What is *Ceratozamia brevifrons* (Zamiaceae)?

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Abstract. The taxonomic status of the Mexican cycad *Ceratozamia brevifrons* is re-assessed based on information from habitat, herbaria and living collections in the Botanic Garden 'Jardín Botánico Francisco Javier Clavijero.' Comparison of gross morphology of *C. brevifrons* with that of *C. mexicana* as well as analysis of leaflet anatomy, including that of *C. robusta,* has provided sufficient evidence for the removal of *C. brevifrons* from synonymy with *C. mexicana*. A neotype is designated for **C. brevifrons** and a description is presented.

Key Words: Neotropical cycads, *Ceratozamia*, Cycadales, nomenclature, leaflet anatomy, girder sclerenchyma, morphometric analyses, Mexico.

Resumen. El estatus taxonómico de la cícada mexicana *Ceratozamia brevifrons* fue revisado con base en información sobre hábitat, especímenes de herbario y las colecciones vivas en el jardín botánico. Al comparar la morfología gruesa de *C. brevifrons* con la de *C. mexicana* así como análisis anatómico de los folíolos, incluyendo *C. robusta* dio suficiente evidencia para remover a *C. brevifrons* de sinonimia con *C. mexicana.* Se designó un neotipo para **C. brevifrons** y se presenta una descripción.

Miquel (1847) described *Ceratozamia brevifrons* Miq. as having ovoid trunks bearing short fronds of unequally lanceolate leaflets. His distinguishing features were short leaves with shorter leaflets and long petioles. Miquel's material was sterile and probably juvenile because there is neither a mention of strobili in the description nor are there illustrations available nor locality information presented. We found plants answering to this description in central Veracruz in the transition zone between cloud forest and oak dominated tropical forest.

Ceratozamia brevifrons was placed in synonymy with *C. mexicana* Brongn. by Dyer (1882–1886), Schuster (1932) and Vovides et al. (1983). *Ceratozamia brevifrons* was later lectotypified by Stevenson and Sabato (1986) designating the Miquel (1847) description owing to the lack of a type specimen. Stevenson and Sabato looked for type specimens of *C. brevifrons* at Utrecht but none were found. Vovides also searched for specimens of *C. brevifrons* at Utrecht, Berlin, and Paris and none were located. Subsequently *C. brevifrons* has been considered part of the *C. latifolia* Miq. species concept by Vovides et al. (2004), mainly based on gross leaf and leaflet morphology. Upon comparison of *C. brevifrons* with *C. mexicana* as well as *C. robusta* Miq. in habitat, greenhouse collections held at the 'Jardín Botánico

Francisco Javier Clavijero' (JBC), and herbarium vouchers, a number of gross morphological differences distinguished between the species.

Methods

Field, Greenhouse and Herbarium observations

Botanical excursions over several years into central Veracruz along the transition zone between cloud forest and tropical forest at around 800–1200 m elevation have resulted in the location of a number of *Ceratozamia* populations. The cycads in one of these populations largely fit Miquel's description of *C. brevifrons* and some specimens, mostly juveniles, fall within the size ranges mentioned by Miquel. These have ovoid trunks 13 cm long and 11 cm diameter bearing short fronds with 8–9 pairs of unequally lanceolate leaflets with upper margin straight and lower margin convex, no longer than 20 cm and 1.5–2.5 cm wide (Miquel, 1847). Living specimens were collected and cultivated at the JBC for several years in order to observe plasticity upon later comparison with habitat plants. Herbarium specimens at XAL, MEXU and especially the type of *C. mexicana* at P were also examined and compared. Table I lists the species and vouchers deposited at XAL of the specimens used for the anatomical preparations and analysis.

