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Molecular Phylogenetics and Evolution 43 (2007) 493–501
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

The phylogeny of yuccas

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Received 13 April 2006; revised 2 December 2006; accepted 18 December 2006
Available online 31 December 2006

Abstract

The genus *Yucca* is widely recognized for its pollination mutualism with yucca moths. Analysis of diversification in this interaction has been hampered by the lack of a robust phylogeny for the genus. Here we attempt the first extensive nuclear DNA based assessment of the phylogenetic relationships of *Yucca*. We used AFLP markers to recover the phylogeny of 87 samples representing 38 *Yucca* taxa. An analysis based on 4322 markers strongly supported a topology consistent with morphological classification at the section level (capsular-fruited Chaenocarpa, fleshy-fruited Sarcocarpa, and spongy-fruited Clistocarpa). Within Sarcocarpa, all but two of the traditional species were monophyletic. Within Chaenocarpa, the morphologically distinct series Rupicolae was strongly supported. In the remaining Chaenocarpa, a western group (Colorado Plateau southward) and an eastern group (Great Plains, central Texas east to Florida) were recovered. Within these groups, where taxonomic circumscriptions are narrow and historically contested, there was at most limited monophyly of traditional taxa, suggesting rapid recent diversification, introgression, or non-monophyletically circumscribed taxa.

Keywords: *Yucca*; Phylogeny; AFLP; Obligate pollination mutualism; Coevolution

1. Introduction

The obligate mutualism between yuccas and yucca moths (Engelmann, 1872; Riley, 1872, 1892; Pellmyr, 2003) was the first reported case in which a pollinator actively provides pollination, and the pollinator's larvae subsequently consume some of the developing seeds. There is evidence of strong specialization in this interaction, with most yuccas being pollinated by one moth species. The interaction between yuccas and yucca moths together with a handful of other associations with similar biological features (Fleming and Holland, 1998; Holland and Fleming, 1999; Weiblen, 2002; Kato et al., 2003; Kawakita et al., 2004; Machado et al., 2005), have proven very useful in studies of the ecology and evolution of species interactions in general, and of mutualism in particular.

An important question in this context is the role of coevolution in trait diversification within species-rich lineages, such as the yuccas and yucca moths. Our understanding of trait evolution has been hampered by the lack of robust phylogenetic data for the respective lineages, and especially so for the plants. A study based on chloroplast restriction site data had too few characters to provide resolution (Hanson, 1993), whereas an analysis based on 400 bp of ITS sequence data (Clary, 1997) included paralogous regions (Clary, pers. comm., Leebens-Mack, unpubl. data). The purpose of this paper is to provide a robust species-level phylogenetic framework for the genus *Yucca* L. The genus has been estimated to conservatively comprise 35–40 species within its native range from Central America northward to southernmost Canada (Matuda and Piña Lujan, 1980; Hess and Robbins, 2002; Pellmyr, 2003). Historically, three sections have been defined based on differences in

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1055-7903/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2006.12.015

fruit morphology. Two sections contain species with indehiscent fruit, including the fleshy-fruited Sarcocarpa and the spongy-fruited Clistocarpa, and the remainder belong in the section Chaenocarpa with dry, dehiscent fruits. Within the Chaenocarpa, there is also consensus in historical, morphology-based treatments for a subset referred to as the Rupicolae. Clistocarpa is monotypic, while the two other sections have roughly equal numbers of taxa. Relationships among these sections remain unsettled.

Species delineation has historically been unstable and contentious for many taxa, in part because of extensive typological species application and widespread horticultural interest in the genus, with its intrinsic narrow definition of taxa. Our purpose here is not to address these taxonomic issues, but rather we aim simply to establish phylogenetic relationships among conservatively defined entities. This will suffice for forthcoming analyses of plant-moth diversification, whereas a genetically based revision of species must await additional data.

2. Methods

We generated AFLP markers from 87 individuals spanning 38 *Yucca* taxa, and the well-defined outgroup *Hesperoyucca whipplei* (Bogler and Simpson, 1996). The latter, while historically placed within *Yucca* (e.g., McKelvey, 1938, 1947; Webber, 1953) is strongly supported as sister together with the small non-moth associated genus *Hesperaloe* to *Yucca*. Between one and seven samples per taxon were used, with the sample size roughly reflecting taxon range (Table 1). We used a protocol developed by M. Gitzendanner (pers. comm.) that was originally modified from the Plant Genome kit (Applied Biosystems, Foster City, CA). Restriction and ligation reactions were carried out in separate steps. Genomic DNA was digested for 3 h at 37 °C with 3 U of *Eco*RI (Promega, Madison, WI), 2.5 U of *Mse*I (New England Biolabs, Beverly, MA) in 10 μL reaction volumes containing sterile water, bovine serum albumin (BSA), and 10× enzyme buffers supplied by the manufacturers. Ligation reactions contained 1.5 U of T4 DNA ligase (Promega), 2 μL of 10× T4 Ligase buffer (Promega), 9 μM *Mse*I adapter (5′-GACGATGAGTCCTGAG-3′ and 5′-TACTCAGGACTCAT-3′), 0.9 μM *Eco*RI adapter (5′-CTCGTAGACTGCGTACC-3′ and 5′-AATTGGTACG CAGTCTAC-3′), and sterile water in 10 μL reaction volumes. The ligation reaction volumes were added directly to the restriction digests and incubated at 25 °C for 3 h.

