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ORIGINAL ARTICLE

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# Allozyme analysis of cryptic species in the *Asplenium nidus* complex from West Java, Indonesia

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**Abstract** In various fern species, a large amount of *rbcL* sequence variation has been reported, and it is possible that these species contain several reproductively isolated cryptic species. In our previous study on *Asplenium nidus* L., it was suggested that the plants growing in Mt. Halimun National Park, West Java, Indonesia, consist of several cryptic species based on the results of crossing experiments among *rbcL* sequence types. In this study, we examined allozyme polymorphisms of five *rbcL* sequence types found in West Java in order to test the hypothesis that the assemblages of *A. nidus* delimited based on the *rbcL* sequences are separate Mendelian populations and gene flow is disrupted by reproductive isolation from one another. The calculated fixation indices suggested that the individuals in each *rbcL* type are randomly crossing at least in the investigated localities. Nevertheless, these *rbcL*-based assemblages were genetically differentiated in allozymes that are encoded in their nuclear genomes, and it is also suggested that gene flow is disrupted even between sympatrically distributed pairs of *rbcL* sequence types. Therefore, our findings support the view that the five *rbcL* sequence types in West Java are potential cryptic species.

**Key words** Allozyme polymorphism · *Asplenium nidus* · Cryptic species · *rbcL*

## Introduction

In systematic research on homosporous ferns, data from cytotaxonomical and enzyme electrophoretic studies have revealed cryptic species in many lineages such as *Adiantum*, *Botrychium*, and *Pityrogramma* (Paris et al. 1989). Recently, in various fern species such as *Asplenium nidus* L., *Hymenasplenium obliquissimum* (Hayata) Sugimoto, *H. cheilosorum* (Kunze ex Mett.) Tagawa (Aspleniaceae), *Stegnogramma pozoi* (Lagasca) K. Iwats. (Thelypteridaceae), *Osmunda cinnamomea* L., *O. claytoniana* L., *O. regalis* L. (Osmundaceae), and *Cheiropleuria bicuspis* (Bl.) Pr. (Dipteridaceae) a large amount of *rbcL* sequence variation has been reported (Murakami et al. 1998a, b; Yatabe et al. 1998, 1999; Kato et al. 2001). Average substitution rates of *rbcL* in the Osmundaceae were estimated to be approximately $7 \times 10^{-8}$ nucleotide substitutions per year (Yatabe et al. 1999).

Considering the slow evolutionary rate of *rbcL*, the large amount of *rbcL* sequence variation in these species may suggest that they contain several reproductively isolated cryptic species. In *Ceratopteris thalictroides* (L.) Brongn. (Parkeriaceae), Masuyama et al. (2002) have revealed three cryptic species, which were suggested to be reproductively isolated from one another and genetically differentiated in *rbcL* sequence as well as in allozyme polymorphism.

*Asplenium nidus* is one of the species assigned to *Asplenium* sect. *Thamnopteris*. *Asplenium* sect. *Thamnopteris* is a group of epiphytic ferns with simple leaves and is found throughout the Old World tropics. In this group, only tetraploids at $2n = 144$ have been recorded (Bir 1960; Abraham et al. 1962; Kawakami 1970; Koul 1970; Tsai and Shieh 1983; Yatabe et al. 2001). Murakami et al. (1999a, b) examined *rbcL* sequences of this group and found that the divergence of *rbcL* sequences reached 4–5% in the plants identified as *A. nidus* sensu Holttum (1974).

In West Java, five *rbcL* sequence types of *Asplenium nidus* were recorded (Murakami et al. 1999b). The phylogenetic analyses based on *rbcL* sequences suggest that these five types are distantly related in *Asplenium* sect. *Thamnopteris*. Three of the five *rbcL* types have been found from Mt. Halimun National Park. The results of crossing experiments suggested that at least two pairs of these three types were not capable of forming F1 hybrids and are reproductively isolated (Yatabe et al. 2001). In addition, the three *rbcL* types from Mt. Halimun National Park were ecologically differentiated. Habitats differ among *rbcL* types with

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respect to altitude or the position where the plants grow on the tree trunks (Murakami et al. 1999b). Such ecological differentiation among the *rbcL* types indirectly suggests that these *rbcL* types keep their separate identities. All of these data support the view that *A. nidus* consists of several cryptic species.

The species boundaries are, however, still uncertain and it remains to be verified that the assemblages that are delimited based on the *rbcL* sequences are separate Mendelian populations and gene flow is disrupted by reproductive isolation from one another. In this study, we examined allozyme polymorphism of *Asplenium nidus* collected from a small area of West Java in order to clarify whether sympatrically distributed *rbcL*-based assemblages are also genetically distinct populations in their nuclear genomes. We addressed the following questions. (1) Are individuals randomly crossing in each *rbcL*-based assemblage? (2) Are these *rbcL*-based assemblages genetically differentiated also in allozyme loci that are nuclear genetic markers? (3) Do any genetic loci other than *rbcL* support the species boundary drawn based on *rbcL*?

