MORPHOMETRIC ANALYSIS OF VARIATION AMONG THREE POPULATIONS OF *DORYOPTERIS LUDENS* (ADIANTACEAE: PTERIDOPHYTA) IN THAILAND

T. BOONKERD

Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
(Email: Thaweesakdi.B@Chula.ac.th)

Keywords: Adiantaceae, *Doryopteris ludens,* cluster analysis, canonical discriminant analysis, multivariate analysis.

ABSTRACT

Morphological variation within populations and among populations was examined in three populations of *Doryopteris ludens* from western and peninsular Thailand. Sixteen quantitative characters of both vegetative and reproductive characters were scored. The field data were analysed by means of cluster analysis and various discriminant analyses. Cluster analysis and canonical discriminant analysis indicated two groups. It is consequently concluded that there are two morphological varieties which that can be distinguished on the basis of sporangium length, sporangium width, fertile-frond sinus-depth, fertile-lamina width and habitat. A conventional identification key is provided, which is based on fertile-frond sinus-depth, sporangium length, and substrate conditions.

INTRODUCTION

Doryopteris is one of the smaller genera of the Pteridophytes. Tryon (1942) included 26 species in his revision of the genus. He also noted that *Doryopteris* is terrestrial, usually growing in rather dry, rocky places. Some species are extremely xeromorphic. Of the 26 species, there is only one species in Thailand, i.e. *Doryopteris ludens* (Wall. ex Hook.) J. Sm. as was enumerated in *Flora of Thailand*, volume 3 part 2 (Tagawa & Iwatsuki, 1985). This species is extremely variable in leaf form. The slender, elongate rhizome separates it from all other reticulate-veined species (Tryon, 1942) and it also differs from the closely related species *D. pedata* (L.) Fée in having a terete stipe. Geographically, this species is Asiatic, since its present distribution is confined to Myanmar, India, southern China and the Malay Peninsula, whilst the majority of *Doryopteris* species are American.

Preliminary studies of herbarium specimens deposited at the following herbaria (abbreviations according to Holmgren *et al.*, 1990):- Bangkok Forest Herbarium (BKF); the Professor Kasin Suvatabhandhu Herbarium, Department of Botany, Chulalongkorn University, Thailand (BCU); the Royal Botanic Gardens, Kew Herbarium (K) and The Natural History Museum, U.K. (BM), suggested that two forms of this species probably occurred, the normal and the dwarf forms. The normal form has a wider distribution throughout the country, occuring naturally in calcareous soils, in shady places in the dry evergreen forest, whilst the dwarf form is confined to the calcareous rocks of the limestone hills or limestone islands in peninsular Thailand. The specimens collected from Langkawi Island, Malaysia, and deposited at Kew

herbarium match well with this dwarf form. However, some additional field studies in peninsular Thailand revealed some morphological variations in characters related to size of frond within these dwarf form populations. There were still some overlaps in stipe length, lamina width and lamina length etc. between normal and dwarf forms, and so it remained unclear whether two forms could be recognized within this species.

The present study is aimed at clarifying the taxonomic status of these two morpho-ecological forms of *D. ludens* by determining the levels of intra- and inter-population variation in the Thai populations.

MATERIALS AND METHODS

Plant materials

Field collections of *D. ludens* were made during the rainy season to permit collection of complete specimens of both fertile and sterile fronds. Eighty-five specimens from three populations were sampled; the location, ecology and number of specimens are listed in Table 1. Sixteen quantitative characters of both vegetative and reproductive structures were measured or counted (Table 2).

TABLE 1: Location, ecology and number of specimens of the three populations of *Doryopteris ludens* in Thailand.

