UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

Instituto de Biología

RADIACIONES EVOLUTIVAS DE LINAJES DE PLANTAS SUCULENTAS EN EL ORDEN CARYOPHYLLALES, CON ÉNFASIS EN LA FAMILIA CACTACEAE

TESIS

QUE PARA OBTENER EL GRADO ACADÉMICO DE
DOCTORA EN CIENCIAS

PRESENTA

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MÉXICO, D.F. **NOVIEMBRE, 2010**

UNAM POSGRADO
Ciencias Biológicas

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Presente

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día 23 de Agosto de 2010, se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la alumna **HERNÁNDEZ HERNÁNDEZ TANIA** con número de cuenta **95318417** con la tesis titulada: **"Radiaciones evolutivas de linajes de plantas suculentas en el orden Caryophyllales, con énfasis en la familia Cactaceae",** realizada bajo la dirección de la **DRA. SUSANA AURORA MAGALLÓN PUEBLA:**

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Sin otro particular, me es grato enviarle un cordial saludo.

Atentamente
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, D.F. a 24 de Noviembre de 2010.

Dr. Juan Núñez Farfán
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c.c.p. Expediente del (la) interesado (a).

AGRADECIMIENTOS

Quiero agradecer, en primer lugar, al Posgrado en Ciencias Biológicas de la Universidad Nacional Autónoma de México por todo el apoyo brindado para la realización de este trabajo y de mis estudios de doctorado, durante los cuales, conté con una beca del Consejo Nacional de Ciencia y Tecnología (CONACYT).

Parte de este trabajo se financió con fondos del proyecto de investigación CONACYT-2004-C01-46475 y del proyecto UNAM, PAPIIT IN202310.

Agradezco a mi tutora, la Dra. Susana Magallón Puebla, y a los miembros de mi Comité Tutoral, el Dr. Héctor Hernández Macías y el Dr. Luis E. Eguiarte Fruns por todo su apoyo y orientación.

El trabajo de cómputo fue realizado en el Instituto de Biotecnología de la UNAM (Macroproyecto de Tecnologías de la Información y la Computación) y en el Centro Nacional de Supercómputo, IPYCYT (San Luis Potosí, México).

AGRADECIMIENTOS PERSONALES

Agradezco profundamente a todas las personas que me apoyaron a todo lo largo de este proceso:

De manera muy especial quiero agradecer a mi tutora, la Dra. Susana Magallón, por toda su confianza, apoyo y orientación. También de manera especial al Dr. Luis E. Eguiarte y al Dr. Héctor Hernández, que fungieron como miembros de mi Comité Tutoral y aportaron valiosos comentarios que enriquecieron este trabajo.

Agradezco al Dr. Daniel Piñero, miembro de mi jurado de examen de candidatura, por llevarme a iniciar la discusión sobre radiaciones adaptativas. El Dr. Piñero y el Dr. Luis E. Eguiarte revisaron una versión temprana del Capítulo I. El Dr. Sergio Cevallos, del Instituto de Geología de la UNAM, fungió también como miembro de mi jurado de candidatura y junto con Emilio Estrada, contribuyeron con importantes datos, comentarios y orientación al enriquecimiento de mi proyecto.

El trabajo de campo para la recolección de cactáceas mexicanas se llevó a cabo con el apoyo de Rosalinda Medina (Instituto de Biología, UNAM), del Dr. Sergio Cevallos y del Laboratorio de Evolución Molecular y Experimental del Instituto de Ecología a cargo del Dr. Luis E. Eguiarte (Instituto de Ecología, UNAM). El Dr. Mark Olson, Rodrigo González, Emilio Estrada, Carlos Castañeda y José Antonio Barba ayudaron en diferentes aspectos de dichas colectas. El Dr. Héctor Hernández (Instituto de Biología, UNAM) y Jerónimo Reyes (Jardín Botánico de la UNAM) proporcionaron material de sus colecciones para este estudio.

La asesoría y orientación de Carlos Gómez Hinostrosa fue invaluable para la adecuada colecta y preparación de los ejemplares de cactáceas. Además, Carlos ayudó a la identificación de la mayoría de los ejemplares colectados y siempre se ha mostrado dispuesto a ayudarme. Para el trabajo de laboratorio recibí el apoyo y orientación de Laura Márquez, Rosalinda Medina, Juan

Carlos Montero y Arturo De Nova. En cuanto al trabajo de cómputo, recibí la valiosa ayuda de Jerome Verleyen del IBT, UNAM para la resolución de problemas técnicos.

Agradezco el gran apoyo del Dr. Raúl Puente, del Jardín Botánico del Desierto en Phoenix, Arizona, quien me permitió hacer una extensa colecta de material sudamericano en dicha institución. También agradezco al Dr. Roberto Kiesling por recibirme y ayudarme a realizar colectas en Argentina. El Dr. Kiesling revisó además la identificación del material sudamericano y me proporcionó información importante para la culminación de la tesis. El Dr. Roberto Kiesling y el Dr. Arias hicieron revisiones valiosas al manuscrito que reporta la filogenia de Cactaceae. Agradezco también los comentarios y discusiones con Violeta Patiño y Monserrat Vázquez que me permitieron culminar dicho trabajo.

Gracias al Dr. Enrique Martínez Meyer por su apoyo y orientación en la realización de parte de mi tesis, y por ayudarme a realizar una estancia en Sudáfrica. El Dr. Matt Buys y el Dr. Guy F. Midgley del South African National Laboratory Institute me recibieron, llevaron a campo y generosamente aportaron valiosos datos e información.

Siempre recibí el apoyo amable del personal del Posgrado en Ciencias Biológicas. En especial me gustaría agradecer al coordinador, el Dr. Juan Núñez, y a Lilia Jiménez, Lilia Espinosa y al Dr. Armando Rodríguez. Gracias también a la Dra. Maria de los Ángeles Herrera, Coordinadora del Posgrado en el IB, UNAM, por todo su apoyo.

Finalmente pero no de manera menos importante, quiero agradecer a mis padres, Isaías Hernández y Beatriz Hernández por apoyarme siempre, sobre todo en los momentos más difíciles. A Edgar Monreal, mi compañero en la vida por estar, y muchas veces padecer, conmigo todo este proceso. A nuestro leal amigo Cufa. A los amigos, invaluable compañía y muchas veces, paño de lágrimas: Andrea González, Rodrigo Macip, Violeta Patiño, Arturo De Nova, Norberto Martínez, Emilio Estrada y Oris Rodríguez. Gracias a ustedes por las comidas, fiestas, risas, pero sobre todo, por brindarme su amistad y por estar conmigo durante este proceso. Gracias a todos los investigadores, tutores y profesores que además de su orientación y asesoría, me brindaron también su amistad.

A EDGAR,

A NUESTRO HIJO QUE VIENE,

A TODOS LOS QUE ME HAN MOSTRADO SU

APOYO, SU CARIÑO Y SU

AMISTAD SINCERA

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PREFACIO

En sus inicios, el proyecto de investigación con el que ingresé al programa de Doctorado en Ciencias Biológicas era limitado, pues implicaba estrictamente la construcción de una filogenia para la familia Cactaceae, enfocando los esfuerzos de muestreo en los linajes mexicanos, para posteriormente estimar las fechas de origen de los principales clados y sus tasas de diversificación. Dicho proyecto estaba enmarcado en un proyecto de investigación más grande y ambicioso, orquestado por mí tutora -la Dra. Susana Magallón- y sus colaboradores, y que buscaba el estudio de las radiaciones vegetales más importantes de México, que posiblemente determinaron los ecosistemas en el País. En este contexto y durante mi estancia en el laboratorio de la Dra. Magallón, tuve la oportunidad de reflexionar mucho sobre las llamadas radiaciones adaptativas y sus fundamentos ecológicos y evolutivos. Además, para la obtención de fechas de origen para la familia Cactaceae y sus principales linajes, fue necesario construir una filogenia para el Orden al que Cactaceae pertenece, las Caryophyllales, dado que la fecha de calibración más adecuada está dada por información proveniente de una infrutescencia fósil de la familia Phytolacaceae que fue encontrada recientemente en Coahuila, México. Fue muy interesante notar que en linajes cercanos a la familia Cactaceae ocurrieron radiaciones similares, representadas por linajes que presentan diferentes tipos de suculencia en zonas áridas de África.

Fue por todo lo anterior que la Dra. Magallón y yo nos dimos cuenta de la necesidad de ampliar mi trabajo de investigación, de manera que la tesis permitiera incluir y reportar todo el trabajo y resultados obtenidos durante mi doctorado, que van más allá de los estudios de la familia Cactaceae. El resultado es la presente tesis, que está estructurada en tres Capítulos, el último de los cuales incluye dos secciones. En congruencia con los requerimientos del Posgrado en Ciencias Biológicas de la UNAM, los capítulos están escritos en formato de artículos científicos, los cuales serán sometidos a dictamen para su eventual publicación en revistas internacionales, y por ello se reportan en inglés; sin embargo, antes de cada capítulo se incluye una breve introducción en español.

El Capítulo I es una revisión sobre el concepto de *radiación adaptativa*, y constituye un importante antecedente teórico para los capítulos subsecuentes que estudian radiaciones particulares en Caryophyllales y en Cactaceae. Además de fundamentar una crítica a la ambigüedad con la que el término se usa en la actualidad, en el Capítulo I se propone el uso de un criterio objetivo para detectar radiaciones, con base en filogenias, fechas de origen de linajes y tasas de diversificación.

Con las bases teóricas y las propuestas metodológicas establecidas en el Capítulo I, el Capítulo II se enfoca en el estudio de las radiaciones de linajes de plantas suculentas al interior de las familias Aizoaceae, Didiereaceae y Cactaceae en el Orden Caryophyllales, como una posible aproximación al estudio del origen de vegetación de zonas áridas. El Capítulo III consta de dos secciones, que conforman un estudio evolutivo más completo de la familia Cactaceae y las radiaciones evolutivas que ocurrieron al interior de ésta. La primera sección de éste último Capítulo ("Marco filogenético y tendencias evolutivas generales") esta conformada por un artículo que ha sido aceptado para su publicación en el *American Journal of Botany*, y que cumple como requisito para la obtención del grado de acuerdo al Posgrado en Ciencias Biológicas. Éste artículo provee de un marco filogenético general para la familia construido con datos moleculares, y en él se discuten tendencias evolutivas generales. Finalmente, la segunda sección consiste en el reporte de fechas de origen y tasas de diversificación de principales linajes en Cactaceae, concentrándose en los linajes de distribución Norteamericana. Es importante mencionar que la amplitud del trabajo aquí presentado y la limitante del tiempo para continuar con las siguientes etapas de mi carrera, me obligaron a dar punto final a ésta Tesis; viéndome en la necesidad de presentar estos trabajos con resultados preliminares, en particular para el Capítulo II y la segunda sección del Capítulo III. El trabajo faltante se discute en la Discusión general, y actualmente me encuentro en proceso de su culminación con la finalidad de someter los trabajos en un futuro a su publicación.

Tania Hernández Hernández,
Noviembre, 2010.

RESUMEN

La evolución explosiva, o el origen geológicamente súbito y la subsecuente estabilidad de morfoespecies paleontológicas, es uno de los patrones más viejos y más robustos observados en el registro fósil. En los linajes vivos, este patrón puede encontrarse en aquellos taxa que incluyen una extraordinaria diversidad en comparación con otros linajes relacionados. Estos patrones han sido explicados usando la teoría Darwiniana de evolución por selección natural y definidos como *radiaciones adaptativas.* Este término se ha usado de manera laxa para designar linajes muy diferentes, con base en diferentes criterios y observaciones empíricas, lo cual ha generado una confusión en su definición. En este trabajo se hace una revisión completa de los orígenes y desarrollo del término, y se sugiere que las discrepancias surgen de un mal entendimiento de la relación que existe entre las tasas de diversificación taxonómica y morfológica. Sugerimos que las radiaciones pueden distinguirse con la detección estadística de incrementos significativos en las tasas de diversificación taxonómica de linajes monofiléticos.

Siguiendo dichos criterios, se estudian las radiaciones de linajes de plantas suculentas que ocurrieron al interior del orden Caryophyllales y más detalladamente, al interior de la familia Cactaceae. La estrecha relación que existe entre los fenotipos de las suculentas de zonas áridas y el clima en el que habitan hace que estos linajes puedan servir como un indicador del posible origen y evolución de vegetación de zonas áridas. Los resultados presentados muestran que las familias Aizoaceae y Cactaceae son relativamente recientes, y los grupos más ricos en especies al interior de ellas tienen las tasas de diversificación taxonómica más elevadas en comparación con linajes cercanos. Es posible que estos linajes se hayan originado en respuesta a los cambios climáticos que provocaron la aridificación de las regiones en que habitan.

Al interior de Cactaceae, sin embargo, los grupos con tasas de diversificación taxonómica más elevadas son las Pachycereeae centrales, las Hylocereeae y las Trichocereeae. Los miembros de estos grupos poseen flores con antésis nocturna. Además, la forma de vida columnar apareció de manera importante en las Pachycereeae y Trichocereeae. Es posible que la radiación de estos linajes haya ocurrido en respuesta a tipos de polinización particulares.

ABSTRACT

Explosive evolution, or the geologically instantaneous origination and subsequent stability of paleontological morphospecies, is one of the oldest and most robust of the paleontological observations. This pattern can also be found in living lineages, when certain taxa include an extraordinary diversity in comparison with related lineages, and has been explained using the Darwinian theory of evolution by natural selection, and defined as *adaptive radiations.* The term has been used in a lax way to designate a large sort of very different lineages, based on distinct criteria and empirical observations, which has generated confusion on its definition. In this study, we review the origins and development of the term, suggesting that discrepancies emerge because of a misunderstanding of the relationship between taxonomical and morphological diversification rates. We suggest that radiations can be distinguished with the statistical detection of significant increases in taxonomical diversification rates of monophyletic lineages.

Following the mentioned criteria, we study the radiation of succulent lineages occurring within the order Caryophyllales, and with more detail, within Cactaceae. The close relationship between the phenotypes of succulents from arid zones and the climate under they inhabit make these lineages a useful indicator of the possible origin and evolution of the vegetation in arid zones. The results presented show that the families Aizoaceae and Cactaceae are relatively recent, and the groups richest in species number within each possess higher taxonomical diversification rates in comparison to related lineages. It is possible that these lineages originated in response to the climatic changes producing the aridification of their distribution areas.

However, within Cactaceae, the groups showing the higher taxonomical diversification rates are the core Pachycereeae, the Hylocereeae and the Trichocereeae. Members of these groups possess flowers with a nocturnal anthesis. In addition, the columnar growth form appeared importantly within the Pachycereeae and the Trichocereeae. It is possible that the radiation of these lineages occurred in response to particular pollination types.

INTRODUCCIÓN GENERAL

La diversidad biológica del planeta es heterogénea en todos sus sentidos. Los linajes se distribuyen de manera heterogénea, y diferentes regiones del planeta tienen diferente riqueza de especies. Existe heterogeneidad en cuanto a la diversidad morfológica de los organismos, que se observa por ejemplo, cuando algunos grupos taxonómicos incluyen un gran número de especies muy parecidas entre ellas, mientras que existen especies que por sus características tan peculiares, son exclusivas de sus propios grupos taxonómicos. La diversidad también se caracteriza por una heterogeneidad temporal, y este fenómeno ha sido objeto de estudio de biólogos evolutivos y paleontólogos por mucho tiempo. Los linajes existentes han aparecido en diferentes momentos de la historia geológica, pero no lo han hecho de manera constante. El patrón más viejo y más robusto que se ha encontrado en el registro fósil es la aparición súbita de elevada diversidad (tanto en número de taxa como en diversidad morfológica) en muchos linajes (Eldredge y Gould 1972; Gould y Eldredge, 1977). El patrón equivalente de éste fenómeno en los linajes vivos ocurre en aquellos que presentan también una extraordinaria diversidad. Los paleontólogos interpretaron estos patrones como evolución explosiva (Zeuner, 1958), que posteriormente fueron explicados usando la teoría Darwiniana de la evolución por selección natural (ver por ejemplo, las definiciones de Osborn, 1902; Simpson, 1953; Eldredge y Cracraft; 1980; Schluter, 2000; Sudhaus, 2004), y fueron definidos como *radiaciones adaptativas* (Givnish y Sytsma, 1997). A partir de entonces se han desarrollado diferentes definiciones de lo que es una radiación adaptativa, algunas de ellas muy parecidas y otras radicalmente distintas; pero todas ellas coinciden en considerar tres aspectos: la diversificación del linaje (espesiación y extinción), la diversificación de caracteres (usualmente morfológicos) y la diversificación ecológica (Sanderson, 1998),

El concepto *radiación adaptativa* ha llegado a cobrar una gran importancia en la actualidad. Una revisión general del número de publicaciones en revistas de temas biológicos ("life sciences") que incluyen el termino "radiación adaptativa" ya sea en su título, resumen o palabras clave; muestra que el termino goza de una extraordinaria popularidad, en particular en los últimos años (Figura 1). Incluso se ha propuesto que la diversidad presente en el planeta es el producto, principalmente, de radiaciones adaptativas (Givnish and Sytsma, 1997). A pesar de su importancia, el concepto ha sido usado de manera laxa, y muchos diferentes linajes de organismos, tanto vivos como extintos, han sido clasificados como radiaciones adaptativas sin criterios objetivos, frecuentemente con base en observaciones empíricas. La recurrencia en el uso irreflexivo del término en tan diversas publicaciones ha generado confusión, lo cual dificulta un mejor entendimiento de los procesos evolutivos.

Las revisiones más importantes disponibles sobre radiaciones adaptativas resultan insuficientes, pues abordan el tema desde una perspectiva exclusivamente ecológica (Sudhaus, 2004), o bien, están basados principalmente en estudios de linajes vivientes (Givnish and Sytsma, 1997; Sudhaus, 2004). Dichos trabajos hacen una revisión histórica del origen y desarrollo del concepto muy escueta, partiendo de las definiciones de Simpson (Sudhaus, 2004; Givnish and Sytsma, 1997), y con una escasa o nula discusión de ideas anteriores, provenientes del campo de la macroevolución y la paleontología. Dada la relevancia del concepto *radiación adaptativa* y su reciente uso indiscriminado, es necesaria una revisión completa y profunda de sus orígenes y las diferentes definiciones que se le han dado, junto con una crítica puntual y fundamentada de los posibles elementos que han generado confusión. El objetivo del primer capítulo de esta tesis es contribuir a llenar este vacío existente en la biología evolutiva, haciendo una revisión completa pero además, proponiendo criterios objetivos para distinguir entre patrones evolutivos, y apuntando hacia la búsqueda de consensos.

En su revisión, Sudhaus (2004) distingue tres explicaciones causales de los patrones de radiación: (1) las radiaciones después de eventos de colonización, (2) las radiaciones de linajes sobrevivientes a extinciones masivas y (3) las radiaciones posteriores a la emergencia de las llamadas “innovaciones clave”. Las primeras dos de estas explicaciones son extrínsecas, pues obedecen primordialmente a la respuesta de los linajes a acontecimientos externos, mientras que la última es intrínseca, ya que asume que una característica inherente a los organismos permitió su radiación.

Los linajes de plantas suculentas pueden representar un modelo para el estudio de radiaciones. Aunque no son exclusivas, las suculentas se asocian comúnmente a las regiones áridas del mundo, pues la limitación en la disposición de agua puede conformar una importante presión adaptativa bajo la cual las plantas evolucionaron tejidos de almacenamiento, u otras estrategias (Axelord, 1972). Se piensa que las zonas áridas del mundo, que abarcan un 26% de la superficie continental de la tierra (McGinnies, 1979), aparecieron recientemente (Berggreb et al., 1995 in Moore and Jansen, 2006), y esto posiblemente fomentó la aparición de linajes de plantas adaptadas a estas nuevas condiciones. La estrecha relación que existe entre el clima y las plantas suculentas que habitan en zonas áridas, hace posible el planteamiento de la hipótesis de que el origen y radiación de esos linajes de suculentas ocurrió en respuesta, principalmente, a factores externos, en particular a la aridificación de sus regiones de distribución.

En el Orden Caryophyllales aparecieron muchos linajes de plantas suculentas que en la actualidad habitan regiones áridas de África y América, y cuya extraordinaria diversidad sugiere que pueden seguir patrones evolutivos de radiación, en particular en las familias Cactaceae y Aizoaceae. En el Capítulo II de la Tesis se hace un estudio de las principales radiaciones de suculentas en Caryophyllales, proveyendo de un marco filogenético con base en datos moleculares; y un marco temporal, con base en estimaciones de fechas para el origen y diversificación de clados. Se discuten las fechas obtenidas para el origen de estos clados, en la búsqueda de posibles coincidencias con las fechas de aridificación de sus zonas de distribución. Además, se analizan las tasas de diversificación de grupos al interior del Orden, como un criterio objetivo claro para detectar radiaciones, y se evalúa si la suculencia extrema puede estar correlacionada con una elevación en las tasas de distribución.

Resulta una tarea sumamente compleja el identificar objetivamente a un linaje como radiación, y posteriormente buscar sus posibles explicación causales, ya que los procesos de espesiación (y de diversificación) son multifactoriales (Futuyma, 1998), además de que generalmente los linajes carecen de un registro fósil completo que nos ayude a entender su historia evolutiva. Adicionalmente, los registros paleoclimáticos de la mayoría de las regiones del mundo, pero en particular de zonas áridas, son muy escasos. En México ocurrieron los eventos de radiación de suculentas más impresionantes del mundo al interior de la familia Cactaceae. En el Capítulo III de la tesis se estudian a profundidad estas radiaciones, buscando lograr una contribución a su entendimiento. Primero, se provee de un marco filogenético robusto con base en datos moleculares que nos muestra que los linajes más ricos en especies con distribución en el País son monofiléticos: Cacteae y Pachycereeae. Usando una estrategia similar a la empleada en el estudio de las radiaciones en Caryophyllales, se comparan los estimados de tasas de diversificación de clados en Cactaceae para identificar verdaderas radiaciones y se discute como las radiaciones identificadas pueden responder, más que a motivos extrínsecos (como por ejemplo, la aridificación de ciertas zonas del País), a razones intrínsecas y a interacciones con otros organismos, como lo es el desarrollo formas de crecimiento y morfologías florales que permiten tipos particulares de polinización.

Espero que los trabajos presentados en esta tesis lleguen a ser una contribución real al entendimiento de patrones evolutivos en general, y también que contribuya al entendimiento de radiaciones de plantas suculentas de zonas áridas, en particular, de una de las radiaciones vegetales más llamativas y más emblemáticas de México, las cactáceas.

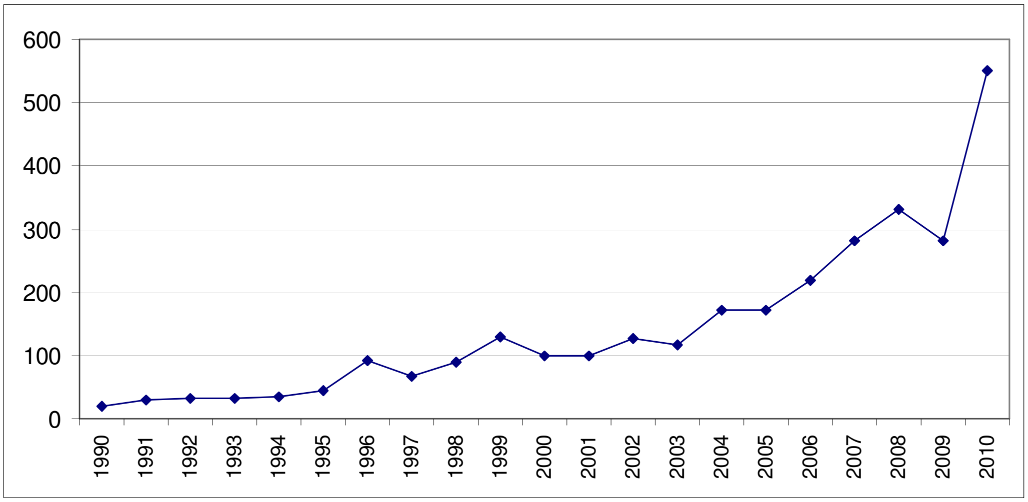

Figura 1. Número de artículos publicados en revistas de contenido biológico ("life sciences") que contienen las palabras "radiación adaptativa" en el título, resumen o palabras clave, en los últimos veinte años (1990-2010). Información tomada de la base de datos de "Scopus" (www.scopus.com)

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CAPÍTULO I

TASAS DE DIVERSIFICACIÓN Y RADIACIONES EVOLUTIVAS, ¿QUÉ QUEDA DE LAS RADIACIONES ADAPTATIVAS?

El termino *radiación adaptativa* se usa recurrentemente para describir los patrones evolutivos que presentan muchos linajes, y se ha propuesto que la diversidad biológica existente es principalmente producto de radiaciones (ver Givnish y Sytsma, 1997; Schluter, 2000). Sin embargo, en la literatura hay muchos desacuerdos en cuanto a los elementos que caracterizan a una *radiación adaptativa* y los criterios más adecuados para circunscribirlas. Las controversias se acentúan cuando los autores usan diferentes criterios para diferenciar un clado que resulta de *radiación adaptativa* de otros clados que evolucionan bajo patrones de especiación "regular" con adaptación; lo cual, según la teoría evolutiva, puede esperarse y predecirse para cualquier linaje. En este capítulo, hago una revisión de las diferentes definiciones y criterios que se han propuesto para distinguir una *radiación adaptativa*. En mi opinión, la principal causa de controversia es el desacuerdo persistente sobre cuáles tasas evolutivas (fenotípicas o taxonómicas) deberían enfatizarse al momento de caracterizar una radiación; una discusión que posiblemente surge de una escasa comprensión de cómo se relacionan las diferentes tasas evolutivas. Otros elementos que también han contribuido a la falta de consensos son la unión de patrón y proceso en el mismo término, el uso de hipótesis nulas inadecuadas y el frecuente uso de comparaciones *ad hoc* entre diferentes linajes. Se discute la importancia de usar rigurosamente términos ya disponibles en la literatura para describir diferentes patrones evolutivos, así como articular criterios sólidos con metodologías objetivas para distinguirlos. Dadas las dificultades para detectar adaptaciones, sugiero tener extremo cuidado cuando se clasifica una radiación como adaptativa. Propongo que la detección estadística de un incremento significativo en la tasa de diversificación taxonómica de un linaje monofilético, sea considerada como la firma distintiva de una radiación. Después de reconocer este patrón, se pueden formular hipótesis causales que lo expliquen, así como correlaciones con otras tasas evolutivas. Los patrones que caracterizan a los linajes sin esta firma pueden ser descritos adecuadamente con terminología disponible.

CHAPTER I

DIVERSIFICATION RATES AND EVOLUTIONARY RADIATIONS: WHAT IS LEFT FROM *ADAPTIVE RADIATIONS*?

The term *adaptive radiation* has been recurrently used to describe evolutionary patterns of several lineages, and has been proposed as the main driver of biological diversification. However, a review of the literature allows identifying disagreements in what characterizes an adaptive radiation and which elements are most appropriate to use for defining its circumscription. Controversies increase when authors use different criteria for supporting their attempts to differentiate a clade under adaptive radiation from clades evolving under ‘regular’ speciation with adaptation, a pattern expected and predicted by the evolutionary theory in any lineage. In this Chapter, I review different definitions and criteria proposed to identify an adaptive radiation, which originate with early interpretations of paleontological observations. In my opinion, the main source of controversy relies on disagreements in relation to which evolutionary rate (either phenotypical or taxonomical) should be considered to identify an adaptive radiation. Such a discussion still prevails possibly due to a weak comprehension about how the different evolutionary rates are related among themselves. The unification of patterns and processes within the same term; the use of inadequate null hypotheses; and the frequent use of *ad hoc* comparisons between different lineages have also contributed to the lack of consensuses. The rigorous use of available terms and the articulation of solid criteria with objective methodologies are imperative to distinguish evolutionary patterns. Given the difficulties to detect adaptation, classifying a radiation as adaptive should be done with caution. I propose that a statistically significant increase in the taxonomic diversification rate of a monophyletic lineage is a distinctive signature of evolutionary radiation. After recognizing such pattern, causal hypotheses explaining them can be stated, as well as correlates with other rates of evolution. Patterns characterizing lineages without this signature can be adequately described with already available terminology.

“*You have loaded yourself with an*
unnecessary difficulty in adopting a Natura
non facit saltum so unreservedly.”
Huxley to Darwin, Nov. 23, 1859, the day
before publication of the Origin

INTRODUCTION

The term adaptive radiation has been used to characterize the evolution of several taxa: horses during the Neogene (Hulbert, 1993), Australian marsupials (Springer et al., 1997), passeriform birds (Ericson et al., 2003), Darwin’s finches (Lack, 1947), cichlid fishes in African lakes (Seehausen, 2006), drosophilid flies of the Hawaiian Islands (Kambysellis et al., 1995), Hawaiian silverswords (Baldwin and Sanderson, 1998), or microbes (Maclean, 2005, and for a summary of several studies see Givnish and Systma, 1997). Lineages that have been classified as adaptive radiations include different number of species that have originated at different ages in different regions of the world, that possess markedly different morphological and ecological characteristics, that may or may not be monophyletic, and some of them are already extinct. Moreover, authors have used different criteria, frequently based on empirical grounds, to define these lineages as radiations. Although the term adaptive radiation was probably used for the first time in the early 1900’s (Osborn, 1902; Givnish, 1997; Gillespie et al., 2001 in Losos and Miles, 2002), the emergence of molecular systematics has promoted its rediscovery and its recent explosive popularity (Gyvnish and Systma, 1997). However, the excessive use of the term has also promoted the emergence of important disagreements and controversies (Givnish, 1997; Sanderson, 1998; Losos and Miles, 2002; Olson and Arroyo-Santos, 2009).

It has been noticed that during enough time most clades will diversify and evolve adaptations, but no quantitative thresholds or objective criteria exist to judge whether a clade constitutes an adaptive radiation (Losos and Miles, 2002), which makes the concept of limited usefulness to distinguish patterns and to understand the evolution of lineages (Losos and Miles, 2002, Olson and Arroyo-Santos, 2009). The elaboration of methodologies to objectively detect distinct evolutionary patterns and radiating lineages is therefore compelling, and although several studies point towards this direction (Magallón and Sanderson, 2001; Losos and Miles, 2002; Gavrilets and Vose, 2005; Magallón and Castillo, 2009; Ackerly, 2009; Gavrilets and Losos, 2009), a consensus rooted in a solid conceptual framework is still pending. This review aspires to gain some advance in this direction.

MANY DEFINITIONS, DISTINCT PATTERNS

The term "adaptive radiation" derives from the integration of two aged ideas: *adaptive evolution* and *explosive evolution*. Evolution by natural selection that moves a population towards a phenotype that best fits the present environment is adaptive evolution (Futuyma, 1998; Eguiarte and Farfán, 2002), and implies that selective pressures over individuals come from the physical or the biotic environment. This kind of evolution was explained by Darwin to be the main force driving diversification. Explosive evolution as early defined by paleontologists, occurs when a taxonomic group appears within a relatively short time interval, and this episode is followed by a longer stretch of time when evolution appears at a quieter pace (Zeuner, 1958).

An early integration of these ideas was proposed by the paleontologist Henry F. Osborn, who defined adaptive radiation as "*the differentiation of habit in several directions from a primitive type*" (Osborn, 1902; pg. 353). But the distinct modern definitions of adaptive radiation are derived from that proposed by Simpson (1953): "… *adaptive radiation strictly speaking refers to more or less simultaneous divergence of numerous lines all from much the same ancestral adaptive type into different, also diverging adaptive zones*" (pg. 223). With a paleontological point of view, Simpson's interest in the study of evolutionary rates took him to consider as characteristics of the phenomenon both an increase in the rate of species generation and an increase in the ecological and phenotypic diversity within the lineage (Simpson, 1953). Also studying patterns in fossil records, Stanley (1979) emphasized the importance of speciation, and defined adaptive radiation as the geometrical or exponential increase in species generation within a lineage, maintaining that speciation accounts for most large-scale phenotypical change (see the discussion on the Punctuated Equilibrium theory below).

Afterwards, ecologists and evolutionary biologists studying extant lineages extended available macroevolutionary definitions of adaptive radiation towards slightly different directions, highlighting different possible aspects of the process. For instance, besides the rapid appearance of numerous species and high morphological diversity, Eldredge and Cracraft (1980) proposed that a monophyletic status of a clade and the confinement to an endemic area are also patterns expected in a clade undergoing an adaptive radiation. Later on, several ecologists took the definition of adaptive radiation towards underlining the importance of morphological and ecological diversity. For instance, Schluter (2000) suggested that an adaptive radiation is the evolution of ecological and phenotypic diversity within a lineage that is rapidly speciating, including their differentiation from a common ancestor in a group of species that inhabit a variety of environments and that differ morphologically and physiologically to exploit these environments. Even with more emphasis on the ecological processes, Sudhaus (2004) established that a radiation is characterized by the relatively rapid origin of diverse ecological niches, established by repetitive speciation events within a lineage, and giving place to a strong evolutionary and ecological divergence in a particular region. In this case, species within the radiation must reflect the different ecological and environmental variables in which they are evolving.

Other proposed definitions give less importance to speciation. From an adaptationist-phenotypical point of view, Losos and Miles (2002) suggested that the number of species within a lineage under adaptive radiation is irrelevant as long as it is more than one, and that the fundamental process is the adaptive disparity (ecological and morphological diversity) between species included in the lineage (see also Givnish, 1997). Other authors consider that monophyly is an irrelevant criterion to distinguish an adaptive radiation (Schluter, 2004), or have questioned the possibility to accurately detect adaptation within radiations (Sanderson, 1998), thus stating as inadequate the labeling of radiations as “adaptive”. Beyond details and particularities of each definition, all of them coincide in considering three fundamental aspects: lineage diversification (speciation and extinction), character diversification (usually morphological) and ecological diversification (Sanderson, 1998), and may differ in emphasizing one or other aspect and in suggesting different criteria and methods to identify them. These differences have determined that several lineages -possibly resulting from different evolutionary processes-, were labeled as adaptive radiations. Lack of consensus reveals the scarcity of objective criteria, strict methods and null hypotheses that could help to distinguish between different evolutionary patterns among distinct lineages.

PUNCTUATED EVOLUTION AND ADAPTIVE RADIATIONS

Explosive evolution -the geologically instantaneous origination and subsequent stability of paleontological morphospecies-, has been considered as the oldest and most robust of the paleontological observations (Eldredge and Gould 1972; Gould and Eldredge, 1977). One example of this pattern was found in the sclearctinian corals or hexacorals, which arose in the mid-Triasic, about 215 MYA (Stanley, 1979). Of the nine living superfamilies, six arose by about the beginning of the Jurassic, after only about 20 MY of evolution (Stanley, 1979). Other typical example can be found in the evolutionary history of Trilobites. The fossil record for this lineage is unknown before the beginning of the Cambrian, but shows a massive diversification during the Early Cambrian, -when about thirty families originated-, in a period that lasted between 15 MY and 30 MY (Harrington, 1959 in Stanley, 1979). The apparently sudden appearance in the fossil record of the diverse classes of invertebrates during the Cambrian (the Cambrian explosion), in an interval lasting approximately 100 MY is perhaps the paradigmatic example of explosive evolution, which Darwin found inexplicable and a valid argument against evolution by natural selection (in Stanley, 1979 pp. 67). This more or less simultaneous appearance of systematic units has been also found in the fossil register of other groups like insects or fishes (Zeuner, 1958), birds and mammals (Feduccia, 1995) or flowering plants (Lidgard and Crane, 1988).

Simpson (1944) described this explosive pattern of lineages generation as “quantum evolution”, and suggested that it might reflect rapid evolution from one adaptive phenotype to another, by way of intermediate stages that were less adaptive and therefore too brief to have left fossil traces. The botanist Verne Grant (1963) (in Futuyma, 1998) coined the term “quantum speciation” to describe the very rapid evolution of some species; whereas Ernst Mayr (1954), suggested that most species originated by rapid evolution in small colonies (peripatric speciation), proposing that this process could account for many of the gaps in the fossil record (Futuyma, 1998). Eldredge and Gould (1972) and Gould and Eldredge (1977) reinterpreted the patterns found in the fossil record with a consideration of the idea of peripatric speciation, and suggested the theory of Punctuated Equilibrium, considering it as the main driver of the evolution of life (Figure 1; Gould and Eldredge, 1977). For these last authors, characters evolve rapidly in concert with speciation, taking Mayr’s ideas on peripatric speciation (Futuyma, 1998).

For decades, the punctuational view have prevailed, grown and gained respect as a complement to the gradualistic view, becoming a useful extension to the evolutionary theory (Schopf, 1977 in Hallam 1977, Gould and Eldredge, 1993). It is possible to argue that this theory explains only patterns of morphological change observed in paleontological morphospecies along the geological history of a lineage, and might not be observed in extant lineages. The punctuational theory suggests that speciation in small populations peripherically isolated from a parental stock, would yield stasis and punctuation when properly scaled into the vastness of geological time (Figure 1; Gould and Eldredge, 1993). However, if an extant lineage is in a stage of increase in its speciation rate (or decrease in its extinction rate, see discussion below), at that stage it would be observed as including more species or taxonomic entities (Figure 1) because there has not been enough time for the expected extinction of several of them. Different studies have shown that species diversity is unequally distributed in different extant lineages (e.g., Sanderson and Donoghue, 1994; Barraclough and Savolainen, 2001; Magallón and Sanderson, 2001; Sims and McConway, 2003; Magallón and Castillo, 2009; Alfaro et al., 2009), importantly because they include different number of species. Taxonomic imbalance, -or the presence of taxonomic entities including a large number of species in comparison to related taxa- is a conspicuous characteristic of nearly all lineages (for instance, angiosperms or insects, see Figure 2). If this increase is not due to the age of the lineage (because an old lineage has more time to accumulate species), it could be taken as evidence of explosive evolution in an extant taxon (Magallón and Sanderson, 2001; Magallón and Castillo, 2009), thus the importance of considering existence time and the extinction probability in the estimation of taxonomic diversity of a lineage, not only its net number of species.

PHYLOGENIES AND MONOPHYLETIC RADIATIONS

Even though not defining it clearly, Simpson (1953) explained profusely the process of an evolutionary radiation. Independently of the causes originating it "*… a radiation does not occur unless a prospectively adapted populations exist, and it is an aspect of their prospective adaptation that they have (as in fact do most populations, but in these cases probably to an unusual degree) large pools of potential genetic variability*" (Simpson, 1953; pg. 228). According to Simpson, an increase in the population size is the first step, followed by a centrifugal pattern of selection away from the ancestral condition to a variety of diverging adaptive zones. These processes would tend to release variation from existing pools of variability and also to increase the probability and rate of fixation of new variability arising from mutation (Simpson, 1953, pg. 228). Thus, the process should start from a single, large population and the emerging phylogenetic pattern after speciation must necessarily be represented as a monophyletic clade within a phylogeny.

In spite of paleontologist's early definitions of radiations like Simpson's or Stanley's, which considered monophyletic clades, monophyly has been questioned as a requisite to identify radiations. Schluter (2000) suggested that monophyly is not necessarily a criterion to define a radiation since several radiations are not monophyletic and some of them do not include all the descendants within a lineage. Paradigmatical examples of adaptive radiations like Darwin's finches, anoles lizards or cichlid fishes were noticed long before the elucidation of their phylogenetic relationships and the assessment of their monophyly (Lack, 1940 in Givnish, 1997; Fryer and Iles, 1972, Greenwood 1974 in Seehausen, 2006; Losos, 1994 in Jackman et al., 1997; Williams, 1972) because radiations frequently lack enough morphological synapomorphies to elucidate their evolutionary history. The increased availability of molecular data makes possible to provide a phylogenetic framework to almost any group, and considering lineages based on their phylogenetic frameworks allows addressing more questions to better understanding their evolution; for instance, the estimation of ages of origin of clades or the study of the dynamics of diversification of a lineage through time (Givnish and Systma, 1997).

The radiation of cichlid fishes in Eastern Africa lakes is an example of the benefits obtained from the study of monophyletic radiations in a phylogenetic framework. Cichlid fish faunas of each of the largest lakes of Eastern Africa, Victoria, Tanganyika and Malawi include several hundreds of species, almost all of them endemic, which makes the Cichlidae one of the species richest vertebrate families (Meyer, 1993). Similar morphological and behavioral solutions to similar ecological problems have arisen in each lake (Stiassny, 1981; Greenwood, 1983 in Reinthal and Meyer, 1997), and the scarcity of morphological synapomorphies and possible convergences characterizing species inhabiting each lake produced a large debate about whether species assemblages were monophyletic, paraphyletic or polyphyletic (see Meyer, 1993; Reinthal and Meyer, 1997). Phylogenetic studies using DNA allowed to elucidate the evolutionary relationships among African cichlids, and proved that Lake Victoria and Lake Malawi species are part of monophyletic clades, the majority included in the tribe Haplochromines, which arose recently within the confines of each lake (Reinthal and Meyer, 1997, Day et al., 2008). On the contrary, Lake Tanganyika represents a very different scenario regarding its oldest geological history and cichlid fauna, assembled from species classified in up to 16 distinct tribes derived from several invasions rather than from a single ancestor (Salzburger, et al., 2005 in Day et al., 2008). With a robust phylogenetic framework available, Seehausen (2006) was able to study cichlid diversification in the Lake Tanganyika by considering each monophyletic tribe independently. He found that five tribes (Limnochromini, Perissodini, Cyprichromini, Bathybatini, and Ectodini) show a speciation burst nearly at the same time followed by a period of stasis. With a similar approach, Day et al. (2008) found that Tanganyika cichlids diversified six times more slowly than endemic radiations in the other lakes, even during peak episodes of diversification possibly coinciding with periods of changing lake levels or with successive invasions and separate radiations.

Radiations are generally associated to geographic regions, and Carlquist (1974) stated that the autochthonous development of a monophyletic clade in an area is a relevant criterion to distinguish an adaptive radiation. This is clear if considering evolutionary radiations that occur on islands. To hypothesize an evolutionary radiation on islands requires that diversification is monophyletic, or to be sure that it is the product of a single invasion event, and not multiple invasion events or polyphily for the area (Carlquist, 1974). However, members of a radiating clade may expand or change the restricted area, which makes difficult to establish a generalization. A phylogenetic framework fostered the understanding of the evolutionary dynamics during the radiation of a paradigmatic lineage inhabiting islands: Caribbean *Anoles* lizards. *Anoles* comprise one of the most diverse vertebrate genera, including nearly 400 species (Losos and Schneider, 2009); however, only the species inhabiting the Caribbean islands (about 150 species) were intensively studied as an emblematic example of adaptive radiation (Williams 1983 in Jackman et al., 1999). On each island of the Greater Antilles (Cuba, Hispanola, Jamaica and Puerto Rico), different *Anoles* species morphologically specialized to a diversity of habitat types (Losos, 1992) and showed high rates of morphological differences (Losos and Miles 2002; Losos et al., 2006). Results on molecular phylogenetic analyses showed that Caribbean *Anoles* represent a paraphyletic assemblage, because early divergent lineages and a rich derived clade within them inhabits mainland, indicating a possible mainland origin and a regression out from the islands (Pinto et al., 2008). It was also shown that lineages that diversified in Central America and northern South America indeed include more species than on the Caribbean islands (Nicholson et al., 2005 in Pinto et al., 2008). Analyses beyond the Caribbean *Anoles* have shown that mainland species display an ecomorphological variety and rates of evolutionary change equal or greater (Irschick et al., 1997; Pinto et al., 2008); suggesting that the *Anoles* radiation includes the entire clade and is not an island phenomenon (Pinto et al., 2008).