## Materials and methods

From 1997 to 2000, a total of 117 individuals, which were all identified as *Asplenium nidus* L., were collected from Mt. Halimun National Park. Within the park, we collected materials haphazardly along the trails climbing up to the top of Mt. South-Kendang and Mt. Botol (Fig. 1). Mt. Botol is about 4 km from Mt. South-Kendeng. At the lower elevation of Mt. Botol, the forest had been destroyed and only the area higher than 1,600 m altitude could be investigated. We also collected 25 and four individuals from Bogor Botanical Garden and on the way from Bogor to Mt. Halimun National Park, respectively. Bogor Botanical Garden is about 50 km from Mt. Halimun National Park (Fig. 1). Vouchers are deposited in the herbarium of the Graduate School of Science, Kyoto University (KYO). Total DNA was extracted from all 146 individuals using a 2 × CTAB (hexadecyl trimethyl ammonium bromide) solution according to Doyle and Doyle (1987).

For *rbcL* sequencing, we used 40 of the individuals in order to investigate the distribution of each *rbcL* type in West Java in addition to the previous investigation (Murakami et al. 1999b). Polymerase chain reaction (PCR) amplification of *rbcL* fragments used the method of Murakami et al. (1999a). The PCR products were purified using a Gene Clean kit (BIO101, Vista, Calif.) after electrophoresis in 1.0% agarose gels, and then were used as templates for direct sequencing. Sequencing reactions were prepared using the Big Dye terminator cycle sequencing kit (Perkin Elmer Applied Biosystems, Foster, Calif.). The reaction mixtures were analyzed on an Applied Biosystems Model 377 automated sequencer (Perkin Elmer). Sequences were aligned using Sequence Navigator software (Perkin Elmer).

For single strand conformation polymorphism (SSCP) analysis (Yap and McGee 1994), we used 116 materials, including ten of the same individuals that were used for *rbcL* sequencing. A newly designed primer set, SSCP-2F CACGGTATGCATTTTCGTGT, and SSCP-2R TAGATACCCAATCTTGAGTG were used for PCR amplification of 193 bp *rbcL* fragment. This fragment contains 11 of 57 variable sites observed in the *rbcL* sequence of *A. nidus* (Fig. 2) and can be used for recognizing any of the five *rbcL* types found from West Java so far. The PCR products were electrophoresed on GenePhor (Amersham Pharmacia Biotech, Buckinghamshire, UK) at 5 °C. After electrophoresis, the gels were stained by the DNA silver staining method of Hoefer (Amersham). Each of the electrophoretic patterns is shown in Fig. 3. After recognizing the *rbcL* type of each individual by the SSCP method, 20 individuals of each *rbcL* type were used as materials for the following allozyme analyses. Fresh leaves were ground in 1.0 ml of cold extraction buffer containing 0.1 mM Tris–HCl, 1 mM EDTA (4Na), 10 mM KCl, 10 mM $MgCl_2$, 0.4% 2-mercaptoethanol, and 10% polyvinyl-pyrrolidone with the pH adjusted to 7.5. Enzymes were resolved on 7.5% poly-

