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Bruguiera Species in Hawai'i: Systematic Considerations and Ecological Implications[1]

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ABSTRACT: At least two mangrove tree species in the genus *Bruguiera* were introduced into Hawai'i from the Philippines in 1922. The two are described in the most current manual on the flora of Hawai'i as *B. gymnorrhiza* (L.) Lamk. and *B. parviflora* (Roxb.) W. & A. ex. Griff. There has, however, been some confusion since its introduction as to the identity of what is currently known as *B. gymnorrhiza.* Early Hawaiian flora manuals (1948 and earlier) and ecological research reports up until at least 1972 referred to the species as *B. sexangula* (Lour.) Poir. Flora manuals published after 1948 and recent ecological papers describe the species as *B. gymnorrhiza.* The reason for the change appears to have been based strictly on an assessment of flower color. In this study we collected specimens of *Bruguiera* from Hawai'i and known samples of *B. sexangula, B. gymnorrhiza,* and *B. exaristata* C. G. Rogers from Australia or Micronesia. Based on a multivariate comparison of flower and hypocotyl morphology of this material, an assessment of other diagnostic attributes, and amplified fragment length polymorphism (AFLP) mapping, we conclude that the primary, and perhaps only, *Bruguiera* species present in Hawai'i is *B. sexangula.* We argue that the current distribution of *Bruguiera* in Hawai'i fits the pattern that might be expected of *B. sexangula,* which is less salt tolerant than *B. gymnorrhiza.* We also conclude that sufficient regional variation occurs to warrant morphological and genetic comparisons of the three species across their whole geographic range.

MANGROVES ARE NOT NATIVE to the Hawaiian Archipelago but, since their introduction in the early part of the twentieth century, they have become well established on most of the main islands. *Rhizophora mangle* L. is by far the most common species, but at least five other mangroves or closely associated species have been introduced, of which two are known to have become naturalized (Wester 1981, Allen 1998). In addition to *R. mangle,* Wagner et al.'s (1990) flora of Hawai'i reported *Bruguiera gymnorrhiza* (L.) Lamk. and *Conocarpus erectus* L. (including *C. erectus* var. *sericeus* Griseb.) as being naturalized within the archipelago. In addition, it is possible that a small number of *B. parviflora* (Roxb.) W. & A. ex Griff. may be present in Hawai'i, although the last herbarium specimens collected for this species were in 1948 (Wagner et al. 1990), and it is unlikely that more than a few individuals still exist.

In the process of reviewing existing literature on the ecological and socioeconomic impacts of mangroves in Hawai'i (Allen 1998), it became evident that there was

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some uncertainty regarding the identity of the *Bruguiera* species currently reported as *B. gymnorrhiza.* Early reports referred to the species as *B. sexangula* (Lour.) Poir. (Degener 1934) and it remained the name used by ecological researchers until the early 1970s (Egler 1942, 1947, Walsh 1967, Lee 1971, Richmond and Mueller-Dombois 1972). However, more recent publications on mangroves in Hawai'i all refer to the species as *B. gymnorrhiza* (Elliott 1981, Wester 1981, Steele 1998).

In this paper, we first briefly review the history of the *Bruguiera* identification issue in Hawai'i. We then compare the morphology of flowers, hypocotyls, and leaves collected from Hawaiian *Bruguiera* with collections of *B. gymnorrhiza, B. sexangula,* and the closely related *B. exaristata* made in Micronesia or Australia. Our interests in undertaking this project are threefold. First, we seek to resolve the question of *Bruguiera* identity in Hawai'i so that the information can be used in future floras. Second, because *B. gymnorrhiza* and *B. sexangula* have different levels of tolerance for salinity (Clough 1992), we believe that proper identification of the species is essential for understanding its current distribution and its ecological impacts within the archipelago. Third, we believe a regional comparison of *B. gymnorrhiza, B. sexangula,* and *B. exaristata* is needed to help clarify questions regarding their morphological and genetic variation; this work represents a first step in that direction.

History of Bruguiera Identification in Hawai'i

In 1922, at least two species of *Bruguiera* were introduced to the island of O'ahu by the Hawaiian Sugar Planters' Association. The propagules of these two species, later reported as *B. sexangula* and *B. parviflora,* were imported from the Philippines (Wester 1981), where *B. sexangula, B. gymnorrhiza,* and *B. parviflora* are native (Ding Hou 1958).

A decade later, the botanist Otto Degener, who was preparing *Flora Hawaiiensis,* sent Felipe M. Salvoza, of the School of Forestry, Agricultural College, Laguna, Philippines, specimens of *Bruguiera* collected from He'eia Swamp, O'ahu, for identification. Salvoza replied (1932, letter to Degener, attached to specimen no. 64319 in Bernice P. Bishop Museum Herbarium, Honolulu) that the specimens were most likely *B. sexangula,* but if the calyces were red when fresh, they were *B. conjugata* (L.) Merr. (a synonym for *B. gymnorrhiza*). Degener then published the taxon as *B. sexangula* in his flora (Degener 1934, 1946).

Raymond Fosberg appears to have been the first botanist to conclude that the specimens collected at He'eia Swamp were *B. conjugata* [*gymnorrhiza*]. Fosberg's conclusion apparently was based on the color of the flowers, which he described as red (Fosberg 1948). His decision may have been influenced by Salvoza's (1932) letter to Degener. Degener eventually agreed that the specimens had been misidentified, and in 1958 Degener and Degener published a replacement page for *Flora Hawaiiensis,* in which they called the species *B. conjugata* and attributed the earlier "error" to the fact that the Hawaiian trees produced yellower floral parts than are generally reported for the species. In Degener and Degener's (1958) paper, Fosberg (1948) is the first reference cited that uses the name *B. conjugata* for the Hawaiian material.

