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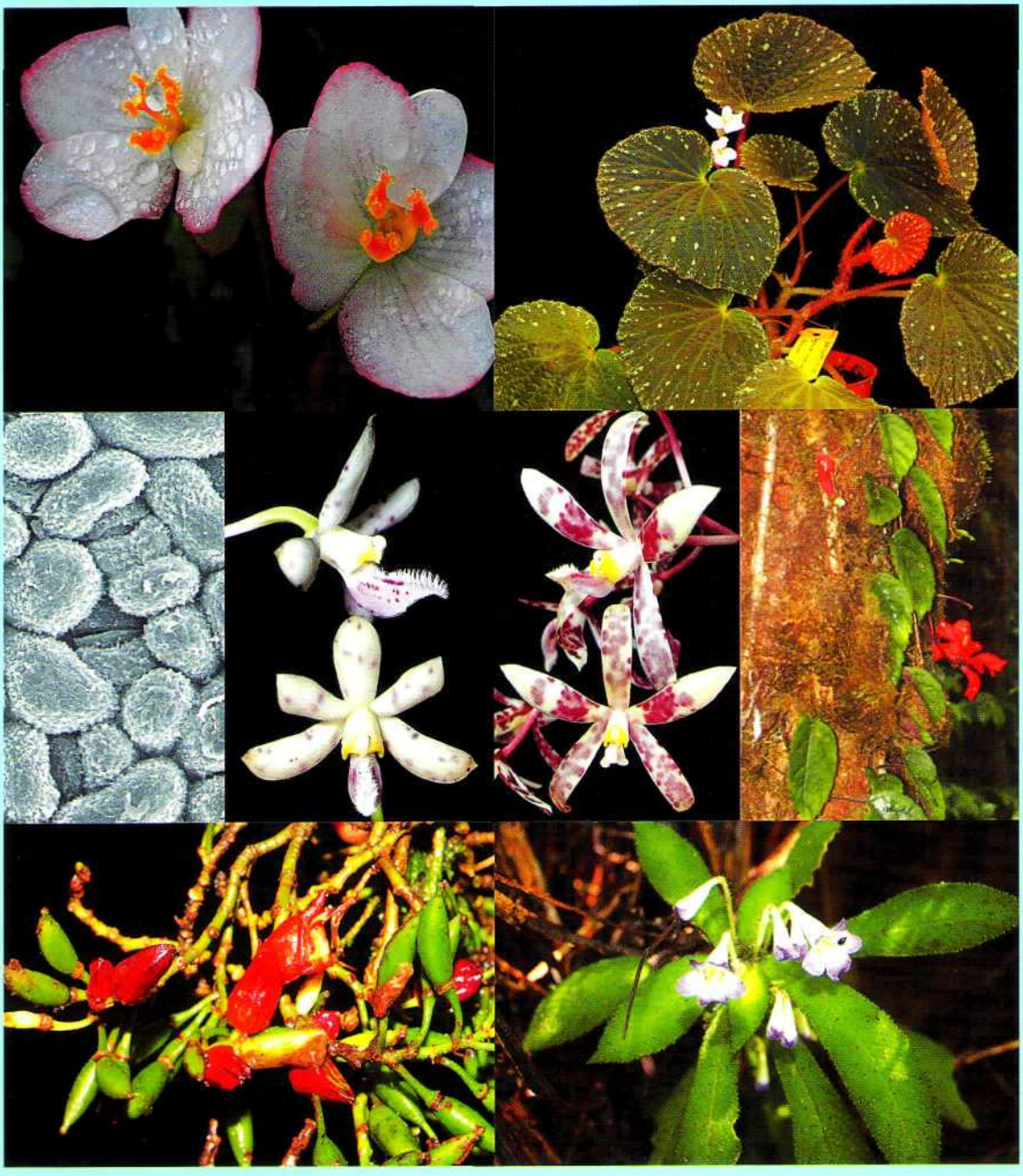

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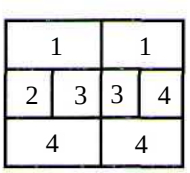

Cover images: 1. *Begonia holosericeoides* (female flower and habit) (Begoniaceae; Ardi *et al.); 2.* Abaxial cuticles of *Alseodaphne rhododendropsis* (Lauraceae; Nishida & van der Werff); *3. Dipodium puspitae, Dipodium purpureum* (Orchidaceae; O'Byrne); 4. *Agalmyla exannulata, Cyrtandra coccinea* var. *celebica, Codonoboea kjellbergii* (Gesneriaceae; Kartonegoro & Potter).

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MOLECULAR PHYLOGENY OF MAIDENHAIR FERN GENUS ADIANTUM (PTERIDACEAE) FROM LESSER SUNDA ISLANDS INDONESIA BASED ON RBCL AND TRNL-F

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ABSTRACT
LESTARI, W. S., ADJIE, B., JARUWATANAPHAN, T., WATANO, Y. & PHARMAWATI, M. 2014. Molecular phylogeny of Maidenhair fern genus *Adiantum* (Pteridaceae) from Lesser Sunda Islands Indonesia based on *rbc*L and *trn*L-F. *Reinwardtia* 14 (1): 143 – 156. — The Lesser Sunda Islands of Indonesia are composed of small islands scattered from Bali to Timor Island. We analyzed a molecular phylogeny of *Adiantum* collected from Lesser Sunda Islands to reveal its phylogenetic relationships. A total of 12 species of *Adiantum* from this region and seven species from Java Island were collected and used in this study. Two cpDNA regions (*rbc*L and *trn*L-F) were chosen as markers and phylogenetic analyses were conducted using Neighbour-Joining (NJ) and Maximum Parsimony (MP) methods. The tree topologies reconstructed by NJ and MP from specimens used in this study and other species downloaded from GenBank are congruent in which trees are divided into five major clades. *Adiantum* species of Lesser Sunda Islands are not monophyletic and comprises three clades, *i. e.* Clade I composed of *A. hispidulum* group, Clade III composed of *A. peruvianum* group and Clade IV or *A. caudatum* group, each together with extra-Lesser Sunda samples. No sample from Lesser Sunda Islands examined is located in Clade II (*A. tenerum* group) and V (*A. capillus-veneris* group).