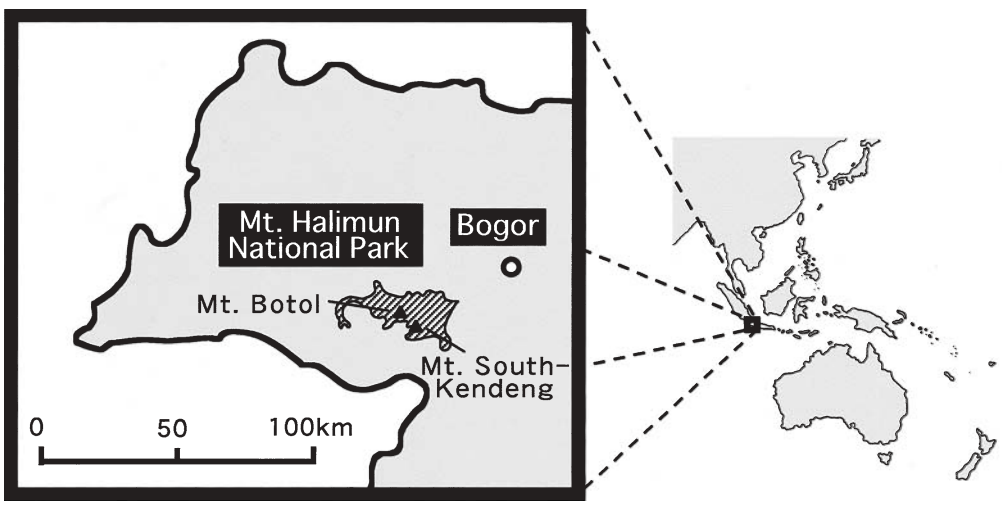


**Fig. 1.** Map showing the investigated localities

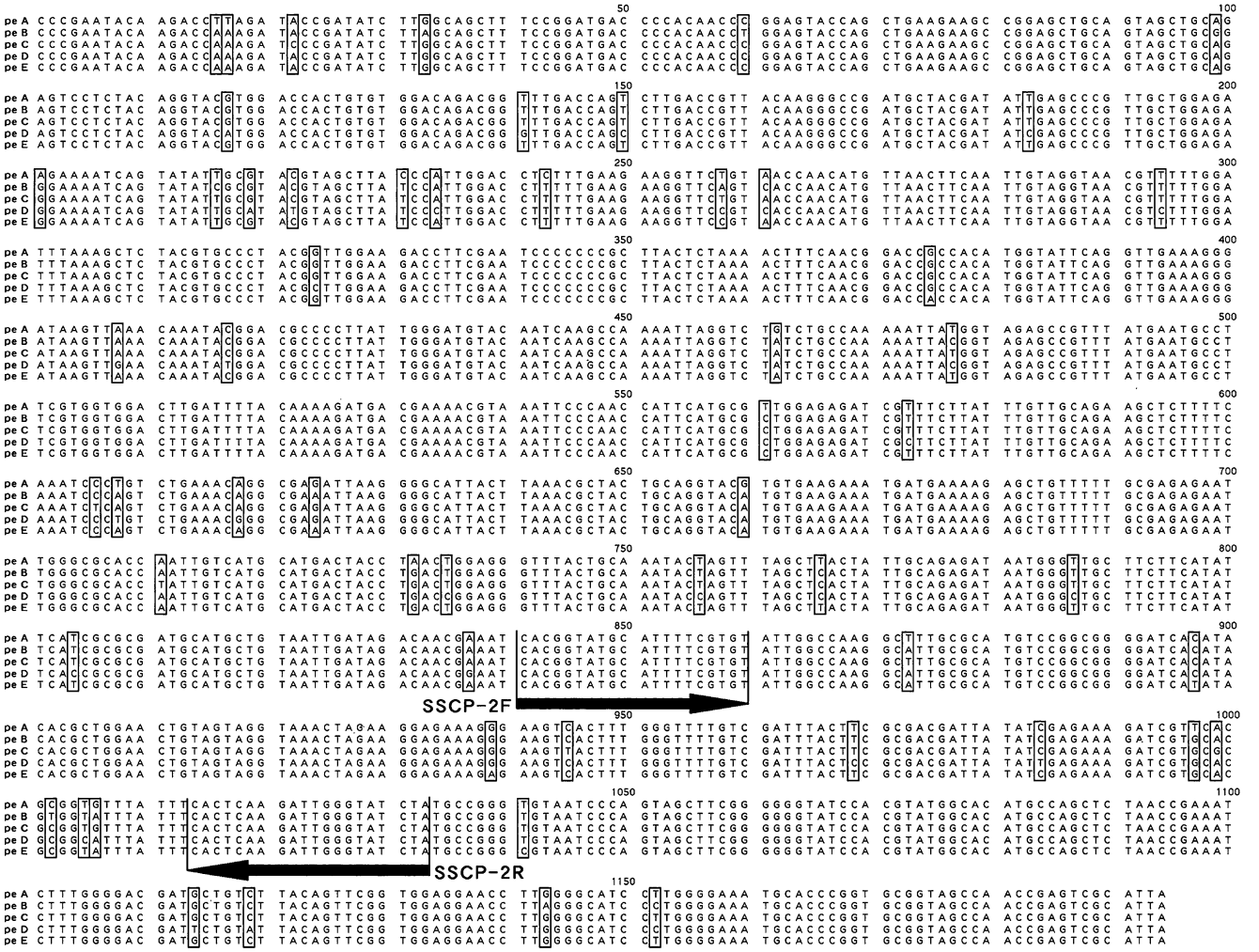


**Fig. 2.** The five *rbcL* sequence types in *Asplenium nidus* from West Java. Variable nucleotide positions are highlighted with *boxes*. Positions of primer set for SSCP analysis are indicated by *arrows*