The restriction/ligation reactions were diluted by a factor of 20 in 1× $TE_{0.1}$ (20 mM Tris–HCl, 0.1 mM EDTA, pH 8.0), and used in a subsequent round of selective amplification. The first selective amplification was conducted in 20 μL reaction volumes containing 4 μL of the diluted restriction–ligation reaction, 1 U *Taq* DNA polymerase (Promega), 10× PCR buffer (10 mM Tris–HCl, 50 mM KCl, pH 8.3), 3.5 mM $MgCl_2$, 0.8 mM dNTPs, 0.3 μM *Eco*RI +1 selective primer (5′-GACTGCGTACCAATT CA-3′), and 0.3 μM *Mse*I +1 selective primer (5′-GACGA TGAGTCCTGAGTAAC-3′). Reactions were heated to 72 °C for 2 min, then cycled 20× at 94 °C for 30 s, 56 °C for 30 s, 72 °C for 120 s, and then held at 60 °C for 30 min. These reactions were diluted by a factor of 14 in 1× $TE_{0.1}$ and used in the final selective amplification step.

The final amplification was performed in 10 μL reactions containing 2.5 μL dilute +1 PCR product, 0.5 U Amplitaq Gold DNA polymerase (Applied Biosystems), 1× Amplitaq PCR buffer (Applied Biosystems), 3 mM $MgCl_2$, 0.8 mM dNTPs, 0.05 μM of each fluorescently labeled *Eco*RI +3 primer (5′-[VIC]GACTGCGTACCAATTCAA C-3′; 5′-[6-FAM]GACTGCGTACCAATTCACA-3′; 5′-[NED] GACTGCGTACCAATTCAAG), and 0.125 μM of one of the following six *Mse*I +3 primers (5′-GACGATG AGTCCTGAGTAACAC-3′; 5′-GACGATGAGTCCTG AGTAACAG-3′. 5′-GACGATGAGTCCTGAGTAACA T-3′; 5′-GACGATGAGTCCTGAGTAACTA-3′; 5′-GA CGATGAGTCCTGAGTAACTG-3′; 5′-GACGATGAG TCCTGAGTAACTT-3′). The reactions were held at 94 °C for 2 min, then cycled 10× starting at 94 °C for 30 s, 65 °C for 30 s, 72 °C for 2 min, with a reduction in the annealing temperature by 1 °C per cycle. Reactions were then cycled 36 times at 94 °C for 30 s, 56 °C for 30 s, 72 °C for 120 s, followed by a 30 min 60 °C hold. For each individual, we performed six +3 selective amplifications using each of the *Mse*I +3 primers listed above. Because each primer was labeled with a fluorescent dye of different wavelength, single reactions contained three primer combinations (i.e., three *Eco*RI +3 primers with one *Mse*I +3 primer). One microliter of the resulting PCR volume was placed in a 10 μL aliquot of deionized formamide and GeneScan®-500 [ROX] size standard mixed according to the manufacturer's instructions (Applied BioSystems). Electrophoresis was conducted on an Applied Biosystems 3100 automated DNA sequencer using the dye set D, GeneScan 36–500 run parameters, and GeneScan 500 analysis parameters. Fragments were scored using the statistically rigorous, objective algorithm developed by Abdo et al. (2006). The resulting matrix used for the analysis in this paper will be deposited in TreeBASE (www.treebase.org).

2.1. Phylogenetic analyses

AFLP data are typically analyzed via parsimony or a distance-based method. We analyzed the *Yucca* AFLP dataset with distance-based methods because many of the AFLP markers were autapomorphies that tended to cause relatively long branches. This also meant that a very small percentage (in some cases, 0.3%) of the markers would determine the branching pattern and monophyly of taxa. Given the anonymous nature of the markers and the fact that relatively few markers would be responsible for determining the phylogeny, we converted the presence/absence of AFLP fragments into Nei–Li distance metric (Nei and Li, 1979) in PAUP 4.0b10 (Swofford, 2002) to provide a composite index of the signal from all AFLP fragments rather than relying on only a very small subset of markers