Marie Neal is another botanist who originally identified the Hawaiian *Bruguiera* as *B. sexangula,* but then later changed to *B. conjugata* [*gymnorrhiza*]. Her first edition of *In Gardens of Hawaii* included only *B. sexangula* and *B. parviflora* (Neal 1948). In 1958, Neal (unpublished notes in Bishop Museum Herbarium), following Watson (1928), concluded that the He'eia specimens were *B. conjugata.* The revised edition of *In Gardens of Hawaii* (Neal 1965) referred to *B. conjugata* as the most common *Bruguiera,* but suggested that *B. sexangula* might also be present in Hawai'i.

In contrast to the botanists working in Hawai'i, Ding Hou (1958) indicated that the species present in Hawai'i is *B. sexangula.* Wagner et al. (1990), though aware of Ding Hou's treatment of the Hawaiian specimens, decided to follow the consensus of the earlier botanists when preparing their flora of Hawai'i.

MATERIALS AND METHODS

Morphological Analysis

A small, preliminary collection of twigs, leaves, mature flower buds, flowers, and hypocotyls from four trees collected from two sites on O'ahu was sent to the Australian Institute of Marine Science, in Townsville, Australia. There, one of us (N.C.D.) determined that the material was *B. sexangula* based on diagnostic characteristics used by Ding Hou (1958).

To support this conclusion, a second, larger collection of specimens from O'ahu, which is the only Hawaiian island known to have *Bruguiera* (Wagner et al. 1990), was made, and a range of morphological characteristics was compared with collections of *Bruguiera* from the islands of Kosrae and Pohnpei in the Federated States of Micronesia and from northern Queensland, Australia. The Micronesian material was collected because *B. gymnorrhiza* is reportedly the only species in the genus that occurs on Kosrae or Pohnpei (Fosberg et al. 1979, Stemmerman 1981), thereby minimizing chances of misidentification. The northern Queensland material included specimens identified by N.C.D. as *B. sexangula* and *B. gymnorrhiza,* both of which are native to the region (McCusker 1984).

The second O'ahu collection consisted of material from a total of 11 trees, with at least two from each of the four locations where *Bruguiera* is known to occur: He'eia Swamp, Ka'alaea Stream, Anahulu River, and Paukauila Stream. The Micronesia collection consisted of a total of 12 trees from four different locations (Utwe River and Lelu on Kosrae and Pohnaulena and Sokehs Island on Pohnpei). Voucher specimens from both the Hawaiian and Micronesian trees were deposited in the Bishop Museum Herbarium in Honolulu, Hawai'i. The Australian collection consisted of six trees identified (by N.C.D.) as *B. gymnorrhiza* from Cairns or along the Daintree River and six trees identified as *B. sexangula* from the Daintree River. In addition, six trees identified as *B. exaristata,* a closely related species, were included in the Australian collection; the trees were located near Cairns or Townsville. The morphological characteristics assessed are listed in Table 1. For each tree, a minimum of nine leaves, two hypocotyls, four mature flower buds, and eight calyces was measured.

Individual quantitative attributes were compared among the Hawaiian, Micronesian, and Australian collections using analysis of variance (ANOVA). Data were first transformed using a square-root transformation to improve homogeneity of variance. Means were separated using Tukey's studentized range test. Discriminant function analysis (DFA) and cluster analysis (CA) were used to investigate multivariate attributes of the data and to classify the Hawaiian material. Data sets for these analyses were square-root transformed mean values per tree of each quantitative attribute; data were standardized before use in the CA. The data from the Australian and Micronesian trees were used as the calibration data set for the DFA. First a stepwise DFA was performed on the calibration data set, and five variables identified as having the greatest ability to discriminate among the four classes (*B. gymnorrhiza* from Australia and Micronesia, *B. sexangula,* and *B. exaristata*) were selected for use in the final DFA and the CA. Statistical Analysis System (SAS Institute 1990) was used for all statistical analyses.

Genetic Analysis

A genetic analysis was conducted on two trees from each of three collections, including *B. gymnorrhiza* from Micronesia, *B. sexangula* from the Daintree River in Australia, and trees from He'eia State Park on O'ahu. Genomic DNA was extracted and purified from young, not yet unrolled leaves, essentially following the method of Porebski et al. (1997). DNA fingerprinting was by amplified fragment length polymorphism (AFLP) technology (Vos et al. 1995) using the Perkin-Elmer plant mapping kit. Restriction fragments of genomic DNA were obtained using the restriction enzymes *Eco*RI and *Mse*I; double-stranded adapters specific for either of these restriction sites were then

TABLE 1

DESCRIPTIONS FOR NUMERIC AND QUALITATIVE ATTRIBUTES USED FOR COMPARISONS OF *Bruguiera* ON O'AHU, HAWAI'I, WITH *B. gymnorrhiza* FROM MICRONESIA AND *B. gymnorrhiza, B. sexangula,* AND *B. exaristata* FROM AUSTRALIA (AFTER DUKE 1990)