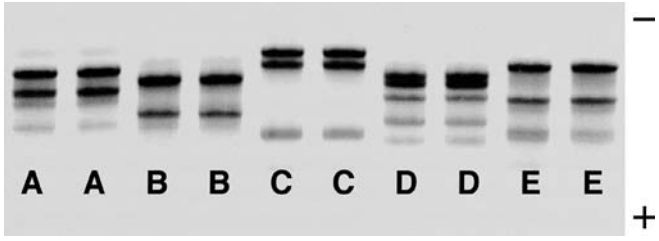


**Fig. 3.** The single strand conformation polymorphisms (SSCP) observed in *rbcL* of *Asplenium nidus*. *A–E* indicate the *rbcL* type

acrylamide gels following the procedures of Shiraishi (1988). We examined aspartate aminotransferase (AAT), leucine aminopeptidase (LAP), phosphoglucose isomerase (PGI), 6-phosphoglconate dehydrogenase (6PGD), glucose-6-phosphate dehydrogenase (G6PD), triosephosphate isomerase (TPI), phosphoglucomutase (PGM), diaphorase (DIA) and hexokinase (HK), also following Shiraishi (1988). Loci were numbered with the most anodal form as "1" and so on, when more than one isozyme was present for an enzyme. Allelomorphs were indicated similarly at each locus, with the most anodal form designated "a" and progressively slower forms as "b", "c", and so on.

After allele frequencies were estimated for each *rbcL* type, indices on allozyme diversity, such as the proportion of polymorphic loci (P), mean number of alleles per locus (A), and mean expected heterozygosities (He) were estimated. For every *rbcL* type, the fixation index (F), that is, selfing rates in homosporous ferns, was also estimated following McCauley et al. (1985). Chi-square tests were also performed in order to assess the goodness of fit to Hardy–Weinberg proportions in each *rbcL* type.

For every pair of *rbcL* types, the probabilities that chance alone could produce a deviation of allele frequencies between *rbcL* types were calculated using Fisher's exact probability test with a program written in Visual Basic. The program is available from the authors. Nei's genetic distance (D) and genetic identity (I) among *rbcL* types were also calculated following Nei (1972). A UPGMA dendrogram was constructed by using PHYLIP v. 3.57 (Felsenstein 1995).

## Results

### Distribution of each *rbcL* type

We determined 1,194 bp nucleotide sequences of *rbcL* for 40 individuals. All five types of *rbcL* sequences recognized so far in West Java (Murakami et al. 1999b) were found also in this study. These five types are designated as A, B, C, D, and E and the sequence of each type is shown in Fig. 2. The DNA database accession numbers of these five types are AB023500, AB023501, AB013245, AB023502, and AB023508. No *rbcL* sequence variants other than these five types were found.

The altitude where the 40 sample individuals for *rbcL* sequencing grew and the distributions of each *rbcL* type are shown in Fig. 4. All five *rbcL* types were found in Mt. Halimun National Park. Only one individual from that location turned out to be type E. Types A, B, C, and E were found along the trail of Mt. South-Kendeng, and types C and D were found from higher elevation of Mt. Botol. Types A and B, A and C, and C and D were found together at the same elevation and sometimes found growing on the same tree trunk. Types B and C were distributed parapatrically at adjacent elevations. The other pairs of the *rbcL* types were found at different elevations and were distributed allopatrically.

Only type E was found in Bogor Botanical Garden. At the roadside between Bogor and Mt. Halimun National Park, types A and E were found.

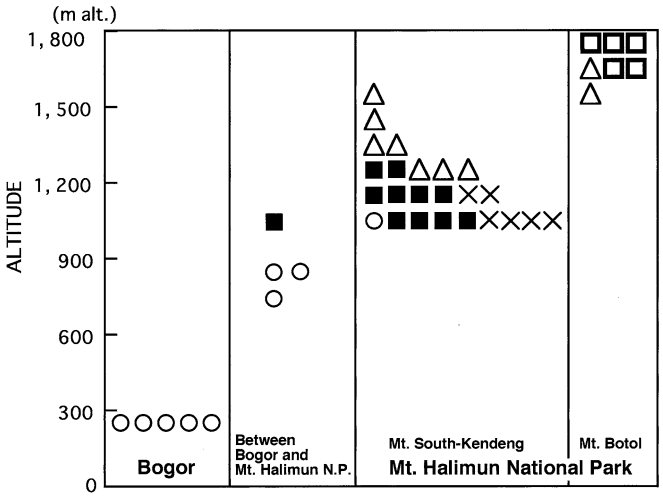


**Fig. 4.** The distribution of each *rbcL* type in the investigated localities. The *rbcL* type is indicated for each individual's 1,194 bp *rbcL* sequence. *Filled box* type A; *cross* type B; *triangle* type C; *empty square* type D; *circle* type E

### Allozyme diversity within *rbcL* types

After recognizing each *rbcL* sequence type by its SSCP profile, we examined allozyme diversity for 20 individuals each of types A, B, C, and D from Mt. Halimun National Park, and 20 individuals of type E from Bogor Botanical Garden. We did not examine the allozyme polymorphism of type E in Mt. Halimun National Park because only one individual of type E was found from there. Eleven putative gene loci of nine enzyme systems were resolvable and polymorphic: *aat*, *lap*, *pgi*, *dia*, *pgm*, *tpi-1, tpi-2, tpi-3*, *hk, g6pd*, and *6pgd*.