Table 1
Samples used in the analysis, including latitude and longitude data

Taxon	Sample site	Lat (N)	Long (W)	Pellmyr DNA accession	*Yucca* section, series[c]
Outgroup					
Hesperoyucca. whipplei (Torr.) Trel.	CA: San Diego Co. Elliott Chaparral Reserve	32.8683	117.1425	S113	
H. whipplei (Torr.) Trel.	CA: Temecula, Santa Margarita Reserve	33.4439	117.1767	S238	
Ingroup: all *Yucca*					
angustissima Engelm. ex Trel.	AZ: Peach Springs	35.561	113.4226	244	CH
angustissima var. *kanabensis* McKelvey	UT: N Coral Sands State Park	37.17932	112.63517	209	CH
arizonica McKelvey	MEX: Son. SON89, km182	30.88058	110.09153	180	S
baccata Torr.	AZ: N Wickenburg	34.2267	113.0699	133	S
baccata Torr.	AZ: Peach Springs	35.5708	113.4283	356	S
baccata Torr.	AZ: Walnut Canyon National Monument	35.1717	111.5097	213	S
baccata Torr.	NM: Las Cruces	32.3077	106.7083	382	S
baccata Torr.	UT: Blanding (Devil's Cyn. Campground)	37.7638	109.4028	203	S
baccata Torr.	UT: Zion Nat'l Park entrance, Kolob Cyn	37.4653	113.1875	208	S
baileyi Wooton and Standley	AZ: St. Johns	34.6666	109.65	202	CH
baileyi var. baileyi Wooton and Standley	AZ: Winona	35.2291	111.425	212	CH
baileyi var. *intermedia* (McKelvey)Reveal	NM: Correo	34.955	107.1841	221	CH
baileyi var. *intermedia* (McKelvey)Reveal	NM: 13.3 km N Cerillos	35.51103	106.06187	121	CH
brevifolia Engelm.	CA: Kingston Mountains	35.77027	115.83557	521	CL
brevifolia Engelm.	CA: Palmdale, Barrel Springs Rd	34.530	118.065	114	CL
brevifolia Engelm.	NV: Las Vegas. Potosi Canyon	36.0238	115.5407	401	CL
capensis Lenz	MEX: BCS. San Pedro de la Soledad	23.24597	109.97407	172	S
capensis Lenz	MEX: BCS. W San Antonio	23.8095	110.06827	169	S
carnerosana Trel.	MEX: Coah. S Saltillo (pass)	25.2442	100.8927	260	S
carnerosana Trel.	TX: Dagger Flat	29.5190	103.0466	146A	S
cernua Keith	TX: 6.4 km W Newton	30.8625	93.8223	400	CH:R
constricta Buckley	TX: Brady	31.0353	99.4227	147	CH
constricta Buckley	TX: E Menard	31.2475	97.1107	220	CH
decipiens Trel.	MEX: Dgo. WSW Durango	23.9865	104.747	182	S
decipiens Trel.	MEX: Dgo. Suchil to Michilia	23.5983	104.0026	268	S
elata Engelm.	AZ: Oak Creek	34.7185	111.7766	228	CH
elata Engelm.	AZ: Sierra Vista, San Pedro River on Rte 80	31.5474	110.1417	229	CH
elata Engelm.	AZ: Willcox Playa	32.2408	109.8181	242	CH
elata Engelm.	AZ: Roosevelt Dam. Bachelor's Cove Camp	33.6986	111.1986	200	CH
elata Engelm.	NM: 2.4 km S Rodeo	31.9508	108.6379	117	CH
elata Engelm.	TX: Hueco	31.8279	105.9419	234	CH
elata var. *utahensis* (McKelvey)Reveal	UT: St. George. Snow Canyon State Park	37.2166	113.6458	207	CH
elephantipes Regel	MEX: Chis. Rizo de Oro	15.9667	92.4833	272	S
elephantipes Regel	MEX: Hgo. 15 km N Yahualica	20.9245	98.5614	184	S
elephantipes Regel[a]	TX: Brownsville. Sabal Palm Grove Sanctuary	25.8484	97.4161	179	S
endlichiana Trel.	MEX: Coah. S Hipolito	25.7	101.4	654	S
filamentosa L.	FL: Archbold Biological Station	27.1882	81.337	279	CH
filamentosa L.	FL: Ocala National Forest, Big Scrub	29.1333	81.5166	278	CH
filifera Chab.	MEX: Hgo. San Vicente	19.9974	98.7005	142	S
filifera Chab.	MEX: Mich. [San Jose] Coapa	19.5549	101.3907	186	S
filifera Chab.	MEX: Mich. 4 km E Morelia	19.8916	101.1149	286	S
filifera Chab.	MEX: SLP. Poza de Santa Clara	23.2502	100.5474	141	S
filifera Chab.	MEX: Qto. Bucareli	21.0517	99.6083	283	S
glauca Nuttall	NE: Hershey	41.1586	101.0026	238	CH
glauca Nuttall	NM: Cuervo	35.03429	104.40029	288	CH
glauca Nuttall	TX: Brownwood	31.3804	99.1725	197	CH
glauca arkansana Trelease	TX: Jefferson	32.7900	94.3674	151	CH
glauca arkansana Trelease	TX: Sarita	27.2166	97.7833	178	CH
harrimaniae Trelease	UT: Wilson Arch	38.2791	109.375	204	CH
harrimaniae Trelease	UT: Interstate 70, exit 102	38.8166	111.1333	206	CH
jaliscensis Trelease[b]	MEX: Jal. Apango	19.7833	103.7	192	S
jaliscensis Trelease[b]	MEX: Jal. El Izote	19.6370	103.6488	297	S
jaliscensis Trelease[b]	MEX: Jal. Mazamitla	19.9188	103.0273	296	S
lacandonica Pompa and Valdez	MEX: Chis. 10 km NE Chiapa de Corzo, km30	16.7521	92.9597	300	S
lacandonica Pompa and Valdez	MEX: Chis. 20 km NNW Ocozocozutla	16.9280	93.4515	189	S
linearifolia Clary	MEX: Coah. Parras	25.4054	102.0500	302	S
linearifolia Clary	MEX: NL. N Galeana	24.9229	100.0678	143	S

(*continued on next page*)

Table 1 (*continued*)