	NO.[a]		DESCRIPTIONS
Numeric attributes	1	Shoot A	Number of leaves per shoot
	2	Shoot B	Apical sheath length
	3	Leaf L	Length of leaf blade
	4	Leaf W	Widest width of leaf blade
	5	Leaf S	Length from blade-petiole junction to widest width
	6	Leaf P	Length of petiole
	*7	Hypocotyl L	Length of mature hypocotyl
	8	Hypocotyl W1	Diameter of mature hypocotyl-plumule junction
	*9	Hypocotyl W2	Diameter of mature hypocotyl at widest width
	10	Hypocotyl S	Length of mature hypocotyl from rooting end tip to widest width
	11	Hypocotyl P	Length of mature hypocotyl plumule
	12	Flower bud A	Number of calyx lobes (from mature bud, flower, or expended fruit)
	13	Flower bud B	Length of open flower bud from peduncle
	14	Flower bud C	Length of open flower bud from base of calyx lobes
	15	Flower bud D	Width of open calyx bowl
	16	Flower bud E	Width of open flower bud at base of calyx lobes
	17	Flower bud F	Inside diameter of open calyx bowl
	18	Flower bud G	Length of closed flower bud from peduncle
	19	Flower bud H	Length of closed flower bud from base of calyx lobes
	20	Flower bud I	Width of closed calyx bowl
	21	Flower bud J	Width of closed flower bud at base of calyx lobes
	22	Flower bud K	Inside diameter of closed calyx bowl
	23	Flower bud L	Depth of flower bud bowl from calyx lobe base
	*24	Flower bud M	Length of peduncle (from mature bud, flower, or expended fruit)
	25	Flower bud N	Width of peduncle
	26	Repro A	Length of style (from bottom of calyx bowl of mature bud or mature flower)
	27	Repro B	Width of style (from mature bud or mature flower)
	28	Repro C	Length of stamen (longer)
	29	Repro D	Width of stamen (filament)
	30	Repro E	Length of anther
	31	Repro F	Width of anther
	32	Corolla A	Length of petal lobe (from lobe groove to tip)
	*33	Corolla B	Length of petal spine
	34	Corolla C	Length of petal
	35	Corolla D	Width of open petal
	36	Corolla E	Width of closed petal
	37	Corolla F	Number of bristles on petal apex
	*38	Corolla G	Length of bristles on petal apex
Qualitative attributes	39	Peduncle color	Color of peduncle (attached to mature bud, flower, or expended fruit)
	40	Mature flower bud position	Position of bud in relation to node
	41	Mature flower position	Position of flower in relation to node
	42	Mature hypocotyl position	Position of mature hypocotyl in relation to node
	43	Mature flower bud color	Color of calyx of mature flower bud
	44	Petal apex	Shape of petal tip
	45	Petal spine	Spine present between petal lobes (Y/N)
	46	Style lobes	Number of lobes on style tip (from mature bud or mature flower)

[a] Attribute numbers 28 to 38 were taken from closed mature buds only.

* Codes for numeric attributes were determined through the use of stepwise discriminant analysis (see Materials and Methods) to be discriminatory for the three *Bruguiera* species in question.

ligated to the fragments. Preselective PCR amplification was with the primer pair *Eco*RI + **A** and *Mse*I + **C**. This was followed by selective PCR using either the *Eco*RI + **AG** and the *Mse*I + **CAA** primer pair or the *Eco*RI + **AC** and the *Mse*I + **CAA** primer pair. The *Eco*RI + **AG** and *Eco*RI + **AC** primers were labeled with the fluorescent dyes TAMRA and FAM, respectively.

Capillary electrophoresis of the labeled fragments was done with a genetic analyzer (Perkin-Elmer ABI Prism 310) equipped with ABI GeneScan 2.1 software. An internal-lane size standard, GeneScan 500, labeled with the fluorescent dye ROX, was included in each run. After fragment sizing using the GeneScan software, presence or absence of a given fragment size was scored as **1** and **0**, respectively. Similarity coefficients were calculated using the SimQual program included in the NTSYSpc 2.02 g software package (Exeter Software Inc., Setauket, New York 11733-2870).

RESULTS

Variation in Individual Attributes

Differences between the Hawaiian material and the *B. gymnorrhiza* material from Micronesia and Australia were statistically significant for many of the numeric morphological attributes assessed (Tables 2 and 3). Of a total of 29 attributes for which statistical comparisons could be made with both *B. gymnorrhiza* collections, 12 (41%) differed significantly between the Hawaiian material and *B. gymnorrhiza* from both Micronesia and Australia. Another 8 attributes (28%) differed from one of the *B. gymnorrhiza* collections.

One of the clearest differences is in hypocotyl length, where there is almost no overlap in length between the Hawaiian specimens and *B. gymnorrhiza* from Micronesia or Australia. Differences in flower morphology are also evident, despite the considerable overlap in the range of the data for most characteristics (Tables 2 and 3). Substantial qualitative differences between the Hawaiian material and the two *B. gymnorrhiza* collections were observed for some attributes, notably color of the mature flower buds/calyces and the shape of the petal apex.

The *B. sexangula* from Australia more closely resembled the Hawaiian material than the *B. gymnorrhiza* from Micronesia and Australia, but nevertheless differed significantly from the Hawaiian collection in 12 of the 33 attributes for which statistical comparisons were possible. Differences between the Australian *B. sexangula* and the Hawaiian material, however, were not significant or were relatively small for most numeric (e.g., hypocotyl length, flower bud length) and qualitative (e.g., flower bud/calyx color, shape of petal apex) attributes that are often regarded as diagnostic for identification of the two species.