It is important to distinguish the process of phenotypic diversification from a common ancestor from the process of convergent evolution of phenotypes from different lineages to similar adaptive types, which can be observed in different regions with similar climatic characteristics. The last have been named species flock (Greenwood, 1984 and Sage, 1984 in Meyer, 1993), usually occurring in diversity hotspots rich in species of different lineages (Myers et al., 2000). According to Sudhaus (2004), the study of an evolutionary radiation should start with a phylogenetic analysis of the study group and the reconstruction of possible characters of the lineage ancestor, in order to find adequate hypothesis about the internal or external causes that promoted the establishment of several econiches. In the case of radiations after colonization events, the event can be searched at the phylogeny node in which the beginning of the radiation is hypothesized, or in which the geological or climatological events that fostered the emergence and diversification of niches is located. Anyhow, several authors point out the need of a generalist or flexible ancestor which allowed the later divergent transformations (Simpson, 1953; Sudhaus, 2004). Focusing on monophyletic radiations by using solid phylogenetic frameworks allows a full consideration of historical processes, and this approach has considerably increased our comprehension of the processes leading to radiating lineages.

EVOLUTIONARY RATES

There exist copious literature on evolutionary rates in different lineages, but it is usually unclear the precise kind of evolutionary rate addressed (Eldredge, 1979). The general pattern of evolution of a lineage can be deconstructed into components of evolutionary rates (Stanley 1979; Gilinsky and Bambach 1987; Vrba 1987; Sepkoski 1998; Rode and Lieberman 2005 in Abe and Lieberman, 2009). The principal author discussing evolutionary rates, -the amount of evolution over time- was Simpson, who proposed different kinds of rates in evolution (Simpson, 1944, 1953). He called “phylogenetic rates” to the amount of morphological or phenotypical changes involved in descendant lineages in relation to their ancestors, and “taxonomic frequency rates” to the changes in numbers or frequencies of taxonomic groups existing in a lineage at a given time (Simpson, 1944 pg.4-5). Considering extant taxa and with slight differences in their definitions, Simpson’s phylogenetic rates have been named *macroevolutionary rates* (Stanley, 1979); *morphological variety* (Foote, 1992), *morphological diversity* (Foote, 1997) or *disparity* (Gould, 1991; Wills et al., 1994; Runnegar, 1987 in Foote, 1997). The taxonomic frequency rates have also been named *richness* (Foote, 1992), or more frequently are referred to only as *diversity*. Modern studies of evolutionary rates followed the two directions early defined by Simpson, one towards the estimation of amounts of phenotypic change over evolutionary time (see for instance Ackerly, 2009; Gingerich, 1983, 2001; Warheit et al., 1999) and the other towards the estimation of taxonomic diversification rates (for example, see Magallón and Sanderson, 2001; Nee, 2006; Rabosky and Lovette, 2008).

Several methods have been developed to measure and compare phenotypic diversification rates among different lineages and characters (Ridley, 1996, see Chapter 20; Ackerly, 2009; Schluter et al., 1997; Gittleman et al., 1996; Foote, 1992). An indirect indicator of the global extent of morphological diversity among taxa can be the change in number of higher taxa they include (generally, phyla, classes, and orders) (e.g. Cherry et al., 1978, 1979, 1982; Foote, 1991; Hafner, 1984; Wilson et al., 1984, all in Foote, 1997). This taxonomic proxy may be criticized on the grounds that taxa are artificial, subjective, non-monophyletic, or stated on the basis of criteria other than morphological distinctiveness; however, the biological meaning of the recognition of higher taxonomic categories relies on the amount of information within lineages (Foote, 1997). Direct measures of phenotypic diversity rely on the measurement and comparison of discrete or continuous variables in a sample of species (Foote, 1997). Haldane (1949) proposed the *darwin* as a standard unit to measure rates of morphological evolution, and other units such as the *felsen* have been proposed later on (Ackerly, 2009) to allow comparisons between different lineages. Determining diversification rates in morphological change can be a difficult task since it requires developing particular methods to measure each character and to compare its change between different lineages in a temporal scale (Gingerich, 1983; Ackerly 2009). Statistically, it requires an adequate sample size (Foote, 1997) usually not available for smaller clades. Moreover, the phenotypic approach to measure diversity is easily criticized as subjective, because rely upon the choice of a finite number of organismic traits under the assumption that they can approximate the total character diversity (Foote, 1997).

The rate of taxonomic diversification within a clade is given by its speciation minus its extinction rate (Box 1). The interplay between these parameters within an interval of time gives the net diversification of a lineage in terms of the number of taxonomic identities it includes, considering a temporal scale (Nee, 2006; Foote, 2000; Magallón and Castillo, 2009). “Birth-Dead”, branching processes and random walk mathematical models have been used to approach the processes of speciation and extinction (Nee, 2006). Some of the models developed take into account the number of species and the absolute age of the clade, allowing to implement a speciation and extinction probability and to consider the possibility of extinct unobserved diversity, by being conditional on the survival of the clade to a given time after its origin (Nee, 2006; Magallón and Castillo, 2009; Doyle and Donoghue, 1993; Magallón and Sanderson, 2001; see Box 1). While an increase in taxonomic diversity can be caused by unusually high rates of speciation, average rates of speciation coupled with exceptionally low rates of extinction could also cause such patterns (Abe and Lieberman, 2009), both cases having different biological explanations and evolutionary meanings.

Molecular data provide another opportunity to study rates of evolution, the molecular rate of change within genetic regions. For Simpson, the ideal measurement of evolution was the amount of genetic change in continuous (ancestral and descendant) populations per year or other unit of absolute time (Simpson, 1944 pg. 4). However, modern studies on the genetical bases of development and comparative genomics have questioned the past belief in a direct linear relation between genetic change, which is usually measured on a restricted number of molecular markers, and morphological change. For example, studies have shown that small single mutational changes in regulatory key genes can produce large morphological changes (Dietrich, 2000; Hintz et al., 2006; Hernández-Hernández et al., 2007; Theissen, 2009).

It is important to remark that all evolutionary rates are correlated (Eldredge, 1979), but each one can depict a different amount of diversity rate (morphological, ecological, taxonomical, molecular) within the same lineage. Some studies have shown for example, the lack of correlation between morphological and molecular rates of evolution within clades (Davies et al., 2004; Bromham et al., 2002; Smith et al., 1992; but see Barraclough and Savolainen, 2001; Jobson and Albert, 2002; Barraclough et al., 1996) or discordances between morphological and taxonomical diversification rates (Foote, 1993). Different authors have defined their study lineages as radiations by remarking the high rates of taxonomic or phenotypic (extended to ecological) evolution. Losos and Miles (2002) proposed to recognize adaptive radiations based on the degree of diversification in ecologically relevant morphological traits, regardless of the number of species the clade may contain. Stanley (1979), on the other hand proposed that adaptive radiation is a phenomenon of geometric or exponential increase in the rate of diversification (speciation minus extinction per unit time). Aiming to clarify this issue, in the next section we review the relation between the phenotypic (considering ecological characters as an extension of it) and taxonomic rates of evolution.

PHENOTYPIC VERSUS TAXONOMIC DIVERSITY

It is important to clarify what is the rate of macroevolution (morphologic or phenotypic rates of evolution according to Stanley, 1979) and how it is related to the rate of speciation, given that this is frequently a discordant factor in the literature concerning radiations.

Detecting high rates of phenotypic diversity among species inhabiting different available habitats has been considered as a basic criterion to determine radiations, because it reflects niche differentiation. From this point of view, Sudhaus (2004) suggested that a radiation should emphasize morphological, physiological or behavioral diversification between descendents of a common ancestor. In the same line of thinking, Carlquist (1974) argues that an adaptive radiation requires the presence of a range of adaptations (morphological changes) as well as discontinuities between the forms within the taxonomical group studied, because a large and morphologically continuous lineage could be the result of constant hybridizations or natural variation expected within a clade given enough evolutionary time. Losos and Miles (2002) restrict the phenotypical criteria even more, defining an adaptive radiation such as "*these clades that exhibit unusually high divergence in adaptive forms*"; and therefore, a fundamental criterion for them is the detection of a high rate of morphological and physiological diversity, -which they call adaptive diversity- (See also Jackman et al., 1997).

Phenotypic definitions of radiations tend to undermine the taxonomic diversification rates as a useful tool to determine radiation patterns. From Givnish's perspective, the number of species within a lineage is irrelevant as long as it is higher than one, and they strongly criticize recent efforts to quantify [taxonomical] diversification rates within lineages arguing that these would reduce an evolutionary radiation to a simple lineage diversification (Givnish, 1997; see also Sanderson, 1998). Barrett and Graham (1997) suggest that the number of lineages emerging in a radiation is less important than the diversification pattern (morphological, anatomical, physiological) of characters within lineages, whereas Jackman et al. (1997) propose that the relevant criterion to define a radiation is not the number of species but the adaptive disparity between them, since even clades with very few species may constitute a radiation if their ecological and morphological disparity is significant.

It has been stressed out that the species number within a clade may not correlate with the range of ecological or morphological diversity it exhibits (Stanley, 1979; Foote 1991, 1992); and indeed, many species-rich clades contain species that differ little ecologically (Warheit, 1999). The studies of lineages without a large number of species but considered as radiations because of their high phenotypical diversity (for example, Darwin's finches or Anoles lizards; see Table 1) frequently lack a consideration of the lineage's age, as well as its speciation and extinction rates; and this is perhaps due to a wrong consideration of phenotypic and taxonomic rates as independent. If speciation, extinction and a temporal framework are not considered, authors miss the point that an increase in morphological diversity may be correlated with the age of the lineage as well as with high extinction rates that can rub out intermediate taxa (see Figure 3). Although several authors have noticed that phenotypic rates during evolutionary radiations are generally associated with high speciation rates (Eldredge and Cracraft 1980; Lieberman et al. 1991; Hulbert 1993; Lieberman 2001; in Abe and Lieberman, 2009), a mechanistic link between these rates has been generally obviated. As Futuyma pointed out, phenotypical change may accumulate anywhere along the geological trajectory of a species, but unless that change be "locked up" by acquisition of reproductive isolation (speciation), it cannot persist or accumulate because it is washed out during the interdigitation through time among varying populations of a species (Futuyma, 1987 in Gould and Eldredge, 1993). "*In the absence of reproductive isolation, differentiation is broken down by recombination … speciation can facilitate morphological change not by liberating a population from genetic homeostasis or accelerating the response to selection, but by enabling a gene pool to remain subject to consistent selection pressures even as it moves about in space. By isolating gene pools that they encounter as they move about, speciation enables them to retain characters that evolved in a localized context. It provides them, as Simpson (1961) pointed out, with a distinct evolutionary trajectory*" (Futuyma, 1987 pg 467). Following these arguments, phenotypical diversification must be inevitably coupled with speciation –considered as reproductive isolation-. It would not be possible to dissociate the phenotypic and the taxonomic diversification rates –estimated considering speciation and extinction -, unless recurring to saltationist explanations (Box 2).

Without considering a time frame and the extinction and speciation rates, it is not possible to discern distinct patterns for the evolution of lineages and to state solid hypotheses about causes leading these patterns. Several methods have been developed to estimate taxonomic diversification rates considering extinction (Hey, 1992; Mooers and Heard, 1997; Raup, 1985; Nee et al., 1994a; Nee, 2006; Ricklefs, 2007; Magallón and Sanderson, 2001) and to compare them to detect radiations from background cladogenesis (Sanderson and Donoghue, 1996; Paradis, 1998; Rabosky, 2006; Chan and Moore, 2002). In the study of evolutionary radiations, once an increase in taxonomical diversification rates is detected (a taxic approach *sensu* Eldredge, 1979), either if the clade possesses high phenotypic rates or not, explanatory hypotheses for the increase in species generation and persistence can be stated. Sudhaus (2004) distinguishes three different types of evolutionary radiations that I believe represent different possible explanations for clades showing an increase in their net taxonomic diversification rates: (1) radiations after successful colonization events, (2) radiation of survivors to mass extinctions and (3) radiations after the evolution of key innovations. The two first causes of radiation are extrinsic and the last one obeys mainly to intrinsic forces, but for the three types of causes a crucial element is the so called "ecological opportunity" (Sudhaus, 2004). Ecological opportunity is given by niche opening (for instance, colonization of a new area), appearance of a character that allows colonizing new niches (key innovation) or environmental heterogeneity within a discrete geographic region that contributes to niche diversity. Developing methods to test and identify these hypotheses is necessary to explain the evolutionary mechanisms leading to each radiation (for examples, see Abe and Lieberman, 2009; Mattila and Bokma, 2008).

TEMPO OF EVOLUTIONARY RADIATIONS AND SELECTION OF NULL HYPOTHESES

A "recent diversification" or "rapid speciation" has been generally implicit in the definition of radiations (e.g. Sudhaus, 2004), given that several model lineages are relatively recent. The Hawaiian islands are less than 800,000 years old, and at least 24 species of *Drosohpila* have apparently originated there (Futuyma, 1998). About 300 species of cichlids in Lake Victoria are thought to have evolved from one ancestor in about 200, 000 years and several species are restricted to a small neighboring lake thought to be only 4000 years old (Fryer and Iles, 1972 in Futuyma, 1998). However, other lineages that have been classified as adaptive radiations are much older; for instance, *Anoles* lizards (~35 MY; Warheit, 1999) or flowering plants (350-130 MY; Magallón and Castillo, 2009). As discussed above, to detect a radiation, the age of a lineage or its number of species alone may be less informative criteria than taking into account its dynamics of evolution via its taxonomic diversification rates, making the mentioned parameters second in importance. For example, flowering plants (angiosperms) have been estimated to be about 241 My old (Magallón and Castillo, 2009), but have a similar diversification rate than *Impatiens* (Balsaminaceae, Ericales); a flowering plant genus estimated to be approximately 22 My old (see Table 1), includes more than 1000 species, and is one of the largest genera of flowering plants (Janssens et al., 2009). Darwin's finches (Geospizini, Fringillidae, Passeriformes) are estimated to have originated about 2.3 My ago (Sato et al., 2001; see Table 1); slightly earlier than the dates of origin of white-eye birds (*Zosterops* [*griseotinctus*] species); a morphologically homogeneous group that is estimated to be 1.4-1.8 My old (Moyle, et al., 2009); however, the latter have a much higher diversification rate (2.24 species per MY; Moyle et al., 2009) compared with Darwin's finches (0.35 species per MY; see Table 1). It can not be ruled out the possibility that Darwin's finches morphologic diversity is the product of high extinction of intermediate morphs, and white-eye birds is the product of increased speciation plus decreased extinction rates. Attributes such as sexual selection, ecological specialization and dispersability have been proposed as promoters of taxonomic diversification; and a powerful approach to test them could be to investigate the diversity patterns among extant groups to infer the nature of the processes operating during their evolution (Barraclough et al., 1998).

Net taxonomic diversification rates have been widely used to detect radiating lineages as a possible comparable measure of the speciation and extinction processes among clades. Baldwin and Sanderson (1998) report a diversification rate of 0.56 ± 0.17 species per MY for the Hawaiian silversword alliance, whereas in a similar study, Klak et al. (2003) estimated rates of 0.77±1.75 (assuming zero extinction) and 0.58±1.32 species per MY (when incorporating a high rate of extinction) for the ice plants in the Succulent karoo. The authors considered these rates to be high compared to average rates of continental radiations such as angiosperm families (0.39 species per MY, Eriksson and Bremer, 1992; or 0.0420-0.0781 species per MY; Magallón and Castillo, 2009), or with rates of other lineages such as fossil Neogene horses (0.5±1.4 species per MY; Hulbert, 1993). However, silversword and ice plant diversification rates would be regular or low if compared to other lineages considered as radiations such as the Hawaiian island drosophilids (1.21 species per MY; Klak et al., 2003) or the Lake Tanganyika cichlids (0.75±1.49 species per MY; McCune, 1997). The lack of thresholds to define a clade as a radiation fosters the report of *ad hoc* comparisons that contribute to generate confusion in the study of radiations.

Comparisons made between distinct lineages assume that their diversification rates, that is, their speciation and extinction dynamics, are equiparable between them. For example, Hughes and Eastwood (2006) studied the diversification rate of the genus *Lupinus*, inhabiting the high-altitude Andes, in order to compare the diversification of a plant group with the cichlid fish radiation (which is faster than any other documented radiations) over a similar time period. Is it reasonable to compare diversification rates between clades with different ecological characteristics and generation times? Different biological aspects characterizing different lineages may affect its taxonomical diversification rates. The geological record shows that there is large variation in the generation of lineages both in space and time (Sepkosky, 1998). Considering extant biodiversity, a relationship between geographic range sizes and speciation has been studied (Gaston, 1998), and it has been suggested that habitat parameters may affect the risk of extinction (Hansky and Gyllenberg, 1997). For example, Mitter et al. (1988) demonstrated an increased species richness in phytophagous insects compared with their non-phytophagous sister groups. Other widely recognized correlation is the one between body size and species richness (see Vogler and Rivera, 2003), or the increased diversity of clades inhabiting the tropics (see Clarke and Crame, 2003). It is important to consider that speciation (and possibly extinction) is a multivariate caused process, in which different internal and external variables characterizing each clade play important roles (Sepkosky, 1998; Barraclough et al., 1998). Dispersal rates, isolation of populations (afforded by topographic or other variables), strong selection, growth rates, competition, ecological opportunities, bottlenecks in population sizes, and differences in genetic and developmental systems are some of the variables that contribute to the process of speciation (Futuyma, 1998).

In this context, what comparisons would be appropriate to detect clades with significant increases in diversification rates? Jablonski's work with fossil mollusks showed that the different rates of speciation and longevity tend to be heritable at the level of clades (Jablonski et al., 1986). In Jablonski's analyses, the underlying cause of characteristic speciation rates is evident from modes of reproduction. In other cases, characteristic speciation rates may relate to ecological factors, such as physiology and function, which affect population structure and density and which are shared by common descent within higher taxa (for example, Martin 1992; Lawton et al., 1994; in Sepkosky, 1998). This information indicates that an appropriate comparison should be made between related lineages (Brooks and McLennan, 1993 in Warheit, 1999) that have similar age and ecological preferences (Losos and Miles, 2002; Warheit, 1999). For example, Mayhew (2002) compared species richness between sister taxa in a Hexapods phylogeny to detect significant increases, under the null hypotheses of equal rates of cladogenesis. Magallón and Sanderson (2001) detected exceedingly species-rich or species-poor angiosperm clades, by comparing their standing diversity with expected species diversity according to a background rate of diversification, obtained by assuming a hypothetical clade that diversifies with a rate equal to that obtained for the angiosperms as a whole (Magallón and Sanderson, 2001). Following this approach, Sauquet et al. (2008) found that Proteaceae, a model plant group distributed in the Mediterranean diversity hotspots of the Southern Hemisphere, diversified under higher rates in the Cape Floristic Region and Southwest Australia than in any other area of their total distribution.

Other studies look for detecting significant increases in diversification rates by analyzing its dynamics in phylogenetic trees (e.g. Sanderson and Donoghue, 1996; Rabosky, 2006) or by analyzing tree shape (Chan and Moore, 2002; see also revision of Mooers and Heard, 1997); under the null hypotheses of random or background cladogenesis (Box 1). The pioneering work of Raup et al. (1973) and Gould et al. (1977) started the idea of random cladogenesis. By using a random model, they built evolutionary trees in which branches split or become extinct with equal probabilities, and obtained patterns strikingly similar to real phylogenetic trees. A model in which speciation and extinction probabilities are constant trough time and within taxonomic groups became a common type of null model of taxonomic diversification (Raup, 1985, Box 2). Afterwards, several studies tried firstly to statistically test homogeneity of diversification rates within the phylogeny, and secondly, to detect clades showing shifts in diversification rates. For instance, Sanderson and Donoghue (1994) found that shifts in diversification rates within angiosperms are not coincident with several hypothesized “key innovations”. By analyzing a phylogeny of primates, Purvis et al. (1995) detected an increase in the diversification rate of the lineage leading to the Old World monkeys, which might be related to habitat changes, land bridges, and dietary adaptations (Fleagle 1988; Conroy 1990 in Purvis et al. 1995). Alfaro et al. (2009) identified nine periods in the history of vertebrates in which the tempo of diversification changes; the most significant of these lying at the base of a clade that includes most of the coral-reef associated fishes as well as cichlids and perches. By analyzing tree symmetry in a dinosaur supertree, Lloyd et al. (2008) rejected the long held belief that dinosaurs radiated during the mid-Cretaceous, when medium to large herbivores and carnivores appeared, suggesting that this hypothesis is a sampling artifact. Models of evolution with phylogenetic trees have proved to be a powerful tool to objectively detect (or reject) radiations as increases in taxonomic diversification rates within lineages.

Lineages through time plots (LTT plots) have also been widely used to detect increases in diversification rates within the same lineage over time, avoiding the possible bias of comparisons with different lineages. Mathematical models considering only speciation generate the null hypotheses of clades growing linearly, however when extinction rate is incorporated, the plot changes, with an apparent accelerated rate of cladogenesis when approaching the present (Figure 4; Nee, 2006). This happens because the more recently generated species have had less time to go extinct (Nee, 2006), originating what have been termed the “pull of the present” (Nee et al., 1994b in Rabosky, 2006), which can mislead researchers concluding that a clade shows an increase in the net taxonomic diversification rate (Nee et al. 1994b, Kubo and Iwasa, 1995 in Rabosky, 2006). Nee et al. (1992) used LTT plots to study the dynamics of the taxonomic diversification rates of birds through time. They found that the Passeriformes (song birds) and the Ciconiiformes (shore birds, waders, flamingos, gulls, etc.) were statistical outliers with exceptionally high diversification rates (Nee et al., 1992; Nee, 2006). More recently, Moyle et al. (2009) used LTT plots to study the dynamics of diversification within the Zosteropidae bird family. They found that its taxonomic diversification significantly increases in the clade including the *Zosterups* genus, considered as a “great speciator”, and that these hyperdiversification occurred early in the Pleistocene and subsisted towards the present.

Several available methods developed have proven useful tools to distinguish clades showing significant increases in their taxonomic diversification rates within their lineages; and are useful to avoid the use of *ad hoc* comparisons. The detection of significant increases in diversification rates with adequate comparisons allows detecting objectively a signature of a radiation, however, the designation of these clades as radiations continues to be debated, because universal methods and thresholds are not yet a consensus.

ADAPTIVE, NON-ADAPTIVE, OR NOTHING?

An important source of confusion in the definition of adaptive radiation is that the term links together a pattern (explosive evolution; morphological or ecological diversity) and a process (adaptive evolution) (Eldredge and Cracraft 1980; Givnish and Sytsma 1997; Vogler and Goldstein 1997; Schluter, 2000; all in Abe and Lieberman, 2005). The presence of adaptation or adaptive diversity within lineages has been proposed for some authors as a criterion to identify an adaptive radiation; however, it has been noticed that studies of adaptation in several cases have been insufficient or even speculative (Sanderson, 1998). An adaptation can be regarded as a phenotypic variant that results in the highest fitness (reproductive success) among a specified set of variants in a given environment (Reeve and Sherman 1993, in Futuyma, 1998), and was early noticed in diverse organism's structures by naturalists of the 18th and 19th Centuries. Nevertheless, its strict detection has shown to be extremely difficult, and several methods and indirect indicators have been proposed to approximate it on populations and lineages (Rose and Lauder, 1999; Conner et al. 2009).

Empirical methods developed to detect adaptation rely on the experimental study of characters or the detection of fitness on populations. One possible approach looks for detecting adaptation by directly studying the function of a trait, and how does its variation affect its function (Conner, et al. 2009). In this case, however, the response variable is not fitness, and therefore it cannot determine whether the trait is really adaptive (Conner et al., 2009). Another approach intends to measure selection on a trait by estimating the relationship between variation in the trait and variation in fitness (Wade and Kalisz, 1990; Conner et al., 2009). These methods are not practical to detect adaptation in radiations, because radiations usually consider numerous species (with several populations each) that diverge in their ecological and morphological characteristics.

In a phylogenetic context, the comparative method has largely been used to detect adaptation in lineages (Martins, 2000; Harvey and Pagel, 1991). The traditional cladistic approach goal is to reconstruct putative ancestral characters and determine whether evolutionary changes in the phenotype have been associated with major shifts in the environment; a method that has been strongly criticized because estimates of ancestral characters can be inaccurate (Losos, 1999; Martins, 2000). A similar approach is the search for statistical relationships between phenotypes and environments, taking phylogenetic information into account (Harvey and Purvis, 1991); however, for a statistical validity these methods require a large number of taxa and evolutionary changes in both phenotypes and environment (Martins, 2000). It has been proposed that the presence of convergence or homoplasy can be an indicator of adaptation, assuming that a strong selective pressure will independently determine the origin of a similar character on different clades (Coddington, 1994). Nevertheless, the detection of convergence is only useful in some cases, because unique adaptive characters could arise within any single clade (Sanderson, 1998).

Methods that compare lineages can be used to evaluate a possible correlation of the presence of a character postulated as adaptation on the diversification rates of lineages. These methods require that the compared clades are monophyletic, one possessing the character and the other lacking it, and both interacting with the environment in a similar way to allow an adequate quantification of the magnitude of the differentiation (Losos and Miles, 2002). One of the main deficiencies of these methods is their poor statistical power, because the number of possible pair wise comparisons (comparable clades) is usually very low. Moreover, these methods do not take into account the amount of evolutionary divergence of sister clades from their common ancestor, the potential differences between the ecological niches they inhabit and the necessity of a functional explanation of the relationships between the organisms within the different lineages and their niches (Sudhaus, 2004). Whichever the method selected, even the most elaborate statistical analysis to detect adaptation or directional natural selection can only determine a correlation and not a causal relation between the environment and the trait (Wade and Kalisz, 1990; Conner, et al. 2009, Martins, 2000), thus producing extreme difficulties in trying to interpret adaptation in radiations, and to determine if it was a cause, a consequence, or a process accompanying speciation.

Beyond the methodological difficulties in detecting adaptation, Gittenberger (2004) defends the use of adaptive and non-adaptive terms applied to radiations, the term adaptive referring to the nature of the speciation events. When speciation in a lineage occurs in allopatry, genetic drift and founder effects may result in divergence, without clear niche differentiation, thus producing what he calls a non-adaptive radiation (Gittenberger, 2004; Rundell and Price, 2009). These non-adaptive radiations have been postulated as null hypotheses of adaptive radiations, and consist of clades that are very rich in terms of number of species but that contain low levels of morphological diversity, suggesting few adaptive differences between species (Gittenberger, 1991). The differences between allopatric speciation in lineages that are rich in species and non-adaptive radiations have not been clearly stated. As it is possible to define any character in adaptive terms (see the criticism to the adaptationist program by Gould and Lewontin, 1979) it is practically impossible to determine that a character or a set of characters occurring in several species are not adaptive, that is, that the characters do not have any function that contributes to the fitness of individuals possessing it (Sudhaus, 2004). As Losos and Miles (2002) have discussed, there are not objective criteria to judge if a clade conforms an adaptive or a non-adaptive radiation, since throughout enough time the majority of clades or lineages will diversify and develop adaptations to their environments and niche differentiation. The term allopatry and parapatry coined by Mayr (1954) adequately describe lineages, in spite of their morphological diversity; and the use of adaptive and non-adaptive radiations may generate confusion.

Arguments discussed above make evident the difficulties to objectively identify characters in radiations as adaptations, or to suggest correlations between phenotypes and environment (adaptive diversity). This explains why the majority of studies on adaptive radiations lack a rigorous detection of adaptation. Unless methodologically detected in a lineage, adaptive evolution can be present in any (if not all) lineages. Operationally, it would be useful to have a simpler measure to distinguish radiating from non-radiating lineages, allowing the possibility to postulate better hypotheses concerning its origins. Considering the importance of a rigorous use of available terms and a strict definition of evolutionary patterns, it would be probably better to avoid the use of the adaptive term applied to radiations, unless adaptations are methodologically demonstrated as a positive correlation. Nevertheless, when studying radiations, it is necessary at least to have a foundered hypothesis of the possible causal agents that provoked the radiation. These hypotheses will always refer to the relation between the organisms and their environment.

CONCLUSIONS

The concept of adaptive radiation links the notions of patterns of explosive evolution and processes of adaptive evolution. In order to construct a strict definition of evolutionary patterns, the use of the term adaptive to classify radiations requires rigorous methods for identifying it. It is recommendable to avoid its use based only on empirical grounds. Using of the terms adaptive and non-adaptive to distinguish radiations does not benefit the understanding of evolutionary patterns, but contribute to generate confusion. Available terminology is suitable to clearly designate the proposed non-adaptive radiations (for instance, allopatric species rich lineages).

Explosive evolution has been documented based on the fossil record of several extinct taxa, and in extant taxa it can be identified as a taxonomic imbalance among related taxa in almost all lineages. Several authors have argued in favor of the increase in phenotypical diversification rate of lineages as a basic criterion to detect radiations, highlighting the importance of the morphological and ecological diversity. This phenotypic definition of radiations usually do not take speciation and extinction into account, and therefore, do not satisfactory explain the processes generating the large morphological diversity. Following the Punctuated Equilibrium theory, we discuss the relationship between taxonomic and phenotypic diversity in a lineage, where morphological diversity is generated in lineages mainly as a consequence of speciation. Large extinction rates can produce lineages with high morphological diversity and few species, because intermediate morphs are eliminated. The consideration of taxonomic diversification rates (speciation minus extinction rates) is crucial to understand the dynamics generating the observed evolutionary patterns. The study of monophyletic lineages in phylogenetic frameworks provides important advantages to understand evolutionary processes, and the estimation of diversification rates is necessary for a better comprehension of the evolutionary processes leading to the observed patterns, as well as to identify radiations.

Major difficulties lie in the detection of lineages with increased taxonomic diversification rates, and much attention must be paid in the lineages selected to compare rates, or in the null hypotheses tested. Several methods have been developed to detect increases in taxonomic diversification rates; however, this is an emerging research area. Efforts in both theoretical and methodological issues are still necessary to discern between evolutionary patterns.

ACKNOWLEDGMENTS

I thank D. Piñero for fostering this discussion and providing interesting comments. I equally thank the participants of the seminar on diversification rates organized by S.M. at IB, UNAM. L.E. Eguiarte kindly revised the manuscript. T.H-H. graduate studies were funded by CONACYT and the Posgrado en Ciencias Biológicas, UNAM.

FIGURES AND TABLES

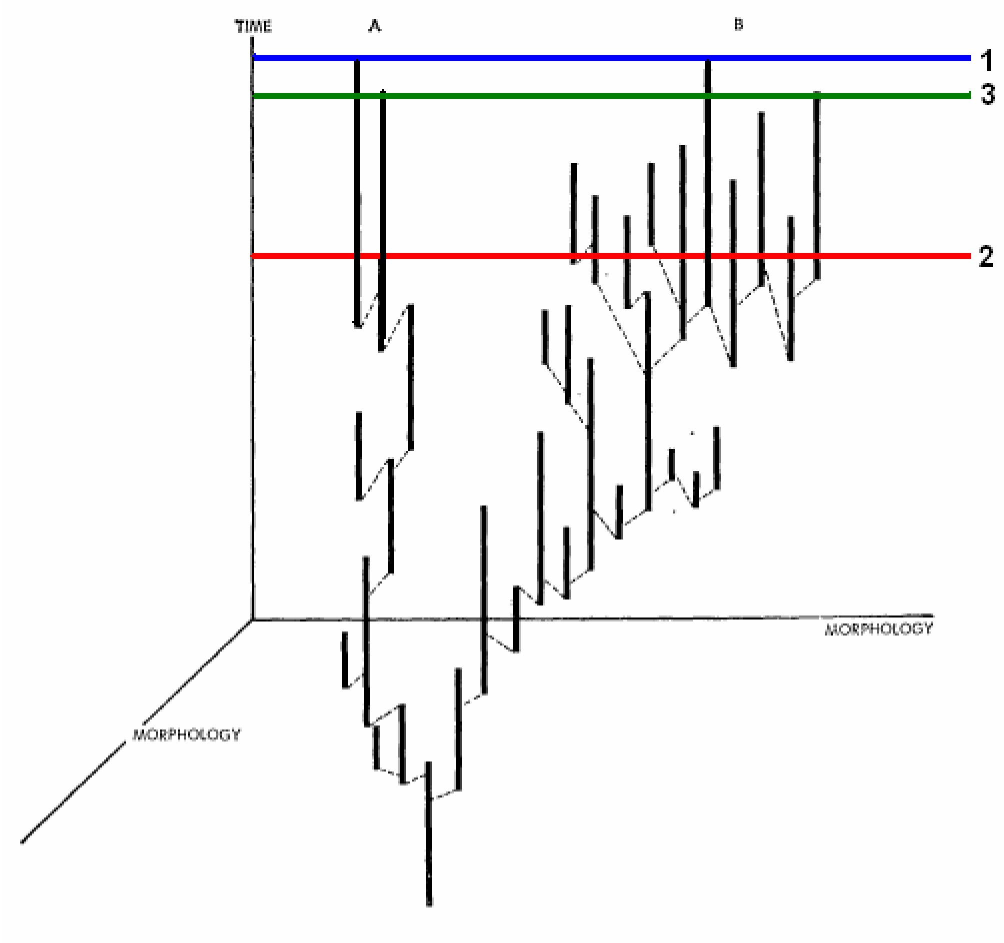

Figure 1. Three-dimensional sketch contrasting a pattern of relative stability (A) with a trend (B), where speciation (dashed lines) is occurring in both major lineages. This figure was modified from Figure 5-10 in Eldredge and Gould (1972), which was presented to explain the theory of Punctuated Equilibrium; where speciation accounts for the morphological trends in each lineage. Lineage (B) has a higher speciation rate, however also a high extinction rate, thus at the hypothesized present 1 (blue line) both lineages (A) and (B) include the same number of species (richness). If we hypothesize the present at 3 (green line) we can see that both lineages include the same number of species, however in (B) the phenotypic diversity (disparity) between them is larger, as a result of the higher speciation and extinction rates of lineage (B). If the present was at 2 (red line) we would see that lineage (B) includes more living species than (A), and represents an extant evolutionary radiation.

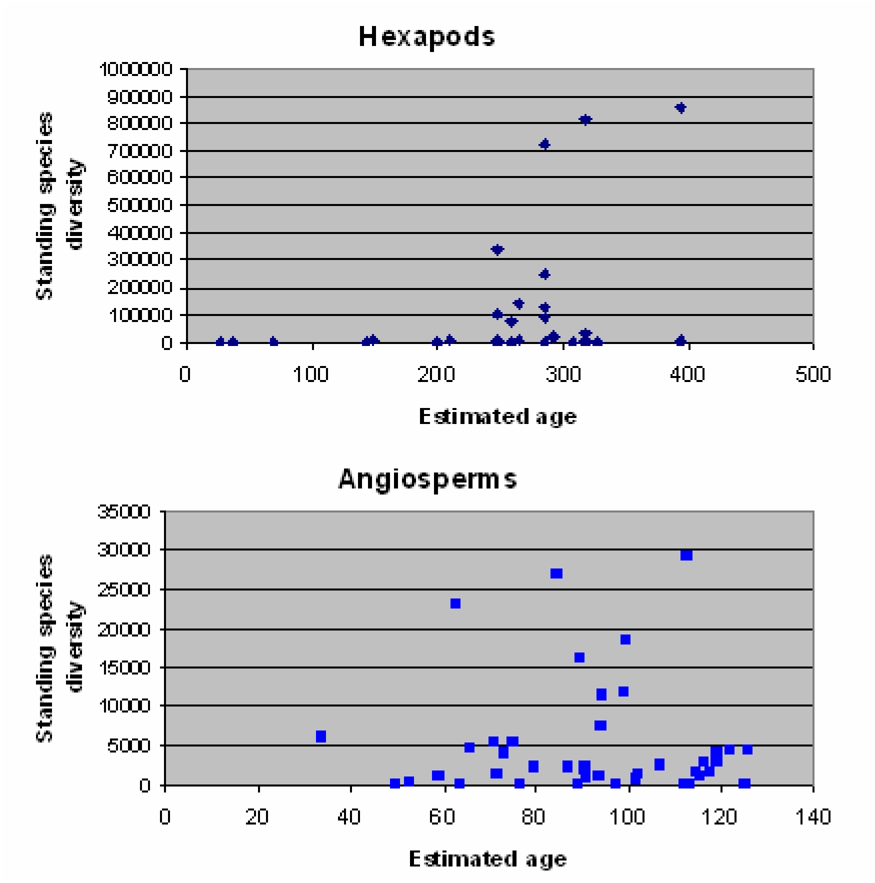

Figure 2. Plots showing estimated age versus number of species included (standing species diversity) in angiosperm and hexapod (insects) orders. No correlation exists between the age of the clade and its standing species diversity. As it can be seen, some orders include an exceedingly large number of species while the majority include a reduced number. Graphs based on data taken from Magallón and Castillo (2009) and Mayhew (2002).

LINEAGE	**DESCRIPTION**	**PLACE**	**SP. NUM.**	**AGE (MY)**	**DIV. RATE**	**METHOD USED**	**REFERENCE**
ANIMALS							
Anolis (Polychrotidae, Squamata, Reptiles)	Iguanian lizards	Caribbean Islands, Central and South America	**400**	**35**	**0.15/0.10***	Birth-Dead (considering zero/high extinction)	Warheit et al., 1999
Sceloporus (Phrynosomatiade, Squamata, Reptiles)	Lizards	North and Central America	**>70**	**20-25**	**0.17/0.10***	Birth-Dead (considering zero/high extinction)	Warheit et al., 1999
Geospizini (Fringillidae, Passeriformes)	Darwin's finches	Galapagos and Cocos Islands, Pacific Ocean	**15**	**2.3**	**0.87/0.35***	Birth-Dead (considering zero/high extinction)	Sato et al., 2001
Lake Malawi and Lake Victoria cichlids (Halpochromini, Cichlidae)	East African cichlid fishes	Lake Malawi, and Lakes Victoria, Kyoga, Rukwa, Kivu, Albert, George, Edward, and surrounding water bodies.	**1135**	**3.1**	**2.09 (C.I. 1.71 to 3.19)**		Day, et al. 2008
Zosterops [*griseotinctus*] species group (Zosteropidae, Passeriformes)	White-eye birds	Islands in the southwest Pacific	**80**	**1.40–1.89**	**2.24 (CI, 1.95–2.63);** **1.95/1.12***	Yule model (Pure Birth); Birth-Dead (considering zero/high extinction)	Moyle, et al. 2009
Nerita (Neritidae, Gastropoda)	Snails	Worldwide, especially tropical oceans	**70**	**55.2**	**0 - 0.049**	Birth-Dead (considering zero/high extinction)	Frey and Vermeji, 2008
Hexapoda (Arthropoda) Orders:	Insects	Worldwide					Mayhew, 2002
Thysanoptera			**5000**	**148.9**	**0.0572**	Pure Birth	“
Psocodea			**5680**	**148.9**	**0.0581**	“	“
Zoraptera			**20**	**317**	**0.0095**	“	“
Dermaptera			**1506**	**317**	**0.0231**	“	“
Grylloblattaria			**13**	**317**	**0.0081**	“	“

Othoptera Coleoptera			**18644** **336893**	**292.5** **247.5**	**0.0336** **0.0514**	“ “	“ “
PLANTS							
Ruschioideae, (Aizoaceae, Caryophyllales)	Ice plants	Succulent Karoo, West coast of Southern Africa	**1563**	**3.8 - 8.7**	**0.77–1.75/0.58–1.32**	Birth-Dead (considering zero/high extinction)	Klak, et al. 2003
Argyroxiphium, Dubautia, Wilkesia; (Asteraceae, Asterales)	Silversword alliance	Hawaiian islands	**28**	**5.2 ± 0.8**	**0.56 ± 0.17**	Pure Birth and Birth-Dead	Baldwin and Sanderson, 1998
Yucca (Agavaceae, Asparagales)	Rosette plants	Arid and semiarid regions of America	**34**	**6.81-6.06**	**0.33±0.06**	Yule model (Pure birth)	Smith et al. 2008
Agave sensu latissimus (Agavaceae, Asparagales)	Rosette succulents	Arid and semiarid regions of America	**240**	**9.46-8.69**	**0.21±0.001**	Yule model (Pure birth)	Smith et al. 2008
Prosopis (Fabaceae, Fabales)	Trees and shrubs	Arid and semiarid regions of America and Africa and west Asia	**45**	**28.96 (26.25–31.68)**	**0.12–0.14**	Per lineage net diversification rate (NDR) sensu Coyne & Orr (2004).	Catalano et al., 2008
Angiosperms	Flowering plants	Worldwide	**269,323**	**241.71-130**	**0.0420-0.0781**	Birth-Dead	Magallón and Castillo, 2009
Impatiens (Balsaminaceae, Ericales)		Tropical and subtropical montane forests	**over 1000**	**22.5 ± 5.6**	**0.03-3.8**	Pure Birth and Birth-Dead	Janssens et al. 2009

Table 1. Diversification rates reported for several lineages of plants and animals. (*) represents diversification rates estimated by us following equation 7 (for hypothesized values of low and high [0 and 0.9] relative extinction rates) in Magallón and Sanderson (2001).

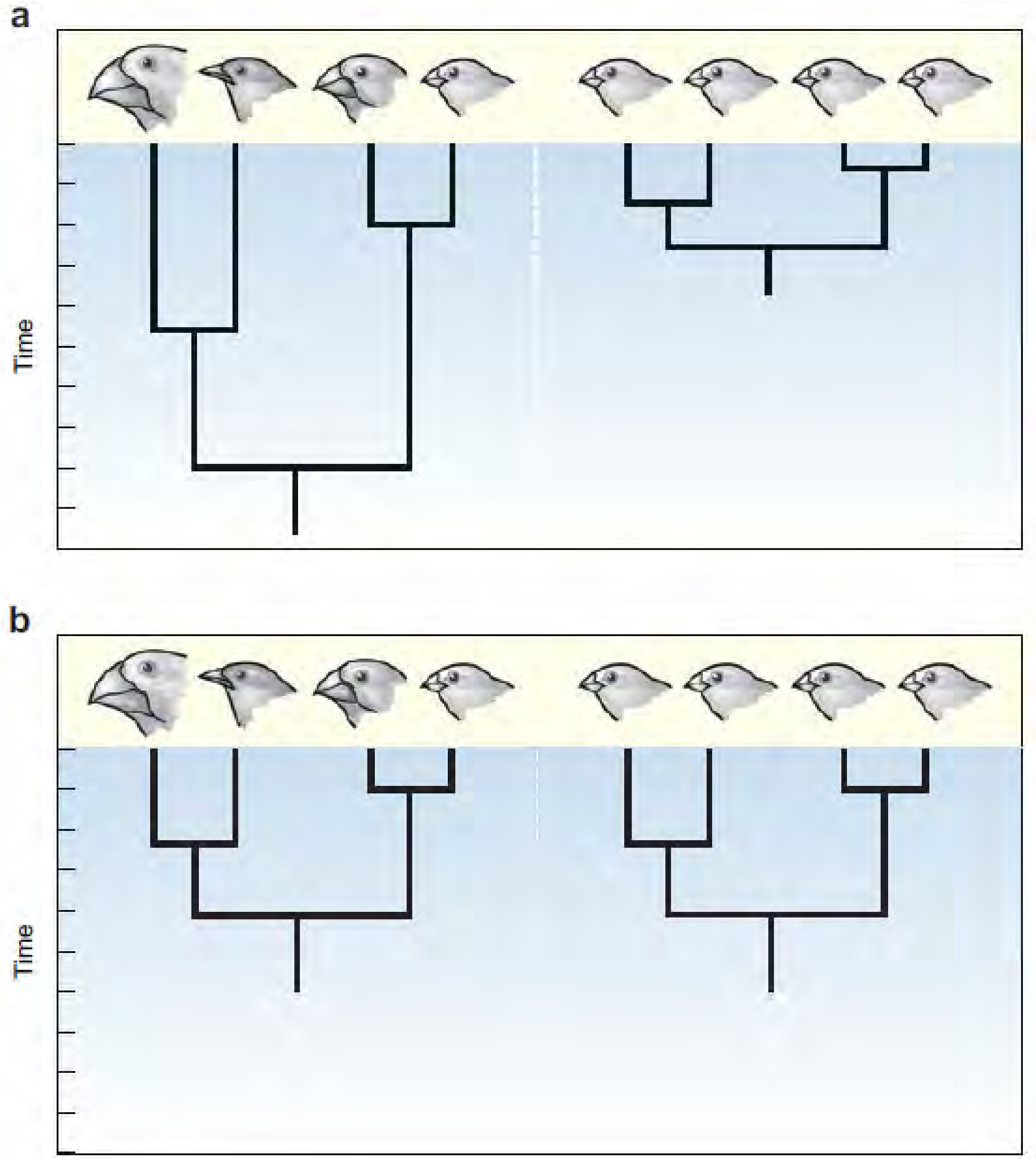

Figure 3. Diagrams illustrating the importance of time in the interpretation of differences in morphological diversity between two monophyletic groups of birds. (a) The group with high phenotypic diversity is much older than the low phenotypic diversity group. In this case the difference in diversity between groups may be due to differences in the amount of time the two groups have had to diversify. (b) Here, the age of the two groups is the same, suggesting that the phenotypic rate of bill evolution in the high-diversity group would have been higher than in the low-diversity group. Taken from Wainwright (2007). Although not shown, it is also important to consider extinction of intermediate states in older groups as a generator of extant morphological diversity.