Leaflet Anatomy

Leaflets for sectioning were taken from plants growing in habitat as well as those cultivated in the Botanic Garden for several years in order to rule out plasticity. The central portion of leaflets taken from the median leaflets of leaves from five individuals of both *Ceratozamia mexicana* and *C. brevifrons* were fixed in FAA and hand sectioned with the aid of a hand microtome. Fresh sections were also taken and stained for lignin using phloroglucinol/hydrochloric acid (Chamberlain, 1932) and for cutin using Sudan III and IV stains. Fixed sections were dehydrated, double-stained with safranin and fast green, cleared and mounted according to Purvis et al. (1966) and photomicrographs taken with a digital camera using clear field light microscopy. Cross-sectional measurements were taken from 11 characters (Table II) using a calibrated eyepiece micrometer; where cells were not isodiametric the measurements were expressed as length and width. Twenty five replicate measurements were taken for each character from each of the five leaflet samples per species. Analysis of variance (ANOVA) and Tukey multiple range analyses were done using JMP version 3.2 statistical software. Discriminant analyses (McCune & Mefford, 1997) were done with Statgraphics software version 2.0. Data were transformed to $\log_{10}$ and Mahalanobis distances were obtained.

Results

General Morphology

Some of the plants in the *Ceratozamia brevifrons* population show a petiole length longer or equal to their rachis length, a feature mentioned by Miquel (1847). This is especially so in young plants grown in the greenhouse, even over a period of over ten years. In contrast, in larger fertile specimens this feature changes where the rachis is longer than the petiole and other measurements overlap or exceed Miquel's ranges. We found leaflets arranged along the rachis at an upward angle forming a keeled leaf bearing 3–9 subopposite pairs in young plants and 9–13 pairs in juvenile plants, and up to 24 pairs in large fertile plants, whereas *C. mexicana* bears longer non-keeled leaves and longer falcate to sub-falcate leaflets. In addition, the adaxial sterile portion of the male sporophylls is longer and narrower in *C.*

Table I
Ceratozamia species and vouchers deposited at XAL of the specimens used for analysis.

Species	State	Herbarium voucher (XAL)				
Ceratozamia brevifrons	Veracruz	S.A.5698	S.A.5749	A.V.682	A.VB.191	A.V.119
Ceratozamia mexicana (Esquilón population)	Veracruz	A.V.15	A. V.18	A.V.72	S.A. 5628	S.A.5619
Ceratozamia mexicana (El Mirador population)	Veracruz	A.V.730	A.V.747	J.R.1688	S.A.5205	S.A.5206
Ceratozamia robusta (San Fernando)	Chiapas	A.V.1267	M.L. s/n	A.V. s/n	M.A.P. s/n	M.A.P.s/n

TABLE II
LEAFLET ANATOMICAL CHARACTERS USED IN ANALYSES.

Character	Character type and cross sectional measurements
EABL	Abaxial epidermal cell length
BULL	Thin walled bulliform-like cell length
CUTAD	Adaxial cuticle thickness
CEADL	Adaxial epidermal cell length
PEL	Palisade parenchyma cell length
PEA	Palisade parenchyma cell width
FPVD	Perivascular fiber diameter
FIVL	Intervascular fiber length
FIVA	Intervascular fiber width
NFPV	Number of perivascular fibers (surrounding vascular bundle)
NFIV	Number of intervascular fibers (between vascular bundles)

brevifrons than in *C. mexicana* (Table III; Figs. 1, and 2). *Ceratozamia robusta* presents much longer and wider leaves and leaflets than either of the two species as well as leaflet anatomical differences that separated the three species (Fig. 4).

LEAFLET ANATOMY

Leaflet cross sections through median part of the leaflet and through the leaflet margins of both *Ceratozamia brevifrons* and *C. mexicana* reveal distinctive anatomical differences between the two species. In *C. brevifrons*, it can be seen that the vascular bundle has associated sclerenchymatous fibers or girder sclerenchyma (Fig. 3A) and the leaflet margin shows up to four layers of sclerenchymatous fibers (Fig. 3B). In contrast, in *C. mexicana* there is no girder sclerenchyma. In addition, *C. mexicana* shows a thicker and more pronounced sub revolute margin with up to four layers of sclerenchymatous fibers but of a greater diameter than those of *C. brevifrons*.

