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Morphometrics and the Identification of *Braunia andrieuxii* and *B. secunda* (Hedwigiaceae, Bryopsida)

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Abstract—Although previous cladistic analyses revealed *Braunia secunda* and *B. andrieuxii* as two lineages and valid species, they have been considered synonyms due to morphological similarities and sympatric distribution. This study was undertaken to evaluate which morphological characters best distinguish these two species. A total of 180 specimens of *B. andrieuxii* and 112 of *B. secunda* from the U.S. and Mexico were examined for morphometric analyses. Digital images of capsules, leaves, and groups of leaf cells were used to extract 31 linear measurements. Specimens were subdivided into seven geographic groups to compare levels of variation within and between species with univariate and multivariate analyses. Most characters are similar among groups within species, except the length of the revolute leaf margin and apical and upper leaf cells, which are significantly different between species (ANOVA and MRT). Partitioned Canonical Variates Analyses on eight variables of leaf cells and seven variables of vegetative leaves identified significant Mahalanobis distances between the two species. These methods also revealed that the revolute leaf margin and upper leaf cells contribute most to the distinction between species.

Keywords—morphological variation, mosses, partitioned CVA, species identification.

Braunia Bruch & Schimp. is the largest genus in Hedwigiaceae, with 23 species. These are mainly distributed in the mountains of subtropical and tropical regions around the world, in five geographic areas: 1) southern United States of America, Mexico, Central America, and the Caribbean Islands, 2) the Andes of Colombia, Ecuador, Peru, Bolivia, and Argentina in South America, 3) eastern and western Africa, 4) the Himalayan mountains in India and China, and 5) Europe. There are few studies concerning taxonomic and systematic problems in *Braunia* (De Luna 1992). Dixon (1920) examined morphological variation in *Braunia diaphana* (Müll. Hal.) A. Jaeger, *B. indica* Paris, and *B. secunda* (Hook.) Bruch & Schimp., and provided a new description of *B. secunda* subsp. *brachytheca* Dixon. Robinson et al. (1977) commented on the variation of *B. secunda* in relation to other South American species. More recently, Biasuso (1992) studied five species of *Braunia* in Argentina. The species of *Braunia* occurring in Mexico have not been investigated. Current floristic treatments by several authors have interpreted *B. andrieuxii* Lor. and *B. secunda* as very similar, and without data or analyses, bryologists have accepted *B. andrieuxii* as a synonym of *B. secunda* (Bartram 1949; Sharp et al. 1994; Churchill and Linares 1995).

A morphological cladistic analysis of all species in Hedwigiaceae indicated that *B. secunda* and *B. andrieuxii* are not sister groups, but two independent lineages (De Luna, 1992). This study was based on 38 terminals and 42 characters of gametophytes and sporophytes. A simplified strict consensus tree of *Braunia* species is presented here (Fig. 1). Several species and varieties traditionally placed as synonyms of *B. secunda* were treated as independent terminal units and proved to be phylogenetically unrelated. Notably, *B. secunda* sensu stricto was placed basal to other species, whereas *B. andrieuxi* occupied a nested position among distal clades. Moreover, the same study suggested that *B. secunda* does not include populations from Africa and India. Reports of *B. secunda* from Africa and India actually correspond to plants that are better placed in *B. macropelma* (Müll. Hal.) A. Jaeger (De Luna 1992). This is a much narrower circumscription of *B. secunda* than conventionally accepted (Dixon 1920; Brotherus 1925). Therefore, *Braunia andrieuxii* is endemic to the southwestern U. S. A. and Mexico (De Luna 1992). Both *B. andrieuxii* and *B. secunda* generally grow on dry boulders and cliffs, in pine-oak forests, in shaded or exposed habitats at high elevations (1700–4100 m). Although they represent two independent lineages, it is very difficult to distinguish *B. secunda* from *B. andrieuxii* morphologically.

The distinction between *B. secunda* and *B. andrieuxii* has not been evaluated in detail. Both species share morphological features such as an ovoid-elliptic capsule and ovate to lanceolate leaf shape. Thériot (1926) first suggested some morphological characters that might differentiate the two species. He pointed out that in *B. andrieuxii* the leaf margin is plane to reflexed, leaf cells are short, and the leaf acumen is gradually differentiated. Bryophyte systematists in the 20th century disregarded his remarks, interpreting such differences as lacking taxonomic value (Bartram 1949; Sharp et al. 1994; Churchill and Linares 1995). Our preliminary observations indicated that leaves are indeed broader, obovate and the upper leaf cells are short in *B. andrieuxii*. Additionally, *B. andrieuxii* has the leaf margins erect or reflexed only at the base. In contrast, *B. secunda* has a revolute leaf margin which extends up half the leaf length, elongate leaf cells, and an acuminate leaf apex. These putative differences have not been studied quantitatively. In the present paper, we address the following goals: 1) evaluate the extent of the morphological variation among populations of *B. andrieuxii* and *B. secunda*, and between species through distance measurements of morphological characters of mature gametophytes and sporophytes, and 2) determine which morphological characters are useful for identifying both species, using univariate and multivariate statistical methods.

Materials and Methods

Specimens—A total of 180 samples of *B. andrieuxii* and 112 of *B. secunda* were included for morphometric analyses. We also evaluated type specimens of both species in order to examine their relative position in the morphospace configured by morphological characters from leaf cells, leaves, and capsules. Most specimens were borrowed from the following herbaria: BM, CAS, DUKE, GE, JE, MEXU, MICH, MO, NY, and US. In addition, we collected populations of both species in new localities from Chiapas, Michoacán, Tlaxcala, and Veracruz (specimens at XAL).

Morphological Characters—We examined seta length, capsule shape, leaf and acumen shape, as well as differences in leaf cells (Table 1), all

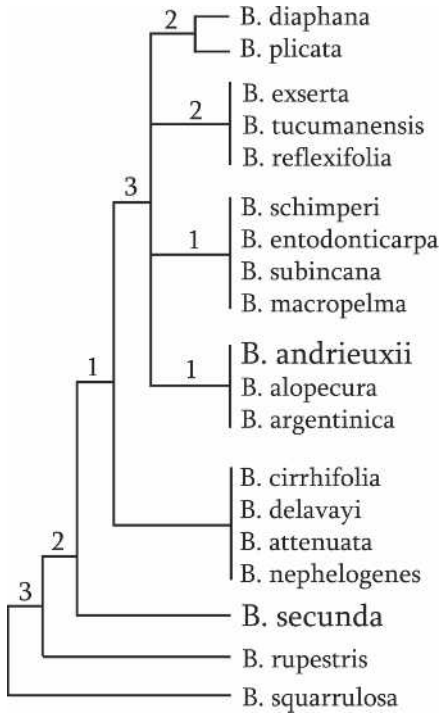

FIG. 1. Phylogenetic relationships among species of *Braunia*. Numbers above branches are Bremer support values as estimated by the morphological cladistic analysis of the Hedwigiaceae by De Luna (1992). This simplified strict consensus tree depicts the position of *B. andrieuxii* and *B. secunda* as separate lineages.