Bruguiera exaristata differed significantly from the Hawaiian material in 25 (76%) of the 33 comparable numeric attributes. Differences between *B. exaristata* and the other collections were readily apparent for a number of numeric and qualitative attributes, including leaf length, hypocotyl width, length of the petal spine, and flower bud/calyx color.

Multiattribute Variation

Five attributes were selected using stepwise DFA for the subsequent multivariate analyses. They include two measures of hypocotyl size, length of the petal spine, length of bristles on the petals, and peduncle length (see the attributes marked with asterisks in Table 1).

A graph of the first two canonical discriminant functions (Figure 1) shows strong separation of the three species. Although not as pronounced as the differences among species, differences between the two collections of *B. gymnorrhiza* are also apparent. The Hawaiian material is most similar to the *B. sexangula* collection from Australia, but again there is clear separation of the two collections. The first discriminant function explains approximately 79% of the total variation; length of the petal spine and length of bristles on the petal apex are the most im-

TABLE 2

MEAN MEASUREMENTS (MM), NUMBER OF SAMPLED TREES [IN BRACKETS], AND TOTAL ATTRIBUTE RANGE (IN PARENTHESES) OF NUMERIC ATTRIBUTES FOR COMPARISONS OF *Bruguiera* ON O'AHU, HAWAI'I, WITH *B. gymnorrhiza* FROM MICRONESIA AND *B. gymnorrhiza, B. sexangula,* AND *B. exaristata* FROM AUSTRALIA

NUMERIC ATTRIBUTES[a]	HAWAI'I *Bruguiera* SP.	AUSTRALIA *B. sexangula*	MICRONESIA *B. gymnorrhiza*	AUSTRALIA *B. gymnorrhiza*	AUSTRALIA *B. exaristata*
Shoot A[NA]	14.6 [11] (8–27)	12.2 [6] (7–16)	12.9 [12] (10–19)	13.2 [6] (7–21)	13.3 [6] (8–18)
Shoot B	49.8 [11]cb (34.3–62.1)	66.8 [6]a (47.0–81.0)	49.2 [12]c (37.2–63.7)	57.6 [6]b (26.0–76.0)	33.4 [6]d (24.0–43.0)
Leaf L	106.7 [11]c (78–154)	146.5 [6]ab (102–202)	143.0 [12]b (100–212)	166.2 [6]a (91–236)	87.3 [6]d (49–122)
Leaf W	42.4 [11]b (29–57)	58.3 [6]a (43–72)	61.7 [12]a (41–87)	65.9 [6]a (34–93)	41.5 [6]b (24–54)
Leaf S	55.4 [11]b (33–85)	74.5 [6]a (49–107)	71.6 [12]a (45–115)	83.0 [6]a (38–117)	46.3 [6]b (26–67)
Leaf P	29.5 [11]b (16–48)	32.2 [6]b (17–42)	39.4 [12]a (22–59)	42.0 [6]a (20–57)	20.6 [6]c (11–27)
Hypocotyl L	73.0 [11]c (65–90)	83.6 [6]c (49–118)	196.3 [12]a (151–246)	160.7 [6]b (86–204)	93.2 [6]c (78–110)
Hypocotyl W1	4.4 [11]b (3.3–5.5)	4.7 [6]b (3.8–6.0)	4.7 [12]ab (3.7–5.8)	5.3 [6]a (4.4–6.1)	3.3 [6]c (2.7–4.0)
Hypocotyl W2	15.3 [11]b (13.4–16.7)	13.5 [6]c (12.0–15.0)	16.4 [12]ab (14.1–19.6)	17.3 [6]a (14.9–20.0)	9.5 [6]d (8.9–10.0)
Hypocotyl S	48.3 [11]c (37.3–58.0)	37.1 [6]d (21.0–56.0)	86.2 [12]a (62.1–111.9)	63.5 [6]b (42.0–77.0)	34.2 [6]d (26.0–47.0)
Hypocotyl P	2.9 [11]a (1.2–4.3)	2.8 [5]a (2.1–3.9)	2.6 [11]a (1.6–3.9)	3.1 [5]a (2.0–4.5)	1.5 [6]b (1.0–2.0)
Flower bud A[NA]	10.9 [11] (9–12)	10.6 [6] (10–12)	12.1 [12] (10–14)	11.2 [6] (9–13)	9.2 [6] (8–10)
Flower bud B	32.1 [11]b (28.2–36.2)	33.0 [4]b (30.1–35.1)	36.8 [12]ab (28.6–47.3)	40.9 [2]a (40.3–41.5)	26.6 [6]c (25.0–27.5)
Flower bud C	18.8 [11]a (16.1–21.1)	17.7 [4]a (16.1–18.8)	19.5 [12]a (14.5–24.8)	20.0 [4]a (16.8–22.9)	12.4 [6]b (11.8–13.2)
Flower bud D	8.1 [11]c (5.6–9.8)	11.0 [4]b (10.5–11.9)	9.8 [12]b (8.2–11.0)	12.7 [2]a (11.4–14.0)	8.2 [6]c (7.3–9.2)
Flower bud E	20.2 [11]a (14.3–27.1)	14.4 [4]b (13.2–16.1)	22.4 [12]a (13.5–31.5)	18.4 [2]ab (13.8–23.0)	13.3 [6]b (10.5–15.9)
Flower bud F	4.2 [11]a (3.5–4.7)	4.6 [4]a (4.0–5.5)	4.9 [1][ID] (4.5–5.2)	—	3.3 [5]b (2.8–3.9)
Flower bud G	31.7 [11]ab (29.0–35.5)	29.9 [6]b (26.5–34.0)	34.3 [12]a (29.1–42.1)	34.1 [5]ab (29.5–41.0)	22.3 [3]c (21.0–24.0)
Flower bud H	19.6 [11]a (17.3–22.3)	17.9 [6]a (15.8–20.7)	19.7 [12]a (16.5–24.6)	19.7 [5]a (17.1–24.6)	12.4 [4]b (11.6–13.1)
Flower bud I	5.8 [11]b (5.0–7.0)	5.3 [6]b (4.1–6.3)	8.0 [12]a (6.3–9.2)	6.2 [5]b (5.1–8.2)	4.4 [3]c (4.0–4.8)
Flower bud J	8.3 [11]c (7.4–9.3)	9.0 [6]bc (7.7–10.5)	9.9 [12]ab (8.2–11.6)	10.5 [5]a (8.5–13.3)	6.2 [3]d (5.8–6.5)
Flower bud K	3.7 [11]a (3.1–4.4)	3.7 [6]a (3.0–4.3)	4.0 [1][ID] (3.6–4.3)	3.8 [5]a (2.8–5.0)	2.5 [4]b (1.9–3.3)
Flower bud L	4.7 [11]b (3.2–5.8)	4.4 [6]b (2.8–5.4)	7.8 [1][ID] (6.5–8.1)	4.3 [5]b (2.7–5.8)	3.7 [6]b (2.3–4.6)
Flower bud M	7.5 [11]c (4.2–12.0)	8.1 [6]cb (5.2–10.8)	16.5 [12]a (11.4–23.0)	15.8 [6]a (6.9–25.5)	10.6 [6]b (4.5–17.0)
Flower bud N	2.3 [11]b (1.8–3.2)	2.3 [6]b (1.9–3.1)	2.5 [12]b (2.0–3.1)	2.9 [6]a (2.2–3.9)	2.0 [6]c (1.5–2.5)
Repro A	18.1 [11]ab (16.9–19.8)	17.0 [6]b (11.9–20.5)	20.5 [12]a (17.1–24.3)	18.9 [5]ab (15.4–22.6)	13.4 [6]c (11.5–15.8)
Repro B	0.6 [11]c (0.5–0.7)	1.2 [6]a (0.9–1.8)	1.0 [12]b (0.5–1.3)	1.3 [5]a (0.9–2.0)	0.8 [6]c (0.6–0.9)