Taxon	Sample site	Lat (N)	Long (W)	Pellmyr DNA accession	*Yucca* section, series[c]
louisianensis Trelease	MS: Mt Olive	31.355	90.9352	193	CH
louisianensis Trelease	TX: Silsbee, Larsen Preserve	30.3893	94.2569	155	CH
mixtecana García-Mendoza	MEX: Oax. Rt125, 7.5 km S Santiago Chazumba	18.1200	97.6816	306	S
mixtecana García-Mendoza	MEX: Pue. S Acantepec	18.2092	97.6343	190	S
pallida McKelvey	TX: Comanche	31.8875	98.6458	156	CH:R
pallida McKelvey	TX: S Dublin	32.0364	98.4005	158	CH:R
periculosa Baker	MEX: Pue. 6 km N Azumbilla	18.683	97.3508	191	S
periculosa Baker	MEX: Pue. S Tehuacan	18.4073	97.4378	310	S
periculosa Baker	MEX: Pue. Zacatepec	19.3663	97.4345	145	S
queretaroensis Piña Lujan	MEX: Qto. Bucareli	21.0517	99.6083	146B	?
reverchonii Trelease	TX: W Sonora, Interstate 10 exit 399	30.5980	100.6697	159	CH:R
rigida (Engelm.) Trelease	MEX: Dgo. Km184 S Torreon	25.1799	103.7166	112	CH:R
rostrata Engelm. ex Trelease	TX: Black Gap Wildlife Management Area	29.55	102.1166	316	CH:R
rostrata Engelm. ex Trelease	TX: E Fort Stockton	30.9578	102.5803	163	CH:R
rupicola Scheele	TX: Johnson City	30.25	98.5166	198	CH:R
rupicola Scheele	TX: Kyle	29.9946	97.8869	165	CH:R
schidigera Roezl ex Ortgies	MEX: BC. N Cataviña	29.8650	114.8427	320	S
schottii Engelm	AZ: Catalina Mountains. Mt Lemmon Rd	32.3583	110.7181	240	S
schottii Engelm	AZ: Santa Ritas Mountains, Florida Canyon	31.7634	110.8459	418	S
schottii Engelm	AZ: Chiricahua National Monument	32.0049	109.3665	241	S
treculeana Carriere	MEX: Dgo. Km156 N Cuencamé	24.9645	103.7275	113	S
treculeana Carriere	NM: Las Cruces	32.3077	106.7083	379	S
treculeana Carriere	TX: Big Bend National Park. Dagger Flat	29.5190	103.0466	167	S
treculeana Carriere	TX: E Fort Stockton	30.9578	102.5803	325	S
treculeana Carriere	TX: Laguna Atascosa National Wildlife Refuge	26.25	97.35	326	S
valida Brandegee.	MEX: BCS. km44 N La Paz	24.0555	110.5747	174	S
valida Brandegee	MEX: BCS. Rte 1, km131 S Cd Constitucion	24.4772	111.2537	175	S

For sample sites, Mexican collections are preceded by MEX, and state abbreviation following Guia Roji (1995). For U.S. collections, state and location given. All ingroup taxa are in the genus *Yucca*.

[a] In relictual tract of native vegetation, with no apparent cultivated source.

[b] Cultivated specimen in village.

[c] S, section Sarcocarpa; CL, section Clistocarpa; CH, section Chaenocarpa; CH:R, Chaenocarpa series Rupicolae.

to estimate the phylogeny. A heuristic search using the minimum evolution criterion and TBR branch swapping was performed in PAUP 4.0b10. Support for the resulting topology was assessed via 10,000 non-parametric bootstrap replicates (Felsenstein, 1985). As a means to provide additional information about support for the resulting topology, we also performed a Bayesian analysis using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) to obtain the posterior probabilities for the resulting clades. We used the restriction site (binary) model, the default mcmc run parameters, and 1,000,000 generations. Convergence was assessed by examining stationarity in the log-likelihood scores and the average standard deviation of split frequencies.

3. Results

We scored a total of 4322 AFLP markers, 4285 (99.1%) of which were variable across taxa. Each individual had an average of 1265 markers (SE ± 7.02, range 1074–1439). The minimum-evolution heuristic search based on the Nei–Li distance measure returned a tree with a tree score of 39349.76 (Fig. 1). The non-parametric bootstrap and Bayesian analyses showed robust support for the three major sections traditionally recognized within the genus (Engelmann, 1873; Trelease, 1902; McKelvey, 1938, 1947; Webber, 1953): Sarcocarpa, which is characterized by the synapomorphy of a fleshy, indehiscent fruit [95% bootstrap, 100% posterior probability (PP)], the monotypic Clistocarpa that is defined by a spongy, indehiscent fruit [100% bootstrap and PP], and Chaenocarpa [78% bootstrap, 100% PP], which retains the ancestral state in Agavaceae of a dry, dehiscent fruit (Bogler and Simpson, 1996). Furthermore, within Chaenocarpa, the morphologically recognized Rupicolae series (McKelvey, 1938, 1947) was recovered with strong support. At the species level there was a marked decrease in resolution, and several taxa with more than one sample were not monophyletic. Bootstrap support within the major clades was generally low with a few notable exceptions. Below, we present a more detailed description of these relationships relative to geography and morphology.