UNIVARIATE ANALYSIS

The ANOVA test on the 11 anatomical variables of the leaflets of the *Ceratozamia* species tested were significantly different at $P < 0.001$ (Table IV) except for palisade parenchyma cell length (PEL). The Tukey multiple range test showed that the number of perivascular fibers (NFPV) were significantly different for the two *C. mexicana* populations, *C. brevifrons* and *C. robusta* separating the four groups with no overlap. *Ceratozamia brevifrons* and *C. robusta* are separated from each other and from the two *C. mexicana* populations by the number of intervascular fibers character (NFIV). The two *C. mexicana* populations and *C. robusta* overlapped for length of the bulliform-like cell (BULL) character separating *C. brevifrons*. For the remaining characters, there was a either continuous overlapping of the four *Ceratozamia* populations studied or overlapping with *C. brevifrons* either with the *C. mexicana* populations or with *C. robusta*.

DISCRIMINANT ANALYSIS

Data derived from the discriminant function analysis of anatomical characters separate four populations (two of *C.mexicana* and one of each *C. brevifrons* and *C. robusta*) coordinately in bi-dimensional space and do not present any overlapping between groups (Fig. 4). The Wilks' Lambda test was highly significant ($P<0.0001$) for the first two factors and marginally significant for the third, $P=0.046$ (Table V), thus showing that all the species were classified correctly. Additionally, the squared Mahalanobis distances (Table VI) were statistically significant in all cases ($P<0.001$). Of the 11 variables included in the standardized discrete canonical function, the two variables with the highest values

TABLE III
DIAGNOSTIC FEATURES SEPARATING *C. MEXICANA* FROM *C. BREVIFRONS*.

	Ceratozamia mexicana	*Ceratozamia brevifrons*
Leaves	Not keeled (flat)	Keeled
Leaflets	Linear lanceolate to lanceolate, falcate to sub falcate	Unequally lanceolate, not falcate to sub falcate
Adaxial sterile portion of microsporophylls	0.35–0.5 cm long	0.7–0.9 cm long
Girder sclerenchyma	Absent	Present

FIG. 1. *Ceratozamia brevifrons*. Habit, showing keeled leaves.

in factor 1, 2 and 3 were the cross sectional length of the thin walled bulliform-like cells (BULL) and the cross sectional width of intervascular fibers (FIVA). The first and second canonical variable showed that 95% of the variation is largely due to anatomical characters. The positive correlations (Table VII) of all the variables show differences between species. As a result of these morphological and anatomical differences we recognize *Ceratozamia brevifrons* as a separate species from *C. Mexicana*; therefore we present a description and designate a neotype.

FIG. 2. Comparison of leaves. **A.** *Ceratozamia mexicana*. **B.** *Ceratozamia brevifrons.*

Description

Ceratozamia brevifrons Miq., Tijdschr. wis-en natuurk. Wet. 1: 41–42. 1847. Type: Mexico. Veracruz: Alto Lucero, Apr 2005, *S. Avendaño R. 5699* (neotype, **designated here**: XAL).