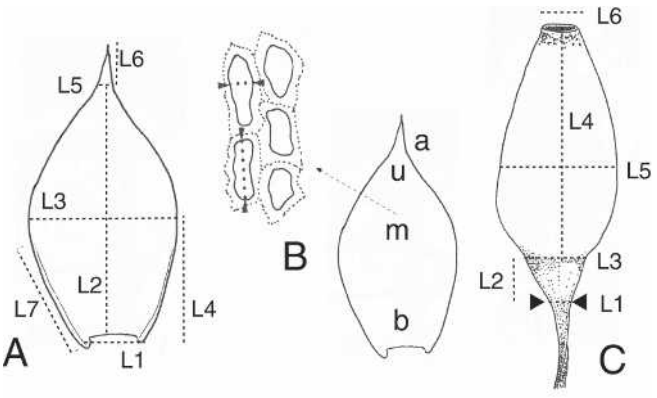

FIG. 2. A-C. Measurements of gametophyte and sporophyte characters of *B. andrieuxii* and *B. secunda* used in this analysis. A. Vegetative leaf: L1 = width at base of leaf lamina, L2 = length of leaf lamina, L3 = width of leaf lamina, L4 = length from base to widest part of leaf lamina, L5 = acumen width, L6 = acumen length, L7 = extension of the revolute leaf margin. B. A group of leaf cells and a leaf showing the location of the four leaf areas sampled for cell features: a = apical, u = upper, m = medial, b = basal. C. Capsule: L1 = neck width, L2 = neck length, L3 = width at urn base, L4 = urn length, L5 = urn width, L6 = capsule mouth width.

previously proposed to diagnose both species (De Luna 1992). We extracted inter landmark distances for morphological characters from vegetative and perichaetial leaves, leaf cells, and capsules. Variation of leaf shape along a module (stem) occurs with young leaves at the tip, mature leaves in the middle, and older juvenile leaves at the base (Mishler and De Luna 1991). Only mature vegetative leaves in the heteroblastic leaf series of the main stem were considered for distance measurements. Five leaves were examined in detail, and six distances were measured per leaf (Fig. 2A). Groups of leaf cells were sampled from four main leaf areas: apical, upper third, medial, and basal. In each leaf area, cell length and width was measured in five cells. At the leaf apex, we excluded the marginal cells, selecting and measuring only cells at the center of the apical area (Fig. 2B). Three to five innermost perichaetial leaves per specimen were examined, measuring length and width. We measured nine sporophytic characters, of which six are measurements estimated from moist capsules (Fig. 2C). Only one sporophyte per plant was analyzed, although not all specimens had capsules. On each capsule, exothecial cell length and width were sampled by measuring five cells from the central area of the dissected and extended urn wall. Table 1 includes characters and acronyms used for purposes of discussion.

TABLE 1. Morphological characters, their acronyms, and the scale unit relevant to each character used in morphometric analyses of *B. andrieuxii* and *B. secunda*.

GAMETOPHYTE—*Leaf cells*. **1. Apical leaf cell length**, AL, μm. **2. Apical leaf cell width**, AW, μm. **3. Upper leaf cell length**, UL, μm. **4. Upper leaf cell width**, UW, μm. **5. Medial leaf cell length**, ML, μm. **6. Medial leaf cell width**, MW, μm. **7. Basal leaf cell length**, BL, μm. **8. Basal leaf cell width**, BW, μm. **9. Length/Width of apical leaf cells**, AL/W, 10. **Length/Width of upper leaf cells**, UL/W, **11. Length/Width of medial leaf cells**, ML/W, **12. Length/Width of basal leaf cells**, BL/W.

Vegetative leaves. **13. Base width of leaf lamina**, LB, mm. **14. Leaf lamina length**, LL, mm. **15. Leaf lamina width**, LW, mm. **16. Length to widest point of leaf lamina**, LWP, mm. **17. Acumen width**, LAW, mm. **18. Acumen length**, LAL, mm. **19. Revolute leaf margin length**, LRM, mm. **20. Leaf lamina length / Revolute leaf margin length ratio**, LL/LRM.

SPOROPHYTE—*Capsule*. **21. Neck width**, CNW, mm. **22. Neck length**, CNL, mm. **23. Urn base width**, CUBW, mm. **24. Urn length**, CUL, mm. **25. Urn width**, CUW, mm. **26. Mouth width**, CMW, mm.

Exothecial cells. **27. Exothecial cell length**, ECL, μm. **28. Exothecial cell width**, ECW, μm.

Seta. **29. Seta length**, SL, mm.

Perichaetial leaves. **30. Perichaetial leaf base width**, PLBW, mm. **31. Perichaetial leaf length**, PLL, mm.

Digital Image Analyses—All measurements were registered on digital images using Image-Pro Plus software. A digital camera (Sony CCD) attached to a compound microscope was used to capture images from slide preparations. We used a glycerin and water solution as mounting medium for semi permanent slides for leaves and capsules. Single vegetative leaves were photographed through a compound microscope with the 2.5× objective. Groups of leaf cells were captured with the 40× dry objective. The perichaetial leaves and capsules were digitized with the camera attached to a stereoscopic microscope. All measurements were transformed from pixels to microns or millimeters using images of scales with the "Calibration: Spatial" command from the "Image" menu in Image-Pro Plus.

Statistical Analyses—All herbarium specimens were grouped a priori into two species and then into as many as four geographic groups as levels of comparison (Fig. 3) in order to evaluate the morphological variation within and between species. Geographic grouping of collecting localities was based on a phytogeographical subdivision according to Rzedowski (1981). Geographic groups 1 (*B. andrieuxii*) and 4 (*B. secunda*) consist of specimens collected in the Sierra Madre Occidental, the northwestern zone (Fig. 3). The two species are also sympatric in the Central Plateau in Mexico; specimens collected in this zone were assigned to groups 2 (*B. andrieuxii*) and 5 (*B. secunda*). Likewise, both species are present along the Neovolcanic Axis in central Mexico; groups of specimens were labeled as 3 (*B. andrieuxii*) and 6 (*B. secunda*). Specimens collected from southern Mexico comprise group 7 (*B. secunda*). Thus, morphological character variation was analyzed through univariate and multivariate statistical methods, by seven geographical groups and by two species.

Coefficient of Variation (V)—In order to make a comparison of the relative variation of each morphological character measured for both taxa, the coefficient of variation (V) was calculated.

This measure expresses the standard deviation of a sample as a percentage of the mean. Highly variable data sets have relatively large values of V, while relatively small values of V indicate less variability (Lewontin 1966; Byrkit 1987).

Univariate Analyses—We calculated descriptive statistics of variation of characters at two levels, by geographic groups and by species. We tested for differences among means of each variable using one-way analysis of variance (ANOVA) on log (base 10) transformed values. In the

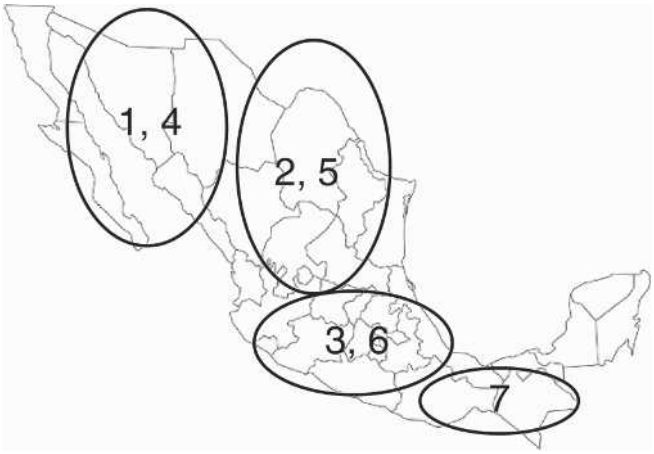

FIG. 3. Map of Mexico showing the seven geographic groupings for *B. andrieuxii* (1, 2 and 3) and *B. secunda* (4, 5, 6 and 7) used in the text and figures. Specimens from localities in the U.S. were either assigned to groups 1 and 2 or 4 and 5, depending on species identification.

analyses by seven geographic groups, if differences among means were statistically significant ($p < 0.05$), then we performed Tukey's Multiple Range Tests (MRT) on log transformed values (Sokal and Rohlf 1981; Zar 1996).