4. Discussion

To date, the phylogenetic relationships of even the major sections of *Yucca* have remained elusive. The present study used a very large AFLP data set, with about an order of magnitude or more markers per sample compared to recent studies, in an attempt to resolve this taxonomically difficult group. An appealing aspect of the resulting phylogeny is

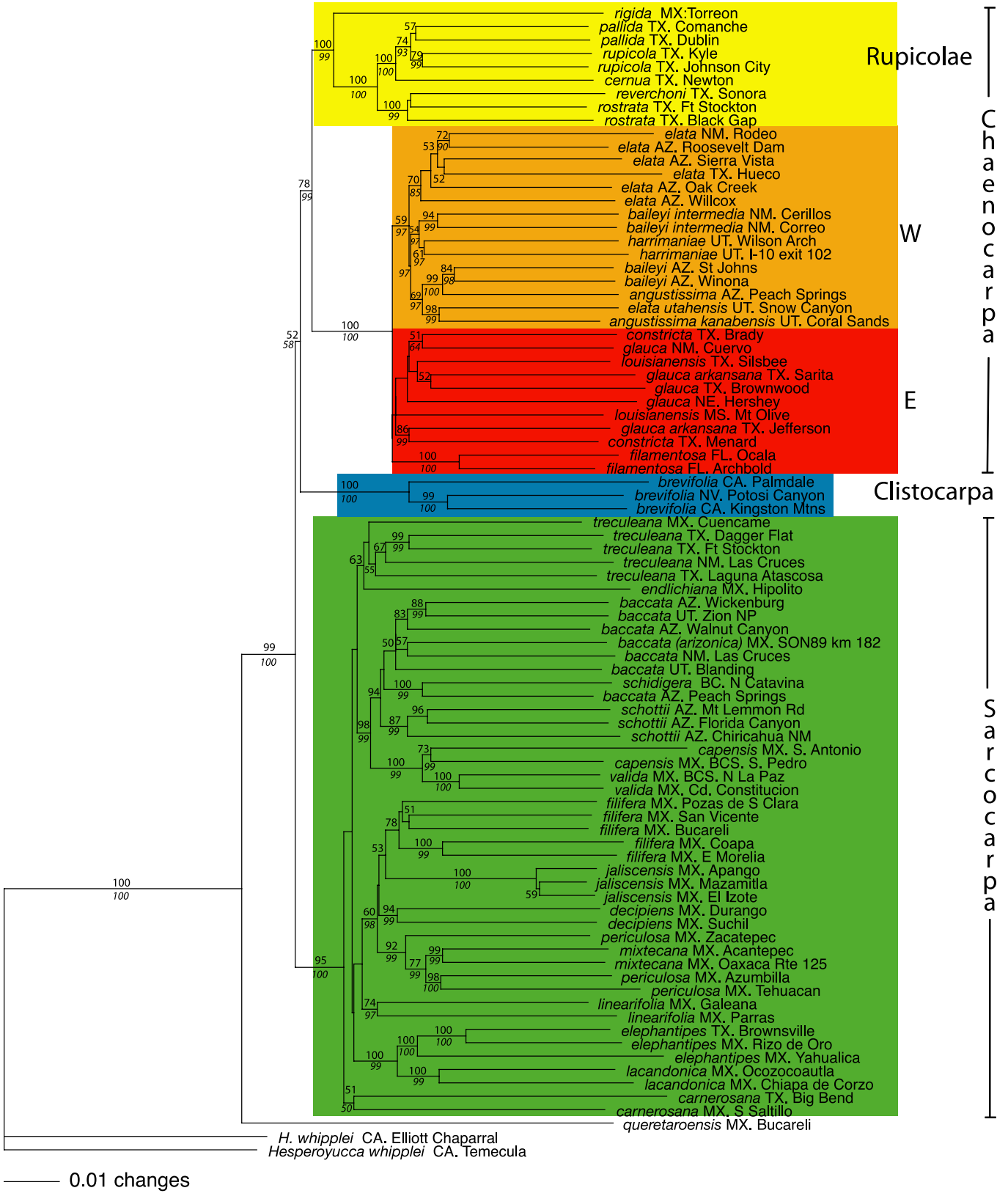

Fig. 1. AFLP-based phylogeny for 38 *Yucca* taxa, based on a minimum-evolution heuristic search using Nei–Li distances with the monobasic *Hesperoyucca whipplei* being used as outgroup. Numbers above the branch indicate proportion of 10,000 non-parametric bootstrap replicates. Numbers below the branch indicate the posterior probability of that node. Labels correspond to the host names used in the yucca moth revision by Pellmyr (1999). Abbreviated locality data are given as part of label, with full data provided in Table 1. Shading identifies the three sections Sarcocarpa, Clistocarpa, and Chaenocarpa, and boxes within Chaenocarpa identify the Rupicolae as well as the western (W) and eastern (E) clusters of remaining capsular-fruited species.