Allele frequencies estimated for each *rbcL* type are listed in the Appendix. Levels of allozyme variability in each *rbcL* type are presented in Table 1. Allozyme variability was high in the populations from West Java, and mean expected heterozygosities (He) range from 0.195 (type B) to 0.361 (type C). The fixation index (F), calculated for every variable locus of each *rbcL* type, is shown in Table 2. Chi-square tests were performed for every applicable locus and the results are also shown in Table 2. The hypothesis that the allele frequencies are in Hardy–Weinberg proportions was not rejected for any locus of any *rbcL* types.

**Table 1.** Levels of allozyme variability in each *rbcL* type. *A* Mean number of alleles per locus; *P* the proportion of polymorphic loci; *He* mean expected heterozygosities; *SD* standard deviation

| | A | SD | P | He | SD |
|---|---|---|---|---|---|
| Type A | 2.818 | (1.601) | 0.909 | 0.325 | (0.229) |
| Type B | 2.455 | (1.128) | 0.818 | 0.195 | (0.221) |
| Type C | 3.182 | (1.328) | 1.000 | 0.361 | (0.167) |
| Type D | 1.909 | (0.831) | 0.636 | 0.251 | (0.227) |
| Type E | 3.000 | (1.844) | 1.000 | 0.270 | (0.183) |
| Mean | 2.672 | (1.415) | 0.873 | 0.280 | (0.207) |

**Table 2.** The fixation index (F) in each *rbcL* type. The number in parentheses indicate the significance probability calculated by the $\chi^2$ test

| *rbcL*type | Allozyme loci | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ATT | LAP | PGI | DIA | PGM | TPI-1 | TPI-2 | TPI-3 | HK | G6P | 6PG | mean |
| A | –0.132 | 0.231 | 0.283 | –0.234 | 0.184 | –0.212 | 0.063 | –0.026 | –0.404 | – | –0.026 | –0.027 |
| | (0.430) | (0.204) | (0.205) | (0.154) | (0.391) | – | – | – | (0.041) | – | – | |
| B | –0.026 | 0.382 | –0.056 | –0.081 | –0.026 | –0.026 | –0.162 | –0.026 | 0.297 | – | – | 0.031 |
| | – | (0.074) | (0.987) | – | – | – | (0.343) | – | (0.182) | – | – | |
| C | 0.031 | 0.002 | –0.081 | 0.024 | –0.088 | 0.177 | 0.161 | –0.254 | –0.013 | –0.053 | 0.165 | 0.006 |
| | (0.964) | (0.658) | – | (0.987) | (0.619) | (0.548) | (0.858) | (0.194) | (0.371) | – | (0.858) | |
| D | – | –0.062 | –0.176 | –0.103 | 0.079 | 0.253 | – | – | 0.177 | –0.068 | – | 0.014 |
| | – | (0.717) | – | – | – | – | – | – | (0.257) | (0.565) | – | |
| E | 0.134 | 0.100 | –0.111 | –0.026 | –0.093 | –0.250 | 0.375 | –0.026 | 0.216 | 0.314 | –0.039 | 0.054 |
| | (0.548) | (0.723) | – | – | (0.523) | – | – | – | (0.201) | – | – | |

**Table 3.** Statistical test for the deviation of allele frequencies at each allozyme locus between five all pairs of *rbcL* types

| Pairs of *rbcL* types | Probabilities | | | | | | | | | | Number of loci significantly differentiated between *rbcL* types $P < 0.0001$ (number of loci entirely differentiated between *rbcL* types) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | AAT | LAP | PGI | DIA | PGM | TPI-1 | TPI-2 | TPI-3 | HK | G6P | 6PG | |
| A–B | 0.0128 | <0.0001 | 0.0238 | [a] | <0.0001 | 0.0284 | <0.0001 | 1.0000 | [a] | [b] | [a] | 6 (3) |
| A–C | <0.0001 | <0.0001 | 0.0012 | 0.0001 | <0.0001 | 0.2589 | <0.0001 | 0.0072 | <0.0001 | 0.2468 | <0.0001 | 6 (0) |
| A–D | 0.0128 | <0.0001 | <0.0001 | [a] | <0.0001 | [a] | [a] | 0.5000 | <0.0001 | 0.0002 | 0.5000 | 7 (3) |
| A–E | 0.0059 | <0.0001 | 0.0012 | [a] | <0.0001 | 0.5000 | <0.0001 | 0.5000 | [a] | 0.0274 | 0.5000 | 5 (2) |
| B–C | <0.0001 | <0.0001 | 0.0012 | <0.0001 | [a] | 0.0284 | 0.0094 | 0.0072 | [a] | 0.2468 | <0.0001 | 6 (2) |
| B–D | 0.5000 | [a] | <0.0001 | <0.0001 | [a] | [a] | [a] | 0.5000 | [a] | 0.0002 | [a] | 8 (6) |
| B–E | 0.0284 | <0.0001 | 0.0012 | 0.1203 | <0.0001 | 0.0144 | <0.0001 | 0.5000 | [a] | 0.0274 | <0.0001 | 5 (1) |
| C–D | <0.0001 | <0.0001 | [a] | <0.0001 | [a] | [a] | [a] | 0.0012 | 0.0001 | 0.0002 | <0.0001 | 8 (4) |
| C–E | <0.0001 | 0.0019 | 0.5000 | <0.0001 | 0.0274 | 0.1741 | 0.0115 | 0.0072 | <0.0001 | 0.0274 | <0.0001 | 4 (0) |
| D–E | 0.0059 | <0.0001 | [a] | <0.0001 | [a] | [a] | [a] | 0.5000 | <0.0001 | 0.0002 | 0.2468 | 7 (4) |