Population		Location	Ecology	Number of specimens
1	Tub Sakae District, Prachuap Khiri Khan Province	Western Thailand	Calcareous soil in shady places in dry evergreen forest	30
2	Had Chao Mai District, Trang Province	Peninsular Thailand	Rock crevices, exposed or partially shaded in limestone hill	30
3	Muang District, Phangnga Province	Peninsular Thailand	Rock crevices, or on humus–rich rocks, exposed or partially shaded in limestone islands	25

Specimens examined

a. Normal form

Habitat: Terrestrial in tropical or evergreen forests at low or medium altitudes.
Altitude : 100-1500 m.
Distribution: India: Type:Wallich 88 (K); Isotype: Cuming 238 (K)
Voucher: Thailand: North: *R. Geesink & C. Phengklai 5949* (BKF), *E. Hennipman 3029* (BKF), *A.F.G Kerr 11365* (BK), *Winit 1074* (BKF); North-Eastern: *T. Boonkerd 23, 269* (BCU), *J.F. Maxwell 76-325* (BK), *M. Tagawa, K. Iwatsuki & N. Fukuoka 1254* (BKF); Central: *Murata, Phengklai, Nagamasu & Nantasaen T-51409*(BKF), *J.F. Maxwell 73-522* (BK), *A.F.G Kerr 6029* (BK); East: *J.F. Maxwell 74-978*(BK), *W. Somprasong 155* (BK); Western: *K. Chandraprasong 61* (BK), *K. Larsen & S.S. Larsen 33971* (BKF), *A. Marcan 2696* (BM), *Put 1430* (BK), *T. Boonkerd 1122* (BCU); Peninsular: *Ch. Charoenphol, K. Larsen & E. Warncke 3634* (BKF), *E. Smith 2418A* (BM), *Put 1027, 1636, 3206* (BK, K),

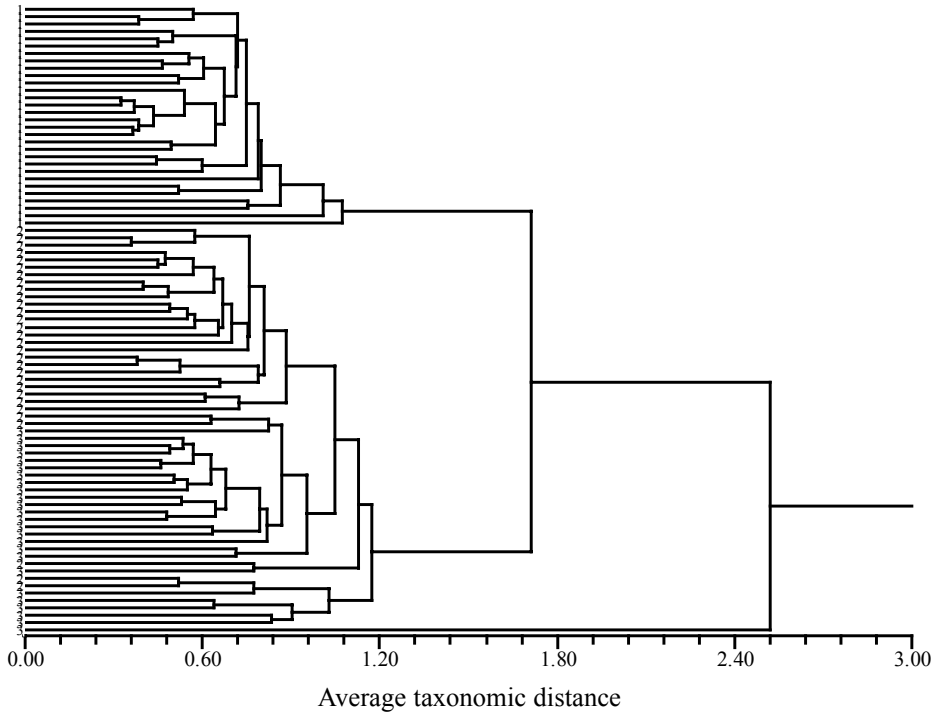

Figure 1. Dendogram of *Doryopteris ludens* specimens

b. Dwarf form

Habitat: Terrestrial in rock crevice or on humus rich rock in limestone islands or limestone hill.

Altitude : sea level to 250 m.