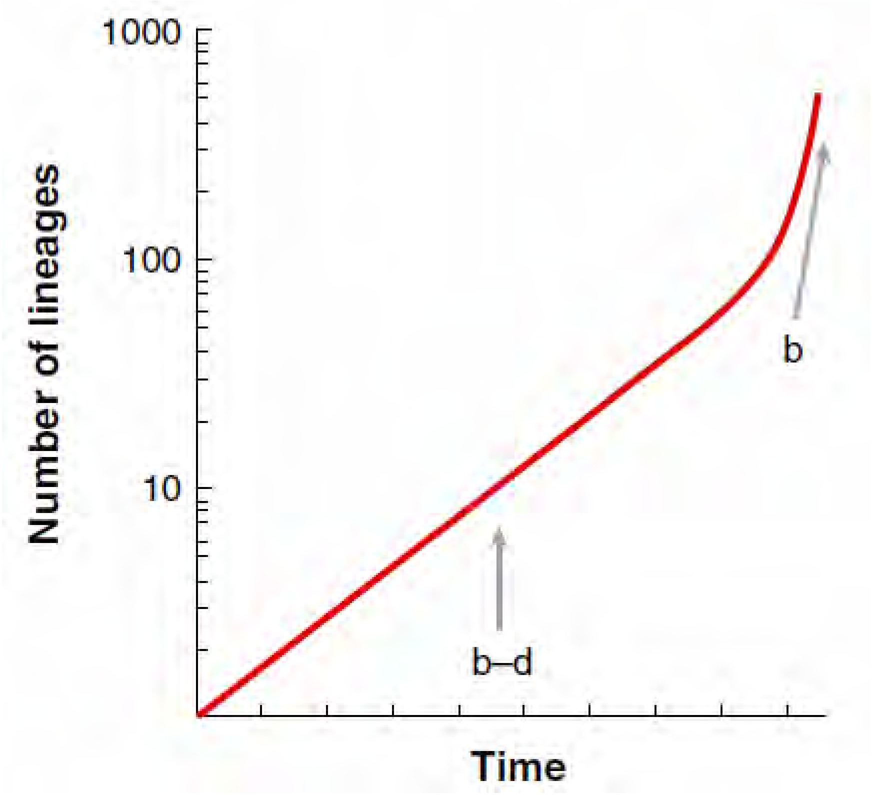

Figure 4. Theoretical plot showing the expected cumulative increase in the logarithm of the number of lineages in a molecular phylogeny growing according to a birth-death process. The theory was developed by Nee et al. (1994) and the Figure was taken from Nee (2006). Arrow close to (b) represents the stage known as "pull of the present", where the slope of the curve is proportional to the speciation rate.

BOXES

BOX 1

(TAXONOMIC) DIVERSIFICATION MODELS

Deterministic branching models

The most basic equation to predict species diversity as a function of branching probability (λ), extinction probability (μ), and time (*t*) is:

$m_t = ae^{(\lambda - \mu)t}$

(Raup, 1985) where *a* is the initial number of species and m_t is the number of species after some elapsed time, *t*. This general model is termed "time homogeneous", because λ and μ do not change through time. It describes an exponential growth if branching exceeds extinction (λ > μ) or exponential decay if extinction exceeds branching (λ < μ). If (λ - μ) is barely larger than zero, growth of diversity will be slow and, and as (λ - μ) increases in numerical value, the rate of cladogenetic diversification approaches the exponential characteristic of radiations (Raup, 1985). Several estimates can be obtained with the described equation, for example, the probability of survival of the clade, the expected number of species at time *t* if the clade survives or the total progeny or the number of lineages produced between 0 and time *t*.

Other models have been elaborated as an extension of the "time homogeneous", which intend to reflect more accurately the empirical data observed in the fossil record, like the "time inhomogeneous" model or the kinetic model, where speciation and extinction rates are density dependent phenomena (Sepkoski 1978, 1979 in Raup, 1985).

Stochastic models of diversification

The "birth-death" model is an analog of the "time homogeneous", but instead of a single value for the number of species at time *t*, there is a probability distribution (Sanderson and Donoghue, 1996). Variations of this model are the "pure birth", "pure death" or the Markov model (Sanderson and Donoghue, 1996; Nee, 2006).

Analyzing diversification dynamics in evolutionary trees

Nee et al. (1994) developed what they called a "reconstructed process", based on the birth-death model. They derived the geometric distribution of lineages at any particular time in the phylogeny and the distribution of waiting times between cladogenetic events, by constructing a likelihood function to estimate birth and death rates. In this context, Rabosky (2006) used the Akaike information criterion to select between a set of rate-variable and rate-constant models of speciation and extinction, to statistically detect temporal variation in diversification rates.

An alternative for the likelihood-based approach is survival analysis (Paradis 1997, 1998), where a speciation event in the reconstructed phylogeny is a failure event in survival. This method has been used to test for temporal increases in net diversification rates (Near et al. 2003); however, because survival models do not include an extinction term, it is unclear whether they can distinguish it from constant background extinction rates (Rabosky, 2006).

Using tree shape

Statistical tests based on tree shape exploit information on the topological distribution of species diversity throughout entire trees (for example, Chan and Moore, 2002). The intention is to detect asymmetry as a fingerprint of imbalanced diversification rates (Agapow and Purvis, 2002).

BOX 2

HOPEFUL MONSTERS

The recent rise of developmental molecular genetics has brought back the discussion of what Goldshmidt termed “hopeful monsters” (Goldsmith 1940; in Dietrich, 2000; Chouard, 2010; Theißen, 2009). Saltationism states the possibility that a mutational event during the reproduction of a single organism can lead to the origin of a new higher taxon as a possible mechanistic explanation of the theories of quantum evolution (Stanley, 1979). The modern interpretation of these ideas rely on experimental evidence showing that subtle genetic changes can have vast effects in the developmental mechanisms of an organism (Chouard, 2010; Hernández-Hernández et al., 2007), thus allowing the possibility of a sudden emergence of characters with high adaptive values (the hopeful monster). Although the possibility of a hopeful monster exist, there is a low probability that it will find a mate and produce fertile offspring, or in the case of self fertilization, that this emerging phenotype will in fact increase instantaneously its fitness. Therefore, it is more probable that a lineage including few species but large morphological diversity be the result of large extinction rates of intermediate lineages over geologic times. On the other hand, the scarce morphological diversity found in a clade rich in number of species may represent a decrease in extinction rates or that the clade is young enough to have time for recently appeared species to go extinct (see Figure 4).

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CAPÍTULO II

RADIACIONES EVOLUTIVAS DE SUCULENTAS EN AIZOACEAE, DIDIEREACEAE Y CACTACEAE (CARYOPHYLLALES, ANGIOSPERMAS) EN REGIONES ÁRIDAS DEL SUR DE ÁFRICA Y NORTE AMÉRICA. EVIDENCIA SOBRE EL ORIGEN DE BIOMAS ÁRIDOS

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Las plantas suculentas se asocian comúnmente a las regiones áridas del mundo. Un ambiente con limitaciones en la disponibilidad de agua puede determinar presiones adaptativas bajo las cuales los linajes de plantas evolucionan características morfológicas que les permiten soportar ambientes secos, siendo el desarrollo de tejido de almacenamiento de agua, o suculencia, uno de los caracteres más evidentes. Diferentes fuentes de evidencia muestran que las zonas secas del mundo se originaron muy recientemente, durante el Cenozoico tardío, y esto debió tener grandes consecuencias para la flora. Sin embargo, por el momento no existe evidencia fósil que indique posibles fechas del origen de vegetación adaptada a condiciones de aridez. Aunque la suculencia se originó de manera independiente en diferentes linajes de angiospermas, todas las formas suculentas de crecimiento aparecieron en diferentes familias dentro del Orden Caryophyllales; lo cual lo hace un excelente grupo taxonómico para estudiar la evolución de la flora de zonas áridas. Los resultados de análisis filogenéticos con base en los datos moleculares disponibles han mostrado que los linajes suculentos al interior del Orden se agrupan en un clado denominado “Caryophyllales centrales superiores”, y dentro de éste existen tres familias con los niveles más altos de suculencia, los cuales han sido reconocidos como monofiléticos por largo tiempo, tales familias son Didiereaceae, Aizoaceae y Cactaceae. Los linajes más ricos en especies al interior de estas familias alcanzan su máxima diversidad en lugares con condiciones ambientales similares, la familia Cactaceae en los desiertos del norte y sur de América, Didiereaceae en las zonas áridas de Madagascar y Aizoaceae en el sur de África. En este estudio integramos resultados de análisis filogenéticos, fechamientos con base en relojes moleculares relajados y estimaciones de tasas absolutas de diversificación para entender mejor la evolución y diversificación de estas radiaciones de suculentas. Adicionalmente, usamos métodos de modelación de nichos ecológicos para obtener las condiciones climáticas más convenientes para las especies incluidas en cada radiación, y para identificar posibles áreas potenciales de riqueza para cada clado en África y América, posibilitando una comparación de las condiciones climáticas en cada una de ellas. Nuestros resultados indican que aunque el origen de las “Caryophyllales centrales superiores” puede remontarse al Cretácico Tardío, los clados monofiléticos que concentran la mayor diversidad de suculentas en Cactaceae, Aizoaceae y Didiereaceae son relativamente recientes, habiéndose diversificando cada uno en diferentes momentos durante los últimos nueve millones de años (MA), cuando las condiciones áridas locales probablemente ya estaban presentes. Encontramos que los clados derivados dentro de cada familia y ricos en especies, tienen también elevadas tasas de diversificación en comparación con otros linajes que incluyen un número similar de especies. Observamos que los nichos ecológicos potenciales de las especies al interior de cada radiación están determinados por diferentes variables climáticas, revelando una posible divergencia ancestral de nicho y la singularidad de cada área de riqueza de suculentas en el sur de África, Norteamérica y Madagascar. Estas radiaciones de suculentas pueden caracterizarse por adaptaciones xerofíticas similares para habitar zonas áridas distintas, reflejando una diversificación a partir de un ancestro común siguiendo trayectorias evolutivas diferentes. Sin embargo, para mejorar las conclusiones sobre la interpretación ecológica de éstas radiaciones de suculentas, se requiere un incremento en el muestreo de datos de localidades para el análisis de nichos ecológicos, y un análisis e interpretación más profunda de las variables climáticas importantes que determinan cada radiación. Discutimos el trabajo adicional que se requiere para mejorar este estudio.

CHAPTER II

EVOLUTIONARY RADIATION OF SUCCULENT AIZOACEAE, DIDIEREACEAE AND CACTACEAE LINEAGES (CARYOPHYLLALES, ANGIOSPERMAE) IN ARID REGIONS OF AFRICA AND AMERICA. INSIGHTS INTO THE ORIGIN OF ARID BIOMES

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Succulent plants are strongly associated with arid regions across the world. A water-stressed environment can confer the adaptive pressure under which several plant lineages evolved morphological characteristics that allow them to withstand dryness, being water storage tissues -succulence-, one of the most evident. Different sources of evidence show that dry areas of the world originated recently (during the late Cenozoic), with great consequences for the flora, however, there is no fossil evidence providing a possible date for the origin of arid-adapted vegetation. Although succulence originated independently in many distantly related lineages, all existent succulent growth forms evolved within different families of the Order Caryophyllales, making it an excellent group to study evolution of arid-adapted flora. Recent molecular phylogenies have shown that caryophyllid succulent lineages belong to a clade named “higher core-Caryophyllales”, and three families (Didiereaceae, Aizoaceae and

Cactaceae) which have been recognized as monophyletic for a long time, show the greatest levels of succulence. The richest lineages within these families reach their highest diversity in places with similar environmental conditions, Cactaceae in deserts of North and South America, Didiereaceae in arid zones of Madagascar and Aizoaceae in Southern Africa. In this study we integrate phylogenetic results, dates from relaxed molecular clocks and estimates of absolute diversification rates to understand the evolution and diversification of these succulent radiations. Additionally, we used ecological niche modeling methods to reconstruct the most suitable environmental conditions for species within each radiation, to identify potential richness areas for radiating clades in Africa and America, and to compare their climatic conditions. Our results indicate that although the origin of the higher core- Caryophyllales date back to the Late Cretaceous, monophyletic clades concentrating the majority of the succulents' diversity within families Cactaceae, Aizoaceae and Didiereaceae are much younger. These clades diversified each at different times during the last 9 million years (MY), probably when local arid conditions were already present. We found that rich derived clades within Aizoaceae and Cactaceae have higher diversification rates than closely related lineages including a similar number of species. We observed that potential ecological niches for species within Aizoaceae, Didiereaceae and Cactaceae rich clades are determined mainly by different climatic variables, revealing a possible ancient niche divergence and the uniqueness on their succulent hotspots in southern Africa, North America and southwestern Madagascar. These succulent radiations might be relying on similar xerophytic adaptations to withstand distinctly arid conditions, reflecting a diversification from a common ancestor following distinct evolutionary trajectories. However, to improve conclusions on the ecological interpretation of these succulent radiations, an increased sampling of locality data for the ecological niches analyses and a deeper analysis and interpretation of the important climatic variables resulting for each radiation are needed. We discuss the additional further work required to improve this work.

INTRODUCTION

Of all the great climatic groups, dry climates are the most extensively developed over the land surface of the earth, occupying 26% of the continental area (McGinnies, 1979). They are due to various causes, the most important being the presence of subsiding or stabilized air masses associated with the descending limbs of Hadley cells in subtropical, low latitudes (Ziegler et al., 2003). Other possible causes are their geographical position in intercontinental regions isolated from moisture sources, or their localization within orographic rain shadows (Ziegler et al., 2003; Tarbuck and Lutgens, 1999). In desertic or arid ecosystems, precipitation is low, highly variable and largely random through the year, and occurs in infrequent discrete events (Noy-Meir, 1973; Von Willert et al., 1992; Evenari, 1985), hence, making water availability the dominant controlling factor of biological processes.

There are some evidences for increases in aridity from the Cretaceous period (Ziegler et al., 2003). Palinological data from the mid Cretaceous support the presence of dry or savanna-like vegetation across the tropics of Africa and South America (Dino et al., 1999 in Ziegler et al., 2003); and reviews of the sediments, soils and megafossils of the Saharan region support an increase in aridity, with pronounced seasonality in rainfall (Lefranc and Guiraud, 1990; Busson and Cornée, 1991 in Ziegler et al., 2003). Although evaporite deposits characterizing deserts and related areas from the Tertiary are located in the same positions as today (Ziegler et al., 2003), -suggesting the presence of dry areas since that time-; there is no fossil record for desert floras.

Desert biomes of the world are considered to have appeared recently, being in most cases no older than the Miocene/Pliocene (Berggreb et al., 1995 in Moore and Jansen, 2006). For example, modern vegetation of the Sonoran desert has been proposed to date from the Pliocene-Pleistocene (2 MYA; Phillips and Comus, 2000), from the Pleistocene (Axelrod, 1979); or from the middle Miocene (15-8 MYA, Van Devender, 2000). Arid conditions in the Chihuahuan desert have been proposed to date from the middle Miocene (Morafka, 1977; in Wilson and Pitts, 2010), suggesting a recent origin for the flora inhabiting there; and fossil grasses found in the Mojave Desert suggest that Mediterranean chaparral-type grasslands had established in that region by he Miocene (Tidwell and Nambudiri, 1989). Evidence from sedimentary deposits suggest that aridity on the west coastal margins of southern Africa was established during the late Cenozoic, and the parallel evolution of aridity in Australia suggest that the climatic changes were global (Tankard and Rogers, 1978).

The origin of water-stressed environments could confer the adaptive pressure under which several plant lineages evolved a variety of specialized structures that enabled them to withstand periods of severe drought (Axelord, 1972; Gibson, 1996), which represent distinct evolutionary routes of morphological changes (Von Willert et al. 1992). For example, the presence of reduced leaf size, specialized leaf covering (scales, trichomes), deciduous habit, deep root system, swollen trunks, or the development of water storage tissues, i.e. succulence (Axelord, 1972). Studies of plant genera adapted to survive in arid environments have revealed insights into the origin of desert biomes. The genus *Prosopis* (Fabaceae) is an important member of arid and semiarid regions of the world, and a recent study estimated that the diversification of main groups within the genus occurred relatively recently, during the Late Miocene, while the diversification of the richest mesquite clade started during the Pliocene (Catalano et al., 2008). Divergence of main lineages within the genus *Tiquilia* (Boraginaceae), which inhabits North American deserts, has been estimated to occur during the early to the late Miocene (Moore and Jansen, 2006). Other examples of xerophytic genera diversifying during the Mid to Late Cenozoic are *Nolana* (Solanaceae) in the coastal deserts of Peru and Chile (Dillon et al., 2009) or *Agave* (Agavaceae) in arid North American regions (Good-Avila et al., 2006).

The order Caryophyllales represents an excellent group to study the evolution of arid-adapted lineages, in the search for clues that help to improve our knowledge about the origin and evolution of arid biomes in Africa and America. Within this order, the subclade named "higher core-Caryophyllales" includes a group of lineages that evolved strategies to inhabit water-stressed environments, succulence being the most evident of them (Cuenoud et al., 2002). As members of this clade, the families Cactaceae, Portulacaceae, Basellaceae, Didieraceae and Halophytaceae were usually placed together on a clade named "succulent clade" (Cuenoud et al., 2002). This clade has been referred also as the Portulacaceous cohort (Nyffeler and Eggli, 2010; Applequist and Wallace, 2001), or the suborder Portulacineae (Engler, 1898 in Nyffeler and Eggli, 2010). Other families including succulent members, such as Aizoaceae and Nyctaginaceae (Cuenoud et al., 2002), are closely related to the suborder Portulacineae. Although the monophyly of several families within the succulent clade has not been confirmed with molecular studies, the succulent families Aizoaceae, Cactaceae and Didiereaceae have long been recognized as monophyletic entities both with molecular and morphological data , and together -particularly lineages within Cactaceae and Aizoaceae-, comprise the largest radiations of succulent plants in terms of species number and morphological diversity (see Figure 1, Cuenoud et al., 2002; Nyffeler, 2002; Nyffeler et al., 2008; Hernández-Hernández et al., in prep.; Klak et al., 2004; Applequist and Wallace, 2000, 2001).

Plant adaptations to arid environments, such as succulence, reveal a strong correlation between organisms and the climatic conditions in which they live (Gibson, 1996) and thus may evolve independently in different lineages in response to similar natural selection regimes. Molecular phylogenies of Cactaceae, Aizoaceae and Didiereaceae indicate that their early diverging members show less degrees of succulence and include fewer species than derived clades (Nyffeler, 2002; Hernández-Hernández et al., 2010; Klak et al., 2003; Applequist and Wallace, 2000). Additionally, in contrast to derived clades, the early divergent slightly- or non-succulent members tend to have broader distributions, while species within derived clades tend to concentrate in localized arid regions, indicating a possible evolutionary trend (Axelrod, 1972). Extremely succulent tissues could have evolved independently in Cactaceae, Aizoaceae and Didiereaceae lineages with few modifications such as increasing the volume of cortex and pith and evolving mechanisms (spines or poisons) that protect stored water (Mauseth, 2004a, b).

In this study, we provide a temporal framework for the radiation of succulent lineages within Cactaceae, Aizoaceae and Didiereaceae within the higher core-Caryophyllales, using molecular phylogenies and relaxed molecular clocks. We estimated diversification rates for families and richest clades, to detect lineages with remarkable increases in speciation rates that might be coincident with the origin of succulence and aridification, as possible indicators of evolutionary radiation patterns (Hernández-Hernández et al., in prep. See Chapter I). Finally, to provide an ecological framework for the xerophytic adaptations that possibly fostered the radiation of succulents in different arid regions of Africa and America, we implemented niche modeling methods and generated potential distributions for species within the richest clades, using distribution data and nineteen climatic variables. Climatic variables are related to temperature and precipitation, and represent a combination of annual trends, seasonality and extreme environmental conditions (Hijmans et al. 2005). Climatic niches have been used to approach evolutionary questions successfully, as an indirect approach to phenotypic evolution (Smith and Beaulieu, 2009) and the integration of phylogenies and niche models is an emerging promising area (Evans et al., 2009; Graham et al., 2004; Kozak et al., 2008, Yesson and Culham, 2006). Here we used a phylogenetic approach, considering monophyletic clades as study entities. This approach allowed us to detect phylogenetic potential richness areas (PPRAs) for clades within Caryophyllales, without relying only on locality data that might be subject to sampling errors. We were able to characterize the most important climatic variables which determine potential distributions for species within radiations and PPRAs, and discuss differences between them and the possible evolutionary trends fostering the local evolution of these arid-adapted clades. Additionally, we projected the potential ecological niches of species of one succulent radiation into the geographical richness area of other radiations, to test for the equivalence of the climatic conditions on each area.

The interpretation of climatic niche information and potential distributions for species, on the framework of dated phylogenies, is a powerful tool to understand evolution in a geographical context (Evans et al., 2009), but further work is still needed to successfully integrate these data. With this approach we studied the evolutionary radiation of three important succulent plant lineages in arid regions of Africa and America, aiming at improving our understanding of the evolution of arid-adapted vegetation.

MATERIALS AND METHODS

Sequences.- We assembled four DNA sequences matrix datasets. One for the order Caryophyllales, and three others for Cactaceae, Aizoaceae and Didiereaceae families separately. For the order Caryophyllales we used *matK*, *atpB*, *rbcL* and 18S genetic regions representatives of the different families. Sequences were downloaded from the GenBank angiosperm nucleotide database, using Blast under default options. Different query sequences were used to ensure full taxonomic coverage on the GenBank database, and were chosen to represent different taxonomic clades following the previous Caryophyllales phylogeny reported by Cuenoud et al. (2002). We excluded sequences from overrepresented genera, by randomly choosing one (or more, depending on the genus size) species and excluding the remaining sequences. Our final Caryophyllales matrix includes at least one species-sequence representative of each genus present in the GenBank database. The final concatenated matrix for Caryophyllales includes 144 taxa and 6,183 characters. Outgroups were selected following Cuenoud et al. (2002).

The same approach was followed to assemble sequences data matrices for Aizoaceae and Didiereaceae families. Query sequences for the Blast searches on GenBank were selected following Klak et al. (2003) and Applequist and Wallace (2000). For Aizoaceae we used *atpB-rbcL*, *psbA-trnH*, *rps16* and *trnL-trnF* genetic regions, and the final concatenated matrix includes 88 taxa and 4182 characters. For Didieraceae we selected *rpl16*, *trnL-trnF* and *trnT-trnL* regions to obtain a concatenated final matrix including 15 taxa and 2007 characters. Outgroups for each family were selected following Klak et al. (2003) and Applequist and Wallace (2000) respectively.

For Cactaceae we used a chloroplast and nuclear sequences matrix dataset including *trnK-matK*, *matK*, *trnL-trnF, rpl16* and *ppc*, which was assembled for a phylogenetic study of the family (Hernández-Hernández et al., 2010, in prep. Chapter III.1). The final matrix includes 226 taxa and 6,664 characters. Outgroups were selected following Hernández-Hernández et al., (2010, see Chapter III.1).

Phylogenetic analyses.- We estimated phylogenies for each of the four assembled matrixes: for the Order Caryophyllales and for the Aizoaceae, Cactaceae and Didiereaceae families. We estimated model parameters for each genetic region in each dataset and for the first, second and third codon positions in the case of coding regions using in PAUP v4.b10 (Swofford, 2002) with the modelBlock provided in Modeltest v3.7 (Posada and Crandall, 1998). A visual examination of these parameters was useful to determine data partitions for phylogenetic analyses. For the Caryophyllales matrix we implemented two data partitions: (1) first and second codon positions in coding regions and (2) third codon positions in coding regions plus the 18S region. For Aizoaceae, Didiereaceae and Cactaceae data matrixes, data partitioning followed the different genetic regions included.

Phylogenetic trees for the Caryophyllales and the Didiereaceae and Aizoaceae matrixes were obtained using Bayesian methods implemented in MrBayes v3.1.2p (Huelsenbeck and Ronquist, 2001). We implemented independent models with unlinked parameters to each partition and variable rate priors. Each model specified six substitution types, a proportion of invariant sites and a gamma distribution for substitution rates between sites with four rate categories. Two independent Metropolis coupled-Markov chain Monte Carlo (MC^3) runs of 10 $\times 10^6$ generations, each consisting of four incrementally heated chains (temp = 0.2), were conducted, sampling one tree every 300 generations. Chain convergence for parameter values was evaluated with Tracer v1.4.1 (Rambaut and Drummond, 2007). The maximum a posteriori (MAP) tree topology for each bayesian analyses was selected as working hypothesis for dating analyses. We obtained branch lengths for trees by randomly selecting one tree out of the group of trees sharing the MAP topology. These phylogenies were used for dating analyses.

The data matrix for Cactaceae showed chain convergence problems in Bayesian analyses, hence, we inferred a phylogeny using maximum Likelihood methods implemented in RAxML v7.0.4 (Stamatakis, 2006), as described by Hernández-Hernández, et al. (2010, in prep; see Chapter III.1). The obtained phylogenies were used as working hypotheses for dating analyses.

Fossil information, calibrations and divergence date estimation.- Estimation of divergence dates was performed in a stepwise basis. First, we obtained dates for the divergence of main groups within Caryophyllales, based on solid calibration with information taken from the fossil record. These dates were subsequently used as calibration dates for analyses conducted on the independent family phylogenies for Cactaceae, Didiereaceae and Aizoaceae.

A review for the fossil record of Caryophyllales yielded several calibration points that could be used given our ingroup and outgroup sampling:

[1] A fossil infructescence assigned to Phytolaccaceae was recently discovered from the Upper Cretaceous (Late Campanian) of Coahuila, México (Cevallos-Ferriz et al. 2008). The infructescence shares reproductive characters with the genus *Phytolacca*, however it has a fixed number of carpels per fruit and a pendulous placentation that support its recognition as a new taxon related to it, thus indicating a possible minimum date for the origin of the group including *Phytolacca*. Although previous molecular phylogenies show that Phytolacaceae is a polyphyletic assemblage (Cuenoud et al., 2002), reported molecular phylogenies as well as our Bayesian analyses showed an association of the Phytolacadoideae sampled members (*Ercilla* and *Phytolacca*) with strong support values (See Fig.1, also Cuenoud et al., 2002), thus making reasonable to consider the fossil as part of this group. The old age assigned to the sediments where the fossil infructescence was found are only slightly earlier than the Santonian-Campanian age reported for pollen of Caryophyllales (Collinson et al., 1993),

falling within the time window for the oldest fossil record of several core eudicot families (Magallón and Sanderson, 2001). We considered this fossil could give an approximation to the stem group minimal date for the entire clade including Phytolacaceae, which also includes members of other families such as Sarcobataceae, Agdestidaceae, Nyctaginaceae and Gisekiaceae, within the Core Caryophyllales.

[2] A fossil inflorescence from the Middle-Late Eocene sediments of northeastern Tasmania also assigned to Caryophyllales. Parsimony analyses suggested this fossil belongs to Caryophyllaceae, either to the subfamily Alsinoideae or Caryophylloideae (Jordan and Macphail, 2003). As the dates assigned to the sediments where this fossil was found are relatively young as to represent a stem group date, we used it as minimal date for the Caryophyllales crown group, which also shows strong support values in our Bayesian analyses.

[3] We also used the date for Caryophyllales derived from fossil pollen of the genus *Aldrovanda* dating back from at least the early Eocene (Krutzsch, 1970 in Yesson and Culham, 2006). The aquatic, monospecific genus *Aldrovanda* has an excellent fossil record (Cameron et al., 2002; Degreef, 1997; Yakubovskaya, 1991; in Yesson and Culham, 2006) based on both pollen (Muller, 1981) and seeds (Yakubovskaya, 1991; in Yesson and Culham, 2006). The fossilized seeds are sufficiently distinct for Yakubovskaya (1991) to recognize two distinct *Aldrovanda* lineages amongst 13 named fossil species. *Aldrovanda* seeds show records from the Pleistocene, Pliocene, Miocene, and Oligocene through to the Eocene (Yakubovskaya, 1991), but at present, the oldest seeds of *Aldrovanda* date from the early to middle Eocene (Chandler, 1961; Reid, 1926). This date was also used as a minimal age for the Droseraceae family.

[4] Finally, given our outgroup sampling, we used the information of fossil pollen grains from the genus *Normapoles* characteristic of the Normapolles group (Fagales), within the core eudicots, which date from the Middle-Cenomanian (Pacltová 1966, 1981).

Divergence times for clades and lineages for the Caryophyllales phylogeny and for the Cactaceae, Aizoaceae and Didiereaceae phylogenies were estimated with penalized likelihood (PL; Sanderson, 2002 , 2004) in r8s version 1.71 (Sanderson, 2003 , 2004). Penalized likelihood is a molecular-based semi parametric method that incorporates among-lineage rate heterogeneity and can use fossil information as auxiliary in divergence time estimation. We transformed fossil stratigraphic information into absolute dates following the International Stratigraphic Chart (ICS, IUGS, www.stratigraphy.org) and used fossil information in r8s v1.71 calibration as follows: [1] The *Phytolacca* infructescence date (Late Campanian, 71.2 MA) as a maximum age constraint for the clade including Phytolacaceae members as well as Sarcobataceae, Agdestidaceae, Nyctaginaceae and Gisekiaceae members within the Core Caryophyllales (See Figure 1), [2] and [3] The *Caryophyllaceae* inflorescence and *Aldrovanda* seeds dates (both Late Eocene, 33.9 MA) as minimum age constraints for the stem group of the Caryophyllales clade and for the origin of the *Aldrovanda* + *Dionaea* + *Drosera* clade respectively, and [4] the *Normapoles* pollen (Middle-Cenomanian, 96.2 MA) date as a fixed age for the Caryophyllales and core Eudicot members present in outgroups (see Figure 1). Penalized likelihood, implemented in r8s, requires a user-defined parameter to specify the level of molecular rate smoothing to be implemented in dating analysis. To identify the smoothing magnitude (λ) that best describes the available data, we used a cross validation procedure that calculates the predictive error associated to molecular rate estimates across the full tree, derived from sequentially pruning terminal branches using the selected MAP topology for Caryophyllales described above (Sanderson, 2004). Each cross validation tested 16 smoothing magnitudes ranging from log λ 10 = − 2 to 5.5 at 0.5 intervals, which comprise a broad spectrum of substitution regimes.

Subsequent to the r8s analyses, we implemented a relaxed uncorrelated lognormal clock model to the Caryophyllales phylogeny using BEAST v1.4.6 (Drummond and Rambaut, 2007). BEAST is a Bayesian Markov chain Monte Carlo method that incorporates a relaxed molecular clock model, thus accounting for the time-dependent nature of the evolutionary process. In BEAST, rates are uncorrelated across the tree, being independently drawn from a parametric distribution (Drummond et al., 2006; Drummond and Rambaut, 2007), while r8s assumes there is an autocorrelation between rates of parental and descendent lineages. Outgroup taxa were removed prior to analyses and a Birth-Death speciation process (Gernhard 2008) was used as prior for the MCMC runs to infer the time to most recent common ancestor. We used BEAST because it allows great flexibility in data analyses and in the implementation of fossil information, allowing a probabilistic calibration into the prior assumptions (Drummond and Rambaut, 2007). We implemented two data partitions (see *Phylogenetic analyses* methods) with independent unlinked General Time Revertible (GTR) substitution models with four gamma categories and a proportion of invariable sites for each. The analysis consisted of two independent runs of 50×10^6 generations, with parameters sampled every 2000 generations. Fossil data can only indicate minimum dates for the species possessing diagnostic characters, however, the origin of the referred taxa must be older than their first occurrence in the fossil record, in order to leave some time for the evolution of the diagnostic characters, speciation, increasing its abundance and the probability of its fossilization. Is has been stressed out that the first appearance of any taxa in the fossil record represents the time it became abundant rather than the time of its emergence (Magallón, 2004; Forest, 2009). For the BEAST analyses, we used the same fossil information for Caryophyllales mentioned above, implementing a lognormal prior distribution for each calibration date; and we arbitrarily added 10 MY to the dates obtained from the fossil record to set the median for each distribution.

To obtain dates for clades within Aizoaceae, Didiereaceae and Cactaceae we implemented independent divergence dating penalized likelihood analyses in r8s v1.7.1, using the phylogeny estimated for each family (see above). We used the stem group dates obtained in the Caryophyllales BEAST analyses for each family (see description above), implementing the corresponding estimated dates as fixed ages at the stem group node of each family, following the procedures explained above for the Caryophyllales r8s analyses.

Diversification rates.- Diversification rates were calculated using method-of-moments estimators (Rohatgi, 1976) in the context of a birth-and-death model (Kendall, 1948) that considers the species taxonomic diversity and age of a clade. These estimators provide absolute estimates of the rate of diversification of a clade, they are conditional on the survival of the clade to a given time *t*, in this case, the present, and they can differentially estimate the diversification rate of a stem clade or of a crown clade (Doyle and Donoghue,1993 ; Magallón and Sanderson, 2001). The conditional estimators of absolute diversification rates were calculated for stem and crown groups following eqs. 6 and 7 in Magallón and Sanderson (2001). The relative extinction rate (ε) is defined as $\varepsilon = \mu / \lambda$. Because absolute speciation and extinction rates for clades are unknown, diversification rates were estimated assuming that the relative extinction rate is bounded within $\varepsilon = 0.0$, which implies no extinction, and $\varepsilon = 0.9$, which implies a very high relative extinction rate. Whereas $\varepsilon = 0.0$ represents an absolute lower bound for the relative extinction rate, the selection of $\varepsilon = 0.9$ as an upper bound is arbitrary (Magallón and Sanderson, 2001).

Locality data and climatic variables.- Although arid regions in South America host several rich cacti lineages, the highest diversity of Cactaceae in terms of number of species and endemisms can be found in certain arid regions of North America (Anderson, 2001, Hunt, 1999; Hunt et al., 2006; Hernández et al., 2001). These regions host the important radiations of columnar and globose cacti species within the tribes Cacteae and core-Pachycereeae (Hernández-Hernández et al., 2010, in prep, see Chapter III.1). Occurrence locality data for 210 species within Cacteae and 93 species within core Pachycereeae tribes were obtained from the REMIB database at CONABIO (http://www.conabio.gob.mx/remib/doctos/remib_esp.html) and the Global Biodiversity Information Facility (GBIF) database (www.gbif.org). These localities were mapped and checked in ArcView v3.2, excluding probable mistaken records following Guzmán and Arias (2003). Occurrence locality data for species included in Didiereaceae were obtained from the GBIF database and from the Tropicos database in the Missouri Botanical Garden (MO) herbarium (http://www.tropicos.org/).

Rischioideae is a leaf succulent tribe within Aizoaceae, which concentrates around 80% of the family's diversity (Klak et al., 2003). Occurrence locality data for 582 species included in the tribe were provided by Dr. Matt H. Buys at SANBI, taken from exemplars at the Compton Herbarium. Localities were checked following Smith et al. (1998).

Environmental data were obtained from WORLDCLIM (version 1.3, http://www.worldclim.org explained in Hijmans et al. 2005), which contains climatic data layers at a spatial resolution of 30 arc seconds (~1 km resolution) obtained by interpolation of climate station records from 1950–2000. From this climate data, 19 climate variables are derived: annual mean temperature [1], mean monthly temperature range [2], isothermality [3], temperature seasonality [4], maximum temperature of warmest month [5], minimum temperature of coldest month [6], temperature annual range [7], mean temperature of wettest quarter [8], mean temperature of driest quarter [9], mean temperature of warmest quarter [10], mean temperature of coldest quarter [11], annual precipitation [12], precipitation of wettest month [13], precipitation of driest month [14], precipitation seasonality [15], precipitation of wettest quarter [16], precipitation of driest quarter [17], precipitation of warmest quarter [18], and precipitation of coldest quarter [19]. These climate variables represent a combination of annual trends, seasonality and extreme environmental conditions.

Niche modeling and potential richness areas.- Our occurrence locality database was divided into two sets, the first one including species with fewer than 20 records and the second set including species with more than 20 records. Species with fewer than 5 records were excluded from the analyses, although they may represent narrowly endemic species. Niche models for each species within the first dataset were obtained without training data, and for the second dataset, 50% of data were used as training. The potential ecological niche for each species was modeled using the MAXENT software (MAXENT; Phillips et al. 2006, available athttp://www.cs.princeton.edu/*schapire/maxent). MAXENT also helped us to evaluate which climatic variable made the greatest contribution to niche models for species. We obtained averages for the estimates of relative contributions of each environmental variable to the MAXENT model for species within each clade. Maps for species predicted distribution were overlapped in ArcView v.3.2 to obtain potential richness areas. We classified areas with low, medium or high number of predicted species and selected the last ones as the richest for each succulent clade.

RESULTS

The phylogeny obtained for the Order Caryophyllales (Figure 2) is highly similar to that reported by Cuenoud et al. (2002); which included slightly less taxa than the one reported here. In general, relationships are well supported with Posterior Probability (pp) values. As reported by Cuenoud et al. (2002), two main sister clades are distinguished after the early *Rhabdodendron* divergence, which have been named “core” and “noncore” Caryophyllales. The non-core Caryophyllales clade includes important families with a high number of species such as Plumbaginaceae and Polygonaceae. The core Caryophyllales is divided into two strongly supported clades (Figure 2). One of these sister clades is composed by two large families, Amaranthaceae and Caryophyllaceae. The other clade has been named “higher core-Caryophyllales” (Cuenoud et al., 2002), and includes the suborder Portulacacineae (succulent clade) as sister to a clade conformed by Aizoaceae, Nyctaginaceae and Phytolacaceae. According to previous studies (Cuenoud et al., 2002; Nyffeler and Eggli, 2010), several families resulted as poly- or paraphyletic, for example, Molluginaceae, Phytolacaceae and

Portulacaceae. Results for Aizoaceae, Didiereaceae and Cactaceae were highly congruent with Klak et al., 2004; Applequist and Wallace, 2000; and Hernández-Hernández et al., 2010 (in prep, see Chapter III.1), respectively.

From the phylogenies obtained, only supported clades were considered to further estimate their possible date of diversification and its absolute diversification rates. These results are shown in Table 1. At the family level, the taxonomic groups with the highest diversification rates are Cactaceae and Aizoaceae, even considering a possible high extinction scenario. Their diversification rates are even higher than other families within the Order which include a similar number of species (for example, Amaranthaceae or Polygonaceae).

We selected clades within each succulent family possessing the highest number of species to estimate their dates of origin and diversification rates. For Aizoaceae, we selected the Ruschioideae subfamily (See Figure 1). This clade represents a large group of about 1,563 species in 101 genera and thus constitutes about 85% of the Aizoaceae (Klak et al., 2004).

For Cactaceae we selected two major lineages representing the largest radiations within the family, which occurred in North America. The Cacteae tribe has been consistently recovered as monophyletic in molecular phylogenetic studies (Nyffeler, 2002; Hernández-Hernández et al., 2010; in prep, see Chapter III.1), and includes around 355 species of globose or barrel cacti, representing about 25.3% of Cactaceae species (Hunt et al., 2006). Another important radiation within Cactaceae occurred in the arborescent or columnar lineages from Mexico and southwestern USA. These lineages are grouped together in the core-Pachycereeae clade, that has also been recovered as monophyletic with good support values (Hernández-Hernández et al., 2010, in prep, see Chapter III.1), and includes 169 species representing nearly 12% of the family's diversity.

Didiereaceae is a small family of species inhabiting the Madagascar Island. Within Didiereaceae we selected the clade which includes members showing the highest levels of succulence, hence excluding the early divergent *Alluaudiopsis* and *Calyoptrotheca*, which are only weakly succulent (See Figure 1). Dates for the origin and diversification of these clades are shown in Table 1.

Richness areas obtained for species within each of the succulent clades are shown in Figures 3 to 6. A table showing a description of each climatic layer used in the potential ecological niches analyses is shown in Table 2, together with the climatic variables that most importantly determined the potential climatic niches for each clade. As shown, the climatic variables that most importantly determine the potential distribution for species within each clade are different, although some coincidences were found. Figures 7 to 10 show the projections of succulent radiations potential niches into each other's geographic richness area.

DISCUSSION

It has been estimated that the Order Caryophyllales includes around 6.3% of the Eudicots diversity (Magallón et al. 1999) and that it probably originated during the Albian (111-104 million years ago, MYA), although Rhabdodendraceae does not split off until 90-83 MYA (Wikström et al. 2001). Anderson et al. (2005: Rhabdodendraceae also included) suggest dates of 116-114 MYA for the stem group Caryophyllales, and 102-99 MYA for the crown group. According to our analyses, we estimated slightly younger dates for the origin of the order, during the Cenomanian in the Upper Cretaceous (Table 2). Most of the extant flowering plant lineages appeared during the Cretaceous (Friis et al., 2006), but it has been proposed that the climate environmental? conditions where they appeared and started diversifying were humid, shady and disturbed (Field et al., 2004).

Some hypotheses on the origin of modern plants adapted to arid conditions suggest that the ancestors of these lineages could have evolved and/or survived in local patches of semi-arid woodland or tropical scrub, even in the complete absence of truly arid sites, because the majority of these desert-adapted plant lineages either occur outside of the deserts or have close relatives that do (Axelord, 1979; quoted by Moore and Jansen, 2006). The "preadaptations" or exaptations following Gould's terminology (Gould and Vrba, 1982; Donoghue, 2005) present in the ancestors of certain xerophytic lineages could have been the preamble for the huge diversification of succulent crown groups, producing a time interval between the dates for the origin of stem ancestral groups and a later radiation or massive diversification of the succulent lineages we see today (Doyle and Donoghue, 1993). Although it has been proposed that the main succulent lineages within Caryophyllales evolved succulence over the last 40 million years (MY) (Landrum, 2002), these dates are not congruent with our results and with data on the origin of arid biomes.

Our results indicate that the higher Core Caryophyllales clade, which includes the succulent and xerophytic lineages, originated early after the origin of the Order, also during the Upper Cretaceous. However, the succulent families such as Didiereaceae, Cactaceae and Aizoaceae are much younger, probably originating during the Miocene and Oligocene (Table 1). As shown by the phylogenetic distribution of taxa within each of these families, extreme succulence might have evolved from a non-succulent ancestor in all of them (see Klak et al., 2004; Applequist and Wallace, 2000; and Hernández-Hernández et al., 2010, in prep, see Chapter III.1). For instance, Cactaceae early diverging members of the genus *Pereskia* inhabit more mesic environments through Central and South America, showing an arboreal or arbustive life form with broaden non succulent leafs (Edwards et al., 2005) and they have been hypothesized as possessing ancestral characters within the family (Mauseth and Landrum, 1997; Edwards et al., 2005; Edwards and Donoghue, 2006). Morphological and taxonomical diversity within Cactaceae is concentrated in the subfamily Cactoideae, particularly in derived clades (Nyffeler, 2002; Hernández-Hernández, et al., 2010 in prep, see Chapter III.1). Although Cactoideae members are widely distributed throughout the continent, arid regions of Mexico and the United States represent the diversity center for this lineage, given the number of species and the amount of endemism in these areas (Ortega-Baes and Godinez-Alvarez, 2006). The most important Cactoideae geographical hotspots in north America are the Chihuahuan Desert, hosting a vast number of globular cacti within the tribe Cacteae (Hernandez et al., 2001; Hernández and Gómez-Hinostrosa, 2005), and the Tehuacán-Cuicatlan Valley and Sierra Madre Oriental in Mexico, hosting the most impressive variety of columnar cacti within the tribe Pachycereeae (Arias et al., 1997).