Key words: *Adiantum*, cpDNA, Lesser Sunda Islands, phylogenetic.

ABSTRAK
LESTARI, W. S., ADJIE, B., JARUWATANAPHAN, T., WATANO, Y. & PHARMAWATI, M. 2014. Hubungan kekerabatan paku suplir marga *Adiantum* (Pteridaceae) dari Kepulauan Sunda Kecil, Indonesia berdasarkan *rbc*L dan *trn*L-F. *Reinwardtia* 14 (1): 143 – 156. — Penelitian mengenai *Adiantum* di Indonesia masih terbatas sehingga informasi mengenai marga ini belum banyak tersedia dan jumlah jenisnya di Indonesia belum dapat dipastikan. Kepulauan Sunda Kecil (Bali dan Nusa Tenggara) sebagai salah satu kawasan yang belum banyak terungkap keanekaragaman floranya diharapkan dapat menjadi langkah awal dalam melakukan studi mengenai marga *Adiantum* di Indonesia. Penelitian ini bertujuan untuk mengetahui keanekaragaman dan hubungan kekerabatan marga *Adiantum* dari Kepulauan Sunda Kecil berdasarkan sekuen dua daerah pada DNA kloroplas yaitu *rbc*L dan *trn*L-F. Dua belas jenis *Adiantum* yang diperoleh dari Kepulauan Sunda Kecil dan tujuh jenis *Adiantum* dari Pulau Jawa digunakan dalam penelitian ini. Analisis filogenetik yang dilakukan pada dua set data secara terpisah menggunakan metode *Neighbour-Joining* (NJ) dan *Maximum Parsimony* (MP) dengan menyertakan beberapa sekuen yang diunduh dari *GenBank* menghasilkan topologi pohon filogeni yang terbagi menjadi lima klad utama. *Adiantum* dari Kepulauan Sunda Kecil tidak mengelompok bersama berdasarkan daerah asalnya, namun terbagi ke dalam tiga klad yaitu Klad I yang terdiri atas grup *A. hispidulum*, Klad III yang terdiri atas grup *A. peruvianum* dan Klad IV atau grup *A. caudatum*. Tidak terdapat sampel *Adiantum* dari Kepulauan Sunda Kecil yang masuk dalam Klad II (grup *A. tenerum*) maupun Klad V (grup *A. capillus-veneris*).

Kata kunci: *Adiantum*, DNA kloroplas, hubungan kekerabatan, Kepulauan Sunda Kecil.

INTRODUCTION

Adiantum (Pteridaceae) is a well known group of ferns and probably the most enthusiastic fern genus (Jones, 1998). The genus called Maidenhairs, is easily recognized by the polished black leaf stalks and the sori covered by specialized reflexed margins of the lamina called false indusia. The members of *Adiantum* are distributed worldwide, mainly in the tropical and subtropical regions (Korpelainen *et al*., 2005). *Adiantum* consist of 200 species (Hoshizaki & Moran, 2002). Many new species of *Adiantum* were published recently and it has been assumsed that the number of *Adiantum* are 280 species globally (Patil *et al*., 2013). Six species occur in Fiji (Brownsey & Perrie, 2011). Seven species are found in New Zealand (three of them are endemic) (Large & Braggins, 1993; Bouma, 2008). About 60 species are native to Asia (Lu *et al.*, 2012). Holttum (1968) recorded seven species in Malaya. Fifteen species were reported in Vietnam (Phan, 2010). Twenty species and two varieties occur in India (Patil *et al*., 2013), while eight species are recorded in Japan (one of them is endemic) (Iwatsuki *et al*., 1995). China is inhabited by 34 species and five varieties (16 species are endemic) (Zhang *et al*., 2013).

Various groupings of *Adiantum* have been proposed, primarily based on regional studies (Afriastini, 2003; Lu *et al*., 2012). There is no up-todate, worldwide revision within this genus, incorporating all findings or comprehensive classification yet (Afriastini, 2003; Korpelainen *et al*., 2005; Bouma, 2008). Recent studies by Bouma (2008) and Lu *et al*. (2012) showed that the result of molecular approach analyses are in incongruence with the previous in-group classification based on morphological characters done by Ching (1957) or Tryon & Tryon (1982).

The genus *Adiantum* is monophyletic (Lu *et al*., 2012). Previous studies showed that it is in need of thorough taxonomic revision since the vittarioid ferns were embedded within this genus and treated as a single subfamily (Schuettpelz *et al*., 2007; Schuettpelz & Pryer, 2007; Bouma, 2008; Christenhusz *et al*., 2011). Recent study by Lu *et al*. (2012) using the combined three-marker (*atp*A, *atp*B, *rbc*L) and the combined five-marker (*atp*A, *atp*B, *rbc*L, *trn*L-F, *rps4-trn*S) showed that the *Adiantum* is monophyletic. Rothfels & Schuettpelz (2013) used six-locus data set (*atp*A, *atp*B, *rbc*L, *atp1*, *nad5*, *gapCp*) to prove the very strong support for the monophyly of *Adiantum*. The relationship between *Adiantum* and the vittarioid ferns were clear as they are sisters to each other and formed the adiantoids clade (Lu *et al*., 2012; Rothfels & Schuettpelz, 2013).