Distribution: Malaysia: Langkawi Island: *H.C. Robinson s.n.* (K)

Voucher: Thailand: South-Eastern: *E. Smith 2417A* (BM); Western: *K. Larsen & S.S. Larsen 33688* (BKF), *Put 250* (BK, BM, K); Peninsular: *A.F.G Kerr 11365* (BK, K), *Rabil 131*(BK, K), *T. Shimizu, N. Fukuoka & A. Nalampoon 7996* (BKF), *T. Boonkerd 151, 1443*(BCU)

Data collection and multivariate analyses

To determine patterns of variation in *D. ludens* both *a priori* and *a posteriori* grouping systems were examined. First, the pattern of variation was examined by cluster analysis using the average taxonomic distance among the 85 specimens (Rohlf & Sokol, 1965). A sequential, agglomerative, hierarchical and nested (SAHN) clustering nested technique (Sneath & Sokal, 1973) was performed using the unweighted pair-group method with arithmetic averages (UPGMA) which is available in NTSYS-pc package version 2.0K (Rohlf, 1998). The purpose of this analysis was to place individual specimens (N=85) into groups (clusters) suggested by the data, but not defined *a priori*. Second, to determine whether morpho-ecological patterns existed from calcareous soil to calcareous rocks, each specimen was assigned to an *a priori* group based on its occurrence in natural habitat.

The SPSSpc-FW (Anonymous, 1997) was used to perform univariate analysis, stepwise discriminant analysis, classification discriminant analysis and canonical discriminant analysis. Stepwise discriminant analysis was used to select a subset of characters that maximized differences among the *a priori* groups. Correct classification rates were used as indicators of separation among the groups. Canonical discriminant analysis was used as a dimension reduction technique to facilitate visualization of the results of the multivariate analysis.

RESULTS

Patterns of variation among specimens of *D. ludens*

The SAHN technique generated a dendrogram which split the specimens into two groups (Figure 1). Specimens classified as group 1 in the cluster analysis consisted of all *D. ludens* from only population 1, whilst group 2 included members from both population 2 and population 3 which are rather separated into two subgroups. These two groups matched the morpho-ecological patterns of this species, i.e. normal form and dwarf form. However, the separation of group 2 into two subgroups suggested intra- and inter-population variation in the dwarf populations. Accordingly, three-clustering grouping were used in subsequent analysis as *a posteriori* groups.

Sixteen characters were determined by stepwise discriminant analysis to be important in discriminating between the three groups. The following nine characters:- 4, 5, 6, 8, 9, 11, 13, 14, 15 were selected as important for giving the best separation of the groups (Table 3). In total, 97.6% of the specimens were classified correctly. These classification rates are extremely high considering that variation within the three populations existed.

Ordination of the 85 specimens by canonical discriminant analysis was presented on the two canonical axes (Figure 2). This shows population 1 clearly separated from closely related population 2 and population 3 on axis 1. Thus the two morpho-ecological forms of *D. ludens* appear distinct. The nature of the group differences is characterized by the within-canonical structure (Table 3). Canonical variable 1 (axis 1) is most highly associated with characters 14, 15, 6, 5, 7, 3, 16 and 12 in descending order of the absolute values of the correlations (Table 3). The canonical correlation of the first canonical discriminant function is 98% correlated with all the variables and the variance explained by it is 93.5%. Thus this axis is effective for separating the two morpho-ecological groups of *D. ludens*.

The F-values (Table 3) indicate by their magnitude the relative order of importance of the characters in general. It is clear that the F-values almost reflect the association of characters with canonical axis 1 because of its high correlation and high variance explained. Basic statistics of the three groups are also summarized in Table 3. It can be concluded that the vegetative characters of the normal form (population 1) were generally larger than the dwarf form (population 2 and 3). In contrast, the reproductive characters of the dwarf form were bigger than the normal form. In general, the means of the most important characters were significantly different, especially the four most important characters for axis 1 as can be seen from boxplots (Figure 3).

DISCUSSION

The results of cluster analysis, and canonical discriminant analysis support the recognition of separating the three populations of *D. ludens* into two distinct entities, probably as two varieties. The four most important characters (Table 3) that separate

TABLE 2. Morphological characters used in the multivariate analysis of *Doryopteris ludens*