One important clade within Cactoideae includes all the globose and barrel cacti from North America: the tribe Cacteae. According to our analyses, although the distribution of members of Cacteae such as *Mammillaria* range from North America to Central America, the richness area resulting for the clade falls in the southernmost portion of the Chihuahuan Desert, reaching the arid regions of Oaxaca and Puebla crossing the Transvolcanic Belt (Figure 3). The North American Chihuahua desert as well as the Sonora and Mojave Deserts are considered as warm deserts (Shreve, 1942, in Riddle and Hafner, 2006), and are also estimated to be of a relatively young age, in most cases not older than the late Miocene/early Pliocene (7–5 MYA; Berggren et al., 1995, in Moore and Jansen, 2006). Our dates are coincident with this scenario, because Cacteae resulted with dates of origin of the stem group from the 10.7 MYA and the crown group from 4 MYA, during the Miocene and early Pliocene.

The Pachycereeae tribe is another important taxonomic group within Cactaceae with a diversity center in Mexico, and includes all the gigantic shrubby, arborescent and columnar cacti (see Chapter III.1). According to our PPRA map (Figure 4), its diversity center is coincident with two important mountain buildings, the Sierra Madre Occidental, along the west coast of Mexico, and the Transvolcanic Belt in central Mexico. The uplift and topographic complexity of these areas might have generated local ecological conditions and geographic isolation that fostered the diversification of the great columnar and arborescent forms. We estimated that the core-Pachycereeae clade originated more recently than the Cacteae clade, from 4.2 to 3.7 MYA during the early Pliocene. The diversification rate of both Cacteae and core-Pachycereeae are high in comparison to other Caryophyllales lineages, however, according to our results the core-Pachycereeae clade diversified more rapidly.

Aizoaceae succulent richest lineages probably evolved also from a non-succulent ancestor. Early diverging members of the family, particularly within Sesuvioideae, Tetragonioideae and Aizooideae subfamilies have only slightly succulent leaves and can be found worldwide in the tropics and subtropics (Smith et al., 1998; Klak et al., 2003; 2004). However, the highest diversity of the family is concentrated within the Ruschioideae subfamily, mainly in the Ruschiae tribe which includes the “stone plants” and several other tongue and rough leaved highly succulent mesembs (Klak et al., 2003; 2004). With few exceptions, members of Ruschioideae are endemic to southern Africa, with the greatest number of species found in the Succulent Karoo, characterized by the dominance of leaf succulents (Ihlenfeldt, 1994; Cowling et al., 1998). Our PPRA map for Ruschioideae suggest a richness area concentrated in western South Africa (Figure 5), as it was clearly expected (Klak et al., 2003, 2004; Linder, 2003). The climate of this region is Mediterranean, with dry summers and rain restricted to the winter months, becoming increasingly arid to the north, with a winter rainfall desert polewards (conforming the ‘Succulent Karoo’ vegetation) and a summer rainfall desert towards the equator (the ‘Nama Karoo’ vegetation) (Linder, 2003, 2005). Evidence from sedimentary deposits as well as vertebrate and invertebrate fossil faunas show that the aridity in Southern Africa was fully established in the Quaternary (Late Cenozoic). The Ruschioideae has been studied as an example of an outstanding radiation in the Cape peninsula, and it has been estimated that the diversification of the crown group started around 3.8 to 8.7 MYA, also during the late Miocene-early Pliocene (Klak et al., 2003); with a per lineage diversification rate of 0.77–1.75 (assuming zero extinction) and 0.58–1.32 (when incorporating a high rate of extinction) (Klak et al; 2004). Here we improved significantly the fossil calibration presented in previous studies dating any Caryophyllales lineage, and estimated a stem group origin for Ruschioideae of 10.7 MYA, and a crown group diversification starting at 4.04 MYA, with a diversification rate for the crown group of 1.6-1.2 species per MY. These data are highly coincident of those reported in Klak et al., (2004); however using the same methodology to estimate diversification rates of distinct lineages allows a straightforward comparison between taxa (see discussion in Chapter I).

Didieraceae is a small family of stem and leaf cactiform succulents that inhabit the southwestern arid regions of Madagascar, with *Alluaudiopsis* and *Calyoptrotheca* as their earliest diverging genera (Applequist and Wallace, 2000). *Alluaudiopsis* and *Calyoptrotheca* are woody shrubs with weakly succulent leafs compared to the crown group, which includes arboreal species with highly succulent stems such as *Didiera*, *Alluaudia*, *Decaryia*, and *Androyella* (Rowley, 1992; Nyffeler et al., 2008). Molecular studies showed that these last four genera are grouped together in a supported monophyletic clade (Applequist and Wallace, 2000). Didiereaceae dominates the vegetation named spiny thicket, which extends across the arid regions of southern and southwestern Madagascar, falling in the extreme rain shadow behind the eastern mountain chain of the Island, far from the prevailing northeastern rains. The climate is dominated by a wet and a dry season with most of the rain falling between October and April, where the rainfall is erratic from year to year, and prolonged periods of drought lasting several years do occur (http://www.eoearth.org/article/Madagascar_spiny_thickets). Although the split of Madagascar from Africa may have occurred as long as 165 MYA (Davis et al., 1994) the vegetation might be younger. Applequist and Wallace (2000) observed that the chloroplast sequences of Didiereaceae and *Calyptrotheca*, the closest relative of Didiereaceae endemic to East Africa, are highly similar, suggesting that the separation of these two taxa is more recent. This, together with the lack of genetic change observed within the family lead them to suggest that the Didieraceae originated from a dispersal of a *Calyptrotheca*-like African ancestor with a subsequent recent evolutionary radiation (Applequist and Wallace, 2000). Our results corroborate these hypotheses, with a stem group date of 28.25 MYA for the separation of *Calypthroteca* and the rest Didiereaceae members, and a crown group diversification of 9.47 MYA, during the late Miocene. The diversification rate of crown Didiereaceae is not high in comparison to other lineages within Caryophyllales, and is much lower to the rates observed in the analyzed Cactaceae and Aizoaceae lineages (Table 2). Although the dispersal capabilities of crown Didiereaceae members are not studied, the fact that their distribution is restricted to Madagascar reveals that they are not able to cross large oceanic masses. The PPRA map obtained for the family (Figure 6) shows that the ecological conditions suitable for succulent Didiereaceae members are not present in continental Africa. The diversification of Didiereaceae might have been slowed by the ecological and geographical limits within the island.

The projection of the PPRA's of the studied lineages revealed the uniqueness of the climatic conditions under species within each radiation inhabit. The projection of Cacteae species's potential ecological niches into Southern Africa shows that the suitable conditions for Cacteae species in that region would be on small patches of the eastern portion of South Africa (Figure 7), in the Eastern Cape and the Northern Transvaal, which possess grassland and savanna vegetation respectively. These areas might be coincident with the distribution of distinct succulent *Euphorbia* species, and our results could be a clue in a possible ecological equivalence of the climatic conditions on the diversity centers of *Euphorbia* in Southern Africa and North and Central Mexico (Zimmermann et al., 2010). The PPRA for Cacteae and the PPRA for Ruschioideae are determined mainly by distinct climatic variables (Table 2), probably reflecting the differences between the Mediterranean weather in the southwestern portion of South Africa, -with a winter rainfall regime-; and the summer rainfall regimes of the Chihuahuan Desert. Both PPRA's are only coincident in one climatic variable, the precipitation of the coldest quarter of the year (Table 2). This is evident when we projected the PPRA of Ruschioideae members into Mexico and North America, where we observe a coincidence with the Mediterranean weather in California and the western USA (Figure 9). Although cacti do distribute in the last area, it is not a particular diversity center of any Cactaceae lineages.

We also projected the potential ecological niches of core Pachycereeae members into Southern Africa and Madagascar (Figure 8), and succulent Didiereaceae members in North America (Figure 10). In these cases we could find small geographic patches of coincidence between the projected PPRA of core Pachycereeae members and the PPRA of crown Didiereaceae (Figure 6, Figure 10), indicating a possible coincidence of the climatic conditions under these groups inhabit. However, as shown in Table 2, the potential ecological niches of species within both radiations are mainly determined by different climatic variables.

CONCLUSIONS

Species within the studied clades in Cactaceae (Cacteae and Pachycereeae tribes), Aizoaceae (Ruschioideae subfamily) and Didiereaceae (*Alluaudia*, *Decarya* and *Didiera*) dominate and characterize the vegetation of the regions they inhabit: the xerophytic scrubs of Mexico and North America, the Succulent Karoo in southern Africa and the spiny thicket of southwest Madagascar respectively (Rowley, 1992; Linder, 2005, Werger, 1978, Rzedowski, 2006). The temporal framework for the diversification of these succulent families and their richest lineages we provide, shows that they diversified recently at different ages during the Miocene, supporting previous hypotheses suggesting a recent origin of arid biomes. Lineages within Cactaceae and Aizoaceae show the highest diversification rates, as a possible signature of an evolutionary radiation pattern (Chapter I).

Our analyses of ecological niches reveal that succulent radiating lineages inhabit (and possibly diversified) in distinct arid climatic conditions, developing similar adaptations such as succulent tissues and reducing their evapo-transpiration surface; however probably evolving particular adaptations to local arid conditions. The knowledge of paleoclimatic and present ecological conditions for these succulent plant radiations in a temporal framework can give a hint into the origin of the vegetation types they characterize and the evolution of adaptation strategies in succulent lineages. Further work is needed to improve this study and the conclusions that can be obtained with reported data.

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FIGURES AND TABLES

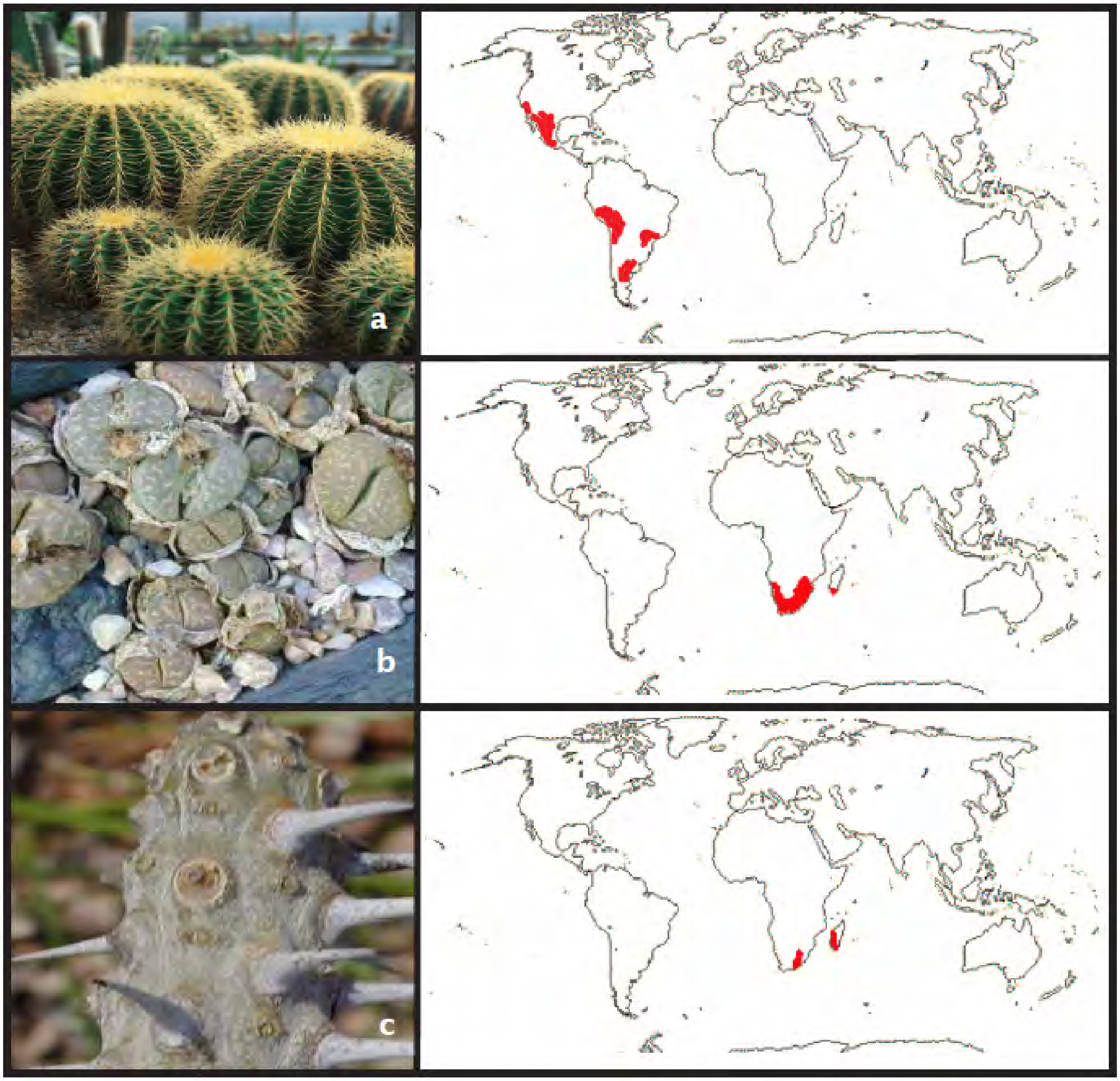

Figure 1. Illustrations and distribution maps of succulent, richest lineages within (a) Cactaceae, (b) Aizoaceae and (c) Didiereaceae.

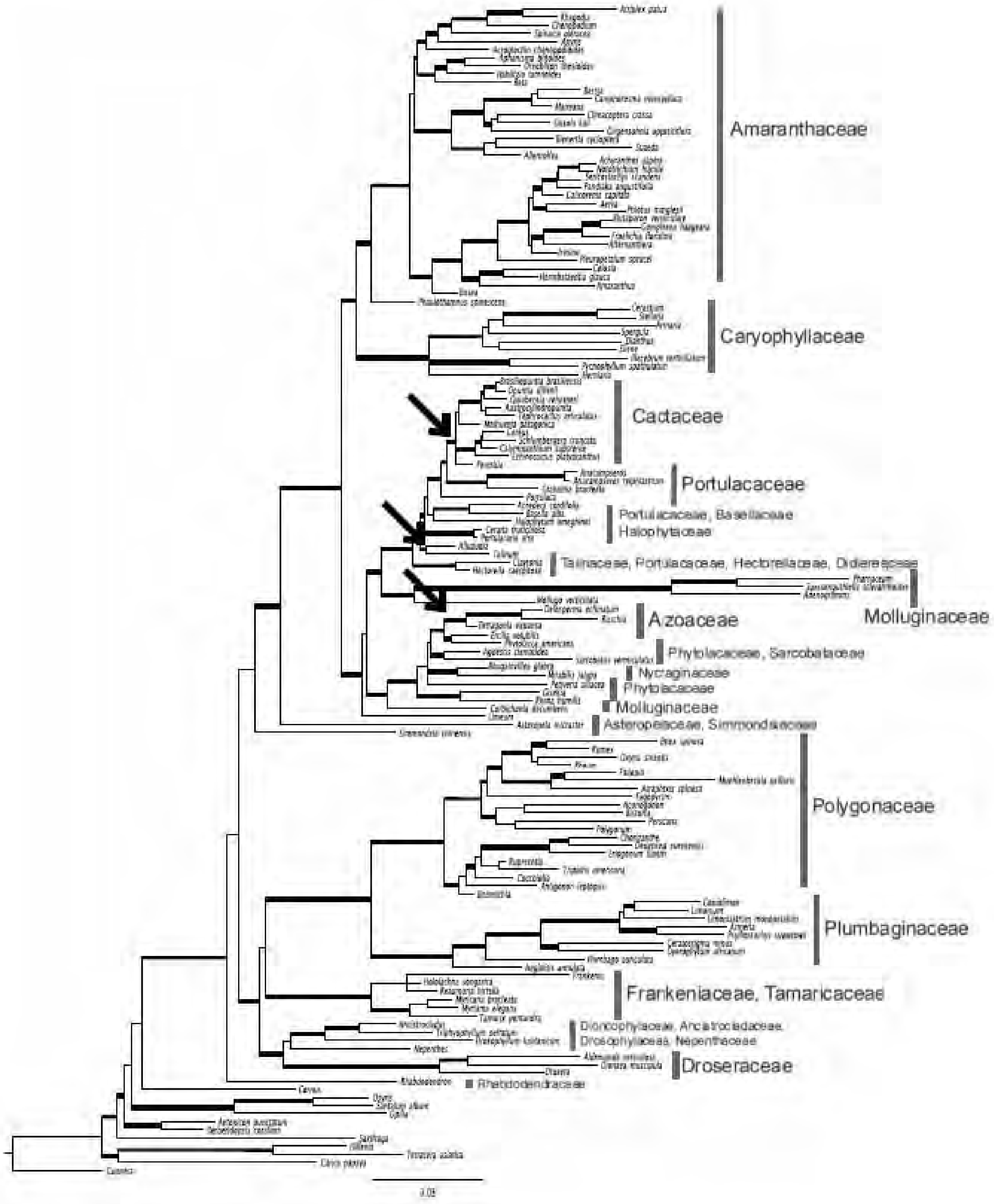

Figure 2. Bayesian molecular phylogeny for Caryophyllales, obtained using the concatenated matrix *atpB*, 18S, *matK*, and *rbcL.* Tick lines represent lineages with pp support values higher than 90%. Arrows show nodes for Cactaceae, Aizoaceae and Didiereaceae.

	# spp.	**Estimated Ages**		**Diversification Rates**			
		Stem Group	**Crown Group**	**Eq.6, Stem Epsilon = 0**	**Eq. 6 Stem Epsilon = 0.9**	**Eq. 7 Crown Epsilon = 0**	**Eq. 7 Crown Epsilon = 0.9**
Angiosperms	269288	350	241	0.035	0.029	0.049	0.042
Caryophyllales	11155	97.83	92.78	0.095	0.071	0.092	0.075
Caryophyllaceae	2200	73.6	56.91	0.104	0.073	0.123	0.093
Amaranthaceae	2050	66.48	55.73	0.114	0.080	0.124	0.094
Aizoaceae	2020	32.3	21.43	**0.235**	**0.164**	**0.322**	**0.245**
Ruschioideae	1563	10.74	4.04	**0.684**	**0.470**	**1.648**	**1.239**
Cactaceae	1500	22.97	17.39	**0.318**	**0.218**	**0.380**	**0.285**
Cacteae	355	10.76	7.96	**0.545**	**0.334**	**0.650**	**0.445**
Core Pachycereeae	140	4.2	3.74	**1.176**	**0.643**	**1.135**	**0.708**
Polygonaceae	1110	67.42	51.27	0.104	0.069	0.123	0.091
Plumbaginaceae	836	67.42	52.75	0.099	0.065	0.114	0.083
Nyctaginaceae	395	49.42	23.8	0.120	0.074	0.222	0.153
Droseraceae	115	66.47	37.76	0.071	0.037	0.107	0.065
Frankeniaceae	90	20.97	-	0.214	0.109	-	-
Tamaricaceae	90	38.24	19.48	0.117	0.059	0.195	0.115
Nepenthaceae	90	59.93	-	0.075	0.038	-	-
Molluginaceae	87	67.16	55.84	0.066	0.033	0.067	0.039
Phytolaccaceae	65	70.52	-	0.059	0.028	-	-
Limeaceae	21	77.61	-	0.039	0.014	-	-
Basellaceae	19	27.49	-	0.107	0.037	-	-
Didiereaceae	16	28.25	-	0.098	0.032	-	-
Crown Didiereaceae	11	28.25	9.47	0.084	0.024	0.180	0.067
Ancisrocladaceae	12	21.24	-	0.116	0.034	-	-
Achatocarpaceae	7	66.48	-	0.029	0.007	-	-
Lophiocarpaceae	6	73.91	-	0.024	0.005	-	-
Gisekiaceae	5	31.83	-	0.050	0.010	-	-
Dioncophyllaceae	3	21.24	-	0.051	0.008	-	-
Rhabdodendraceae	3	92.71	-	0.011	0.001	-	-
Sarcobataceae	2	33.8	-	0.020	0.002	-	-
Halophytaceae	1	27.49	-	0	0	-	-
Drosophylaceae	1	40.85	-	0	0	-	-
….Simmondsiaceae	1	88.16	-	0	0	-	-

Table 1. Results for the estimation of dates and diversification rates analyses.

CLIMATIC LAYERS WORLDCLIM	RUSCHIAE (AIZOACEAE)	DIDIEREACEAE	PACHYCEREAE (CACTACEAE)	CACTEAE (CACTACEAE)
BIO1 = Annual Mean Temperature				■
BIO2 = Mean Diurnal Range (Mean of monthly (max temp - min temp))	■			
BIO3 = Isothermality (P2/P7) (* 100)		■		
BIO4 = Temperature Seasonality (standard deviation *100)			■	
BIO5 = Max Temperature of Warmest Month				■
BIO6 = Min Temperature of Coldest Month		■		
BIO7 = Temperature Annual Range (P5-P6)				■
BIO8 = Mean Temperature of Wettest Quarter	■	■		
BIO9 = Mean Temperature of Driest Quarter	■			
BIO10 = Mean Temperature of Warmest Quarter				■
BIO11 = Mean Temperature of Coldest Quarter				
BIO12 = Annual Precipitation				
BIO13 = Precipitation of Wettest Month				
BIO14 = Precipitation of Driest Month		■	■	
BIO15 = Precipitation Seasonality (Coefficient of Variation)			■	
BIO16 = Precipitation of Wettest Quarter		■	■	
BIO17 = Precipitation of Driest Quarter				
BIO18 = Precipitation of Warmest Quarter	■			■
BIO19 = Precipitation of Coldest Quarter	■		■	

Table 2. Description of the climatic layers used in this study and the most important variables determining the potential ecological niches for species within each tested clade.

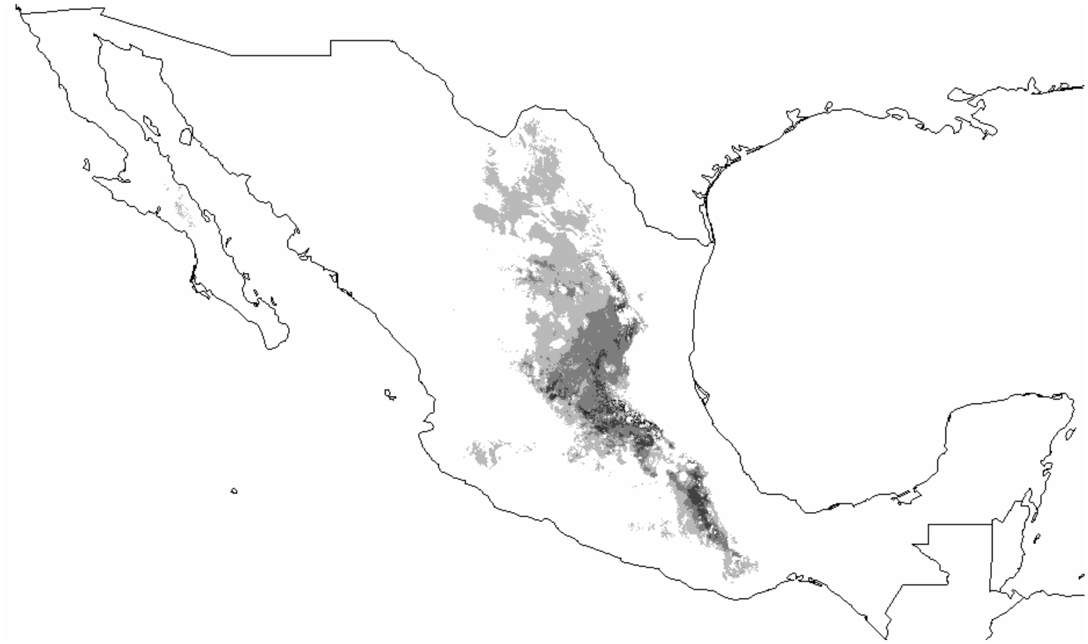

Figure 3. Potential Phylogenetic Richness Area (PPRA) for the Cacteae Clade (Cactaceae).

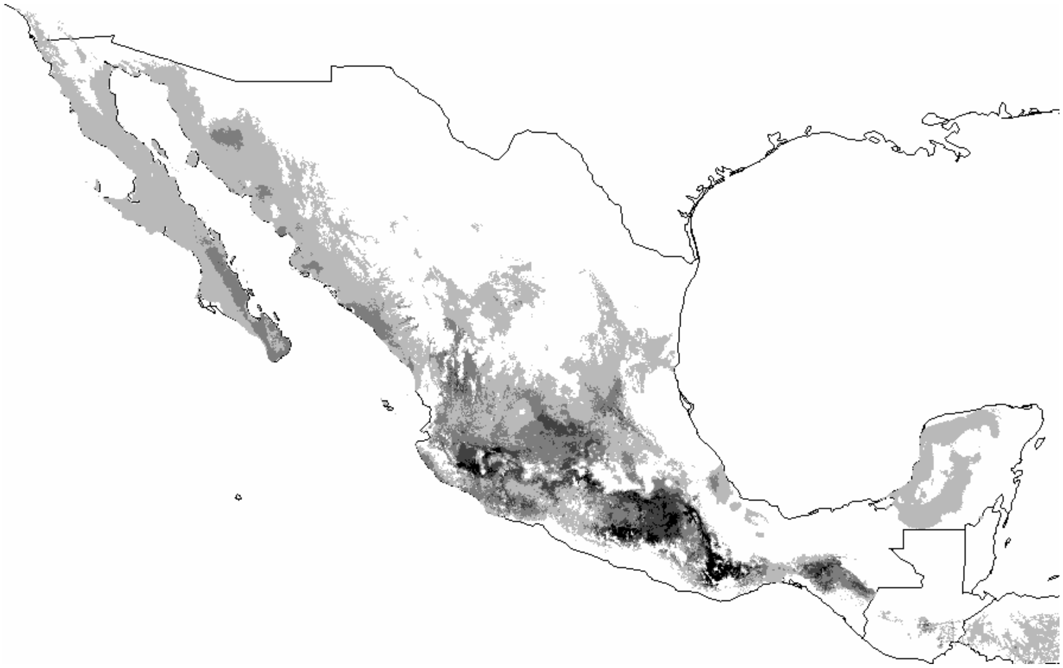

Figure 4. Potential Phylogenetic Richness Area (PPRA) for the core Pachycereeae Clade (Cactaceae).

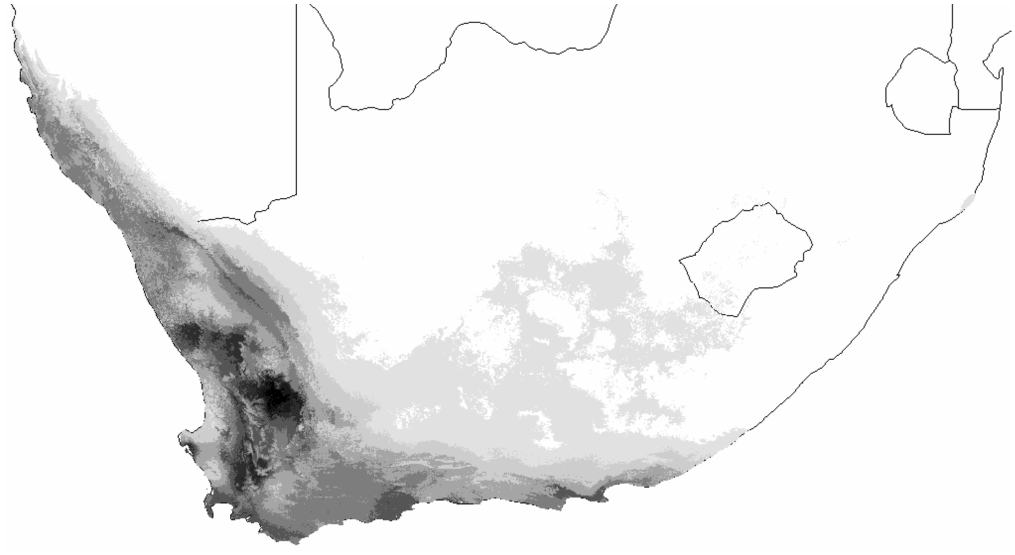

Figure 5. Potential Phylogenetic Richness Area (PPRA) for the subfamily Ruschioideae clade (Aizoaceae).

Figure 6. Potential Phylogenetic Richness Area (PPRA) for succulent Didiereaceae.

Figure 7. Projection of potential ecological niches estimated for Cacteae members in Southern Africa and Madagascar.

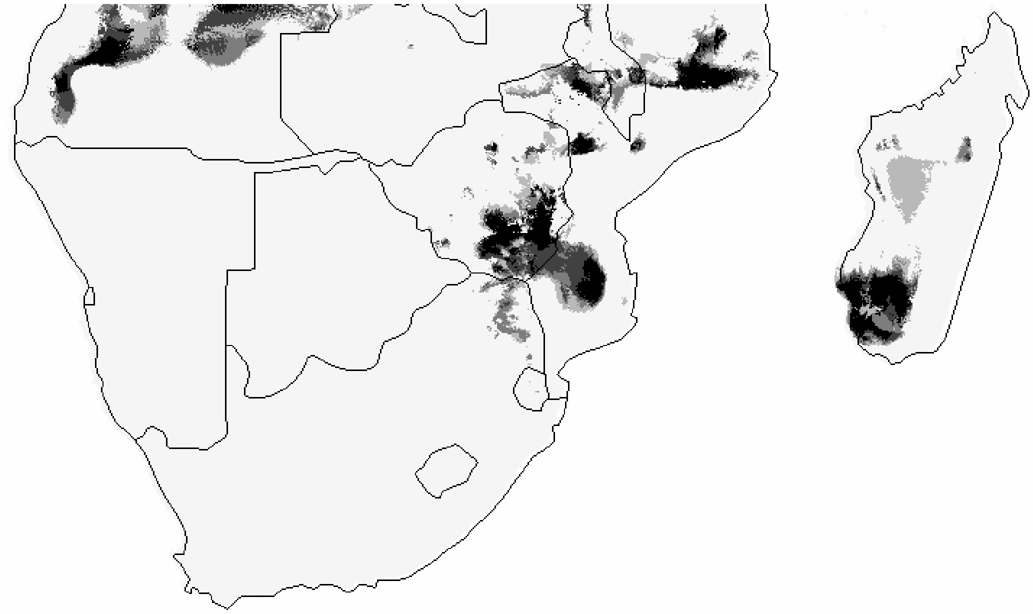

Figure 8. Projection of potential ecological niches estimated for core Pachycereeae members in Southern Africa and Madagascar.

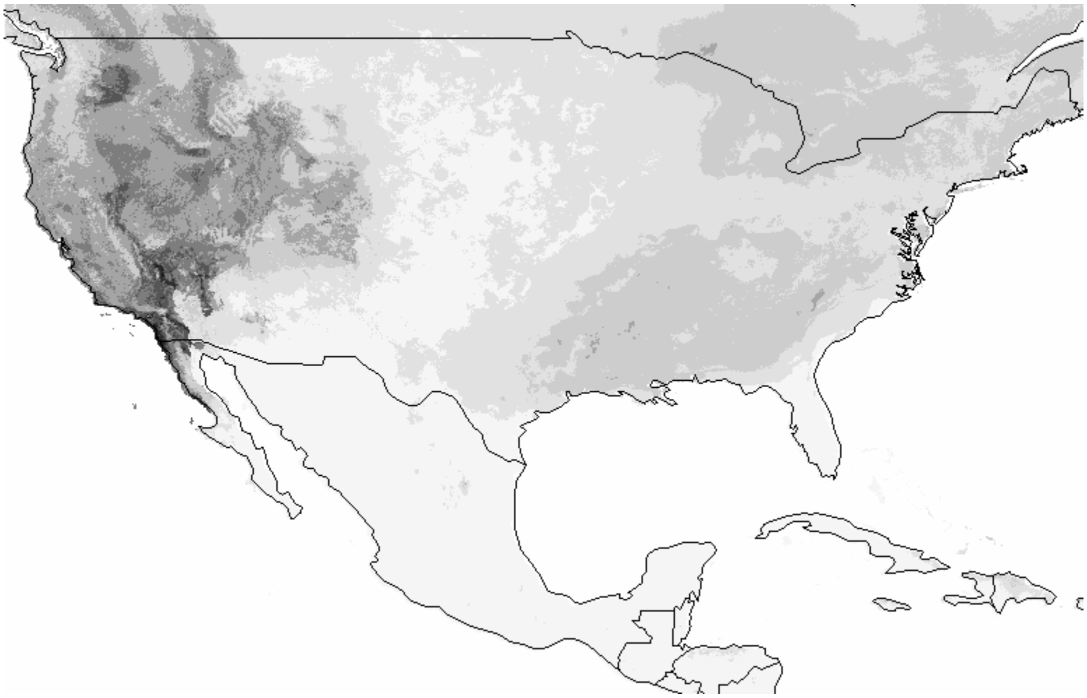

Figure 9. Projection of potential ecological niches estimated for the Ruschioideae members in North America.

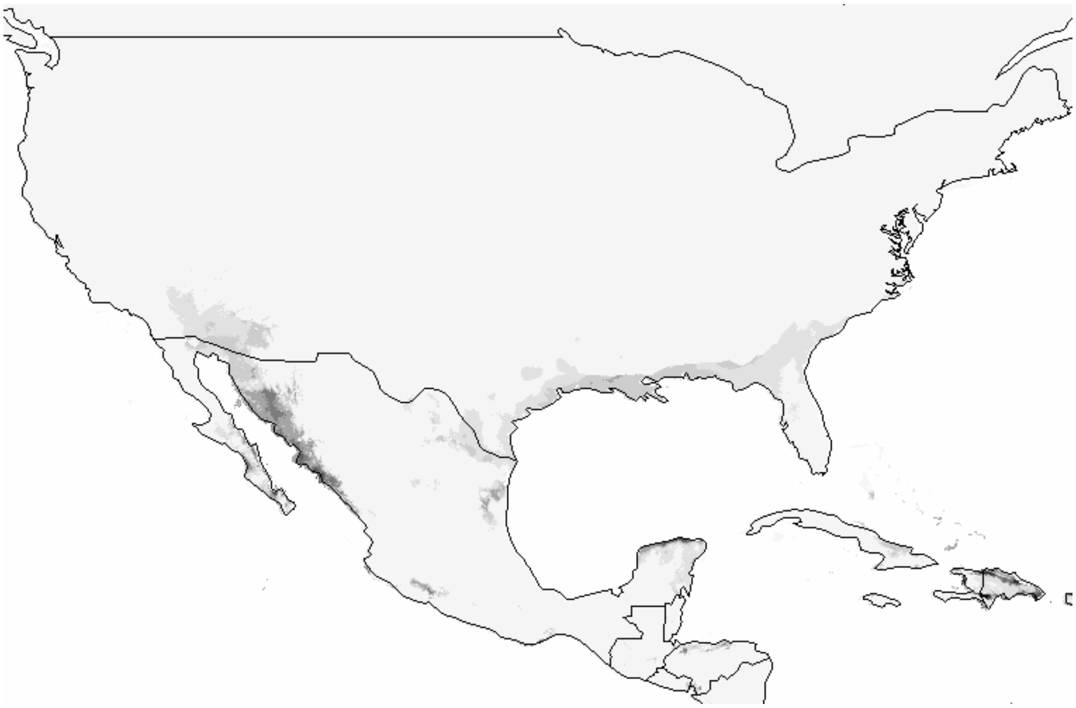

Figure 10. Projection of potential ecological niches estimated for succulent Didiereaceae members in North America.

CAPÍTULO III

RADIACIONES EVOLUTIVAS EN CACTACEAE

Las cactáceas son plantas suculentas muy llamativas por las modificaciones morfológicas y fisiológicas que muchas presentan como respuestas adaptativas a las condiciones de aridez en que habitan, además de que son elementos característicos típicos de paisajes desérticos americanos. En particular destacan en muchas especies sus tallos fotosintéticos suculentos con producción retardada de corteza, por lo general globosos, columnares o aplanados y con una superficie reducida en relación con su volumen (lo que evita una excesiva evapotranspiración), espinosos y con hojas usualmente rudimentarias o vestigiales (Barthlott y Hunt, 1993). Sin embargo, en los géneros más primitivos como *Pereskia* hay tallos leñosos (no-fotosintéticos, no-suculentos) y hojas formadas por limbo y pecíolo, lo cual es más parecido a la mayoría de las angiospermas.

Las cactáceas incluyen cerca de 1500 especies formando alrededor de 127 géneros (Barthlott y Hunt, 1993; Hunt et al., 2006) y tienen una distribución amplia en una diversidad de ambientes, desde el norte de Canadá hasta la Patagonia; desde el nivel del mar en las dunas costeras, hasta los 5100 m de altitud en Perú. Crecen desde zonas áridas y subtropicales, hasta tropicales húmedas, donde algunas viven como epifitas (Bravo Hollis y Sheinvar, 1999). La familia representa una radiación evolutiva espectacular. El centro de diversidad actual de las cactáceas se encuentra en México, en donde habitan 40% de las especies conocidas y de éstas aproximadamente 73% de los géneros y 78% de las especies son endémicos (Hernández y Godínez, 1994) y se distribuyen en casi todos los tipos de vegetación mexicana descritos por Rzedowski (1978) a excepción de la vegetación acuática (Bravo Hollis y Scheinvar, 1999). Dada la amplia gama de ambientes en que habitan, las cactáceas han desarrollado una gran diversidad de formas de vida, desde globulares, cilíndricas y toneliformes hasta arborescentes, candelabriformes, erectas e inclusive epifitas. Alcanzan su máxima especialización y diversidad en los matorrales xerófilos y en los bosques tropicales caducifolios, donde existen condiciones de aridez más o menos extremas (Bravo Hollis y Scheinvar, 1999).

México es no sólo el centro de diversidad mundial de las cactáceas sino también de muchos otros grupos de plantas, con una relevancia particular por la presencia de elevados niveles de endemismo y la disparidad morfológica entre taxa. Se piensa que algunas de las causas de la riqueza de especies de la flora mexicana son la accidentada fisiografía del país, las grandes cadenas montañosas, la numerosa variedad de tipos de suelo, los amplios litorales, los altiplanos, los valles, las cuencas fluviales, etc. (Bravo Hollis y Scheinvar, 1999). Sin embargo, no es claro aún si la diversidad de plantas en México se debe a elevadas tasas de especiación recientes o al mantenimiento de linajes muy viejos, extintos en el resto del mundo, debido a bajas tasas de extinción locales.

En el presente capítulo, se examinan la historia evolutiva y los patrones de diversificación de la familia Cactaceae, principalmente en México, explorando los mecanismos evolutivos que han determinado las radiaciones de sus principales linajes. Como primer paso se llevó a cabo un análisis filogenético con base en datos moleculares, el cual constituye un marco de referencia para plantear hipótesis sobre los procesos de diversificación y evolución de cambios morfológicos. Por ejemplo, la filogenia obtenida fue utilizada para evaluar la importancia de la convergencia en caracteres de forma de crecimiento y hábito, o la serie de cambios que dieron lugar a las cactáceas más especializadas. Estos resultados se reportan en la primera parte del Capítulo, el cual está conformado por un manuscrito titulado “Phylogenetic relationships and growth form evolution in Cactaceae (Caryophyllales)”; que ha sido aceptado para su publicación en el *American Journal of Botany.* En la segunda parte del Capítulo se muestran resultados de la estimación de fechas de origen de la familia y de la diversificación de sus subgrupos, así como del origen de grupos predominantemente mexicanos. Se analizan sus tasas de diversificación, comparando con los resultados disponibles para otros grupos de plantas en México y con la historia de cambios en la fisiografía del país a lo largo del tiempo geológico, generando así hipótesis acerca de cuándo las cactáceas poblaron el paisaje mexicano y explorando las razones de su radiación y gran diversidad en México.

III. 1. MARCO FILOGENÉTICO Y TENDENCIAS EVOLUTIVAS GENERALES

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La familia Cactaceae es particularmente interesante debido a la suculencia que las caracteriza y la extraordinaria diversidad de formas de vida de sus integrantes. Aunque los cactos son elementos conspicuos de los ecosistemas áridos y semiáridos a lo largo del continente Americano, y son sistemas modelos para estudios ecológicos y anatómicos; las relaciones evolutivas entre sus linajes han sido difíciles de reconocer dada la alta incidencia de convergencia morfológica y la escasez de sinapomorfias fenotípicas al interior de la familia. Para inferir relaciones evolutivas de linajes al interior de Cactaceae, en este estudio llevamos a cabo análisis filogenéticos usando parsimonia "ratchet" y métodos de verosimilitud, usando marcadores de núcleo y cloroplasto (*trnK/matK*, *matK*, *trnL-trnF*, *rpl16*, y *ppc*). Incluimos un muestreo de 224 especies, que representan aproximadamente el 85% de los géneros de la familia. La filogenia obtenida fue usada para llevar a cabo una reconstrucción de caracteres ancestrales al interior de la subfamilia Cactoideae, la más rica en especies y diversidad morfológica, con la finalidad de evaluar posibles tendencias evolutivas en las formas de crecimiento. La matriz final concatenada incluye 6148 pb, de los cuales 3400 (55.3%) son invariantes y 1597 (26.2%) son parsimoniosamente informativos. Los resultados de nuestros análisis filogenéticos corroboran la clasificación tradicional en subfamilias. La subfamilia Cactoideae esta compuesta de (1) la tribu Cacteae, formando un clado con altos valores de apoyo, y que incluye a los cactos globosos norteamericanos, y (2) el clado de las Cactoideae centrales, que incluye a las especies previamente clasificadas en las tribus restantes. Nuestros resultados indican que los principales clados en Cactoideae probablemente tenían formas de crecimiento ancestrales distintas, y que la convergencia hacia formas globosas, arborescentes o columnares ocurrió en diferentes linajes. Nuestro estudio propone hipótesis filogenéticas detalladas para la familia Cactaceae, siendo el marco filogenético general más completo al momento para entender tendencias evolutivas en la familia.

III. 1. PHYLOGENETIC FRAMEWORK AND GENERAL EVOLUTIONARY TRENDS[1]

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Cactaceae is one of the most charismatic plant families because of the extreme succulence and outstanding diversity of growth forms of its members. Although cacti are conspicuous elements of arid ecosystems in the New World and are model systems for ecological and anatomical studies, the high morphological convergence and scarcity of phenotypic synapomorphies make the evolutionary relationships and trends among lineages difficult to understand.

We performed phylogenetic analyses implementing parsimony ratchet and likelihood methods, using plastid and nuclear markers (*trnK/matK*, *matK*, *trnL-trnF*, *rpl16*, and *ppc*), obtaining a concatenated 6148 bp matrix. We included 224 species representing approximately 85% of the family's genera. Likelihood methods were used to perform an ancestral character reconstruction within Cactoideae, the richest subfamily in terms of morphological diversity and species number, to evaluate possible growth form evolutionary trends.

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[1] El título original de este artículo es "Phylogenetic relationships and growth form evolution in Cactaceae (Caryophyllales)", y fue aceptado en noviembre de 2010 para su publicación en el *American Journal of Botany.*

Our phylogenetic results support previous studies showing the paraphyly of subfamily Pereskioideae, and the monophyly of subfamilies Opuntioideae and Cactoideae. After the early divergence of *Blossfeldia,* Cactoideae splits in two clades: Cacteae, including North American globose and barrel members, and core Cactoideae, including the largest diversity of growth forms distributed throughout the American continent. Para- or polyphyly is persistent in different regions of the phylogeny. Main Cactoideae clades were found to have different ancestral growth forms; and convergence towards globose, arborescent or columnar forms occurred in different lineages. Our study provides a detailed hypothesis of relationships among cacti lineages, representing the most complete general phylogenetic framework available to date to understand evolutionary trends within Cactaceae.