No study has been made about this genus in Indonesia since Posthumus (1944) who enumerated 11 species from Lesser Sunda Islands of Indonesia. Lesser Sunda Islands (LSI) are composed of small islands scattered from Bali to Timor Island. This eastern region is quite different from other parts of Indonesia in the drier and more seasonal climate, resulting in a different flora and fauna (de Lang, 2011).

In the present study we analyzed a molecular phylogeny of *Adiantum* occurring in Lesser Sunda Islands of Indonesia by using the *rbc*L gene and *trn*L-F region of the chloroplast genome to reveal the phylogenetic relationship. *rbc*L is a gene (more than 1.400 bp) that encodes the large subunit of ribulose 1,5-biphosphate carboxylase/oxygenase (RUBISCO) and being the most characterized plastid coding region in GenBank with sufficient variation to discriminate among species (Soltis & Soltis, 1998; Avise, 2001; Newmaster *et al*., 2006). The other plastid marker, the *trn*L-F, is a region between *trn*L (UAA) 5' exon and *trn*F (GAA) (Adjie *et al.,* 2008). This region is the most variable across ferns because it contains an intron of *trn*L and an intergenic spacer between *trn*L and *trn*F, which displays relatively high rates of mutation, making it an ideal locus for detecting variation at the interspecific and intraspecific taxonomic levels (Taberlet *et al*., 1991; Bouma, 2008; Li *et al.,* 2009). The combination between these two plastid marker *rbc*L and *trn*L-F possesses all the necessary qualities to form a powerful barcode for species identification of pteridophytes (de Groot *et al*., 2011).

MATERIALS AND METHODS

Taxon Sampling

A total of 12 species (22 specimens) of *Adiantum* were collected from Lesser Sunda Islands (Bali, Lombok, Sumbawa, Sumba and Timor Island), and seven species (eight specimens) were collected from Java. Three species (five specimens) of *Antrophyum* and one species (two specimens) of *Vittaria* collected from Java, Bali, Lombok, Sumbawa and Molucca Island were used as outgroup based on the previous study (Lu *et al*., 2012). The sequences of non-Lesser Sunda Islands taxa registered at GenBank were also added to the dataset. All taxa included in this study with voucher information, locality and sequence are listed in Table 1. The figure of some specimens are presented in Appendix 1 (Figs. 3, 4 & 5). Living specimens and vouchers were deposited as living and herbarium collection in Bali Botanic Garden, Indonesia (THBB).

Table 1. List of specimens examined

No.	Voucher	Species	Locality	Length of		Accession Number	
				*rbc*L (bp)	*trn*L-F (bp)	*rbc*L	*trn*L-F
1.	AG 326	*Adiantum* sp. AG326*	Sumbawa[1]	1142	924	LC004106	LC004376
2.	WN 118	*Adiantum* sp. WN118*	Bali[1]	-	926	-	LC004378
3.	WN 114	*A. capillus-veneris* L.	Java[1]	1148	-	LC004118	-
4.	WN 154	*A. capillus-veneris* L.	Java[1]	1380	814	LC004119	LC004377
5.	WN 140	*A. caudatum* L.	Bali[1]	-	898	-	LC004375
6.	WN 117	*A. concinnum* Humb. & Bonpl. ex Willd.*	Bali[2]	-	913*	-	LC004382
7.	SH 1129	*A. concinnum* Humb. & Bonpl. ex Willd.*	Lombok[2]	-	923*	-	LC004383
8.	WN 150	*A. concinnum* Humb. & Bonpl. ex Willd.*	Java[2]	1367*	966*	LC004120	LC004381
9.	WN 112	*A. diaphanum* Blume	Bali[1]	-	868	-	LC004386
10.	BA 754a	*A. diaphanum* Blume	Bali[1]	-	949	-	LC004385
11.	BA 742	*A. edgeworthii* Hook.	Timor[1]	1182	843	LC004107	LC004387
12.	WN 157	*A. hispidulum* Sw.	Bali[1]	1062	858	LC004108	LC004393
13.	WN 158	*A. hispidulum* Sw.	Timor[1]	1376	910	LC004109	LC004392
14.	WT 797	*A. hispidulum* Sw.	Lombok[1]	-	852	-	LC004394
15.	WN 120	*A. hispidulum* Sw.	Bali[1]	1365	934	LC004114	LC004391
16.	BA 706	*A. hispidulum* Sw.	Timor[1]	1380	900	LC004115	LC004388
17.	BA 809	*A. hispidulum* Sw.	Sumba[1]	1336	901	LC004116	LC004389
18.	WN 144	*A. hispidulum* Sw.	Java[1]	1366	906	LC004121	LC004390
19.	WN 119	*A. peruvianum* Klotzsch	Bali[3]	1373	858	LC004113	LC004399
20.	WN 128	*A. philippense* L.	Bali[1]	1173	-	LC004110	-
21.	WN 142	*A. philippense* L.	Lombok[1]	1221	-	LC004111	-
22.	SH 1130	*A. philippense* L.	Lombok[1]	1244	-	LC004112	-
23.	WN 153	*A. philippense* L.	Java[1]	1156	-	LC004122	-
24.	WN 116	*A. polyphyllum* Willd.*	Java[3]	-	909*	-	LC004397
25.	WN 111	*A. raddianum* C. Presl.	Bali[2]	1380	910	LC004117	LC004379