	Number	Character	Details of measurements and counts
Vegetative	1	fertile stipe diameter (mm)	diameter at base of stipe of fertile frond
	2	sterile stipe diameter (mm)	diameter at base of stipe of sterile frond
	3	fertile lamina length (cm)	length of lamina of fertile frond
	4	fertile lamina lobe number	numbers of lobes of lamina of fertile frond
	5	fertile lamina width (cm)	width of lamina of fertile frond
	6	fertile frond sinus depth (cm)	distance between margin and base of sinus of fertile frond
	7	fertile stipe length (cm)	length of stipe of fertile frond
	8	sterile lamina length (cm)	length of lamina of sterile frond
	9	sterile lamina lobe number	numbers of lobes of lamina of sterile frond
	10	sterile lamina width (cm)	width of lamina of sterile frond
	11	sterile frond sinus depth (cm)	distance between margin and base of sinus of sterile frond
Reproductive	12	sterile stipe length (cm)	length of stipe of sterile frond
	13	annulus thick (μm)	distance from top to base of an annulus cell situated at the top of sporangium
	14	sporangium length (μm)	longest distance measured from base to top of sporangium (without stalk)
	15	sporangium width (μm)	longest distance measured from side to side of sporangium at the equatorial axis
	16	spore diameter (μm)	length of spore from polar view or polar diameter

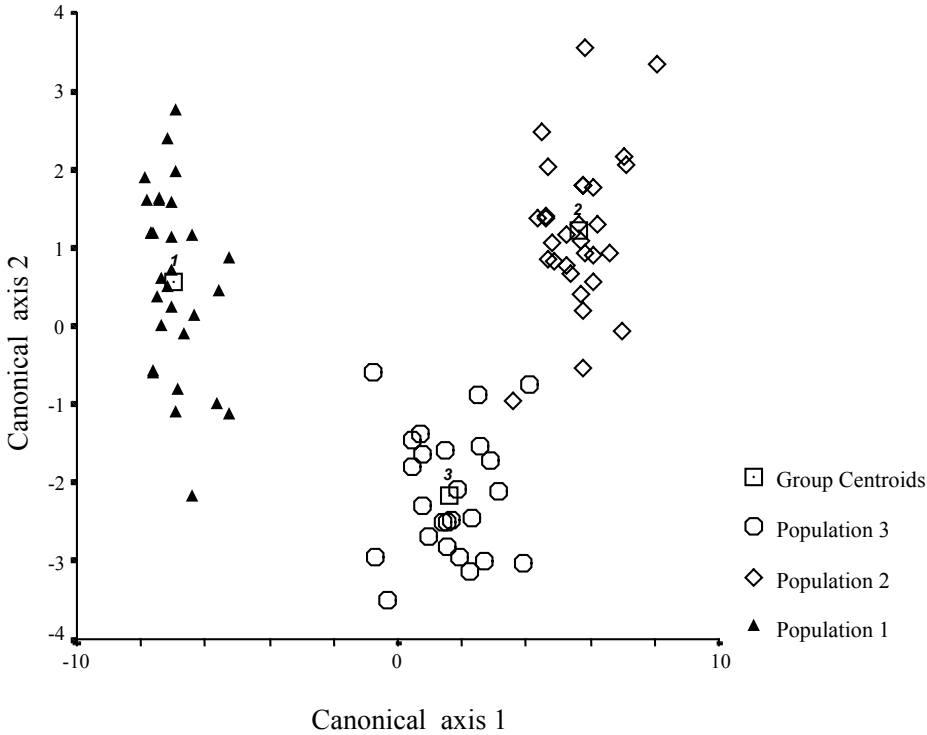

Figure 2. Canonical discriminant analysis

these two varieties are sporangium length (14), sporangium width (15), fertile-frond sinus-depth (6), and fertile-lamina width (5). However, fertile-frond sinus-depth (6) and fertile-lamina width (5) tend to be more important in the field for separating normal and dwarf forms of *D. ludens* as sporangium length (14) and sporangium width (15) are microscopic characters, only suitable for laboratory herbarium determination. These two vegetative characters are characters of leaf dissection as mentioned in Tryon (1942) in his revision of the genus. He noted that most of the species are quite variable in leaf dissection and should not be used alone as a diagnostic character in key construction, even as infraspecific determination, unless supported by additional characters. However, Tryon (1942) recognized the value of the sporangium stalk-length together with the leaf dissection characters to separate the species.