INTRODUCTION

Cactaceae (Caryophyllales, Eudicotyledoneae, Angiospermae; Cantino et al., 2007) is a well known plant family possessing several adaptations for aridity. The family is remarkable due to the evolution of extreme succulence of most of its members, their conspicuous presence in New World dry regions and its outstanding diversity of growth forms. Several cacti species are commercialized as ornamental plants on a worldwide scale, and some of them are important as food source, giving this family a strong economical relevance (e.g. Nobel, 1994, 2002; De Kock, 2001; Stintzing and Carle, 2005; Feugang et al., 2006). In addition to being distinctive elements of arid and semiarid biomes, Cactaceae species play fundamental ecological roles (e.g., Parker, 1989; Mandujano et al., 1996; Drezner et al., 2002; Godínez-Álvarez et al., 2003). Different cacti species have been studied as models in plant anatomical and physiological studies (e.g., Mauseth 1999, 2004, 2006, 2007; Shishkova et al., 2008), and some cacti lineages are persistent invasive in different biomes in Africa, Australia, and elsewhere (Nobel 1994).

The cacti family includes over 1450 species belonging to approximately 127 genera (Barthlott and Hunt, 1993; Hunt et al., 2006). Its greatest species richness is concentrated primarily in Mexico, with secondary centers in the southwestern Andean region and in eastern Brazil. The morphological features that characterize members of Cactaceae are the presence of short shoots modified into areoles, a shoot apical meristem organized into four zones, and nearly all cacti possess inferior ovaries covered by bracts or areoles (Gibson and Nobel, 1986; Nyffeler, 2002). At the molecular level, the family is characterized by the inversion of a chloroplast genetic region including *atpE*, *atpB*, and *rbcL* genes (Downie and Palmer, 1993), and its monophyly has been supported in molecular phylogenetic studies based on different loci (e.g. Applequist and Wallace, 2001a; Cuenoud et al., 2002; Nyffeler, 2002).

Taxonomic studies since the 19th Century have recognized Pereskioideae, Opuntioideae and Cactoideae as distinct subfamilies within Cactaceae (Anderson, 2001; Metzing and Kiesling, 2008). The genus *Maihuenia* has been typically considered as a member of Pereskioideae, however, its placement in a monogeneric subfamily has been suggested on the basis of its unique ecological and morphological attributes (Anderson, 2001), and molecular phylogenetic analyses (Wallace, 1995a, b). In a recent molecular phylogeny of Cactaceae (Nyffeler, 2002), species of *Pereskia* and *Maihuenia* were found to form an early diverging grade within Cactaceae, with Cactoideae and Opuntioideae being well supported clades.

Species of *Pereskia* have been recognized as morphologically plesiomorphic within Cactaceae (Gibson and Nobel, 1986; Leuenberger, 1986; Metzing and Kiesling, 2008), displaying characters such as broad flattened leaves with C3 photosynthesis, arborescent growth form, areoles with leaf production, dense and fibrous wood, simple cortex lacking cortical bundles, poorly developed stem epidermal and hypodermal layers, non-succulent tissues, and occupation of relatively mesic environments (Leuenberger, 1986; Mauseth and Landrum, 1997). In a molecular-based analysis of relationships among early Cactaceae lineages, Edwards et al. (2005) found that members of *Pereskia* were grouped in two distinct early diverging lineages that formed consecutive sister groups of a clade formed by (*Maihuenia*, Cactoideae) and Opuntioideae.

Subfamilies Opuntioideae and Cactoideae have long been recognized as monophyletic on the basis of morphological and molecular data (Barthlott and Hunt, 1993; Nyffeler, 2002; Wallace and Dickie, 2002; Griffith and Porter, 2009). Members of Opuntioideae share a number of structural synapomorphies, such as areoles with glochids (small deciduous barbed spines); polyporate pollen grains with peculiar exine structures (Leuenberger, 1976); and seeds surrounded by a funicular cover – frequently described as a bony aril (Barthlott and Voit, 1979). In addition, studies based on molecular data show that Opuntioideae is characterized by a deletion in the *acc*D region of the chloroplast genome (Wallace, 1995b; Wallace and Dickie, 2002; Griffith and Porter, 2009).

Subfamily Cactoideae has been strongly supported as monophyletic in molecular phylogenies (Nyffeler, 2002). Members of this subfamily are characterized by the presence of tubercles or ribs on the stems, by possessing reduced or suppressed leaves subtending each areole (Wallace and Gibson, 2002), and by the loss of an intron in the *rpoC1* chloroplast gene (Wallace and Cota, 1995). Based on detailed morphological observations, Buxbaum (1958), and Endler and Buxbaum (1982) subdivided Cactoideae in nine tribes, with several subtribes and "lines", and Barthlott and Hunt (1993) derived a tribal arrangement for Cactoideae following previous classifications and morphological data (e.g. Buxbaum, 1958; Britton and Rose, 1919-1923); however, this classification has been recently modified (Anderson, 2001). Morphological phylogenetic reconstruction and taxonomic classification for Cactoideae has proved to be difficult due to the frequent convergence of characters and lack of clear synapomorphies for tribes, hence, molecular-based phylogenetic analysis might represent a useful alternative for recognizing main lineages and their relationships (Applequist and Wallace, 2001b). Recent molecular phylogenetic results for Cactoideae conflict with traditional morphological classifications (Applequist and Wallace, 2001b; Nyffeler, 2002). Except for Cacteae, the traditionally defined tribes have been found to be para- or polyphyletic (e.g. Applequist and Wallace, 2001b; Nyffeler, 2002; Wallace and Gibson, 2002).

Cactoideae includes the largest number of genera and species, and the largest diversity of growth forms among Cactaceae subfamilies (see Barthlott and Hunt, 1993; Anderson, 2001). Its members diversified throughout the American continent, becoming adapted to different environments and evolving a large variety of forms and habits (Gibson and Nobel, 1986). For example, dry tropical forests in central Mexico host a large radiation of columnar and arborescent cacti, and these growth forms also occur in some lineages from several regions of South America. Epiphytes with cylindrical or flattened stems inhabit Central and South American humid tropical forests, and a variety of globose or spherical species growing solitary or forming clumps can be found in the arid and semiarid regions of both South and North America; in the latter ranging in size from few millimeters above the ground to gigantic barrel cacti over two meters in height (Gibson and Nobel, 1986).

Efforts have been made to describe and explain the profuse diversity of forms within Cactoideae. According to Gibson and Nobel (1986), cacti stem forms within the subfamily can be barrel, globose, or cylindrical-shaped. Barrel-shaped forms include species with a globular or spherical stems, with a maximum height of 0.5 to 2 m, whereas globose cacti possess spherical stems less than 0.5 m in height (Gibson and Nobel, 1986). The cylindrical (or columnar, according to the authors) form includes species whose stems are 10 or more times longer than wide (Gibson and Nobel, 1986). According to their branching patterns, cylindrical stemmed species can be classified in different growth forms (Arias and Terrazas, 2006). If the cylindrical stem is not branched, the form can be classified as a columnar simple; while arborescent forms are tree-like in appearance, with a trunk branching above its base; and shrubby forms are characterized by the absence of a main trunk, or with a basitonic branching (Anderson, 2001; Arias and Terrazas, 2006; Buxbaum, 1951). Sometimes, cacti with a cylindrical stem form can grow prostrated, clambering, scrambling or climbing (Anderson, 2001), due to the lack of mechanical support of the stem. Instead of growing erect, these cacti grow over the soil, or supported by other plants or neighboring vegetation; and when cacti grow on other plants, they are classified as epiphytes (Anderson, 2001). An important adaptation in the stem of species of Cactoideae is the presence of a folded surface, which allows them to swell without tearing, and to absorb large quantities of water when available (Mauseth, 2006). The stem surface in Cactoideae species can be folded longitudinally into ribs, or both longitudinally and transversely into tubercles. Whereas ribs allow the inner cortex to expand and shrink radially without damaging the shoot surface, tubercles allow the entire shoot to shorten or lengthen as water content changes (Mauseth, 2006). The complex and large diversity of forms and habits present in Cactaceae -and particularly within Cactoideae-, have complicated the achievement of a consensed classification of cacti growth forms; and together with the lack of stable phylogenetic hypotheses for relationships among lineages, hinder our understanding of the evolution of the family's diversity of forms.

The main goal of our study is to reconstruct the phylogenetic relationships within Cactaceae based on an expanded and more comprehensive taxonomic sample across the family, and molecular sequence data from the plastid and nuclear genomes. Our results will be compared with reported studies; to test for the monophyly of previously proposed groups -particularly within Cactoideae-, to provide independent evidence to show relationships among them, and to identify lineages where further work is needed. Our taxonomic sample includes 224 species belonging to 108 genera of Cactaceae (of approximately 127; Barthlott and Hunt, 1993; Hunt et al., 2006), representing all traditionally recognized subfamilies, including an increased sample of the North American tribes Cacteae and Pachycereeae, and several South American lineages. We performed phylogenetic analyses with parsimony ratchet and maximum likelihood methods, based on four protein coding and non-coding plastid loci, and one low-copy nuclear locus. Although one of the most arresting characteristics of the Cactaceae family is the extraordinary diversity of structure of its members (Mauseth, 2006), the extensive convergence of growth form among distanctly related lineages difficult the understanding of the evolution of cacti structure. Although authors have postulated hypotheses based on observations (Britton and Rose, 1919-1923; Buxbaum, 1951; Gibson and Nobel, 1986; Barthlott and Hunt, 1993; Mauseth, 2006), a formal analysis of character evolution is pending. We evaluated possible trends in the evolution of growth form within subfamily Cactoideae, by scoring and reconstructing ancestral character states at main, supported nodes, using likelihood methods. Our results provide a complete framework to understand evolutionary relationships within Cactaceae, based on an extensive sampling of lineages; and provide insights into the evolution of growth form diversity within Cactoideae.

MATERIALS AND METHODS

Taxa and data – Our taxonomic sampling includes 224 species belonging to 108 genera of Cactaceae, which represent approximately 85% of the generic diversity and 15.4% of the species diversity of the family. Species sampling followed Barthlott and Hunt (1993) and Anderson (2001), aiming to achieve a nearly complete generic representation, and to increase the representation of North American taxa belonging to tribes Cacteae and Pachycereeae, as well as several South American lineages. We included at least one species for each genus within Cacteae and Pachycereeae, but more than one for larger genera such as *Mammillaria*, *Echinocereus*, *Pachycereus*, and *Opuntia*. Mexican taxa were collected mainly from natural populations, but several endangered species were sampled from the living collection at the Jardín Botánico, Universidad Nacional Autónoma de México (UNAM). Tissues of several South American taxa were obtained from the living collection at the Desert Botanical Garden

(DBG, Phoenix, Arizona), with species identifications subsequently verified. Tissue of *Blossfeldia liliputana* was collected from natural populations. Four additional species of Anacampserotaceae, the closest relatives of Cactaceae (Nyffeler, 2007; Nyffeler and Eggli, 2010), were included as outgroups. Vouchers of collected specimens are deposited at the National Herbarium of Mexico (MEXU, UNAM), and listed in Appendix 1.

Phylogenetic analyses were based on the nucleotide sequences of five loci from the plastid and nuclear genomes. Plastid markers are one protein coding gene (*matK*), an intron (*rpl16*), and two intergenic spacers (*trnL-trnF* and *trnK-matK*), which have been used successfully in previous phylogenetic analyses of Cactaceae (Nyffeler, 2002; Arias et al., 2003, 2005; Edwards et al., 2005). The nuclear marker is the fourth intron of the phosphoenolpyruvate carboxylase (*ppc*) gene, and is here used for the first time to infer phylogenetic relationships in Cactaceae.

***DNA extraction, PCR amplification and sequencing* –** Genomic DNA was extracted from silica-gel dried tissue following the procedures of the DNeasy Plant Mini kit (QIAGEN, CA, USA) with slight modifications (information available upon request). Amplifying and sequencing primers for *rpl16* and *trnK/matK* loci were designed by us (Table 1). To amplify the *trnL-trnF* intergenic region we used primers trnL-c and trnL-f (Taberlet et al., 1991). The *ppc* locus was amplified using primers PPCX4F and PPCX5R (Olson, 2002). PCR amplification reactions were performed following an initial denaturation step at 94˚C for 5 min., followed by 34 cycles at 94˚C for 60 seconds, different annealing temperatures (depending on the primers) for 60 seconds, 72˚C elongation temperature for different time durations depending on the length of the product, and a finishing step at 72˚C for 4 min. Detailed conditions of each reaction are shown in Table 2. For several species, gel electrophoresis of PCR products of *ppc* yielded two bands. These two copies were extracted using the QIAquick gel extraction protocol (QIAGEN, CA, USA), and sequenced. Sequencing was performed at The Genome Center, Washington University (http://genome.wustl.edu/) and Macrogen, Seoul, Korea (http://www.macrogen.com).

Phylogenetic analyses – Datasets were assembled by combining the newly obtained sequences with those downloaded from Genbank for the *trnL-trnF*, *trnK-matK, matK* and *rpl16* regions (Appendix 1). For each locus, sequences were preliminarily grouped in several datasets following tribal classifications of Barthlott and Hunt (1993) and Anderson (2001), automatically aligned using MUSCLE (Edgar, 2004), and manually refined with BioEdit v5.0.6 (Hall, 1999). Preliminary alignments were subsequently joined into a single matrix using the profile alignment option in MUSCLE, followed by a second round of manual refinement with BioEdit (Data submitted to TreeBASE, www.treebase.org, submission number 10345). Incomplete sequence fragments at the 5' and 3' ends of each aligned data matrix were excluded from subsequent analyses (516 base pairs [bp] in total). The final concatenated matrix is 6148 bp long.

Preliminary heuristic parsimony analyses were conducted independently for each locus, except for *trnK-matK* and *matK*, which were analyzed jointly (results for independent analyses are not shown, available upon request). We performed 1000 replicates implementing a random-order-entry starting tree, and tree-bisection-reconnection (TBR) branch swapping with MULTREES, using PAUP* v4.0b10 (Swofford, 2002). Strict consensus trees for each locus were obtained, and after observing that differences among them, especially among chloroplast markers, are not strongly supported, the five loci were combined in a single dataset. This combined data set was analyzed with the parsimony ratchet (Nixon, 1999) in

PAUP* using PAUPRat (Sikes and Lewis, 2001). Parsimony uninformative and invariant sites were excluded, and 20 independent runs of 500 iterations each were conducted. Most parsimonious (MP) trees were filtered from the set of resulting trees on each iteration, and used to construct a strict consensus tree. Clade support was assessed with 1000 replicates of a non-parametric bootstrap analysis following a parsimony ratchet, implemented in TNT v1.1 (Goloboff et al., 2000).

We estimated the likelihood (-lnL), the proportion of invariant sites and the alpha parameter of the gamma distribution for each of the four non-coding loci (*trnK-matK*, *trnL-trnF*, *rpl16* and *ppc*), for the coding *matK*, and for codon position partitions in *matK* using Modeltest v3.7 (Posada and Crandall, 1998). We selected best fitting models for each mentioned dataset using the Akaike information criterion (AIC). To determine adequate data partitioning in our concatenated matrix for further phylogenetic analyses, we visually examined the parameters and the models obtained for each region and codon positions as a proxy to their molecular evolutionary dynamics. These examinations indicated that the data could be appropriately divided into five partitions: (1) chloroplast intergenic *trnK-matK* (best fit model: K81uf+I+G); (2) chloroplast coding *matK* (best fit model: TVM+I+G); (3) chloroplast intergenic *trnL-trnF* (best fit model: GTR+I+G); (4) chloroplast *rpl16* intron (best fit model: TIM+I+G); and (5) the nuclear *ppc* region (best fit model: TVM+I+G).

Maximum likelihood (ML) phylogenetic analyses were performed with RAxML v7.0.4 (Stamatakis, 2006) for the matrix consisting of the five concatenated loci, for the plastid matrix (four concatenated loci: *trnK-matK*, *matK*, *trnL-trnF* and *rpl16*), and for the nuclear matrix (one locus: *ppc*). For the five loci matrix and the plastid matrix, we implemented an independent general time reversible model (GTR), and a gamma distribution for site rates for each data partition, described before. We set 25 rate categories for the gamma distribution in both cases, because an exploratory analysis in RAxML showed this number of categories to improve the likelihood values obtained. We performed 500 independent searches starting from different MP initial trees. The ML tree was selected from the entire set of resulting trees on each search. Clade support was assessed with 1000 replicates of a non-parametric bootstrap analysis for the five loci matrix, the plastid matrix, and the nuclear *ppc*, also conducted with RAxML.

Ancestral character reconstruction within subfamily Cactoideae – To address the evolution of growth form within subfamily Cactoideae, we used the ML phylogeny obtained for the five loci matrix to perform a reconstruction of ancestral character states with the program BayesMultistate implemented in BayesTraits (Pagel, 1999; Pagel et al., 2004; program available at www.evolution.rdg.ac.uk), under an ML approach. This method reconstructs the most probable character states at each tested node, maximizing the probability of the character states observed in terminal taxa; allowing the incorporation of phylogenetic branch lengths by implementing a continuous-time Markov model of character evolution; with the number of rate parameters depending on the number of character states (Pagel, 1994, 1999; Pagel et al., 2004).

We scored four different characters describing the growth form of species within subfamily Cactoideae following discussions in Buxbaum (1951), Loup (1983), Gibson and Nobel (1986), Anderson (2001) and Arias and Terrazas (2006). Character states for Cactoideae species included in this study were obtained from published descriptions and photographs (Kattermann, 1994; Schulz and Kapitany, 1994; Leuenberger and Eggli, 2000; Porter et al., 2000; Anderson, 2001; Taylor and Zappi, 2004; Hunt et al., 2006). Scored characters are: (1) main growth form, (2) stem support, (3) stem features and (4) habit. For main growth form we distinguished six possible character states: arborescent (with a trunk branching above the base), shrubby (absence of a main trunk or with a basitonic branching), columnar (not branched), globose solitary (plants with spherical stems less than 0.5 m in height, growing single), globose caespitose (plants with spherical stems less than 0.5 m in height, growing in clumps), and barrel (plants with spherical stems with more than 0.5 m in height). As noticed by Gibson and Nobel (1986, p. 141), in the flat-stemmed epiphytes of the subfamily Cactoideae the pith is small and encircled by a vascular cylinder like that found in narrow-stemmed, ribbed, terrestrial cacti; hence, we classified epiphytes (for example, species in *Epiphyllum*, *Rhipsalis* or *Hylocereus*) as having a shrubby growth form. For the stem support character we distinguished two possible states: erect or non-erect. For stem features we distinguished two character states: ribbed or tubercled; and finally, for habit, we distinguished two character states: terrestrial or epiphytic.

RESULTS

The final five loci data matrix includes 6148 bp, of which 3400 (55.3 %) are invariant and 1597 (26.2%) are parsimony informative. Table 3 summarizes the information content of each genomic region. The alignment of *trnK-matK* plus *matK* was the longest, and provided the majority of parsimony informative sites.

In spite of the need to visually inspect and edit each alignment carefully, particularly for some highly variable regions within *trnL-trnF*, our alignment strategy yielded an adequate global alignment in a reasonable time. The final five loci matrix was partially complete, due to unavailable plant tissues, or unsuccessful lab techniques. For some taxa, only fragmentary sequences could be obtained (Appendix 1).

The parsimony ratchet analysis for the five loci matrix resulted in 83 most parsimonious trees of 10476 steps each (CI=0.5137, RI=0.7521). The ML analysis identified a most likely tree of lnL = -55205.24 (Figs. 1-4). ML bootstrap values (bML) were generally higher than those obtained with parsimony (bMP; Figs. 1-4); however parsimony and ML analyses failed to find strong support for derived nodes, particularly within core Cactoideae (see Discussion). Parsimony and ML trees differed mostly in relationships close to the tree tips within this clade. Analyses with maximum likelihood methods provided better results than parsimony ratchet in terms of tree resolution and computing time.

The ML phylogeny obtained for the plastid matrix (*trnL-trnF, trnK-matK, matK, rpl16*), its topology and support values are highly similar to the ones obtained with the five loci matrix, probably due to the overwhelming amount of plastid data in relation to the amount of nuclear data. The nuclear region *ppc* was difficult to amplify for several species with our experimental lab strategy, and we were only able to obtain sequences for a portion of our sample including 118 taxa. Although some main clades present in the five loci and in the plastid resulting trees were also recovered with the *ppc* nuclear region (see Discussion); several relationships were not recovered, and support values obtained with *ppc* were very low, particularly for deep nodes. The percentage of variable sites in *ppc* (Table 3) as well as the low support values for deeper nodes and better support values for derived nodes in the *ppc* ML phylogeny, indicate that this marker by itself is not adequate to infer relationships among main Cactaceae clades. However, in spite of the technical difficulties in its amplification and isolation, the *ppc* marker might be useful to infer relationships within derived cacti lineages.

Phylogenetic relationships are here discussed on the basis of the ML tree obtained for the five loci matrix, with comparisons to the MP strict consensus obtained from the same matrix, and with references to the ML nuclear and plastid trees.

To infer the ancestral character states and evolutionary trends of growth form and habit within Cactoideae we used the ML phylogeny for the five loci matrix. Methods used provide a probability value for each character state at each tested node. We reconstructed the ancestral characters of main clades and clades with high support values, identifying the most probable character state at each selected node. A representation of results is shown in Figure 5.

DISCUSSION

***Phylogenetic analyses* –** Previous phylogenetic studies have revealed difficulties to resolve phylogenetic relationships within Cactaceae (Nyffeler, 2002; Arias et al., 2003, 2005). Although the consensus tree obtained with the resultant MP phylogenies for the five loci matrix provided solid hypotheses about relationships among main lineages; it usually lacked resolution (resulted in polytomies) among derived lineages within Cactoideae, particularly in the South American lineages within the core Cactoideae clade (see Discussion below).

In the search of alternatives to MP, we explored ML phylogenetic methods. RAxML is a heuristic parallelized program that uses ML as optimization criterion to infer phylogenetic relationships. It implements a novel algorithmic optimization of the likelihood function to estimate large phylogenies in short times (Stamatakis, 2006). Experimental studies on large real-world datasets have shown that RAxML can find trees with higher likelihood values in less time and with less computer memory requirements than other programs, for example, GARLI or PHYML (Hordijk and Gascuel, 2005;). Stamatakis (2006) also reports a better performance of RAxML in comparison to MrBayes, nonetheless he recognizes the comparison is not fair because it considers the algorithm of the latter as an ML heuristic. We mostly base our discussion of phylogenetic relationships and evolution of lineages within Cactaceae on the obtained ML tree for the five loci matrix (Figs. 1-4). Previous molecular phylogenetic studies have provided insights about relationships among Cactaceae (Nyffeler, 2002), and within its subclades (e.g. Butterworth et al., 2002; Arias et al., 2003, 2005). The larger taxon sampling and sequence data included in this study, particularly of the North American tribes Pachycereeae and Cacteae, allows an independent assessment of relationships among major lineages within Cactaceae, as well as greater resolution within particular clades. The obtained phylogenetic hypothesis represents a framework to evaluate evolutionary trends within the family, and we used it to trace the evolution of growth forms within subfamily Cactoideae, which possesses the largest diversity of forms within Cactaceae.

Evolutionary relationships: early diverging Pereskia and Maihuenia – *Pereskia* and *Maihuenia* have usually been placed within subfamily Pereskioideae, because they lack many derived characters of Cactaceae (e.g. Cactoideae members, Edwards et al., 2005). *Maihuenia*, however, has also been placed within Opuntioideae (Britton and Rose, 1919-1923; Butterworth and Wallace, 2005) or within its own subfamily, Maihuenioideae (Anderson, 2001). Our study confirms the paraphyly of *Pereskia* (Fig. 1), whose members have been found to form an early diverging grade within Cactaceae in molecular phylogenies (Butterworth and Wallace, 2005; Edwards et al., 2005). *Maihuenia* is a cushion- or mat-forming shrub from the southern Andes and Patagonia. Species of *Maihuania* have persistent leaves; and their relationship to either *Pereskia* or Opuntioideae has been suggested (Leuenberger, 1997; Anderson, 2001). In contrast to *Pereskia*; *Maihuenia* has anatomical adaptations to xeric environments in cold high-Andean habitats (Leuenberger, 1997; Mauseth, 1999). Previous molecular phylogenies have placed *Maihuenia* as sister of Cactoideae (Edwards et al., 2005), Opuntioideae (Nyffeler, 2002), or showed no support to any relationship to either of the two subfamilies (Butterworth and Wallace, 2005). In the five loci ML tree, *Maihuenia* is weakly supported as the sister of ((Opuntioideae, Cactoideae) *P. lychnidiflora*) (Fig. 1). Our results suggest the placement of *Maihuenia* among the branches of the *Pereskia* grade (Fig. 1).

Subfamily Opuntioideae – The monophyly of Opuntioideae has long been recognized (Barthlott and Hunt, 1993; Griffith and Porter, 2009). Although the inclusion of all described genera within a single genus, *Opuntia*, has been proposed (Hunt, 1999), they are morphologically diverse (Fig. 1) and occupy a wide range of habitats through the American continent. Our study confirms the monophyly of Opuntioideae with the five loci matrix and with the nuclear marker (100 bML/100 bMP; Fig. 1). The five loci ML phylogeny shows that it is composed of three main clades: one including the cylindrical-stemmed *Quiabentia*, *Grusonia*, *Pereskiopsis* (Fig. 1e) and *Cylindropuntia* (100 bML/96 bMP); another with species characterized by rather possessing spherical stems, including *Maihueniopsis*, *Tephrocactus* (Fig. 1c) and *Pterocactus* (Fig. 1d) (78 bML/63 bMP); and a third, which contains *Miqueliopuntia* and *Tunilla* -with cylindrical to spherical stems-, as early divergent members, and also *Opuntia* and *Nopalea*, with flattened stems (cladodes; Figs. 1a-1b) (100 bML/99 bMP). These results differ slightly from those of Griffith and Porter (2009) who, based on ITS and *trnL-trnF* and a denser taxonomic sampling, found *Pterocactus* to be separated from (*Maihueniopsis*, *Cumulopuntia*).

***Cactoideae* –** Members of subfamily Cactoideae possess succulent stems, usually with ribs or tubercles (Wallace and Gibson, 2002; Figs. 2-4). The common ancestry of all of its members has been supported by morphological, anatomical, and molecular data (Wallace, 1995b; Wallace and Cota, 1995; Terrazas and Arias, 2003). Previous molecular phylogenies (Nyffeler, 2002), and our results (Fig. 2) confirm this monophyly with high support values (94 bML/85 bMP). Cactoideae encompasses the greatest species richness and growth form diversity within Cactaceae, including approximately 80% of the species in the family (Anderson, 2001; Hunt et al., 2006). It has been subdivided into several tribes, but morphological studies have proven insufficient to clarify relationships within them and even within genera, presumably because of convergent evolution at the species level (Applequist and Wallace, 2001b; Nobel, 2002). According to our five loci ML tree, Cactoideae consists of two large sister clades, Cacteae and core Cactoideae; and *Blossfeldia* as sister to this pair (Fig. 2). Based also on molecular phylogenetic studies, Crozier (2004) suggests to divide Cactoideae into two subfamilies, Cactoideae and Rhipsalidoideae (see Discussion below), and to place *Blossfeldia* within its own subfamily, Blossfeldioideae.

***Blossfeldia* –** As in previous studies (Nyffeler, 2002; Butterworth, 2006), we found *Blossfeldia*, a monotypic genus, as the strongly supported sister of the rest of Cactoideae (96 bML/85 bMP for its inclusion in Cactoideae and 95 bML/84 bMP for the monophyly of all other Cactoideae; Fig. 2). *Blossfeldia*, the smallest cactus, is widely distributed in Argentina and Bolivia, where it grows in rock crevices (Anderson, 2001). It has morphological and ecological features rarely found in other cacti, and has been generally recognized as a clearly distinct genus within tribe Notocacteae (Gibson and Nobel, 1986; Barthlott and Hunt, 1993; Anderson, 2001) or even proposed to be placed in its own subfamily, Blossfeldioideae

(Croizer, 2004). Nyffeler (2002) reported for the first time the intriguing early diverging position of *Blossfeldia* within Cactoideae. This lead to a controversy in the literature trying to explain *Blossfeldia*'s morphologically derived characters (Gorelick, 2004; Butterworth, 2006). Gorelick (2004) stated a series of hypotheses to explain Nyffeler's results. He noticed that *Blossfeldia liliputana* is a hexaploid species, and suggested that this could indicate its possible hybrid origin. In this case, chloroplast sequences would not show the evolutionary history of *Blossfeldia* because they are maternally inherited. Our analyses based on the five loci data set and on independent loci, including the nuclear *ppc*, confirm *Blossfeldia* as the sister to all other Cactoideae (Fig. 2).

***Cacteae* –** Cacteae is the only traditionally recognized tribe of Cactoideae that is strongly supported as monophyletic in our ML analyses (100 bML/100 bMP, Fig. 2). This tribe comprises all the North American globose, barrel-shaped and short-cylindrical cacti (Fig. 2), and reaches its greatest morphological diversity and species richness in Mexico, particularly in the southeastern Chihuahuan Desert (Hernández, et al. 2001; Hernández and Gómez-Hinostrosa, 2005). Previous molecular phylogenies also support the monophyly of Cacteae (Butterworth et al., 2002; Nyffeler, 2002). Buxbaum (1958) described it as "a clear-cut phylogenetic unit", and divided it into subtribes based mainly on seed morphology. Croizer (2004) even proposed to taxonomically raise the tribe Cacteae to the level of subfamily, recognizing it as subfamily Cactoideae; and placing the remaining tribes in other subfamily, Rhipsalidoideae.

Our five loci ML tree provided resolution of four main lineages within Cacteae with well supported relationships (Fig. 2). Within these lineages, we recognized some clades similar to the previous ones reported by Butterworth et al. (2002); which they found with a molecular MP phylogenetic analyses based on *rpl16*. However, our results show different relationships among them. Butterworth et al. (2002) found a clade they called "*Aztekium*" (*Aztekium*, *Geohintonia*) as the earliest divergent within Cacteae (Butterworth et al., 2002). We consistently recovered the same "*Aztekium*" clade (93 bML/84 bMP); however; in our results it is associated with *Echinocactus* and *Astrophytum*, and both clades together form a poorly supported clade sister of all remaining Cacteae (Fig. 2). An association between *Echinocactus* and *Astrophytum* was also found in our nuclear *ppc* analyses, as the earliest divergent clade within Cacteae. Although in the five loci ML tree *E. grusonii* appears to be the earliest diverging branch within the *Aztekium* clade, this position is not recovered in the plastid ML phylogeny. The *Aztekium* clade includes solitary globose to barrel cacti with strongly ribbed stems.

The next clade to diverge within tribe Cacteae includes *Sclerocactus brevihamatus* and *Echinomastus* (100 bML/95 bMP); although its earliest diverging position from remaining Cacteae members is poorly supported (58 bML). This clade includes solitary globose plants with low ribs. The next clade to diverge is only moderately supported in our ML analyses (81 bML), and is similar to the "*Ferocactus*" clade found by Butterworth et al. (2002). This clade includes *Stenocactus spp.*, *Thelocactus spp.*, *Leuchtenbergia principis, Ferocactus spp.* and *Sclerocactus uncinatus*, and a similar association, although poorly supported, was found also in our *ppc* analyses. Species within this clade are solitary or clustering, and possess stems with ribs, tuberculated ribs (*S. uncinatus*) or long tubercles (*L. principis*). The position of *S. uncinatus* as sister to *Ferocactus latispinus* and *F. haematacanthus* species was recognized in our five loci ML and MP trees (Fig. 2). *Sclerocactus uncinatus* and *S. uncinatus* ssp. *crassihamatus* are synonyms of *Glandulicactus uncinatus* and *G. crassihamatus*, respectively.

Some authors (e.g., Barthlott and Hunt, 1993; Hunt, 1999; Anderson, 2001) do not recognize *Glandulicactus* as a distinct genus. Considering vegetative and floral morphology, and molecular phylogenies, this genus has been suggested to be closer to *Ferocactus* and *Thelocactus* than to *Sclerocactus* (Ferguson, 1991; Butterworth et al., 2002). Our results, although weakly supported, suggest that *Ferocactus* is polyphyletic, and suggest the close relation of *Sclerocactus uncinatus* with members of *Ferocactus* (Fig. 2).

The genus *Sclerocactus* has been subject to much controversy. Our analyses included two of its species (*S. brevihamatus* and *S. uncinatus*), but they did not group together, suggesting that this genus is also polyphyletic. Based on molecular and morphological data, Porter (1999) concluded that *Sclerocactus* should be subdivided, and that *Echinomastus* should be recognized as distinct. In spite of our limited intrageneric sampling, our results support Porter (1999) and Porter et al. (2000) in proposing that *Sclerocactus* should be revised, and that *Echinomastus* and *Glandulicactus* should be recognized as distinct taxa, supporting the inclusion of *S. brevihamatus* within the genus *Echinomastus* (Anderson, 2001).

A sister pair consisting of (*Ariocarpus*, *Strombocactus*, *Turbinicarpus*, and *Epithelantha*), and the "Mammilloid" clade (Butterworth et al., 2002) is strongly supported in the five loci ML tree (94 bML), but weakly so in the MP tree (Fig. 2). A clade similar to the former was recognized by Butterworth et al. (2002; their "ATEP" clade), except for the absence of *Strombocactus* and the presence of *Pediocactus*. The "Mammilloid" clade (Butterworth et al., 2002) includes (*Acharagma, Lophophora*, *Obregonia*) plus (*Coyphantha*, *Mammillaria*, (*Cochemiea, Neolloydia, Ortegocactus*)). Previous studies, as well as our results, suggest that *Mammillaria* is polyphyletic (see position of *M. picta* in Fig. 2, and Butterworth and Wallace, 2004). An association among *Acharagma, Lophophora* and

Obregonia was also found by Butterworth and collaborators (the "*Lophophora*" clade; Butterworth et al. 2002) but in their study, this group was distantly related from the "Mammilloid" clade. Although *Cochemiea*, a genus from the Baja California Peninsula in Mexico, has been placed within *Mammillaria* in recent taxonomic reports (Hunt et al., 2006) and MP phylogenetic analyses (Butterworth and Wallace, 2004), here we confirm that it is independent from *Mammillaria*, suggesting that it should be regarded as taxonomically distinct (Barthlott and Hunt, 1993; Anderson, 2001). The "Mammilloid" clade represents one of the most spectacular radiations of Cactaceae in the arid and semiarid regions of North America. This clade comprises small globose members with tuberculated stems and dimorphic areoles (Butterworth et al., 2002), and includes the richest genera within Cacteae, namely *Mammillaria* (ca. 145 species), *Coryphantha* (55 species) and *Escobaria* (23 species; Hunt, 1999). The derived position of the "Mamilloid" clade within Cacteae may be consistent with a recent diversification.

Core Cactoideae – The strongly supported clade here referred to as core Cactoideae (94 bML, Fig. 3) is another species-rich clade within Cactaceae including species distributed throughout the American continent (Nyffeler, 2002). Core Cactoideae has been previously recognized with molecular data (Nyffeler, 2002; Croizer, 2004). It includes members of tribes Pachycereeae, Hylocereeae, Browningieae, Trichocereeae, Cereeae, Rhipsalideae, and Notocacteae; which, as in previous results (Nyffeler, 2002; Arias et al., 2005; Ritz et al., 2007), were found here to be para- or polyphyletic. Core Cactoideae encompasses a vast diversity of growth forms (Figs. 3-4), including gigantic arborescent forms (such as species of *Echinopsis*, *Pachycereus* or *Neobuxbaumia*), solitary or clumped globose forms (such as species of *Parodia* and *Copiapoa*, respectively), and hanging epiphytes with cylindrical spineless or flattened two-ribbed stems (for example, different members of *Rhipsalis* or *Hylocereus*); distributed throughout America.

Early divergent genera Copiapoa, Calymmanthium and Frailea – *Copiapoa*, *Calymmanthium* and *Frailea* are weakly supported as the three earliest diverging branches within core Cactoideae in the five loci ML tree (Fig. 3). This position was not found in the corresponding MP tree, where *Frailea* grouped with members of Rhipsalideae, and *Calymmanthium* grouped with *Pfeiffera*. The position of these genera was uncertain in the study of Nyffeler (2002).

Copiapoa is endemic to the Atacama Desert in northern Chile, and includes twenty six species of globose to short cylindrical cacti that can form massive clumps, and are highly adapted to aridity (Schulz and Kapitany, 1994; Anderson, 2001). The monophyly of *Copiapoa* is well supported (100 bML/100 bMP). Its position as sister to all other core Cactoideae was also found in our MP analyses (Fig. 3), but not in the *ppc* ML tree, however its relationships may be reconsidered given the weak support of the branches diverging after it, especially in MP analyses (see also Nyffeler, 2002).

Calymmanthyum is a monotypic arborescent genus endemic to northern Peru, with unique anatomical and morphological features, like a uniseriate to biseriate collenchymatous hypodermis with relatively thin walls, with no mucilage cells in either cortex or pith; and its flower, with an inner perianth partially shielded by an outer floral tube (Anderson, 2001, Nobel, 2002, Wallace, 2002). It has been suggested that it retains numerous plesiomorphic features within Cactoideae, for example, few ribbed juvenile shoots (Nobel, 2002; Wallace, 2002; Wallace and Gibson, 2002). The position of *Calymmanthium* in the five loci ML tree is weakly supported, and unresolved within Cactoideae in the *trnK/matK*, *matK* and *ppc* independent analyses. The genus *Frailea* was established by Britton and Rose (1919-1923) to include eight species of dwarf globular cacti from southern Brazil and northeast Argentina. Anatomical studies show that *Notocactus*, *Blossfeldia* and *Frailea* share features in common with *Parodia* (see references in Eggli and Nyffeler, 1998), but our results, as well as those of Nyffeler (2002), suggest that *Frailea* is an unrelated, independent lineage. Its position in the ML tree (Fig. 3) is weakly supported.

Core Cactoideae I – Following the divergence of *Frailea*, our ML tree shows a pair of large sister clades, here referred to as core Cactoideae I and core Cactoideae II. Core Cactoideae I is composed of *Pfeiffera ianthothele*; a strongly supported *Eulychnia* and *Austrocactus* clade (100 bML/84 bMP); and a clade containing Pachycereeae, Hylocereeae and Browningieae members, here termed the PHB clade (85 bML; Fig. 3).

Austrocactus and *Eulychnia*, together with *Corryocactus*, were considered early diverging members of Notocacteae (Buxbaum, 1969). *Austrocactus* comprises five or six species of globose to short cylindrical cacti from southern Argentina and Chile, thought to be closely related to *Corryocactus* (Anderson, 2001). *Eulychnia* on the other hand includes arborescent candelabriform, sometimes gigantic, species (e.g., *E. breviflora*; Anderson, 2001) that inhabit the coastal deserts of Chile and southern Peru. The highly supported (*Austrocactus*, *Eulychnia*) clade is a good example of the high divergence of growth forms that can be found among closely related taxa within core Cactoideae. In our five loci ML and MP phylogenies, the (*Austrocactus*, *Eulychnia*) clade is sister to the remaining members of core Cactoideae I (Fig. 3).

A clade similar to the PHB was previously recognized by Nyffeler (2002), who referred to it as the AHCLP clade. The PHB clade (94 bML) includes members of tribe

Pachycereeae, Hylocereeae and three genera formerly included in Browningieae: *Castellanosia, Neoraimondia* and *Armatocereus*. *Corryocactus*, which includes shrubby to arborescent columnar species from Peru, western Bolivia and northern Chile, is sister to the PHB clade (85 bML; Fig. 3). Whereas its tribal placement has been subject of controversy (Anderson, 2001), its position as the sister branch to the PHB clade (Fig. 3) was also found in previous molecular phylogenies (Nyffeler, 2002; Wallace, 2002; Wallace and Gibson, 2002).

A weakly supported clade containing *Armatocereus, Neoraimondia, Castellanosia* (Browningieae), and *Dendrocereus, Leptocereus* and *Pseudoacanthocereus* (formerly in Pachycereeae) was recognized in our five loci ML and MP analyses (Fig. 3). *Castellanosia, Neoraimondia, Armatocereus* and *Leptocereus* were unresolved in the AHCLP clade of Nyffeler (2002). The geographical distribution of these genera makes their association intriguing. *Leptocereus* and *Dendrocereus* share a Caribbean distribution (Anderson, 2001), but whereas the former contains sprawling cylindrical forms, the latter contains arborescent species with woody trunks. Their sister species, *Castellanosia caineana* from lowland Bolivia, is also arborescent. *Castellanosia* was considered as part of *Neoraimondia* (Mauseth and Kiesling, 1998; Kiesling and Mauseth, 2000), however in our five loci ML tree, their representative species do not form a clade (Fig. 3). *Neoraimondia* includes arborescent candelabriform cacti up to 15 m tall, from the Peruvian coast and the Peruvian and Bolivian Andes (Anderson, 2001). *Armatocereus* is a massive shrubby genus with segmented, cylindrical stems, mainly from the Andes of Colombia, Ecuador and Peru (Anderson, 2001). *Pseudoacanthocereus* is distributed in Brazil and Venezuela, and includes sprawling to upright shrubs (Anderson, 2001). The finding of a South American/Caribbean clade as early divergent within the PHB clade supports previous suggestions regarding the possibility that the closest relatives of North American columnar Pachycereeae and the primarily epiphytic Hylocereeae are South American (Wallace, 2002).

Pachycereeae is the second largest tribe of North American cacti, and represents a diversification that gave rise to columnar and arborescent forms that can reach gigantic sizes, such as the saguaro (*Carnegiea gigantea*) and cardón (*Pachycereus pringlei*) from northern Mexico and southwest USA. In their anatomical, chemical and morphological study of Mexican columnar cacti, Gibson and Horak (1978) and Gibson (1982) recognized two major lineages within Pachycereeae: subtribe Pachycereinae (*Pachycereus*, *Neobuxbaumia*, *Cephalocereus*, *Carnegiea, Mitrocereus* and *Lophocereus*) and subtribe Stenocereinae (*Escontria, Myrtillocactus, Polaskia* and *Stenocereus*). Our results are congruent with this hypothesis, as we recovered clades similar to the described subtribes. The North American columnar genera are well supported as a monophyletic group (100 bML/74 bMP), here referred to as core Pachycereeae. The deepest split within this clade separates the clades we named Pachycereinae (99 bML/82 bMP) and Stenocereinae (99 bML; Fig. 3) following Gibson and Horak (1978) and Gibson (1982). However; in addition, and as in previous studies (Arias et al., 2005), we found a strongly supported *Echinocereus* clade as sister to the remaining members of Stenocereinae (Fig. 3).

Peniocereus had been typically considered as closely associated with Pachycereeae or Echinocereeae (Barthlott and Hunt, 1993; Anderson, 2001), and molecular and morphological studies showed this genus to be paraphyletic (Arias et al., 2005; Gómez-Hinostrosa and Hernández, 2005); with some of its species more closely associated to Hylocereeae. Our results confirm these previous results in that *Peniocereus* subgen. *Pseudoacanthocereus* (*P. castellae* and *P. chiapensis*) is closely related to *Acanthocereus tetragonus* and to tribe

Hylocereeae, forming a clade here named “expanded Hylocereeae”; whereas other species of *Peniocereus* (e.g., *P. greggii*, *P. johnstonii*, *P. serpentinus* and *P. viperinus*) are closer to Pachycereinae.

Hylocereeae is one of only two tribes within Cactoideae that includes epiphytes (see below). Its members are scandent or epiphytic shrubs with flattened or few-ribbed stems, mainly distributed in Central America. It was considered to be related to Echinocereeae (*Harrisia, Acanthocereus*) and Cereeae (Barthlott and Hunt, 1993). As recognized by Arias et al. (2005), we found that all included species of Hylocereeae form a weakly supported monophyletic group within the PHB clade (Fig. 3).

Core Cactoideae II – Core Cactoideae II includes all remaining South American members of core Cactoideae as well as members of tribe Rhipsalideae. This clade is recognized, but weakly supported, in our five loci ML and MP analyses (Fig. 4). Species included within this clade were scarcely sampled in our nuclear *ppc* analyses, and did not form the same association as in the phylogenetic results obtained with the plastid or the five loci matrices.