26.	WN 149	*A. raddianum* C. Presl.	Java[2]	-	896	-	LC004380
27.	BA 808	*A. silvaticum* Tind.*	Sumba[1]	-	882*	-	LC004395
28.	Drapemmu 103	*A. silvaticum* Tind.*	Sumba[1]	-	915*	-	LC004396
29.	WN 155	*A. tenerum* Sw.	Java[2]	-	824	-	LC004384
30.	AG 329	*A. trapeziforme* L.*	Sumbawa[2]	-	839*	-	LC004398
31.	RS 54	*Antrophyum* sp.	Molucca[1]	-	910	-	LC004404
32.	WN 146	*Antrophyum callifolium* Blume	Java[1]	-	865	-	LC004403
33.	DEE 37	*Antrophyum callifolium* Blume	Bali[1]	-	975	-	LC004401
34.	SH 1163	*Antrophyum callifolium* Blume	Lombok[1]	-	951	-	LC004402
35.	RS 51	*Antrophyum latifolium* Blume	Molucca[1]	1231	851	LC004123	LC004400
36.	SO 002	*Vittaria zosterifolia* Willd.	Bali[1]	1177	-	LC004124	-
37.	SO 002a	*Vittaria zosterifolia* Willd.	Sumbawa[1]	1377	-	LC004125	-

Species number 31 - 37 are outgroup. Asterix (*) show the species of which chloroplast DNA sequences are firstly reported in this study. [1]native, [2]possibly naturalized, [3]cultivated.

DNA Extraction, PCR Amplification and Sequencing

Total genomic DNA was extracted from silica-gel-dried leaf tissue using a modification of the CTAB extraction procedure (Doyle & Doyle, 1987). The *trn*L-F region was amplified and sequenced with primers ”cF” and ”fR” (Taberlet *et al*., 1991), and the *rbc*L gene was amplified and sequenced using primers ”aF” and ”cR” (Hasebe *et al*., 1994). All amplification were performed in a 25 µl reaction-mixture volume, contained 17.375 µl distilled deionized water, 2.5 µl 10x Buffer, 2 µl dNTP, 1 µl primer (F) 10 µM, 1 µl primer (R) 10 µM, 0.125 µl *Ex Taq*™ (TaKaRa Bio) and 1 µl DNA sample. For *trn*L-F, reactions were incubated at 95°C for 3 min, then cycled 35 times (94°C for 1 min; 55°C for 1 min; and 72°C for 2 min), followed by a final extension for 10 min at 72°C (Taberlet *et al*., 1991). For *rbc*L, reactions were incubated at 95°C for 3 min, then cycled 35 times (95°C for 45 s; 55°C for 45 s; and 72°C for 90 s), followed by a final extension for 10 min at 72°C.

The PCR products were evaluated with 1% agarose gel electrophoresis and purified using ExoSAP-IT® PCR Product Cleanup (Affymetrix), 1 µl ExoSAP-IT/10 µl sample, then incubated (37ºC, 1.5 h; 80ºC, 15 min). All cycle sequencings were performed in a 10 µl reaction-mixture volume, containing 6.44 µl distilled deionized water, 1.98 µl 5× Sequencing Buffer, 0.08 µl primer (F/R) 10 µM, 0.5 µl Big Dye 3.1 and 1 µl sample. Reactions were performed at 96ºC for 1 min, then cycled 30 times (96ºC for 10 s; 50ºC for 5 s; 60ºC for 4 min), followed by a final extension for 7 min at 60ºC. Each sample then added with 1 µl 125 mM EDTA and 26 µl ethanol : 3 M sodium hydroxide (50:2), and centrifuged (12.500 rpm, 25ºC, 60 min). Precipitate was then added with 50 µl 70% ethanol, centrifuged (12.500 rpm, 25ºC, 45 min) and air-dried, then incubated for 2 min 95ºC and added with formamide then re-incubated (95ºC, 3 min). After cold shock on an ice cube for 3 min, the sequencing reactions were run on an ABI 3500 Genetic Analyzer.

The sequence fragments were analyzed using Sequencing Analysis (Applied Biosystems, Foster City, California, USA), then assembled using Auto Assembler 2.1.1. A total of 50 sequences were determined as part of this study and eight sequences of it are new (Table 1). In order to complete the data, the *rbc*L and/or *trn*L-F sequences of 22 specimens were downloaded from GenBank (Appendix 2). Sequence aligned

