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ARTICLE

The last Patagonian cycad, *Austrozamia stockeyi* gen. et sp. nov., early Eocene of Laguna del Hunco, Chubut, Argentina[1]

Peter Wilf, Dennis Wm. Stevenson, and N. Rubén Cúneo

Abstract: The cycads pose classic problems in evolutionary biogeography, owing to their far-flung extant distributions and the sparse fossil records of living genera. A noteworthy example is Tribe Encephalarteae of Family Zamiaceae, today consisting of *Encephalartos* (Africa) and the Australian genera *Lepidozamia* and *Macrozamia*. Numerous petrified trunks of Encephalarteae described from the Cretaceous of Patagonia, Antarctica, and India indicate far larger past distributions across Gondwana and subsequent extinctions. The only fossils close to the current range are Paleogene leaf fragments from Australia assigned to *Lepidozamia* and *Macrozamia*. Here, we report a large frond piece and several isolated leaflets of a compressed cycad, along with an associated spiny petiole, from the late-Gondwanan, 52.2 Ma Laguna del Hunco flora of Patagonia, Argentina. ***Austrozamia stockeyi*** gen. et sp. nov., has the novel combination of an *Encephalartos*-type leaf and *Lepidozamia*-type cuticle. The Australian *Lepidozamia* fossils, widely used for molecular clock calibrations, could also represent an extinct genus. *Austrozamia stockeyi* demonstrates survival of the Encephalarteae in Patagonia and presumably across Gondwana until its terminal phase, adding a striking new component to the growing list of South American plant extinctions associated with Antarctic separation and related climate changes.

Key words: cycads, Eocene, *Encephalartos*, *Lepidozamia*, Patagonia, Zamiaceae.

Résumé : Les cycadophytes posent des problèmes classiques en biogéographie de l'évolution à cause de leurs distributions étendues des espèces toujours existantes et des rares registres de fossiles de genres vivants. Un exemple notable consiste en la tribu des Encephalarteae de la famille des Zamiaceae, qui comprend aujourd'hui *Encephalartos* (Afrique) et les genres australiens *Lepidozamia* et *Macrozamia*. Plusieurs troncs pétrifiés d'Encephalarteae du Crétacé de Patagonie, Antarctique, et de l'Inde indiquent des distributions passées beaucoup plus larges à travers le Gondwana et des extinctions subséquentes. Les seuls fossiles près de la série actuelle consistent en fragments foliaires du Paléogène de l'Australie assignés à *Lepidozamia* et *Macrozamia*. Les auteurs présentent ici un large morceau de fronde et plusieurs petites feuilles isolées d'un cycadophyte comprimé ainsi qu'un pétiole spinifère associé, provenant du Gondwana tardif, 52.2 Ma, de la flore de la Laguna del Hunco, Patagonie, Argentine. ***Austrozamia stockeyi*** gen. et sp. nov., possède la combinaison inédite de feuilles de type *Encephalartos* et le cuticule de type *Lepidozamia*. Les fossiles de *Lepidozamia* d'Australie, largement utilisés pour les calibrations de l'horloge moléculaire, pourraient aussi représenter un genre éteint. *Austrozamia stockeyi* témoigne de la survie des Encephalarteae en Patagonie et vraisemblablement à travers le Gondwana jusqu'à sa phase terminale, ajoutant une nouvelle composante frappante à la liste croissante d'extinctions de plantes en Amérique du Sud associées à la séparation de l'Antarctique et aux changements climatiques reliés. [Traduit par la Rédaction]

Mots-clés : cycadophytes, Éocène, *Encephalartos*, *Lepidozamia*, Patagonie, Zamiaceae.

Introduction

The cycads are a living Paleozoic seed-plant lineage that may be closely related to the long-extinct medullosan pteridosperms (Crane 1985; Norstog and Nicholls 1997; Brenner et al. 2003). Today, they number only 10 genera and ca. 344 species distributed in the warm regions of the globe, and the vast majority of them are under moderate to severe threat (Jones 2002; Donaldson 2003; Calonje et al. 2013–2016). Cycads participate in plant–animal associations of noted evolutionary significance (Farrell 1998; Stevenson et al. 1998; Terry 2001; Bayliss et al. 2010; Peñalver et al. 2012) and play promi-

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P. Wilf. Department of Geosciences, Pennsylvania State University, University Park, PA 16802, USA.
D. Wm. Stevenson. New York Botanical Garden, Bronx, NY 10458, USA.
N.R. Cúneo. CONICET, Museo Paleontológico Egidio Feruglio, Avenida Fontana 140, 9100 Trelew, Chubut, Argentina.
Corresponding author: Peter Wilf (email: pwilf@psu.edu).

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nent roles in landscape ecology and human cultures (Heibloem 1999; Jones 2002; Goel and Khuraijam 2015). Despite their rich fossil record, extending at least to the Early Permian and peaking in diversity during the Mesozoic (Taylor et al. 2009; Pott et al. 2010), the cycads present some of the most challenging problems in plant evolution and evolutionary biogeography.

Reliable fossil evidence for the living cycad genera is rare and limited to comparatively young strata. Only a few descriptions exist from the modern era of paleobotany, including Paleogene Australian fossils of *Bowenia*, *Lepidozamia*, and *Macrozamia* (Cookson 1953; R.S. Hill 1978, 1980, 1998; Carpenter 1991); Eocene *Cycas* fossils from China (Su et al. 2014) that are contested (Condamine et al. 2015); and Oligocene to Miocene European fossils of *Ceratozamia* (Kvaček 2002, 2004, 2014). In corollary, most cycad fossils appear to represent extinct genera, even as late as the Paleogene and Neogene. Examples include the Cenozoic occurrences of *Ctenis*, *Dioonopsis*, *Eostangeria*, *Pseudodioon*, and *Pterostoma* foliage (R.S. Hill 1980; Horiuchi and Kimura 1987; Hill and Pole 1994; Uzunova et al. 2001; Erdei et al. 2010, 2012; Erdei and Manchester 2015). Several molecular phylogenetic studies have concluded that these patterns could reflect reality in that molecular age estimates of the crown nodes of the cycad genera mostly correspond to the late Paleogene or Neogene in the geologic time scale (Moretti et al. 1993; Crisp and Cook 2011; Nagalingum et al. 2011; Salas-Leiva et al. 2013; Condamine et al. 2015).