Multivariate Analyses—Canonical Variates Analysis (CVA) was performed on log transformed values (base 10) to examine the pattern of variation among groups. Separate CVAs were estimated for specimens grouped into two species, as well as with data preclassified into seven geographic groups of specimens (1–7). The distinction between species or among geographic groups was assessed using an F test on the Mahalanobis distance (D^2) among group centroids. We also examined how well the discriminant functions classify the individuals into their preassigned groups. As we were interested in patterns of character variation, in addition to a global CVA including all characters, we also executed a "partitioned CVA" for each set of variables describing leaves, leaf cells, or capsules separately. We also performed a Principal Components Analysis using the covariance of all 26 quantitative characters of leaf cells, vegetative and perichaetial leaves, and sporophytes (ratios not included) to estimate the contribution of each morphological character to total variation of all specimens. All statistical analyses were carried out using Statistica v. 6 (StatSoft 1998).

RESULTS

Relative Variability of Characters—Nine of the 31 characters were highly variable in both *B. secunda* and *B. andrieuxii* (Table 2). Apical leaf cell length (AL, V = 0.24, V = 0.28), the length/width ratio of apical leaf cells (AL/W, V = 0.29, V = 0.30), and seta length (SL, V = 0.25, V = 0.25) showed the highest coefficients of variation. For these characters, values of V are similar between species (Table 2). Coefficient of variation of revolute leaf margin length (LRM) was higher in

TABLE 2. Morphological variation between *B. andrieuxii* and *B. secunda*. Basic statistics (N = sample size, M = mean, SD = standard deviation), values of Coefficient of Variation (V), and results of analysis of variance (ANOVA, F-ratio, $p < 0.05$) for 31 morphological characters. Asterisks indicate characters where means differ between species.

	B. andrieuxii				*B. secunda*				
Character	N	M	SD	V	N	M	SD	V	**F-ratio**
Leaf cells									
AL	180	17.41	± 4.87	0.28	112	17.89	± 4.36	0.24	1.22
AW	180	6.39	± 0.84	0.13	112	6.13	± 0.86	0.14	7.0*
UL	180	11.13	± 1.91	0.17	112	13.94	± 2.90	0.21	92.18*
UW	180	6.03	± 0.71	0.12	112	5.68	± 0.79	0.14	17.43*
ML	180	10.91	± 1.66	0.15	112	12.18	± 2.64	0.22	21.95*
MW	180	6.39	± 0.76	0.12	112	5.75	± 0.75	0.13	52.40*
BL	180	61.31	± 7.91	0.13	112	63.23	± 9.04	0.14	3.29
BW	180	5.34	± 0.62	0.12	112	5.50	± 0.65	0.12	4.48*
AL/W	180	2.77	± 0.83	0.30	112	2.99	± 0.86	0.29	4.68*
UL/W	180	1.87	± 0.37	0.20	112	2.47	± 0.50	0.20	140.92*
ML/W	180	1.72	± 0.29	0.17	112	2.14	± 0.51	0.24	84.18*
BL/W	180	11.64	± 1.95	0.17	112	11.62	± 1.99	0.17	0
Vegetative leaves									
LB	180	0.31	± 0.05	0.16	112	0.32	± 0.05	0.17	0.27
LL	180	1.57	± 0.12	0.08	112	1.68	± 0.14	0.08	42.70*
LW	180	0.94	± 0.11	0.11	112	0.91	± 0.12	0.13	8.43*
LWP	180	0.61	± 0.06	0.10	112	0.60	± 0.06	0.11	1.06
LAW	180	0.11	± 0.01	0.10	112	0.11	± 0.01	0.11	5.68*
LAL	180	0.20	± 0.03	0.15	112	0.21	± 0.03	0.15	28.06*
LRM	180	0.69	± 0.18	0.26	112	1.47	± 0.18	0.13	1031.38*
LL/LRM	180	2.40	± 0.49	0.20	112	1.15	± 0.09	0.08	1078.02*
Capsule									
CNW	73	0.27	± 0.05	0.17	35	0.28	± 0.05	0.18	2.35
CNL	73	0.30	± 0.06	0.20	35	0.31	± 0.07	0.22	0.57
CUBW	73	0.55	± 0.09	0.16	35	0.61	± 0.11	0.18	6.52*
CUL	73	2.10	± 0.30	0.14	35	2.14	± 0.33	0.15	0.30
CUW	73	1.12	± 0.17	0.15	35	1.18	± 0.13	0.11	3.57
CMW	73	0.38	± 0.09	0.22	35	0.47	± 0.10	0.22	20.14*
Exothecial cells									
ECL	67	37.63	± 5.85	0.16	36	41.77	± 5.58	0.13	12.41*
ECW	67	24.78	± 4.21	0.17	36	25.23	± 4.84	0.19	0.16
Seta									
SL	80	9.75	± 2.42	0.25	41	12.41	± 3.13	0.25	26.33*
Perichaetial leaves									
PLB	106	0.58	± 0.09	0.15	50	0.6	± 0.08	0.14	0.04
PLL	106	2.62	± 0.29	0.11	50	2.7	± 0.34	0.13	2.13

B. andrieuxii (V = 0.26) than in *B. secunda* (V = 0.13). Variability was also different between species in the ratio of lamina length (LL/LRM) and revolute leaf margin length (V = 0.08 for *B. secunda*, V = 0.20 for *B. andrieuxii*).

Leaf Cell Variation Between and Within Species—Width and length of four groups of leaf cells were analyzed individually and also as length/width ratios (Table 2). Based on eight leaf cell dimensions, the two species differ in at least six characters where the F ratio is significant. Most of the difference between species is in the upper and medial leaf cells (Fig. 4A–C); this is revealed also in the comparison using the length/width ratio for each group of cells.

When the comparison is at the level of seven geographic groups, there are no differences among groups of specimens within species. Differences are detected only among geographic groups of the two species (Table 3). An ANOVA test ($p < 0.05$) among groups 1–7 shows that *B. andrieuxii* specimens (groups 1–3) are most similar to each other rather than to any of the four groups of specimens of *B. secunda* (groups 4–7). For all eight characters from leaf cells, F ratios are significant only in cross comparisons between geographic groups of the two species. Within species, morphological variation for most characters has a high degree of overlap (Table 3). In contrast, differences are higher between species than within species (geographic groups). The three geographic groups of *B. andrieuxii* (groups 1–3) have significantly shorter upper leaf cells (UL, Fig. 4A) and wider medial leaf cells (ML, Fig. 4C) than any of the four groups of *B. secunda* (groups 4–7).