TABLE 2 (continued)

NUMERIC ATTRIBUTES[a]	HAWAI'I *Bruguiera* SP.	AUSTRALIA *B. sexangula*	MICRONESIA *B. gymnorrhiza*	AUSTRALIA *B. gymnorrhiza*	AUSTRALIA *B. exaristata*
Repro C	13.5 [11]a (11.3–15.5)	10.6 [6]b (7.8–12.7)	14.1 [12]a (11.8–19.0)	13.0 [4]a (9.5–16.3)	8.9 [3]b (8.0–10.0)
Repro D	0.1 [11]b (0.1–0.2)	0.3 [6]a (0.3–0.4)	0.3 [12]a (0.2–0.5)	0.4 [5]a (0.3–0.5)	0.3 [3]a (0.3)
Repro E^{NS}	5.3 [11] (3.2–6.5)	4.7 [6] (3.8–5.6)	5.0 [1] (4.2–5.8)	6.0 [5] (4.5–7.9)	4.8 [3] (4.3–5.4)
Repro F	0.4 [11]b (0.1–0.6)	0.6 [6]ab (0.3–0.8)	0.4 [1]^{ID} (0.3–0.5)	0.8 [5]a (0.6–0.9)	0.6 [3]ab (0.5–0.7)
Corolla A	4.6 [11]b (3.4–7.7)	4.9 [6]ab (4.1–5.7)	5.5 [12]ab (4.1–7.8)	6.2 [5]a (4.3–7.7)	3.2 [3]c (2.5–3.8)
Corolla B	2.9 [11]c (2.2–3.9)	3.2 [6]c (2.8–3.9)	5.8 [12]a (4.0–7.4)	4.4 [6]b (4.0–4.8)	0.2 [3][b]d (0.0–0.5)
Corolla C	13.7 [11]bc (12.4–15.3)	13.0 [6]c (9.4–14.8)	15.1 [12]ab (12.8–18.1)	16.0 [5]a (13.5–18.5)	9.3 [3]d (8.9–9.6)
Corolla D	3.4 [11]b (2.2–4.2)	3.7 [6]ab (3.0–4.5)	3.5 [12]b (2.8–4.3)	4.3 [5]a (3.3–5.7)	3.4 [3]b (2.9–3.6)
Corolla E^{NS}	1.9 [11] (1.6–2.3)	2.2 [6] (1.8–2.7)	1.8 [1] (1.6–2.0)	2.3 [5] (1.9–2.7)	1.9 [2] (1.8–2.2)
Corolla F^{NA}	3.1 [11] (2–4)	2.1 [6] (0–3)	2.1 [12] (0–3)	2.8 [6] (2–4)	0.3 [3] (0–1)
Corolla G	1.3 [11]c (0.7–2.1)	<5 [6]d[c] (0.3–0.5)	2.9 [12]a (1.4–4.3)	2.2 [5]b (2.0–2.5)	<0.1 [3]e[c] (0.0–0.3)

[a] All numeric attributes, except Corolla D ($P = 0.0035$) and Flower bud L ($P = 0.0025$), that were significantly different were so at $P < 0.001$. Mean values by collection followed by the same letter for a particular numeric attribute are not significantly different at the $P = 0.05$ level.