Baum and Bailey (1994) used a series of discriminant analyses to determine taxonomic status of *Hordeum caespitosum* Scribner from different geographical ranges. They pointed out that for *Hordeum* a group of characters must be used together for identification, at least of the most important ones in the above sense. Speer and Hilu (1999) evaluated taxonomic status and determined quantitatively the importance of morphological characters that contribute to the discrimination between var. *latiusculum* (Desv.) W.C. Shieh and var. *pseudocaudatum* (Clute) A. Heller of Bracken fern, *Pteridium aquilinum* (L.) Kuhn (Dennstaedtiaceae) which is usually treated as a single species. They concluded that the treatment of the eastern North American bracken as var. *latiusculum* and var. *pseudocaudatum* seemed justifiable from the results of their study.

TABLE 3: Pooled within-canonical structure and F-values with their probabilities. Correlations between the two canonical variables and the original variables. Summary of mean, SD in the three populations of *Doryopteris ludens*

Character	F-value	Prob.	Axis 1	Axis 2	Population 1		Population 2		Population 3	
					mean	SD	mean	SD	Mean	SD
1. fertile-stipe diameter[a]	43.83	0.00	0.01	0.18*	1.89	0.18	1.36	0.25	1.40	0.28
2. sterile-stipe diameter[a]	15.91	0.00	-0.13	0.41*	1.32	0.21	1.33	0.18	1.05	0.23
3. fertile-lamina length[a]	143.58	0.00	-0.19*	0.16	20.79	2.27	9.53	3.23	11.25	2.64
4. fertile-lamina-lobe number	2.67	0.75	0.04	-0.09*	13.67	2.26	16.13	5.68	17.00	7.94
5. fertile-lamina width	87.48	0.00	-0.26*	0.25	18.26	1.88	9.76	3.43	10.33	2.70
6. fertile-frond sinus-depth	151.80	0.00	-0.34*	0.32	11.47	1.31	5.14	1.85	5.59	1.42
7. fertile-stipe length[a]	44.83	0.00	-0.22*	0.10	39.34	12.04	16.68	6.95	24.74	8.19
8. sterile-lamina length	113.23	0.00	-0.28	0.43*	14.50	2.07	8.20	1.52	7.58	2.18
9. sterile-lamina-lobe number	52.96	0.00	0.19	0.29*	7.03	1.16	15.67	3.47	9.92	4.59
10. sterile-lamina width[a]	1.42	0.25	-0.04	0.14*	10.83	2.02	7.89	1.55	9.18	2.23
11. sterile-frond sinus-depth	59.92	0.00	-0.19	0.41*	5.66	1.57	3.15	0.71	2.46	0.99
12. sterile-stipe length[a]	26.02	0.00	-0.10*	0.10	22.48	6.91	13.25	4.95	13.16	4.71
13. annulus-thick	230.33	0.00	0.41	0.46*	5.04	0.56	9.01	0.86	6.69	0.72
14. sporangium-length	294.69	0.00	0.48*	0.26	2.03	0.12	3.47	0.22	2.82	0.32
15. sporangium-width	146.61	0.00	0.34*	0.13	1.50	0.14	2.40	0.20	2.02	0.25
16. spore-diameter[a]	150.92	0.00	0.16*	-0.04	5.39	0.71	9.40	1.14	8.13	0.83

[a] : character not used in the stepwise analysis,
* : largest absolute correlation between each variable and any discriminant function