The losses of so many cycad taxa to extinction and the accumulating evidence for comparatively young crown groups pose extraordinary difficulties for reconstructing cycad evolutionary history (Hermsen et al. 2006; Zgurski et al. 2008; Salas-Leiva et al. 2013; Lu et al. 2014; Condamine et al. 2015). Also, fossil reproductive organs are very scarce (Archangelsky and Villar de Seoane 2004; Watson and Cusack 2005), and most cycad remains are either "stem fossils" or "leaf fossils," yielding non-overlapping character sets that hinder phylogenetic analyses (Hermsen et al. 2006). Currently, there is little agreement among topologies based on morphological data, including from fossils, and those primarily based on molecular data from extant taxa (Hermsen et al. 2006; Cúneo et al. 2010; Salas-Leiva et al. 2013; Condamine et al. 2015).

For all the reasons given above, there is considerable potential value in each new cycad fossil. In particular, new discoveries of early Cenozoic cycads that represent undersampled clades, regions, and organ types are needed to fill the large temporal and geographic gaps in lineage histories and to test the idea that crown groups of cycad genera are not ancient.

The Patagonian region of southern South America is notably rich in cycad remains, primarily from the Mesozoic as for the rest of the world, but it lacks any living cycads (Archangelsky 1963; Artabe and Stevenson 1999; Tang 2006; Taylor et al. 2009; Cúneo et al. 2010). One classic Gondwanan group that is long extinct from South America but well-represented there as fossil stems is Tribe Encephalarteae of Family Zamiaceae. The living representatives of this tribe are disjunct in Africa (*Encephalartos*, ca. 65 species) and Australia (*Lepidozamia*, two species; *Macrozamia*, ca. 41 species; Johnson 1959; Goode 1989; K.D. Hill 1998; Calonje et al. 2013–2016).

There are numerous Patagonian records of Late Cretaceous to possibly early Paleocene petrified-stem genera allied with Encephalarteae, including *Menucoa*, *Neochamberlainia*, *Wintucycas*, and *Worsdellia* (Petriella 1969; Artabe and Stevenson 1999; Artabe et al. 2004, 2005; Cúneo et al. 2010; Martínez et al. 2012). If it is Paleocene, *Menucoa* is also the heretofore "youngest Patagonian cycad." Petrified trunks of *Fascisvarioxylon* (Jain 1962; Artabe et al. 2005; Salas-Leiva et al. 2013), from Early Cretaceous strata of the Rajmahal Hills, Amarjola, India (Banerji 2000), and *Cetricycas*, from the Late Cretaceous of James Ross Island, Antarctica (Cantrill 2000), further demonstrate the glorious Gondwanan history of Encephalarteae.

The youngest fossils of the tribe, which are also the only previous foliar remains and the only Encephalarteae fossils located in any proximity to extant representatives, are Australian Paleogene leaf fragments with cuticle assigned to two species of *Lepidozamia* and one of *Macrozamia* (Cookson 1953; R.S. Hill 1980; Carpenter 1991). The oldest of these is *Lepidozamia foveolata* R.S. Hill (1980) from the middle Eocene Nerriga flora (Hill 1982), described from material with no preserved leaflet margins, bases, or apices. However, the cuticle of *L. foveolata* is nearly identical to living *Lepidozamia* (Thomas and Bancroft 1913; Cookson 1953; Pant and Nautiyal 1963; Greguss 1968). For this reason, *L. foveolata* is frequently used as a calibration for the *Encephalartos–Lepidozamia* divergence in molecular dating work (Nagalingum et al. 2011; Lu et al. 2014; Condamine et al. 2015). The other Australian fossils assigned to Encephalarteae are apparently post-Gondwanan, as summarized by R.S. Hill (1998): *Lepidozamia hopeites* (Cookson) L. Johnson is "probably Oligocene" (Bacchus Marsh site), and *Macrozamia australis* R.J. Carpenter comes from the early Oligocene Cethana site.

The outstandingly preserved and extremely diverse Laguna del Hunco biota from Chubut, Argentina, currently stands as the most informative paleofloral record for the entire South American continent during the global warmth of the early Eocene (Berry 1925; Wilf et al. 2003, 2013). The assemblage represents the early Eocene climatic optimum, the terminal phase of Gondwana, and the western end of an extinct, trans-Antarctic rainforest biome; thus, the Laguna del Hunco flora is uniquely relevant to studies of Gondwanan paleobiogeography and evolutionary timing, among many other topics (Romero 1978; Wilf et al. 2013; Kooyman et al. 2014; Merkhofer et al. 2015; Wilf and Escapa 2015). Numerous fossils of extant Old World rainforest taxa that are now extinct in South America have been recently discovered at Laguna

del Hunco. Most of these are unknown from any other South American fossil sites, except in several cases from the nearby, early middle Eocene Río Pichileufú locality. Examples include the fern *Todea* (Osmundaceae; Carvalho et al. 2013); diverse gymnosperms detailed below; two Laurales with respective affinities to Australian *Wilkiea* (Monimiaceae) and *Daphnandra* (Atherospermataceae; Knight and Wilf 2013); and the eudicots *Akania* (Akaniaceae), *Eucalyptus* (Myrtaceae), and *Gymnostoma* (Casuarinaceae; Romero and Hickey 1976; Gandolfo et al. 1988, 2011; Zamaloa et al. 2006; Hermsen et al. 2012).

The Laguna del Hunco flora is well-dated, coming from a 170 m stratigraphic section that is temporally calibrated to ^{40}Ar–^{39}Ar ages from three analyzed volcanic tuffs and two paleomagnetic reversals, all located within the fossiliferous caldera-lake beds (Wilf et al. 2003). The most reliable radiometric age is from analyses in two different labs of multiple single crystals of sanidine from a tuff (sample 2211A) located in the middle of the most fossiliferous interval (Wilf et al. 2003, 2005). The most recently revised age from those analyses is 52.22 ± 0.22 Ma (Wilf 2012), which represents a working age for the entire flora (see also the section on Materials and methods). Thus, the Laguna del Hunco biota represents both the final moments of Gondwana, just before the separation of South America and Antarctica began (Lawver et al. 2011), and the sustained global warmth of the early Eocene climatic optimum (Zachos et al. 2008).