[b] One corolla possessed petals with very minute spines (0.4–0.5 mm).

[c] Actual means are not presented for Corolla G measurements from this location because of the low level of accuracy associated with measuring minute bristles.

^{NA}, not part of quantitative analysis; ^{NS}, not significant; ^{ID}, insufficient data to include in analysis.

portant variables (i.e., they have the largest canonical coefficients). The second discriminant function explains 13% of the total variation and is most influenced by hypocotyl width, followed by hypocotyl length and peduncle length. Most of the separation on the second axis appears to be due to the relatively large differences between *B. exaristata* and the other two species, especially in hypocotyl width.

In the DFA, resubstitution of individual observations into the four classes was perfect, and cross validation resulted in only two misclassifications, both involving the two populations of *B. gymnorrhiza* (Table 4 a). Of the 11 trees from Hawai'i (i.e., the "unknowns"), 10 were classified as *B. sexangula* (Table 4 b). If one tree from the Australian *B. gymnorrhiza* collection with unusually small hypocotyls is removed from the data set, all the Hawaiian material classifies as *B. sexangula.* The close relationship between the Hawaiian material and the *B. sexangula* is further demonstrated by the results of CA (Figure 2).

Genetic Relationships

A total of 101 restriction fragments was amplified and detected on gels, ranging in length from 70 to 460 base pairs. There were no detectable differences between the two individuals from the same collections. Of the 101 fragments, 69 were present in both the Australian *B. sexangula* and the *Bruguiera* collected in Hawai'i, whereas only 5 fragments were unique to one or the other of the two collections (Table 5 a). The Micronesian *B. gymnorrhiza* and the Hawaiian *Bruguiera* had a lower number of fragments in common (48) and much greater numbers that were present in only one of the collections (Table

TABLE 3

SUMMARY OF QUALITATIVE ATTRIBUTES MEASURED FOR COMPARISONS OF *Bruguiera* ON O'AHU, HAWAI'I, WITH *B. gymnorrhiza* FROM MICRONESIA AND *B. gymnorrhiza, B. sexangula,* AND *B. exaristata* FROM AUSTRALIA

ATTRIBUTES	HAWAI'I *Bruguiera* SP.	AUSTRALIA *B. sexangula*	MICRONESIA *B. gymnorrhiza*	AUSTRALIA *B. gymnorrhiza*	AUSTRALIA *B. exaristata*
Peduncle color[a]	Mostly green; few green with orange undersides	Green	Red with green undersides; few green	Pale yellow green to green	Green
Mature flower bud position	Nodes 2–3	Nodes 1–2	Nodes 1–2	Nodes 1–2	Nodes 1–2
Mature flower position	Nodes 2–3	Nodes 1–3	Nodes 1–2	Nodes 1–2	Nodes 2–3
Mature hypocotyl position	Nodes 4–6	Nodes 3–4(–5)[b]	Nodes (4)5–7[b]	Nodes 4(5–6)[b]	Nodes 4–5
Mature flower bud color[c]	Orange/yellow with red tinge	Variable[d]	Mostly red with patches of green	Mostly red with patches of green	Pale green and yellow
Petal apex	Obtuse	Obtuse	Acute	Acute	Obtuse
Petal spine (>0.5 mm)	Y	Y	Y	Y	N
Style lobes[a]	3	3(–4)[b]	3–4	3–4	2–3

[a] Measured on either a mature bud or a mature flower; number of style lobes is listed as a qualitative attribute because of the small, discrete range present for this character.

[b] Parentheses indicate one occurrence at that node.

[c] Mature buds from *B. gymnorrhiza* in Micronesia are represented by a full range of color forms, from completely pale green ("white," which were not sampled) to mostly red with patches of green. The "white" color morphs were seen mostly on Yap, Micronesia; however sightings were made on Chuuk and Kosrae, Micronesia, as well.

[d] Colors are variable and can range from pale orange to fleshy colored with pale green patches to pinkish orange blushes on otherwise green calyces.

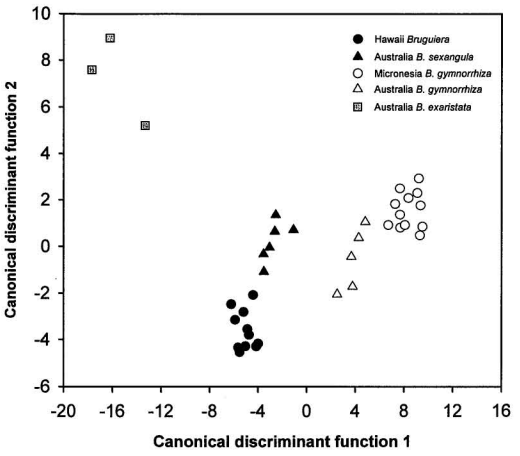

FIGURE 1. Plot of the first two axes for the discriminant function analysis of *Bruguiera* from O'ahu, Hawai'i; *B. gymnorrhiza* from Micronesia; and *B. gymnorrhiza, B. sexangula,* and *B. exaristata* from Australia. Discriminant function analysis was performed collectively on numeric attributes 7, 9, 24, 33, and 38, as indicated in Table 1. The sample size for some collections has been reduced because of missing values for one or more of the attributes.