Multivariate analysis of leaf cell variation within and between species was carried out using CVA calculated from the same eight leaf cell characters (ratios not included). The first discriminant function explains 75% of the total variance among seven geographic groups (Table 4). Characters with higher absolute loadings are upper leaf cell length (UL, −0.722), and medial leaf cell width (MW, 0.538). The second discriminant function axis explains an additional 10% of the total variance. Characters with higher absolute loadings are basal leaf cell width (BW, 0.687) and medial leaf cell width (MW, −0.58). Scatter plots of the first two canonical variates (Fig. 5A–B) show preclassified specimens by geographic groups clustered together by species.

Pair wise Mahalanobis Distances (D^2) were not significant between pairs in geographic groups 1–3 (*B. andrieuxii*) and between pairs from groups 4–7 (*B. secunda*) (Table 5). The mean Mahalanobis distance ($D^2 = 0.6597$) between groups 1–3 is smaller that the mean distance between groups 4–7 ($D^2 = 2.1901$). In contrast, the mean D^2 of comparisons between groups across species is almost twice the distance within species ($D^2 = 3.6727$). Significant D^2 (Wilks' Lambda $p \leq 0.0001$) occur only in pair wise comparisons between groups of different species. The scatter plot of the first two discriminant function axes (Fig. 5B) from the CVA with preclassified specimens into two species shows some overlap.

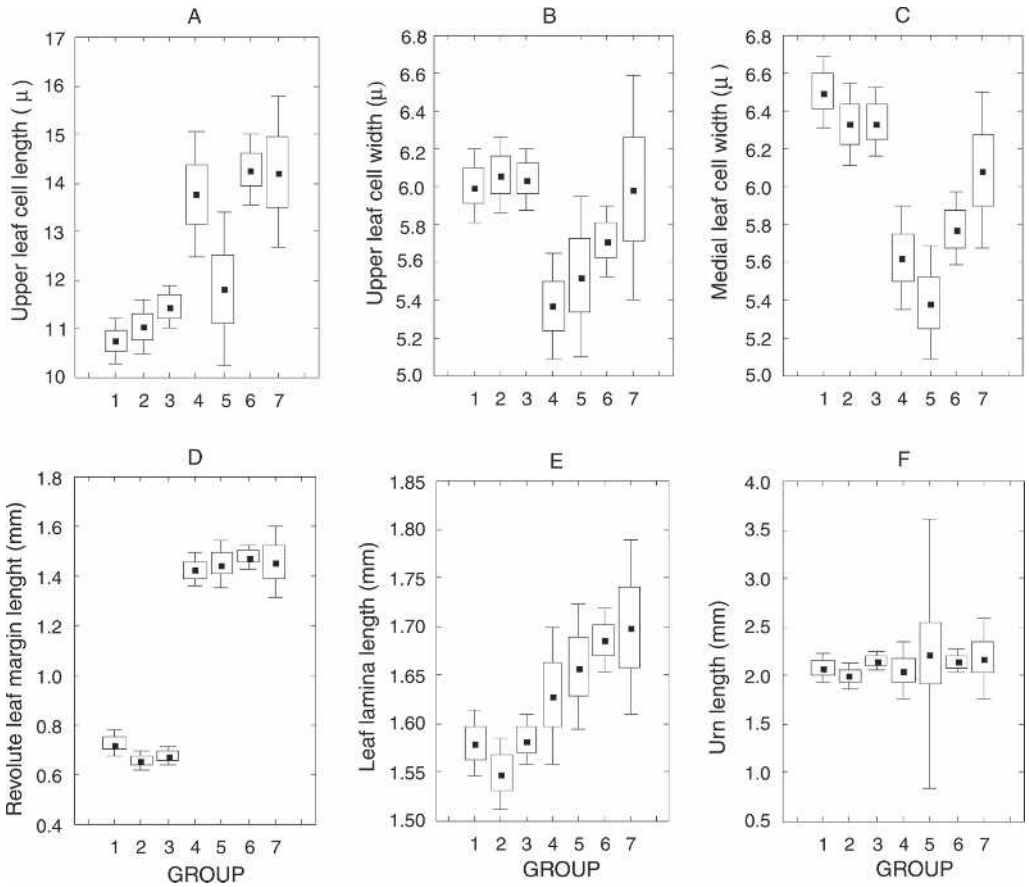

FIG. 4. A-F. Morphological variation in leaf cells, vegetative leaves, and capsules among seven geographic groups (Fig. 3). In all box-and-whisker plots, the black square is the median, the open rectangle is one quartile, and the line is the range. Only six characters are presented as examples of variation patterns in *B. andrieuxii* (groups 1–3) and *B. secunda* (groups 4–7). A. upper leaf cell length; B. upper leaf cell width; C. medial leaf cell width; D. revolute leaf margin length; E. leaf length; F. urn length.

TABLE 3. Character variation within species estimated by univariate descriptive statistics for seven groups of specimens of *B. andrieuxii* and *B. secunda*. Specimens of *B. andrieuxii* were subdivided into three geographical groups (1–3, see Fig. 3). Specimens of *B. secunda* were assigned to four geographical groups (4–7, see Fig. 3). Character means (M), sample sizes (N), and F-ratio are given for each geographic group 1–7. Asterisks represent significant differences (ANOVA, $p \leq 0.05$). Groups sharing codes (C) indicate results of pair wise comparisons (MRT).