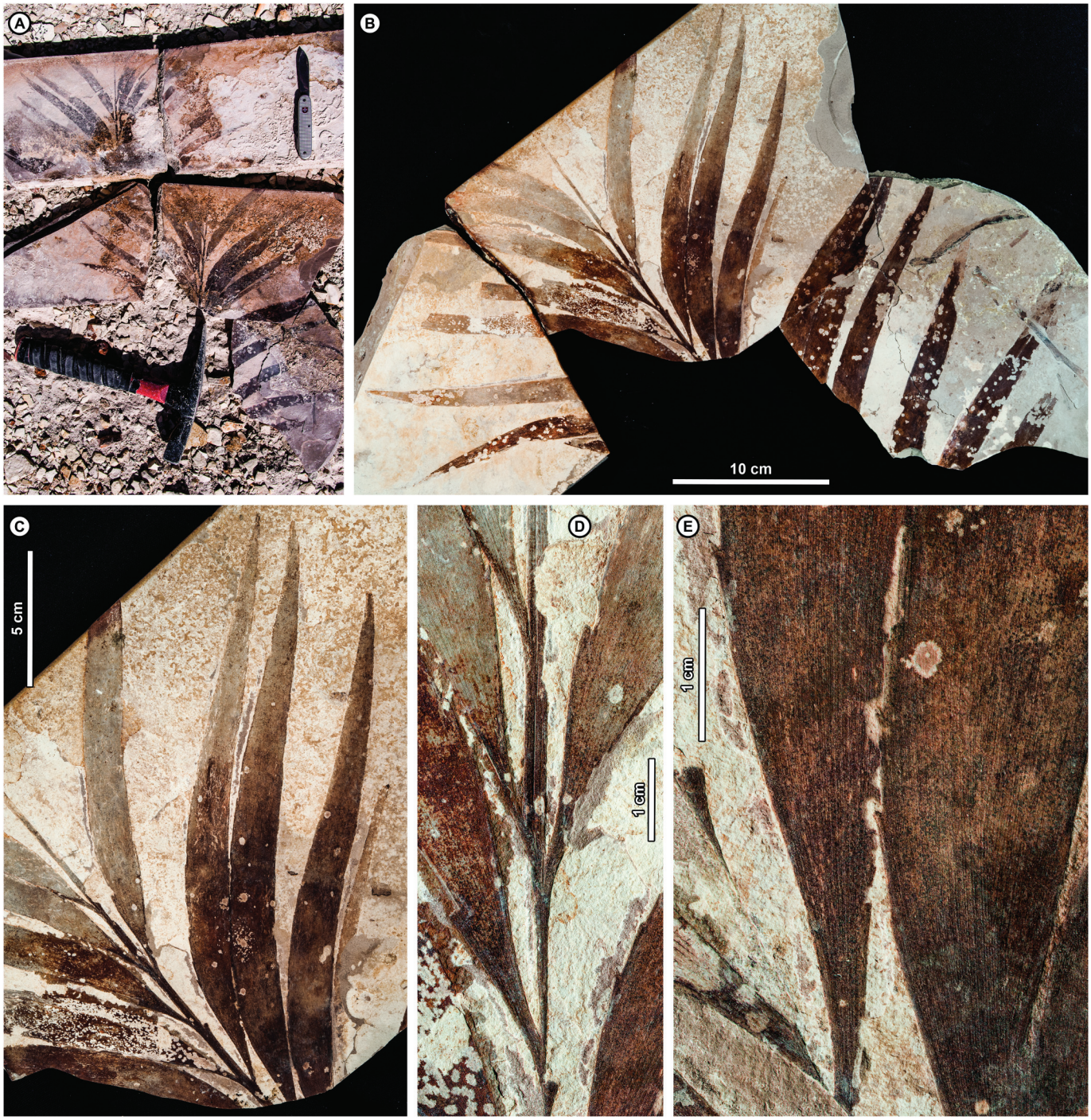
The Laguna del Hunco flora was originally presented as a small, single-drawer type collection of putatively Miocene angiosperm remains (Berry 1925). However, it is now recognized as an assemblage of >200 species and morphotypes of early Eocene plant organs from ca. 6000 recently collected specimens, dominated by angiosperms but also including ferns and gymnosperms (Wilf et al. 2005; Carvalho et al. 2013; Merkhofer et al. 2015). The gymnosperm flora, which we contribute to here, primarily represents living Old World rainforest taxa that are also known as fossils elsewhere in Gondwana, especially southeastern Australia (e.g., Hill and Brodribb 1999). The conifers include *Papuacedrus prechilensis* (Berry) Wilf, Little, Iglesias, Zamaloa, Gandolfo, Cúneo et Johnson (Cupressaceae); *Araucaria pichileufensis* Berry of *Araucaria* Section *Eutacta* and *Agathis zamunerae* Wilf (Araucariaceae); and diverse Podocarpaceae including *Acmopyle engelhardti* (Berry) Florin, *Dacrycarpus puertae* Wilf, *Podocarpus andiniformis* Berry, and an undescribed species of *Retrophyllum* (Wilf et al. 2005, 2009, 2014; Wilf 2012). All of these conifer taxa are also found at reduced relative abundance in the early middle Eocene Río Pichileufú flora. Significantly, several of the recent conifer revisions, as for many of the flora's other components, have been from New to Old World taxa. Of particular interest here, the leafy branches of *Agathis zamunerae* were once considered to be fronds of the New World cycad genus *Zamia* (Berry 1938; Wilf et al. 2014). In addition to the conifers, Villar de Seoane et al. (2015) recently revised *Ginkgoites patagonicus* (Berry) Villar de Seoane, Cúneo, Escapa, Wilf et Gandolfo from Laguna del Hunco and Río Pichileufú; this species represents the last South American occurrence of the ginkgophytes, another living seed-plant lineage with Paleozoic origins (e.g., Taylor et al. 2009). *Ginkgoites patagonicus* is a potential close relative of *Ginkgo australis* (McCoy) Drinnan et Chambers, which last appears in the Paleogene of Tasmania (Hill and Carpenter 1999).

The final, extremely rare (ca. 0.001% relative abundance) component of the Laguna del Hunco gymnosperm flora as so far known had been fragments of toothed cycad leaflets with no cuticle or attachments preserved. One of these was briefly illustrated in an overview paper (Wilf et al. 2003) as "cycad leaf similar to extant *Dioon*." However, during a 2009 expedition to Laguna del Hunco, a large frond piece was recovered that preserves multiple leaflets attached to a rachis (Fig. 1). Macroscopically, this specimen closely resembles *Encephalartos*, which has no reliable fossil record (Kvaček and Velitzelos 2000). However, the same specimen preserves abraded, in-situ adaxial cuticle that cannot be removed for mounting, but whose coarse features can be studied using epifluorescent light. This cuticle's morphology is not distinguishable from that of *Lepidozamia*. We here present all these cycad remains, the youngest known from Patagonia by more than 10 million years (*Menucoa*), as an extinct genus and species of Zamiaceae and consider their evolutionary and biogeographic significance.