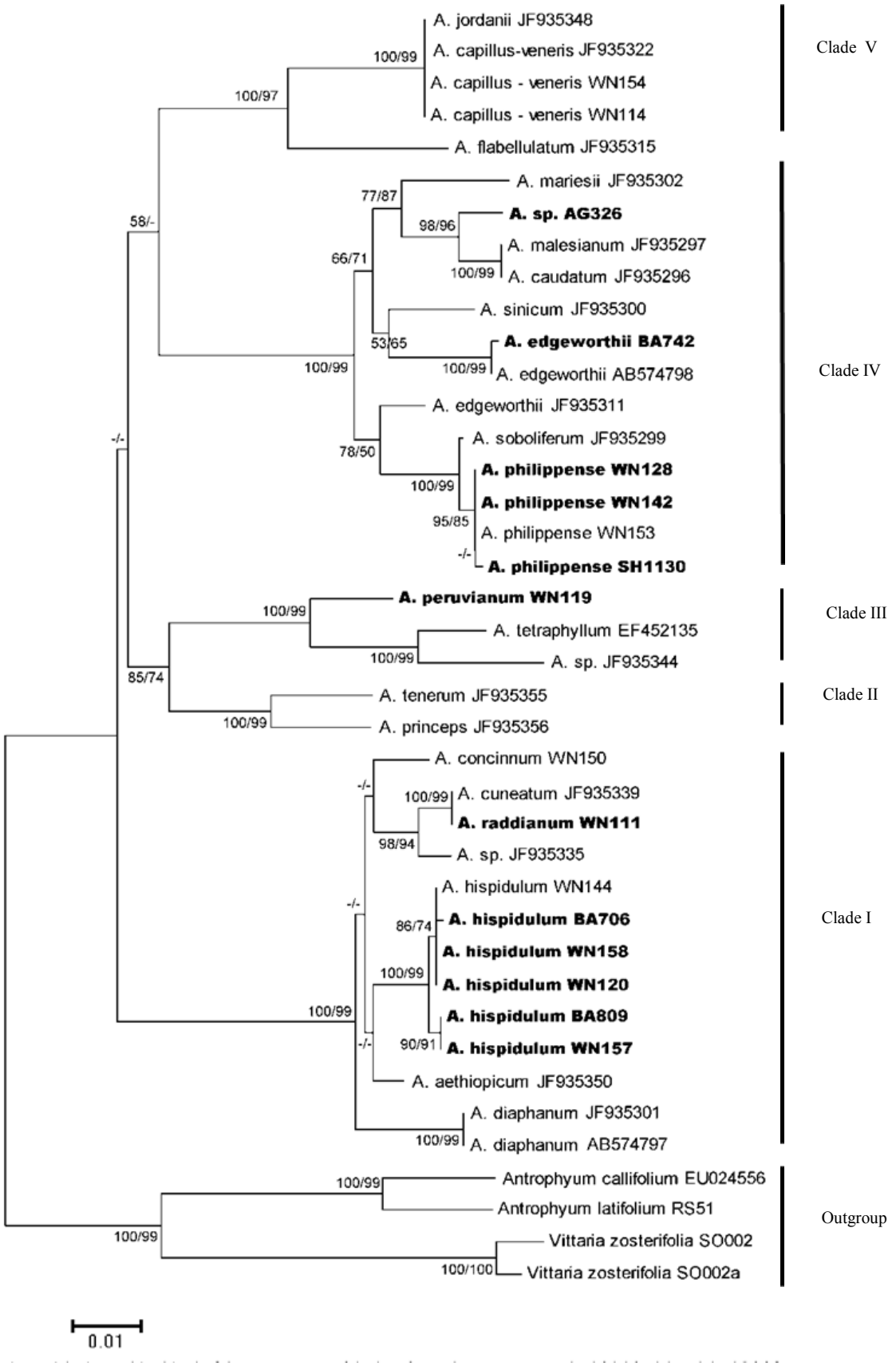

Fig. 1. Neighbour-Joining tree of the *rbc*L sequences. Figure at the nodes indicate bootstrap values (NJ/MP; >50%). Support values under 50 are shown as hyphens (-). Bold : nucleotide sequences from LSI.

automatically using ClustalW (Larkin *et al*., 2007) as implemented in MEGA 5.05 (Tamura *et al*., 2011) and followed by manual adjustment.

Phylogenetic Analysis

Phylogenetic analyses were conducted separately for the two data set (*rbc*L and *trn*L-F),using Neighbour-Joining (NJ) and Maximum Parsimony (MP) on MEGA 5.05. The NJ tree was constructed with genetic distance set according to Jukes-Cantor Model (Jukes & Cantor, 1969) and bootstrapping of 1,000 replicates. MP trees was calculated with the following options: Close-Neighbour-Interchange (CNI) on Random Trees with 1.000 replicates. All characters were equally weighted where indels are coded as missing data. A 50% majority-rule consensus tree was calculated to obtain topology with average branch lengths for all resolved nodes (Adjie *et al*., 2008).

RESULTS

Sequence Characteristics

Among 37 specimens used in this study, 13 specimens can be sequenced for both *rbc*L and *trn*L-F regions (Table 1). Seven other specimens can be sequenced for the *rbc*L region. For the remaining 17 specimens, only *rbc*L sequences were obtained. The nucleotide sequences obtained in this study were deposited in DNA Data Bank of Japan (DDBJ) under accession number LC004106 to LC004125 for *rbc*L and LC004375 to LC004404 for *trn*L-F (Table 1.).

The length of the *rbc*L sequence obtained in *Adiantum* varied from 1.062 bp in *Adiantum hispidulum* (WN157) to 1.380 bp in *A. capillus-veneris* (WN154), *A. hispidulum* (BA706) and *A. raddianum* (WN111). No insertion or deletion was observed in any sequences for this gene. The alignment of 40 *rbc*L sequences including 20 sequences downloaded from GenBank of the *Adiantum* and allied genera, included 317 (30%) variable sites and 267 (25.2%) were parsimony informative.

The length of the sequence of *trn*L-F region obtained in *Adiantum* also varied between 814 bp in *Adiantum capillus-veneris* (WN154) to 966 bp in *A. concinnum* (WN150). Several indels were found in this region and alignment of 45 sequences (of which 15 sequences were downloaded from GenBank) produced 1176 characters in a matrix (available upon request), of which 748 (63.6%) were variable sites and 623 (53.0%) were parsimony informative.

Phylogenetic Analysis

Phylogenetic analysis was conducted for each dataset employing NJ and MP. These two reconstruction methods generated mostly congruent topologies for each data set. In the *rbc*L analysis, 40 sequences were included in dataset and the MP method recovered 15 most parsimonious trees of 653 steps (CI = 0.550000; RI = 0.862525). Thus, NJ tree with bootstrap values for the *rbc*L is shown in Fig 1. as representative.