Trunks erect to procumbent, globose to cylindrical, semi-hypogeous 9–28 cm long 8–19 cm diam., rarely branching, covered with light brown persistent scale-like leaf and cataphyll bases especially near apex. *Cataphylls* long triangular pale pubescent 2.9–4.5 cm long, 2.1–4.1 cm wide at the base. *Leaves* ptyxis erect, green-emergent, keeled, ascending to decurrent forming an open crown of 3–17 leaves, glabrous at maturity; rachis and leaflets sparsely pubescent at emergence, rachis armed with few short prickles, 76–120×39–69 cm. *Leaflets* unequally-lanceolate but less so in older plants, 3–9 subopposite pairs in young plants and 9–13 pairs in juvenile plants, and up to 24 pairs in large fertile plants, 14.5–34.5×2.7–3.7 cm, apex acute, base attenuate, articulation 0.8–1.2 cm wide. *Petiole* terete to subterete 31–39 cm long, 0.5–0.9 cm diam., armed with long (> 2 mm) stout prickles becoming less in number and shorter along rachis, base stipulate light brown tomentose. *Pollen strobili* conical, light green glabrous, 45–59 cm long, 5–5.5 cm diam., peduncle 6 cm long, 1.5–1.9 cm diam. *Microsporophylls* cuneiform, bicornate 1.8–2.5 cm× 0.7–0.9 cm, non-fertile section 0.7–0.9 cm long, distance between horns 0.35–0.5 cm. *Ovulate strobili* cylindrical, olive green, glabrous 24 cm long, 9.5 cm diam., peduncle 8 cm long, 1.5 cm diam. *Megasprophylls* peltate, face hexagonal bicornate, scarce white tomentum beneath peripheral edge, 2.9–3.3 cm long, face long axis 3.3–3.4 cm, face short axis 1.4–1.5 cm. *Seeds* angular ovoid 2.2–2.4 cm long, 1.4–1.7 cm diam., sclerotesta beige smooth, sarcotesta brown when mature. *Chromosome number* $2n=16$.

Additional specimens examined. MEXICO. VERACRUZ: Alto Lucero, April 2005, *S. Avendaño R. 5694* (MEXU), *S. Avendaño R. 5696* (NY), *S. Avendaño R. 5813* (K); Apr 1981, *G. Castillo-Campos & F. Vázquez B. 1297* (XAL); Dec 1974, *J. Rees 1636* (XAL), *J. Rees 1642* (XAL).