		B. andrieuxii			*B. secunda*				
		1	2	3	4	5	6	7	F-ratio
Character									
Leaf cells (N)		**60**	**43**	**77**	**16**	**12**	**71**	**13**	
AL	M	17.13	16.01	18.4	16.6	15.99	18.39	18.52	2.47
AW	M	6.32	6.49	6.39	5.55	6.14	6.27	6.08	3.22*
	C	01	0	01	1	01	01	01	
UL	M	10.76	11.06	11.46	13.77	11.83	14.28	14.24	18.30*
	C	0	0	01	1	01	1	1	
UW	M	6.01	6.06	6.04	5.37	5.53	5.72	5.99	3.89
ML	M	10.54	10.48	11.45	12.12	11.34	12.29	12.41	5.90*
	C	0	0	01	01	01	1	01	
MW	M	6.51	6.33	6.34	5.62	5.39	5.78	6.09	10.21*
	C	0	01	01	1	1	1	01	
BL	M	59.21	60.26	63.54	62.58	60.81	64.27	60.59	3.09*
	C	0	01	01	01	01	1	01	
BW	M	5.27	5.34	5.38	4.99	5.79	5.61	5.23	4.11*
	C	0	01	01	01	012	1	012	
AL/W	M	2.74	2.52	2.92	3.06	2.64	3.02	3.08	2.68
UL/W	M	1.81	1.85	1.92	2.57	2.13	2.52	2.42	25.68*
	C	0	0	0	1	01	1	1	
ML/W	M	1.64	1.67	1.82	2.17	2.09	2.16	2.05	16.61*
	C	0	0	01	1	01	1	01	
BL/W	M	11.36	11.37	11.99	12.66	10.54	11.57	11.69	2.08
Vegetative leaves (N)		**60**	**43**	**77**	**16**	**12**	**71**	**13**	
LB	M	0.32	0.29	0.32	0.29	0.32	0.32	0.32	2.77
LL	M	1.58	1.55	1.58	1.63	1.66	1.69	1.7	7.98*
	C	0	01	0	01	01	1	01	
LW	M	0.93	0.91	0.98	0.84	0.88	0.93	0.9	4.93*
	C	01	01	0	1	01	01	01	
LWP	M	0.6	0.6	0.62	0.58	0.62	0.61	0.59	1.64
LAW	M	0.11	0.11	0.12	0.1	0.1	0.11	0.11	5.61*
	C	01	0	0	1	01	0	01	
LAL	M	0.19	0.19	0.21	0.2	0.19	0.22	0.22	12.72*
	C	0	01	01	012	01	02	012	
LRM	M	0.73	0.66	0.68	1.43	1.45	1.48	1.46	173.86*
	C	0	0	0	1	1	1	1	
LL/LRM	M	2.3	2.43	2.45	1.15	1.15	1.15	1.18	181.73*
	C	0	0	0	1	1	1	1	
Sporophyte Capsule (N)		**23**	**17**	**33**	**9**	**3**	**17**	**6**	
CNW	M	0.25	0.24	0.29	0.24	0.26	0.31	0.28	6.88*
	C	0	0	1	01	01	1	01	
CNL	M	0.31	0.29	0.3	0.34	0.27	0.33	0.25	2.37
CUBW	M	0.58	0.5	0.56	0.6	0.55	0.64	0.56	3.17*
	C	01	0	01	01	01	1	01	
CUL	M	2.08	2	2.16	2.05	2.23	2.15	2.19	0.8
CUW	M	1.07	1.02	1.2	1.13	1.02	1.22	1.19	5.12*
	C	0	0	1	01	01	1	01	
CMW	M	0.39	0.37	0.39	0.41	0.4	0.5	0.51	4.58*
	C	0	0	0	01	01	1	01	
Exothecial cells (N)		**19**	**13**	**35**	**9**	**4**	**17**	**6**	
ECL	M	36.11	34.99	39.44	41.71	45.03	41.83	39.52	3.97*
	C	01	0	01	01	01	1	01	
ECW	M	23.67	24.45	25.5	22.44	29.37	25.76	25.19	1.65
Setae (N)		**22**	**19**	**39**	**9**	**4**	**21**	**7**	
SL	M	8.64	8.42	11.03	13.22	12.75	11.52	13.86	10.03*
	C	0	0	1	1	01	1	1	
Perichaetial leaves (N)		**36**	**26**	**44**	**12**	**6**	**26**	**6**	
PLB	M	0.59	0.59	0.57	0.56	0.61	0.6	0.55	0.8
PLL	M	2.55	2.43	2.79	2.47	2.79	2.74	2.96	8.33*
	C	0	0	01	0	01	01	01	

However, D^2 between species centroids (2.925) is significant (Wilks' Lambda $p \leq 0.0001$).

Leaf Variation Between and Within Species—Seven distances from leaves and the proportion of leaf length and revolute leaf margin were analyzed individually. The two species differ in five leaf characters, where the F ratio is significant (Table 2). Most of the difference between species is in the length of the revolute margin (LRM, F = 1031.38).

TABLE 4. Character variation within species estimated by three partitioned canonical variates analyses (CVA) for seven groups of specimens of *B. andrieuxii* and *B. secunda*. Each partitioned CVA was based on eight characters from leaf cells, seven distances describing vegetative leaves, or six variables from capsules. Geographical groups 1–3 of *B. andrieuxii* and 4–7 of *B. secunda* were used as grouping variables to calculate variance within groups. The first two discriminant functions were extracted and interpreted. Eigenvalues, standardized canonical coefficients for each variable and percent of the total cumulative variance explained are given for each partitioned CVA for leaf cells, for vegetative leaves, and for capsules.

	Discriminant function I	Discriminant function II
CVA for leaf cells		
AL	0.149	−0.028
AW	−0.188	0.4
UL	−0.722	−0.415
UW	0.315	0.326
ML	−0.385	0.022
MW	0.538	−0.58
BL	−0.204	0.062
BW	−0.257	0.687
Eigenvalue	0.797	0.111
% of total cumulative variance explained	75.2	85.7
CVA for vegetative leaves		
LB	−0.088	−0.632
LL	0.347	0.741
LW	−0.504	−0.531
LWP	−0.348	−0.246
LAW	0.076	0.103
LAL	0.116	−0.911
LRM	1.014	−0.042
Eigenvalue	4.894	0.287
% of total cumulative variance explained	92.5	97.9
CVA for capsules		
CNW	−0.764	0.06
CNL	−0.379	0.341
CUBW	1.195	−0.677
CUL	0.535	−0.501
CUW	−1.333	0.492
CMW	0.077	−0.892
Eigenvalue	0.764	0.32
% of total cumulative variance explained	55.9	79.4

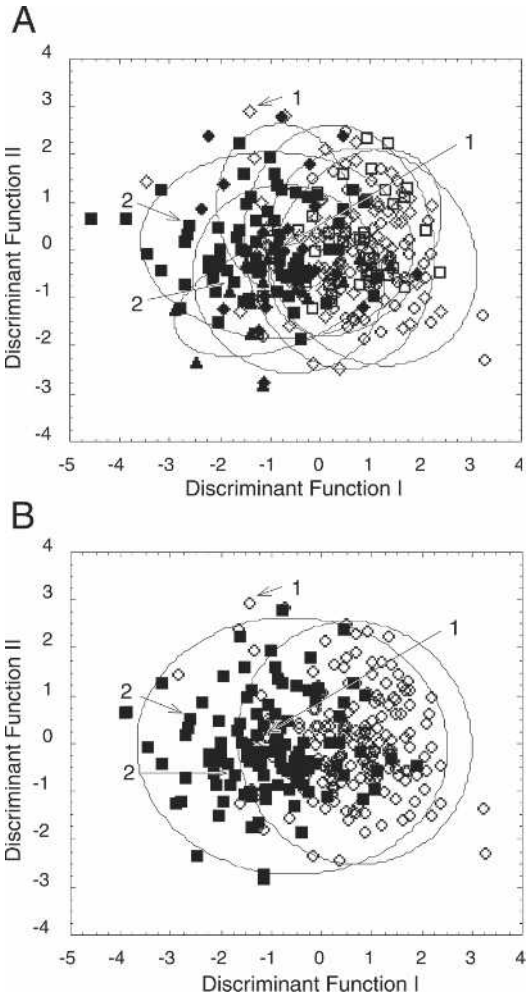

FIG. 5. A-B. Canonical Variates Analyses of eight leaf cell variables (lengths and widths) from 180 specimens of *B. andrieuxii* and 112 of *B. secunda*. A. Plot with the geographical groups 1–7 as grouping variables. Open symbols = *B. andrieuxii*. White circles = 1, Sierra Madre Occidental; white squares = 2, Central Plateau; white diamonds = 3, Neovolcanic Axis. Dark symbols = *B. secunda.* Dark triangles = 4, Sierra Madre Occidental. Dark circles = 5, Central Plateau; dark squares = 6, Neovolcanic Axis, and dark diamonds = 7, Southern Mexico (Sierra Madre del Sur). B. Plot with the species as grouping variables: circles = *B. andrieuxii*; squares = *B. secunda*. 1 = *B. andrieuxii* isotypes, 2 = *B. secunda* isotypes.