The NJ and MP trees of the *rbc*L sequences generated five major clades. Clade I (NJ: 100/ MP: 99) is composed of *Adiantum hispidulum* group. *Adiantum hispidulum* and *A. raddianum* from Lesser Sunda Islands formed a clade with *A. hispidulum*, *A. cuneatum* and *A. concinnum* from Java, *A. diaphanum* from Taiwan and China, *A. aethiopicum* from New Zealand and an unidentified specimen from Bolivia.

Clade II (100/99) is composed of *Adiantum tenerum* and *A. princeps* from Mexico, and did not include any sample from Lesser Sunda Islands. Clade III or *A. peruvianum* group, sister to Clade II, is composed of *A. peruvianum* from Lesser Sunda, *A. tetraphyllum* (cultivated) and an unidentified specimen from Malaysia. This clade is highly supported (100/99) and the relationships between Clade II and Clade III was also resolved in NJ (85), but was not well supported in MP (74).

Clade IV (100/99) or the *Adiantum caudatum* group, is strongly supported, formed by *A. philippense* from Lesser Sundas and Java, an unidentified specimen from Lesser Sundas (AG326), *A. soboliferum*, *A. caudatum*, *A. malesianum*, *A. mariesii* and *A. sinicum* from China, *A. egdeworthii* from Lesser Sundas, Japan and China.

Clade V (*Adiantum capillus-veneris* group) is also strongly supported (100/97) and perhaps sister to Clade IV with low support. This clade was composed by *A. capillus-veneris* from Java and China, *A. jordanii* from USA and *A. flabellulatum* from China. This clade does not include any sample from Lesser Sunda Islands examined.

For the *trn*L-F, 45 sequences were included in dataset and the MP method recovered 15 shortest trees of 884 steps (CI = 0.622328; RI = 0.901058). All phylogenetic trees of the *trn*L-F dataset recovered the same five clades with similar memberships as shown in the *rbc*L dataset, with some additional specimens whose *rbc*L sequences could not be determined. The NJ tree with bootstrap values for the *trn*L-F is shown in Fig 2.

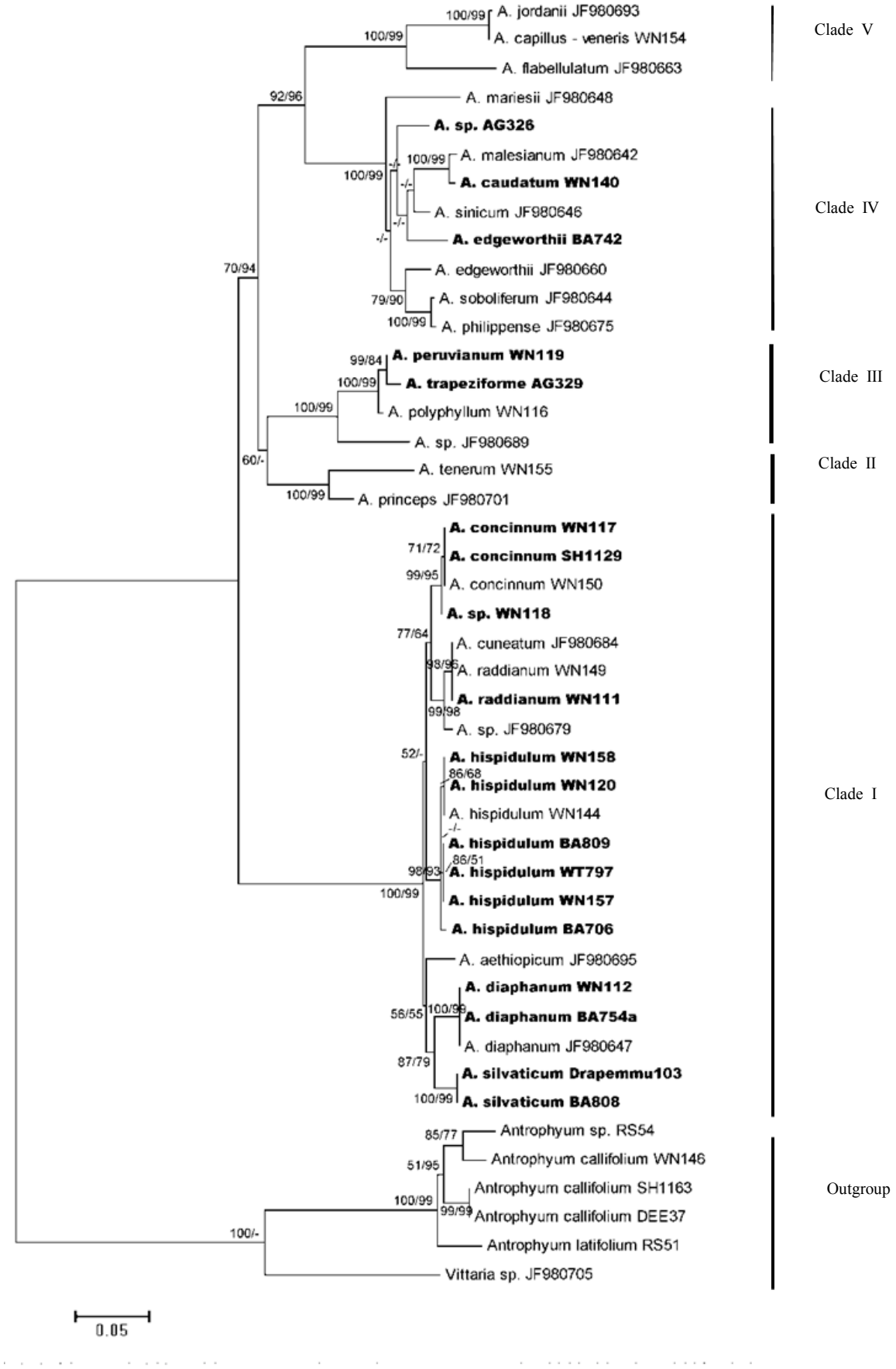

Fig. 2. Neighbour-Joining tree of the *trn*L-F sequences. Numbers at the nodes indicate bootstrap values (NJ/MP; >50%). Support values under 50 are shown as hyphens (-). Bold : nucleotide sequences from LSI.