Rhipsalideae, mostly distributed in eastern Bolivia and southeastern Brazil (Barthlott and Hunt, 1993), is another Cactoideae tribe that includes epiphytes and lithophytes. A clade comprising members of tribe Rhipsalideae (100 bML/99 bMP) was found in our five loci ML tree as the sister of the remainder of core Cactoideae II, but with weak support (Fig. 4). Nyffeler (2002) found an equivalent relationship, but with stronger support, suggesting a close relationship between some Rhipsalideae members and the remaining South American tribes (i.e., Cereeae, Notocacteae, Trichocereeae, Browningieae) within core Cactoideae II. Nevertheless, in our five loci MP tree, the Rhipsalideae clade is sister of (Core Cactoideae I, Core Cactoideae II) and in the nuclear *ppc* analyses, a clade of (*Hatiora salicornoides*, *Rhipsalis baccifera*) is sister to representative species of Core Cactoideae I.

Pfeiffera, regarded as “transitional” between *Corryocactus* and Rhipsalideae (Gibson and Nobel, 1986), is the only traditional member of Rhipsalideae that did not group with other members of this tribe. Instead, *Pfeiffera* was found to be closely related to North American members of core Cactoideae (Fig. 3), as previously recognized by Nyffeler (2002). This position needs to be confirmed with sequences obtained from other specimen and species, given that we used Nyffeler’s data for our single representative of this genus. Besides *Pfeiffera,* the epiphytic habit hence seems to have evolved independently in two distantly related lineages of Cactaceae: in Hylocereeae, within core Cactoideae I; and in Rhipsalideae, within core Cactoideae II.

A clade containing *Eriosyce*, *Parodia* and *Neowerdermannia vorwerkii* (93 bML/80 bMP) was here recognized (Fig. 4). Nyffeler (2002) found an equivalent clade, and referred to it as core Notocacteae. *Eriosyce* includes 33 to 35 species of globular to elongated, rarely columnar cacti distributed in central Chile, southern Peru and northwest Argentina (Anderson, 2001). It was previously allied to *Austrocactus*, *Eulychnia, Copiapoa*, *Corryocactus* and *Neowedermannia* (Kattermann, 1994). *Parodia* includes approximately 29 accepted and 76 provisional species, which are small to moderately-sized, solitary or clustering, generally with globose stems, and inhabit the east slope of the Andes in southern Brazil, Uruguay and Paraguay to the northern half of Argentina (Eggli and Nyffeler, 1998; 2007). *Neowerdermannia* consists of two globose species disjunctly distributed in southwestern Chile (*N. chilensis*), and Bolivia and northern Argentina (*N. vorwerkii*; Kattermann, 1994). It has been previously associated with *Gymnocalycium* (Anderson, 2001). Our results, together with those of Nyffeler (2002), indicate that Notocacteae sensu Anderson (2001) and Barthlott and Hunt (1993) is a polyphyletic assemblage with *Austrocactus* and *Eulychnia* closely related to Pachycereeae; *Copiapoa* as an early diverging independent lineage within core Cactoideae; and *Eriosyce, Parodia* and *Neowedermannia* forming a clade within core Cactoideae II.

A clade including all sampled members of tribe Trichocereeae, and some members of Cereeae and Browningieae, conform another major clade within core Cactoideae II (Fig. 4). A similar association has been referred to as the BCT clade (Nyffeler, 2002; Wallace, 2002), and includes several South American columnar or arborescent species, with *Uebelmannia* as its earliest diverging branch (Fig. 4, see also Nyffeler, 2002). Our results recover this BCT clade as strongly supported (100 bML/91 bMP), and it was also recovered with good support values in the nuclear *ppc* analyses. After *Uebelmannia*, several taxa formerly assigned to Browningieae and Cereeae, as well as *Discocacuts*, *Rebutia* and *Sulcorebutia* (Trichocereeae), form an early diverging grade within BCT (Fig. 4). *Browningia* and *Stetsonia* (Browiningieae) are shrubby or large arborescent forms (Fig. 4e), mainly from the central Andean region (northern Chile, Bolivia and Peru), and presumably display plesiomorphic floral attributes (Wallace, 2002). In contrast to the western South America diversity-centered taxa (i.e., *Gymnocalycium*, *Parodia*, *Eriosyce*), former members of Tribe Cereeae are most diverse in eastern South America. This tribe includes cylindrical-shaped species ranging from large, robust trees to thin-stemmed shrubs, but also includes globular forms, such as *Melocactus* (Fig. 4c). Tribe Trichocereeae consists of long stemmed cylindrical cacti displaying a wide variety of habits, including sprawling, shrubby, large arborescents or candelabriforms, and even globoses (possibly reduced; Wallace, 2002) forms. In our five loci ML analysis, members of Trichocereeae, except for *Discocacuts*, *Rebutia* and *Sulcorebutia*, belong to a weakly supported clade nested within BCT (Fig. 4). Our analyses indicate that *Echinopsis* sensu lato is polyphyletic, and requires to be taxonomically revised.

***Evolutionary trends of growth form and habit within Cactoideae* –** Probably no other plant family exceeds Cactaceae in diversity of structure (Mauseth, 2006), making difficult to propose an inclusive, simple group of categories for the extraordinary diversity of growth forms and habits present in Cactaceae (Anderson, 2001). Subfamily Cactoideae is probably the richest in terms of species number and diversity of growth forms (see Barthlott and Hunt, 1993; Anderson, 2001). Within the Subfamily, the Cacteae clade includes globose cacti with ribbed or tubercled stems, which range in sizes from few centimeters (*Escobaria*) to a couple of meters above the ground (*Echincactus platyacanthus*); while the predominant growth forms in the core Cactoideae clade are scandent, shrubby or arborescent cacti with cylindrical stems. The arborescent and the columnar growth form evolved in derived clades both in Core Cactoideae I and Core Cactoideae II; and both clades include also globose forms, apparently by stem reduction.

To trace the evolution of growth forms and habits within subfamily Cactoideae, we performed a reconstruction of ancestral characters using ML methods. According to our results (Fig. 5), the ancestor of Cactoideae was a solitary globose cacti with a ribbed stem. However, the probability values for this growth habit at this node is not high (0.30). The ancestral growth form of the Cacteae clade was inferred to be also a ribbed, solitary globose, with a probability of 0.99 and 0.93 respectively (Fig. 5). Nevertheless, the ancestral condition for the ("Mamilloid" clade and "*Lophophora*" clade) plus the ATEP clade appears to be clustering or caespitose, globose, with tubercled stems, which evolved towards a solitary stem within the ATEP clade (Fig. 5).

According to our results; in contrast to the globose ancestral condition in Cacteae, the ancestor of Core Cactoideae was probably a ribbed barrel (Fig. 5). This condition probably changed in the ancestral node for Core Cactoideae I, where the shrubby character state had the highest probability (Fig. 5). The main clade within Core Cactoideae I is the PHB clade. The prevailing growth forms observed among early diverging lineages within the PHB clade are arborescent or shrubby (e.g., *Armatocereus*, *Neoraimondia*), or more frequently, sprawling epiphytes or lithophytes (e.g., *Selenicereus, Hylocereus, Pseudoacanthocereus, Disocactus, Peniocereus*) distributed in Central and South America. The columnar or candelabriform forms within this clade acquired their most conspicuous expression in highly nested clades, such as in core Pachycereeae, suggesting a trend towards the evolution of a large columnar and arborescent growth forms. Our reconstruction suggests shrubby cacti with non-erect stems as ancestral to the PHB clade. This non-erect, shrubby condition apparently evolved into an arborescent ancestor in the Stenocereinae clade, and a columnar ancestor in the Pachycereinae clade (both with probabilities of 0.4, see Fig. 5). Our results suggest that the large columnar and arborescent North American cacti probably evolved from a Central or South American shrubby, non-erect ancestor (but see below).

The ancestor of the majority of the evaluated nodes was most probably terrestrial, including the “expanded Hylocereeae” clade. Our results confirmed that the epiphytic condition is derived, and originated independently in two evolutionary lineages: the Rhipsalideae clade, and the Hylocereeae sensu stricto clade (Fig. 5 and Table S4.1).

The globose solitary, ribbed condition found to be most probable at the base of the Cacteae clade, appeared again in the ancestral node of Core Notocacteae, possibly as a reduction of a barrel ancestor (Fig. 5). However, the ribbed, barrel-shaped condition prevailed as the most probable in the BCT and Trichocereeae ancestral nodes tested. Our results indicate that, on the contrary of the evolution of columnar and arborescent North American cacti, the columnar and arborescent South American cacti (for example, *Echinopsis chiloensis* or *Browningia candelaris*) evolved from a barrel ancestor (Fig. 5). Unfortunately, the poor support values and taxonomic sampling within the BCT and the Trichocereeae clades in our study do not allow a more detailed reconstruction and comparison.

Within core Cactoideae II, and particularly in the BCT clade, distinct trends in growth form evolution are not apparent, possibly due to the high variability and convergence of the character within lineages. The absence of clearly distinct trends may be also the consequence of insufficient taxonomic representation, particularly of South American lineages.

CONCLUSIONS

The phylogenetic relationships presented in this study corroborate previous results, and provide further resolution of evolutionary relationships and tendencies within Cactaceae, in the context of a denser generic representation, molecular sequence data from the plastid and nuclear genomes, and parsimony and maximum likelihood phylogenetic analyses. The onset of diversification within Cactaceae gave rise to a phylogenetic grade that includes *Pereskia*, and possibly *Maihuenia*. Following that early grade, the subfamilies Opuntioideae and Cactoideae, each strongly supported as monophyletic, form a sister pair.

Opuntioideae consists of a clade of flat-stemmed *Opuntia* and *Nopalea*, a clade including genera with spherical stems, such as *Pterocactus* and *Maihueniopsis*, and a clade of cylindrical or spherical stemmed genera, such as *Cylindropuntia* and *Quiabentia*. Within Cactoideae, the placement of the monotypic *Blossfeldia* as the sister to (Cacteae, core

Cactoideae), is confirmed. This relationship was obtained both in analyses of the five loci matrix, the plastid matrix and of individual loci, including a nuclear marker. Cacteae, strongly supported as monophyletic, is a cohesive lineage that comprises the globose and barrel cacti from North America. Our ancestral character reconstruction analyses showed a possible trend within this clade from a globose ribbed ancestor towards a tubercled, caespitose or solitary cacti present in derived nodes. Our results indicate possible cases of paraphyly and polyphyly in the circumscriptions of several genera within Cacteae (*Mammillaria*, *Sclerocactus*, *Echinocactus*, *Ferocactus, Turbinicarpus*).

Core Cactoideae is a well supported clade comprising species of North and South America that display a wide variety of growth forms. Its earliest diverging lineages appear to be South American globose forms (*Copiapoa*, *Frailea*), but their relationships are weakly supported. Our results suggest a barrel, ribbed condition as the ancestor of core Cactoideae; which changed to a shrubby ancestor in the core Cactoideae I clade. The predominant growth forms within core Cactoideae I are shrubs or trees with cylindrical stems, possibly derived from a South American ancestor. Our results suggest that the North American columnar or arborescent growth forms within the core Cactoideae I clade evolved from a shrubby, non-erect (sprawling, prostrated, clambering) ancestor; while arborescent South American lineages of the Trichocereeae clade evolved from a barrel-shaped ancestor. The barrel condition possibly became reduced towards a globose form in Core Notocacteae and other lineages within the BCT clade. Our results show the high diversity and frequent convergence in the evolution of growth form in this major clade.

Evolutionary relationships in Cactaceae, mainly in lineages within subfamily Cactoideae, have been difficult to elucidate only on the basis of morphological attributes, possibly because of substantial convergent evolution. Our phylogenetic results further document the complexities in resolving evolutionary relationships within Cactaceae using molecular data, even in the context of a substantial representation at the generic level. These molecular results provide solid information at the subfamilial level; however, relationships at derived phylogenetic levels generally lack strong support, including those of less-intensely studied South American lineages. A full understanding of phylogeny and evolutionary tendencies within Cactaceae will require a further increase in the taxonomic sampling, in the context of greater concerted efforts to improve data matrices by filling existing gaps.

ACKNOWLEDGMENTS

The authors are deeply thankful to R. Kesling and S. Arias, who kindly revised the manuscript. R. Kiesling also hosted a collecting trip to Argentina, and verified identifications of South American sampled cacti. C. Gómez-Hinistrosa provided valuable help in species identification and voucher preparation. We thank S. Cevallos, R. González, L. Márquez, R. Medina, M. Olson, J. Reyes and R. Tapia, for help in different aspects of this project. The Instituto de Biotecnología, UNAM (Macroproyecto de Tecnologías de la Información y la Computación) and Centro Nacional de Supercómputo, IPYCYT (San Luis Potosí, México) provided computing resources. T. H.-H. doctoral studies were funded by the Consejo Nacional de Ciencia y Tecnología (CONACYT), México and by the Posgrado en Ciencias Biológicas (UNAM). This research was partially funded by CONACYT-2004-C01-46475 research grant.

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APPENDIX 1

Taxa, voucher or plant register (if collected for this study, else, refer to GenBank number), geographic origin (lc if collected from a living collection), collection or herbarium (DBG AZ: Desert Botanical Garden, Phoenix, AZ; JB UNAM: Jardín Botánico de la Universidad Nacional Autónoma de México, MEXU: Herbario Nacional de México, CORD: Herbario de la Universidad de Córdoba, Argentina) and GenBank accession number (*trnK-matK* , *trnL-trnF*, *rpl16*, *ppc* or na when sequence is not available). GenBank numbers in boldface indicate sequences obtained by us for this study.

Acanthocalycium spiniflorum* (Schumann) Backeberg**,1977 1079 0101 G,lc,DBG AZ,**HM041644**,**HM041222**,**HM041377**,na;Acanthocereus tetragonus* (Linnaeus) Hummelinck**,T.Hernández 5,México: Guerrero,MEXU,**HM041645**,**HM041223**,**HM041378**,**HM041534**;***Acharagma aguirreana* (Glass & R.A.Foster) C.E.Glass**,J. Reyes 4442,lc,JB UNAM,**HM041646**,**HM041224**,**HM041379**,**HM041535**;***Acharagma roseana* (Boedeker) E.F. Anderson**,J. Reyes 4411,lc,JB UNAM,**HM041647**,**HM041225**,**HM041380**,na;***Ariocarpus agavoides* (Castañeda) Anderson**,H. Hernández 2560,México: San Luis Potosí,MEXU,**HM041648**,**HM041226**,**HM041381**,**HM041536**;***Ariocarpus bravoanus* Hernandez & Anderson**,J. Reyes s.n.,lc,JB UNAM,na,**HM041227**,**HM041382**,**HM041537**;***Ariocarpus fissuratus* (Engelmann) Schumann**,C. Gómez 1758,México: Coahuila,MEXU,**HM041649**,**HM041228**,**HM041383**,**HM041538**;***Armatocereus godingianus* (Britton & Rose) Backeb.**,gb,-,-,AY015296,AY015346,na,na;***Armatocereus laetus* (Kunth) Backeberg ex A.W.Hill**,gb,-,-,na,DQ099923,DQ099992,na; ***Arrojadoa rhodantha* (Guerke) Britton & Rose**,1993 0548 0101 G,lc,DBG AZ,**HM041651**,**HM041230**,**HM041385**,**HM041539**;***Astrophytum capricorne* (Dietrich) Britton & Rose**,H. Hernández 2095,México: Coahuila,MEXU,**HM041652**,**HM041231**,**HM041386**,**HM041540**;***Astrophytum ornatum* (De Candolle) Britton & Rose**,T. Hernández H04,México: Hidalgo,MEXU,**HM041653**,**HM041232**,**HM041387**,**HM041541**;***Austrocactus bertinii* (Cels ex Henricq) Britton & Rose**,gb,-,-,AY015300,AY015300,na,na;***Aztekium ritteri* (Boedeker) Boedeker**,gb,-,-,AY015290,na,AF267923,na;***Bergerocactus emoryi* (Engelmann) Britton & Rose**,1969 9558 0101,lc,DBG AZ,**HM041654**,**HM041233**,**HM041388**,**HM041542**;***Blossfeldia liliputana* Werdermann**,T. Hernández p104,Argentina: Mendoza,MEXU,**HM041655**,**HM041234**,**HM041389**,**HM041543**;***Browningia candelaris* (Meyen) Britton & Rose**,1999 0004 0105,lc,DBG AZ,**HM041656**,**HM041235**,**HM041390**,**HM041544**;***Browningia hertlingiana* (Backeberg) Buxbaum**,gb,-,-,AY015315,AY015403-AY015362,na,na;***Calymmanthium substerile* Ritter**,gb,-,-,AY015291,DQ099926,AF267924-AY851614-DQ099995,na;***Carnegiea gigantea* (Engelmann) Britton & Rose**,s.n.,lc,JB UNAM,**HM041657**,**HM041236**,**HM041391**,**HM041545**;***Castellanosia caineana* (Cárdenas) D.R.Hunt**,gb,-,-,AY015298,AY015389-AY015348,na,na;***Cephalocereus apicicephalium* Dawson**,gb,-,-,na,DQ099927,DQ099996,na;***Cephalocereus columna-trajani* (Karwinsky ex Pfeiffer) Schumann**,T. Hernández 22,México: Puebla,MEXU,**HM041658**,**HM041237**,**HM041392**,**HM041546**;***Cephalocereus nizandensis* (Bravo & Macdougall) Buxbaum**,gb,-,-,na,DQ099928,DQ099997,na;***Cephalocereus senilis* (Haworth) Pfeiffer**,T. Hernández H12,México: Hidalgo,MEXU,na,**HM041238**,**HM041393**,**HM041547**;***Ceraria fruticulosa* H.Pearson & Stephens**,gb,-,-,AY875371,AF094846,na,na;***Cereus aethiops* Haworth**,1987 0458 2101 Z,lc,DBG AZ,**HM041659**,**HM041239**,**HM041394**,**HM041548**;***Cereus alacriportanus* Pfeiff.**,gb,-,-,AY015313,AY015313,na,na;***Cereus hildmannianus* Schumann**,1950 2885 0102 G,lc,DBG AZ,**HM041660**,**HM041240**,**HM041395**,**HM041549**;***Cleistocactus icosagonus* (Kunth) Weber ex Roland-Gosselin**,1950 2845 0102 G,lc,DBG AZ,**HM041661**,**HM041241**,**HM041396**,**HM041550**;***Cleistocactus parviflorus* (Schumann) Roland-Gosselin**,1994 0226 1006 W,lc,DBG AZ,**HM041662**,**HM041242**,**HM041397**,**HM041551**;***Cleistocactus tupizensis* (Vaupel) Backeberg**,1994 0227 1002 W,lc,DBG AZ,**HM041663**,**HM041243**,**HM041398**,**HM041552**;***Cochemiea pondii* (Greene) Walton**,V. Alvarado 36,México: Baja California Sur,MEXU,**HM041664**,**HM041244**,**HM041399**,**HM041553**;***Cochemiea poselgeri* (Hildmann) Britton & Rose**,T. Hernández p106,México: Baja California Sur,MEXU & JB UNAM,**HM041665**,**HM041245**,**HM041400**,**HM041554**;***Coleocephalocereus fluminensis* (Miquel) Backeberg**,gb,-,-,AY015318,AY015318,na,na;***Copiapoa cinerascens* (Salm-Dyck) Britton & Rose**,2002 0155

0101 W,lc,DBG AZ,**HM041666**,**HM041246**,**HM041401**,**HM041555**;***Copiapoa coquimbana*** **(Karwinski ex Ruempler) Britton & Rose**,1992 0994 0102 W,lc,DBG AZ,**HM041667**,**HM041247**,**HM041402**,**HM041556**;***Copiapoa humilis*** **(Philippi) Hutchison**,1992 0237 0101 Z,lc,DBG AZ,**HM041668**,**HM041248**,**HM041403**,**HM041557**;***Corryocactus aureus*** **(Meyen) Hutchison ex Buxbaum**,2003 0364 0101 G,lc,DBG AZ,**HM041669**,**HM041249**,**HM041404**,**HM041631**;***Corryocactus brevistylus*** **(Schumann) Britton & Rose**,gb,-,-,AY015302,AY015393-AY015352-AY566650,na,na;***Corryocactus tenuiculus*** **(Backeberg) Hutchison ex Buxbaum**,gb,-,-,AY015303,AY015394-AY015353,na,na;***Coryphantha salinensis*** **(Poselger) A.Zimmerman ex Dicht & A.Luethy**,T. Hernández 65,México: Nuevo León,MEXU,**HM041670**,**HM041250**,na,**HM041558**;***Coryphantha durangensis*** **(Schumann) Britton & Rose**,H. Hernández 2300,México: Coahuila,MEXU,**HM041671**,**HM041251**,**HM041405**,**HM041559**;***Coryphantha erecta*** **(Pfeiffer) Lemaire**,T. Hernández 97,México: Guanajuato,MEXU,**HM041672**,**HM041252**,**HM041406**,**HM041560**;***Coryphantha pycnacantha*** **(Martius) Lemaire**,T. Hernández 47,México: Oaxaca,MEXU,**HM041673**,**HM041253**,**HM041407**,na;***Cylindropuntia imbricata*** **(Haworth) F.M. Knuth**,T. Hernández 20,México: Puebla,MEXU,**HM041739**,**HM041320**,**HM041475**,na;***Dendrocereus nudiflorus*** **(Engelmann ex Sauvalle) Britton & Rose**,gb,-,-,na,DQ099929,DQ099998,na;***Denmoza rhodacantha*** **(Salm-Dyck) Britton & Rose**,1939 0374 0101 G,lc,DBG AZ,**HM041674**,**HM041254**,**HM041408**,**HM041561**;***Discocactus boomianus*** **(Buining & Brederoo) N. P. Taylor & Zappi**,1992 0232 0102 G,lc,DBG AZ,**HM041675**,**HM041255**,**HM041409**,**HM041562**;***Disocactus amazonicus*** **(Schumann) Hunt**,gb,-,-,AY015312,AY015400-AY015359,na,na;***Disocactus flagelliformis*** **(Linnaeus) Barthlott**,T. Hernández 44,México: Oaxaca,MEXU,**HM041676**,**HM041256**,**HM041410**,**HM041563**;***Echinocactus grusonii*** **Hildmann**,H. Hernández 2442,México: Queretaro,MEXU,**HM041677**,**HM041257**,**HM041411**,**HM041640**;***Echinocactus horizonthalonius*** **Lemaire**,T. Hernández 61,México: San Luis Potosí,MEXU,**HM041678**,**HM041258**,**HM041412**,**HM041642**;***Echinocactus platyacanthus*** **Link & Otto**,T. Hernández H08,México: Hidalgo,MEXU,**HM041679**,**HM041259**,**HM041413**,**HM041641**;***Echinocereus cinerascens*** **(De Candolle) Lemaire**,T. Hernández H21,México: Hidalgo,MEXU,**HM041680**,**HM041260**,**HM041414**,**HM041564**;***Echinocereus enneacanthus*** **Engelmann**,C. Gómez 2156,México: Zacatecas,MEXU,**HM041681**,**HM041261**,**HM041415**,**HM041565**;***Echinocereus leucanthus*** **Taylor**,gb,-,-,na,DQ099932,DQ100001,na;***Echinocereus parkeri*** **Taylor**,gb,-,-,na,DQ099933,DQ100002,na;***Echinocereus pectinatus*** **(Scheidweiler) Engelmann**,H. Hernández 3403,México: Coahuila,MEXU,**HM041682**,**HM041262**,**HM041416**,**HM041566**;***Echinocereus pentalophus*** **(De Candolle) Lemaire**,gb,-,-,AY015307,AY015396-AY015355,na,na;***Echinocereus poselgeri*** **Lemaire**,gb,-,-,na,DQ099935,DQ100004,na;***Echinocereus schmollii*** **(Weingart) Taylor**,gb,-,-,na,DQ099936,DQ100005,na;***Echinocereus triglochidiatus*** **Engelmann**,gb,-,-,na,DQ099937,DQ100006,na;***Echinomastus intertextus*** **(Engelm.) Britton & Rose**,1993 0823 1001,lc,DBG AZ,**HM041683**,**HM041263**,**HM041417**,**HM041567**;***Echinomastus unguispinus*** **(Engelm.) Britton & Rose**,J. Reyes 5145,lc,JB UNAM,**HM041684**,**HM041264**,**HM041418**,**HM041568**;***Echinopsis chiloensis*** **(Colla) Friedrich & Rowley**,gb,-,-,AY015322,AY566653-AY015409-AY015368,na,na;***Echinopsis formosa*** **(Pfeiffer) Jacobi ex Salm-Dyck**,1998 0056 1003 W,lc,DBG AZ,**HM041685**,**HM041265**,**HM041419**,na;***Echinopsis leucantha*** **(Gillies ex Salm-Dyck) Walpers**,1995 0358 1001,lc,DBG AZ,**HM041686**,**HM041266**,**HM041420**,na;***Echinopsis pachanoi*** **(Britton & Rose) Friedrich & Rowley**,2003 008 0102 Z,lc,DBG AZ,**HM041687**,**HM041267**,**HM041421**,na;***Echinopsis pasacana*** **(F. A. C. Weber) Navarro**,1952 3603 0104,lc,DBG AZ,**HM041688**,**HM041268**,**HM041422**,na;***Echinopsis sucrensis*** **Cardenas**,1992 0237 0101 Z,lc,DBG AZ,**HM041689**,**HM041269**,**HM041423**,na;***Epiphyllum phyllanthus*** **(Linnaeus) Haworth**,T. Hernández p107,México: Chiapas,MEXU,**HM041690**,**HM041270**,**HM041424**,**HM041569**;***Epithelantha micromeris*** **(Engelmann) Weber**,H. Hernández 3402,México: Coahuila,MEXU,**HM041691**,**HM041271**,**HM041425**,**HM041570**;***Eriosyce aurata*** **(Pfeiffer) Backeberg**,gb,-,-,AY015336,AY015336,na,na;***Eriosyce islayensis*** **(Foerster) Kattermann**,gb,-,-,AY015337,AY015337,na,na;***Eriosyce napina*** **(Philippi) Kattermann**,gb,-,-,AY015339,AY015339,na,na;***Eriosyce subgibbosa*** **(Haworth) Kattermann**,gb,-,-,AY015338,AY015338,na,na;***Eriosyce taltalensis*** **(Hutchison) Kattermann**,2002 0150 0101 W,lc,DBG AZ,**HM041692**,**HM041272**,**HM041426**,na;***Escontria chiotilla*** **(Weber ex Schumann) Rose**,gb,-,-,AY015308,AY015397-AY015356-AY181622,AY181608,na;***Espostoa nana*** **Ritter**,1994 0247 1012 W,lc,DBG AZ,**HM041693**,**HM041273**,**HM041427**,na;***Espostoopsis dybowskii*** **(Roland-Gosselin) Buxbaum**,1979 0374 0101 G,lc,DBG AZ,**HM041694**,**HM041274**,**HM041428**,na;***Eulychnia castanea*** **Philippi**,1993 0231 0101

W,lc,DBG AZ,na,**HM041275**,**HM041429**,na;***Eulychnia iquiquensis*** **(Schumann) Britton & Rose**,gb,-,-,AY015301,AY015301,na,na;***Ferocactus echidne*** **(De Candolle) Britton & Rose**,T. Hernández H09,México: Hidalgo,MEXU,**HM041695**,**HM041276**,**HM041430**,**HM041571**;***Ferocactus haematacanthus*** **(Salm-Dyck) Bravo ex Backeberg & F.Knuth**,T. Hernández 72,México: Nuevo León,MEXU,**HM041696**,**HM041277**,**HM041431**,**HM041572**;***Ferocactus latispinus*** **(Haworth) Britton & Rose**,T. Hernández 51,México: Queretaro,MEXU,**HM041697**,**HM041278**,**HM041432**,**HM041573**;***Frailea pumila*** **(Lemaire) Britton & Rose**,1981 0090 0101 G,lc,DBG AZ,**HM041698**,**HM041279**,**HM041433**,na;***Geohintonia mexicana*** **Glass & Fitz Maurice**,J. Reyes 3226,lc,JB UNAM,**HM041699**,**HM041280**,**HM041434**,na;***Grahamia bracteata*** **Gill**.,gb,-,-,AY015273,na,na,na;***Grahamia coahuilensis*** **(S. Watson) G.D. Rowley**,gb,-,-,DQ855854,na,na,na;***Grusonia bradtiana*** **(J. M. Coult.) Britton & Rose**,H. Hernández 3342,México: Coahuila,MEXU,**HM041700**,**HM041281**,**HM041435**,**HM041638**;***Gymnocalycium denudatum*** **(Link & Otto) Pfeiffer ex Mittler**,gb,-,-,AY015317,AY015404-AY015363,na,na;***Gymnocalycium guanchinense*** **A. Berger**,1993 0183 0101 G,lc,DBG AZ,**HM041701**,**HM041282**,**HM041436**,na;***Gymnocalycium saglionis*** **(Cels) Britton & Rose**,1999 033 0101 W,lc,DBG AZ,**HM041702**,**HM041283**,**HM041437**,na;***Gymnocalycium uruguayense*** **(Arechavaleta) Britton & Rose**,1993 0179 0101 G,lc,DBG AZ,**HM041703**,**HM041284**,**HM041438**,na;***Haageocereus pseudomelanostele*** **(Werdermann & Backeberg) Backeberg**,gb,-,-,AY015329,AY015415-AY015374,na,na;***Haageocereus decumbens*** **(Vaupel) Backeberg**,1994 0284 1006 W,lc,DBG AZ,**HM041704**,**HM041285**,**HM041439**,na;***Haageocereus limensis*** **(Vaupel) Backeb**.,1994 0249 1001 W,lc,DBG AZ,**HM041705**,**HM041286**,**HM041440**,na;***Harrisia earlei*** **Britton & Rose**,gb,-,-,na,DQ099939,DQ100008,na;***Harrisia martinii*** **(Labouret) Britton**,1996 1255 0102,lc,DBG AZ,**HM041706**,**HM041287**,**HM041441**,na;***Harrisia pomanensis*** **(Weber) Britton & Rose**,gb,-,-,AY015324,AY015411-AY015370,na,na;***Hatiora salicornioides*** **(Haworth) Britton & Rose ex Bailey**,T. Hernández p108,personal colection,MEXU,**HM041707**,**HM041288**,**HM041442**,**HM041636**;***Hylocereus trigonus*** **(Haworth) Safford**,H. Hernández 2236,México: Yucatan,MEXU,**HM041708**,**HM041289**,**HM041443**,na;***Hylocereus undatus*** **(Haworth) Britton & Rose**,T. Hernandez 06 (M002),México: Oaxaca,MEXU,**HM041709**,**HM041290**,**HM041444**,**HM041574**;***Isolatocereus dumortierii*** **(Scheidweiler) Backeberg**,J. A. Barba 1,México: San Luis Potosí,MEXU,**HM041749**,na,**HM041485**,**HM041602**;***Lepismium cruciforme*** **(Vellozo) Miquel**,gb,-,-,AY015344,AJ583237,na,na;***Leptocereus leonii*** **Britton & Rose**,gb,-,-,AY015297,AY015388-AY015347,na,na;***Leptocereus quadricostatus*** **(Bello) Britton & Rose**,gb,-,-,na,DQ099942,DQ100011,na;***Leuchtenbergia principis*** **Hooker**,H. Hernández 2009,México: Nuevo León,MEXU,**HM041710**,**HM041291**,**HM041445**,**HM041575**;***Lobivia pentlandii*** **(Hook.) Britton & Rose**,gb,-,-,AY015323,AY015369-AY015410,na,na;***Lophocereus schottii*** **(Engelm) Britt. & Rose**,gb,-,-,AY015309,AY181620,AY181613,na;***Lophophora williamsii*** **(Lemaire ex Salm-Dyck) J.Coulter**,T. Hernández 62,México: San Luis Potosí,MEXU,**HM041711**,**HM041292**,**HM041446**,**HM041576**;***Maihuenia patagonica*** **(Philippi) Spegazzini**,Las Peñas & Uñates 14,Argentina: Neuquen,CORD,**HM041712**,**HM041293**,**HM041447**,na;***Maihuenia poeppigii*** **(Pfeiffer) Schumann**,gb,-,-,AY015282,AY015282,AY851609 AF191656,na;***Maihueniopsis atacamensis*** **(Philippi) F.Ritter**,2004 0354 0101 W,lc,DBG AZ,**HM041713**,**HM041294**,**HM041448**,**HM041577**;***Mammillaria candida*** **Scheidweiler**,gb,-,-,na,AJ583218,AF267945 AY545250,na;***Mammillaria carnea*** **Zuccarini ex Pfeiffera**,T. Hernández 32,México: Puebla,MEXU,**HM041714**,**HM041295**,**HM041449**,**HM041578**;***Mammillaria geminispina*** **Haworth**,T. Hernández 93,México: Queretaro,MEXU,**HM041715**,**HM041296**,**HM041450**,**HM041579**;***Mammillaria haageana*** **Pfeiffer**,gb,-,-,AY015289,AY545268-AF267953,na,na;***Mammillaria magnimamma*** **Haworth**,T. Hernández H10,México: Hidalgo,MEXU,**HM041716**,**HM041297**,**HM041451**,**HM041580**;***Mammillaria picta*** **Meinshausen**,T. Hernández 63,México: Nuevo León,MEXU,**HM041717**,**HM041298**,**HM041452**,**HM041581**;***Mammillaria senilis*** **Loddiges ex Salm-Dyck**,gb,-,-,na,AJ583212,AY545318-AF267956,na;**Matucana madisoniorum (Hutchinson) Rowley**,1994 0702 0102, Knize 456,lc,DBG AZ,**HM041718**,**HM041299**,**HM041453**,**HM041639**;***Melocactus curvispinus*** **Pfeiffer**,1995 0053 1008 W,lc,DBG AZ,**HM041719**,**HM041300**,**HM041454**,na;***Melocactus intortus*** **(Miller) Urban**,1985 0172 1004 W,lc,DBG AZ,**HM041720**,**HM041301**,**HM041455**,**HM041582**;***Micranthocereus albicephalus*** **(Buining & Brederoo) Ritter**,gb,-,-,AY015314,AY015314,na,na;***Mila caespitosa*** **Britton & Rose**,1996 0168 1006 W,lc,DBG AZ,**HM041721**,**HM041302**,HM041456,na;***Miqueliopuntia miquelii*** **(Monville) Ritter**,2001 0107 0102 FK1098,lc,DBG AZ,**HM041722**,**HM041303**,**HM041457**,**HM041643**;***Monvillea spegazzinii*** **(F.A.C. Weber) Britton & Rose**,2005 0028 0101 G,lc,DBG AZ,**HM041723**,**HM041304**,**HM041458**,na;***Myrtillocactus geometrizans***

(Martius) Console,gb,-,-,na,DQ099943,DQ100012,na;***Myrtillocactus schenckii*** **(J.Purpus) Britton & Rose**,gb,-,-,na,AY181633,AY181607,na;*Mytrocereus fulviceps* **(F. A. C. Weber) Backeb**.,T. Hernández 45,México: Oaxaca,MEXU,**HM041724**,**HM041305**,**HM041459**,**HM041583**;*Neobuxbaumia mezcalaensis* **(Bravo) Backeberg**,T. Hernández 37,México: Oaxaca,MEXU,**HM041725**,**HM041306**,**HM041460**,**HM041584**;***Neobuxbaumia polylopha*** **(De Candolle) Backeberg**,T. Hernández 91,México: Queretaro,MEXU,**HM041726**,**HM041307**,**HM041461**,**HM041585**;*Neolloydia conoidea* **(De Candolle) Britton & Rose**,T. Hernández 85,México: Tamaulipas,MEXU,**HM041727**,**HM041308**,**HM041462**,**HM041634**;***Neoraimondia arequipensis*** **(Meyen) Backeberg**,gb,-,-,AY015299,AY015390-AY015349,na,na;***Neoraimondia herzogiana*** **(Backeberg) Buxbaum & Krainz**,1954 4849 0102 G,lc,DBG AZ,**HM041728**,**HM041309**,**HM041463**,**HM041586**;***Neowerdermannia vorwerkii*** **(Fric) Backeberg**,gb,-,-,AY015340,AY015340,na,na;***Nopalea cochenillifera*** **(L.) Salm-Dyck**,1997 0395 0101,lc,DBG AZ,**HM041729**,na,**HM041464**,**HM041587**;***Nopalea dejecta*** **Salm-Dyck**,2002 0342 0103 Z,lc,DBG AZ,**HM041730**,**HM041310**,**HM041465**,**HM041588**;***Nopalea gaumeri*** **Britton & Rose**,1999 0027 0101,lc,DBG AZ,**HM041731**,**HM041311**,**HM041466**,**HM041589**;***Nopalea karwinskiana*** **(Salm-Dyck) Schum**,T. Hernández 7,México: Oaxaca,MEXU,**HM041732**,**HM041312**,**HM041467**,**HM041590**;***Nopalea lutea*** **Rose**,2002 0044 0101 W,lc,DBG AZ,**HM041733**,**HM041313**,**HM041468**,**HM041591**;***Nopalea sp***,T. Hernández P036,México: Oaxaca,MEXU,**HM041734**,**HM041314**,**HM041469**,**HM041635**;*Notocactus concinnus* **(Monv.) A.Berger**,1985 0339 0101,lc,DBG AZ,**HM041735**,**HM041315**,**HM041470**,**HM041592**;***Obregonia denegrii*** **Fric**,L. White 26,México: Tamaulipas,MEXU,na,**HM041316**,**HM041471**,na;***Opuntia echios*** **Howell**,1994 0007 0103 Z EFA 2560,lc,DBG AZ,**HM041736**,**HM041317**,**HM041472**,**HM041593**;***Opuntia excelsa*** **Sanchez-Mejorada**,T. Hernández 3,México: Guerrero,MEXU,**HM041737**,**HM041318**,**HM041473**,**HM041594**;***Opuntia helleri*** **Schumann ex Robinson**,1995 0316 0101 YD 24743,lc,DBG AZ,**HM041738**,**HM041319**,**HM041474**,**HM041595**;***Opuntia lasiacantha*** **Hort. Vindob. ex Pfeiffer**,T. Hernández H11,México: Hidalgo,MEXU,**HM041740**,**HM041321**,**HM041476**,**HM041596**;***Opuntia leptocaulis*** **De Candolle**,T. Hernández 87,México: Tamaulipas,MEXU,**HM041741**,**HM041322**,**HM041477**,na;***Opuntia macbridei*** **Britton & Rose**,1990 0601 0101,lc,DBG AZ,**HM041742**,**HM041323**,**HM041478**,**HM041597**;***Opuntia megasperma*** **Howell**,1994 0019 0201 Z,lc,DBG AZ,**HM041743**,**HM041324**,**HM041479**,**HM041630**;***Opuntia microdasys*** **(Lehmann) Pfeiffer**,T. Hernández 59,México: San Luis Potosí,MEXU,**HM041744**,**HM041325**,**HM041480**,**HM041598**;***Opuntia palmadora*** **Britton & Rose**,1997 0187 0102,lc,DBG AZ,**HM041745**,**HM041326**,**HM041481**,**HM041599**;***Opuntia tunicata*** **(Lehmann) Link & Otto ex Pfeiffer**,1993 0198 0101 FK 352,lc,DBG AZ,**HM041746**,**HM041327**,**HM041482**,**HM041600**;*Oreocereus celsianus* **(Salm-Dyck) Riccobono**,1987 0468 2108 W,lc,DBG AZ,**HM041747**,**HM041328**,**HM041483**,na;***Ortegocactus macdougallii*** **Alexander**,J. Reyes 4007,lc,JB UNAM,**HM041748**,**HM041329**,**HM041484**,**HM041601**;***Pachycereus gaumeri*** **Britton & Rose**,gb,-,-,na,AY181626,AY181606,na;***Pachycereus marginatus*** **(De Candolle) Britton & Rose**,T. Hernández 14,México: Puebla,MEXU,na,**HM041330**,**HM041486**,**HM041603**;***Pachycereus pecten-aboriginum*** **(Engelmann) Britton & Rose**,T. Hernández 8,México: Oaxaca,MEXU,**HM041750**,**HM041331**,**HM041487**,**HM041604**;***Pachycereus sp***,T. Hernández H03,México: Hidalgo,MEXU,**HM041751**,**HM041332**,**HM041488**,**HM041605**;***Parodia buenekeri*** **(Buining) Ritter**,gb,-,-,AY015331,AY015331,na,na;***Parodia erinacea*** **(Haworth) Taylor**,1965 8091 0101 G,lc,DBG AZ,**HM041752**,**HM041333**,**HM041489**,**HM041625**;*Parodia haselbergii* **(Haage ex Ruempler) Brandt**,gb,-,-,AY015330,na,AF267975,na;***Parodia magnifica*** **(Ritter) Brandt**,1985 0436 0101 G,lc,DBG AZ,**HM041753**,**HM041334**,**HM041490**,na;***Parodia ottonis*** **(Lehmann) Taylor**,gb,-,-,AY015335,AY015380,na,na;***Peniocereus castellae*** **Sánchez-Mejorada**,gb,-,-,na,DQ099945,DQ100014,na;***Peniocereus chiapensis*** **Bravo**,C. Gómez 2100,México: Chiapas,MEXU,**HM041754**,**HM041335**,**HM041491**,**HM041606**;***Peniocereus greggii*** **(Engelmann) Britton & Rose**,J. Reyes 4688,lc,JB UNAM,**HM041755**,**HM041336**,**HM041492**,**HM041607**;*Peniocereus johnstonii* **Britton & Rose**,gb,-,-,na,DQ099951,DQ100020,na;***Peniocereus serpentinus*** **(Lagasca & Rodrigues) Taylor**,T. Hernández 92,México: Queretaro,MEXU,**HM041756**,**HM041337**,**HM041493**,**HM041608**;***Peniocereus viperinus*** **(Weber) Kreuzinger**,gb,-,-,na,DQ099964,DQ100033,na;***Pereskia aculeata*** **Miller**,T. Hernández p111,lc,JB UNAM,**HM041757**,**HM041338**,**HM041494**,na;***Pereskia diaz-romeroana*** **Cárdenas**,gb,-,-,AY875353,na,AY851592,na;***Pereskia lychnidiflora*** **De Candolle**,gb,-,-,AY875358,na,AY851594,na;***Pereskia weberiana*** **Schumann**,gb,-,-,AY875357,na,AY851593,na;***Pereskiopsis sp***,T. Hernández p109,México: Chiapas,MEXU,**HM041758**,**HM041339**,**HM041495**,**HM041627**;*Pfeiffera ianthothele* **(Monville) Weber**,gb,-,-,AY015304,AY015354,na,na;***Pilosocereus chrysacanthus*** **(Weber) Byles & Rowley**,T. Hernández 17,México:

Puebla,MEXU,**HM041759**,**HM041340**,**HM041496**,na;***Polaskia chichipe*** **(Roland-Gosselin) Backeberg**,T. Hernández 16,México: Puebla,MEXU,**HM041760**,**HM041341**,**HM041497**,**HM041615**;***Portulacaria afra*** **Jacq**.,gb,-,-,AY875368,na,na,na;***Pseudoacanthocereus brasiliensis*** **(Britton & Rose) Ritter**,gb,-,-,na,DQ099967,DQ100036,na;***Pseudoacanthocereus sicariguensis*** **(Croizat & Tamayo) Taylor**,gb,-,-,na,DQ099968,DQ100037,na;***Pterocactus gonjianii*** **Kiesling**,1997 0260 0103,lc,DBG AZ,**HM041761**,**HM041342**,**HM041498**,na;***Pterocereus foetidus*** **Th. MacDoug. & Miranda**,T. Hernández p110,México: Chiapas,MEXU,**HM041762**,**HM041343**,**HM041499**,**HM041616**;***Pygmaeocereus bylesianus*** **Andreae & Backeberg**,1994 0519 0101,lc,DBG AZ,**HM041763**,**HM041344**,**HM041500**,na;***Quiabentia chacoensis*** **Backeberg**,1985 0461 0101,lc,DBG AZ,**HM041764**,**HM041345**,**HM041501**,na;***Quiabentia verticillata*** **Vaupel**,1992 1063 0101,lc,DBG AZ,**HM041765**,**HM041346**,**HM041502**,na;***Rauhocereus riosaniensis*** **Backeberg**,gb,-,-,AY015326,AY015413-AY015372,na,na;***Rauhocereus sp***,93-2010,lc,DBG AZ,**HM041766**,**HM041347**,**HM041503**,na;***Rebutia arenacea*** **Cardenas**,1992 0082 0102 Z,lc,DBG AZ,**HM041767**,**HM041348**,**HM041504**,**HM041633**;***Rebutia fiebrigii*** **(Guerke) Britton & Rose ex Bailey**,1992 0068 0202,lc,DBG AZ,**HM041768**,**HM041349**,**HM041505**,na;***Rhipsalis baccifera*** **(J.S. Mueller) Stearn**,T. Hernandez 12,México: Hidalgo,MEXU,**HM041769**,**HM041350**,**HM041506**,**HM041619**;***Samaipaticereus corroanus*** **Cardenas**,gb,-,-,AY015321,AY015408-AY015367,na,na;***Schlumbergera truncata*** **(Haworth) Moran**,gb,-,-,AY015343,AJ583238,na,na;***Sclerocactus brevihamatus*** **(Engelmann) Hunt**,T. Hernández 68,México: Nuevo León,MEXU,**HM041770**,**HM041351**,**HM041507**,**HM041620**;***Sclerocactus uncinatus*** **(Galeotti) Taylor**,H. Hernández 3597,México: Coahuila,MEXU,**HM041771**,**HM041352**,**HM041508**,na;***Selenicereus boeckmannii*** **(Otto ex Salm-Dyck) Britton & Rose**,gb,-,-,AY015311,AY015399,na,na;***Selenicereus donkelaarii*** **(Salm-Dyck) Britton & Rose ex Bailey**,H. Hernández 2226,México: Yucatán,MEXU,**HM041772**,**HM041353**,**HM041509**,**HM041621**;***Selenicereus sp***,T. Hernández 04 (G31),México: Guerrero,MEXU,**HM041773**,**HM041354**,**HM041510**,**HM041632**;***Stenocactus coptonogonus*** **(Lemaire) Berger ex Hill**,H. Hernández 1773,México: San Luis Potosí,MEXU,**HM041774**,na,**HM041511**,**HM041626**;***Stenocactus sp***,T. Hernández H19,México: Hidalgo,MEXU,**HM041775**,**HM041355**,**HM041512**,**HM041622**;***Stenocereus beneckei (Ehrenberg) Buxbaum***,1980 0297 0102 G,lc,DBG AZ,**HM041776**,**HM041356**,**HM041513**,na;***Stenocereus eruca*** **(T.Brandegee) Gibson & Horak**,1939 0425 0111 W,lc,DBG AZ,**HM041777**,**HM041357**,**HM041514**,**HM041623**;***Stenocereus gummosus*** **(Engelmann) Gibson & Horak**,1966 8584 0101 G,lc,DBG AZ,**HM041778**,**HM041358**,**HM041515**,**HM041624**;***Stenocereus pruinosus*** **(Otto ex Pfeiffer) Buxbaum**,T. Hernández 43,México: Oaxaca,MEXU,**HM041779**,**HM041359**,**HM041516**,**HM041617**;***Stenocereus stellatus*** **(Pfeiffer) Riccobono**,T. Hernández 19,México: Puebla,MEXU,**HM041780**,**HM041360**,**HM041517**,**HM041618**;***Stetsonia coryne*** **(Foerster) Britton & Rose**,1939 0402 0103 G,lc,DBG AZ,**HM041781**,**HM041361**,**HM041518**,na;***Strombocactus disciformis*** **(De Candolle) Britton & Rose**,H. Hernández 1522,México: Queretaro,MEXU,**HM041782**,**HM041362**,**HM041519**,**HM041637**;***Sulcorebutia candiae*** **(Cardenas) Buining & Donald**,1992 0082 0102 Z,lc,DBG AZ,**HM041783**,**HM041363**,**HM041520**,**HM041609**;***Tacinga funalis*** **Britton & Rose**,ex Zurich. SS HU 748,lc,DBG AZ,**HM041784**,**HM041364**,**HM041521**,na;***Talinum paniculatum*** **(Jacq.) Gaertner**,gb,-,-,AY015274,na,AY851610,na;***Tephrocactus alexanderi*** **(Britton & Rose) Backeberg**,2001 0055 0101,lc,DBG AZ,**HM041785**,**HM041365**,**HM041522**,na;***Tephrocactus articulatus*** **(Pfeiffer) Backeberg**,1993 0335 0202,lc,DBG AZ,**HM041786**,**HM041366**,**HM041523**,na;***Thelocactus hastifer*** **(Werdermann & Boedeker) F.Knuth**,C. Gómez 1608,México: Queretaro,MEXU,**HM041787**,**HM041367**,**HM041524**,**HM041610**;***Thelocactus tulensis*** **(Poselger) Britton & Rose**,T. Hernández 55,México: San Luis Potosí,MEXU,**HM041788**,**HM041368**,**HM041525**,na;***Trichocereus taquimbalensis*** **Cardenas**,999 0011 0101 Z,lc,DBG AZ,**HM041789**,**HM041369**,**HM041526**,na;***Tunilla corrugata*** **(Salm-Dyck) D.R.Hunt & J.Iliff**,2003 0022 0102 Z,lc,DBG AZ,**HM041790**,**HM041370**,**HM041527**,**HM041628**;***Turbinicarpus gielsdorfianus*** **(Werdermann) John & Riha**,J. Reyes 6168,lc, JB UNAM,**HM041791**,**HM041371**,**HM041528**,**HM041611**;***Turbinicarpus pseudomacrochele*** **(Backeberg) Buxbaum & Backeberg**,J. Reyes 5016,lc, JB UNAM,**HM041792**,**HM041372**,**HM041529**,**HM041612**;***Turbinicarpus schmiedickeanus*** **(Boedeker) Buxbaum & Backeberg**,H. Hernández 2362,México: San Luis Potosí,MEXU,**HM041793**,**HM041373**,**HM041530**,**HM041613**;***Turbinicarpus viereckii*** **(Werdmann) John & Riha**,C. Gómez 1368,México: San Luis Potosí,MEXU,**HM041794**,**HM041374**,**HM041531**,**HM041614**;***Uebelmannia pectinifera*** **Buining**,1981 0226

0101 G,lc,DBG AZ,**HM041795**,**HM041375**,**HM041532**,na;***Weberbauerocereus johnsonii* Ritter**,HBG 95204 ISI 2007-9,lc,DBG AZ,**HM041796**,**HM041376**,**HM041533**,na;***Weberocereus glaber* (Eichlam) Rowley**,gb,-,-,na,DQ099984,DQ100053,na;

FIGURES AND TABLES

TABLE 1. Primers designed and used to amplify the plastid fragments *rpl16* and *trnK/matK*.