Materials and methods

One frond segment and six leaflet fragments of cycads (Figs. 1, 2A–2F and 3A–3E) were recovered from previously described quarries LH04, LH13, and LH27 (Wilf et al. 2003; Gandolfo et al. 2011) of the Tufolitas Laguna del Hunco (Aragón and Mazzoni 1997), Huitrera Formation, early Eocene of Chubut Province, Patagonia, Argentina, during several expeditions from 1998 to 2009 launched from Museo Paleontológico Egidio Feruglio (MEF) in Trelew, Chubut. Geographic and stratigraphic data for all Laguna del Hunco fossil sites have been given in recent papers (Wilf et al. 2003; Wilf 2012), and additional locality data are available to qualified researchers from P. Wilf and the Museo Paleontologico Egidio Feruglio (MEF) Collections staff. Quarries LH13, LH04, and LH27 sit near the bottom, middle, and top, respectively, of the ca. 60 m densely fossiliferous middle interval of the volcanic lake sequence (Wilf et al. 2003, 2005), showing that cycad plants were present around the ancient caldera lake throughout its interval of deposition. Also, a spiny petiole fragment that appears to represent a cycad (Fig. 3F–3H) was found at quarry LH16, which is at the same stratigraphic horizon as quarry LH04 (Wilf et al. 2003). Quarries LH04 and LH16 lie only 0.4 m beneath the

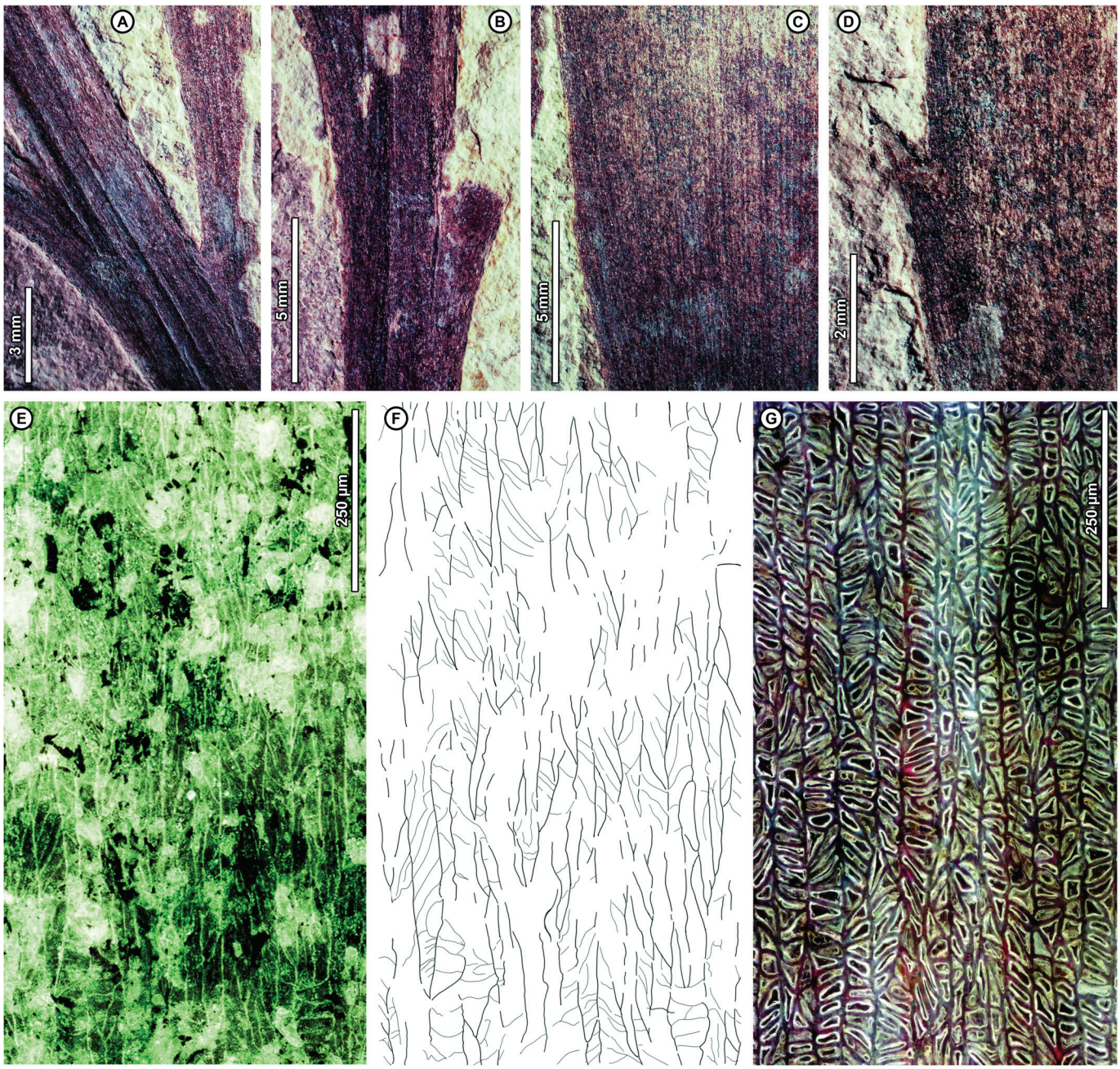
Fig. 1. ***Austrozamia stockeyi*** gen. et sp. nov., holotype, MPEF-Pb 8340. (A) Discovery photograph, 21 November 2009, showing all parts and counterparts (counterpart is the slab at top underneath penknife); (B) cleaned and prepared frond portion from center of (A); (C) detail of frond portion at center of (B) preserving leaflet attachments and the most complete leaflets; (D) detail of lateral, decurrent leaflet insertion, finely tapered bases, and dichotomizing parallel venation of the distalmost leaflets shown in (C); (E) detail of the proximal leaflet bases shown in (C), showing a well-preserved, spiny tooth at upper left (also Fig. 2D), portions of several other teeth, and closely spaced, dichotomizing parallel veins. [Colour online.]



aforementioned volcanic ash that yielded the ^{40}Ar–^{39}Ar age of 52.22 ± 0.22 Ma on sanidine (Wilf et al. 2003; Wilf 2012). All specimens are accessioned at MEF under repository acronym MPEF-Pb.