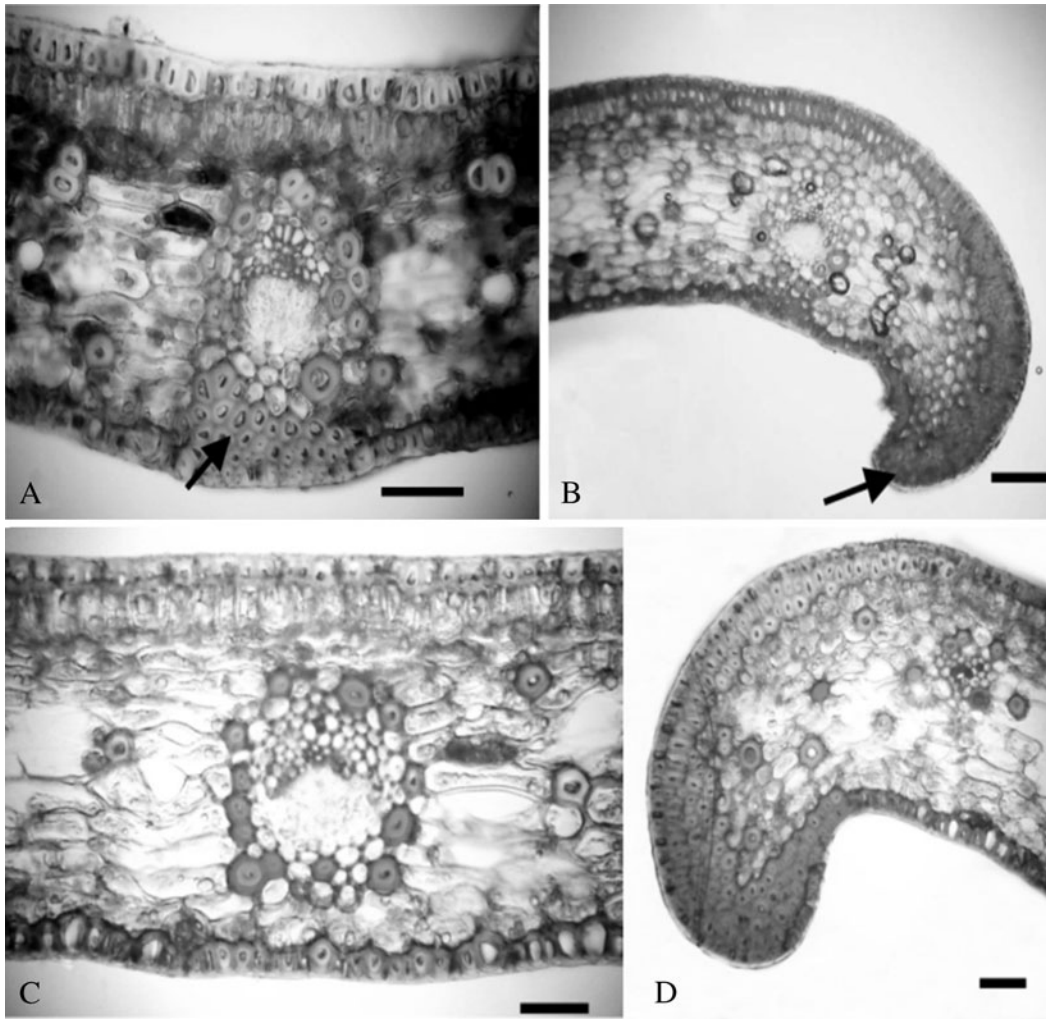

Fig. 3. A. *Ceratozamia brevifrons*, transverse section of the central region of a leaflet showing vascular bundle and girder sclerenchyma, arrow. **B.** *Ceratozamia brevifrons*, section through subrevolute margin, arrow. Bars=100 μm. **C.** *Ceratozamia mexicana*, transverse section of the central region of a leaflet showing vascular bundle (note absence of girder sclerenchyma). **D.** *Ceratozamia mexicana*, section through subrevolute margin. Bars=100 μm.

Ceratozamia mexicana Brongn. Ann. Sci. Nat. Bot., ser. 3, 5: 7–8, Tab. 1. 1846. Type: Mexico. From Ghiesbrecht, cult. in Hort. Bot. Parisiensis, 1845, *Brongniart s.n*. (holotype: P).

Additional specimens examined. MEXICO. **Veracruz**: Coacoatzintla, Nov 2004, *S. Avendaño R. 5628* (XAL), Jilotepec, *5619* (XAL); Jilotepec, Aug 1976, *R. Ortega O. & M. Ortíz T. 525* (MEXU, XAL); Jilotepec, Feb 1980, *A.P. Vovides 470* (XAL); Jilotepec, Aug 1976, *M. G. Zolá B. 667* (MEXU, XAL).

Discussion

This study based on both gross morphology and leaflet anatomy has helped to define the status of *C. brevifrons*, which was previously under synonymy with *C. mexicana*. The leaflet shape of *C. brevifrons* always corresponds to Miquel's description of "foliolis suboppositis subdimidiato-lanceolatis, margine superiore recto, inferi-ore convexo recurvo, subacuminatis, pungentibus, basi attenuatis (supra eam haud constrictis),"

TABLE IV
SUMMARY OF ANALYSIS OF VARIANCE OF THE 11 ANATOMICAL CHARACTERS R^2 (CORRELATION COEFFICIENT), F (F VALUE), P (PROBABILITY).

Character	R^2	F	P
EABL	0.6354	9.2958	0.0009
BULL	0.6209	8.7364	0.0012
CUTADGROS	0.5776	7.2929	0.0027
CEADL	0.6266	8.9516	0.0010
PEL	0.3546	2.9300	0.0655
PEA	0.7367	14.9243	<.0001
FPVL	0.8343	26.8484	<.0001
FIVL	0.7813	19.0494	<.0001
FIVA	0.8121	23.0598	<.0001
NFPV	0.9735	196.1378	<.0001
NFIV	0.9518	105.2225	<.0001

TABLE V
SUMMARY OF THE DISCRIMINANT ANALYSIS RESULTS FROM THE ANALYSIS OF FOUR TAXA IN *CERATOZAMIA* χ^2 = CHI SQUARE TEST, DF: DEGREES OF FREEDOM, P = PROBABILITY.