[a] Locus entirely differentiated, where no allele is shared by the two different *rbcL* types
[b] Locus where no variation was found

**Table 4.** Genetic identity (above diagonal) and genetic distance (below diagonal)

| | A | B | C | D | E |
|---|---|---|---|---|---|
| A | – | 0.599 | 0.702 | 0.596 | 0.659 |
| B | 0.513 | – | 0.621 | 0.379 | 0.693 |
| C | 0.353 | 0.476 | – | 0.401 | 0.806 |
| D | 0.517 | 0.970 | 0.913 | – | 0.516 |
| E | 0.417 | 0.366 | 0.215 | 0.663 | – |




**Fig. 5.** UPGMA dendrogram of *Asplenium nidus* from West Java based on Nei's genetic distance

### Allozyme diversity between *rbcL* types

The extent of the genetic differentiation based on allozyme data between the *rbcL* types is shown in Table 3. For every pair of *rbcL* types, we found some loci where the allele frequencies deviated significantly from each other. In most of the loci, however, alleles were shared by several different *rbcL* types. In eight of ten pair-wise comparisons, at least one locus showed complete differentiation in alleles. Nei's genetic distance (D) and genetic identity (I) calculated among *rbcL* types are shown in Table 4. A dendrogram based on Nei's genetic distance is shown in Fig. 5.

## Discussion

The calculated fixation index and the results of chi-square tests (Table 2) suggest that the individuals are randomly crossing in each *rbcL* type of *Asplenium nidus* from West Java (types A–E). *Asplenium nidus* can be considered as out-crossing species. Therefore, we can test the hypothesis that these *rbcL* sequence types are separate Mendelian populations and gene flow is disrupted by reproductive isolation from one another by examining sympatrically distributed populations and verifying whether these *rbcL* types are also genetically distinct or differentiated in nuclear genomes. Although the allozyme data on *Asplenium* sect. *Thamnopteris* were provided by Murakami et al. (1999a), this data did not include the locality where several *rbcL* types grow sympatrically. Therefore, we could not test this hypothesis or discuss the species status in *Asplenium* sect. *Thamnopteris*.

In this study, we collected the materials from the same locality, Mt. Halimun National Park, for allozyme analyses

of four of the five *rbcL* types found from West Java. Three of the six pairs of these four *rbcL* types, types A and B, A and C, and C and D, were found from the same elevation (Fig. 4) and sympatrically distributed. Nevertheless, significant genetic differentiation was also observed in the allozyme data between the *rbcL* types (Table 3). Therefore, some factors other than geographical isolation were expected to prevent the gene flow from occurring among *rbcL* types. Yatabe et al. (2001) performed crossing experiments and reported that at least two pairs of types A, B, and C are incapable of forming hybrids between them (A × B and A × C; other pairings were not tested). Therefore, one of the factors that prevent gene flow was thought to be reproductive isolation.

It was confirmed that the rest of the pairs, types B and C, A and D, and B and D, were distributed parapatrically or allopatrically because they were found in adjacent or different elevations (Fig. 4). Their distributions were, however, continuous and no geographical isolation was recognized among their habitats. Significant differentiation was observed in six, seven and eight loci between the pairs of types B and C, A and D, and B and D, respectively (Table 3). Considering the high abilities of spore dispersal in homosporous ferns, it is still possible that reproductive isolation may prevent gene flow even between these pairs of *rbcL* types.

Significant differentiation in allele frequencies of the allozyme loci was also observed between type E and the other types (Table 3). It is, however, still not clear whether type E and the other *rbcL* types are reproductively isolated because the materials for type E were collected from Bogor Botanical Garden and did not originate from the same locality as the other *rbcL* types did.