Specimens were prepared and photographed at MEF using the same techniques that were detailed in recent papers on fossils from this site (e.g., Wilf 2012), including manual removal of matrix using standard air and hand tools; DSLR photography using several cameras (Nikon D90, Nikon D700, Canon EOS 7D, and Canon EOS Rebel T2i; Canon Inc. and Nikon Inc., both Tokyo, Japan); and examination and microphotography using Nikon SMZ

Fig. 2. ***Austrozamia stockeyi*** gen. et sp. nov., holotype, MPEF-Pb 8340 (A–F) and adaxial cuticle of extant *Lepidozamia peroffskyana* Regel (G). (A–D) Selected details of areas shown in Figs. 1D and 1E, including striated rachis and lateral, decurrent leaflet insertion (A–B, insertion best seen in left leaflet of each pair, right-hand leaflets damaged), parallel venation (C), and a well-preserved tooth (D); (E) in-situ adaxial cuticle remains under epifluorescence, oriented apex up, showing obliquely oriented cells organized in elongate rows with asymmetrically tapered ends; light and dark areas indicate abrasion or other degradation; (F) overlay drawing of epidermal cell-wall remains visible in (E); (G) adaxial cuticle of *L. peroffskyana* under epifluorescence, for comparison to (E–F); Little & Stevenson 1050 (NY). [Colour online.]



1000 binocular and Eclipse 50i compound microscopes, each with dedicated Nikon DS-Fi1 cameras. Long-pass green filter epifluorescence was used on the Eclipse 50i, especially for imaging cuticle in-situ on the holotype designated below with no preparation (Fig. 2E; filter specifications: exciter HQ470/40, dichroic Q495LP BS, emitter HQ500LP). Removal and mounting of the highly fragmented, thinly preserved cuticle for further study was not feasible. An overlay drawing (Fig. 2F) of the epidermal cell walls visible in Fig. 2E was done by R. Wilf using a Cintiq Companion graphics tablet (Wacom Technology, Portland, Oregon, USA). Material from New York Botanical Garden (NY) of both extant *Lepidozamia* species was examined at the Penn State Paleobotany Laboratory using an epifluorescent filter with specifications identical to that described above on a Nikon LV100 compound

Fig. 3. ***Austrozamia stockeyi*** gen. et sp. nov., selected leaflet fragments (A–E) and associated spiny petiole (F–H). (A–B) Leaflet with characteristic asymmetrical, finely tapered base (insertion not preserved), spiny teeth on both margins, and venation displayed with good contrast (large cracks that traverse the whole leaflet surface are preservational; light area at top of both images is weathered matrix), MPEF-Pb 470, the specimen illustrated in Wilf et al. 2003; (C–D) leaflet with spiny teeth well preserved on both margins and detail (D) under epifluorescence of middle tooth on right margin from (C), MPEF-Pb 8342; (E) leaflet with one large spiny tooth at lower right and at least two additional fragmentary teeth, MPEF-Pb 8343; (F–H) spiny petiole and selected details of the spines, MPEF-Pb 8346, note the dark, unweathered, extensively prepared axis portion to upper left and the white, weathered, unprepared portion at bottom right of (F). [Colour online.]

microscope with a Nikon DSRi1 CCD camera; *z*-stacking was used for imaging to accommodate uneven surfaces (Fig. 2G; Adobe Photoshop CC Align and Blend tools; Adobe Systems Inc., San José, California, USA). Standard whole-image adjustments of exposure, contrast, color temperature, and white and black levels were performed using Adobe Camera Raw CC Editor to maximize visibility of diagnostic features.

We attempted several cladistic analyses, using a matrix modified from that of Hermsen et al. (2006) to reflect newly described characters for the Patagonian fossils and to include several Cenozoic Australian cycad fossils. Unfortunately, the new fossils represent portions of compressed foliage without stomatal preservation; thus, they could not be scored for 90% of the 72 characters in the matrix, including all those related to stem anatomy, stomatal configuration, or reproductive organs. Moreover, as mentioned earlier, resolution of cycad fossils from morphological trees is limited because whole plants are not known, and most fossil cycad genera are preserved as either foliage or stems. The combination of missing data for the new taxon and mutually exclusive characters among cycad fossils, in general, prevented any meaningful cladistic assessment of phylogenetic position in relation to the critical taxa *Dioon*, *Encephalartos*, *Lepidozamia*, and *Macrozamia*, along with their fossil relatives. The eventual discovery of specimens preserving sets of the missing characters is probable at the prolific Laguna del Hunco site or elsewhere and would be likely to remedy this situation. In the meantime, the new fossils provide ample character information for the traditional systematic treatment that appears here.

Cycad taxonomy follows Stevenson (1992), which remains the most widely used formal classification. We acknowledge ongoing, significant revisions of cycad relationships from molecular phylogenetic analyses (e.g., Salas-Leiva et al. 2013; Condamine et al. 2015). However, the Encephalarteae clade of significance here and its three constituent genera (*Encephalartos*, *Lepidozamia*, and *Macrozamia*) are well-supported in both Stevenson (1992) and most molecular phylogenies, and our results do not otherwise depend on the complete cycad topology. For paleogeographic discussion, we use the separation times of ca. 114 Ma for India–Antarctica, 112–106 Ma for South America–Africa, ca. 50 Ma for South America–Antarctica, and ?middle Eocene for Australia–Antarctica (Wilf et al. 2013, per Lawver et al. 2016).

Results

Systematics

Family	Zamiaceae Horan.
Subfamily	Encephalartoideae D. Stevenson
Tribe	Encephalarteae Miquel

Austrozamia Wilf, D. Stevenson, et Cúneo, gen. nov.

DIAGNOSIS: Leaflets sparsely toothed, the tooth apices pungent to slightly rounded; bases acute and asymmetrical, tapering smoothly to a narrow, lateral, decurrent insertion on the rachis. Venation parallel, dense, and dichotomizing with no midvein. Adaxial epidermal cells arranged in elongate rows that parallel venation, the ends of the rows asymmetrically tapering to a point, the long axes of the cells oriented markedly obliquely to the direction of venation and with thicker walls at row boundaries than between rows.

TYPE SPECIES: ***Austrozamia stockeyi*** Wilf, D. Stevenson, et Cúneo, sp. nov. (Figs. 1, 2A–2F, and 3A–3E).

TYPE MATERIAL AND PROVENANCE: As for the type species, owing to monotypy.

ETYMOLOGY: Indicating the southern provenance of the material as well as the affinity to Family Zamiaceae. The early Eocene paleolatitude of Laguna del Hunco was approximately 47°S (Wilf et al. 2005).

DESCRIPTION: As for the type species, owing to monotypy.