that the deeper nodes are consistent with morphologically based groups recognized in five classic comprehensive studies (Engelmann, 1873; Trelease, 1902; McKelvey, 1938, 1947; Webber, 1953; although Trelease treated one species as a separate genus). Specifically, the capsular-fruited Chaenocarpa, fleshy-fruited Sarcocarpa, and the spongy-fruited Clistocarpa were reciprocally monophyletic; Clistocarpa appeared as sister to the Chaenocarpa, but support was modest [55%], suggesting rapid diversification of the three sections and uncertain relationships among them. Furthermore, within Chaenocarpa, the morphologically well-defined Rupicolae was also strongly supported [100%]. The utility of AFLP as a tool to recover phylogenies, especially at lower phylogenetic levels, where uniparentally inherited or single nuclear markers are more prone not to reflect organismal history, has been amply documented in species-rich clusters (Hodkinson et al., 2000; Beardsley et al., 2003; Goldman et al., 2004; Sullivan et al., 2004; Furini and Wunder, 2004; Gottlieb et al., 2005; Mendelson and Shaw, 2005; Spooner et al., 2005) and clades with diffuse species boundaries (Marhold et al., 2004; Koopman, 2005; Kauseryd et al., 2006). The concordance of AFLP and classical morphology-based higher taxa stands in contrast to a previously attempted phylogenetic analysis of *Yucca*; in a parsimony-based phylogeny based on 55 variable morphological characters, the series Rupicolae was recovered, but none of the three sections were monophyletic (Clary, 1997). This analysis showed <50% bootstrap support for all except three taxon pairs, and likely reflects the more limited number of characters common to morphological data matrices. Clary (1997) also compiled a 400-bp nucleotide data set from the nuclear ribosomal ITS region for comparative phylogenetic purposes, but subsequent analyses have shown this data set to include paralogous copies (Clary, pers. comm., J. Leebens-Mack, unpubl. data), so these results must be set aside.

An unexpected result in the current analysis was very strong support for a basal position of *Y. queretaroensis* at a position below the three sections. The fruit type of this poorly known species from the Sierra Gorda region of east-central Mexico (Piña Lujan, 1989) is not known; visual examination of a photograph of a collection (Piña Lujan, 1990; p. 62) is inconclusive. Whereas leaf and floral morphology is consistent with this being a true *Yucca* species, sampling of the genus *Hesperaloe*, which together with *Hesperoyucca* constitute the sistergroup of *Yucca* (Bogler et al., 1995; Bogler and Simpson, 1996) or a basal clade (Bogler et al., 2005), will be needed to determine whether the species actually belongs in a lineage other than *Yucca*.

In Sarcocarpa, which dominates the southern part of the composite genus range (Fig. 2), the morphologically distinctive *Y. carnerosana* constituted a separate lineage, and the two southernmost taxa of the genus, *Y. elephantipes* and *Y. lacandonica*, diverged next. All remaining fleshy-fruited taxa comprised a clade. One subclade of seven arborescent Mexican taxa had *Y. linearifolia* as the basal taxon. The widespread *Y. filifera*, with a characteristic pendant inflorescence, showed two clades reflecting northeastern and southern population clusters, respectively. Three samples of *Y. jaliscensis* were the sister group of *Y. filifera*. These *Y. jaliscensis* samples were obtained from long-established cultivated plants in three villages, where they are vegetatively propagated and used for their fibers (oral comm., local resident of El Izote, Jal.) and the plant populations lacked any of the entomofauna regularly associated with yuccas within their native ranges, suggesting that they had been transplanted outside of the native range and habitat. This was confirmed by a local resident at one sample site, El Izote. Herbarium sheets of plants collected in moist native forest near Talpa de Allende, ~120 km WNW of the present sample sites (McVaugh 21435, 14352; MICH) include fruits with feeding damage consistent with pollinator larvae (OP, unpubl. data). The allopatric *Y. decipiens* of northwest Mexico, which differs from *Y. filifera* chiefly in having an erect rather than pendant inflorescence, was sister to these taxa. *Yucca mixtecana* was recently extracted as a separate taxon from within *Y. periculosa* (García-Mendoza, 1998), but the nested position of the two samples within *Y. periculosa* does not support the distinctiveness of this taxon.

Also within Sarcocarpa, we found a two-taxon clade that contained the endemic yuccas of the Baja California peninsula, *Y. valida* and *Y. capensis*. *Yucca schottii* of the northeast Madrean woodlands, which diverged next, shares biological features with *Y. capensis* in that they are montane woodland taxa, flower relatively late in the season, and are host to species of *Parategeticula*, the other clade of pollinating yucca moths (Powell, 1984; Pellmyr and Balcázar-Lara, 2000; Pellmyr and Balcazar-Lara, in prep.). The extensively sampled northernmost species of Sarcocarpa, *Y. baccata*, incorporated a single sample of the dubiously distinct *Y. arizonica*, and, interestingly, also the single available sample of *Y. schidigera*. Lastly, the widespread *Y. treculeana* of the Chihuahuan desert and Tamaulipan grasslands was inferred to be the sister taxon of *Y. endlichiana*, a highly derived species with small maroon-colored flowers growing as clonal circles on the desert floor in a small segment of the north-central Chihuahuan desert.

