely that each is more closely related to other genera of the group than it is to the other subgenus, strongly support that subgenus *Calochlaena* has been elevated correctly to generic rank (White & Turner 1988).

Calochlaena (Maxon) M.D. Turner & R.A. White is a genus of large terrestrial ferns with creeping to semi-erect stems or rhizomes and fronds as long as 1.5 m. The rhizomes seldom forms a true trunk, and *Calochlaena* can only be marginally regarded as a tree fern. The stipe is hairy, and the sori are marginal with bivalved indusia. The sporangium has a long stalk and a well-defined oblique structure (the annulus) situated along one edge. Spores of all species are trilete, with coarse tubercles covering the surface. Chromosome number $n = 55-58$ for *C. dubia* (Large & Braggins 2004).

White & Turner (1988) recorded five species of *Calochlaena* namely *C. dubia* (R. Br.) M.D. Turner & R.A. White, *C. javanica* (Blume) M.D. Turner & R.A. White, *C. novae-guineae* (Rosenst.) M.D. Turner & R.A. White, *C. straminea* (Labill.) M.D. Turner & R.A. White, and *C. villosa* (C. Chr.) M.D. Turner & R.A. White. *Calochlaena* is distributed from Malesia (Java to the Philippines

and New Guinea), Melanesia, and Polynesia to eastern Australia. Plants grow in damp, open area in tropical forest, on hillsides and banks. Several species colonize disturbed sites such as road cuts (Large & Braggins 2004).

Korall *et al.* (2006) have made phylogenetic analysis of major tree ferns using four protein-coding loci. They included two species of *Calochlaena* (*C. dubia* and *C. villosa*), both species nested in the same clade in the core of *Dicksoniaceae*. This paper analyzes the placement of *C. javanica* in the phylogenetic tree based on *rbcL* gene solely.

MATERIALS AND METHODS

DNA Isolation, Amplification, and Sequencing

Genomic DNA was extracted from silica-dried leaf material from living collection of Bali Botanic Garden originated from Mutis NR, Timor island (NTT) using a modified Doyle & Doyle (1987). A region of cpDNA (*rbcL* gene) was amplified using the polymerase chain reaction (PCR) following established protocols (Hasebe *et al.* 1994). The PCR products were purified using the GeneClean III kit (Qbiogene, Irvine, CA, USA) after electrophoresis in 1% agarose gel and used as templates for direct sequencing. Sequencing reactions were carried out with a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). All sequencing reactions

were processed using ABI 310 Genetic Analyzer (Applied Biosystems). Sequence fragments were analyzed using the Sequencing Analysis v5.2 (Applied Biosystems) and assembled by use of SeqScape v2.5 (Applied Biosystems).

Alignment and Phylogenetic Analysis

The *rbcL* sequences were aligned automatically by use of ClustalW (Higgins *et al.* 1994). Aligned sequence was then used for phylogenetic analysis using MEGA 5.1 software (Tamura *et al.* 2011). The distance method (Neighbor-Joining tree) and parsimony (Maximum Parsimony) were used to construct the tree. Reliable tree was calculated with bootstrap test (Felsenstein 1985) in 1000 replicates. For this purposes several *rbcL* sequences of *Dicksoniaceae* family downloaded from GenBank and used as ingroup. *Cibotium ba-*

rometz was chosen as outgroup for rooting based on previous study (Korall *et al.* 2006)

Spore Morphology

The spore morphology was taken by scanning electron microscope at University of Toho, Japan. The SEM was set to 20 kV and photographs taken in 1,500x–5000x magnification.

RESULTS

A 1246 bp of partial *rbcL* gene was successfully sequenced and stored in GenBank with accession number HQ334992. Alignment of *rbcL* sequences with other dicksonioid ferns produced 1183 bp with no indels that used for analysis. The topology of NJ tree was identical with MP tree as presented in Figure 1 with bootstrap value shown along the branches.