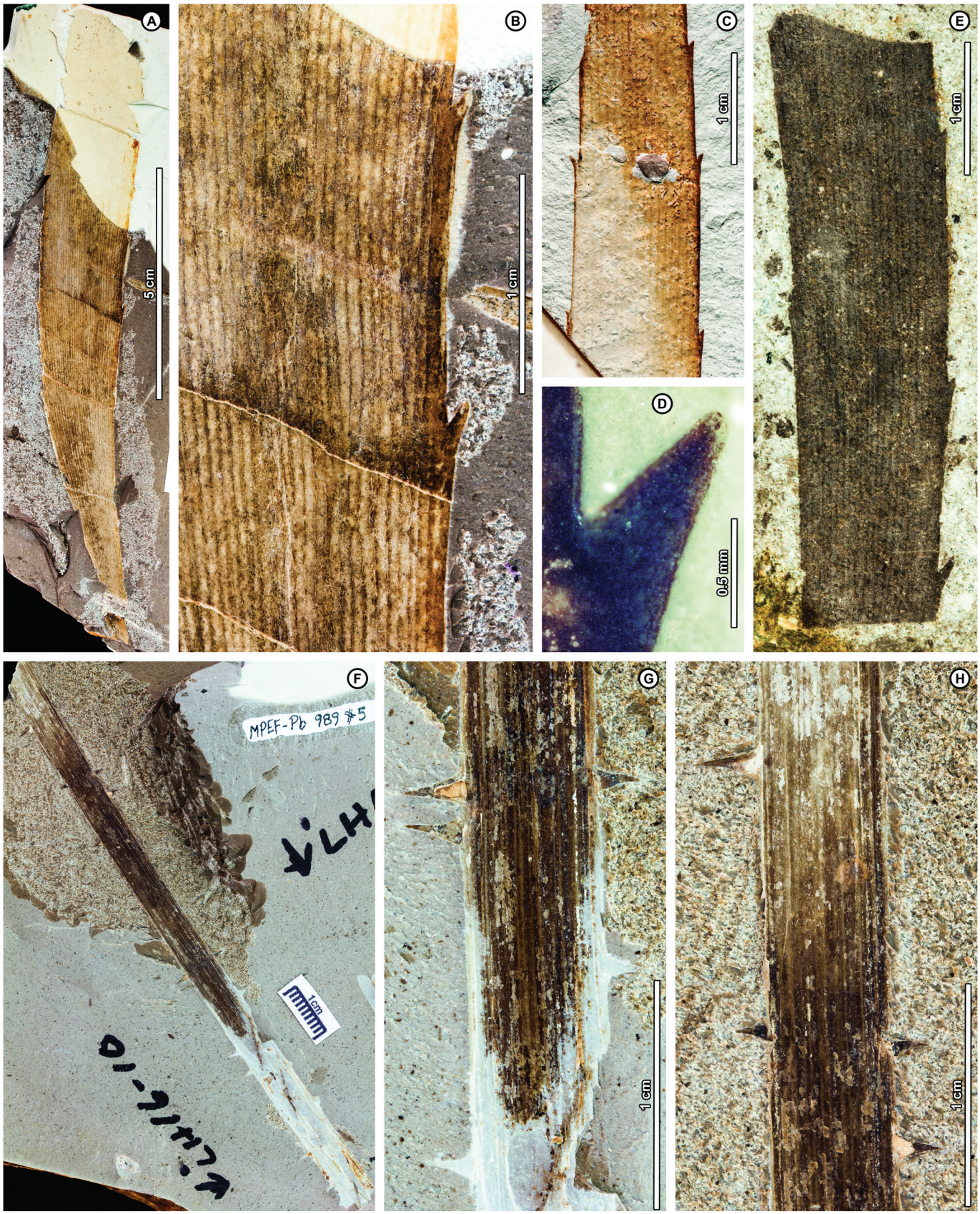
Austrozamia stockeyi Wilf, D. Stevenson, et Cúneo sp. nov.

DIAGNOSIS: As for the genus, owing to monotypy.

HOLOTYPUS HIC DESIGNATUS: MPEF-Pb 8340 (Figs. 1 and 2A–2F), from quarry LH27, early Eocene Tufolitas Laguna del Hunco, Huitrera Formation, Chubut, Argentina. Collected by P. Wilf, 21 November 2009.

PARATYPES: MPEF-Pb 470 (Figs. 3A and 3B) and 8344 from quarry LH4 or same stratigraphic level; MPEF-Pb 8341 and 8342 (Figs. 3C and 3D) from quarry LH4; MPEF-Pb 8343 (Fig. 3E) from quarry LH13; and MPEF-Pb 8345, found as float from an unknown source level. All from the early Eocene Tufolitas Laguna del Hunco, Huitrera Formation, Chubut, Argentina.

ETYMOLOGY: In honor of Dr. Ruth A. Stockey and her numerous seminal paleobotanical and neobotanical contributions to knowledge of gymnosperm evolution, including Patagonian fossil gymnosperms.



DESCRIPTION: Leaf pinnate, with a grooved rachis to 3.3 mm width, at least 13 leaflet pairs preserved on the holotype. Leaflet arrangement opposite to subopposite, insertion decurrent and lateral on rachis, adjacent leaflets slightly overlapping, length to 213 mm, width to 22 mm. Base very narrow and subpetiolulate at insertion, width to 2.0 mm at intersection of distal margin with rachis, shape asymmetrical with distal flank markedly more convex than basal flank. Blade asymmetrical, ovate, with maximum width at ca. one-third the length, then tapering gradually and with increasingly straight margins to a narrow-acute but not pungent apex. Teeth triangular, irregularly spaced and well separated, to 2.3 mm length, expressed on both margins, apices pungent to slightly rounded. Venation parallel, dense, and dichotomizing, with no midvein; veins and fibers apparently both present but not reliably distinguishable, anastomoses not observed (potentially due to preservation). Oblique linear features of uncertain origin traverse the vein fields (Figs. 3B, 3C, and 3E). Adaxial epidermal cells (Figs. 2E and 2F) arranged in elongate, mostly uniseriate rows that are parallel to venation, row length variable to 540 μm, row width variable to 40 μm, the ends of rows asymmetrically tapered and pointed; cells irregularly angled with long axes markedly oblique to venation, cell walls thicker at row boundaries than between rows. Cell pattern not varying over veins. Pitting, stomatal architecture, and abaxial epidermis not preserved or too degraded to recognize.

Notes

Previous informal references to specimens here assigned to *Austrozamia stockeyi* are: "cycad leaf similar to extant *Dioon*" (Wilf et al. 2003: Fig. 1I; that specimen, MPEF-Pb 470, shown here in Figs. 3A and 3B); "cycad aff. *Dioon*," morphotype TY016 (Wilf et al. 2005: Table A2); and "this cycad closely resembles the African genus *Encephalartos*" (Wilf et al. 2014: 173).