Soltis and Soltis (1989) reported the range of genetic identity (I) between congeneric species and that between conspecific populations of homosporous pteridophytes to be 0.00–0.85 and 0.78–0.996, respectively. All genetic identities (I) estimated in this work fall into the range of that between congeneric species (Table 4). Only the value estimated between types C and E falls into the range of that between conspecific populations as well (Table 4). Our findings support the view that the five *rbcL* sequence types in West Java are separate cryptic species.

Types B and D were entirely differentiated from the others in one or two allozyme loci (Appendix, Table 3). The other three *rbcL* types were, however, less distinct populations according to allozyme alleles. The pairs of types A and C, and C and E shared some alleles in every locus and no other genetic loci other than *rbcL* support the species boundary drawn based on *rbcL* in these three types. Types A, C, and E formed a cluster in the obtained UPGMA dendrogram (Fig. 5). These three types also formed a clade with 93% bootstrap reliability in the molecular phylogenetic analysis based on the *rbcL* sequence although many other plants, collected from Thailand, China, Japan and so on, were nested within this clade (Yatabe et al. 2001).

There are three possible reasons for the ambiguous boundaries of these assemblages of types A, C, and E. The first is the formation of F1 hybrids or introgression between the *rbcL* types. The extent of introgression between these *rbcL* types, however, should be small enough that these *rbcL* types are still significantly differentiated at several loci (Table 3). Indeed, types A and C did not form F1 hybrids in the crossing experiments (Yatabe et al. 2001), suggesting that the frequency of the formation of a F1 hybrid cannot be high in nature either. The second is the difference in the effective population size between *rbcL* and allozymes. The effective population size for *rbcL* is expected to be one-quarter that of allozymes because *rbcL* is coded on the maternally inherited chloroplast DNA (Gastony and Yatskievych 1992) whereas allozymes are encoded on nuclear genomes. It has been suggested theoretically that the smaller the effective population size is, the more seriously genetic drift causes a loss in variation of alleles (Wright 1931). The larger effective population size also may cause incomplete lineage sorting (Moore 1995). This means that chloroplast DNA sequence data can be more useful than allozyme data as a first approximation for recognizing reproductively isolated assemblages in a locality. The last possible reason is the presence of cryptic alleles of allozyme not recognized during electrophoresis. In order to discuss which reason is most feasible, further investigations will be required.

In *Asplenium* sect. *Thamnopteris*, sporophyte morphology does not provide good qualitative taxonomic characters for species recognition. Holttum (1974) monographed 15 species of sect. *Thamnopteris* using gross morphological characters. However, species delimitation is unclear and the naturalness of these taxa as species is questionable because most of the characters that he adopted as keys to the species were quantitative characters such as frond width and frond apex shape. In cases such as this, in which morphology fails to yield clear hypotheses, the characters of chloroplast DNA sequences can provide useful alternative information for species recognition. When we combine DNA sequence data and nuclear genomic markers such as allozyme polymorphism, we are able to clarify whether the assemblages based on the *rbcL* sequence are reproductively isolated and to recognize the cryptic species more effectively in homosporous ferns.

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**Appendix** Allele frequencies for 11 allozyme loci