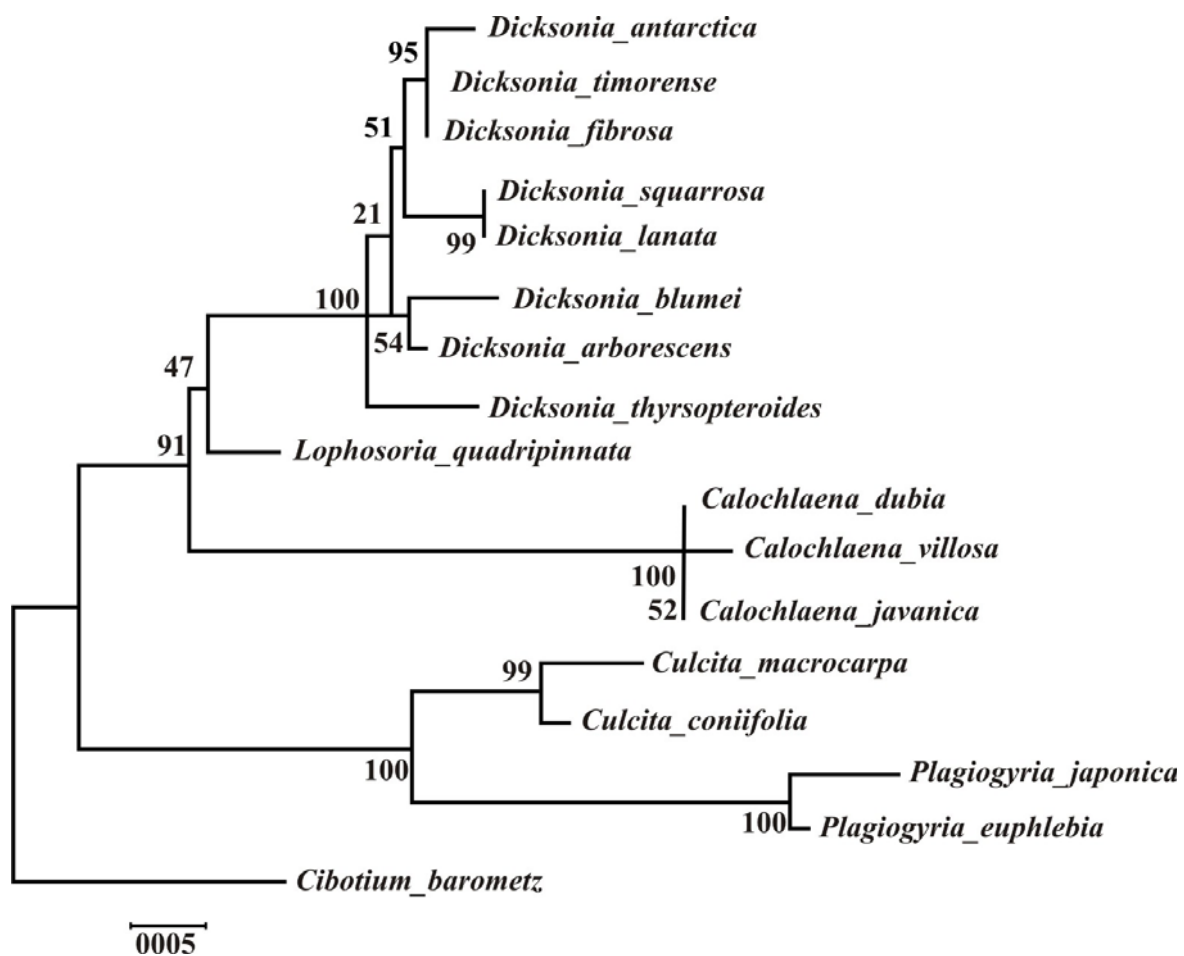


Figure 1. The phylogenetic tree showing the position of *C. javanica*. The tree was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown. The evolutionary distances were computed using the K2P method.

The spore size is about 37 μm , spheroidal in shape. Aperture trilete, the arm $\frac{3}{4}$ the radius, obscured by adjacent ridges (Figure 2).