In the capsular-fruited Chaenocarpa, the morphologically well-defined series Rupicolae was strongly supported as sister group of all other chaenocarps (Fig. 1). The six named taxa are internally allopatric in the Chihuahuan desert, and on and to the east of the Edwards Plateau of Texas (Fig. 2), and differ primarily in vegetative structures and growth form. Pellmyr and Balcázar-Lara (2000) noted that one of the few proposed differences between *Y. rigida* and *Y. rostrata*—namely presence/absence of medial constriction of the fruit—is an artifact of oviposition by different pollinators and, thus, may raise doubt on species status. The basal position of the *Y. rigida* sample does not support this argument, indicating that they are likely separate entities. The relationships among the five other taxa reflect geographical proximity. *Yucca pallida* and *Y. rupicola*, which differ primarily in leaf morphology, have abutting ranges on the eastern edge of the Edwards Plateau, and their sister

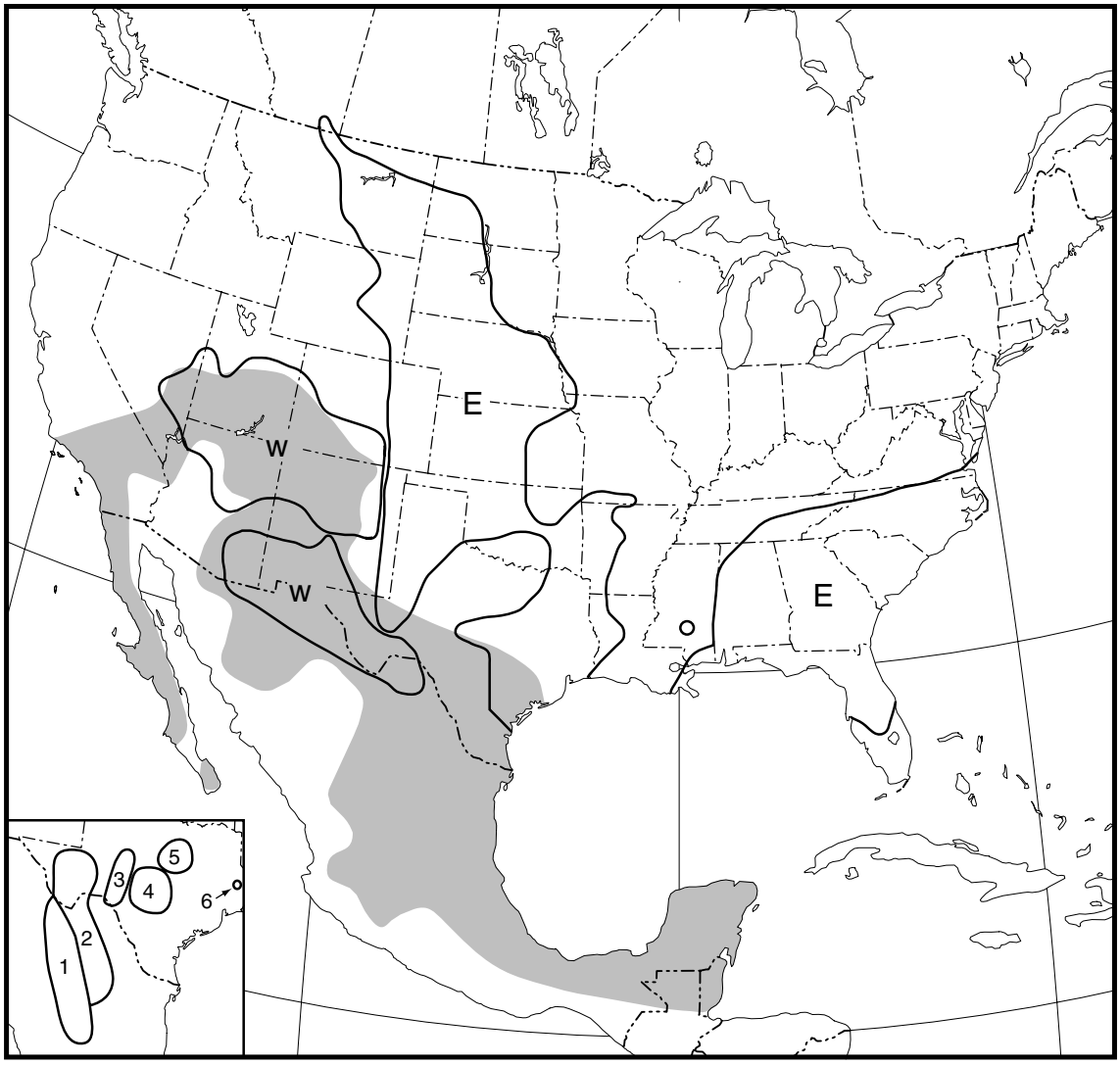

Fig. 2. Schematic map showing the composite range outline of Sarcocarpa (shaded gray), and the composite ranges of the eastern (E) and western (W) clades of Chaenocarpa except Rupicolae. Lower right inset map of NE Mexico and Texas shows ranges of species in the Rupicolae: (1) *rigida*, (2) *rostrata*, (3) *reverchoni*, (4) *rupicola*, (5) *pallida*, (6) *cernua*. Data compiled from confirmed collection records in McKelvey (1938, 1947), Webber (1953), Great Plains Flora Association (1977), Matuda and Piña Lujan (1980), Piña Lujan (1990), Clary (1995), Welsh et al. (1993), García-Mendoza (1998), herbarium records from University of Michigan (MICH), Universidad Autonóma de México National Herbarium (MEXU), and the Missouri Botanical Garden (MO), and unpublished data from field work across the range of the genus, and especially in Mexico by OP and MBL.

taxon *Y. cernua* is confined to easternmost Texas. Meanwhile, *Y. rostrata* of the northern Chihuahuan desert approaches *Y. reverchonii*, with both being geographically separate from the *pallida–rupicola–cernua* triad.