| Locus | Allele | *rbcL* type | | | | | Locus | Allele | *rbcL* type | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | | | A | B | C | D | E |
| AAT | a | 0.000 | 0.025 | 0.000 | 0.000 | 0.175 | TPI-1 | a | 0.000 | 0.000 | 0.000 | 0.625 | 0.000 |
| | b | 0.850 | 0.975 | 0.425 | 1.000 | 0.825 | | b | 0.000 | 0.000 | 0.075 | 0.000 | 0.000 |
| | c | 0.100 | 0.000 | 0.550 | 0.000 | 0.000 | | c | 0.000 | 0.000 | 0.000 | 0.375 | 0.000 |
| | d | 0.050 | 0.000 | 0.025 | 0.000 | 0.000 | | d | 0.825 | 0.975 | 0.825 | 0.000 | 0.800 |
| LAP | a | 0.000 | 0.050 | 0.000 | 0.000 | 0.000 | | e | 0.175 | 0.025 | 0.100 | 0.000 | 0.200 |
| | b | 0.000 | 0.025 | 0.000 | 0.000 | 0.050 | TPI-24 | a | 0.000 | 0.050 | 0.000 | 0.000 | 0.000 |
| | c | 0.050 | 0.500 | 0.100 | 0.000 | 0.025 | | b | 0.000 | 0.000 | 0.150 | 0.000 | 0.400 |
| | d | 0.025 | 0.000 | 0.050 | 0.000 | 0.050 | | c | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| | e | 0.000 | 0.000 | 0.025 | 0.000 | 0.000 | | d | 0.200 | 0.825 | 0.625 | 0.000 | 0.600 |
| | f | 0.150 | 0.000 | 0.000 | 0.000 | 0.025 | | e | 0.800 | 0.125 | 0.225 | 0.000 | 0.000 |
| | g | 0.000 | 0.425 | 0.650 | 0.000 | 0.575 | TPI-3 | a | 0.000 | 0.000 | 0.200 | 0.000 | 0.025 |
| | h | 0.000 | 0.000 | 0.100 | 0.000 | 0.000 | | b | 0.975 | 0.975 | 0.775 | 1.000 | 0.975 |
| | I | 0.275 | 0.000 | 0.000 | 0.000 | 0.000 | | c | 0.025 | 0.025 | 0.025 | 0.000 | 0.000 |
| | j | 0.425 | 0.000 | 0.050 | 0.925 | 0.225 | HK | a | 0.000 | 0.050 | 0.000 | 0.000 | 0.000 |
| | k | 0.025 | 0.000 | 0.000 | 0.025 | 0.025 | | b | 0.000 | 0.475 | 0.000 | 0.000 | 0.000 |
| | l | 0.050 | 0.000 | 0.000 | 0.050 | 0.025 | | c | 0.000 | 0.450 | 0.000 | 0.000 | 0.000 |
| | m | 0.000 | 0.000 | 0.025 | 0.000 | 0.000 | | d | 0.000 | 0.025 | 0.000 | 0.000 | 0.000 |
| PGI | a | 0.000 | 0.000 | 0.075 | 0.000 | 0.100 | | e | 0.600 | 0.000 | 0.000 | 0.000 | 0.000 |
| | b | 0.000 | 0.125 | 0.000 | 0.000 | 0.000 | | f | 0.025 | 0.000 | 0.000 | 0.000 | 0.000 |
| | c | 0.775 | 0.775 | 0.925 | 0.000 | 0.900 | | g | 0.375 | 0.000 | 0.200 | 0.625 | 0.000 |
| | d | 0.000 | 0.050 | 0.000 | 0.000 | 0.000 | | h | 0.000 | 0.000 | 0.000 | 0.000 | 0.025 |
| | e | 0.225 | 0.050 | 0.000 | 0.850 | 0.000 | | I | 0.000 | 0.000 | 0.750 | 0.350 | 0.250 |
| | f | 0.000 | 0.000 | 0.000 | 0.150 | 0.000 | | j | 0.000 | 0.000 | 0.000 | 0.000 | 0.700 |
| DIA | a | 0.025 | 0.000 | 0.000 | 0.000 | 0.000 | | k | 0.000 | 0.000 | 0.050 | 0.025 | 0.025 |
| | b | 0.025 | 0.000 | 0.000 | 0.000 | 0.000 | G6P | a | 0.000 | 0.000 | 0.050 | 0.000 | 0.000 |
| | c | 0.300 | 0.000 | 0.025 | 0.000 | 0.000 | | b | 0.000 | 0.000 | 0.000 | 0.050 | 0.000 |
| | d | 0.650 | 0.000 | 0.775 | 0.000 | 0.000 | | c | 1.000 | 1.000 | 0.950 | 0.725 | 0.875 |
| | e | 0.000 | 0.000 | 0.000 | 0.000 | 0.025 | | d | 0.000 | 0.000 | 0.000 | 0.225 | 0.000 |
| | f | 0.000 | 0.925 | 0.200 | 0.525 | 0.975 | | e | 0.000 | 0.000 | 0.000 | 0.000 | 0.125 |
| | g | 0.000 | 0.075 | 0.000 | 0.475 | 0.000 | 6PG | a | 0.025 | 0.000 | 0.000 | 0.000 | 0.025 |
| PGM | a | 0.000 | 0.025 | 0.000 | 0.000 | 0.000 | | b | 0.975 | 0.000 | 0.625 | 1.000 | 0.950 |
| | b | 0.000 | 0.000 | 0.000 | 0.000 | 0.025 | | c | 0.000 | 1.000 | 0.200 | 0.000 | 0.025 |
| | c | 0.375 | 0.975 | 0.000 | 0.000 | 0.050 | | d | 0.000 | 0.000 | 0.175 | 0.000 | 0.000 |
| | d | 0.000 | 0.000 | 0.000 | 0.425 | 0.000 | | | | | | | |
| | e | 0.000 | 0.000 | 0.900 | 0.000 | 0.875 | | | | | | | |
| | f | 0.550 | 0.000 | 0.000 | 0.575 | 0.000 | | | | | | | |
| | g | 0.000 | 0.000 | 0.000 | 0.000 | 0.050 | | | | | | | |
| | h | 0.075 | 0.000 | 0.075 | 0.000 | 0.000 | | | | | | | |
| | I | 0.000 | 0.000 | 0.025 | 0.000 | 0.000 | | | | | | | |

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