Analyses by geographic groups 1–3 (*B. andrieuxii*) and groups 4–7 (*B. secunda*) indicated most of variation in leaf characters is between species (Table 3). Multiple comparisons revealed that all leaf characters are not significantly different ($p < 0.05$) among groups of specimens of *B. andrieuxii* (groups 1–3). Also, leaves are similar among any of the four groups of specimens of *B. secunda* (groups 4–7). Differences are detected only in comparisons between geographic groups of the two species. Analyses by geographic groups indicate *B. secunda* and *B. andrieuxii* differ in five leaf characters where the F ratio is significant (Table 3). The three geographic groups belonging to *B. andrieuxii* (groups 1–3) have a significantly shorter revolute leaf margin (LRM, Fig. 4D) than any of the four groups of *B. secunda* (groups 4–7).

Multivariate analyses of leaf variation within and between species were carried out using the same seven leaf lamina morphological characters. Results show the first two discriminant functions accounted for 98% of the total variance of specimens grouped by geographic groups (Table 4). The first discriminant function explains 92% of total variance. Characters that contribute most to the difference as indicated by the higher absolute loadings are revolute leaf margin length (LRM) and leaf lamina width (LW). The second discriminant function explains an added 6% of the total variance. It has high negative loadings for acumen length (LAL) and base width of leaf lamina (LB) contrasted with moderately low negative and positive loadings for the remaining characters (Table 4).

The scatter plot of the first two discriminant functions (Fig. 6A) shows that geographic groups of each species are separated along the first discriminant function. Pair wise D^2 between centroids of *B. andrieuxii* (groups 1–3) showed no significant differences (Wilks' Lambda $p \leq 0.0001$, Table 5). Also, all the six values of D^2 between group centroids of *B. secunda* (groups 4–7) were not statistically significant (Wilks' Lambda $p \leq 0.0001$). In contrast, most pair wise D^2 between groups across species are significant (Table 5). A similar variation pattern is perceived from the CVA with preclassified specimens into two species (Fig. 6B). There is minimal overlap, and D^2 between species centroids (2.925) is signifi-

TABLE 5. Squared Mahalanobis Distances (D^2) among three geographical groups of specimens of *B. andrieuxii* (1–3) and four of *B. secunda* (4–7). Multivariate distances are calculated from three separate partitioned CVAs for leaf cells, vegetative leaves and capsules. Statistically significant differences (Wilks' Lambda $p \leq .0001$) are marked with an asterisk.

B. andrieuxii			*B. secunda*			
Leaf cells						
1	2	3	4	5	6	7
0	0.37	0.847	4.683*	4.784*	5.360*	3.382
		0.762	4.199*	3.371	4.021*	2.811
			3.378*	3.213	2.889*	1.987
				3.603	1.809	1.244
					1.836	3.535
						1.112
Vegetative leaves						
1	2	3	4	5	6	7
0	0.773	1.238*	19.355*	16.633*	18.118*	18.811*
		2.032*	22.979*	20.421*	22.153*	22.952*
			26.341*	22.981*	22.717*	24.261*
				0.986	2.011	1.383
					2.178	2.281
						0.413
Capsules						
1	2	3	4	5	6	7
0	1.201	4.612*	1.021	1.864	3.786*	5.439
		2.975	1.584	3.364	4.503*	4.8
			3.587	7.745	2.084	4.707
				4.929	2.981	5.008
					5.941	5.042
						3.213

cant (Wilks' Lambda $p \leq 0.0001$). Thus, multivariate analyses of leaves suggest the most useful character for discriminating between *B. andrieuxii* and *B. secunda* is the revolute leaf margin length (LRM).

Capsule Variation Between and Within Species—Based on capsule variation, *B. andrieuxii* and *B. secunda* are very similar (Table 2). Most characters show no significant difference ($p < 0.05$) between species and also at the level of seven geographic groups (Table 3). Sample size of each geographic zone was smaller as compared to gametophyte characters, because most herbarium specimens lack capsules. Between and within species, variation in six characters from capsules overlap (Fig. 4F), with F ratios not significant in comparisons between geographic groups of the two species.

Multivariate analyses of capsule variation within and between species were carried out using the same six characters. Results from CVA of specimens grouped by geographic groups 1–7 show the first two discriminant functions accounted for 80% of the total variance (Table 4). The first axis explains 56% of the total variance. The highest absolute loadings are for urn width (CUW), urn base width (CUBW) and neck width (CNW), contrasted with low loadings for mouth width (CMW), neck length (CNL) and urn length (CUL). The second discriminant function explains a further 24% of total variance. It had moderately high loadings for mouth width (CMW) and urn base width (CUBW, Table 4). The scatter plot of the first two discriminant functions shows a high degree of overlap among groups (Fig. 7A) and between species (Fig. 7B). All pair wise D^2 (Table 5) among groups of each taxa and between species were not significant (Wilks' Lambda $p \leq 0.0001$).

Variation of all Gametophyte and Sporophyte Characters Between and Within Species—Ordination analyses (PCA) detected the first component explaining 20.5% of total variance of all specimens (unclassified). Characters with higher absolute loadings on PC1 are urn width (CUW), leaf lamina length (LL), capsule neck width (CNW), and urn base width (CUBW). The second principal component explains a further 12.5% of the total variance. It has positive loadings for leaf lamina width (LW) and apical leaf cell width (AW). A scatter plot (not shown) of the first two principal components shows considerable overlap among the seven geographic groups.

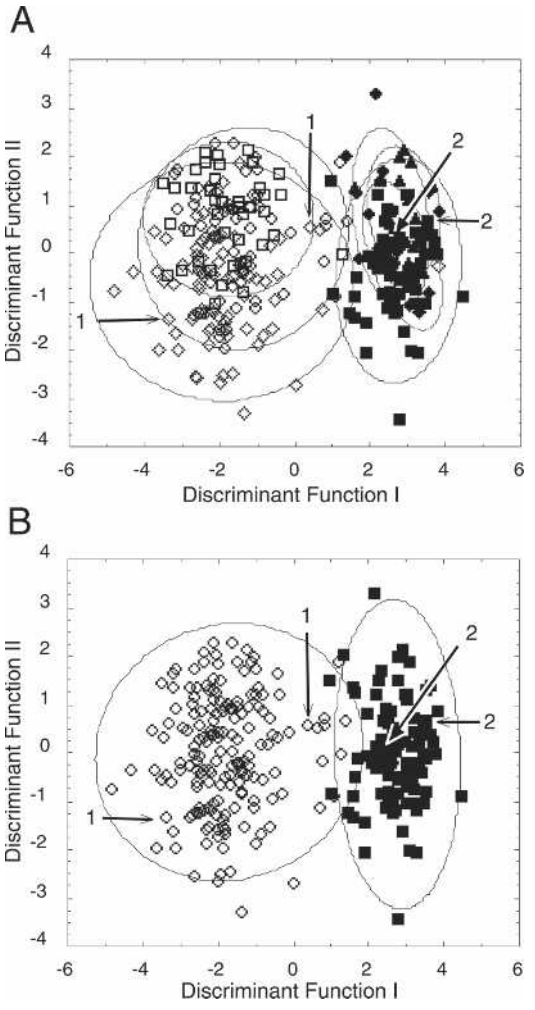

FIG. 6. A-B. Canonical Variates Analyses of seven variables (lengths and widths) from leaves of 180 specimens of *B. andrieuxii* and 112 of *B. secunda*. A. Plot with the geographical groups 1–7 as grouping variables. As in Fig. 5, open symbols = *B. andrieuxii*; dark symbols = *B. secunda*. B. Plot with the species as grouping variables: circles = *B. andrieuxii*; squares = *B. secunda*. 1 = Isotypes of *B. andrieuxii*; 2 = *B. secunda* isotypes.