Derivate Functions	Wilks' Lambda	χ^2	DF	P-Value
1	0.000169	99.8608	33	0.0000
2	0.0176084	46.4529	20	0.0007
3	0.224494	17.1799	9	0.0460

which is subopposite and unequally lanceolate, with the upper margin straight and the lower margin convex. We found leaflets arranged along the rachis at an upward angle forming a keeled leaf, a character that would be lost in herbarium specimens after the pressing and drying process.

A tentative molecular phylogeny of the genus *Ceratozamia* by González and Vovides (2002) has shown that *C. brevifrons* is sister species to *C. mexicana* var. *robusta* (= *C. robusta*, see Walters & Osborne (2004)). The leaflet anatomy of the latter does show a presence of girder sclerenchyma, but there are other major morphological differences separating the two species as well as the anatomical differences as shown by the ANOVA and discriminant analyses (Fig. 4). The discriminant analyses showed that 95% of the variation was due to the cross sectional length of the thin walled bulliform-like cells (BULL) and the cross sectional width of intervascular fibers (FIVA). The Tukey multiple range test showed that *C. brevifrons* was separated from the other *Ceratozamia* spp. by the number of perivascular fibers (NFPV), number of intervascular fibers (NFIV) and the cross sectional length of the thin walled bulliform-like cells. The possibility of these differences arising through phenotypic plasticity has been eliminated since the specimens used for the investigation have been taken from the field as well as plants grown under similar conditions in the Botanic Garden for over ten years. Further work is underway to elucidate the status of the *C. mexicana* population at El Esquilón since the analysis has shown a marked separation of this population from the one at El Mirador. In summary, our observations show that *Ceratozamia mexicana* differs from *C. brevifrons* by having much longer and wider non-keeled or flat leaves with linear-lanceolate to lanceolate, falcate to subfalcate leaflets, whereas *C. brevifrons* has shorter and narrower keeled leaves with generally unequally lanceolate leaflets, leaf and leaflet characters not reported by Miquel (1847).

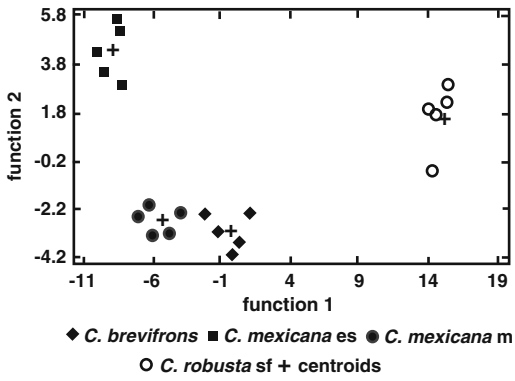

FIG. 4. Scatter plot score derived from the functions produced by stepwise discriminant analysis of 11 anatomic characters from *Ceratozamia brevifrons, C. mexicana* Esquilon population (es), *C. mexicana* El Mirador population (m), and *C. robusta* San Fernando Population (sf).

It could be argued that the name *C. brevifrons* be of uncertain application and that a new specific epithet could be applied to the plants of the wild population. However, because Stevenson and Sabato (1986) lectotypified *C. brevifrons* by the Miquel description and this description largely fits these plants, and because these plants differ from *C. mexicana,* we chose to use Miquel's specific epithet. Additionally the name *C. brevifrons* is in current use and has been applied to plants from this population by González

TABLE VI
SUMMARY OF THE SQUARED MAHALANOBIS DISTANCE, F VALUES AND STATISTICAL SIGNIFICANCE (P). ESQ = ESQUILÓN POPULATION, MIR = MIRADOR POPULATION.