After observing the linear features that traverse fields between primary veins obliquely in *Austrozamia* (Figs. 3B, 3C, and 3E), we quickly found similar features in many living cycads, apparently never before described. They superficially resemble the cross-veins of monocots, but no cross-veins are visible in any cleared cycad foliage known to us, and they are far too large to be related to the oblique epidermal cells. Also, we cleared and examined leaflets of both extant *Lepidozamia* species and several *Encephalartos* species, and no veins or other transverse features were visible. Thus, we conclude that these lineations are not veins and are instead related to other leaf tissues requiring further anatomical study.

Associated petiole

The petiole specimen MPEF-Pb 8346 (Figs. 3F–3H) is from quarry LH16 of Wilf et al. 2003, a lateral quarry in the same fossiliferous bed as quarry LH4, which preserved several of the *Austrozamia* paratypes listed above. This material is likely to represent a petiole of the *Austrozamia* plant, but without organic attachment, we do not formally assign it to any taxon. The petiole fragment is 122 mm long (broken at both ends, presumably much longer in life) and to 6.5 mm wide, with at least 13 laterally-inserted spines preserved along one margin and at least six on the other. The spines are pungent and slightly curved, ranging to 2.1 mm basal width and 3 mm in length.

Discussion

Austrozamia stockeyi has numerous features that indicate it is a cycad, including its large, pinnately compound frond with unlobed, minutely toothed, densely parallel-veined, decurrent leaflets and its distinctive epidermal cell pattern. These and other features can also be used to readily discriminate *Austrozamia* from other taxa that have parallel-veined foliage in the rich Laguna del Hunco flora. *Agathis zamunerae* leaves differ from *Austrozamia* leaflets in being simple, dehiscent, and untoothed and in their opposite (subopposite)-decussate arrangement, with distichous deployment via basal twisting, on branches (not rachises; Wilf et al. 2014). Although *Agathis zamunerae* leaves have strongly narrowed bases somewhat like *Austrozamia* leaflets, these are comparatively more symmetrical and straight-sided near insertion in the fossil *Agathis*, giving the appearance of a short petiole (false petiole). *Ginkgoites patagonicus* leaves are easily distinguished from *Austrozamia* leaflets because they are compound-lobed, untoothed, and usually preserved with thick cuticles whose epidermal cell pattern is quite different from that of *Austrozamia* (Berry 1935; Traverso 1964; Villar de Seoane et al. 2015). Several monocot leaf morphotypes are present in this flora, preserved as strap-shaped pieces of leaves and leaflets from palms and potentially other taxa not yet diagnosed (Wilf et al. 2005). These differ from *Austrozamia* leaflets in having midveins, a much more elongate aspect, and often oblique cross-veins.

Austrozamia qualifies as a new, extinct genus because it has the unique combination of *Lepidozamia*-type cuticle and macromorphology most similar to *Encephalartos*, as discussed below. There are no previously described extinct cycad genera that are remotely similar to *Austrozamia* (see, e.g., R.S. Hill 1980; Hill and Pole 1994; Watson and Cusack 2005; Erdei and Manchester 2015).

Among the living cycads, the new fossil taxon most resembles the three Encephalarteae genera and *Dioon* (15 living species, Central America). Close relationships of the fossils to the six other living cycad genera can be easily excluded (for cycad morphology see, e.g., Greguss 1968; Stevenson 1990; Jones 2002). Only two of these six have any living species with toothed leaflets (*Bowenia* and *Zamia*), and none has the distinctive epidermal cell pattern shared by the fossils and *Lepidozamia* (discussed below). In addition, *Cycas* and *Stangeria* leaflets have

midveins and many other differences from the fossils in venation, and *Stangeria* also has distinctive fern-like foliage. *Bowenia* leaflets, unlike the fossils, do not overlap along the rachis, and they have considerably more convex leaf margins as well as strongly falcate asymmetry. Their only similarity to the fossils is the presence of subpetiolulate leaflets. In the sole toothed species, *B. serrulata*, the teeth are much more densely distributed along the margin than in the fossils, and the tooth morphology, including relatively pronounced, often rounded apical sinuses, is quite unlike that of the fossils. Fossil *Bowenia* foliage from several early and middle Eocene Australian sites (R.S. Hill 1978, 1998) resembles that of extant *Bowenia* species and is not similar to *Austrozamia*. *Ceratozamia*, *Microcycas*, and *Zamia* have articulated, non-decurrent leaflets, unlike the inarticulate and decurrent leaflets of the fossils.

In its macromorphology, *Dioon* is the most similar genus to the fossils that is outside Encephalarteae, whereas its cuticle is completely different (e.g., Barone Lumaga et al. 2015). As mentioned above, the taxon described here was first illustrated from a leaflet fragment as "cycad leaf similar to extant *Dioon*" (Wilf et al. 2003). However, the *Austrozamia* holotype presented here was subsequently discovered (Fig. 1); its leaflet bases are laterally decurrent, as in *Dioon*, but they are also finely tapered and subpetiolulate at insertion, quite unlike the broad, often expanded insertion of all living *Dioon* species and of their extinct relatives in *Dioonopsis*. The differences in leaflet base morphology, along with the dissimilar cuticle, appear to rule out any close affinity of *Austrozamia* and *Dioon*.

Within extant Encephalarteae, the fossils show more convincing affinities in gross morphology to *Encephalartos* than to the Australian genera *Lepidozamia* and *Macrozamia*. All three of these genera feature decurrent leaflets without midveins that display some degree of basal narrowing (unlike *Dioon*), but only *Encephalartos* has species with toothed leaflets, as in the fossils. Moreover, *Lepidozamia* leaflets are inserted adaxially on the rachis, and *Macrozamia* leaflets have basal, raised callosities; both of these features are quite conspicuous on those genera but absent in the fossils and *Encephalartos*, where the leaflets insert laterally and lack callosities. The marked basal narrowing of the fossil leaflets is most similar to *Encephalartos* in its degree and pronounced asymmetry. In many *Encephalartos* species, the leaflet bases are abruptly pinched rather than smoothly tapered as in the fossils; however, several species and ecotypes do have smoothly tapered bases, although these do not narrow so finely as the fossils (e.g., *Encephalartos villosus* Lem.; Giddy 1984; Goode 1989). Thus, without the information from cuticle, the fossils would best align with *Encephalartos* based on a characteristic combination of leaflet features, including lateral insertion, asymmetrical and strongly narrowed bases, absence of basal callosities, and presence of teeth.