Variation in all characters between species was examined with a CVA on specimens preclassified in two groups. Mahalanobis distance ($D^2 = 48.312$) between species centroids was significant and the scatter plot shows no overlap between *B. andrieuxii* and *B. secunda* (Fig. 8A). Also, the same pattern is revealed in the global CVA based on specimens preclassified by geographic groups 1–7. The first discriminant function explains 72% of the total variance given by 26 characters of gametophytes and sporophytes (Table 6). Characters with high loadings are the revolute leaf margin length (LRM, -0.971) and leaf lamina length (LL, -0.694).

Scatter plots of the first two discriminant functions (Fig. 8A–B) show that seven geographic groups are clearly sepa-

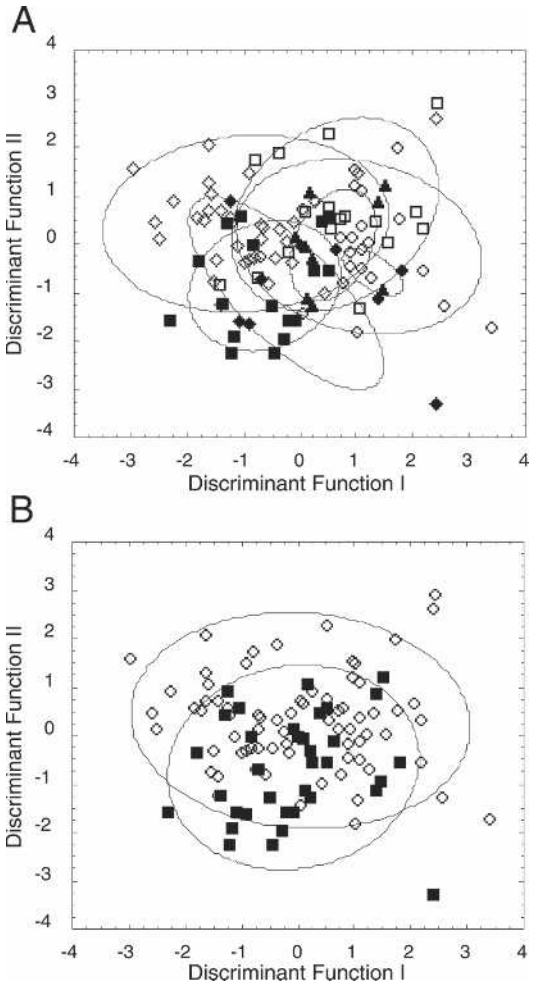

FIG. 7. A-B. Canonical Variates Analyses of six variables (lengths and widths) from capsules of 73 specimens of *B. andrieuxii* and 35 of *B. secunda*. A. Plot with the geographical groups 1–7 as grouping variables. As in Fig. 5, open symbols = *B. andrieuxii*; dark symbols = *B. secunda*. B. Plot with the species as grouping variables: circles = *B. andrieuxii*; squares = *B. secunda*.

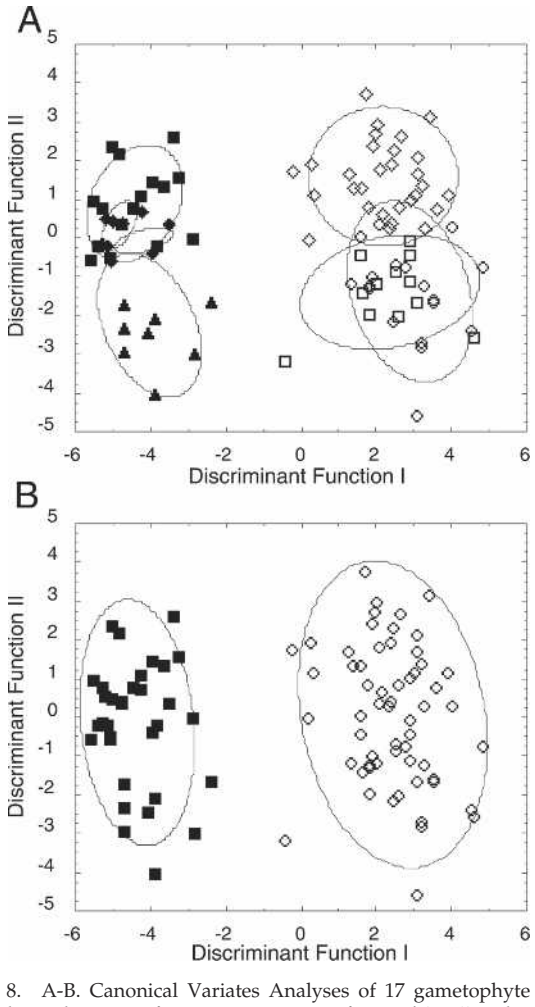

FIG. 8. A-B. Canonical Variates Analyses of 17 gametophyte and 9 sporophyte characters from 59 specimens of *B. andrieuxii* and 33 of *B. secunda*. A. Plot with the geographical groups 1–7 as grouping variables. As in Fig. 5, open symbols = *B. andrieuxii*; dark symbols = *B. secunda*. B. Plot with the species as grouping variables: circles = *B. andrieuxii*; squares = *B. secunda*.

rated by taxa along the first discriminant function. Pair wise D^2 between centroids of groups 1–3 (*B. andrieuxii*) showed no significant differences (Wilks' Lambda $p \leq 0.0001$, Table 7). Also, values of D^2 between group centroids of groups 4–7 (*B. secunda*) were not significant (Wilks' Lambda $p \leq 0.0001$). The mean Mahalanobis distance ($D^2 = 11.279$) between groups 1–3 is about the same magnitude as the mean between groups 4–7 ($D^2 = 19.174$). In contrast, all pair wise D^2 between groups 1–3 and 4–7 are significant (Wilks' Lambda $p \leq 0.0001$, Table 7). The mean D^2 calculated for comparisons between groups across species ($D^2 = 62.5267$) is more than three times the mean distance within species.

DISCUSSION

Our univariate and multivariate morphometric analyses of gametophyte and sporophyte variation coincide in revealing the most crucial characters for differentiation of *B. andrieuxii* and *B. secunda* were the extension of the revolute margin (LRM) and features of upper leaf cells (UL, Table 3). Our statistical analyses are also congruent with previous interpretations concerning the similarity between *B. andrieuxii* and *B. secunda* in capsule dimensions, some gametophyte features, and perichaetial leaves. These conclusions about character variation derived from estimations of ANOVA and CVA stand whether specimens are classified by species or by geographic groups 1–7.

Relative variation among characters as revealed by coefficients of variation (V) indicates that both the gametophyte and sporophyte are very variable, since the highest V values in both species were found in leaves, leaf cells, and capsules. This pattern is also detected in the PCA analysis; the first axis (20.5% of total variance) is mostly correlated with features of leaves and capsules (urn width, leaf lamina length, capsule neck width, urn base width). This finding is reasonable, since characters with highest variances are those expected to be associated with the first principal component. Thus, univariate V values and character loadings from PCA identify the same characters of gametophytes and sporophytes as highly variable.