In this tree, *Adiantum silvaticum*, *A. diaphanum*, *A. concinnum* and an unidentified sample from Lesser Sundas (WN118) are included in Clade I. Again, Clades II and V do not include any sample from Lesser Sunda Islands examined. The Brittle Maidenhair from Java, *A. tenerum*, included in Clade II, while *A. trapeziforme* from Lesser Sunda and *A. polyphyllum* from Java included in Clade III, and *A. caudatum* from Lesser Sunda Islands was included in Clade IV.

DISCUSSION

This study examined 12 species of the Genus *Adiantum* as representative species in Lesser Sunda Islands. Six species are considered as native species, *i. e. A. caudatum*, *A. diaphanum*, *A. edgeworthii*, *A. hispidulum*, *A. philippense* and *A. silvaticum* based on their distribution record. The other species, *A. concinnum*, *A.raddianum* and *A. trapeziforme* considered as an introduced and naturalized species, while *A. peruvianum* considered as cultivated species. The two unidentified specimens, *Adiantum* sp. AG326 and WN118 are morphologically distinct from any species reported in Malay Archipelago so far and tentatively considered as native taxa in the present study.

Both phylogenetic trees of *rbc*L and *trn*L 5'exon to *trn*F (*trn*L-F) are comprised of five major clades. In the trees, Lesser Sunda's samples are not monophyletic. Some species of Lesser Sunda Islands have wide distribution and were grouped with the samples of the same species from other regions. The Lesser Sunda's species are referable to the three clades, *i. e.* Clade I (the *Adiantum hispidulum* group), Clade III (the *A. peruvianum* group) and Clade IV (the *A. caudatum* group).

Clade I (*Adiantum hispidulum* group) consists of seven to nine species (of which six are from Lesser Sunda). Intraspecific variation in *rbc*L and *trn*L-F sequences were observed in *Adiantum hispidulum*. The nucleotide substitution occurs in several sites and the *A. hispidulum* samples were divided into two subclades in the *rbc*L tree.

Adiantum hispidulum has been considered to be a polymorphic species, due to the variation at several morphological characters. Large and Braggins (1993) described two polymorphic members of the New Zealand *A. hispidulum* complex, *A. hispidulum s.s.* and *A. pubescens* Schkuhr., which distinguished based on their pinnule hairs. One subgroup with short (63-815 μm), stiff, often pigmented hairs with enlarged basal cells was treated as *A. hispidulum* var. *hispidulum*, while the other subgroup with long (251-1003 μm), soft, pale hairs with narrow basal cells is given varietal status as *A. hispidulum* var. *pubescens*. Bouma (2008) found another type of New Zealand's *A. hispidulum* pinnule's hair called type 3, but did not describe it clearly. Morphological examination of the above two subclades in the *rbc*L tree do not accord with the subdivision based on pinnule hair character.

Adiantum hispidulum is distributed in southern India, eastern Africa and the Pacific Islands (Hoshizaki & Moran, 2002) and also found in New Zealand (Large & Braggins, 1993), Australia (Bostock *et al*., 1998), Thailand (Boonkerd & Pollawatn, 2013), Indonesia (Posthumus, 1944; Lu *et al*., 2012) and China (Lu *et al*., 2012). The cpDNA variation observed in *Adiantum hispidulum s.l.* would be applicable for future studies on phylogeography or on gene flow patterns among continents and islands.

In the *trn*L-F tree (Fig. 2), *Adiantum silvaticum* from Lesser Sunda is included in Clade I. *Adiantum silvaticum* was reported native in eastern Australia. This species is closely related in *A. cunninghamii* Hook., *A. fulvum* Raoul. and *A. viridescens* Col., endemic to New Zealand (Parris & Croxall, 1974). In our result, *A. silvaticum* was also related to *A. diaphanum*. This family Maidenhair is native to Asia, Australia, New Zealand and the Pacific Islands (Hoshizaki & Moran, 2002).

As shown in a recent study by Bouma (2008) and Lu *et al.* (2012), the result of molecular analysis incongruent with the previous ingroup classification based on morphological characters. *Adiantum concinnum* and *A. raddianum* native to tropical America (Hoshizaki & Moran, 2002) were similar to *A. capillus-veneris* morphologically. However, the chloroplast sequence data shows that *A. capillus-veneris* is distantly related to the two. In both *rbc*L and *trn*L-F trees (Figs. 1 & 2.), *A. raddianum* was grouped together with *A. cuneatum* which was recorded as a synonym of *A. raddianum* (Scamman, 1960; Hoshizaki & Moran, 2002).