5 a). There were no cases where a given fragment was present in both the *B. gymnorrhiza* from Australia and the *Bruguiera* collected in Hawai'i but *not* the Australian *B. sexangula,* which might have been interpreted as evidence of introgressive hybridization. Calculated similarity coefficients clearly demonstrate the strong resemblance of the Australian *B. sexangula* and the *Bruguiera* material collected in Hawai'i (Table 5 b).

DISCUSSION

Identity of the Hawaiian Bruguiera

Morphological comparisons of the material from Hawai'i with the other collections strongly suggest that it is *B. sexangula,* a result that is supported by AFLP mapping. Although we cannot rule out the presence of both *B. gymnorrhiza* and *B. sexangula* in Hawai'i, we have seen no trees that appear to be different from those from which we collected material, despite extensive searching of all stands known to have *Bruguiera,* as well as several other stands on O'ahu, Kaua'i, and Moloka'i. There were no outliers in the Hawaiian material that more closely resembled the *B. gymnorrhiza* from Micronesia and Australia (Figure 1 and Table 5). Based on these results and personal observations in the field, we conclude that *B. sexangula* is the predominant, and perhaps only, species of *Bruguiera* currently present in Hawai'i. [At the time the manuscript for this paper was being developed, the revised edi-

TABLE 4

CROSS VALIDATION (a) AND CLASSIFICATION (b) SUMMARIES USING DISCRIMINANT FUNCTION ANALYSIS BASED ON THE FIVE ATTRIBUTES MARKED IN TABLE 1

		PREDICTED CLASSIFICATION			
ACTUAL CLASSIFICATION	MICRONESIA *B. gymnorrhiza*	AUSTRALIA *B. gymnorrhiza*	AUSTRALIA *B. sexangula*	AUSTRALIA *B. exaristata*	TOTAL
a Micronesia *B. gymnorrhiza*	10	2	0	0	12
Australia *B. gymnorrhiza*	0	5	0	0	5
Australia *B. sexangula*	0	0	6	0	6
Australia *B. exaristata*	0	0	0	3	3
b Hawai'i *Bruguiera* sp.	0	1	10	0	11

Note: Sample size for some collections has been reduced because of missing values for one or more of the attributes.

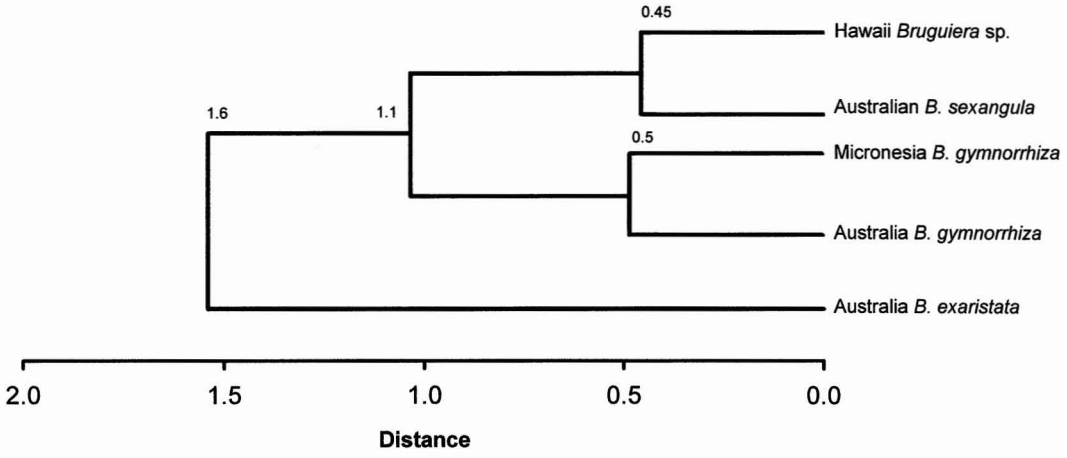

FIGURE 2. Classification of *Bruguiera* species collected from Hawai'i, Australia, and Micronesia resulting from cluster analysis. Cluster analysis was performed collectively on numeric attributes 7, 9, 24, 33, and 38, as indicated in Table 1. The sample size for some collections has been reduced because of missing values for one or more of the attributes.

TABLE 5

(a) PAIRWISE COMPARISONS OF PRESENCE OR ABSENCE OF GIVEN DNA FRAGMENTS AND (b) GENETIC SIMILARITY COEFFICIENTS FOR COLLECTIONS OF *Bruguiera* ON O'AHU, HAWAI'I; *B. gymnorrhiza* FROM MICRONESIA; AND *B. sexangula* FROM AUSTRALIA

a COMPARISON[a]	NO. OF FRAGMENTS	b SIMILARITY COEFFICIENT			
Both BRGY and BRSE	50				
BRGY but not BRSE	22				
BRSE but not BRGY	24		BRGY	BRSE	BR sp.
Both BRSE and BR sp.	69	BRGY	1	—	—
BRSE but not BR sp.	5	BRSE	0.685	1	—
BR sp. but not BRSE	5	BR sp.	0.658	0.932	1
Both BRGY and BR sp.	48				
BRGY but not BR sp.	24				
BR sp. but not BRGY	26				

[a] BRGY, *B. gymnorrhiza*; BRSE, *B. sexangula*; BR sp., *Bruguiera* sp. (the Hawaiian material).

tion of Wagner et al.'s (1990) *Manual of the Flowering Plants of Hawai'i* was also being prepared. The revised edition (Wagner et al. 1999) does not change the text on *Bruguiera,* but, based on our results, contains a statement in its supplement (p. 1890) recognizing the treatment of *B. gymnorrhiza* as a misidentification.]