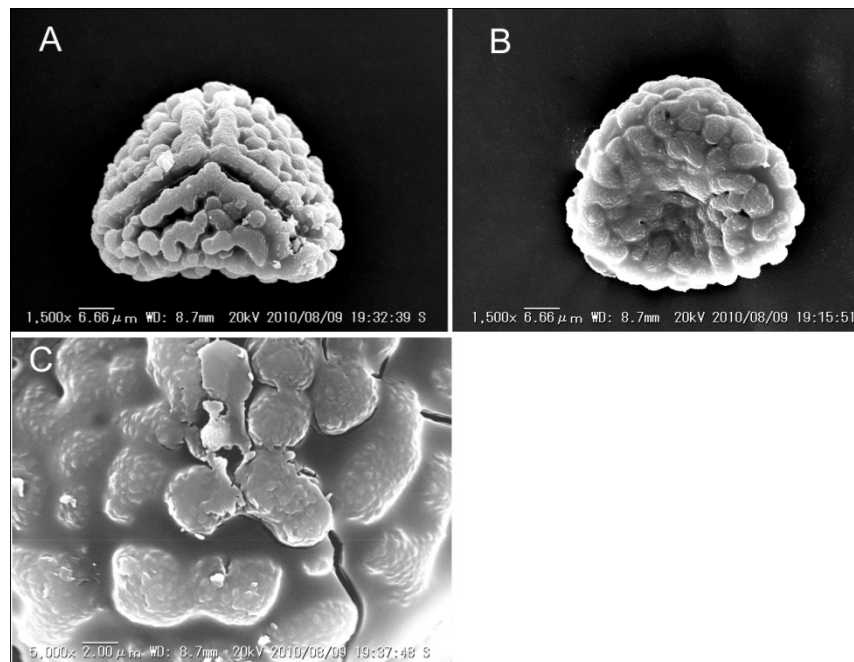


Figure 2. Spore of *C. javanica*. A. Polar view, proximal face, showing laesurae with projected margo and irregularly tuberculate; B. Polar view, showing somewhat depressed distal face; C. part of distal face, showing prominent irregular tuberculate.

DISCUSSION

The *rbcL* sequence of *Cibotium barometz* was used to root the tree, and the core of *Dicksoniaceae* family is monophyletic and clustered into three monophyletic clades. First clade is the genus *Calochlaena*, second *Lophosoria* and third *Dicksonia*. *Dicksonia* sisters to *Lophosoria*, with the dicksonioid genus *Calochlaena* as sister to these two (Fig.1). A close relationship among these three taxa was previously shown by Korall *et al.* (2006). The *Calochlaena* clade supported with very high bootstrap value (100 %), within the clade *C. javanica* was closely related to *C. dubia* and *C. villosa*.

C. javanica and *C. dubia* share similar *rbcL* sequence, however they have structural differences, *C. javanica* have typically dicksoniaceous bivalved sori. The sorus of *C. dubia* appears to be superficial and inframarginal with a single indusial flap (White & Turner 1988). *C. javanica* distributed in Java, Lesser Sunda Islands, Sarawak and North Borneo to Philippines (Holtum 1963), while *C. dubia* is limited to eastern Australia.

The spore of *C. javanica* is remarkably si-

milar and indistinguishable from the other three species of the genus (*C. straminea*, *C. villosa* and *C. dubia*). The spores support recognition of *Calochlaena* as clearly distinct from *Culcita*. The spheroidal shape and prominent tuberculate to verrucate surface differ from spore of *Culcita* and most other genera of *Dicksoniaceae* (Tryon & Lugardon 1991).

Significant differences between *Culcita* and *Calochlaena* have been frequently reported. These include differences in anatomy, spore morphology, and chromosome number (White & Turner 1988). Molecular evidence also suggested separation between these two genera (Korall *et al.* 2006). *Calochlaena* is included in *Dicksoniaceae* and *Culcita* is more closely to *Plagiogyria*. The genus *Culcita* then elevated to become an independent family *Culcitaceae* with two species (Smith *et al.* 2006, Christenhusz *et al.* 2011).

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