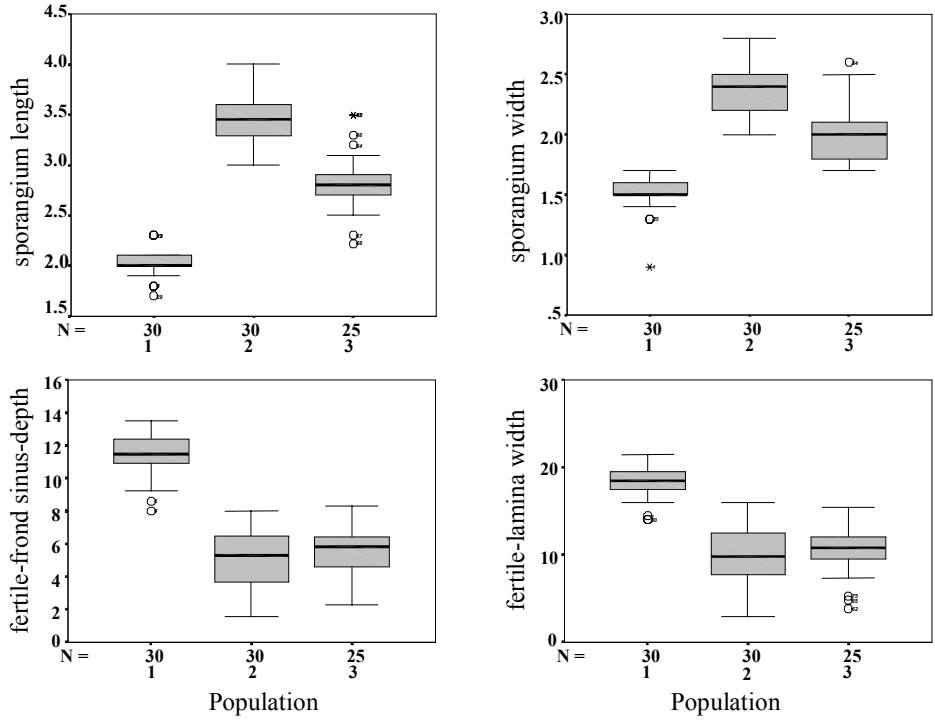

Figure 3. Boxplots of the four most important characters

Key to identify the infraspecific taxa of *D. ludens*.

Fertile-frond sinus-depth more than 7 cm; sporangium length 1.9-2.1 mm; plant growing in calcareous soils, in dense dry evergreen forest var. A (*ludens*)

Fertile-frond sinus-depth less than 7 cm; sporangium length 2.5-3.7 mm; plant growing in rock crevices, exposed or partially shaded var. B

CONCLUSION

The results provided justification for the recognition of infraspecific variation among the three populations of *D. ludens*. In most cases they can be distinguished morpho-ecologically by their sporangium length, sporangium width, fertile-frond sinus-depth, fertile-lamina width and by their habitats (calcareous soils or calcareous rocks). However, this study is exploratory and further verification is required.

ACKNOWLEDGMENTS

I am extremely grateful to Dr. Bernard R. Baum for his kind valuable comments on the manuscript. The work was partially supported by a grant from Chulalongkorn University, Bangkok, Thailand. I appreciate the field assistance of Rossarin Polawatn and assistance of Wanachai Chatan in the measurement of characters. This paper improved markedly from the comments of anonymous reviewers, to whom I am very grateful.

REFERENCES

ANONYMOUS 1997. SPSS for Windows Release 7.5.2, Standard version, SPSS Inc.,Chicago.

BAUM, B.R. & BAILEY, L.G. 1994. Taxonomy of *Hordeum caespitosum*, *H. jubatum*, and *H. lechleri* (Poaceae: Triticeae). Pl. Syst. Evol. 190: 97-111.

HOLMGREEN, P.K., HOLMGREN, N.H. & BARNETT, L.C. 1990. Index Herbariorum. Part I: The Herbaria of the World. 8 Edn. I.A.P.T., New York Bot. Gard., New York:

ROHLF, F.J. 1998. NTSYS-pc version 2.0k Numerical Taxonomy and Multivariate Analysis System. Setauket, Exeter Software, New York.

ROHLF, F.J. & SOKAL, R.R. 1965. Coefficients of correlation and distance in numerical taxonomy. Univ. Kansas Sci. Bull. 45:3-27.

SNEATH, P.H.A. & SOKAL, R.R. 1973. Numerical Taxonomy: The principles and practice of numerical classification. W.H. Freeman and Company, San Francisco.

SPEER, W.D. and HILU, K.W. 1999. Relationships between two infraspecific taxa of *Pteridium aquilinum* (Dennstaedtiaceae). I. Morphological evidence. Syst. Bot. 23(3): 305-312.

TAGAWA, M. & IWATSUKI, K. 1985. Pteridophytes. In: LARSEN, K. & SMITINAND, T. (Eds) Flora of Thailand of Thailand, vol. 3, part 2, The Chutima Press, Bangkok.

TRYON, A.F. 1942. A revision of the genus *Doryopteris*. Contr. Gray Herb. 143: 1-80.