Region	Primer name: Sequence
rpl16	rpl161F: 5′-GCTATGCTTAGTGTGTGACTCGTT-3′
	rpl163R: 5′-CTTCTATTTGTCTAGGCGTGATCC-3′
trnK/matK	matK3F: 5′-GGATGAAGCAAGGAATTCGTCTAC-3′
	matK4R: 5′-TTCATTGCACACGGCTTTCCCTA-3′
	matK9R: 3′-TAGCCAACGATCCAACCAGAG-5′
	matK13F: 3′-GGGCACAAGCACAAGAAGAAT-5′

TABLE 2. PCR conditions for the amplification of the different genomic regions used in this study.

Genomic region	Primers[a] forward/reverse (μL of each)	Annealing temp. (°C)	Elongation time (min)	Final volume (μL)	PCR buffer (μL)	$MgCl_2$ (μL)	DNTPs (μL)	*Taq* (μL)
rpl16	rpl161F/rpl163R (1.25)	63	2min	25 μl	2.5 μl	0.75 μl	0.5 μl	0.125
trnK/matK (long)	matK3F/matK4R (1.625)	66.5°C	4min	65μl	6.5μl	1.95μl	1.625μl	0.4
trnK/matK (short)	matK3F/matK9RmatK13F/ matK4R (1.25)	65°C/63°C	2.25	50μl	5μl	1.5μl	1.25μl	0.312
trnL-trnF	trnL-c/trnL-f (2.5)	62°C	2min	50 μl	5 μl	1.5 μl	1 μl	0.25
ppc	PPCX4F/PPCX5R (1)	60°C	3min	30μl	2.5μl	0.75μl	0.5μl	0.125

[a] Notes: Primer concentration:10 mmol/L; *Taq* concentration: 1 mmol/L (5 units/μL).

TABLE 3. Characteristics and information for alignments of sequences from the different loci used in this study

Region	Source	Length (bp)[a]	Constant sites	Parsimony informative	Percentage info. sites (%)[b]
trnK/matK	chloroplast	2514	1616	534	33.4
trnL-trnF	chloroplast	1530	809	406	25.4
rpl16	chloroplast	1546	766	458	28.6
ppc	nucleus	558	231	227	14.2

[a] Length of aligned individual matrixes after removing 5′ and 3′ low quality regions.

[b] Percentage of parsimony informative sites from the total concatenated matrix.

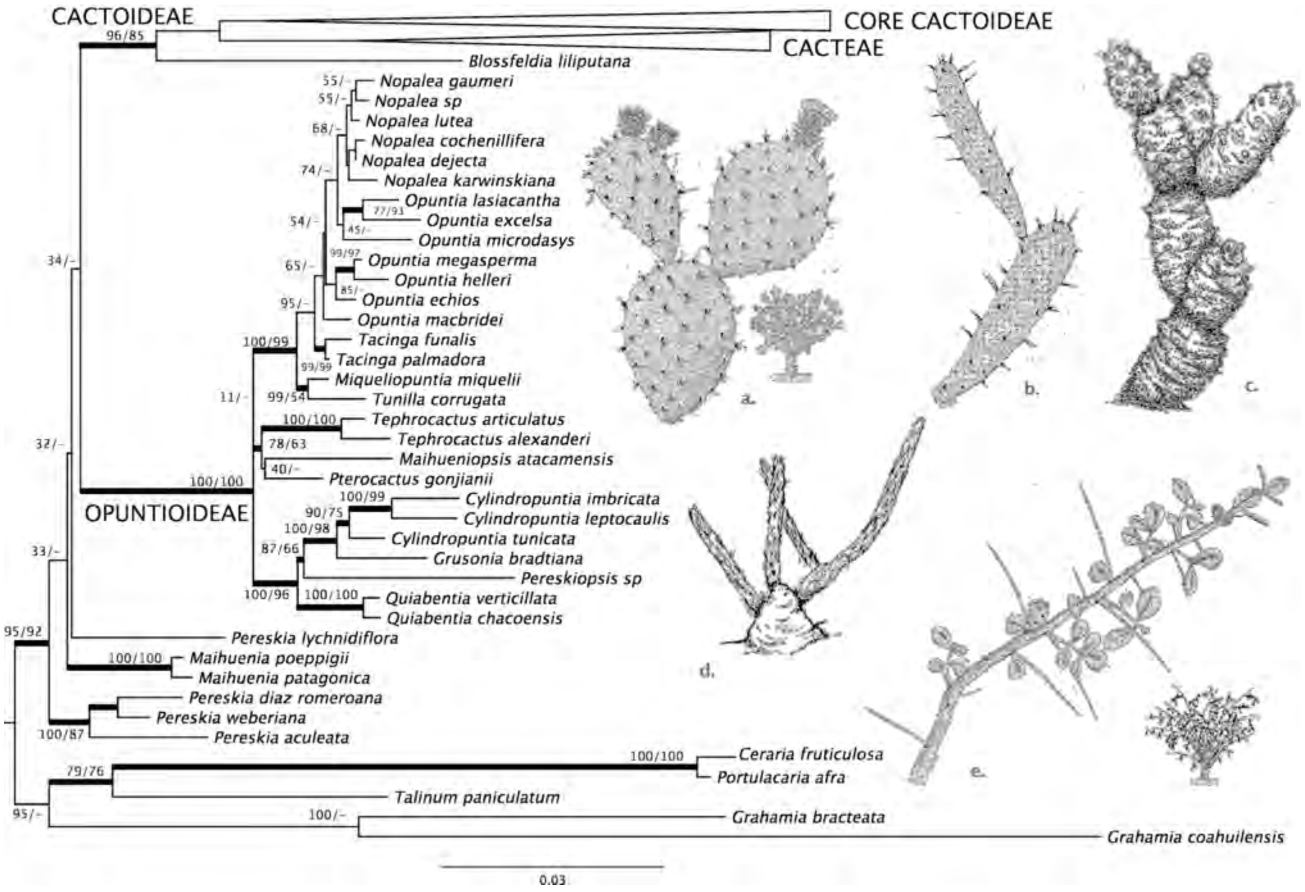

Figure 1. Maximum likelihood tree for Cactaceae derived from the concatenated analyses of *trnK-matK*, *matK*, *trnL-trnF*, *rpl16* and *ppc* (five loci data matrix) showing early diverging lineages. Bootstrap percentage values derived from maximum likelihood and parsimony analyses (bML/bMP) are shown associated to branches. Thick lines indicate the most strongly supported clades. Figures (not on scale) represent growth forms of (a) *Opuntia velutina;* (b) *Nopalea;* (c) *Tephrocactus*; (d) *Pterocactus*; and (e) *Pereskiopsis rotundifolia*. Figures (a) and (e) were modified from Arias, et al. (1997).

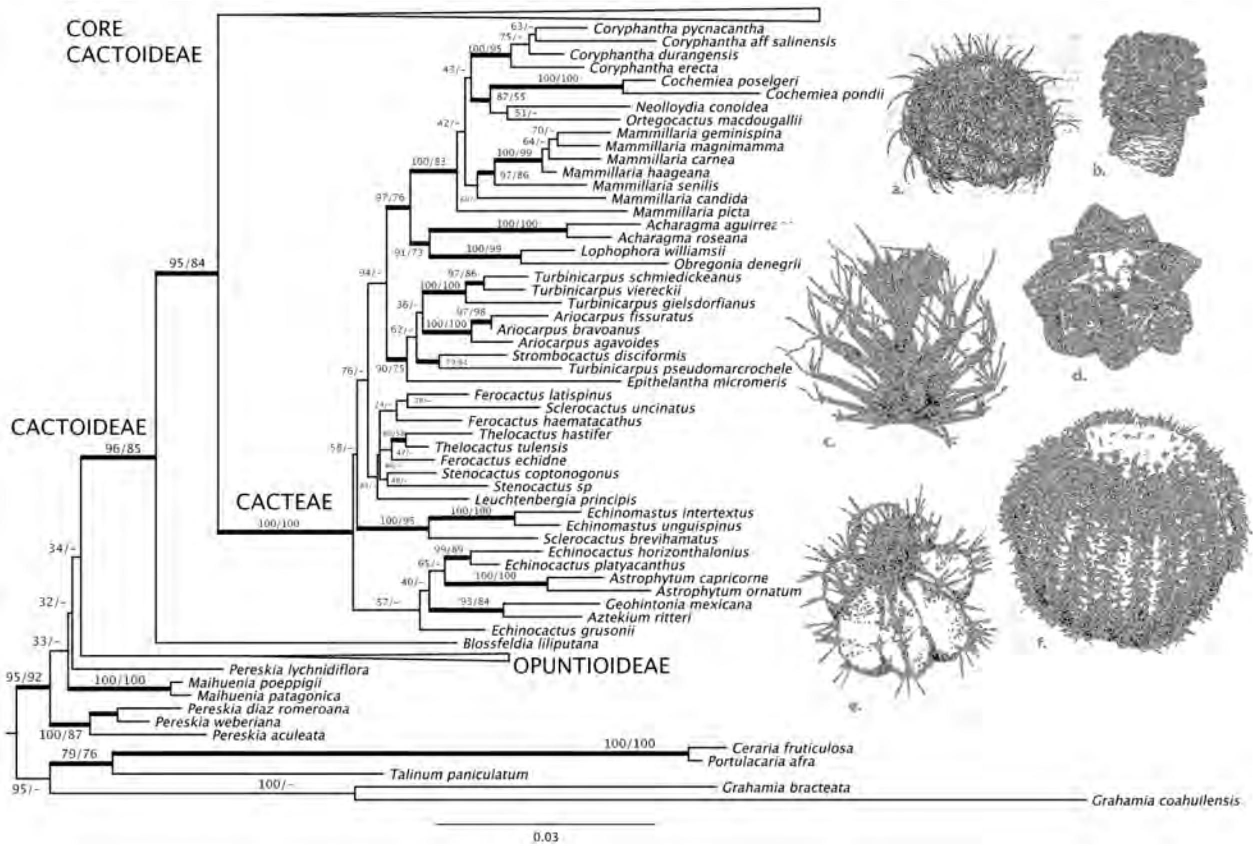

Figure 2. Maximum likelihood tree for Cactaceae derived from the concatenated analyses of *trnK-matK*, *matK*, *trnL-trnF*, *rpl16* and *ppc* (five loci data matrix) showing the Cacteae clade and relationships within it. Bootstrap percentage values derived from the ML and MP analyses respectively (bML/bMP) are shown above or next to clades. Thick lines indicate the most strongly supported clades. Figures (not on scale) represent growth forms of (a) *Coryphantha pycnacantha*; (b) *Mammillaria hernandezii*; (c) *Leuchtenbergia principis*; (d) *Ariocarpus fissuratus*; (e) *Astrophytum ornatum*; and (f) *Echinocactus grusonii.* Figures (a) and (b) were modified from Arias, et al. (1997).

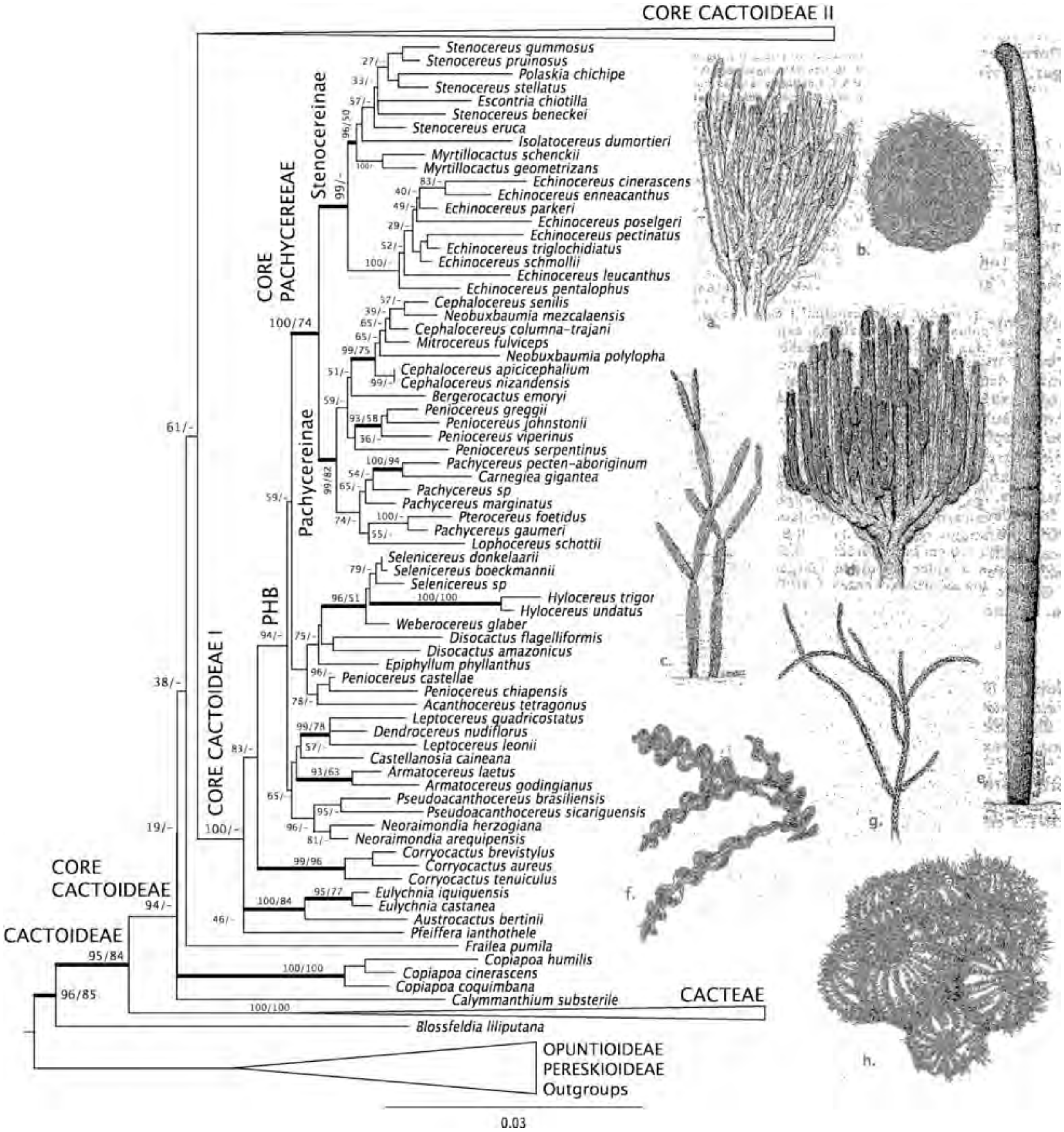

Figure 3. Maximum likelihood tree for Cactaceae derived from the concatenated analyses of *trnK-matK*, *matK*, *trnL-trnF*, *rpl16* and *ppc* (five loci data matrix) showing the core Cactoideae I clade and relationships within it. Bootstrap percentage values derived from the ML and MP analyses respectively (bML/bMP) are shown above or next to clades. Thick lines indicate the most strongly supported clades. Figures (not on scale) represent growth forms of (a) *Stenocereus pruinosus*; (b) *Echinocereus pulchellus*; (c) *Acanthocereus subinermis*; (d) *Pachycereus weberi*; (e) *Cephalocereus columna-trajani*; (f) *Epiphylum anguliger*; (g) *Pterocereus viperinus*; and (h) *Copiapoa cinerea*. Figures (a) to (e), and (g) were modified from Arias, et al. (1997).

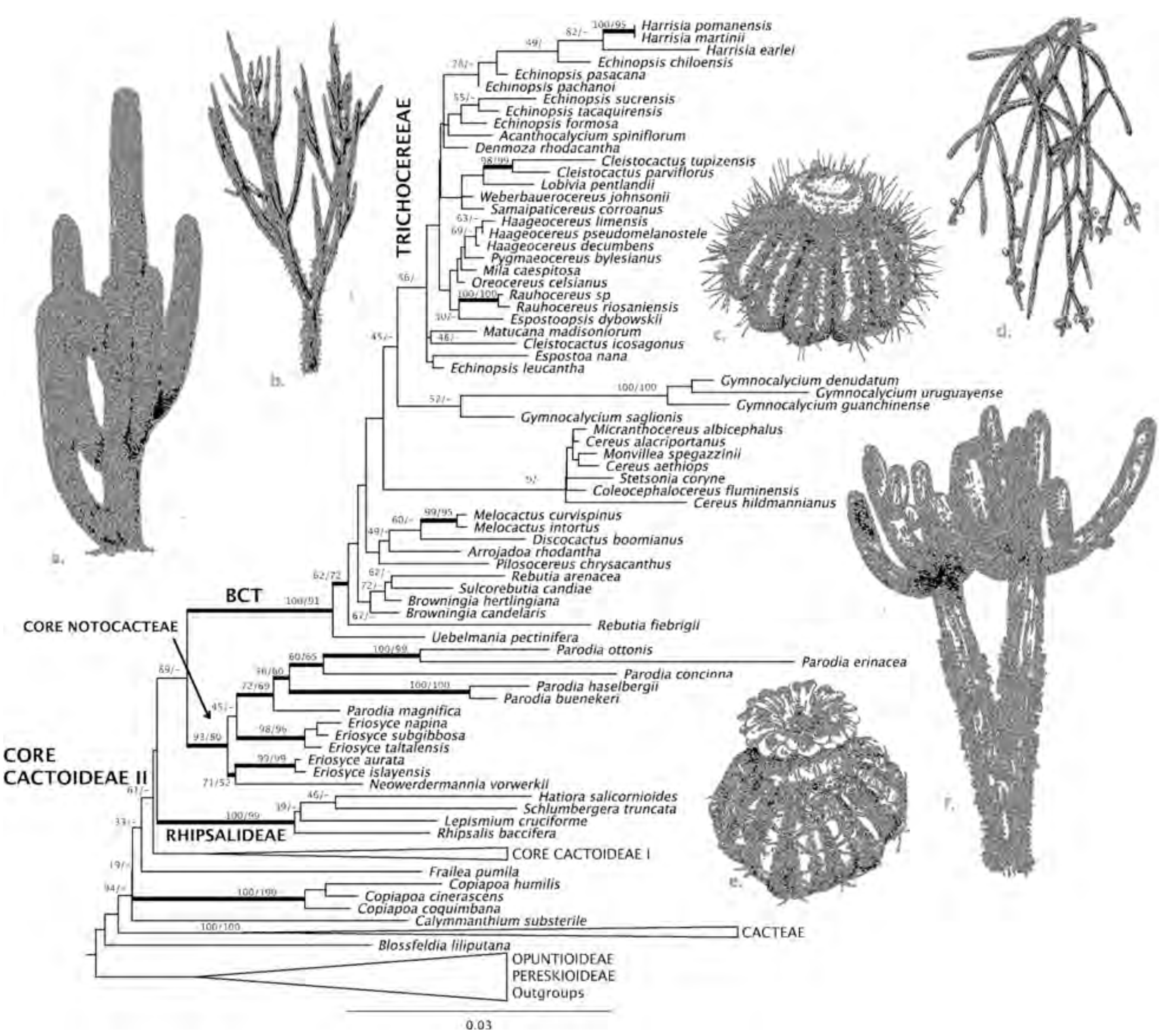

Figure 4. Maximum likelihood tree for Cactaceae derived from the concatenated analyses of *trnK-matK*, *matK*, *trnL-trnF*, *rpl16* and *ppc* (five loci data matrix) showing the core Cactoideae II clade and relationships within it. Bootstrap percentage values derived from the ML and MP analyses respectively (bML/bMP) are shown above or next to clades. Thick lines indicate the most strongly supported clades. Figures (not on scale) represent growth forms of (a) *Echinopsis atacamensis*; (b) *Harrisia eriophora* (c) *Melocactus intortus*; (d) *Rhipsalis baccifera*; (e) *Parodia erinaceae*; (f) *Browningia candelaris*.

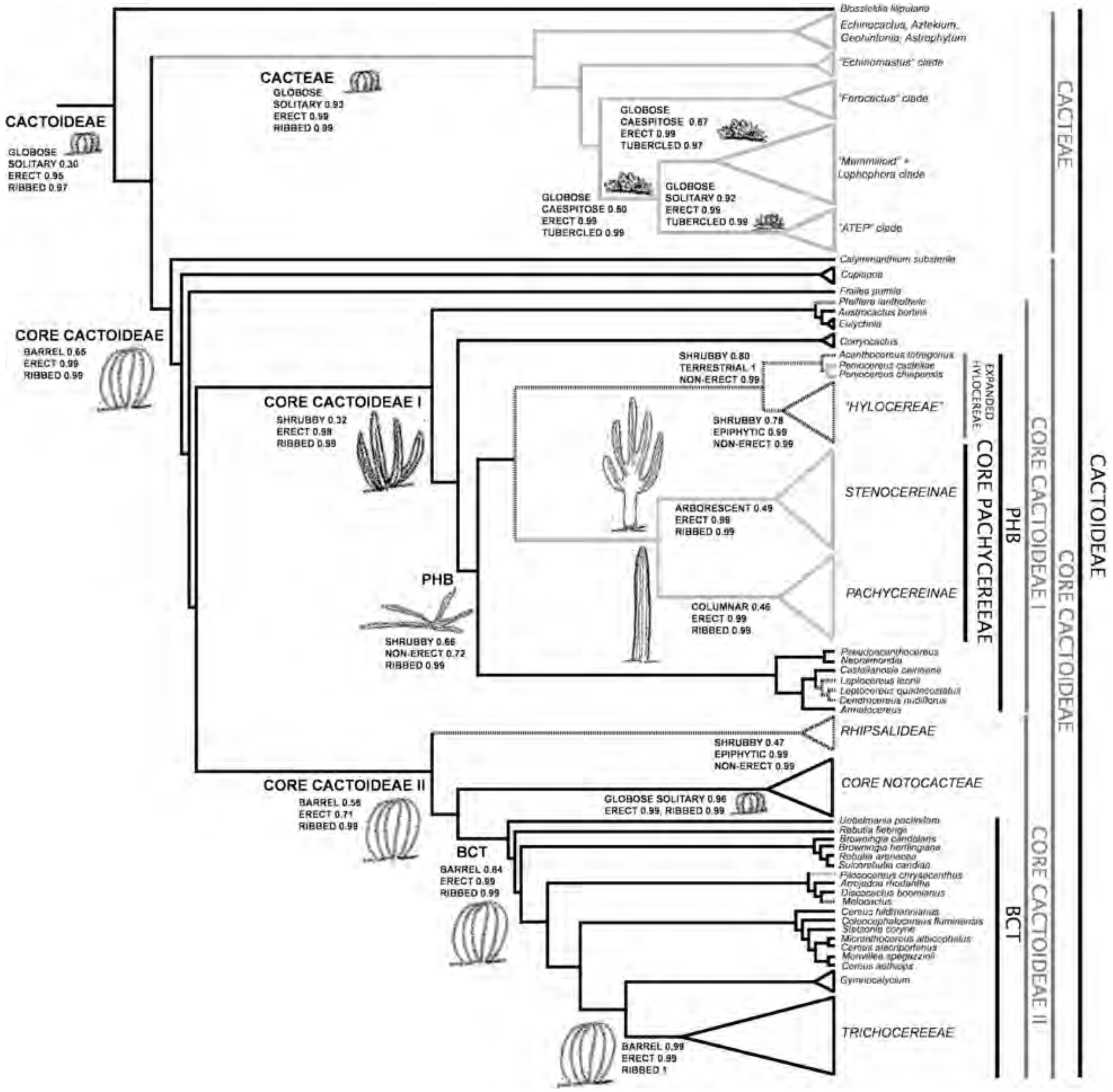

Figure 5. Maximum likelihood reconstruction of ancestral characters for growth form and habit within Cactoideae. Character states found to have the highest probability, and probability values, are shown next to each node. Figures represent the combination of the most probable character states found at tested nodes. Dark lines indicate lineages distributed mainly in South America, gray lines indicate linages distributed mainly in North America and dashed lines indicate linages distributed mainly in Central America and the Caribbean.

III. 2. TIEMPOS Y TASAS DE DIVERSIFICACIÓN EN CACTACEAE

La familia Cactaceae (Caryophyllales, Eudicotyledoneae) es muy carismática debido a las adaptaciones para habitar en condiciones de aridez que poseen muchos de sus miembros, así como su extraordinaria diversidad de formas de crecimiento. Las especies incluidas en la familia se distribuyen en todo el continente Americano, habitando en casi todos los tipos de vegetación. A pesar de su extensa distribución, los cactos son elementos conspicuos característicos de los paisajes áridos y semiáridos del norte y sur de América, donde alcanzan su máxima diversidad taxonómica. La filogenia molecular reportada más recientemente para la familia corrobora la clasificación en Subfamilias, y muestra que Cactoideae, la subfamilia más rica en número de especies y diversidad morfológica, está dividida en dos clados principales: Cacteae, un linaje de cactáceas globosas y toneliformes distribuidas en Norteamérica, y las Cactoideae Centrales, que incluyen especies con la mayor diversidad de formas de crecimiento distribuidas en Norte y Sudamérica. En Norteamérica habitan especies incluidas en las dos radiaciones más importantes dentro de Cactoideae: Cacteae, con su centro de diversidad en el Desierto Chihuahuense, y las Pachycereeae centrales; con un centro de distribución que se expande desde el centro de México a través de la Sierra Madre Occidental hasta el Desierto Sonorense. En este estudio, aportamos un marco temporal a la evolución y diversificación de los principales linajes al interior de Cactaceae, que contribuyeron a moldear las comunidades vegetales en los biomas áridos de América. Nuestros resultados muestran que Cacteae y las Pachycereeae centrales son relativamente jóvenes (Mioceno Tardío, Plioceno Medio); resultados que concuerdan con las posibles fechas de aridificación de sus zonas de distribución y el origen de otros linajes característicos de zonas áridas en América.

III. 2. TIMES AND DIVERSIFICATION RATES IN CACTACEAE

Cactaceae (Caryophyllales, Eudicotyledoneae) is an impressing plant family due to the adaptations of the majority of its lineages to inhabit arid environments, and its extraordinary diversity of growth forms. Cactaceae members are distributed all throughout the American continent and inhabit almost all existing vegetation types. However, they are particularly conspicuous elements in arid and semiarid landscapes of North and South America, where they attain its maximum taxonomical diversity. Recent molecular phylogenetic results supported the subfamilial classification of the family, and showed that Cactoideae, the richest subfamily, is divided in two main clades: Cacteae, a lineage of North American globose and barrel forms, and Core Cactoideae, which includes the greatest diversity of growth forms from North and South America. North America harbors two major radiations within Cactoideae: Cacteae, with a diversity center in the Chihuahuan desert and core Pachycereeae, with arborescent and columnar species with a diversity center spanning from central Mexico through the Sierra Madre Occidental to the Sonoran Desert. In this study, we provide a temporal framework for the evolution and diversification of main lineages within Cactaceae, which contributed to shape plant communities in the New World arid biomes. Our results show that Cacteae and Pachycereeae, are relatively young (Late Miocene to Mid Pliocene), in concordance with the aridification of their geographic distribution regions and the origin of other arid adapted lineages. The North American core Pachycereeae, a clade that includes the columnar and arborescent cacti; the Central American clade Hylocereeae, including epiphytic cacti and the South American clade Trichocereeae, which also includes columnar and arborescent forms, possess the highest diversification rates within Cactaceae. These lineages are pollinated by nectarivorous bats, and the evolution of this fertilization mechanism might be the cause of their increased diversification rates.

INTRODUCTION

Cacti are conspicuous elements of almost all floristic regions of the New World. Their greatest diversity is recorded in Mexico, with 586 species, followed by Brazil, Argentina, Bolivia and Perú, which are also rich in endemic species (Ortega-Baes et al., 2010). Cactaceae has been traditionally divided into three subfamilies, Pereskioideae, Opuntioideae and Cactoideae; the latter including the largest number of species and morphological diversity. Cactoideae members are distributed all through the New World, from the Patagonian to Canada, inhabiting all existing vegetation types and displaying an outstanding diversity of life forms. Different clades within Cactoideae have radiated in different regions of America. For instance, tribe Cacteae, -a monophyletic group according to phylogenetic molecular results-, includes all the globose and barrel North American cacti with a diversity center in the southernmost portion of the Chihuahuan Desert (Hernández and Gómez-Hinostrosa, 2005). Tribe Hylocereeae sensu stricto was also found to be monophyletic, and it includes epiphytic cacti with a diversity center in Central to North America. An important radiation of columnar, arborescent or large shrubby cacti occurred in the core Pachycereeae clade, with its area of greatest richness ranging from central Mexico, across the Sierra Madre Oriental to the Sonoran Desert. These life forms evolved also in a radiating South American clade, the Trichocereeae (Hernández-Hernández et al., 2010, in prep. See Chapter III.1).

To distinguish a clade as a radiation, it is important to detect a significant increase in its diversification rates, making adequate comparison against ecologically similar and closely related lineages; and if this signature is found, causal hypotheses can be stated (see discussion in Chapter I). It has been pointed out the potential of the evolution of particular pollinating systems in the radiation of xerophytic lineages with increased diversification rates like *Agave* (Good-Avila et al., 2006), and this may be relevant also for the radiation of several lineages within Cactaceae (Mandujano et al., 2010), particularly for the radiation of columnar cacti in North America (Valiente-Banuet, 1996). In order to provide a temporal framework to the evolution of Cactaceae and to search for clades that represent radiations within the family, we implemented a relaxed molecular clock to obtain dates for the origin of main clades, and used them to estimate absolute diversification rates for strongly supported monophyletic clades. Our study indicates that main cacti radiations occurred during the Miocene, and the lineages with the highest diversification rates are the core Pachycereeae, Hylocereeae, and Trichocereeae. These lineages include arborescent, columnar, shrubby, or sprawling species with a nocturnal anthesis, showing bat or moth pollination. The accelerated evolution of these lineages might be driven by the evolution of adaptations to a generalized pollination syndrome but with an important contribution of bats in the North American and moths in the South American lineages.

MATERIALS AND METHODS

Data set and phylogenetic analyses.- We used the same dataset and phylogeny reported in Hernández-Hernández et al., 2010 (in prep. see Chapter III.1), which includes DNA sequences data for 240 cacti species and five molecular markers from the chloroplast and nuclear genomic regions. Those are the *rpl16*, *trnL-trnF, trnK-matK, matK* (chloroplast) and *ppc* (nuclear). The phylogenetic analyses were performed using maximum likelihood methods in RAxML (see details in Hernández-Hernández et al., 2010 in prep. Chapter III.1).

Divergence dates estimation.- Divergence times for clades and lineages were estimated using penalized likelihood (PL; Sanderson, 2002, 2004) in r8s v1.71 (Sanderson, 2003, 2004). Penalized likelihood is a molecular-based semi parametric method that incorporates among-lineage rate heterogeneity and can use fossil information as auxiliary in divergence time estimation. Penalized likelihood, implemented in r8s, requires a user-defined parameter to specify the level of molecular rate smoothing to be implemented in dating analysis. To identify the smoothing magnitude (λ) that best describes the available data, we used a cross validation procedure that calculates the predictive error associated to molecular rate estimates across the full tree, derived from sequentially pruning terminal branches using the selected maximum likelihood topology for Cactaceae described above (Sanderson, 2004). Each cross validation tested 16 smoothing magnitudes ranging from log λ 10 = $-$ 2 to 5.5 at 0.5 intervals, which comprise a broad spectrum of substitution regimes. We used the resulting stem group dates obtained in the Caryophyllales BEAST divergence date analyses reported in the Chapter II of this thesis. For the stem Cactaceae node we set a fixed age at 22.9 MYA, and a minimum age constraint for its crown group at 17.39 MYA (See Table 1, Chapter II).

***Diversification rates*.-** Diversification rates were calculated using method-of-moments estimators (Rohatgi, 1976) in the context of a birth-and-death model (Kendall, 1948) that consider the species taxonomic diversity and age of a clade. These estimators provide absolute estimates of the rate of diversification of a clade, they are conditional on the survival of the clade to a given time *t*, in this case, the present, and they can differentially estimate the diversification rate of a stem clade or of a crown clade (Doyle and Donoghue,1993; Magallón and Sanderson, 2001). The conditional estimators of absolute diversification rates were calculated for stem and crown groups following Eqs.6 and 7 in Magallón and Sanderson (2001). The relative extinction rate (ε) is defined as $\varepsilon = \mu / \lambda$. Because absolute speciation and extinction rates for clades are unknown, diversification rates were estimated assuming that the relative extinction rate is bounded within $\varepsilon = 0.0$, which implies no extinction, and $\varepsilon = 0.9$, which implies a very high relative extinction rate. Whereas $\varepsilon = 0.0$ represents an absolute lower bound for the relative extinction rate, the selection of $\varepsilon = 0.9$ as an upper bound is arbitrary. Data on species numbers for Cactaceae are shown in Table 1, and were obtained from Hunt et al., (2006).

RESULTS

Chronograms resulting from the dating analyses in Figures 1 to 3 show that the origin of the family Cactaceae could date from the Early Miocene, however the majority of extant lineages radiated much later, during the Late Miocene or Pliocene. The estimated dates and diversification rates (Table 2) indicate that whereas the onset of diversification into living species of Cactoideae started 11.69 MYA, the diversification of Opuntioideae occurred later, at 5.33 MYA. The crown group of Cacteae is 7.96 MY, also older than Opuntioideae. Within the Core Cactoideae I clade, the lineages showing the highest diversification rates are those included within the core Pachycereeae clade (ranging from 0.6 to 1.2 species per MY) and in the Hylocereeae clade, both *sensu stricto*, and the Hylocereeae clade including *Peniocereus* subgen. *Pseudoacanthocereus* and *Acanthocereus* (ranging from 0.59 to 1.2 species per MY, see Table 2, and see description of phylogenetic results in Hernández-Hernández et al., 2010 in prep. Chapter III.1). Within the Core Cactoideae II clade, which includes the majority of the South American lineages, it was difficult to estimate dates and diversification rates, because the majority of clades lack bootstrap support. Additionally, in this region of the phylogeny, the majority of classically described tribes were shown to be poli- or paraphyletic (Hernández-Hernández et al., 2010, in prep. Chapter III.1), which, together with the relatively scarce taxonomic sampling of South American lineages, complicated the estimation of the number of species in each clade. Nevertheless, the dated phylogenies suggested that South American lineages could also be the result of a recent diversification, particularly in the case of derived clades such as the BCT and the Trichocereeae (estimated dates for their crown group diversification are 8.8 and 4.5 MYA respectively). Trichocereeae in particular includes a vast array of life forms and was found to have high diversification rates (ranging from 0.7 to 1.2 species per MY. See Table 2).

DISCUSSION

The time of origin of many lineages of Cactaceae substantially precedes their diversification. According to our results, the divergence of Opuntioideae and Cactoideae occurred around 13.69 MYA (middle Miocene), and within Cactoideae, the divergence between Cacteae and Core Cactoideae occurred around 10.76 MYA (late Miocene). Cacteae is the only classically defined Cactaceae tribe recognized as monophyletic in phylogenetic analyses (Hernández-Hernández et al., 2010, in prep. Chapter III.1) and it includes all the globose and barrel North American cacti, with a diversity center in the southernmost portion of the Chihuahuan Desert (Hernández and Gómez-Hinostrosa, 2005). The Core Cactoideae includes all the cacti belonging to remaining tribes, with a vast diversity of growth forms and distributed all throughout the American continent. According to our results, the Cacteae and the Core Cactoideae clades started diversifying closely in time: Cacteae around 7.96 MYA and the Core Cactoideae around 9.7 MYA, both during the late Miocene.

As it can be seen in Table 1, there are several Cactaceae lineages including a large number of species, like the tribe Cacteae, with 356 species, or the genus *Mammillaria*, the largest within Cacteae and the largest of the cacti genera, including 163 species. However, these lineages do not have substantially high diversification rates (Table 2). According to our results, the lineages with accelerated diversification rates, especially considering a possible scenario of low extinction rates, are the Hylocereeae and the Core Pachycereeae clades, both within the PHB clade (Core Cactoideae I), and the Trichocereeae clade, within the Core Cactoideae II clade (see description of clades and phylogenetic relationships in Hernández-Hernández et al., 2010, in prep. Chapter III.1).

The Hylocereeae was found to have diverged recently (3.4-3.12 MYA), and according to our results, this clade diversified approximately simultaneously to the diversification of both the Pachycereinae (3.08 MYA) and Stenocereinae (3.15 MYA) subtribes. Hylocereeae includes thin, sprawling or epiphytic cacti with a main diversity center spanning throughout Central America to North America (Hernández-Hernández et al., 2010, in prep. Chapter III.1). Epiphytism evolved twice within Cactaceae, in the Hylocereeae clade and in the Rhipsalideae (Hernández-Hernández et al., 2010, in prep. Chapter III.1); however diversification rates of the latter are lower than those of Hylocereeae, particularly considering low extinction rates (Table 2), indicating that the evolution of the epiphytic habit might not be responsible for the accelerated speciation rates of Hylocereeae. Genera within Hylocereeae such as *Hylocereus*, *Epiphylum* and *Selenicereus* produce large, nocturnal, white disc shaped flowers, which are partially zygomorphic (most of the stamens are placed on one side of the flower), have a large nectarial chamber, and are commonly visited by moths (Ramírez-Mireles 1999; Rowley, 1980; in Nobel, 2002). However these floral attributes are also characteristic of chiropterophilous plants (Munguía-Rosas et al., 2009), and *Hylocereus* and *Selenicereus* species has been reported as bat pollinated species (Fleming et al., 2009).