Results from univariate and multivariate analyses also concur in detecting that most of morphological variation is between species rather than geographical variation within both species (groups 1–3 and 4–7). Partitioned CVA of leaf cells and leaf lamina variation indicated significant differences between species. Group centroids and pair wise D^2 of *B. an-*

TABLE 6. Overall morphological variation within species estimated by a multivariate analysis for seven groups of specimens of *B. andrieuxii* and *B. secunda*. The discriminant analysis (CVA) was based on 17 gametophyte and 9 sporophyte variables. The first two discriminant functions were extracted and interpreted; Eigenvalues, standardized canonical coefficients for each variable and percent of the total cumulative variance explained are given.

Characters	Discriminant function I	Discriminant function II
Leaf cells		
AL	0.430	0.243
AW	0.041	0.399
UL	−0.127	0.403
UW	0.096	−0.400
ML	−0.136	0.060
MW	0.248	−0.131
BL	0.097	0.009
BW	−0.031	0.317
Vegetative leaves		
LB	0.234	0.387
LL	−0.694	−0.302
LW	0.168	0.548
LWP	0.570	0.075
LAW	0.412	−0.294
LAL	−0.155	0.335
LRM	−0.971	−0.200
Capsule		
CNW	0.001	0.501
CNL	0.223	0.152
CUBW	0.221	−0.782
CUL	0.134	−0.726
vCUW	−0.184	0.858
LMW	−0.396	0.090
Exothecial cells		
ECL	−0.223	−0.008
ECW	−0.088	0.485
Seta		
SL	−0.257	−0.042
Perichaetial leaves		
PLB	0.019	0.130
PLL	−0.131	0.390
Eigenvalue	11.591	2.243
% total cumulative variance explained	72.4	86.5

drieuxii (geographical groups 1–3) compared to group centroids of *B. secunda* (groups 4–7) suggested a distinction between species rather than variation among geographic groups of each species.

All statistical analyses agree in detecting characters useful to distinguish both species. Univariate analyses indicated significant differences between species in upper and medial leaf cells (UL, ML). Similarly, univariate and partitioned multivariate analyses of leaf variation suggest that the most useful character for discriminating between *B. andrieuxii* and *B. secunda* is the length of the revolute margin (LRM). A global CVA of all characters from gametophytes and sporophytes indicated also that the revolute leaf margin length is correlated with the first canonical variate.

Historically, *B. secunda* was first described based on specimens collected in Toluca, central Mexico. Later, *B. andrieuxii* was described based on specimens collected in Oaxaca. Thériot (1926) argued for the recognition of *B. andrieuxii* as different from *B. secunda*. Our multivariate analyses situated the type specimens of both species in the morphospace of all specimens for partitioned analyses of leaf cells and leaf variation (Fig. 5–6). In terms of leaf variation, two isotypes of *B. secunda* are near the centroid of the species (label #2, Fig. 6), but the isotypes of *B. andrieuxii* are in the outer ring of the corresponding cloud of specimens (label #1, Fig. 6). Isotypes of both species are even closer to each other in the morphospace configured by leaf cell variation (Fig. 5). It is therefore logical to understand in retrospective that such leaf differences among type specimens were interpreted as part of a morphological continuum.

In conclusion, univariate and multivariate analyses show that the revolute margin and the upper leaf cells are diagnostic characters that allow the distinction between *B. andrieuxii* and *B. secunda* (Fig. 9). A leaf margin plane or narrowly revolute half way up the leaf length identifies *B. andrieuxii*. It is exceptional that the extent of the revolute margin reaches the acumen of the leaf lamina. In this case, medial and upper leaf cells are short, subquadrate or rarely rectangular. In contrast, *B. secunda* has a strongly revolute

TABLE 7. Squared Mahalanobis Distances (D^2) among three geographical groups of specimens of *B. andrieuxii* (1–3) and four of *B. secunda* (4–7). Multivariate distances are calculated from a combined canonical variates analysis for 17 gametophyte and 9 sporophyte variables. Statistically significant differences (Wilks' Lambda $p \leq .0001$) are marked with an asterisk.

B. andrieuxii			*B. secunda*			
1	2	3	4	5	6	7
0	7.509	12.302*	59.175*	71.503*	65.598*	74.136*
		14.029	54.853*	62.433	58.976*	70.035*
			59.714*	61.011	50.569*	62.311*
				26.716	18.286	21.106
					19.039	18.585
						11.314

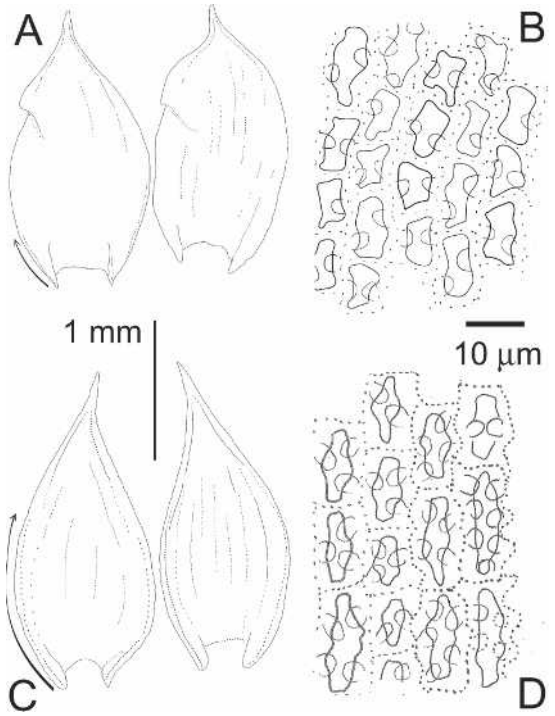

FIG. 9. Leaf characters that distinguish *B. andrieuxii* and *B. secunda*. A-B. *B. andrieuxii*. Leaves and upper leaf cells (*Andrieux 23*, BM). C-D. *B. secunda*. Leaves and upper leaf cells (*Humboldt & Bonpland*, BM). Arrows indicate the revolute leaf margin extending up from the leaf base.

leaf margin extending from the leaf base all the way to the leaf acumen. Also, the upper and medial leaf cells are narrowly rectangular, with cell walls moderately to highly sinuose.

The following key is presented to help in the identification of *Braunia secunda* and *B. andrieuxii*, as well as other two species, *B. plicata* (Mitt.) A. Jaeger and *B. squarrulosa* (Hampe) Müll. Hal., also known from Mexico.

1. Leaf apex strongly dentate, hyaline; capsule cylindrical ..... *B. plicata*
1. Leaf apex entire or scabrous 2
   2. Leaf apex long acuminate, narrow, flexuose; capsule turbinate, globose; seta (3–)5–6(–7) mm long ..... *B. squarrulosa*
   2. Leaf apex short acuminate; capsule cylindrical, narrow; seta (5–)9–14(–20) mm long 3
      3. Leaf margin revolute from the base up 3/4 the leaf length; upper leaf cells rectangular, (8.2–)11–16.8(–21.9) μm long, (3.7–)4.9–6.5(–8.2) μm wide, 2–3: 1, cell walls sinuose ..... *B. secunda*
      3. Leaf margin plane, or revolute from the base up 1/3 the leaf length; upper leaf cells subquadrate, (7.2–) 9.2–13(–17.2) μm long, (4.4–)5.3–6.7(–8) μm wide, 1.5–2: 1, cell walls straight ..... *B. andrieuxii*

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