Among the remaining Chaenocarpa, all are effectively confined to the USA except for a modest southward extension of *Y. elata* into Mexico and a <10-km extension of *Y. glauca* into Alberta, Canada. The chaenocarps have created the most discord among systematists in species delineation (e.g., Trelease, 1902; McKelvey, 1938, 1947; Webber, 1953, 1960; Reveal, 1976; Welsh et al., 1993; Hess and Robbins, 2002), and the AFLP-based phylogeny reflects this in part. One clade (marked W in Fig. 1) contains all taxa of the Colorado Plateau and areas of lower elevation to the south of the Plateau. Five taxa were monophyletic, but *Y. angustissima kanabensis* and *Y. elata utahensis* were strongly supported as sister taxa, and did not group with either proposed major taxon. The latter variety has been considered most closely related to *Y. a. kanabensis* on morphological grounds (Welsh et al., 1993), and this opinion better conforms to the genetic data presented here. The more eastern sister clade (marked E in Fig. 1) contained a monophyletic *Y. filamentosa*, isolated on the Florida peninsula and adjacent coastal areas from all other taxa. For a set of four other, morphologically poorly distinguished taxa of the Great Plains, Texas east of the Chihuahuan desert, and coastal pine barrens of Louisiana and Mississippi, there was no evidence of reciprocal monophyly. These taxa differ primarily in degree of leaf rigidity and degree of clonal growth form, and are not readily demarcated.

One of the prominent features of the phylogeny was that many species (particularly in Chaenocarpa) with more than one sample were not monophyletic, a circumstance that can reflect incomplete lineage sorting due to rapid diversification, non-monophyletically delineated species, or introgression. We hypothesize that all three may be applicable in different instances. For example, the relatively short internal branch lengths for the major clades suggests that the three sections likely evolved in quick succession during a rapid series of speciation events. The results also lend little support for the monophyly of many of the named taxa in Chaenocarpa, and may indicate that fewer phylogenetic species are involved. Introgression could in principle also explain some of these patterns, but is not required. Furthermore, the hypothesis could be entertained that the placement of the *Y. baccata* sample from Peach Springs as strongly supported sister of *Y. schidigera* reflects introgression. Whereas speculation about naturally occurring introgression in yuccas has been widespread, if not rampant (McKelvey, 1938, 1947; Webber, 1953, 1960; Lenz and Hanson, 2000a,b), the only case for which supporting genetic data exists is that of *Y. baccata* and *Y. schidigera*. Hanson (1993) documented an extended zone across northwestern Arizona of unidirectional introgression of *Y. schidigera* into *Y. baccata*, and documented extensive introgression into phenotypic *Y. baccata* at a site only ~50 km away from Peach Springs. This hypothesis can readily be tested once additional *Y. schidigera* samples become available. The resolution of these issues will require more extensive sampling across ranges and genetic analyses of the involved taxa.

5. Conclusion

The establishment of a robust phylogeny for the yuccas based on AFLP data provides more than the platform for studies of plant-pollinator diversification. The results also direct attention to two geographically confined clusters of capsular-fruited taxa where the current classification may not reflect evolutionary history or, alternatively, are suggestive of recent diversification. A detailed understanding of the evolution of these species will require further sampling and analysis. Finally, the congruence of the AFLP phylogeny with previous accounts of the morphology and geographic distribution of these species attests to the utility of these markers in phylogenetic studies of rapidly evolving species groups.

Acknowledgments

We thank Karen Clary, Eric Keith, Abisai García-Mendoza, Carlos Beutelspacher, and Rogers McVaugh for site information, Paul Wilson for *Y. brevifolia* tissue, and Huntington Botanical Garden for a *Y. endlichiana* sample. Big Bend National Park, Black Gap Wildlife Management Area, Laguna Atascosa National Wildlife Refuge, Ocala National Forest, Torrey Pines State Park, the Nature Conservancy at Larsen Sandyland Sanctuary, Mojave National Preserve, Sabal Palm Grove Sanctuary, Snow Canyon State Park, Walnut Canyon National Monument, the Santa Margarita and Elliott Chaparral Reserves of the University of California Nature Reserves system, and the Coronado National Forest of Arizona gave permission to gather plant tissue. Matt Gitzendanner provided helpful information in setting up the AFLP protocol. Tony Reznicek and the staff at the University of Michigan herbarium (MICH) kindly permitted examination of their holdings of *Y. jaliscensis*. The curators of the herbaria at Universidad Nacional Autónoma de México and at Missouri Botanical Garden also provided access to all collections. We thank an anonymous reviewer for helpful suggestions that improved the manuscript.

Field work was funded by the National Geographic Society, and field and lab work by Grants DEB 0242783 and DEB 0321293 from the National Science Foundation. Computing facilities were supported by NIH Grants P20RR016454 and P20RR016448 from the INBRE and COBRE Programs of the National Center for Research Resources.

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