The core Pachycereeae clade (including Stenocereinae and Pachycereinae) was also found to have high diversification rates. It includes all the columnar, arborescent, shrubby and sometimes scandent cacti from North America. Columnar species within Pachycereeae are frequently pollinated by bats (Badano and Schlumpberger 2001; in Mandujano et al., 2010); and this pollination system has been particularly studied in different members of the Pachycereeae tribe, for example, species of *Stenocereus* (Nassar et al. 1997), *Neobuxbaumia* (Valiente-Banuet et al. 1997a), *Pachycereus* and *Carnegia* (Fleming et al. 2001); which have white large flowers that produce copious amounts of nectar and pollen as rewards, and bloom at night. It appears that the plesiomorphic pollination system in the Core Pachycereeae was chiropterophily, as in *Neobuxbaumia mezcalaensis* and *Stenocereus martinezii* (Fleming et al. 2001), however, species like *Carnegia gigantea* and *Stenocereus thurberi* in the Sonoran desert have modified the original characters of timing of anthesis and a specialized chiropterophily pollination system, to generalist systems that allow both nocturnal and diurnal floral visitors. It has also been reported that *Polaskia chende*, *P. chichipe*, *Escontria chiotilla*, and *Myrtillocactus schenckii* other arborescent species, are pollinated by bees (Cruz and

Casas, 2002; Otero-Arnaiz et al. 2003; Oaxaca-Villa et al. 2005; Ortíz et al. 2010). *Echinocereus* is an important element of the core Pachycereeae radiation. This large genus includes about 67 species of dwarf globose to cylindrical cacti, although some of them might have long sprawling stems (Anderson, 2001). Species within *Echinocereus* are mainly pollinated by bees, and few of them are pollinated by birds (Cota-Sanchez, 1993). Bee pollination is the most common pollination system in cacti and is considered to be the plesiomorphic condition in the family (most Pereskioideae, Opuntionideae and many Cactoideae; Cota-Sanchez, 1993; Barthlott and Hunt, 1993). The derived position of the *Echinocereus* clade within the core Pachycereeae suggests the growth forms and pollination syndromes within this genus might be a regression to an ancestral condition.

The Trichocereeae (including *Gymnocalycium*) is another clade found to have high diversification rates (Table 2). Although the traditionally defined Trichocereeae tribe was found to be polyphyletic in phylogenetic analyses (Hernández-Hernández et al., 2010, in prep. Chapter III.1), the Trichocereeae plus the *Gymnocalycium* clades described in phylogenetic analyses by Hernández-Hernández et al. (2010, in prep. Chapter III.1. See Figure 3) conform a monophyletic group which includes the majority of the genera traditionally included in tribe Trichocereeae (Table 1). The taxonomic sampling in that phylogeny, which is used here, includes at least all the largest genera within Trichocereeae (Hernández-Hernández et al., 2010, in prep. Chapter III.1), thus, we were able to obtain an estimate of the possible number of species in this clade by excluding *Discocactus*, *Rebutia,* and *Sulcorebutia* species, which fell outside the Trichocereeae+Gymnocalycium clade in the phylogeny (Figure 3). Generally speaking, we could refer to the Trichocereeae tribe as the South American equivalent of the Pachycereeae. Trichocereeae includes several columnar cacti with nocturnal flowers, which inhabit the extra-tropical arid regions of South America (e.g. *Echinopsis* species). It has been suggested that these species probably have generalized pollination systems, similar to their ecological Pachycereeae counterparts in the extra-tropical deserts of North America (Fleming et al., 2001), with moths (but not bats) as primary pollinators (Ortega-Baes et al., 2010b).

Unfortunately, little is known about the phylogenetic relationships, ecology and pollination biology among members of Trichocereeae. For instance, *Harrisia* comprises 18 species of thin stemmed, few ribbed sprawling shrubby to arborescent cacti, 14 of which are restricted to the Caribbean and four to the southeastern South America (Anderson, 2001).

They have large, whitish, hermaphroditic flowers that open at night and produce a strong odor and large quantities of pollen and nectar (Proctor, 1984; Liogier, 1994; in Rojas-Sandoval and Meléndez-Ackerman, 2009). Based on these floral characteristics, a chiropterophilous pollination syndrome has been suggested for four *Harrisia* species (USFWS, 2001; González-Oliva and Urquiola, 2005; in Rojas-Sandoval and Meléndez-Ackerman, 2009); however, for most species pollination mode is unknown. *Weberbauerocereus* includes seven species, and studies with *W. weberbaueri*, a gigantic arborescent cactus from southwestern Perú, documented that it is pollinated by bats and hummingbirds (Sahley, 1996). The genus *Echinopsis* is the largest within Trichocereeae (Table 1). It includes species with a wide diversity of life forms, from small solitary or clustering globose forms to gigantic trees. Phylogenetic results indicate *Echinopsis* is a polyphyletic assemblage (Hernández-Hernández et al., 2010, in prep. Chapter III.1). Studies on the pollination of *Echinopsis* are also very scarce. The reproductive biology of two gigantic arborescent species, *E. terscheckii* and *E. atacamentis* has been studied. *E. terscheckii* from the northwest Argentina has nocturnal flowers, which are pollinated by night moths but are visited by bees and occasionally birds during the day (Ortega-Baes et al., 2010b). *E. atacamentis* also has nocturnal flowers visited by moths, and that remain open until the following day, when they are visited by bees and hummingbirds (de Viana et al., 2001; Schlumpberger and Badano 2005; in Ortega-Baes et al., 2010b). In the case of *E. chiloensis*, another arborescent cactus from central Chile, it was observed that both diurnal bees and nocturnal moths contributed equally to fruit set, suggesting a generalized pollination system (Walter, 2009). Moths have also been reported as important pollinators of South American *Cereus* (Cereeae tribe) species (e.g. *Cereus hildmannianus* and *C. fernanbucensis*; Silva and Sazima 1995; Locatelli and Machado 1999; in Ortega-Baes et al., 2010b); and their role in the pollination of North American arborescent species in the Sonoran Desert in North America has also been reported, for species such as *Lophocereus schotti* (Fleming and Holland 1998; Holland and Fleming 1999), *Stenocereus eruca* (Clark-Tapia and Molina-Freaner 2004) and other *Peniocereus* species (Suzán et al. 1994; Raguso et al. 2003). Based on studies of species within Trichocereeae, it has been suggested that species of columnar cacti with nocturnal flowers in this tribe might have evolved towards moth pollination, particularly in the extra-tropical deserts of South America, where nectar-feeding bats are not present (Otrgea-Baes et al., 2010b).

Species within Cactaceae display different sexual strategies for successful reproduction, and several theories have attempted to explain the origin and causes of such diversity based on the facts that plants are sessile, modular, many are cosexual, and need to develop strategies in order to reach mates (Lovett-Doust and Lovett-Doust 1988; Waser 1983 in Mandujano et al., 2010). Floral visitors of cactus blossoms include bats, birds, bees, wasps, moths, beetles, grasshoppers and ants (Mandujano et al., 2010), however, cacti species have been thought of as a group specialized in animal pollination because floral traits are well adapted to animal behavior (Gibson and Nobel 1986; Mandujano et al., 1996; Valiente-Banuet et al., 1996, 2002). Bird, bat, and moth pollinations, all derived conditions, are less common and tend to occur in some tribes such as Pachycereeae and Trichocereeae (Gibson and Nobel, 1986; Grant and Grant, 1979b in Cota-Sanchez, 1993). Studies on the genus *Agave* (Agavaceae), a genus with a geographic distribution highly coincident with columnar Pachycereeae members, was also found to have high diversification rates (Good-Avila et al., 2006); possibly as a result of the evolution of a generalistic pollination system highly dependent on bats. Some authors have suggested that the coincidence in the geographic distribution of *Agave* and columnar Pachycereeae might be explained by the evolution of similar ecological mechanisms (Good-Avila et al., 2006; Molina-Freaner and Eguiarte, 2003). The time when pollinators are active has been thought to exert selective pressures under floral anthesis, and a nocturnal or crepuscular anthesis is a trait typical of moth or bat pollinated flowers (Van der Pijl 1960; Wyatt 1983; in Miyake and Yahara, 1999). A nocturnal-crepuscular system that allows a more diverse array of species to pollinate flowers may signify extra advantages, for example, in relation to places with distinct pollinator's availability (Valiente-Banuet et al., 1997a, b). In addition to the evolution of particular floral pollinating systems, the evolution of the columnar or arborescent form in the context of the dwarf vegetation arid and semiarid regions in the New World might be helpful to facilitate the pollination by moths, bats or birds (Fleming et al., 2009). In both, Core Cactoideae I and II the most conspicuous growth forms are shrubs (Figure 4), however, both in core Pachycereeae and in Trichocereeae this growth form reaches gigantic sizes and the arborescent and columnar form appeared in both clades.

CONCLUSIONS

Lineages showing the fastest diversification rates within Cactaceae are the core Pachycereeae (North America), the Hylocereeae (Central to North America) and the Trichocereeae (South America). The more conspicuous growth forms within these lineages are columnar, arborescent or large shrubs (Pachycereeae and Trichocereeae), or epiphytic or sprawling (Hylocereeae). They possess flowers with a nocturnal to crepuscular anthesis.

The possession of a columnar vegetative body and nocturnal flowers might favor the visit of pollinators such as bats or birds (and possibly moths), and might provide important benefits to plants: the deposition of large amounts of pollen and a variety of pollen genotypes on plant stigmas and, compared with pollinators such as ants of bees, they are long-distance pollen dispersers (Fleming et al., 2009). Nectar-feeding bats are the most effective pollinators of several cactus species within the core Pachycereeae (Nassar et al., 1997; Fleming et al., 2001, 2009; Munguía-Rosas et al., 2009b; in Ortega-Baes et al., 2010). Some of these species have generalized pollination systems, and are also effectively pollinated by diurnal animals such as birds and bees (Fleming et al., 2001; Munguía-Rosas et al., 2009a, b; in Ortega-Baes et al., 2010). In columnar cacti with bat-pollination systems, the relative contribution of diurnal visitors to fruit set increases with increasing latitude; hence, cactus populations in extra-tropical deserts are more dependent on diurnal visitors for seed production (Munguía-Rosas et al., 2009b). Columnar cacti with nocturnal flowers are pollinated not only by nectar-feeding bats, but also by moths, which are possibly the main pollinator of South American columnar cacti (Fleming and Holland 1998; Holland and Fleming 1999; Locatelli & Machado 1999; Clark-Tapia and Molina-Freaner 2004; in Ortega-Baes et al., 2010). The outstanding diversification of the Core Pachycereeae, Hylocereeae and Trichocereeae might be related with their nocturnal-crepuscular pollination system, with a possible trend towards a specialization for bats in North America and moths in South America, both facilitated by a columnar life form.

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FIGURES AND TABLES

	#SPP
CACTACEAE	**1503**
PERESKIOIDEAE	
Pereskia	**17**
MAIHUENIOIDEAE	
Maihuenia	**2**
OPUNTIOIDEAE	
Corynopuntia	14
Austrocylindropuntia	8
Opuntia	75
Brasiliopuntia	1
Pereskiopsis	6
Consolea	3
Pterocactus	9
Cumulopuntia	4
Quiabentia	2
Cylindropuntia	33
Tacinga	6
Grusonia	1
Tephrocactus	7
Maihueniopsis	7
Tunilla	5
Miqueliopuntia	1
Nopalea	4
TOTAL	**186**
CACTOIDEAE	
TOTAL	**1298**
Calymantheae	
Calymmanthium	1
Hylocereae	
Disocactus	11
Epiphyllum	12
Hylocereus	14
Pseudorhipsalis	6
Selenicereus	12
Strophocactus	3
Weberocereus	8
TOTAL	**66**
Cereeae	
Arrojadoa	5
Brasilicereus	2
Cereus	25
Cipocereus	5
Coleocephalocereus	8
Melocactus	37
Micranthocereus	9
Pierrebraunia	2
Pilosocereus	41
Praecereus	2
Stephanocereus	2
Uebelmania	3
TOTAL	**207**
Trichocereae	
Acanthocalycium	?
Arthrocereus	4
Brachycereus	1
Cephalocleistocactus	?
Cleistocactus	38
Denmoza	1
Discocactus	11
Notocacteae	
Austrocactus	3
Blossfeldia	1
Cintia	?
Copiapoa	21
Eriosyce	32
Eulychnia	4
Frailea	12
Neowedermania	2
Parodia	58
Yavia	1
TOTAL	**134**
Rhipsalideae	
Hatiora	6
Lepismium	6
Rhipsalis	35
Schlumbergera	6
Pfeiffera	9
TOTAL	**62**
Browningieae	
Armatocereus	7
Browningia	8
Castellanosia	1
Jasminocereus	1
Neoraimondia	2
Stetsonia	1
TOTAL	**20**
Pachycereae	
Acanthocereus	1
Bergerocactus	1
Carnegiea	1
Cephalocereus	3
Corryocactus	12
Dendrocereus	2
Echinocereus	67
Escontria	1
Isolatocereus	?
Leptocereus	11
Myrtillocactus	4
Neobuxbaumia	8
Pachycereus	13
Peniocereus	20
Polaskia	2
Pseudoacanthocereus	2
Stenocereus	24
TOTAL	**172**
Cacteae	
Acharagma	2
Ariocarpus	7
Astrophytum	6
Aztekium	2
Cochemiea	?
Coryphantha	42
Cumarinia	1
Echinocactus	6
Echinomastus	?

Echinopsis	77	*Epithelantha*	2
Espostoa	11	*Escobaria*	19
Espostoopsis	1	*Ferocactus*	28
Facheiroa	3	*Geohintonia*	1
Gymnocalycium	49	*Leuchtenbergia*	1
Haageocereus	9	*Lophophora*	3
Harrisia	9	*Mammillaria*	163
Lasiocereus	2	*Mammilloydia*	1
Leocereus	1	*Neolloydia*	2
Matucana	14	*Obregonia*	1
Mila	1	*Ortegocactus*	1
Oreocereus	6	*Pediocactus*	7
Oroya	2	*Pelecyphora*	2
Pygmaeocereus	2	*Sclerocactus*	20
Rahuocereus	1	*Stenocactus*	8
Rebutia	29	*Strombocactus*	1
Samaipaticereus	1	*Thelocactus*	14
Weberbauocereus	7	*Turbinicarpus*	16
Yungasocereus	1	TOTAL	**356**
TOTAL	**281**		

Table 1. Cactaceae classification sensu Anderson (2001), with genera included in each subfamily and tribe, and the number of species included on each

		Estimated Ages		**Diversification Rates**			
	# spp.	**Stem Group**	**Crown Group**	**Eq.6, Stem Epsilon = 0**	**Eq. 6 Stem Epsilon = 0.9**	**Eq. 7 Crown Epsilon = 0**	**Eq. 7 Crown Epsilon = 0.9**
ANGIOSPERMS	269288	350	241	0.035	0.029	0.049	0.042
CARYOPHYLLALES	11155	97.83	92.78	0.095	0.071	0.092	0.075
OPUNTIOIDEAE	186	13.69	5.33	0.381	0.216	0.850	0.547
Cylindrical-stemmed opuntias (*Quiabentia, Pereskiopsis, Grusonia, Cylindropuntia* clade)		5.33	4.38				
Spherical-stemmed opuntias (*Maihueniopsis, Tephrocactus, Pterocactus*)		5.33	4.45				
Flattened stemmed opuntias (including *Mihueliopuntia+Tunilla*)		5.33	2.71				
Flattened stemmed opuntias (*Opuntia, Nopalea, Tacinga*)		2.71	2				
Nopalea		1.68	0.81				
CACTOIDEAE	1298	13.69	11.69	0.523	0.355	0.553	0.412
CACTEAE	356	10.76	7.96	0.545	0.334	0.650	0.445
Aztekium clade		7.66	5.22				
Astrophytum		4.66	2.38				
Sclerocactus+Echinomastus clade		7.96	4.71				
Thelocactus+Ferocactus+Leuchtenbergia clade		7.37	4.5				
Ariocarpus+Turbinicarpus+Epithelantha clade		7.08	5.7				
Mammilloid clade		7.08	6.7				
Coryphantha	42	5.4	3.22	0.692	0.301	0.945	0.489
Mammillaria	163	5.33	4.47	0.955	0.533	0.984	0.624
CORE CACTOIDEAE	942	10.76	9.7	0.636	0.423	0.634	0.464
Copiapoa	21	8.77	2.53	0.347	0.125	0.929	0.413
Rhipsalideae	62	9.46	6.29	0.436	0.207	0.545	0.303
Corryocactus	12	4.66	6.29	0.533	0.159	0.995	0.382
PHB clade		4.29	4.2				
Core Pachycereeae [a]	155	4.2	3.74	**1.200**	**0.666**	**1.163**	**0.734**
Pahycereinae	44	3.74	3.08	**1.011**	**0.445**	**1.003**	**0.524**
Stenocereinae	98	3.74	3.15	**1.225**	**0.633**	**1.235**	**0.736**
Echinocereus	67	3.15	2.22	**1.334**	**0.643**	**1.581**	**0.890**
Hylocereae (sensu stricto)	66	3.4	3.12	**1.232**	**0.592**	**1.120**	**0.629**
Hylocereae (plus Peniocereus and Acanthocereus)	69	4.2	3.4	**1.008**	**0.489**	**1.041**	**0.588**
Core Notocacteae		8.81	7.18				
BCT clade		8.81	5.27				
Gymnocalycium	49	4.5	3.77	0.864	0.390	0.848	0.452
Trichocereeae [b]	241	4.56	4.5	**1.202**	**0.705**	**1.064**	**0.703**

[a] Traditional Pachycereeae excluding *Acanthocereus*, *Peniocereus subgen. Pseudoacanthocereus*, *Corryocactus*, and *Pseudoacanthocereus.*

[b] Traditional Trichocereeae excluding *Discocactus*, *Rebutia*, *Sulcorebutia*

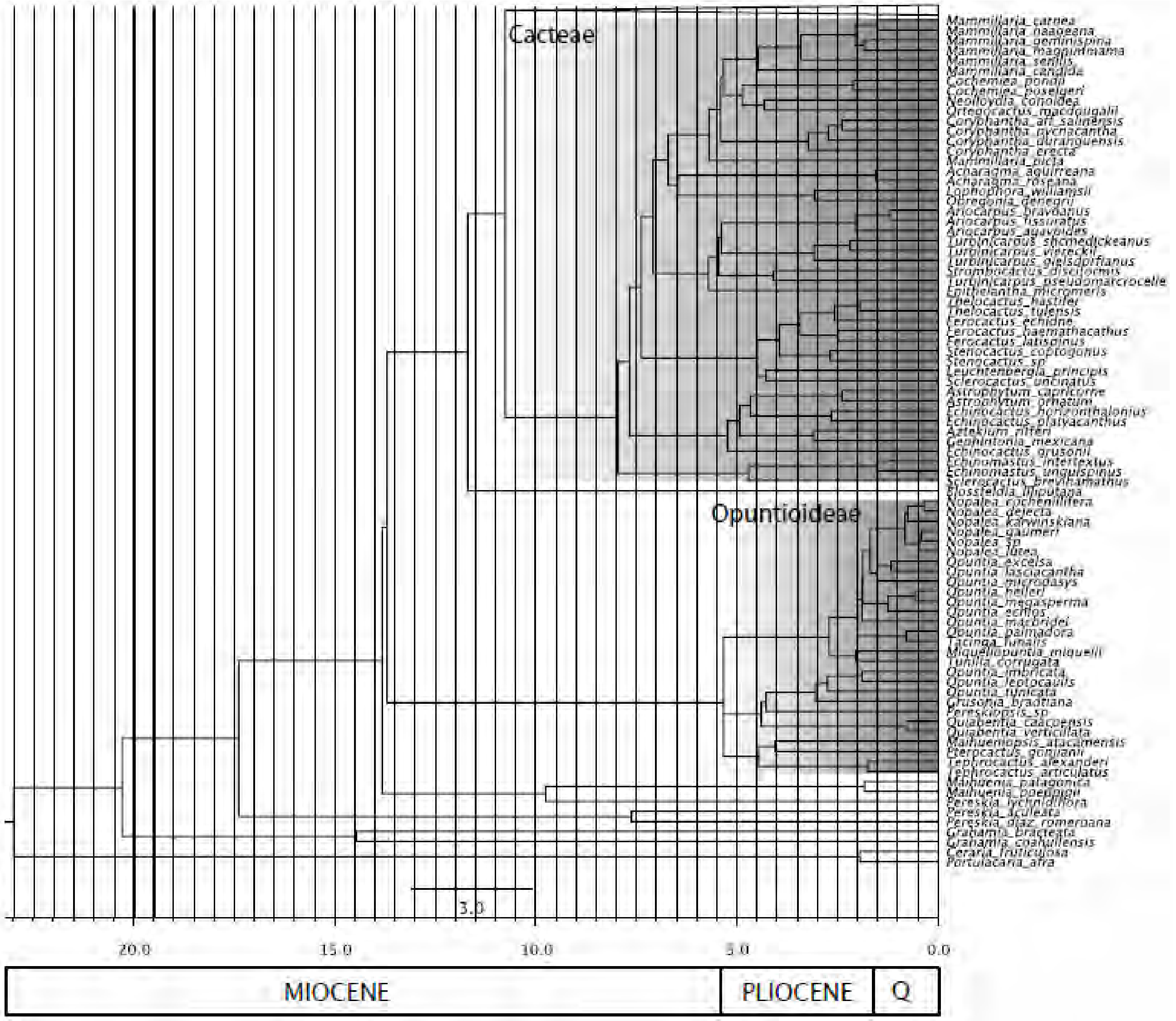


Figure 1. Chronogram resulting from the PL analyses in r8s. Some clades are collapsed to better show the Opuntioideae and the Cacteae clades.

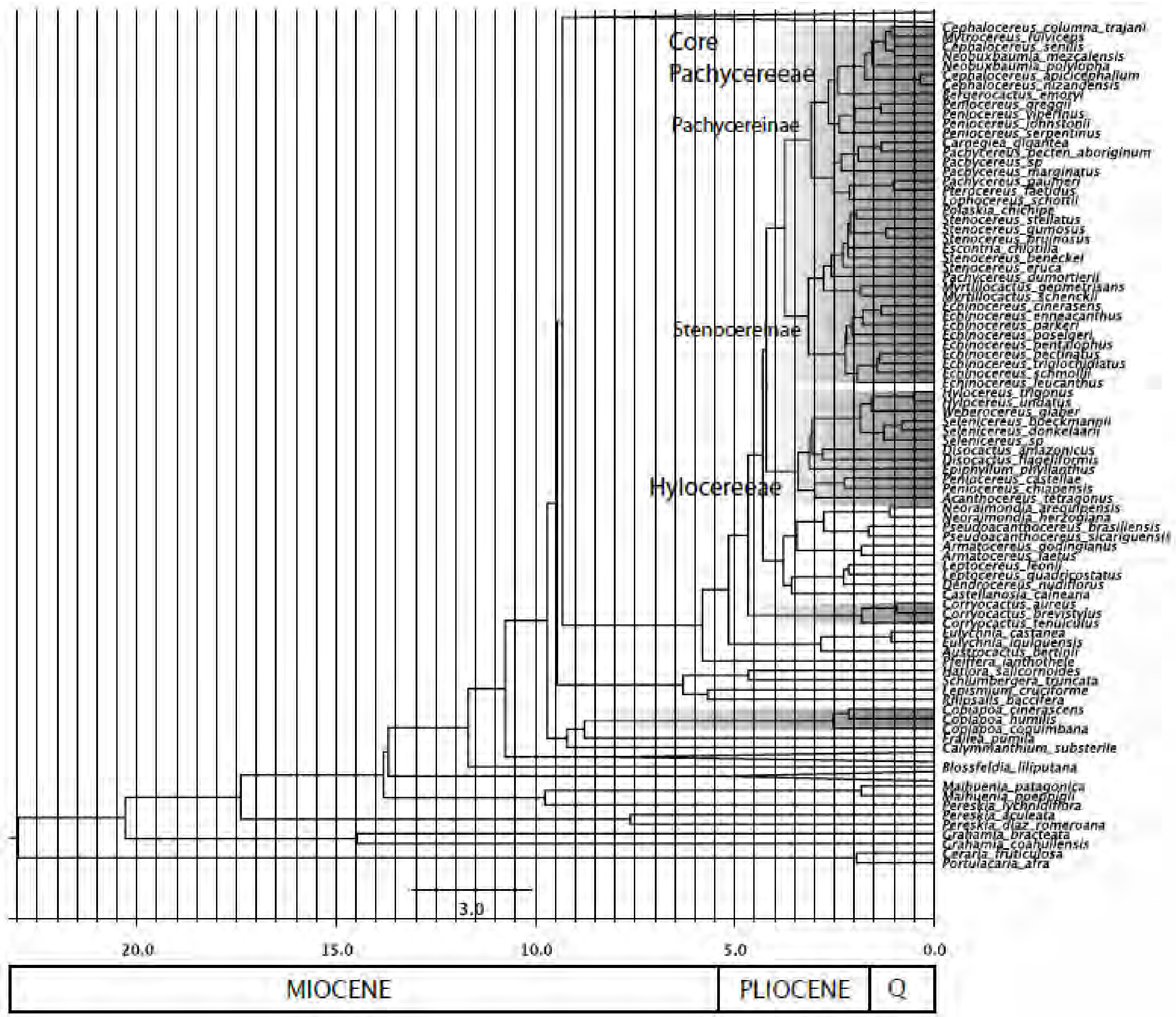


Figure 2. Chronogram resulting from the PL analyses in r8s. Some clades are collapsed to better show the Core Cactoideae I clade.

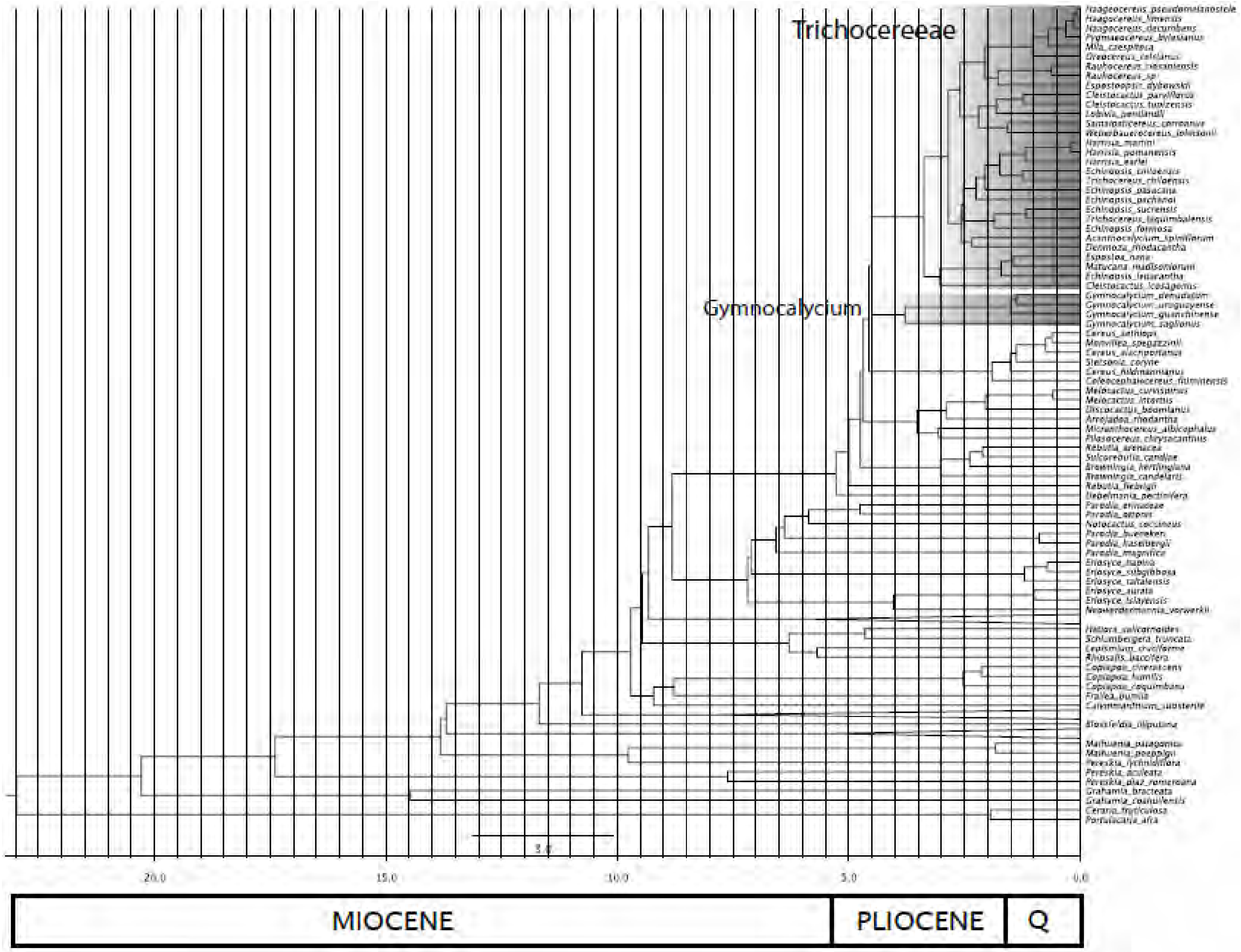
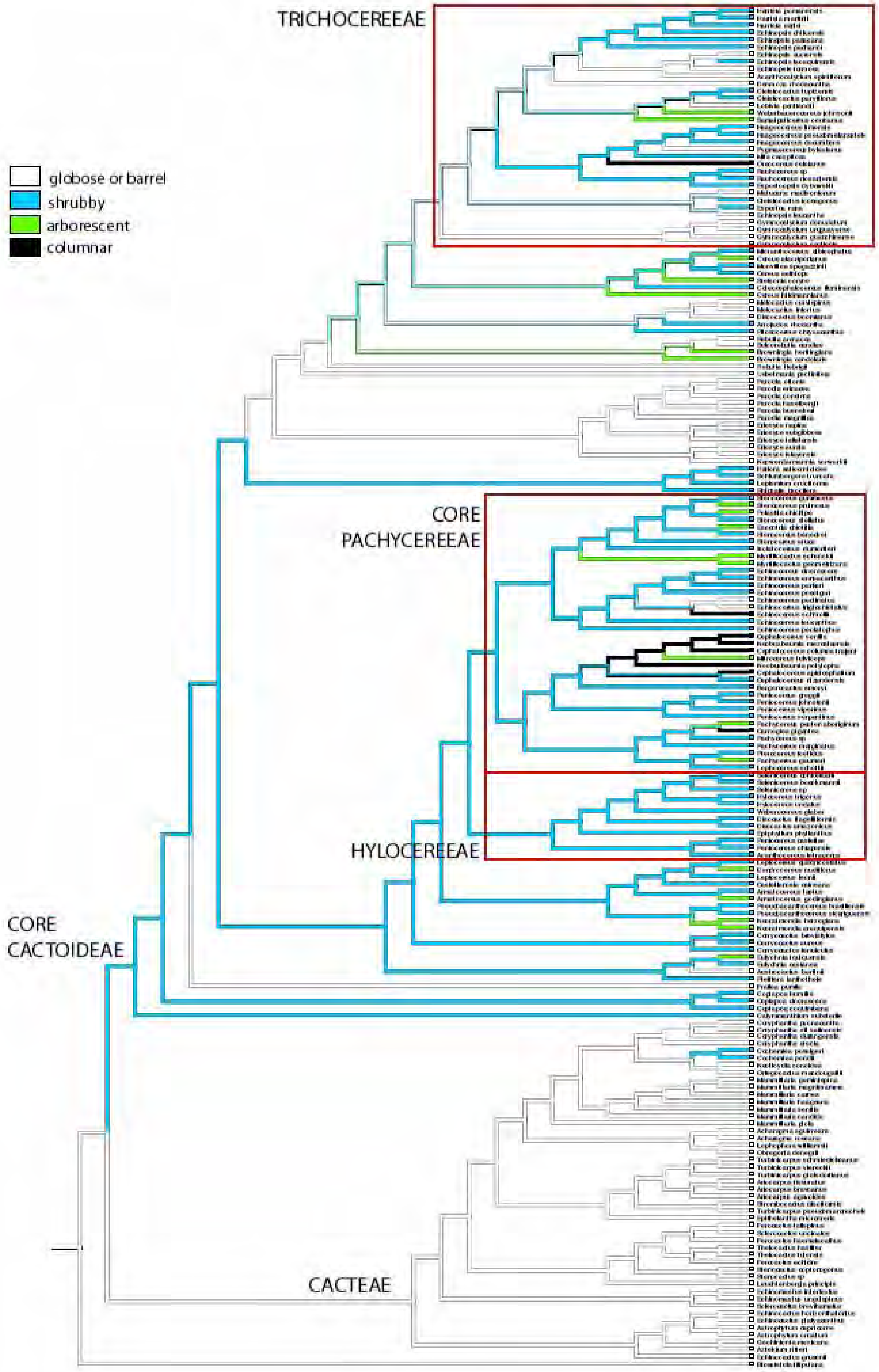


Figure 3. Chronogram resulting from the PL analyses in r8s. Some clades are collapsed to better show the Core Cactoideae II clade.

Figure 4. (Opposite page). Phylogeny of Cactoideae showing the growth form character. To see details on growth form classification see Hernández-Hernández et al., 2010 (in prep.).


TRICHOCEREEAE
globose or barrel
shrubby
arborescent
columnar
CORE
PACHYCEREEAE
HYLOCEREEAE
CORE
CACTOIDEAE
CACTEAE

Discusión general

El término *radiación adaptativa* ha sido usado indiscriminadamente y de manera muy laxa por biólogos evolutivos que estudian una gran diversidad de linajes. Esto ha llevado a una ambigüedad en su definición, de manera que en la actualidad el concepto aporta poco a nuestro conocimiento sobre la evolución de los organismos. Las revisiones disponibles y las recientes críticas al concepto son insuficientes, ya que carecen de una discusión profunda de los orígenes y motivos del surgimiento concepto, basándose principalmente en estudios de linajes vivos desde el campo de la ecología (Givnish and Sytsma, 1997; Sudhaus, 2004). Además, tal vez por que se ha dejado a un lado a las ideas provenientes del campo de la paleontología y la macroevolución, se ha propuesto incluso la inexistencia de los patrones de evolución explosiva y por tanto, la invalidez del concepto *radiación adaptativa* (Olson y Arroyo-Santos, 2009). En el Capítulo I se presenta una revisión del concepto, ubicando los orígenes de la idea de radiación adaptativa quizás hacia finales del siglo XIX o principios del XX, con las primeras observaciones paleontológicas de la aparición súbita de linajes fósiles y la propuesta de la idea de evolución adaptativa. A través del tiempo, diferentes investigadores han propuesto distintas definiciones de radiación adaptativa, enfatizando diferentes características del proceso, como por ejemplo, la diversidad ecológica, la diversidad fenotípica, el número de especies o la velocidad en la aparición de nuevas especies. Proponemos que mucha de la confusión existente puede radicar en la incomprensión de la relación que existe entre las tasas de evolución taxonómicas y fenotípicas. Con base en la teoría del Equilibrio Puntuado (Eldredge y Gould 1972; Gould y Eldredge, 1977), se discute dicha relación, y se propone que una manera no ambigua de detectar una radiación es identificando un aumento significativo en las tasas de diversificación taxonómica del linaje estudiado. Ante las dificultades metodológicas en la detección de adaptación (ver por ejemplo, Wade y Kalisz, 1990; Conner et al., 2009), se sugiere usar preferentemente el término *radiación evolutiva*. De esta manera, se propone que las radiaciones evolutivas son linajes monofiléticos que se originaron en un proceso de diversificación acelerada en comparación con linajes cercanos, ecológicamente similares. Pueden existir varias explicaciones sobre la aceleración en las tasas de diversificación presentes en dichos linajes, que van desde las internalistas como la aparición de las llamadas innovaciones clave, o las externalistas, como la apertura de nichos ecológicos por extinciones masivas o los cambios climáticos, como la gradual aridificación de ciertas regiones (ver Sudhaus, 2004).

El orden Caryophyllales representa un grupo taxonómico que facilita el estudio de radiaciones evolutivas de linajes de plantas suculentas, que pueden servir como indicadores del origen y evolución de floras de zonas áridas. En el Capítulo II estudiamos las radiaciones de suculentas al interior del Orden usando filogenias moleculares, y estimados de las fechas de origen y tasas de diversificación. Dentro del Orden, los linajes que presentan mayores tasas de diversificación son la subfamilia Ruschioideae (Aizoaceae), y las tribus Cacteae y Pachycereeae (Cactaceae), representando dos radiaciones espectaculares de suculentas en el Sur de África y en Norte América (ver Klak et al, 2004; Hernández-Hernández et al, 2010 en el Capítulo II.1). El estudio de sus nichos ecológicos potenciales muestra que las condiciones climáticas en las cuales estas plantas habitan son muy distintas, lo cual indica que a pesar de provenir de un ancestro común, posiblemente han evolucionado adaptaciones morfológicas y fisiológicas similares, pero en distintas condiciones de aridez, siguiendo trayectorias evolutivas distintas. La subfamilia Ruschioideae (Aizoaceae), y las tribus Cacteae y Pachycereeae (Cactaceae) representan ejemplos claros de radiaciones evolutivas, posiblemente en respuesta a factores externos, como la aridificación de las regiones en que habitan.

A pesar de los resultados obtenidos, y para tener una coherencia con las propuestas metodológicas establecidas en el Capítulo I, es necesario llevar a cabo un análisis estadístico riguroso para detectar objetivamente si los incrementos en las tasas de diversificación de la subfamilia Ruschioideae (Aizoaceae) y de las tribus Cacteae y Pachycereeae (Cactaceae) son significativos. Un posible método fue desarrollado por Magallón y Sanderson (2001), en el que los autores obtuvieron intervalos de confianza de la diversidad esperada para las angiospermas, detectando así los linajes con tasas de diversificación significativamente altas. Otro método recientemente publicado es MEDUSA (Alfaro et al., 2009), desarrollado en el lenguaje de programación R. Este método usa el criterio de información de Akaike para detectar múltiples cambios significativos en las tasas de crecimiento y muerte a lo largo de una filogenia, y es útil para detectar incrementos en las tasas de diversificación en un contexto filogenético, en vez de usar comparaciones entre tasas absolutas de diversificación, que asumen una constancia en las tasas de diversificación a través del tiempo. Para entender mejor el origen de los linajes suculentos en Caryophyllales, es necesario además discutir más profundamente las fechas estimadas de origen de esos linajes en un contexto paleoclimático y geológico. El análisis y discusión de los nichos ecológicos potenciales de los linajes suculentos en África y América puede mejorarse con un análisis estadístico más formal de las variables que determinan los nichos potenciales de manera más importante al interior de los clados. La discusión del significado ecológico de las diferencias en éstas variables climáticas entre linajes puede ser mejorada, por ejemplo, con más datos sobre las posibles adaptaciones particulares que tiene cada linaje. Finalmente, para proveer de una liga conceptual entre las condiciones ecológicas actuales de los linajes de suculentas que se distinguieron como radiaciones y su historia evolutiva, se pueden estimar nichos ecológicos potenciales ancestrales usando métodos de reconstrucción de caracteres ancestrales. Cuando los nichos ecológicos son interpretados como una extensión de los fenotipos, el análisis de los nichos ecológicos puede ayudar a entender la evolución de adaptaciones particulares.

Las cactáceas incluyen cerca de 1500 especies incluidas en alrededor de 127 géneros (Barthlott y Hunt, 1993; Hunt et al., 2006) con una distribución amplia en una diversidad de ambientes, y con una gran diversidad de formas de vida (Bravo Hollis y Sheinvar, 1999). El centro de diversidad actual de las cactáceas se encuentra en México, con gran cantidad de endemismos (Hernández y Godínez, 1994). En el Capítulo III se analiza a mayor profundidad la evolución de linajes y las radiaciones más importantes que ocurrieron al interior de la familia Cactaceae. Dentro de ésta familia, los linajes que presentan tasas de diversificación más altas son las Pachycereeae centrales, Hylocereeae y Trichocereeae. Sus miembros tienen en común la posesión de flores con antésis nocturna, polinizadas posiblemente por murciélagos en Norte América y por mariposas nocturnas durante la noche. En las Pachycereeae centrales y las Trichocereeae, la forma de crecimiento columnar podría también facilitar la polinización por animales como aves o murciélagos. Las Pachycereeae centrales, Hylocereeae y Trichocereeae son linajes que representan radiaciones evolutivas importantes, posiblemente en respuesta a factores internos, como la evolución de flores con antésis nocturna y la forma de crecimiento columnar.

De la misma manera que con el trabajo realizado para las radiaciones de suculentas en Caryophyllales, la detección de radiaciones al interior de la familia Cactaceae requiere de un análisis estadístico riguroso. También es necesario discutir a mayor profundidad el contexto paleoclimático y geológico de las fechas estimadas para el origen de principales linajes. La estimación de fechas de origen y diversificación de las familias Cactaceae, Aizoaceae and Didiereaceae en el Orden Caryophyllales y de linajes al interior de Cactaceae puede mejorarse usando el programa BEAST (Drummond and Rambaut, 2007). Este programa ofrece muchas ventajas, como la implementación de fechar de calibración como distribuciones de probabilidad, además de que provee márgenes de error para las fechas estimadas.

Los miembros de los linajes de suculentas en el Orden Caryophyllales estudiados (subfamilia Ruschioideae, familia Didiereaceae y linajes al interior de Cactaceae) conforman elementos constitutivos de las vegetaciones de las zonas áridas en que habitan: el Karoo suculento en el sur de África, los matorrales espinosos del sur de Madagascar y los matorrales xerófilos y selvas bajas de México y zonas áridas de Sudamérica respectivamente. El estudio del origen y evolución de estos linajes puede aportar información importante para el entendimiento de los patrones de radiaciones evolutivas, y aportar elementos para la comprensión del origen de los ecosistemas en que habitan.

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Conclusiones generales

Un criterio objetivo para la determinación de radiaciones evolutivas es la detección de incrementos significativos en las tasas de diversificación taxonómica de linajes monofiléticos. Después de detectar dicho patrón, se pueden fundamentar hipótesis sobre posibles explicaciones causales de las radiaciones, que pueden ser extrínsecas, como por ejemplo los cambios climáticos; o intrínsecas, como la evolución de innovaciones clave.

Los estimados obtenidos para las fechas de origen de diferentes linajes de suculentas dentro el Orden Caryophyllales y de linajes al interior de la familia Cactaceae son relativamente recientes. En particular los clados monofiléticos que corresponden a la subfamilia Ruschioideae en Aizoaceae y las tribus Pachycereeae, Hylocereeae y Trichocereeae en Cactaceae presentan además elevadas tasas de diversificación, lo cual muestra que siguen un patrón de radiación evolutiva. Los miembros de dichos linajes son elementos constitutivos de los paisajes florísticos en zonas áridas de África y América, y determinantes de los procesos ecológicos en sus diferentes ecosistemas, y es posible que su diversificación responda a elementos extrínsecos como la aridificación de las regiones geográficas en dónde se distribuyen o bien, en particular en el caso de los linajes en Cactaceae, a la evolución de formas particulares de crecimiento y de características florales que permiten tipos de polinización particulares.

Los análisis de los nichos ecológicos potenciales de los miembros de las radiaciones en Aizoaceae, Didiereaceae y Cactaceae muestran que, a pesar de habitar en ambientes aridos similares y haber evolucionado suculencia; han seguido trayectorias evolutivas diferentes que les permiten habitar condiciones aridas distintas. Es necesario mejorar los análisis de fechación, estimación de tasas de diversificación y detección de incrementos significativos y de comparación de nichos ecológicos potenciales; asi como mejorar la discusión de los resultados, para lograr una contribución más significativa en el entendimiento de la evolución de linajes de suculentas que puedan indicar algunas pistas del origen de la vegetación de zonas áridas.

Espero que los trabajos presentados en esta tesis conformen una contribución al desarrollo y esclarecimiento del concepto de radiación adaptativa, y al reconocimiento de patrones evolutivos en linajes. También pueden contribuír al entendimiento de las relaciones filogenéticas dentro del Orden Caryophyllales y dentro de la familia Cactaceae, y al entendimiento de la evolución de las principales radiaciones que ocurrieron al interior de dichos grupos.