Some features of the Hawaiian material, such as hypocotyl length, were clearly different from *B. gymnorrhiza* collections and exhibited virtually no size overlap. These results are in close agreement with published descriptions of the two species. Ding Hou (1958), for example, reported the hypocotyl as 15–25 cm long for *B. gymnorrhiza* and 6–8 cm long for *B. sexangula.* Percival and Womersley (1975) recorded the hypocotyl lengths as 7–25 cm for *B. gymnorrhiza* and 4–8 cm for *B. sexangula.* Propagules of the size we collected in Hawai'i were mature (i.e., the hypocotyls separated easily from the

calyx and were capable of developing into established seedlings), and we have not observed propagules similar in appearance to *B. gymnorrhiza* material in Hawai'i. Hypocotyl characteristics were apparently not considered by Fosberg or Degener.

The flowers collected in Hawai'i were also substantially different from the two *B. gymnorrhiza* collections. The first canonical discriminant function clearly separated the three species (Figure 1) and was based primarily on two flower attributes (peduncle and petal spine lengths). Other key attributes, such as the color and number of mature flower bud/calyx lobes, also differed and closely resembled published descriptions for *B. sexangula* (Ding Hou 1958, McCusker 1984, Tomlinson 1986). It is interesting to note, however, that the Hawaiian material appears to have some characteristics that are unusual for *B. sexangula.* Of greatest interest in this regard, because they are regarded as diagnostic attributes, is that both the length and number of bristles on the petal tip are somewhat greater than described for *B. sexangula* by Ding Hou (1958) and found in the Australian *B. sexangula.*

One possible explanation for the slight flower character divergence observed in the Hawaiian material is introgressive hybridization between *B. sexangula* and any *B. gymnorrhiza* that is now, or once was, present in Hawai'i. The genetic evidence, however, suggests that this is unlikely, at least for the two trees sampled. Another possibility, given that the Hawaiian material comes from a single introduction, is a founder effect, but we cannot address this without a direct comparison of the Hawaiian material and *Bruguiera* material collected from the area of origin in the Philippines.

The decision of some botanists to identify the species in Hawai'i as *B. gymnorrhiza* appears to have been based predominantly on flower color. Fosberg (1948 : 8), for example, stated that the *Bruguiera* collected from He'eia Swamp "has red flowers and is undoubtedly *B. conjugata* [*gymnorrhiza*] in spite of occasionally having mostly ten-lobed calyces." In Fosberg's case, we are uncertain whether he meant that the whole calyx surface was red or, as we have observed, merely red-tinged. Our observations on the color of the mature flower buds/calyces of the Hawaiian, Micronesian, and Australian material (Table 3) are in agreement with current descriptions of the two species, which indicate substantial color variation but specify that *B. sexangula* is never bright red (e.g., Ding Hou 1958, Melana and Gonzales n.d.).

Leaf characteristics are also in general agreement with published descriptions, which indicate that *B. gymnorrhiza* has somewhat larger leaves. The differences, although statistically significant, were not dramatic, and there was considerable overlap between the Hawaiian material, the Australian *B. sexangula,* and the *B. gymnorrhiza* collections (Table 2). Descriptions such as Ding Hou's (1958) and McCusker's (1984) also indicate a sizeable degree of overlap in size. Because leaves vary with their environment (Duke 1990), we do not consider the differences observed in our study to be reliable diagnostic indicators, despite the general agreement with published descriptions.

Ecological Implications

Proper identification of the *Bruguiera* species in Hawai'i should lead to a better understanding of the distribution of mangrove species within the archipelago. Currently, *Bruguiera* is known to occur at only four sites, all on the island of O'ahu; most mangroves in Hawai'i are monospecific stands of *R. mangle* (Allen 1998). Only in the upper reaches of He'eia Swamp, where the salinity is nearly that of fresh water (Walsh 1967), can *Bruguiera* be considered common. Based on observations of the distribution of *B. gymnorrhiza* in Micronesia (Fujimoto et al. 1995, Ewel et al. 1998) and elsewhere in the Pacific (Kuraishi et al. 1985, Steele 1998), which includes sites physically similar to ones in Hawai'i occupied by only *R. mangle,* we would expect *Bruguiera* to be more widespread in Hawai'i if it were indeed *B. gymnorrhiza. Bruguiera sexangula,* however, is less tolerant of salinity than *B. gymnorrhiza* and is among the least salt tolerant of the true mangroves (Bunt et al. 1982, Clough

1992). We believe that the current distribution of *Bruguiera* in Hawai'i fits the pattern that would be expected of *B. sexangula* much better than *B. gymnorrhiza* and lends further support to our conclusion that the primary species present in Hawai'i is *B. sexangula.*

The Need for a Broader Morphological and Genetic Comparison

Confusion regarding the identification of *B. gymnorrhiza, B. sexangula,* and *B. exaristata* is by no means unique to Hawai'i. Watson (1928), Symington (1940), Wyatt-Smith (1960), Mitra and Banerjee (1979), and McCusker (1984) all indicated that identification of two or more of the species is problematic. McCusker (1984) stated that the three *Bruguiera* species considered in this paper need to be compared carefully across their whole geographic range. Our results, which demonstrate considerable overlap in many attributes and also the probability of significant intraspecific, regional variation, lead us to agree strongly with that recommendation. Recent observations (by N.C.D.) of putative hybrids of *B. gymnorrhiza* and *B. sexangula* in Australia also indicate the need for a closer look at these species.

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