	C. brevi	*C. mex* Esq	*C. mex* Mir	*C. robust*
Squared Mahalanobis Distances				
C. brevi		167.086	58.809	323.355
C. mex Esq	167.086		86.176	719.406
C. mex Mir	58.809	86.176		536.065
C. robust	323.355	719.406	536.065	
F-values; df=11, 6				
C. brevi		11.392	4.010	22.047
C. mex Esq	11.392		5.876	49.050
C. mex Mir	4.010	5.876		36.550
C. robust	22.047	49.050	36.550	
P-values				
C. brevi		0.004	0.051	0.0006
C. mex Esq	0.001		0.020	< 0.0001
C. mex Mir	0.051	0.020		0.0001
C. robust	0.0006	< 0.0001	0.0001	

and Vovides (2002) in a molecular phylogeny of the genus. Also by Pant and Nautiyal (1963) in a cycad cuticle study though no source information was given for *C. brevifrons*, by Vovides et al. (2004) within a concept of species complexes in *Ceratozamia*, and Osborne et al. (in press) in the world list of cycads.

Species delimitation for cycads has relied heavily on gross morphological characters, many of which tend to be lost after voucher preparation. This has added to taxonomic confusion regarding 19th century descriptions of cycads that are often partial and based mostly on sterile material cultivated in temperate region greenhouses. The use of anatomical characters to help delimit cycad species can be helpful in spite of their paucity. Though anatomical descriptions have been done in the past, these have been limited to describing the various features of just a few species selected from the known living genera (Lamb, 1923; Papadopoulos, 1928; Pant & Mehra, 1958; Pant & Nautiyal, 1963; Greguss, 1966, 1968). More recently Tang et al. (2004) have described pinnae venation of ten species within six genera of Zamiaceae and hypothesize an evolutionary trend toward a reduction in mucilage canals and an increase in girder sclerenchyma. They also commented that few anatomical characters were used in morphology-based phylogenies. Morphological phylogenies at the genus level by Crane (1988) and Stevenson (1990) used 23 and 30 morphological characters respectively, but only seven of which were anatomical and were identical in both matrices. We conclude that there is a need to search for and include more anatomical characters along with gross morphology to complement future species delimitations and cycad phylogenies.

TABLE VII
STANDARDIZED DISCRIMINANT FUNCTION VALUES FOR EACH OF THREE FACTORS USED IN THE ANALYSIS OF TAXA.

Discriminant function	1	2	3
EABL	−0.03	0.59	0.34
BULL	1.78	−1.60	−1.29
CUTAD	0.71	0.17	−0.47
CEADL	0.21	−0.56	−0.35
PEL	0.03	0.49	−0.19
PEA	1.77	−1.55	−0.32
FPVD	0.13	−1.35	0.03
FIVL	−0.83	1.34	1.52
FIVA	2.12	−1.66	−1.17
NFPV	−1.36	−0.64	0.27
NFIV	−1.33	1.46	−0.31
EIGENVALUE	102.98	11.75	3.45
AMONG GROUP VARIANCE	87.14	9.94	2.92
CANONICAL CORRELATION	1.00	0.96	0.88

HABITAT

Ceratozamia brevifrons is found in the transition zone between cloud forest and oak dominated tropical forest between 800 and 1200 meters elevation in clay soils with abundant basalt stones. Dominant tree species are *Casearia corymbosa* Kunth, *Diospyros*

riojae Gómez-Pompa, *Leucaena pulverulenta* Benth., *Quercus laurina* Humb. & Bonpl., and *Q. oleoides* Schtdl. & Cham. Common shrub and herbaceous species are *Anthurium* spp., *Bouvardia ternifolia* Schltdl., *Cnidoscolus aconitifolius* I. M. Johnst. *Faramea* sp., *Malvaviscus arboreus* Cav., *Philodendron* spp., *Psychotria oerstediana* Standl., and *Tabernaemontana alba* Mill.

Conservation Status

There are two known populations of *Ceratozamia brevifrons* close by and we consider them to be the remnants of a once larger population that has been fragmented. We consider the species to have small populations, collectively of less than 5000 individuals. The cycad occurs in disturbed forest and in some places on steep slopes unsuitable for agriculture but accessible to cattle. The main threats are habitat loss for agricultural expansion, cattle grazing and new road works. Owing to the small population size and restricted distribution of this species we recommend the IUCN (2010) category of Vulnerable (VU), subcategory D2 restricted area of occupancy.

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