In contrast, the adaxial epidermal cell pattern of the fossils (Figs. 2E and 2F) is considerably more similar to *Lepidozamia* (Fig. 2G) than to *Macrozamia* or *Encephalartos* (Thomas and Bancroft 1913; Cookson 1953; Pant and Nautiyal 1963; Greguss 1968; R.S. Hill 1980). Although *Encephalartos* has several species with oblique adaxial epidermal cells (*Macrozamia* lacks this trait), these are usually deployed in extended longitudinal rows and not configured in shorter, asymmetrically tapered rows. Non-random clustering of both thin- and thick-walled cells, as seen at the thickened walls of row boundaries in the fossils, has been observed in *Lepidozamia*, specifically, which also shows epidermal cell organization in asymmetrically tapered rows as in the fossils (Fig. 2G; Greguss 1968: Plate CXX, Fig. 1). The adaxial epidermal cells of the Australian fossil *Lepidozamia foveolata* (R.S. Hill 1980: Fig. 4) are also strongly organized into tapered rows, with thickened cell walls at row boundaries.

Our discovery of cuticle characters typical of *Lepidozamia* on an extinct genus shows that these features are plesiomorphic, not apomorphic for *Lepidozamia* as previously considered. Nevertheless, we find that the most reasonable interpretation of the fossils, pending more character data becoming available, is that the combination of *Encephalartos*-type frond morphology and *Lepidozamia*-type cuticle allies *Austrozamia* with Encephalarteae, presumably at a position below the crown node.

The isolated spiny petiole (Figs. 3F–3H) is compatible with many cycad genera, including *Cycas*, *Dioon*, *Macrozamia*, and *Encephalartos*, but not *Lepidozamia*, which does not have spiny petioles. *Encephalartos* and *Dioon* more commonly display a graded leaf-reduction series towards the base, but several species, such as *Encephalartos ituriensis* Bamps et Lisowski and *Encephalartos laurentianus* De Wild., can feature extended petiole sections with spines and no leaves as in the fossil petiole (Goode 1989; Heibloem 1999).

The Paleogene *Lepidozamia* and *Macrozamia* fossils described from Australia were based on leaflet fragments with cuticle, and their general frond morphology is mostly unknown (Cookson 1953; R.S. Hill 1980; Carpenter 1991). Based only on comparable leaflet fragments, the Patagonian fossils presented here could also have been assigned to *Lepidozamia*, but their *Encephalartos*-like frond morphology clearly places them outside that genus. Thus, the discovery of the *Lepidozamia*-type epidermal cell pattern in a demonstrably extinct genus raises the possibility that the Australian *Lepidozamia* fossils could also represent extinct taxa. In this light, *L. foveolata* (and *Austrozamia*) should now be used to constrain more basal nodes of the cycad phylogeny than the *Lepidozamia–Encephalartos* divergence as commonly practiced (Nagalingum et al. 2011; Lu et al. 2014; Condamine et al. 2015).

The living Encephalarteae inhabit a range of environments, including humid subtropical and montane rain-

forests in both Africa and Australia (Goode 1989; K.D. Hill 1998; Heibloem 1999) that broadly fit recent paleoenvironmental interpretations of ancient Laguna del Hunco (Wilf et al. 2009; Carvalho et al. 2013). Both *Lepidozamia* species and several *Macrozamia* species inhabit Australian rainforest areas that Merkhofer et al. (2015) identified as particularly rich in Laguna del Hunco "survivor" taxa. Remarkably, the fossil osmundaceous fern *Todea amissa* M. Carvalho associated with *Austrozamia* at Laguna del Hunco (Carvalho et al. 2013), and extant *Todea barbara* (L.) T. Moore associates not only with *Lepidozamia* and *Macrozamia* in Australia (K.D. Hill 1998) but also with *Encephalartos* on African mountains (Goodier and Phipps 1960).

Austrozamia is a new Gondwanan contribution to the growing list of extinct cycad taxa with deceptive similarities to living genera (e.g., Horiuchi and Kimura 1987; Hill and Pole 1994; Kvaček and Manchester 1999). The discovery broadly supports the idea that cycad diversity has declined drastically not only since the Mesozoic, but even since the early Cenozoic, and that crown groups of living cycad genera are the geologically young descendants of the depleted survivor pool (e.g., Salas-Leiva et al. 2013; Condamine et al. 2015).

The presence of early Eocene *Austrozamia stockeyi* in Patagonia and middle Eocene *Lepidozamia foveolata* in Australia suggests that the Encephalarteae occurred across Gondwana until its terminal phase, presumably including Antarctica in their range. This inference is similar to recent interpretations of numerous other genera now known as Paleogene fossils from Patagonia, southern Australia, and occasionally Antarctica (Hill and Brodribb 1999; Wilf et al. 2009, 2013; Carpenter et al. 2014; Kooyman et al. 2014). For the Encephalarteae, as for so many other examples (R.S. Hill 1994; Kooyman et al. 2014), Australia holds a singularly significant role as both fossil archive and refuge for surviving, formerly widespread Gondwanan plant lineages. The diversification of *Encephalartos* in Africa remains enigmatic without relevant African fossils. However, vicariant origins of *Encephalartos* from a Gondwanan ancestral stock appear very likely given the presence of Encephalarteae in India during the Early Cretaceous (*Fascisvarioxylon*), when India and Africa were still adjacent (Lawver et al. 2016).

Conclusions

Early Eocene ***Austrozamia stockeyi*** is the youngest cycad known from southern South America by more than ten million years. The new foliage fossils confirm the presence of Encephalarteae in South America, formerly known from Late Cretaceous and possible early Paleocene petrified trunk material, and their survival until the terminal phase of Gondwana. With *Austrozamia*, the Encephalarteae join many other taxa with significance to Gondwanan biogeography that make their last (or only) South American appearances at Laguna del Hunco or the early middle Eocene Río Pichileufú site. The living relatives of these associated taxa have defined the modern analog environments for *Austrozamia stockeyi* as relatively cool, everwet rainforests like those found in the subtropical Border Ranges of Australia, Southeast Asian tropical mountains, and elsewhere (Wilf et al. 2009; Kooyman et al. 2014; Merkhofer et al. 2015).

Austrozamia is a noteworthy new contribution to the accumulating evidence showing that environmental changes in Patagonia associated with Antarctic separation caused the regional extinction of the ancient Gondwanic rainforest biome and its characteristic flora, now including the once-diverse Patagonian cycads. The new extinct genus adds significant support to the emerging idea that there may have been no crown groups of cycad genera extant during the early Paleogene nor, by extension, on Gondwana.

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