Among the samples examined, there was no sample from Lesser Sunda Islands in Clade II. In Clade III or the *Adiantum peruvianum* group, *A. peruvianum* and *A. trapeziforme* from Lesser Sunda were grouped together with *A. polyphyllum* from Java. *Adiantum peruvianum* is recorded native to Ecuador, Peru and Bolivia, *A. trapeziforme* is native to Central America and the West Indies, and *A. polyphyllum* occurs from Venezuela to Peru (Hoshizaki & Moran, 2002). Members of this group can be identified by having clathrate rhizome scales with minutely denticulate margins (McCarthy, 2012).

Clades IV (*Adiantum caudatum* group) is highly supported, although the relationships among its members are not yet well understood. This clade is characterized by the pinnate fronds with the rachis prolonged into a prolifereous whip. *Adiantum edgeworthii* is apparently polyphyletic, where Chinese *A. edgeworthii* form a sub clade with *A. soboliferum* and *A. philippense*, while Japanese and Lesser Sunda's form a sub clade with *A. sinicum*. But the supports are very low. Besides China, Japan and Indonesia, *A. edgeworthii* are also recorded from Bhutan, India (north), Malaysia, Myanmar, Nepal, Philippines, Thailand (north) and Vietnam (Zhang *et al*., 2013). Extensive analysis is necessary to settle the relationships of the species.

Finally, Clade V corresponds to Lu *et al.*'s *Adiantum capillus-veneris* clade (Lu *et al*., 2012). *A. capillus-veneris* is a worldwide distributed species, from warm-temperate to subtropical area (Hoshizaki & Moran, 2002). Even the morphological plasticity in frond is very high, all samples from their distribution showed no sequence variation for *rbc*L. Lu *et al.* (2012) suggested morphological heterogenity of this species may by due to its wide-spread distribution and broad ecological range, suggesting a recent geographic migration.

CONCLUSION

Adiantum species of Lesser Sunda Islands are not monophyletic and the relationships are in incongruent with previous morphological in-group classification either. Lesser Sunda's *Adiantum* divided into three main clades based on two plastid markers (*rbc*L, *trn*L-F). Clade I of Lesser Sunda's *Adiantum* is composed of *Adiantum hispidulum*, *A. silvaticum*, *A. diaphanum*, *A. raddianum* and *A. concinnum*, Clade III is composed of *A. peruvianum* and *A. trapeziforme*, and Clade IV is composed of *A. caudatum*, *A. philippense* and *A. edgeworthii*. No sample from Lesser Sunda Islands examined is placed in Clades II (*A. tenerum* group) and Clades V (*A. capillus-veneris* group).

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Appendix 1.

Fig. 3. A. *Adiantum* sp. WN118 (Bali). B. *Adiantum* sp. AG326 (Sumbawa). C. *A. capillus-veneris* WN114 (Java). D. *A. caudatum* WN140 (Bali). E. *A. concinnum* WN117 (Bali). F. *A. diaphanum* WN112 (Bali). G. *A. edgeworthii* BA742 (Timor). H. *A. hispidulum* WN144 (Java).

Appendix 1. Continued

Fig. 4. A. *Adiantum hispidulum* WN120 (Bali). B. *A. hispidulum* WN157 (Bali). C. *A. hispidulum* WT797 (Lombok). D. *A. hispidulum* WN158 (Timor). E. *A. hispidulum* BA706 (Timor). F. *A. hispidulum* BA809 (Sumba). G. *A. peruvianum* WN119 (Bali). H. *A. philippense* WN128 (Bali). I. *A. polyphyllum* WN116 (Java).

Appendix 1. Continued.

Fig. 5. A. *Adiantum raddianum* WN111 (Bali). B. *A. raddianum* WN149 (Java). C. *A. silvaticum* BA808 (Sumba). D. *A. tenerum* WN155 (Java). E. *A. trapeziforme* AG329 (Sumbawa). F. *Antrophyum callifolium* DEE37 (Bali). G. *Antrophyum latifolium* RS51 (Molucca, Doc. : N. K. E. Undaharta). H. *Vittaria zosterifolia* SO002 (Bali).

Appendix 2.

List of nucleotide sequences used for phylogenetic analyses

No.	Species	Origin	Accessions No.	
			***rbc*L**	***trn*L-F**
1.	*Adiantum* sp.	Bolivia	JF935335	JF980679
2.	*Adiantum* sp.	Malaysia	JF935344	JF980689
3.	*A. aethiopicum*	New Zealand	JF935350	JF980695
4.	*A. capillus-veneris*	China	JF935322	-
5.	*A. caudatum*	China	JF935296	-
6.	*A. cuneatum*	Indonesia	JF935339	JF980684
7.	*A. diaphanum*	Taiwan	AB574797	-
8.	*A. diaphanum*	China	JF935301	JF980647
9.	*A. edgeworthii*	Japan	AB574798	-
10.	*A. edgeworthii*	China	JF935311	JF980660
11.	*A. flabellulatum*	China	JF935315	JF980663
12.	*A. jordanii*	USA	JF935348	JF980693
13.	*A. malesianum*	China	JF935297	JF980642
14.	*A. mariesii*	China	JF935302	JF980648
15.	*A. philippense*	Phillipines	-	JF980675
16.	*A. princeps*	Mexico	JF935356	JF980701
17.	*A. sinicum*	China	JF935300	JF980646
18.	*A. soboliferum*	China	JF935299	JF980644
19.	*A. tenerum*	Mexico	JF935355	-
20.	*A. tetraphyllum*	Cultivated	EF452135	-
21.	*Antrophyum callifolium*	-	EU024556	-
22.	*Vittaria* sp.	China	-	JF980705

(Schuettpelz *et al*., 2007; Ruhfel *et al*., 2008; Ebihara *et al*., 2010; Lu *et al.*, 2012).

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